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Each article should include an abstract of not more than 250 words. The abstract should give the rationale for the study, describe the methods, present the significant results and state succinctly the interpretation of the data.

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A review of laboratory safety in clinical chemistry

Osuji KC
Department of Chemical Pathology, Irrua Specialist Teaching Hospital, Irrua, Edo State Nigeria.

Abstract
Commitment to safety in the clinical laboratories has evolved over the years, there is an increasing awareness and commitment by both the employers and employees in the laboratories to ensure adequate safety and wellbeing of laboratory staff while at work. Provision of adequate laboratory safety costs money, in terms of: the cost of employment of personnel, cost of training and retraining of personnel on provision and maintenance of safety, and the cost of provision of equipment/instruments required to ensure the safety of staff at work. However, the benefits of ensuring safety of workers far outweighs the cost, thus safety should be a prerogative of everyone engaged in the clinical laboratory, either as a management staff or a technical staff. Available literature on laboratory safety in clinical chemistry was sourced for, using both manual and online library search. There are various regulations and guidelines in place in most countries of the world, guiding the safety of every laboratory worker. In most developed countries, these laws and guidelines are strictly enforced by various institutions and organs of government. Provision of safety in the laboratory requires a lot of commitment and support from all those involved, it also has all the advantages desired for improved productivity at work.

Keywords: Laboratory, safety, clinical, chemistry

Introduction
A daily commitment from all individuals in an institution is required for an effective safety and security program. It is important for all individuals at all levels to work together to eliminate the risk of exposure to hazardous materials and conditions in the laboratory.1 While it is recognized that laboratory safety is the duty of all, it is also accepted that the initiative for laboratory safety should come from all employers of labour in the laboratories and laboratory directors, who apart from being obligated, are expected to be liable for violations of safety or non-provision of safety measures in the laboratory. Safe operation of a clinical chemistry laboratory is multipronged, however the key elements required include (i) A formal safety plan, (ii) Documented policies and effective use of mandated plans and programmes in areas of chemical hygiene, control of exposures to blood borne pathogens, tuberculosis control and ergonomics. (iii) Identification of occupational hazards such as biological, chemical, fire and electrical hazards and clear identification and documentation of policies for employees to deal with each type of hazard. (iv) Recognition of additional important relevant safety areas of concern such as housekeeping, waste management/disposal, bioterrorism and chemical terrorism.2 In the United States of America, various bodies of the government, have developed guidelines and standards which guide the provision of safety of staff in the laboratory, they include: the Occupational Safety and Health Administration (OSHA), the Centre for Disease Control (CDC), the College of American Pathologists (CAP) and the Joint Commission (JC). It is important to note that the “Universal Precautions” widely used in laboratory all over the world is a derivative of the CDC guidelines on biosafety.3,4,5

Safety Program
It is advised that every clinical laboratory should implement its own formal safety program by either setting up a safety committee, if the laboratory is big or have a designated staff as a safety officer if the laboratory is small.
The safety officer or the safety committee has the responsibility of preparing and updating manuals that address safety policies and procedures, maintenance of records of training and continuing education and maintenance of records of exposure to hazardous materials. The safety officer is also responsible for ensuring that protective devices are available for use and are being properly and consistently used by all laboratory staff and that the laboratory is functioning as a safe working environment. An integral part of a laboratory safety program includes, education and motivation of all laboratory employees in all matters related to safety. All new staff should be trained and retrained on safety during orientation.

Other integral parts of the safety program include, proper handling, labeling, storage and disposal of chemicals, which could be incorporated in a chemical hygiene plan, types and location of fire extinguishers, provision of good chemical and fire hoods, safety of electrical fittings and installations, and ergonomic issues such as provision of appropriate furniture and equipment to protect laboratory staff against development of musculoskeletal disorders.

Another important component of the safety plan, is proper waste management and disposal. Regulatory bodies such as OSHA requires that the laboratory should provide its employees with protective equipment such as laboratory coats, scrubs, gowns, gloves, Google and eye protective devices, eye washers, safety showers, heat resistant hand gloves for handling hot items and a chemical fume hood which should be used to mix reagents, that are capable of producing toxic fumes and heat flammable solvents. Apart from providing safety equipment it is important that the laboratories, ensure that employees use them, this is achievable with proper education and institution of punitive measures for employees who refuse to use them. It is also advocated that, there is a need for regular internal safety inspections, by laboratory staff who are not necessarily members of the safety committee to independently assess, the level of safety and compliance to safety measures put in place by the laboratory management, as a form of internal quality control measure. Aside from this, safety inspection by statutory government and accreditation agencies at regular intervals are also encouraged.

Clinical chemistry laboratories in most developed countries are mandated by regulating agencies to have different plans to handle some major categories of hazards or potential sources of hazards in the laboratory. For instance, in the United States of America, the OSHA and CAP mandates all clinical chemistry laboratories to have a chemical hygiene plan, an exposure control plan, and an ergonomics plan, while the CDC insists on the institution of a tuberculosis control plan and a pandemics plan.

The Chemical Hygiene Plan: is aimed at protecting and educating of laboratory employees, it must have some major elements, such as a standard operating procedure for the handling of all chemicals used in a particular laboratory and a materials safety data sheet (MSDS) which defines the category of hazards associated with each chemical as supplied by the manufacturer. The chemical hygiene plan must also contain information on the list of all chemicals in the laboratory inventory, storage requirements, labeling requirements, list of personnel protective equipment, information on chemical waste removal, requirements for employee physicals and medical consultations, training and record keeping requirements. Furthermore each MSDS is expected to give information on the different components of a chemical substance, hazards associated with each component and recommended handling and disposal modalities. All this information should be made available to all staff. The chemical hygiene plan is usually coordinated by a designated chemical hygiene officer or the laboratory’s safety officer in the situation where the laboratory is a small one.

The Exposure Control Plan: This seeks to protect laboratory staff against exposure to blood borne pathogens and ensure that waste produced by the laboratory is properly handled. The plan is structured out with the active involvement and cooperation all laboratory staff and involves the classification of laboratory staff into groups ranging from I to III depending on their level of exposure to potentially infectious material, employees whose duty exposes them to infectious materials always are classified as group I, while those who are exposed sometimes are classified as group II and those whose duties never expose them to infectious materials are classified as group III. The exposure control plan must also have a data sheet of injuries sustained in the laboratory as well as interventions and outcome.

The tuberculosis control and the pandemic control plans, are meant to protect laboratory staff from
tuberculosis and public breakout of infections, while being more relevant in the medical microbiology laboratory, regulatory bodies often insist that they should be available in the clinical chemistry laboratory as well. Basically both plans contain measures to ensure early identification and isolation of cases among laboratory staff, environmental control measures, appropriate use of protective gears, education of laboratory staff and the early initiation of therapy.\textsuperscript{2,13}

The CAP requires laboratories to have an ergonomic plan, whose aim is to ensure that the work equipment, design and furniture, are constructed in such a way as to ensure that they do not predispose workers to musculoskeletal disorders. Elements of the plan include: routine laboratory activities, functionality of the workplace including, floor matting, lighting, noise level, equipment design etc.\textsuperscript{2}

Types of hazards in the clinical chemistry laboratory.

Four major categories of hazards are encountered in the clinical chemistry laboratory, they are chemical hazards, biological hazards, Fire hazards and electrical hazards.

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<td>- Concentrated acids and strong alkali should be dispensed with automatic dispensers.</td>
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<td>- Mix chemicals in a sink if possible.</td>
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<td>- Store minimal quantities in the laboratory.</td>
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<td>- Volatile chemical should be stored in a fume hood</td>
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<td>- Dispose properly</td>
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<td>- Ensure that the laboratory has an exposure control plan</td>
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<td>- All staff must adhere to the universal precautions.</td>
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<td>- Barrier protective measures such as hand gloves, laboratory coats, gowns, face shields and masks, must be made available to all staff.</td>
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<td>- Keep hands away from eyes, mouth and nose while in the laboratory</td>
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<td>- Keep centrifuges, water baths and ovens closed when in use</td>
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<td>- Decontaminate all surfaces and re-useable devices after use</td>
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<td>- Decontaminate all human fluids before disposal</td>
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<td>- Eating, drinking and smoking should be prohibited in the laboratory</td>
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| FIRE HAZARDS | - Provision of emergency exist in the laboratory |
| | - The appropriate type of fire extinguishers should be |
provided and stationed at strategic points in the laboratory.

- Safety showers should be provided
- Provision of safety blankets
- Every staff should be instructed on how to use the five protective devices.

**Chemical Hazards**

It is important that laboratory staff should have a good knowledge of all the chemicals they work with both routinely and occasionally, this is desirable for their own safety. All chemicals should be properly labeled, transported, and handled with care, for example chemicals in glass bottles should be transported in a plastic container so that in an event of breakage or spillage, the chemicals will still be retained within the plastic container, bottles containing chemicals should never be held by the neck but by the body and if possible with both hands. Caustic and concentrated acids should be mixed in a sink, with the staff wearing facemasks and eye protective devices, while flammable and potentially explosive chemicals should be handled in a protective glass hood. Water must never be added to acid but rather acid should be slowly and gently poured into cold water during dilution. Also great care should be taken when disposing of chemicals. Volatile, caustic or acidic chemicals are advisable disposed of commercially. Some chemicals such as perchloric acid which has the potentials to cause explosions when it comes in contact with organic materials should be disposed of cautiously.\(^2\)\(^,\)\(^8\) while sodium azide a component of some reagents used in the clinical chemistry laboratory has the potential to cause explosions, when it comes in contact with metals such as lead, copper and iron.\(^6\) It is also advised that only a minimal quantity of chemicals should be stored in the laboratory, this is to minimize risks of spillage, and vaporization of these chemicals which could lead to formation of toxic chemical fumes in the laboratory. In laboratories where compressed gases from refillable cylinders are used, say for electrolyte measurement and calibration gases for blood gas assessment, it is recommended the gas cylinders should be placed firmly in support bases securely strapped to the wall outside the laboratory and that they should be checked regularly for leakages.\(^2\)

**Biological Hazards**

Laboratory workers are exposed to hazards from infections biological agents such as hepatitis virus, human immunodeficiency virus (HIV), viruses causing viral hemorrhagic fever (VHF) and oftentimes drug resistant bacteria. Exposures could arise from accidental cuts and needle punctures, spilling of infected samples on the bench top, centrifuge accidents, or aerosol production from uncovered centrifuges and cuts or scratches from contaminated instruments.\(^2\)\(^,\)\(^6\) Handling of biological hazards is ordinarily encompassed in the exposure control plan, which each laboratory is expected to have. Also this is encompassed in the universal precautions document from the CDC which is use all over the world. This document mandates all laboratory staff to treat all human blood and bodily fluids as if they contain infectious agents.\(^1\)\(^,\)\(^4\) The universal precautions also specify that adequate, appropriate barrier protective gear such as hand gloves, laboratory coats, gowns, face masks, mouth piece resuscitation bags etc. are provided for use by laboratory staff. In addition recommendations were made for staff to protect themselves by engaging in safe laboratory practices such as: not indulging in mouth pipetting, wearing protective gear while in the laboratory, avoiding or minimizing spillage, washing of hands whenever gloves are changed, disposing needles and sharp objects in a sharps container, keeping hands away from their mouth, eyes and nostrils while in the laboratory, not eating drinking or smoking in the laboratory work area, not mixing infectious materials by bubbling air through it, avoiding the use of damaged laboratory wares like test tubes, pipettes or flasks, ensuring regular cleaning and decontamination of work area.\(^7\)\(^,\)\(^1\)\(^1\) In addition, it is mandatory that all laboratory staff be provided the option of receiving a free hepatitis B vaccination. This is in addition to training on work practice control.\(^2\)\(^,\)\(^6\) It is also recommended that all used samples should be decontaminated before disposal and should never be disposed into open drainages.\(^6\)\(^,\)\(^1\)\(^5\)

**Fire hazards**

Three classes of fire can occur in the clinical chemistry laboratory, they are: (i) class A fire, which is fire affecting ordinary combustibles such as wood, clothes, and paper, (ii) class B fire which involves inflammable liquids and gases and (iii) class C Fire which involves
operating electrical equipment, however once the electrical equipment is turned off, the fire reverts back to a class A or B. This classification is important because different types of fire require different types of fire extinguishers to put them off, available fire extinguishers include: the triclass dry chemical fire extinguisher which is particularly favoured for use in the laboratory, because apart from being effective against all the classes of fire, it poses limited hazard to personnel, the halotron fire extinguisher which though effective against all the classes of fire, has the disadvantage of being ineffective against deep seated fires, in addition to possessing some level of toxicity to laboratory personnel. Other types of fire extinguishers are the carbon dioxide, and the regular dry chemical extinguishers. Apart from fire extinguishers, safety showers and fire blankets are also recommended in every laboratory.2,6

**Electrical Hazards**

All sockets and electrical fitting, in the laboratory should be fused and grounded. Sockets should be about a foot above the bench top levels far away from sinks, while the use of power extension cords are prohibited. It is also advocated that electrical appliances should never be handled with wet hands, in events of liquid spills on electrical equipment, they should be turned off and dried properly before use.2

Proper housekeeping procedures such as cleaning of work benches with a 1:10 dilution of sodium hypochlorite at regular intervals, routine cleaning of waste cans and other receptacles, decontamination and cleaning of spillages as well as proper cleaning of contaminated laundry should also be put in place to ensure safety in the laboratory.

**Conclusion**

Working in a clinical chemistry laboratory, comes with a lot of exposure to different forms of hazard, great efforts are being made by regulatory agencies, laboratory directors and laboratory staff to ensure safety in the laboratory.

**References**


Automation in clinical chemistry: a review

Osuji KC

Department of Chemical Pathology, Irrua Specialist Teaching Hospital, Irrua, Edo State Nigeria.

Abstract

Sample analysis in most clinical chemistry laboratories are fast changing from a totally manual analytical platform to an automated/semi-automated platform, both in the developed and the developing climes. This is borne out of the necessity to improve turnaround time (TAT), minimize the numerous human errors inherent in the manual methods of sample analysis which in turn negatively influences the reliability of and clinical utility of produced results, reduce the overall the cost of production through reagent and manpower maximization, maximize profits and improve the total efficiency of the clinical chemistry laboratory. However, despite the desire and enthusiasm shown by pathologists and laboratorians, clinical laboratories especially in developing countries are still a long way from achieving optimal laboratory automation. Available literature on automation in clinical chemistry was sourced for using both manual and online library search. Automated laboratory platforms though initially capital intensive, are on the long run cheaper and more cost effective than manual platforms, their attraction and desirability is further horned by the constantly evolving novel technologies and improvements in design and function of automated systems. Many clinical laboratories are either introducing them or upgrading from one version to another superior one because of their benefits which far outweighs the cost. Laboratory automation is in fact the future trend of clinical laboratories and laboratory medicine.

Keywords: Automation, Clinical Chemistry

Introduction

Health care costs are becoming an increasingly important issue for both the health care provider and the patient, these cost are both in financial and time quantifications and they have adverse implications for the patient. In a bid to reduce these costs, laboratories are consistently being asked to reduce their own costs, improve turnaround time (TAT), expand their test menu and reduce laboratory errors. To meet these demands the laboratory has to be on the cutting edge of technology, and automation is the key to achieving these goals. Automation in clinical chemistry is defined as the process whereby an analytical instrument is used to carry out many tests with only minimal involvement of an analyst. Olsen K in 2012, observed that “laboratory automation today is a complex integration of robotics, computers, liquid handling and numerous other technologies”.

The evolution of automation in the clinical laboratories generally is in an almost equal competition with manufacturing industries globally. Its introduction into clinical laboratories has come with numerous advantages which includes improved turnaround times for all investigations, elimination of variability of results and errors of analysis through the eliminations of the tasks that are repetitive and monotonous for most individuals and reduction of cost of personnel because fewer staff are required when most laboratory processes are automated.

Furthermore by providing rapid turnaround time for critical tests, intra laboratory tracking of specimens, and preventing errors in specimen aliquoting, the benefits of automation can reach outside the laboratory to provide a positive impact on patient safety. Unlike in the past when automation was geared towards assisting the laboratory technologist to perform his duties, automation now includes processing and transport of specimens, loading of specimens into the automated analyzers, assessment of the results of the test performed and storage of specimens.
The primary goals of automation in the clinical chemistry laboratory include reduction in costs, expansion of laboratory testing to generate more revenue, reduction in turnaround time, reduction, in laboratory errors and improvement in laboratory safety, all of which will eventually positively impact on the care of patients.\textsuperscript{1,8} To achieve these goals, obtaining of an automation system requires the input of some core people who are the operators and the user of the laboratory, these includes the laboratory director, the laboratory technologists, hospital administrators and the medical staff who utilize the laboratory.\textsuperscript{1,7}

Automated analyzers generally incorporate the mechanized versions of basic manual laboratory techniques and procedures; thus modern instrumentation is packaged in a wide variety of configurations for the purpose of automation. The most common configuration is the random-access analyzer where analyses are performed sequentially on a collection of specimen with each specimen being analyzed for a different selection of tests. This approach allows measurement of a variable number and variety of analytes in each specimen.\textsuperscript{2}

This is a marked improvement from the continuous flow and centrifugal analyzers which were the earlier auto analyzers in use. Continuous, flow analyzers were the first automated analyzers developed, they were initially used in a single channel analysis, where they did the same single analysis on all samples. Later they were developed into the multiple channel analysis versions where they, performed the same multiple analyses on all samples whether desired or not, such that results of investigations not desired on any of the samples is discarded causing great wastage. It is an inflexible method and has long been replaced.

Then came the centrifugal analyzers which were an improvement over the continuous flow analyzers. They use discrete pipetting to load aliquots of specimen and reagents sequentially into the discrete chambers in a rotor and the specimens subsequently are analyzed in parallel by spinning the rotor to exert centrifugal force to mix the specimens and rotors and to drive the mixtures into cuvettes located on the periphery of the rotor. They are not versatile enough for use in the laboratory and has been replaced by the random access analysers.\textsuperscript{1,2}

**Laboratory automation**

The first practical and completely automated system for measuring urea, glucose and calcium, was developed by Leonard Skeggs in 1956. It performed blood analysis from start to finish without manual intervention by a technologist, with original models using a single channel continuous flow auto analyzer and later models using a multichannel continuous flow auto analyzer to analyze the samples.\textsuperscript{9,10,11,12} This innovation opened up a vigorous floodgate of interests in automating laboratory processes, with the production of the Robot Chemist by the Research Specialties Company in 1959. The Robot Chemist used discreet analysis with conventional cuvettes and automatic pipetting and mixing essentially automating all the manual steps carried out by technologists, it was however too mechanically complex to be practical at that time and went out of production in 1969, but not before charting the future of laboratory automation.\textsuperscript{11,13} Another approach to automation was introduced in 1968 by Norman Anderson with the advent of Centrifugal analyzers, which proved successful for about 20 years in clinical laboratories.\textsuperscript{13} The success of laboratory automation was further broadened by Sigma Chemical Company in St Louis Missouri, with the introduction of prepackage ready to use assay reagents, with instructions for use, this being a big boost considering that majority of analyzers use liquid reagent for analysis.\textsuperscript{11}

Three broad types of automation systems are currently in use clinical chemistry, they are (i) total laboratory automation (TLA), (ii) modular integrated automation and (iii) modular or stand-alone systems for front end sample processing and archiving and sample retrieval.

**Total Laboratory Automation (TLA)**

Total laboratory automation was defined by Hawker CD\textsuperscript{14} as automation that includes pre analytical and post analytical functions combined with analytical activities that are interfaced directly with the automation system. While Zaninotto M and Plebani M.\textsuperscript{15} defined it as” Human-less robotic laboratories that could allow better operations with less human errors”. TLA is by far the most innovative, and expensive of all laboratory automation systems, in terms of space, size and weight of the equipment, power requirement, complexity, and cost. TLA employs an integrated track system that
links all laboratory work stations including clinical chemistry, haematology, microbiology and immunology, thus creating a continuous comprehensive network. It has turnkey systems that take care of each stage of analysis such as sample sorting, centrifugation, aliquoting and analysis. At each stage errors such as poor sample labeling, inadequate samples presence of clots etc. are detected and such samples are passed into an exemption tray for manual analysis. Sorting is commonly by bar coding, each sample is labeled with a bar code which contains necessary information on the patients such as patient’s name, age, sex, clinic, hospital, requesting clinician and the investigations required. A bar code scanner within the automation unit is able to decipher this information and sorts out the samples accordingly. Improperly placed bar code labels will lead to sample exemption. Samples are then conveyed to a centrifuge for centrifugation after which they are sent to the aliquoting station where aliquots are made and sent to the necessary work stations such as chemistry, microbiology, haematology, coagulation etc. After analysis, the specimens are transported to the archiving workstation where they are unloaded from the sample carriers, scanned placed in racks recapped and stored in a refrigerated stockyard for sample retrieval in the case of a repeat testing or additional requests on the sample. At intervals samples in the stockyard are emptied into larger refrigerating units for long term storage, due to the limited space in the onsite stockyard. The advantages of TLA include the standardization of testing to improve patient care, almost total elimination of the always present human errors in the handling and testing of samples, reduction in sample handling steps, increase in laboratory productivity, decrease and standardization of TAT and most importantly it is adapted to handle large workloads. Because of the cost implications of TLA, a large test volume is required to justify it.1,3,11,16

It is estimated that a laboratory needs to process more the 2,500 samples per day to justify its use.1,8 TLA systems have been shown to decrease labeling errors by 27% and reduce turnaround times by 17% to 23%.5,17,18,19,20

Modular integrated systems (MIS)

Modular integrated systems (MIS) offer an alternative to total laboratory automation, MIS link together multiple laboratory disciplines into a single testing platform that is interconnected by a track. The track consists of two lanes with one lane allowing for rapid processing of stat specimens while the second lane handles the routine specimens. Its component segments are in modules or sections according to the choice or requirements of individual laboratories, such segments will include any of the following: specimen input area, bar code reading station, transport system, automated centrifuge, decapper station, recapper station, aliquoter, interface to automated analyzers, sorter, take out station, storage and retrieval stations and laboratory information system (LIS).1,2 Modular integrated systems are cheaper than TLA in terms of cost of installation, space requirement, training requirement, cost of use and flexibility. It also enables a more relaxed interface with laboratory information systems.

Stand-alone systems

Here specific segments of the laboratory processing of samples are automated and carried out specific equipment while other segments may be carried out manually an example is the front end sample processing unit in which samples are sorted, centrifuged uncapped and divided into aliquots if test for more than one work station has been requested and then manually fed into an auto-analyzer, thus making the auto-analyzer a stand-alone system.1 In this system, each aspects of the overall sample analysis, which are carried out manually, are bound to introduce errors and cause a prolongation of turnaround time. Studies have shown that the manual aspect of sample processing could be responsible for up to 40% of the elongation of the turnaround time associated with this system and for most of the laboratory errors and lost specimens/specimen wastages associated with them.18,23

Various approaches have been employed to automate the manual steps of the front end sample processing, in a bid to improve on the inadequacies noted above. These are either by using modular systems that automate the entire front end processes or stand, alone systems that automate only one aspect of the front end processes at a time. The modular systems automation of front end processes though costlier than the stand
alone systems have the advantage of improved turnaround time and flexibility. Stand-alone systems are often used to automate sample sorting, sample uncapping and aliquot functions of the front end sample processing.\textsuperscript{24}

Some individual processes need to take place for a complete analysis of a sample to occur and they collectively referred to as unit operations. These include: specimen identification, specimen delivery, specimen processing, sample introduction and internal transport, sample loading and aspiration, reagent handling and storage, reagent delivery, chemical reaction phase, measurement approaches, Signal processing, data handling and process control.

**Specimen identification**

Usually the identifying link between the patient and the specimen is generated at the bed side the nurses’ station or the clinic and that link is maintained until the final results are imputed back into the patients’ medical record. The identifier could be a serial number or a laboratory number. However, the use of electronically detectable methods is now often advocated and are as a matter of fact more commonly in use. Such methods include: barcoding, magnetic strips, smart cards, radiofrequency identification, voice identification etc.\textsuperscript{2,7}

In most clinical laboratory practice, barcode labeling is employed, here specimen is labeled with a barcode generated by the laboratory information system which may be interphasing with the hospital’s electronic medical records system if the laboratory is hospital based for instance. The bar code contains requisite information about the patient and the desired investigations, which optical scanners in the automation unit are able to read and transmit to the automation unit for necessary actions. The code is kept open until all the results of requested investigations are entered, reported and vetted by the designated authority, who then certifies the code closed.\textsuperscript{2,25}

**Specimen preparation delivery and aspiration**

Manual preparation of specimen is time consuming and is a major cause of delay in turnaround time for most laboratory investigations especially where sample preparation is strictly required before sample analysis. This has necessitated the need for its automation in most systems or an outright elimination of the need for it in some systems and a good example is the use of whole blood for the measurement of plasma electrolyte in some ion selective electrode, automated.

Specimen delivery is often by methods such as pneumatic tube systems, electric track vehicles and mobile robots.\textsuperscript{2,25} The specimen/samples are delivered to the loading zone in small cups or test tubes or in some cases in sample bottles with gel separators. The loading zone is often a tray like structure with numbered holes which hold the sample cups. It rotates in sequence to enable sample uptake by preset micropipettes for analysis.

**Reagent handling and storage**

Many automated systems use liquid reagents stored in containers and kept onboard the equipment at 4-10 °C, they equipped with sensors that would detect reagent reserves. Reagents are identified by reagent bar codes which contain information such as the name of the reagent, its volume and number of tests it would be able to run, its lot number and expiration date. The reagents when required for use in an assay, are aspirated and delivered to mixing chambers, for dilution, mixing or constitution as the case may be, before further delivery to reaction chambers by positive displacement syringe devices.\textsuperscript{1}

**Analytic approach**

Traditionally, automated analyzers have relied on photometers and spectrophotometers to read the absorbance of resultant products of chemical reactions. But more innovative optical reading devices such as fluorimeters, reflectance photometers and illuminometers have been introduced into most auto analyzers used in general chemistry. These innovations stand to improve the accuracy, precision and limit of detection of assays carried out with these equipment. The sensitivity of immunoassay techniques was in turn improved by the introduction of detection methods such as chemiluminscence, electro chemiluminscence and fluorescence. It is however important to note that the assay methodology being used largely determines the mode of automation of the analytic segment of a laboratory automation system. Signals produced from the analytic segment are passed on to detectors which converts them to values in concentration units which may be printed out or transferred to a laboratory information system which often interfaces with the
patients’ electronic medical record.\textsuperscript{1} This is often carried out via laboratory process management software also called middleware.\textsuperscript{9}

Middleware was initially a simple interface engine between the laboratory and the LIS, but it is today a powerful process control and management tool, with the ability to carry out most supervisory and quality control, tasks on behalf of the laboratorian. It can collate in a single screen all the information related to a patient and related work orders, physicians, and results integrated with tools to obtain additional information, make annotations, order reprocessing, or new tests and much more.\textsuperscript{9}

Conclusion

Laboratory automation is an innovation with a promising past, a robust present and a limitless future, it has become a well-accepted technology that allows high quality, efficient and patient centric operation with low operating costs. The availability of different systems will avail individual laboratories to select what suits them best, every laboratory cannot afford TLA and everyone does not need to go for TLA. The technology will continue to evolve and laboratories will have to position themselves to adapt to the evolution, for the benefit of their patients.

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Antibiotic Resistance of *Streptococcus pneumoniae* isolated from patients attending University of Ilorin Teaching Hospital, Ilorin

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Abstract

Between January and December 2014, one hundred and fifty-eight isolates of *Streptococcus pneumoniae* isolated from patients attending Medical Microbiology Laboratory of the University of Ilorin Teaching Hospital were studied in order to determine their antimicrobial susceptibility pattern. All the isolates were recovered from clinical samples and identified by their alpha-haemolytic reaction on sheep blood agar, bile solubility and their sensitivity to optochin. Susceptibility testing was carried out using the stokes-disc diffusion method. Majority of the *Streptococcus pneumoniae* isolates were recovered from cerebrospinal fluid (78.4%), sputum 18(11.3%), throat swab 14(9.0%) and eye swab 2(1.3%). Eighty-three percent of the isolates were resistant to Penicillin G but sensitive to Imipenem which are third generation cephalosporins and quinolones. The imipenem, cephalosporins and quinolones remained effective and are therefore recommended.

Key words: *Streptococcus pneumoniae*, Penicillin G, Resistance

Introduction

The genus *Streptococcus* belongs to the non-spore forming gram positive lancet shaped cocci. They are usually arranged in pairs or in short chains and are known as diplococcic. *Streptococcus pneumoniae* can induce a diverse spectrum of diseases associated with considerable morbidity and mortality. *Streptococcus pneumoniae* are leading causes of community acquired pneumonias and very frequent causes of otitis media, sinusitis and meningitis. Humans are the natural hosts of pneumococci as there is no known animal reservoir, and a proportion of the healthy population harbour virulent organism in the oropharynx. In the past, most Pneumococcal strains were susceptible to Penicillins G with minimum inhibitory concentration (MIC) less than 0.06 μg/ml allowing most physicians to treat persons who had severe infection of *Streptococcus pneumoniae* with Penicillins G alone. However, resistance to Penicillin G and other antimicrobial agents evolved and spread rapidly. The problem of increasing resistance among previously sensitive bacteria species to common antimicrobial agent has become a worldwide problem. Penicillins G resistance in *Streptococcus pneumoniae* was first reported in Australia in 1967, in New Guinea in 1969, in South Africa in 1977, in New Guinea in 1969 in South Africa in 1977, and in the Africa, Asia and Europe.

Pneumococcal resistance to β-lactam antibiotics occurs due to structural alterations in the penicillin binding proteins (PBP). Though typically, resistance to β-lactam antibiotics by most organisms is usually due to the production of β-lactamase enzyme, which is able to hydrolyse penicillins G compounds. However, resistance in *Streptococcal pneumoniae* to Penicillins G and other β-lactams is due to expression of low affinity to PBPs and not β-lactamase production.

The present study was carried out to determine the resistance patterns of *Streptococcus pneumoniae* in this...
environment with a view to provide valuable information to be used by physicians for therapy.

**Material and Methods**

The study was carried out from January to December 2014 at Medical Microbiology Department of University of Ilorin Teaching Hospital, Ilorin, Nigeria.

A total of 158 isolates were characterized by standard bacteriological technique\(^1\). All *Streptococci* provisionally identified by the alpha hemolysis on blood agar were sub-cultured on to sheep blood agar. A 6 mm size filter paper disc impregnated with 5µg optochin was placed on the blood agar and incubated aerobically at 35\(^\circ\)C for 18-24 hours. Sensitivity to optochin confirms *Streptococcus pneumoniae* while resistant shows *Streptococcus viridans*. All the *Streptococcus pneumoniae* isolates also had positive bile solubility test. The 158 confirmed *Streptococcus pneumoniae* isolates were tested against the following antibiotics using the Stoke’s disc diffusion method\(^1\): Ampicillin (10µg), Penicillin (1unit), Erythromycin (10ug), Ciprofloxacin (5µg), Ofloxacin (5µg), Ceftriazone (30µg), Ceftazidime (30µg). A standard inoculum adjusted to 0.5 Macfarland was swabbed on the sensitivity agar. Antibiotic discs were dispensed after drying the plate for 3-5min and incubated at 37\(^\circ\)C for 24 hours. *Streptococcus pneumoniae* NCTC 10319 and Viridian *Streptococcus* NCTC 10712 served as control organisms (Akanbi II et al., 2004).

**Result**

A total of one hundred and fifty eight (158) *Streptococcus pneumoniae* were isolated during the period of study from different clinical specimens. One hundred and twenty four (124) isolates were recovered from cerebrospinal fluid (78.4%), 18 (11.3%) from sputum, 14 (9%) from throat swab and 2(1.3%) from eye swabs (Table 1). The difference of the clinical isolates between the specimens is statistically significant (p=0.0042).

A total of 131 (83.0%) isolates were resistant to Penicillin G, 117 (73.8%) to Ampicillin and 89 (56.6%) to Erythromycin. The level of resistance of the isolates on the antibiotics is statistically significant (p<0.05).

<table>
<thead>
<tr>
<th>Specimen</th>
<th>No of isolates</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSF</td>
<td>124</td>
<td>78.4</td>
</tr>
<tr>
<td>Sputum</td>
<td>18</td>
<td>11.3</td>
</tr>
<tr>
<td>Eye swab</td>
<td>2</td>
<td>1.3</td>
</tr>
<tr>
<td>Throat swab</td>
<td>14</td>
<td>9.0</td>
</tr>
<tr>
<td>Total</td>
<td>158</td>
<td>100.0</td>
</tr>
</tbody>
</table>

The difference of the clinical isolates between the specimens is statistically significant

Table 2. In-vitro antibiotic susceptibility pattern of *Streptococcus pneumoniae* isolates at University of Ilorin Teaching Hospital (N=158 in each case)

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>No sensitive (%)</th>
<th>No resistant (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Imipenem</td>
<td>158(100.0)</td>
<td>0(0.0)</td>
</tr>
<tr>
<td>Ceftazidine</td>
<td>131 (83.0)</td>
<td>27 (17.0)</td>
</tr>
<tr>
<td>Cefuroxime</td>
<td>130 (82.0)</td>
<td>28 (18.0)</td>
</tr>
<tr>
<td>Ceftriazone</td>
<td>114 (72.0)</td>
<td>44 (28.0)</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>126 (80.0)</td>
<td>32 (20.0)</td>
</tr>
<tr>
<td>Ofloxacin</td>
<td>122 (77.2)</td>
<td>36 (22.8)</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>69 (43.4)</td>
<td>89 (56.6)</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>41 (26.2)</td>
<td>117 (73.8)</td>
</tr>
<tr>
<td>Penicillin</td>
<td>27 (17.0)</td>
<td>131 (83.0)</td>
</tr>
</tbody>
</table>

**Discussion**

Antibiotic resistance is an increasing problem among isolates of medical importance particularly in *Streptococcus pneumoniae* infection. In the past *Streptococcus pneumoniae* was almost uniformly susceptible to Penicillin G, allowing most infection to be treated with Penicillin G, thus making the susceptibility testing of pneumococci unnecessary. Bacteria resistance has been reported to almost every antibiotic currently available. Many bacteria now exhibit simultaneous resistance to two or more
antibiotics and are called multiple drug resistance isolates. Pneumococcus resistance may occur alone or in combination with resistance to other antimicrobial agents.\textsuperscript{15,16}

In this study, the pneumococcal isolates were largely resistant to Penicillin G. This correlates with the findings of other workers\textsuperscript{17,18}. There is no doubt Penicillin G are the most widely used antibiotic in the developing countries more especially among the general medical practitioners. The greater the quantity and the longer the drug have been in use, the more likely it is that strains resistant to the antibiotics will develop and spread\textsuperscript{16}. In the present study, 44.3\% of the isolates were also resistant to both Penicillin G and Erythromycin while 12.7\% were resistant to more than three antibiotic groups. This shows the gradual increase in the level of resistance of the isolates in agreement with the trend worldwide\textsuperscript{15}. However, contrary to other reports\textsuperscript{15,16,17} the resistance to Cephalosporins was low and all the isolates were sensitive to Imipenem. This is probably as a result of the fact that these drugs are expensive and they are injectables and less abused in this environment. Previous studies in this centre have shown many pathogens to be relatively sensitive to Cephalosporins\textsuperscript{18,19}. In the first line empirical treatment of infections due to \textit{Streptococcus pneumoniae} in our environment, Penicillin G should no longer be advocated. We recommend the use of second or third generation Cephalosporins or the Fluoroquinolones and the Coarbapenems where indicated; in the empiric treatment of serious infections due to \textit{Streptococcus pneumoniae}. Reducing the impact of drug resistance in \textit{Streptococcus pneumoniae} may be achieved through a policy that serves to reduce indiscriminate antibiotic use in the community and increase understanding of the factors that contribute to the development of resistance such as under dosage, which is a common practice in our communities.

References

Clinical predictors of childhood pneumonia cases seen at University of Ilorin teaching hospital, Nigeria

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Abstract

**Background/Objectives:** Pneumonia continues to be a major contributor to childhood mortality and morbidity in developing countries. Nigeria ranks 5th among countries with the highest estimated absolute number of new cases of clinical pneumonia and an annual incidence of 6.1million new cases. This study is aimed at evaluating the sensitivity and specificity of clinical symptoms and signs as predictors of pneumonia in children aged two months to fifty-nine months.

**Methodology:** This was a cross-sectional study of the clinical predictors of pneumonia among children aged two to fifty-nine months. Two hundred children were recruited consecutively. All the patients had chest x-ray. The study hypothesis was “pneumonia can be predicted clinically by assessing the various symptoms and signs associated with it”.

**Results:** Pneumonia accounted for 15.9% (200 out of 1254) of all the admissions during the period. Bronchopneumonia was seen in 168(84%) and lobar pneumonia in 32(16%) children. Cough, difficulty in breathing, fast breathing and fever were the most common symptoms, while age-related tachypnoea and chest wall recession were recognized as the most common clinical signs in the study population. Tachypnoea was the clinical sign that showed the highest sensitivity (98.8%) and specificity(67%).

**Conclusion:** Tachypnoea used as the only clinical sign is useful for identifying pneumonia in children, with no significant variations for age. The presence of either a respiratory rate ≥50/min or chest in-drawing, or of both signs, was a good indication of pneumonia, with a predictive power of 46% for a positive test and 83% for a negative test.

**Introduction**

Pneumonia continues to be a major contributor to childhood mortality and morbidity in developing countries. It is responsible for a quarter of all deaths in under-five children. Pneumonia is second only to malaria as a cause of admission and deaths among children seen at the University of Ilorin Teaching hospital (UITH). Of the 15 countries with the highest estimated absolute number of new cases of clinical pneumonia, Nigeria ranks 5th with an annual incidence of 6.1million new cases. An estimated 20-25 percent of pneumonia-related death occur among children below 5 years of age in Nigeria. Many of the deaths occur in those less than 24 months especially in infants. In developing countries, the operational definition of pneumonia adopted by WHO is based on the presence of clinical parameters like fast breathing (tachypnoea) or chest in-drawing/retraction, in a child with cough and/or difficult breathing of less than 28 days. Early detection and treatment are important aspects in the management of children with pneumonia.

This study was carried out over a period of 12 months, between 1st April 2015 and 31th March 2016, to document the clinical predictors of pneumonia in children seen at University of Ilorin Teaching Hospital, Ilorin, North-Central Nigeria. It is based on the hypothesis that pneumonia can be predicted clinically by assessing the various symptoms and signs associated with it.

**Materials and Method**

**Study Design:** This was a prospective, cross-sectional study of children aged two months to fifty-nine months who were admitted to the Emergency Paediatric Unit...
(EPU) of the UIITH which is a tertiary hospital in North-Central Nigeria. UIITH serves Kwara state, with a population of 2.4 million\textsuperscript{5}, and adjoining states of Ekiti, Osun, Oyo and Niger. The EPU caters for children from beyond the neonatal period to the age of 14 years. The study was done between 1st April 2015 and 31st March 2016.

**Sample size:** The formula used for estimating the minimum sample size is the Fisher’s formula and with reference to the prevalence of 11.1% from a previous study.\textsuperscript{6} A minimum of 187 subjects were calculated for the study and at the end of study period 200 patients were enrolled.

**Ethical clearance:** Ethical clearance was duly obtained from the Ethics and Research Committee of the UIITH. In addition, informed consent was obtained from the individual parent/guardian as appropriate, after a clear explanation of the objectives and logistics of the study to them.

**Subject recruitment:** All consecutive children who presented initially with less than 28-day old symptom complex of cough, fever, difficulty with breathing were assessed. Relevant clinical history of the illness was obtained and a thorough examination was conducted, including age, sex and duration of disease according to a questionnaire prepared for the purpose of the study before treatment with the most appropriate medication according to the current institutional guidelines. The clinical examination included documentation of the following: height, weight, body temperature, heart rate, respiratory rate, presence or absence of chest in-drawing, cyanosis, nose flaring, grunting and wheezing. The respiratory rate was measured by observing the thorax for a full minute for chest movements, with the child awake, calm(without crying), and lying down. To measure the respiratory rate accurately, a one-minute timer, distributed by UNICEF was used. Tachypnoea was defined according to criteria recommended by the WHO: in children between 2 and 12 months old, > 50/minute; and in children older than 12 months, >40/minute.\textsuperscript{4} All children had a chest x-ray taken the same day as the consultation. The radiographs were evaluated by a single radiologist, who was blind to the clinical diagnosis the presence of one or more of the chest radiographic features of patchy, segmental or lobar consolidation, with or without a positive air bronchogram and with or without pleural effusion was used to confirm the diagnosis of pneumonia.

Patients were excluded from the study if a murmur was detected on auscultation of the heart indicating the presence of heart disease.

**Data analysis**

Data was collected with the aid of a pre-coded study proforma and analysis was carried out with a micro-computer using the Epi info SPSS software packages.\textsuperscript{7} The chi-square and student t-test were used to identify significant differences for categorical and continuous variables respectively. Fisher’s exact test was used if the frequencies were less than 5. The sensitivity and specificity of each clinical sign in its ability to predict pneumonia was also calculated. A *p*-value of <0.05 was considered significant.

Nutritional status was assessed according to weight for age deficit taking standard deviations to classify mild, moderate, or severe weight for age losses, corresponding to $-1$ Z-score, $-2$ Z-score, or $-3$ Z-score, respectively.\textsuperscript{8}

**Results**

Two hundred patients were recruited, and all patients completed the study. The male: female ratio was 1.5:1, and 121(60.5%) of the children were infants. The mean (SD) age of the children with pneumonia was 14.3 (13.5) months. 91 (45.5%) children were aged <12 months, 56 (28.0%) were aged 12 - <24 months, 34 (17.0%) were aged 24 - <36 months, 5 (2.5%) were 36 - <48 months, and 14 (7.0%) were 48 - <60 months as shown in Table I.

Pneumonia accounted for 15.9% (200 out of 1254) of all the admissions during this period. Out of the 200 children, 186 (93%) presented with cough and 167 (83.5%) presented with fever (\(T^o>37.5\)). Other common symptoms present were difficult breathing 181(90.5%), nasal discharge 53 (26.5%), and fast breathing 174 (87.0%). The common findings on clinical examination were age-related tachypnoeia 191 (95.5%), reduced intensity of breath sounds 176 (88.0%), and the presence of adventitious sounds 161 (81.0%), as highlighted in Table II.
The least common findings were the presence of central cyanosis (3.0%) and wheeze (2.5%). With regards to type of pneumonia, 32 (16%) of the children had lobar pneumonia, and 168 (84%) had bronchopneumonia. Complications were present among 92 (46.0%) of the children. The most common were heart failure 61 (30.5%) and pleural effusion 16 (8.0%), whereas pyopneumothorax and pneumomediastinum were the least common with one occurrence each. Seventeen of the children died, giving a case fatality of 8.5%. Ten of the dead children were aged less than 12 months, while seven were aged between 12 and 59 months. Twelve of the children that died were male while 5 were female as shown in Table III.

### Table III: Distribution and clinical outcome of pneumonia in the 200 patients

<table>
<thead>
<tr>
<th>Type of Pneumonia</th>
<th>Frequency</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bronchopneumonia</td>
<td>168</td>
<td>84</td>
</tr>
<tr>
<td>Lobar Pneumonia</td>
<td>32</td>
<td>16.0</td>
</tr>
<tr>
<td><strong>Case Fatality</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12 Months</td>
<td>10</td>
<td>5.0</td>
</tr>
<tr>
<td>≥ 12 - 59 Months</td>
<td>7</td>
<td>3.5</td>
</tr>
<tr>
<td><strong>Mortality by sex</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>12</td>
<td>6.0</td>
</tr>
<tr>
<td>Female</td>
<td>7</td>
<td>3.5</td>
</tr>
</tbody>
</table>

### Table I: Clinical characteristics of the 200 children studied

<table>
<thead>
<tr>
<th>Age (Months)</th>
<th>Number</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-5</td>
<td>57</td>
<td>28.5</td>
</tr>
<tr>
<td>6-11</td>
<td>34</td>
<td>17.0</td>
</tr>
<tr>
<td>12-23</td>
<td>56</td>
<td>28.0</td>
</tr>
<tr>
<td>24-35</td>
<td>34</td>
<td>17.0</td>
</tr>
<tr>
<td>36-47</td>
<td>5</td>
<td>2.5</td>
</tr>
<tr>
<td>48-59</td>
<td>14</td>
<td>7.0</td>
</tr>
</tbody>
</table>

### Anthropometry

<table>
<thead>
<tr>
<th>Frequency</th>
<th>Percentage</th>
<th>Range</th>
<th>Mean±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (Kg)</td>
<td>200</td>
<td>100</td>
<td>3.0-20.0</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>200</td>
<td>100</td>
<td>50.0-114.5</td>
</tr>
</tbody>
</table>

### Table II: Clinical features of pneumonia in the 200 children studied

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>Frequency n(%)</th>
<th>OR (95%CI)</th>
<th>p-value</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cough</td>
<td>186 (93.0)</td>
<td>1.21 (1.15-1.27)</td>
<td>0.001*</td>
<td>92.0</td>
<td>4.0</td>
</tr>
<tr>
<td>Difficulty with breathing</td>
<td>181 (90.5)</td>
<td>3.16 (1.30-8.11)</td>
<td>0.001*</td>
<td>98.8</td>
<td>14.5</td>
</tr>
<tr>
<td>Fast breathing</td>
<td>174 (87.0)</td>
<td>89.5</td>
<td>40.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Noisy breathing</td>
<td>60 (30.0)</td>
<td>1.63 (0.59-4.53)</td>
<td>0.285</td>
<td>13.3</td>
<td>3.5</td>
</tr>
<tr>
<td>Fever</td>
<td>167 (83.5)</td>
<td>80.2</td>
<td>23.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nasal discharge</td>
<td>53 (26.5)</td>
<td>0.60 (0.32-1.1)</td>
<td>0.77</td>
<td>36.1</td>
<td>21.3</td>
</tr>
<tr>
<td>Age-related tachypnoea</td>
<td>191 (95.5)</td>
<td>13.82 (2.08-589.5)</td>
<td>0.001*</td>
<td>98.8</td>
<td>67.0</td>
</tr>
<tr>
<td>Intercostal/subcostal recession</td>
<td>187 (93.5)</td>
<td>Undefined</td>
<td>86.7</td>
<td>53.3</td>
<td></td>
</tr>
<tr>
<td>Reduced intensity of breath sound</td>
<td>176 (88.0)</td>
<td>Undefined</td>
<td>0.00*</td>
<td>15.4</td>
<td></td>
</tr>
<tr>
<td>Adventitious Breath sounds</td>
<td>162 (81.0)</td>
<td>1.69 (0.76-3.94)</td>
<td>0.168*</td>
<td>85.5</td>
<td>22.2</td>
</tr>
<tr>
<td>Febrile (≥37.5°C)</td>
<td>145 (72.5)</td>
<td>1.09 (0.55-2.17)</td>
<td>0.791*</td>
<td>73.5</td>
<td>28.2</td>
</tr>
<tr>
<td>Hepatomegaly</td>
<td>127 (63.5)</td>
<td>48.2</td>
<td>46.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pallor</td>
<td>64 (32.0)</td>
<td>2.94 (1.53-5.72)</td>
<td>0.000*</td>
<td>45.8</td>
<td>15.8</td>
</tr>
<tr>
<td>Grunting</td>
<td>59 (29.5)</td>
<td>4.46</td>
<td>81.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abnormal Percussion findings</td>
<td>46 (23.0)</td>
<td>4.02 (1.91-8.79)</td>
<td>0.000*</td>
<td>37.3</td>
<td>87.2</td>
</tr>
<tr>
<td>Splenomegaly</td>
<td>22 (11.0)</td>
<td>7.2</td>
<td>86.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bronchial breath sounds</td>
<td>19 (9.5)</td>
<td>4.51 (1.45-16.72)</td>
<td>0.003*</td>
<td>16.9</td>
<td>95.7</td>
</tr>
<tr>
<td>Central cyanosis</td>
<td>6 (3.0)</td>
<td>0.097</td>
<td>20.5</td>
<td></td>
<td>12.8</td>
</tr>
<tr>
<td>Wheeze</td>
<td>5 (2.5)</td>
<td>4.87</td>
<td>97.4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

OR= odds ratio; CI= confidence interval; *=chi square test; #= Fisher exact test
Clinical features of pneumonia that were statistically significant on examination include pallor, grunting, intercostal recession and chest in-drawing (p=0.000, 0.000, 0.000, and 0.000 respectively). Other significant findings on clinical examination were the presence of abnormal percussion notes, bronchial breath sounds and tender hepatomegaly (p=0.000, 0.003 and 0.001 respectively). The clinical features in the children with pneumonia were included in a linear regression model. A linear regression analysis was thereafter carried out to exclude their relative contribution list wise. The clinical features that were still significant were lower chest wall in-drawing (p=0.000), bronchial breath sounds (p=0.002) and tender hepatomegaly (p=0.002).

The sensitivity and specificity of age-related tachypnoea for detecting pneumonia were 98.8% and 14.5% while those of lower chest wall in-drawing were 86.7% and 53.3% respectively. The presence of bronchial breath sounds had a 16.9% and 95.7% sensitivity and specificity, respectively while the corresponding values for tender hepatomegaly were 48.2% and 82.9% as shown in Table II.

Discussion

Clinical pneumonia is best diagnosed by chest radiograph (chest X-ray) which is often regarded as the gold standard. Nevertheless, the present study suggests that certain inexpensive and suitable clinical criteria could be useful for prompt identification of clinical signs that best predict pneumonia in children under 5 years of age, particularly in the rural area of a developing country where chest X-ray was not feasible in all cases. Although all our subjects had chest x-ray on admission, we should remember that the radiological picture of pneumonia appears 24–48 hours after the onset of disease. Therefore, it is likely that some cases that were considered false positives may have been in their earliest stages and therefore not detected by x rays.

The WHO developed an algorithm to aid medical and non-medical health care workers in diagnosing lower respiratory tract infection without radiological confirmation. This algorithm was designed for use in the developing countries. Our findings showed that age-related fast breathing and difficulty with breathing are the symptoms that showed significant association with pneumonia. This was similar to the findings of Taylor et al and WHO algorithm, Lozano does not find an association with the symptoms. This was probably due to the method of his sampling or differences in the definition and interpretation of symptoms. The individual symptoms of cough, difficult with breathing, fast breathing and fever were all sensitive indicators of pneumonia with adequate sensitivity but their specificity was low. Similar results had also been reported in other studies. This will reduce the probability of missing the diagnosis. Several individual signs in the present study showed significant association with pneumonia. The most useful signs are age-related tachypnoea, intercostal/subcostal recession (chest in drawing), grunting, reduced breath sounds, abnormal percussion notes and pallor. Age-related tachypnoea was the best predictor of pneumonia with adequate sensitivity and specificity simultaneously. We used criteria suggested by the WHO to classify tachypnoea, according to different age groups. We found that tachypnoea, by itself, had a sensitivity of 98.8% and a specificity of 67% to identify pneumonia, as has been reported by other authors. Palafox found that tachypnoea (as defined by WHO) had a 74% sensitivity and 67% specificity for radiologically defined pneumonia. It is important to note that tachypnoea as a sign of pneumonia must also be used with caution in children with co-morbid conditions such as asthma where tachypnoea is a sign of deterioration of the underlying condition. Intercostal/subcostal recession (chest in drawing) and grunting were also good indicators of pneumonia. They had high sensitivity, specificity with adequate predictive value. These results confirm the findings from previous studies.

Grunting and nasal flaring increase the likelihood of pneumonia, but their absence cannot be relied upon to rule out pneumonia. Other signs that relate to the severity of the pneumonia are nasal flaring, and cyanosis. But these signs alone are not sensitive or specific for the diagnosis of pneumonia. Fever as defined in this study (≥37.5°C) was not a good predictor of pneumonia. Other studies found high fever (≥38.5°C) in young children (aged up to 3 years) with bacterial pneumonia, to be a sign of pneumonia. The use of multiple indicators at the same time in parallel (considering the presence of any criterion as a positive diagnosis) increases the sensitivity and reduces the false negative diagnosis. This would increase the
negative predictive value and improve the negative likelihood ratio. Conversely, the use of multiple indicators serially (considering the absence of any indicator as a negative diagnosis) maximizes the specificity and minimizes the false positive diagnosis. In this manner, the positive predictive value and positive likelihood ratio would increase. The absence of clinical signs is sometimes more helpful to a clinician than their presence. If all clinical signs are negative, pneumonia is deemed to be unlikely. However, if signs are present, they can be used in combination to guide the clinician to consider a diagnosis of pneumonia but do not secure a definitive diagnosis.

Conclusion

We found that tachypnoea was the single most sensitive and specific sign for making a clinical diagnosis of pneumonia. In view of this, we encourage health workers, particularly in the primary health centres of low resource setting countries where chest X-ray may not be readily available, to use tachypnoea routinely to identify pneumonia in children.

References:

Comparative review of prolapsed and unprolapsed intussusception in children

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2Department of Surgery, Delta State University Teaching Hospital, Oghara, Delta State, Nigeria

Abstract

Introduction: Intussusception is a common cause of intestinal obstruction in childhood. Prolapse of the intussusception through the anus is a rare form of presentation. Treatment of unprolapsed and prolapsed intussusception is associated with some degree of morbidities and mortalities.

Aim: To evaluate the pattern of prolapsed intussusceptions (PI) and to compare the outcome of its management with that of unprolapsed intussusception (UPI) in children. To serve as a basis for proactive management of UPI.

Patients and methods: The study was a prospective study of children with prolapsed and unprolapsed intussusception presenting to the children emergency unit of Delta State University Oghara, Nigeria. The study was over a 2-year period 2014 and 2015.

Results: 22 patients were seen, 28.3% had PI. 66.6% of patients with PI were females however more males were seen with UPI (83.3%). 100% of patients with PI presented to the hospital within 48 hours. Patients with PI did not have an increased risk of having bowel resection (33.3% - 37%).

Discussion: PI in children was common in this study with a rate of 28.3%. Early presentation was noted (66.6% within 24 hours) majorly because of the unpleasant sight. The high rate of postoperative complications seen in the prolapsed group accounted for the longer duration of hospital stay.

Keywords: Prolapse, intussusception, transanal, protrusion

Introduction

Intussusception is a common cause of childhood intestinal obstruction requiring urgent attention which may be by operative or non-operative intervention. Untoward sequelae could accompany intussusception if intervention is delayed. Intussusception is defined at the invagination of one segment of bowel into an adjacent segment. It is commonly the proximal segment invaginating or telescoping into the distal segment; the reverse could be the case in few rare instances. It occurs commonly between the ages of 3 months to 3 years with the peak occurrence at 6 months to 9 months. Only 30 percent of all cases occur in children older than 2 years. PI is defined as the invagination of a segment of the intestine into the adjacent segment with the exteriorization of the head intussusception through the anus. The bowel is seen prolapsing through the anus. It is a rare form of presentation of intussusception, believed to be commoner in the developing countries, perhaps reflecting a longer duration of symptoms. Ogundoyin et al reported that intussusception is the commonest cause of intestinal obstruction in children in Nigeria accounting for 29.3%. Other causes of intestinal obstruction in children were anorectal malformation 22.5% and Hirschsprung disease 13.8%. The incidence of PI is about 8% of children with intussusception.

The etiology of intussusception is majorly idiopathic with only about 10% presenting with an identifiable precipitating factor (lead point). The non-idiopathic causes may be due to congenital gastrointestinal...
abnormalities such as Merckels diverticulum and intestinal duplication cysts. It may also be due to harmatomas, polyps and lipomas which serve as lead points. With increasing age, the non-idiopathic (secondary causes) tend to become more prevalent.7

Most children with intussusception will present with sudden onset of abdominal pain, exhibited by drawing up of the knees, screaming and lethargy between painful bouts. The onset of pain is shortly followed by obstructive symptoms such as bilious vomiting and abdominal distention. Half of the cases progress to bloody, mucoid currant jelly stools within 12 hours. However, the classic triad of pain, a palpable mass and currant jelly stool is seen in less than 15 percent. In developed countries the diagnosis is made early whereas in developing countries, children are received late at the regional centers.9, 10, 11

It is impossible to tell which child with intussusception that will eventually have prolapse of the bowel through the anus. Though an uncommon presentation, it is not unusual to find prolapse through the anus.8, 9 PI may present without any cardinal symptoms of intussusception6, it may actually present with the first few episodes of colicky pains the child experiences. PI could also be seen following several hours or even days following the onset of symptoms.

Children with prolapsed and UPI require urgent surgical attention to maintain homeostasis and release the obstruction. These interventions may carry some complications based on the state of the patient and timing of presentation.

It is believed that children with prolapsed intussusception are at a higher risk of bowel gangrene. This however has not been proven in recent studies.

This article evaluates and compares the outcome of management of patients with prolapsed and unprolapsed intussusception. It will help to serve as a template in helping to prognosticate outcome of management in these categories of patients.

Patients and Methods

This was a prospective study of patients with intussusception over a 2-year period. The patients were seen as emergency presentations to the children emergency unit of Delta State University Teaching Hospital Nigeria, from January 2014 and December 2015. Relevant information was obtained by structured history taking from the parents or care givers. The data was analyzed using simple statistical methods and SPSS 20 version.

Result

22 cases of intussusception were seen over the period. 12(54.5%) cases were males and 10(45.5%) females. M: F ratio was approximately 1:1.

Figure 1: Sex distribution

16(72.7%) were unprolapsed cases and 6(28.3%) were prolapsed.

Table 1: Sex distribution in the two groups

<table>
<thead>
<tr>
<th></th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unprolapsed</td>
<td>10(83.3%)</td>
<td>6(60.0%)</td>
</tr>
<tr>
<td>Prolapsed</td>
<td>2(16.7%)</td>
<td>4(40.0%)</td>
</tr>
</tbody>
</table>

Table 2: Age at presentation

<table>
<thead>
<tr>
<th>Age</th>
<th>Unprolapsed</th>
<th>Prolapsed</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-7 months</td>
<td>7(43%)</td>
<td>2(33.3%)</td>
</tr>
<tr>
<td>8-10 months</td>
<td>5(31.2%)</td>
<td>2(33.3%)</td>
</tr>
<tr>
<td>11-12 months</td>
<td>2(12.5%)</td>
<td>1(16.6%)</td>
</tr>
<tr>
<td>&gt;1 year</td>
<td>2(12.5%)</td>
<td>1(16.6%)</td>
</tr>
<tr>
<td>TOTAL</td>
<td>16(100%)</td>
<td>6(100%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Unprolapsed (n/%)</th>
<th>Prolapsed (n/%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Simple reduction</td>
<td>10(62.5%)</td>
<td>4(66.6%)</td>
</tr>
<tr>
<td>Resection and anastomosis</td>
<td>6(37.5%)</td>
<td>2(33.3%)</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>6</td>
</tr>
</tbody>
</table>
Discussion

Prolapsed intussusception (PI) is also referred to as transanal protrusion (TP) by some authors. It refers to the protrusion of intussusception through the anus. It is a rare form of presentation of intussusception.

In this study a total of 22 children with intussusception were seen over the 2-year period. Of this 6 were cases of prolapse through the anus bringing the prolapse rate to 28.8%. PI is an uncommon manifestation of childhood intussusception with rates from 8%, 10.8%, 11% to 29%. Obioru EU et al in Enugu, Nigeria reported a prolapse rate of 16%. The prolapse rate in this study is one of the highest recorded in literature, close to the 29% reported by Ameh EA et al. These studies were both carried out in Nigeria. PI in children is higher in Nigeria in comparison to other studies elsewhere.

The male to female ratio of intussusception in this study was approximately 1.1.

However, more females in this study had prolapse accounting for 66.6% of cases in the prolapsed group. Though in the unprolapsed group more males, 83.3% were seen. This female preponderance in the prolapse group is seen in similar studies.

By 10 months, 66.6% of PI and 74.2% UPI presented, p value 0.05. 83.2% of PI and 86.7% of UPI presented by 1 year. The occurrence of prolapse as a form of presentation of intussusception is not affected by the age of the patients. 2(12.5%) of the unprolapsed group and 1(16.6%) of the prolapsed group presented after 1 year.
4(66.6%) of prolapsed cases presented to the hospital less than 24 hours of onset of symptoms of obstruction. This is compared to 6(37.5%) of unprolapsed cases. 100% of all prolapsed cases had presented within 48 hours. Patients with prolapsed intussusception are more likely to present earlier to the surgeon compared to the unprolapsed cases. The prolapse from the anus could possibly frighten the parents thus prompting early medical consultation. A mean of 1 day was observed as time of onset of symptoms to presentation for prolapsed cases. Obiora EU et al reported a mean of 5.9 days in their study.

Surgery through laparotomy was the preferred treatment option in many previous reports but in a series by Ramachandran et al many patients had air enema reduction and only 50% had laparotomy with manual reduction or bowel resection. In this study all the patients had laparotomy. 46.6% of the prolapsed cases and 10(62.5%) of unprolapsed cases had simple reduction. Bowel resection was done for 2(33.3%) of the prolapsed cases and 6(37.3%) of unprolapsed cases, p value > 0.05. Patients with PI do not have a significantly increased risk of having bowel resection in this study (33.5% to 37.5%).

Postoperative complications where more common in cases of PI than in the unprolapsed category. Postoperative pyrexia of over 38°C lasting more than 24 hours was seen in 50% of prolapsed cases and 25% of unprolapsed cases. Wound infection was seen in 85% of prolapsed cases and 31% of unprolapsed cases. Wound infection was suggested by erythema of the wound, serous or purulent discharge or breakdown of the wound. Prolapse of intussusception through the anus predisposes the prolapsed bowel to coming in contact with pathogenic organisms around the anal region. This may account for the higher risk of postoperative complications seen in these patients.

Patients in the prolapsed group have a higher duration of stay in the hospital. 50% of them spent more than 1 week on admission compared to 12.5% of unprolapsed cases. The high rate of post-operative complications could account for this pattern.

One mortality was observed in this study and was in the unprolapsed group. The cause of the mortality was severe sepsis.

Recurrence was not seen in any of the group of patients within the study period.

**Conclusion**

Prolapse from the anus could occur in children with intussusception. They tend to present earlier. It could carry an increased risk of wound infection and postoperative pyrexia.

**References**

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Abstract

Aim: This study was designed to assess the electrolytes and azotemic index in pre and post hemodialysis patients with chronic kidney disease in National Hospital Abuja. A total of 92 participants were recruited at random into two groups. The study group comprised of 46(50%) chronic kidney disease patients and the control group, also of 46(50%) of apparently healthy individuals.

Methods: Electrolytes were analyzed by ion selective electrode method while calcium, phosphate, urea, creatinine and uric acid were analyzed using Automatic analyzer.

Result: The mean electrolytes levels in pre-dialysis compared to post-dialysis showed significant difference for sodium (p<0.0001), calcium (p<0.0001) and phosphate (p<0.0001) while there was no significant difference for potassium (p>0.0001) and chloride (p>0.005). Also there were significant difference in the mean urea, creatinine and uric acid levels in pre and post-dialysis patients (p<0.0001). The post-dialysis and control showed no significant difference in sodium, chloride, calcium and phosphate levels (p>0.001). However, there were significant difference for urea, creatinine and uric acid levels (p <0.0001).

Conclusion: These findings reveal the need to routinely screen individuals for electrolytes and azotemic indices with more attention paid particularly to those ≥50yrs of age. We also suggest that though hemodialysis seems to restore levels of serum electrolytes and azotemic index, a significant number of subjects still present with post-dialysis imbalance. Therefore, we wish to strongly recommend that multiple dialysis should be encouraged in chronic kidney disease patients for more efficient and stable homeostasis.

Key words: Electrolytes, Azotemic indices, Chronic Kidney Disease, Dialysis.

Introduction

The kidneys act as regulators of many of the body’s function and control complex metabolic processes that maintain homeostasis (1,2). The kidneys also receive about 20-25% of cardiac output per minute filtered through the nephrons with each kidney having a million nephrons which enable homeostasis to be maintained even when the nephrons are damaged (3,4). However, when 90% of the nephrons are lost, renal function is significantly impaired resulting in end-stage renal disease (ESRD) order-wise known as chronic renal disease (CKD) (5,6,7).

CKD is defined as a gradual and usually permanent loss of kidney function over time, which takes usually months to years (8). Johnson CA et al;8 in 2004 stated that CKD is divided into five stages of increasing severity. Accordingly stage five chronic kidney failure is also referred to as ESRD with a total or near total loss of kidney function. Johnson CA et al;8 in 2004 also suggested that such patients need dialysis or transplantation to stay alive. Forley et al;10 in 1996 had reported the multiple electrolyte levels alteration in patients with CKD. Potassium may be normal until late in CKD because of the inability of the kidney to excrete potassium due to decreased glomerular filtration rate (GFR) (9,10). It is also known that when metabolic acidosis is present, potassium ions shift from the intracellular compartment to the extracellular space in exchange for hydrogen ions (H+), in order to
maintain extracellular electrochemical neutrality. Porth\textsuperscript{11}in 2002; Sharon and Judith\textsuperscript{12}in 2006, also reported that serum phosphorous and calcium levels are altered in CKD. Sharon and Judith\textsuperscript{12}in 2006 stated that in CKD, nitrogenous waste products from protein metabolism are retained in the body, resulting in azotemia. Since patients with CKD most often undergo estimation of electrolytes, therefore, this study was designed to have a comparative evaluation of pre and post- dialysis of routine biochemical markers of kidney function namely; electrolytes and urea, creatinin and uric acid in patients with CKD with a view to establishing the effectiveness of dialysis on these parameters.

Materials and Methods:

The study which was a cross-sectional and analytical survey was done at the National Hospital, Abuja- Federal Capital Territory, Nigeria.

Sample Population: The sample population comprised of a control group, made up of apparently healthy individuals and a case group who were patients of known and established cases of Chronic Kidney Disease (CKD).

Sample Size: A total of 92 subjects were studied; made up of 46 cases of CKD and 46 Controls with a ratio of case to control of 1:1, with the power of the study being 70%. The patients used in this study are those that were on dialysis and their age ranged between 21yrs to 60yrs.

Sample Collection: Blood samples (about 5cm\textsuperscript{3}) were collected from the selected subjects using a 5cm\textsuperscript{3} sterile disposable syringe and needle. Each blood sample was transferred into separate lithium heparin tubes and mixed gently. At the end of each day’s collection, the heparinized blood samples were centrifuged using Mega centrifuge 1.0 Heraus instrument of German made. The instrument was set at 5,000 rpm for 5 minutes, followed by subsequent separation of each of the plasma samples into plain tubes and labeled appropriately. Plasma samples were analyzed on the same day of collection. The pre-dialysis samples were collected before dialysis commenced and immediately after dialysis, the post-dialysis samples were taken. The control samples were collected from apparently healthy individuals and were treated as the study group.

Sample Analysis: Sodium, potassium and chloride were analyzed by ion selective electrode method as developed by Levy\textsuperscript{13}in 1981. Ion-selective electrodes make possible the potentiometric measurement of specific ions when incorporated in an electrode. When an ion-specific membrane separates two solutions that differ in concentration of that ion, a potential is developed across the membrane, the size of the potential depends on the difference in the ion concentration. The light then, measure sodium, potassium and chloride using ion selective electrode-technology. The flow through sodium electrode contains glass tubes specially formulated to be sensitive to sodium ions, the flow through potassium electrode employs a plastic tube incorporating valinomycin as the selective element and the flow through chloride electrode include a plastic tube specially formulated to be selective to chloride. Calcium was determined by the method of chelation with O-cresolphthelein complexone as established by Lorenz\textsuperscript{14} in 1932. The dye O-cresolphthelein complexone, binds calcium tightly in an alkaline solution to form a highly coloured complex whose absorbance was measured at 578nm. Inorganic phosphate was measured by the method of Fiske and Subbarow\textsuperscript{15}in 1925. Serum proteins are precipitated by trichloroacetic acid and the phosphate is converted to a phosphomolybybdate (MOi\textsuperscript{+}) complex by addition of sodium molybdate. The addition of p-Methylaminophenol reduces the MOi\textsuperscript{+} in the complex to yield an intensely blue-coloured phosphomolybdate complex (MO\textsuperscript{3+}) by 10 months whose absorbance was measured at 700nm. Creatinine was determined by modified Jaffe-kinetic method as developed by Moss et al;\textsuperscript{16}in1975. At alkaline pH, creatinine reacts with picric acid to produce a coloured compound of alkaline picrate solution which was measured at 520nm. Urea was measured by Berthelot’s method\textsuperscript{17} 1859. Urea in plasma was hydrolyzed by urease into ammonia and carbon dioxide. The ammonia generated then reacts with alkaline hypochlorite and sodium salicylate in the presence of nitroprusside to yield a blue chromophore whose absorbance was measured colorimetrically. While uric acid was analyzed by uricase method as developed by Duncan et al;\textsuperscript{18} in 1982. In the presence of peroxidase, hydrogen peroxide reacts oxidatively with 3,5-dichloro-2-hydroxybenzenesulfonic acid and 4-aminophenazone to form a red dye. Potassium ferricyanide was included in the reagent in order to
oxidize ascorbate. The colour formed was measured spectrophotometrically.

Data Analysis: All data were analyzed using Epinfo version 3.5.1, 2008. Means, standard deviations and proportions were determined as applicable. The proportions were compared using Z-score while a probability value (P value) less than 0.05 was taken as statistically significant.

Results

Table 1 below shows the comparison of mean concentrations of electrolytes and azotemic indices in the control and pre-dialysis groups. From the table there is a significant difference in the mean levels of sodium (p<0.0001), potassium (p<0.001), phosphate (p<0.001) urea (p<0.0001), creatinine (p<0.0001) and uric acid (p<0.001). Table 2 shows the comparison of mean of electrolytes and azotemic indices in pre and post-dialysis groups. From this analysis, there are significant difference in the mean levels of potassium (p<0.0001), phosphate (p<0.0001), urea (p<0.0001), creatinine (p<0.0001) and uric acid (p<0.0001) in pre and post-dialysis groups. Table 3 is the comparison of the mean concentrations of electrolytes and azotemic indices in post-dialysis and control groups. From the table whereas only potassium is significant (p<0.003) amongst the electrolytes, urea, creatinine and uric acid are all significant (p<0.0001; p<0.001) amongst the azotemic indices.

Table 1: Comparison of Means of Electrolytes and Azotemic Indices in the Control and Pre-Dialysis Group

<table>
<thead>
<tr>
<th>Analytes</th>
<th>Predialysis</th>
<th>Control</th>
<th>z score</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na (mmol/l)</td>
<td>130.5 ± 6.8</td>
<td>141.3 ± 2.3</td>
<td>10.3</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>K</td>
<td>4.9 ± 1.2</td>
<td>3.6 ± 0.4</td>
<td>7.2</td>
<td>&lt; 0.001*</td>
</tr>
<tr>
<td>Cl</td>
<td>99.6 ± 5.8</td>
<td>100.6 ± 2.9</td>
<td>0.9</td>
<td>0.3</td>
</tr>
<tr>
<td>Ca</td>
<td>2.2 ± 0.4</td>
<td>2.3 ± 0.3</td>
<td>1.7</td>
<td>0.09</td>
</tr>
<tr>
<td>Po4</td>
<td>2.0 ± 0.9</td>
<td>1.0 ± 0.2</td>
<td>7.5</td>
<td>&lt; 0.001*</td>
</tr>
<tr>
<td>Urea</td>
<td>24.6 ± 8.9</td>
<td>4.2 ± 1.6</td>
<td>15.2</td>
<td>&lt; 0.0001*</td>
</tr>
<tr>
<td>Creatinine</td>
<td>1038.6 ± 288.1</td>
<td>62.1 ± 20.5</td>
<td>22.9</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>Uric acid (mg/dl)</td>
<td>7.4 ± 2.1</td>
<td>3.6 ± 2.2</td>
<td>8.7</td>
<td>&lt; 0.001*</td>
</tr>
</tbody>
</table>

P < 0.5 (Significant)

Table 2: Means of Pre and Post-Dialysis Concentrations of Electrolytes and Azotemic Indices

<table>
<thead>
<tr>
<th>Analytes</th>
<th>Predialysis</th>
<th>Postdialysis</th>
<th>z score</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na (mmol/l)</td>
<td>130.5 ± 6.8</td>
<td>134.9 ± 4.9</td>
<td>3.6</td>
<td>0.06</td>
</tr>
<tr>
<td>K</td>
<td>4.9 ± 1.2</td>
<td>3.1 ± 1.0</td>
<td>7.9</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>Cl</td>
<td>99.6 ± 5.8</td>
<td>101.8 ± 5.6</td>
<td>1.9</td>
<td>0.06</td>
</tr>
<tr>
<td>Ca</td>
<td>2.2 ± 0.4</td>
<td>3.5 ± 0.4</td>
<td>2.0</td>
<td>0.05</td>
</tr>
<tr>
<td>Po4</td>
<td>2.0 ± 0.9</td>
<td>1.2 ± 0.7</td>
<td>4.9</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>Urea</td>
<td>24.6 ± 8.9</td>
<td>7.9 ± 5.1</td>
<td>10.9</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>Creatinine</td>
<td>1038.6 ± 288.1</td>
<td>321 ± 189.5</td>
<td>14.1</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>Uric acid (mg/dl)</td>
<td>7.4 ± 2.1</td>
<td>2.3 ± 1.5</td>
<td>13.6</td>
<td>&lt; 0.0001*</td>
</tr>
</tbody>
</table>

P < 0.5 (Significant)

Table 3: Means of Control and Post-Dialysis Concentrations of Electrolytes and Azotemic Indices

<table>
<thead>
<tr>
<th>Analytes</th>
<th>Control</th>
<th>Postdialysis</th>
<th>Z score</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na (mmol/l)</td>
<td>141.3 ± 2.3</td>
<td>134.9 ± 4.9</td>
<td>1.8</td>
<td>0.07</td>
</tr>
<tr>
<td>K</td>
<td>3.6 ± 0.4</td>
<td>3.1 ± 1.0</td>
<td>3.0</td>
<td>&lt; 0.003*</td>
</tr>
<tr>
<td>Cl</td>
<td>100.6 ± 2.9</td>
<td>101.8 ± 5.6</td>
<td>1.4</td>
<td>0.2</td>
</tr>
<tr>
<td>Ca</td>
<td>2.3 ± 0.3</td>
<td>3.5 ± 0.4</td>
<td>1.8</td>
<td>0.07</td>
</tr>
<tr>
<td>Po4</td>
<td>1.0 ± 0.2</td>
<td>1.2 ± 0.7</td>
<td>1.9</td>
<td>&lt; 0.06</td>
</tr>
<tr>
<td>Urea</td>
<td>4.2 ± 1.6</td>
<td>7.9 ± 5.1</td>
<td>4.7</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>Creatinine</td>
<td>62.1 ± 20.5</td>
<td>321 ± 189.5</td>
<td>9.2</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>Uric acid (mg/dl)</td>
<td>3.6 ± 2.2</td>
<td>2.3 ± 1.5</td>
<td>3.4</td>
<td>&lt; 0.001*</td>
</tr>
</tbody>
</table>

P < 0.5 (Significant)

Discussion

This study revealed, that 22(47.8%) were within the age bracket of 51-60yrs out of the total 46 CKD patients in the study group. This is consistent with the works of Mulder and Hillen19; Rowe JW et al20 who in 2001 and in 1996 respectively reported a substantial reduction in kidney functions with aging and also that CKD is commoner in subjects ≥ 50yrs. Also in this study, 29 (63%) were males while 17 (37%) were females. This findings, is in line with other studies (Strangel B et al21; Strangel B et al22) who both in 2003 stated that males are non-modifiable risk factor for CKD. The significant difference observed in the mean concentrations of sodium (p<0.0001), potassium (p<0.001) and phosphate (p<0.001) in pre-dialysis and control groups is in agreement with the report of Forleyet al10 who in 1996 noted that multiple electrolytes levels are altered in patients with CKD and
hence the need to establish dialysis. Equally the significant difference in potassium also agrees with the findings that progression of CKD is usually associated with worsening hyperkalaemia that may require dialysis (23,24). Also the statistical difference (p<0.001) observed in phosphates conforms with the works of Porth11; Stein IH et al26 who in 2002 and 2006 both stated that because of reduced GFR as seen in CKD, phosphorus excretion is impaired leading to phosphatemia. Since phosphorus and calcium has a reciprocal relationship, it means that hyperphosphatemia invariably leads to hypercalcemia. Equally the significant difference that were observed for pre-dialysis urea (p<0.0001), creatinine (p<0.0001) and uric acid (p<0.001) when compared with control are in agreement with the study of Sharon and Judith12 who in 2006 reported significant increase in the values of azotemic indices in CKD patients. Comparative evaluation of pre and post-dialysis revealed an increase in the mean levels of post-dialysis sodium, calcium and chloride with a post-dialysis reduction in the mean levels of potassium and phosphate at a significance level of p<0.0001. This observation is in agreement with the studies of Michael IB et al2; Stein IH et al26 who in 2005 and in 2006 respectively stated that the levels of post-dialysis sodium, calcium and chloride are slightly higher than in pre-dialysis, though not at a significant level. The variation recorded in phosphate and calcium levels is consistent with the statement of Porth11 who in 2002 stated that as calcium level increases there is a corresponding decrease in the level of phosphate in extracellular fluid.

The phenomenon of increase in the mean post-dialysis sodium concentration can be explained by the fact that in CKD, due to the failure of the renal tubules, sodium reabsorption is hindered causing much to be excreted in urine while the reverse is the case in post-dialysis. The same also applies to chloride. Also the significance difference observed in mean concentration of potassium levels in pre and post-dialysis can be explained by the Na\(^{2+}\)-K\(^{2+}\) ATPase ion pump. The system exchanges 3 sodium ions for 2 potassium ions. Therefore, as the post-dialysis sodium was increasing, potassium was going down in order to maintain homeostasis. Equally the significant difference (p<0.0001) observed in the mean concentrations of azotemic indices (urea, creatinine and uric acid) in pre and post-dialysis agrees with the work of Sharon and Judith12 who in 2006 stated that in CKD, the GFR is drastically reduced which leads to the accumulation of these nitrogenous compounds in the body. Equally, the outcome in the post-dialysis and control groups does not differ as such with the observations in the pre-dialysis and post-dialysis groups, as the two statistical analysis both gave credence that some electrochemical equilibrium is maintained through dialysis in CKD patients.

**Conclusion**

Our findings in this study encourage the present practice of periodic/multiple regimen of dialysis as a means of maintaining electrochemical equilibrium in the absence of renal transplantation in CKD patients. Also, we wish to suggest the need to routinely screen individuals for electrolytes and azotemic indices with more attention paid particularly to those ≥50yrs of age.

**References**

Menstrual hygiene knowledge and practices among adolescent girls in secondary schools in Ilorin, Kwara State

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1Department of Community Medicine, College of Health Sciences, University of Ilorin
2Department of Community Medicine, College of Health Sciences, Kogi State University

Abstract

Introduction: Menstruation is the periodic vaginal bleeding that occurs as a result of shedding of the uterine mucosa. Poorly managed, menstrual period may be accompanied by discomfort, reproductive tract infections, odour and embarrassment among others.

Methods: This descriptive study assessed knowledge and practice of menstrual hygiene among adolescent girls in senior secondary schools in Ilorin Metropolis, Kwara State, Nigeria. It also identified factors influencing the knowledge and practice of menstrual hygiene. A self-administered questionnaire was used to assess the knowledge and practice of 200 girls. Multistage sampling technique was used to select respondents. Data were analyzed using SPSS software version 16.0. Chi-square test of significance and ordinal logistic regressions were used in statistical analysis; and level of significance was pre-determined at p-value < 0.05 at confidence level of 95%. Knowledge on menstruation and menstrual hygiene were graded as good, fair and poor. Practice of menstrual hygiene was graded as good and poor respectively. The maximum score on knowledge was 14 points. Respondents who scored 0-4 points for knowledge were rated as poor knowledge, while those who scored 5-9 and 10-14 were rated fair and good knowledge respectively. Maximum score for practice was 10 points. Respondents who scored 6-10 and 0-5 were rated good and poor practice respectively.

Results: Only few respondents had good knowledge of menstruation and menstrual hygiene; 67(33.5%). The proportion of girls who practiced good menstrual hygiene was 87(43.5%). Factors negatively associated with knowledge and practice of good menstrual hygiene in this study included: low educational level of parents, high cost of sanitary pads and lack of toilet facilities in school for changing absorbents, p< 0.05. Late stage of adolescents (adolescents between the ages of 17 – 19 years) and high educational attainment of parents were however found to be positively associated with good knowledge and good practice of menstrual hygiene, p<0.05. The only independent predictor of knowledge of menstrual hygiene was socioeconomic class of parents (class one), parents of respondents who belonged to socioeconomic class one (high socioeconomic class) are three times more likely to have good knowledge of menstruation compared to other classes.

Conclusion: This study showed poor knowledge and practice of menstrual hygiene among adolescent school girls in Ilorin metropolis in the short term. Government should provide sanitary pads free of charge or at subsidized rates to adolescent school girls in order to improve their practice of menstrual hygiene.

Key Words: Adolescent, menstrual hygiene, knowledge, practice, peer education

Introduction

More than half of the world’s population is below the age of 25 years, and one in every two young people in the world is an adolescent. The universal definition of adolescence is best restricted to a ‘period of transition’, in which ‘although no longer considered a child, the young person is not considered as an adult’. United Nations defined an adolescent as anybody within the ages of 10-19 years; the young as people within the ages of 10-24 years and youth as people within the ages of 15-24 years.

Adolescence is a stage where young people develop their adult identity, move toward physical and physiological maturity, and commence the journey towards economic independence. Physiologically, the hypothalamus produces growth hormone and
gonadotropins which initiates pubertal changes. Sequential events of puberty in the female adolescent include; appearance of breast bud, pubic hair, axillary hair, and attainment of menarche.

Menstruation is the periodic vaginal bleeding that occurs as a result of shedding of the uterine mucosa; it is one of the signs of puberty and occurs one or two years following appearance of secondary sexual characteristics. Once established, every mature female menstruates on the average of 3-5 days (range 2-7 days) each month until menopause except during pregnancy and the immediate postpartum period. A woman’s period can be light, moderate or heavy. If poorly managed, menstrual period may be accompanied by discomfort, reproductive tract infection, odour and embarrassment among others.

Menstrual hygiene deals with the special healthcare needs and requirements of women during monthly menstrual cycle. Menstruation and menstrual practices are still clouded by taboos and socio-cultural restrictions resulting in adolescent girls remaining ignorant of the scientific facts and hygienic health practices, which sometimes result in adverse health outcomes. Learning about hygiene during menstruation is a vital aspect of health education for adolescent girls as patterns developed during adolescence are likely to persist into adult life. Although menstrual hygiene is an issue that every girl and woman has to deal with in her life, there is still inadequate provision of information on the process of menstruation, the physical and psychological changes associated with puberty and proper requirements for managing menstruation especially in the developing countries. Many caregivers, especially mothers lack correct information and skills to communicate about menstrual hygiene to their adolescent girls leading to negative attitudes, belief and practices in this regard. This may result in incorrect and unhealthy behaviour during their menstrual periods. A study from South-South Nigeria reported that a woman’s culture, reference group, educational status and religious inclination largely influence her perception of menstruation and menstrual hygiene which in turn affect the type of information passed to their children. A similar study from North Western Nigeria revealed that most of the respondents knew that poor hygiene predisposes to infection and that menstrual hygiene has a place in prevention of menstrual pain. However, a study from Nepal reported that knowledge and practices related to menstruation and menstrual hygiene were not satisfactory among rural adolescent girls. Other studies among high school girls, caregivers, and primary school girls revealed that health education is an effective intervention for promoting menstrual hygiene amongst adolescent school girls. These researchers suggested that health education by teachers, caregivers, peer group and the media are important in addressing misconceptions about menstrual health.

Menarche, or the onset of menstruation, is a landmark feature of female puberty and signals reproductive maturity. Anxiety, fear, confusion, and depression are frequently reported experiences of menarche. While the anatomy of the genital tract and physiology of menstruation are taught in schools in Nigeria, the practical management of menstruation has often been regarded as inappropriate for public discussion. The burden of reproductive tract infections is a major public health concern worldwide and RTIs are particularly widespread in low income settings.

A study conducted in Onitsha, South East Nigeria revealed that only 32.7% of Nigeria girls use sanitary materials as absorbents during menstruation. Other areas of special concern apart from the choice of the best feminine hygiene products include: how often and when to change the feminine hygiene products, bathing and care of the vulva and vagina during menstruation.

According to research, 60% of women and girls in South Africa do not have access to traditional sanitary ware (pads and tampons). And as an alternative to pads and tampons, they have no option but to use rags, toilet paper, newspaper, leaves, recycled tampons, pads and disposable nappies. This raises concern regarding the experiences of women and girls in terms of health and hygiene, productivity, as well as dignity and confidence to be active members of a society.

A study in South-eastern Nigeria revealed that most girls (69%) used sanitary pads. The use of cloth and tissue paper pads, however, was more common among girls who had no pre-menarcheal training. Over half of the respondents changed their menstrual absorbent three or more times a day and there was no difference.
by pre-menarcheal training. In a similar study in South-south Nigeria approximately 33.6% of the girls used sanitary materials (sanitary pad, and tampon) as menstrual absorbents, as high as 55.7% used unsanitary methods. This was similar to the observation of Abioye-Kuteyi who reported the use of unsanitary methods of menstrual absorbencies in 66.3% of girls studied in South western Nigeria. However, the study in North western Nigeria revealed that three hundred and forty eight (93.8%) of the school girls that have commenced menstruation used sanitary pads as absorbents during their last menstrual period. The remaining 23 (6.2%) used either designated pieces of cloth that they washed/boiled, dried and re-used; or used any available piece of cloth that they discarded after use. Reasons given for not using sanitary pads were that it was too expensive in about (91%), the remaining (8.7%) claimed that sanitary pad causes vaginal discharge.

### Menstrual Hygiene

Menstrual hygiene deals with the special health care needs and requirements of women during their monthly menstrual cycle. Areas of special concern include choice of best feminine hygiene products, how often and when to change the hygiene products, bathing, care of the vulva and the vagina during menstruation. Women have used several methods including homemade remedies like pieces of cloth which are either placed on a woman’s undergarment or on a homemade belt that wraps around the waist. These cloths are washed, dried and used again. Available commercial products for women’s hygiene during menstruation include pads, tampons and cups. It is essential to maintain strict hand-washing practices before and after changing sanitary products. Any bacteria on the hands and fingers prior to fitting a sanitary product can be transferred to the vaginal canal and cause infection since menstrual blood can act as culture medium for bacterial growth. Likewise, any bacteria on the fingers following the changing of a product can be transferred to other items. Many women feel uncomfortable and unclean during their menstrual cycle and may wish to bathe more often. There are no rights and wrongs for washing and bathing during menstruation, each individual will adopt practices that are acceptable to them. It should be noted though, that there is no need to clean inside the vagina during the periods as this can disturb the normal body flora and increase the risk of infection. It is fine to gently cleanse around the external labia of the vagina and pat dry.

Components of menstrual hygiene practices can be summarised as follows:

#### Safe menstrual practices: Changing sanitary material at least three times a day or when soaked, changing underwear/panties frequently, washing hands before and after changing absorbents, bathing at least twice daily/ frequently during menstruation, use of sanitary pads or clean cotton materials/cloth that have been preserved specifically for menstruation every month, avoiding the use of reused cloths and toilets rolls as absorbents, regular washing of under wears and proper disposal of used absorbents. Safe methods of disposing used absorbents at household level include: deep burial, burning/incineration, and use of disposal chute and composting pit latrines.

#### Poor menstrual practices: Use of toilet tissue, drying previously used cloth inside dark corners of the house and reusing it, washing of used sanitary cloth in streams or rivers, use of dirty/unclean underwear / panties. Effects of poor menstrual hygiene practices include: reproductive tract infections, discomfort, offensive odour and low self-esteem. Potential health risks of poor menstrual hygiene practices are highlighted in the table below.

A recent community based study in Ungogo, a peri-urban community of Kano on sexual and reproductive health communication between mothers and their adolescent daughters reported that a greater proportion of daughters used sanitary pads during menstruation compared with their mothers (81% vs. 39%).

Poverty, low maternal education and low social class which is highly prevalent in Nigeria were reported to play a major role towards the use of unsanitary materials as menstrual absorbents among Nigeria girls, and women in general.

Unfortunately, information on menstruation and menstrual hygiene given by mothers are often incomplete and incorrect, usually being based on cultural myths, and therefore probably constitutes a major factor towards the negative and distorted
perception and practice of menstruation and menstrual hygiene.\textsuperscript{22, 23}

This research was carried out with the hope that it will contribute immensely to the improvement of menstrual hygiene knowledge and practices among adolescent girls and to produce reliable data on this subject in North central Nigeria. The general objective was to assess menstrual hygiene knowledge and practices among adolescent girls in senior secondary schools in Ilorin, Kwara State, Nigeria.

**Materials and Methods**

Ilorin, the capital of Kwara State is strategically located within the North Central geopolitical zone of Nigeria. The city is located on latitude 8°N and longitude 4°S and situated about 302 kilometers North of Lagos, 602 kilometers South of Kaduna and about 475 kilometers South of Abuja, the Federal Capital Territory (FCT).\textsuperscript{24} The projected population based on 2006 Census using an annual growth rate of 3.2% is 805,396.\textsuperscript{24} It has diverse population because of its strategic location in between the Northern and Western Nigeria. Ilorin metropolis is made up of three Local Government Areas namely Ilorin East, Ilorin West and Ilorin South LGAs with their headquarters at Oke-Oyi, Aleniboro area and Fufu respectively.

The tertiary educational institutions in Ilorin are University of Ilorin, Kwara State University, Kwara State Polytechnic, Kwara State College of Education, College of Arabic and Islamic Legal Studies Kwara State, United Missionary Theological College of Africa (UMTCA), School of Nursing and Midwifery and Al-Hikmah University which is a private University.

This descriptive study included adolescent girls within the ages of 10 – 19 years attending day public senior secondary schools in Ilorin Metropolis. The inclusion criteria were in-school adolescent girls that have started menstruation and in senior secondary classes.

**Sample Size Determination**

**Study Population**

The study population included adolescent girls within the ages of 10 – 19 years attending day public senior secondary schools in Ilorin Metropolis.

The minimum sample size required was estimated using the formula: \textsuperscript{25}

\[
 n = \frac{(Z_{\alpha} + Z_{\beta})^2 (p_1q_1 + p_2q_2)}{p_2 - p_1}^2
\]

Where, \(n\) = Minimum sample size required per group

\(Z_{\alpha}\) = the standard normal deviate corresponding to 5% level of significance. The value obtained from the normal distribution table is 1.96

\(Z_{\beta}\) = the standard normal deviate corresponding to the power of the test to detect differences, 95% power was used for this study. The value obtained from the normal distribution table is 1.64

\(P_1\) = Proportion of adolescent girls with good menstrual hygiene 33.7% (study done in south west Nigeria).\textsuperscript{20}

\(q_1\) = complementary probability to \(p_1\) = 1 - 0.337 = 0.663

\(P_2\) = Proportion of adolescent girls with good menstrual hygiene in an intervention study = 53.7% (Effect of peer education intervention on secondary school adolescents' reproductive health knowledge in Saki, Nigeria).\textsuperscript{20}

\(q_2\) = complementary probability to \(p_2\) = 1 - 0.537 = 0.463

\[
 n = \frac{(1.96+1.64)^2 (0.337x0.663) + (0.537x0.463)}{(0.537-0337)^2} = 152.
\]

**Adjusting for Attrition**

The minimum sample size; \(N = n/ (100 - r\%)\)

Where \(\%\) is the anticipated attrition rate, which is 10%

Substituting; \(N = 152/(100\% - 10\%)\)

152/0.9 = 169

The minimum sample size required for this study was 169 participants. However, 200 students were recruited.

**Sampling Technique**

A multistage sampling method was used for the selection of respondents. This was done in three stages

STAGE 1: Selection of LGA. One LGA out of the three LGAs in Ilorin metropolis were selected using simple random sampling technique by balloting.
without replacement. Ilorin South Local Government Area was selected.

STAGE 2
Step 1- Selection of schools: The list of all the senior secondary schools in Ilorin South LGA and their students’ population were obtained (Appendix 1)\(^{24}\). Four schools were selected. This was done using simple random sampling technique (Balloting). The selected schools were at least 30-50km far away from each other to prevent cross contamination of information during the study. (Appendix 2)

Step 2- The required sample size for each selected school was obtained by proportional allocation based on the population of female students in each school, total number of females in all selected schools and the calculated sample size. (Appendix 3)

\[
N = \frac{n \times 200}{N_t - 1}
\]

N= Required sample size for each selected school
n= No of females in each school
Nt= Total number of females in all the selected schools

STAGE 3
Step 1: Proportionate allocation was carried out to determine number of respondents needed per arm. This was done based on the number of female in each arm, total no of females in all the arms and allocated sample size for each school. (Appendix 4)

\[
R=\frac{n}{N} \times S
\]

R= no of respondents to be chosen in each arm
n= no of female in each arm
N= total no of female in all the arms
S= required sample size for that particular school.

Step 2: finally, eligible respondents in each arm were selected using systematic random sampling technique. Sampling ratio and interval for each arm were obtained using ni/Ni

ni= female respondents in each arm
Ni= estimated sample size for each arm

Simple random sampling technique (balloting method) was used to choose the first respondent. Subsequently, calculated sampling interval was added to the picked number, until the final respondent was obtained.

Data was collected using a pre-tested, self-administered questionnaire to collect information from eligible respondents. Questionnaire used was adapted from previous studies. \(^{10-12}\) Four research assistants were recruited for this study.

Self-administered questionnaires were administered to all the 200 eligible respondents selected from each of the study schools to generate. Administration of these questionnaires was done during break time and over a period of two weeks.

Data collected was presented in prose, frequency tables, charts and graphs. Frequency distribution and other relevant summary statistics were generated. Chi Square test was used to test for association between the categorical variables. Parents were grouped into five socioeconomic classes using classification by Olusanya et al.\(^{26}\) Similarly, multivariate analysis, specifically ordinal logistic regression analysis was used to identify main predictive factors of the knowledge and practice of menstrual hygiene. A confidence limit of 95% was used in this study and a p- value of < 0.05 was considered significant.

Ethical clearance to conduct the study was obtained from the ethical review committee of the University of Ilorin Teaching Hospital before the commencement of the study. Group informed consent was obtained from the parents of respondents during the Parents Teacher Association meetings before the commencement of the study, while written informed assent was obtained from respondents before administration of the questionnaire.

Data Management

**Measurements of Variables**

The data obtained was entered into a personal computer. Analysis was done using SPSS software version 16.0. The outcome measures in this study included:

a) Knowledge on menstruation and menstrual hygiene: This was assessed using specific questions 13-27 on the questionnaire, each correct response attracted one point, while any wrong or don’t know answer attracted no mark. The maximum score on knowledge was 14 points. Respondents who scored 0-4 points for knowledge were rated as poor knowledge, while
those who scored 5-9 and 10-14 were rated fair and good knowledge respectively.\textsuperscript{10}

b) Practice of menstrual hygiene: specific practice assessed were; use of sanitary products like pads as absorbents during menses, reduction in use of re-used cloth, numbers of times absorbents are changed in a day during menses, increase in the number of baths during menses, proper disposal of absorbents, etc. Each correct response attracted 2 points, while any wrong or don’t know answer attracted no mark. Maximum score for practice was 10 points. Respondents who scored 6-10 and 0-5 were rated good and poor practice respectively as used in a previous study\textsuperscript{10}

Results

Socio-demographic characteristics of respondents
A total of two hundred questionnaires were administered to respondents in all schools. All questionnaires were correctly completed. The ages of respondents ranged from 10 to 19 years. The mean age of students was 15.3 ±1.60. Respondents in the age group 14-16 years had the largest representation in the study, 107 (53.5%). Over half of the respondents were Muslims in the study, 111 (55.5%).

Table 1: Distribution of respondents according to socio-demographic characteristics

<table>
<thead>
<tr>
<th>Variables</th>
<th>N = 200 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age group</td>
<td></td>
</tr>
<tr>
<td>10 – 13</td>
<td>20 (10.0)</td>
</tr>
<tr>
<td>14 - 16</td>
<td>107 (53.5)</td>
</tr>
<tr>
<td>17 – 19</td>
<td>73 (36.5)</td>
</tr>
<tr>
<td>Mean age ± SD (years)</td>
<td>15.3 ± 1.60</td>
</tr>
<tr>
<td>Respondent’s Class</td>
<td></td>
</tr>
<tr>
<td>SS 1</td>
<td>23 (11.5)</td>
</tr>
<tr>
<td>SS 2</td>
<td>91 (45.5)</td>
</tr>
<tr>
<td>SS 3</td>
<td>86 (43.0)</td>
</tr>
<tr>
<td>Religion</td>
<td></td>
</tr>
<tr>
<td>Islam</td>
<td>111 (55.5)</td>
</tr>
<tr>
<td>Christianity</td>
<td>88 (44.0)</td>
</tr>
<tr>
<td>Traditional</td>
<td>1 (0.5)</td>
</tr>
</tbody>
</table>

Ninety-seven (48.5%) and 45.5% of respondent’s father and mother had tertiary education respectively as shown in Table 2

Table 2: Level of education of respondents’ parents

<table>
<thead>
<tr>
<th>Level of Education</th>
<th>N = 200 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Father’s level of education</td>
<td></td>
</tr>
<tr>
<td>No Formal</td>
<td>16 (8.0)</td>
</tr>
<tr>
<td>Primary</td>
<td>16 (8.0)</td>
</tr>
<tr>
<td>Secondary</td>
<td>71 (35.5)</td>
</tr>
<tr>
<td>Tertiary</td>
<td>97 (48.5)</td>
</tr>
<tr>
<td>Mother’s level of education</td>
<td></td>
</tr>
<tr>
<td>No Formal</td>
<td>18 (9.0)</td>
</tr>
<tr>
<td>Primary</td>
<td>16 (8.0)</td>
</tr>
<tr>
<td>Secondary</td>
<td>75 (37.5)</td>
</tr>
<tr>
<td>Tertiary</td>
<td>91 (45.5)</td>
</tr>
</tbody>
</table>

Table 3: Distribution of respondents according to socio-demographic characteristics

<table>
<thead>
<tr>
<th>Variables</th>
<th>N = 200 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethnicity</td>
<td></td>
</tr>
<tr>
<td>Yoruba</td>
<td>166 (83.0)</td>
</tr>
<tr>
<td>Hausa</td>
<td>15 (7.5)</td>
</tr>
<tr>
<td>Igbo</td>
<td>9 (4.5)</td>
</tr>
<tr>
<td>Others</td>
<td>10 (5.0)</td>
</tr>
<tr>
<td>Age at Menarche</td>
<td></td>
</tr>
<tr>
<td>10 – 12</td>
<td>94 (47.0)</td>
</tr>
<tr>
<td>13 – 15</td>
<td>95 (47.5)</td>
</tr>
<tr>
<td>≥ 16</td>
<td>11 (5.5)</td>
</tr>
<tr>
<td>Mean age ± SD (years)</td>
<td>12.7 ± 1.5</td>
</tr>
<tr>
<td>Range (years)</td>
<td>10 – 16</td>
</tr>
<tr>
<td>Marital status</td>
<td></td>
</tr>
<tr>
<td>Single</td>
<td>193 (96.5)</td>
</tr>
<tr>
<td>Married</td>
<td>7 (3.5)</td>
</tr>
<tr>
<td>Socio-economic class of parents</td>
<td></td>
</tr>
<tr>
<td>Class 1</td>
<td>19 (9.5)</td>
</tr>
<tr>
<td>Class 2</td>
<td>33 (16.5)</td>
</tr>
<tr>
<td>Class 3</td>
<td>101 (50.5)</td>
</tr>
<tr>
<td>Class 4</td>
<td>32 (16.0)</td>
</tr>
<tr>
<td>Class 5</td>
<td>15 (7.5)</td>
</tr>
</tbody>
</table>

Knowledge of menstrual hygiene
Only 16.0% of respondents could describe menstruation correctly. Majority of the girls knew the expected age at menarche, 96.5%. Almost half of respondents knew the correct source of menstruation, 45.5%. Over half, 55.0% of respondents knew the
normal duration of menstrual flow. Only 9.0% of respondents could define menstrual cycle correctly.

Table 3: Respondents’ knowledge of menstruation

<table>
<thead>
<tr>
<th>Respondent’s knowledge on menstruation</th>
<th>YES n (%)</th>
<th>NO n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Description of menses Physiological process</td>
<td>32 (16.0)</td>
<td>168 (84.0)</td>
</tr>
<tr>
<td>Source of menses Uterus</td>
<td>91 (45.5)</td>
<td>109 (54.5)</td>
</tr>
<tr>
<td>Expected age of menarche 9 – 14 years</td>
<td>193 (96.5)</td>
<td>07 (3.5)</td>
</tr>
<tr>
<td>Duration of menstrual flow 3 – 7 days</td>
<td>110 (55.0)</td>
<td>90 (45.0)</td>
</tr>
<tr>
<td>Definition of menstrual cycle First day of one period to the first day of the next period</td>
<td>38 (19.0)</td>
<td>152 (81.0)</td>
</tr>
<tr>
<td>Duration of menstrual cycle 21 – 35 days</td>
<td>18 (9.0)</td>
<td>182 (91.0)</td>
</tr>
<tr>
<td>Age at menopause 45 – 55 years</td>
<td>71 (35.5)</td>
<td>129 (65.5)</td>
</tr>
</tbody>
</table>

Source of information about menstruation
Friends (42.3%) and mothers (41.8%) were however the most common source of information about menstruation for respondents. Majority (98.0%) of the respondents had received some form of information on menstruation before the attainment of menarche.

Respondents’ knowledge of the components of menstrual hygiene practices
Majority of girls described the components of menstrual hygiene practices as the use of toilet rolls and reused cloth during menses, 97.0% and 90.0% respectively. Only few respondents (31.0%) described component of menstrual hygiene practices as the use of sanitary pads. The percentage of respondents who described the components of menstrual hygiene practices as bathing at least twice per day was 37.0%, regular cleaning of vagina (48.0 %) and regular washing of under wears (66.0%).

Level of awareness of products for absorbing menstrual blood was 80.5% and 55.5% on knowledge of good menstrual hygiene in reducing vaginal discharge. Only 67 (33.5 %) of girls in had good knowledge of menstruation and menstrual hygiene.

Practice of menstrual hygiene
About 43.5% of the respondents used sanitary pad during their last menses. Out of 124 girls that used cloth, 84(68.0%) washed and reused the cloth during subsequent menses. Only 28.0% of respondents changed their absorbents at least thrice in a day. About half (52.0%) of the girls did not increase the number of times they bath during menses. 51.0% washed their hands before and after changing absorbents while 77.5% cleans their vagina before changing absorbents. 87(43.5%) of respondents practiced good menstrual hygiene.

Factors influencing the knowledge and practice of menstrual hygiene among respondents
Majority (95.5%) of the respondents do not have toilet in their schools for changing absorbents.

Although 95% of the respondents in study schools have toilet at their homes, 55.8% of the toilets do not have water supply. The most common type of absorbent used by respondents was cloths, 45.0%, this was closely followed by sanitary pads (30.0%) and toilet roll (23.0%). The major reasons for not using sanitary pads among respondents were because it was expensive, this was closely followed by not comfortable and causes discharge. Majority of the respondents disposed of their used absorbents with domestic refuse, this was closely followed by burning and flushing in water closet. Majority (96.5%) of the respondents attended schools during menses.However, most (70.5%) of the girls do not change their absorbents in school.

At bivariate level, age groups, classes of respondents, and age at menarche were significantly associated with respondent’s knowledge of menstruation and menstrual hygiene among respondents. A high proportion of respondents in early adolescent stage had fair knowledge of menstrual hygiene (75%) compared with other stages. Respondents in SSS 3 classes had the highest knowledge of menstruation and menstrual hygiene (40.7%) while respondents in SS1 classes had the lowest knowledge (26.1%). A higher proportion of girls who started menstruation after the age of 16 years (86.7%) had good knowledge of menstruation and menstrual hygiene compared with other age groups. These were all found to be statistically significant, p <0.05.
At bivariate level, socioeconomic status of parents and mother educational level were also found to be statistically associated with the knowledge of menstrual hygiene. Respondents whose parents fell within socioeconomic class one (95.7%) had the highest knowledge of menstruation and menstrual hygiene compared with other classes, this was found to be statistically significant, p< 0.05. The analysis showed that 43.9% of girls whose mothers had tertiary education had good knowledge of menstruation and menstrual hygiene, compared to other groups, this was found to be statistically significant, p<0.05.

Table 4: Socio demographic factors influencing knowledge of menstrual hygiene among respondents

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Good n (%)</th>
<th>Fair n(%)</th>
<th>Poor n (%)</th>
<th>Total n (100%)</th>
<th>$\chi^2$</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age groups</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>10 – 13</td>
<td>1 (5.0)</td>
<td>15 (75.0)</td>
<td>4 (20.0)</td>
<td>20</td>
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<td></td>
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<tr>
<td>14 – 16</td>
<td>39 (36.5)</td>
<td>30 (28.0)</td>
<td>38 (35.5)</td>
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<td></td>
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<td>17 – 19</td>
<td>27 (38.0)</td>
<td>32 (43.8)</td>
<td>14 (19.2)</td>
<td>73</td>
<td>20.54</td>
<td>&lt;0.001*</td>
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<td>Class</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>SS 1</td>
<td>6 (26.1)</td>
<td>8 (34.8)</td>
<td>9 (39.1)</td>
<td>23</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SS 2</td>
<td>26 (28.6)</td>
<td>55 (60.4)</td>
<td>10 (11.0)</td>
<td>91</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SS 3</td>
<td>35 (40.7)</td>
<td>14 (16.3)</td>
<td>37 (43.0)</td>
<td>86</td>
<td>38.994</td>
<td>&lt;0.001*</td>
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<td>Religion</td>
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<tr>
<td>Islam</td>
<td>39 (33.6)</td>
<td>41 (35.3)</td>
<td>36 (31.1)</td>
<td>116</td>
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<td>Christianity</td>
<td>28 (33.3)</td>
<td>36 (42.9)</td>
<td>20 (23.8)</td>
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<td>1.624</td>
<td>0.443</td>
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</tr>
<tr>
<td>Hausa</td>
<td>4 (33.3)</td>
<td>5 (41.7)</td>
<td>3 (25.0)</td>
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<td></td>
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<tr>
<td>Igbo</td>
<td>8 (47.1)</td>
<td>6 (35.3)</td>
<td>3 (17.6)</td>
<td>17</td>
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<td></td>
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<tr>
<td>Yoruba</td>
<td>55 (32.2)</td>
<td>66 (38.6)</td>
<td>50 (29.2)</td>
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<td>1.884</td>
<td>0.757</td>
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<td>Marital status</td>
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<td>Single</td>
<td>65 (34.4)</td>
<td>73 (38.6)</td>
<td>51 (27.0)</td>
<td>189</td>
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<tr>
<td>Married</td>
<td>2 (22.3)</td>
<td>4 (44.4)</td>
<td>5 (33.3)</td>
<td>11</td>
<td>1.114</td>
<td>0.572*</td>
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<td>Age at menarche</td>
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<td>10 – 12</td>
<td>26 (26.5)</td>
<td>52 (53.1)</td>
<td>20 (20.4)</td>
<td>98</td>
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<tr>
<td>13 – 15</td>
<td>28 (32.2)</td>
<td>23 (26.4)</td>
<td>36 (41.4)</td>
<td>87</td>
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<td></td>
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<tr>
<td>≥16</td>
<td>13 (86.7)</td>
<td>2 (13.3)</td>
<td>0 (0.0)</td>
<td>15</td>
<td>30.925</td>
<td>&lt;0.001*</td>
</tr>
</tbody>
</table>

$\chi^2$: Chi square; *: Yates corrected p value; *: statistically significant (i.e. p value)

Table 4b: Socio-demographic factors influencing knowledge of menstrual hygiene among respondents

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Good n (%)</th>
<th>Fair n(%)</th>
<th>Poor n (%)</th>
<th>Total n (100%)</th>
<th>$\chi^2$</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Socio-economic class of parents</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Class 1</td>
<td>22 (95.7)</td>
<td>1 (4.3)</td>
<td>0 (0.0)</td>
<td>23</td>
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<tr>
<td>Class 2</td>
<td>9 (32.1)</td>
<td>10 (35.8)</td>
<td>9 (32.1)</td>
<td>28</td>
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<tr>
<td>Class 3</td>
<td>10 (11.9)</td>
<td>46 (54.8)</td>
<td>28 (33.3)</td>
<td>84</td>
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</tr>
<tr>
<td>Class 4</td>
<td>25 (40.4)</td>
<td>19 (30.6)</td>
<td>18 (29.0)</td>
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<tr>
<td>Class 5</td>
<td>1 (33.3)</td>
<td>1 (33.3)</td>
<td>1 (33.3)</td>
<td>3</td>
<td>17.76</td>
<td>0.023*</td>
</tr>
<tr>
<td>Fathers’ Educational level</td>
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<td></td>
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<td></td>
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<tr>
<td>No Formal</td>
<td>4 (25.0)</td>
<td>6 (37.5)</td>
<td>6 (37.5)</td>
<td>16</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary</td>
<td>5 (31.2)</td>
<td>2 (12.5)</td>
<td>9 (56.2)</td>
<td>16</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Secondary</td>
<td>20 (28.2)</td>
<td>35 (49.3)</td>
<td>16 (22.5)</td>
<td>71</td>
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<td></td>
</tr>
<tr>
<td>Tertiary</td>
<td>38 (39.1)</td>
<td>34 (35.1)</td>
<td>25 (25.8)</td>
<td>97</td>
<td>10.387</td>
<td>0.238</td>
</tr>
<tr>
<td>Mothers’ Educational level</td>
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<td></td>
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</tr>
<tr>
<td>No Formal</td>
<td>2 (11.1)</td>
<td>9 (50.0)</td>
<td>7 (38.9)</td>
<td>18</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary</td>
<td>5 (31.25)</td>
<td>6 (37.5)</td>
<td>5 (31.25)</td>
<td>16</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Secondary</td>
<td>20 (26.7)</td>
<td>27 (36.0)</td>
<td>28 (37.3)</td>
<td>75</td>
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</tr>
<tr>
<td>Tertiary</td>
<td>40 (43.9)</td>
<td>35 (38.5)</td>
<td>16 (17.6)</td>
<td>91</td>
<td>15.585</td>
<td>0.040*</td>
</tr>
</tbody>
</table>

$\chi^2$: Chi square; *: Yates corrected p value; *: statistically significant (i.e. p value)
Table 5: Predictors of knowledge of menstrual hygiene among respondents from multivariate logistic regression analysis

<table>
<thead>
<tr>
<th>Predictor</th>
<th>OR (95% CI)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age groups</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 – 13</td>
<td>0.496 (0.068 - 3.602)</td>
<td>0.480</td>
</tr>
<tr>
<td>14 – 16</td>
<td>1.014 (0.563 - 1.827)</td>
<td>0.963</td>
</tr>
<tr>
<td>17 – 19(RC)</td>
<td></td>
<td>0.282</td>
</tr>
<tr>
<td><strong>Classes</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SS 1</td>
<td>1.319 (0.539 - 3.226)</td>
<td>0.543</td>
</tr>
<tr>
<td>SS 2</td>
<td>1.374 (0.762 - 2.477)</td>
<td>0.290</td>
</tr>
<tr>
<td>SS 3 (RC)</td>
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<td>0.310</td>
</tr>
<tr>
<td><strong>Socio-economic class of parents</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Class 1</td>
<td>3.384 (0.159 - 0.925)</td>
<td>0.029*</td>
</tr>
<tr>
<td>Class 2</td>
<td>0.991 (0.449 - 2.188)</td>
<td>0.982</td>
</tr>
<tr>
<td>Class 3</td>
<td>0.908 (0.329 - 2.509)</td>
<td>0.852</td>
</tr>
<tr>
<td>Class 4</td>
<td>0.885 (0.393 - 1.995)</td>
<td>0.769</td>
</tr>
<tr>
<td>Class 5 (RC)</td>
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<td>0.451</td>
</tr>
<tr>
<td><strong>Age at Menarche</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 – 12</td>
<td>1.292 (0.717 - 2.328)</td>
<td>0.393</td>
</tr>
<tr>
<td>13 – 15</td>
<td>0.775 (0.429 - 1.399)</td>
<td>0.397</td>
</tr>
<tr>
<td>≥16 (RC)</td>
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<td>0.199</td>
</tr>
<tr>
<td><strong>Mothers’ Educational level</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No Formal</td>
<td>0.231 (0.056 – 0.421)</td>
<td>0.568</td>
</tr>
<tr>
<td>Primary</td>
<td>0.422 (0.132 – 0.689)</td>
<td>0.363</td>
</tr>
<tr>
<td>Secondary</td>
<td>0.532 (0.214 – 0.929)</td>
<td>0.866</td>
</tr>
<tr>
<td>Tertiary (RC)</td>
<td></td>
<td>0.845</td>
</tr>
</tbody>
</table>

OR= Odd Ratio; CI= Confidence Interval; RC = Reference Category; *p<0.05

Table 6: Socio demographic factors influencing practice of menstrual hygiene among respondents in the study schools

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Good n (%)</th>
<th>Poor n (%)</th>
<th>Total N=200 (100 %)</th>
<th>χ²</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age group</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 – 13</td>
<td>6 (31.3)</td>
<td>14 (68.7)</td>
<td>20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14 – 16</td>
<td>34 (31.8)</td>
<td>73 (68.2)</td>
<td>107</td>
<td></td>
<td></td>
</tr>
<tr>
<td>17 – 19</td>
<td>47 (64.4)</td>
<td>26 (35.6)</td>
<td>73</td>
<td>20.421</td>
<td>0.000*</td>
</tr>
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<td><strong>Religion</strong></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Islam</td>
<td>48 (43.2)</td>
<td>63 (56.8)</td>
<td>111</td>
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</tr>
<tr>
<td>Christianity</td>
<td>39 (43.8)</td>
<td>50 (56.2)</td>
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<td><strong>Ethnicity</strong></td>
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<tr>
<td>Hausa</td>
<td>5 (33.3)</td>
<td>10 (66.7)</td>
<td>15</td>
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<td></td>
</tr>
<tr>
<td>Igbo</td>
<td>13 (68.4)</td>
<td>6 (31.6)</td>
<td>19</td>
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</tr>
<tr>
<td>Yoruba</td>
<td>69 (41.6)</td>
<td>97 (58.4)</td>
<td>166</td>
<td>3.842</td>
<td>0.323</td>
</tr>
<tr>
<td><strong>Classes</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SS 1</td>
<td>7 (30.4)</td>
<td>16 (69.6)</td>
<td>23</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SS 2</td>
<td>31 (34.1)</td>
<td>60 (65.9)</td>
<td>91</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SS 3</td>
<td>39 (45.3)</td>
<td>47 (54.7)</td>
<td>86</td>
<td>2.344</td>
<td>0.309</td>
</tr>
<tr>
<td><strong>Marital status</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single</td>
<td>83 (43.5)</td>
<td>108(56.5)</td>
<td>191</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Married</td>
<td>4 (44.4)</td>
<td>5 (55.6)</td>
<td>9</td>
<td>0.082</td>
<td>0.774</td>
</tr>
<tr>
<td><strong>Age at Menarche</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 – 12</td>
<td>41 (44.0)</td>
<td>53 (56.0)</td>
<td>94</td>
<td></td>
<td></td>
</tr>
<tr>
<td>13 – 15</td>
<td>45 (44.1)</td>
<td>57 (55.9)</td>
<td>102</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥16</td>
<td>1(25.0)</td>
<td>3(75.0)</td>
<td>4</td>
<td>0.077</td>
<td>0.994</td>
</tr>
</tbody>
</table>

χ²: Chi square; *: statistically significant
Socio-demographic variables that were significant for knowledge of menstruation and menstrual hygiene at bivariate level were further subjected to logistic regression analysis to determine the predictors of knowledge of menstruation and menstrual hygiene among respondents in the study schools. The socioeconomic class one remained the only significant predictor of knowledge of menstruation and menstrual hygiene among respondents in the study schools. Girls whose parents belonged to socioeconomic class one (high socioeconomic class) is three times more likely to have good knowledge of menstrual hygiene compared with other groups.

Table 6b: Socio demographic factors influencing practice of menstrual hygiene among respondents

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Good (%)</th>
<th>Poor (%)</th>
<th>Total</th>
<th>$\chi^2$</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Socio-economic class of parents</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Class 1</td>
<td>8 (42.1)</td>
<td>11 (57.9)</td>
<td>19</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Class 2</td>
<td>20 (60.6)</td>
<td>13 (39.4)</td>
<td>33</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Class 3</td>
<td>39 (38.6)</td>
<td>62 (61.4)</td>
<td>101</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Class 4</td>
<td>12 (37.5)</td>
<td>20 (62.5)</td>
<td>32</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Class 5</td>
<td>8 (53.3)</td>
<td>7 (46.7)</td>
<td>15</td>
<td>5.984</td>
<td>0.200</td>
</tr>
<tr>
<td><strong>Fathers’ Educational level</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No Formal</td>
<td>9 (56.3)</td>
<td>7 (43.7)</td>
<td>16</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary</td>
<td>6 (37.5)</td>
<td>10 (62.5)</td>
<td>16</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Secondary</td>
<td>14 (19.7)</td>
<td>57 (80.3)</td>
<td>71</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tertiary</td>
<td>58 (59.8)</td>
<td>39 (40.2)</td>
<td>97</td>
<td>28.194</td>
<td>0.000*</td>
</tr>
<tr>
<td><strong>Mothers’ Educational level</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No Formal</td>
<td>8 (44.4)</td>
<td>10 (55.6)</td>
<td>18</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary</td>
<td>5 (31.3)</td>
<td>11 (68.7)</td>
<td>16</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Secondary</td>
<td>23 (30.7)</td>
<td>52 (69.3)</td>
<td>75</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tertiary</td>
<td>51 (56.0)</td>
<td>40 (44.0)</td>
<td>91</td>
<td>11.891</td>
<td>0.018*</td>
</tr>
</tbody>
</table>

$\chi^2$: Chi square; *: statistically significant

Table 7: Predictors of practice of menstrual hygiene among respondents from multivariate logistic regression analysis

<table>
<thead>
<tr>
<th>Predictor</th>
<th>OR (95% CI)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age groups</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 – 13</td>
<td>0.639 (0.125 – 2.702)</td>
<td>0.540</td>
</tr>
<tr>
<td>14 – 16</td>
<td>1.945 (0.473 – 0.334)</td>
<td>0.432*</td>
</tr>
<tr>
<td>17 – 19 (RC)</td>
<td>0.037</td>
<td></td>
</tr>
<tr>
<td><strong>Fathers’ Educational level</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No Formal</td>
<td>0.021 (0.056 – 0.311)</td>
<td>0.332</td>
</tr>
<tr>
<td>Primary</td>
<td>0.310 (0.221 – 0.882)</td>
<td>0.127</td>
</tr>
<tr>
<td>Secondary</td>
<td>0.514 (0.401 – 0.874)</td>
<td>0.885</td>
</tr>
<tr>
<td>Tertiary (RC)</td>
<td>0.102</td>
<td></td>
</tr>
<tr>
<td><strong>Mothers’ Educational level</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No Formal</td>
<td>0.245 (0.142 – 0.415)</td>
<td>0.647</td>
</tr>
<tr>
<td>Primary</td>
<td>0.398 (0.099 – 0.728)</td>
<td>0.573</td>
</tr>
<tr>
<td>Secondary</td>
<td>0.879 (0.688 – 1.034)</td>
<td>0.741</td>
</tr>
<tr>
<td>Tertiary (RC)</td>
<td>0.578</td>
<td></td>
</tr>
</tbody>
</table>

OR = Odd Ratio; CI = Confidence Interval; RC = Reference Category; *p<0.05
Predictors of practice of menstrual hygiene among respondents from multivariate logistic regression analysis

Socio-demographic variables that were significant for practice of menstrual hygiene at bivariate level were further subjected to logistic regression analysis to determine the predictors of menstrual hygiene among respondents in the study schools. Age groups of respondents remained the only predictor of menstrual hygiene. Respondents within the age group 14-16 are two times more likely to practiced good menstrual hygiene compared to other age groups.

Table 8: Relationship between knowledge and practice of menstrual hygiene among respondents

<table>
<thead>
<tr>
<th>Practice</th>
<th>Good (%)</th>
<th>Fair (%)</th>
<th>Poor (%)</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Good Practice</td>
<td>48 (71.6)</td>
<td>28 (36.4)</td>
<td>11 (19.6)</td>
<td>87 (43.5)</td>
</tr>
<tr>
<td>Poor Practice</td>
<td>19 (28.4)</td>
<td>49 (63.6)</td>
<td>45 (80.4)</td>
<td>113 (56.5)</td>
</tr>
<tr>
<td>Total</td>
<td>67 (100.0)</td>
<td>77 (100.0)</td>
<td>56 (100.0)</td>
<td>200 (100)</td>
</tr>
</tbody>
</table>

\[ X^2 = 36.15 \text{ df}=2 \quad p<0.00 \]

There was statistically significant association in the knowledge and practice of menstrual hygiene among respondents in the study schools (p<0.00). Respondents who had good knowledge (71.6%) of menstrual hygiene practiced good menstrual hygiene, while 80.0% of girls who had poor knowledge of menstrual hygiene practiced poor menstrual hygiene.

Discussion

Respondent’s age ranged from 10-19 years. About half (53.3 %) of the respondents were in their mid-adolescence (14-16 years). This is not surprising because majority of the students in senior secondary schools usually fall within this age group. The mean ages of the students in this study was 15.3 ±1.60 years. This is also in keeping with the findings of a study conducted in Kano where the mean age of respondents was 15.2 ±2.0 years.9 The finding here is also congruent with what was obtained in a study conducted in Iran on promoting menstrual health among Persian adolescent girls from low socioeconomic backgrounds, where the mean age of respondent was 15.7 ±1.08 years.3

In this study, 50.5% of girls in the study had the socioeconomic class of their parents within class 3 socioeconomic groups. This was similar to the findings of Moloud et al where the socioeconomic class of parents of majority of the participants belonged to class 3 socioeconomic group (83.3%).3 This study revealed that 48.5% of fathers of the respondents in the study schools attained tertiary level of education while, 45.5% of the mothers of respondents in the study schools had tertiary level education.

The level of awareness of menstruation among respondents was quite high with 98% of respondents being aware of menstruation before they attained menarche. This high level of awareness is not surprising because this study was conducted among secondary school female students who are expected to have some exposure to this subject through school teachers, parents, mass media, peers, etc. This high level of awareness of menstruation is similar to what was found in a study conducted in Kano where 97.2% of the respondents had heard of menstruation before they attained menarche.10

Friends (42.3%) closely followed by mothers (41.8%) were the main source of menstrual information for girls. This finding is similar to that observed in a study conducted in Enugu, South East Nigeria on the impact of pre-menarcheal training on menstrual practices and hygiene of Nigerian school girls, where mothers (74.7%) were the main source of menstrual information.26 However, in a related study in Egypt, 92.2% of the girls accessed menstrual information primarily from the mass media.23 The Mass media have also been observed to also play a prominent role in the dissemination of reproductive health information.27

Although majority of the respondents were aware of menstruation before the attainment of menarche, only 16% of girls in the study schools and 15% of girls in the control could describe menstruation as a normal physiological process. This observation may be due to lack of proper health education programs that focused on the menstrual health and hygiene among girls in the schools. Most teachers are also not well equipped in delivering detailed lecture on reproductive health issues, and may feel uncomfortable when topics on reproductive health are taught in class especially in the presence of male students.20 This is also consistent
with what was found in India where only 18.35% of the girls believed menstruation to be physiological process. This finding is also comparable with the studies of Adinma in Onitsha, South East Nigeria and Adika et al in Bayelsa, South South, Nigeria where 19.3% and 22.0% of the respondents in the studies perceived menstruation to be a physiological process respectively. A large proportion (65.5%) described menstruation as assured fertility and cleansing of the womb. The finding here is also congruent with Adinma’s study in Onitsha, where 73.1% of the respondents perceived menstruation from the point of view of cleaning-up of the uterus and assured fertility. This perhaps may not be un-related to the premium the average African attaches to fertility and child bearing. They will therefore go to any length to seek for the needed assurance on fertility, or remedies for infertility.

Although, majority of the respondents (96.5%) knew the expected age range for attainment of menarche, more than half (54.5%) did not know the source of menstrual bleeding and gave sources like kidney, liver, etc. This may also be due to lack of proper health education programmes in the schools. This finding is comparable with what was found in Malaysia where only 2.5% of the study girls stated that menstrual bleeding came from the uterus and 76.2% were unaware of the source of the menstrual bleeding.

Only 19% and of girls could describe menstrual cycle correctly and 9.0% knew the normal duration of menstrual cycle. Despite the high level of awareness of menstruation, the aggregate knowledge score was low. Only 33.5% of girls had good knowledge of menstruation and menstrual hygiene and many lacked detailed information about specific aspects of menstruation and menstrual hygiene.

Poor menstrual hygiene and self-care practices are synonymous to poor health outcome and health status of the adolescent female. The practice score was 43.5%. A high proportion of girls, 43.5%, used sanitary pads during their last menstrual period. This is also similar to the observation of Abioye-Kuteyi who reported the use of unsanitary methods of menstrual absorbencies in 66.3% of girls studied in Southwestern Nigeria. This study also noted that most respondents not using sanitary pads did so because they could not afford it, thereby prefer other absorbents like clothes and toilet rolls. The implication of using clothes is the tendency towards their being re-cycled, which was observed to be the case in 68.0% of the girls, a situation which may highly predispose them to pelvic infection. Lack of finance was also noted to be responsible for non-usage of sanitary pads and hence the use of other absorbents such as clothes and toilet roll among adolescents’ girls in Amassoma community of Bayelsa State, Nigeria. In this study, 43.5% of girls practiced good menstrual hygiene. Socioeconomic status of parents of respondents was responsible for the insignificant change in use of sanitary pads for menses as majority of the parents of respondents in both groups fell within class three socioeconomic groups and stated financial constraint as mitigating factor for not using sanitary pads.

It was noted that a high proportion, 40.5% of girls who had good knowledge of menstruation and menstrual hygiene were in SS3 classes. This is however not surprising because most students in SS3 classes will be in their mid or late adolescent stage and would probably have been exposed to various information concerning menstruation and menstrual hygiene from teachers, peers, mass media, parents etc.

Also, a significant proportion of girls whose parents belonged to class one socioeconomic group had good knowledge of menstruation and menstrual hygiene. This is also not surprising because class one socioeconomic groups include professionals like doctors, lawyers, accountants, engineers, etc. They are more educated and equipped with adequate information on health issues which can probably be passed to their children. This finding is also congruent with what was found in Saudi Arabia where 78% of girls whose parents fell within class one socioeconomic group had good knowledge of menstrual hygiene.

The only independent predictor of knowledge of menstrual hygiene in this study was socioeconomic class one. Respondents whose parents belonged to class one socioeconomic group were three times more likely to have good knowledge compared with others. Their parents having a wealth of information on health
may have transferred information on various aspects of health including puberty and menstruation to them. This was similar to the findings of Dongre et al in India on the effects of community-based health education intervention on management of menstrual hygiene among rural Indian adolescent girls where respondents whose parents fell in the high socioeconomic group had good knowledge of menstrual hygiene.12

Bivariate analysis of the socio-demographic factors associated with the practice of menstrual hygiene among respondents showed statistically significant relationship between the practice of menstrual hygiene and the age group of respondents. Over half of respondents that fell within ages 17-19 years (late adolescent), 54.0% practiced good menstrual hygiene compared with other age groups. This may be due to the fact that they are already in their late adolescent and they may have acquired experience over time.

Educational attainment of respondents’ parents was also found to be significantly associated with knowledge and practice of menstrual hygiene among respondents in this study with p < 0.05. Educational attainment of parents has been found to influence the economic strength of the family and individuals’ social exposure hence reducing the negative impact of harmful local practices.26,33 In this study, 43.9% of respondents whose mothers attained tertiary education had good knowledge of menstrual hygiene. Also, 59.8% of respondents whose fathers attained tertiary education practiced good menstrual hygiene among respondents. This is comparable with a study in South East Nigeria on age at menarche and the menstrual pattern of Igbo women where pre-menarcheal training was significantly related to the educational attainment of the respondent’s parents.19 This also corroborates the finding of a study in South Western Nigeria on menstrual knowledge and practices amongst secondary school girls in Ile-Ife which revealed that parental education was positively associated with girls’ menstrual knowledge.20

About 71.6 % of girls who had good knowledge of menstrual hygiene practiced good menstrual hygiene among respondents. Also, 80.4% of respondents who had poor knowledge practiced poor menstrual hygiene among respondents. This association between knowledge and practice among respondents was found to be statistically significant with p < 0.05.

Conclusion

This study revealed that although most of the respondents had received some form of information on menstruation before attaining menarche, their knowledge and practice of menstrual hygiene were found to be poor. High socio-economic class was found to be a predictor of good menstrual hygiene practice.

Recommendations

In view of the findings from this study, the following recommendations are therefore made

1. Sanitary pads should be made available free of charge or subsidized by the government and non-governmental organisations to all adolescent females as it is presently being done by the Ugandan government.

2. Menstrual health should be incorporated into health education sessions in family planning and antenatal clinics. This will help in equipping mothers with adequate knowledge of menstrual hygiene which could be passed to their daughters, since they also constitute a major source of information about menstrual hygiene.

3. Peer education should be included in the educational and school health programmes by kwara State Ministry of Education, this will help to equip adolescent girls with adequate information and skills on menstrual hygiene and other adolescents’ reproductive health issues as demonstrated by this study and other previous studies.

4. Relevant stakeholders such as Ministry of Health, in collaboration with Ministry of Education, should embark on ‘training of trainers’ project as regard menstrual hygiene. This is to ensure quality of training.

References


Nigella sativa oil improved oxidative stress parameters in streptozotocin-induced diabetes in wistar rats.

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2Department of Physiology, Faculty of Basic Medical Sciences, College of Health Sciences, University of Ilorin, Ilorin, Kwara State, Nigeria.

Abstract

**Background:** Hyperglycemia is known to cause oxidative stress, resulting mainly from enhanced production of mitochondrial reactive oxygen species (ROS). In diabetic patients, the persistence of hyperglycemia has been implicated in causing increased production of oxygen-free radicals through glucose autoxidation and non-enzymatic glycation. Such imbalance in oxidative redox resulting from hyperglycemia may account for the cellular pathologies in vital tissues of diabetic patients. In this study, we have investigated the effects of black seed (*Nigella sativa*) oil on oxidative redox states in streptozotocin (STZ)-induced diabetes in rats by assessing anti-oxidant enzyme activities and lipid peroxidation.

**Methodology:** Thirty adult Wistar rats (weighing 160-200 g) were used. Rats were assigned at random into five groups (control (normal saline); untreated STZ-diabetic (70 mg/kg BW, IP); treated STZ-diabetic with *Nigella Sativa* oil (NGS) (1 ml/kg BW, oral); treated STZ-diabetic with NGS (2 ml/kg BW oral); NGS (1 ml/kg B.W, oral)). After 28 days of treatment, the animals were fasted overnight, anesthetized and sacrificed. Subsequently, levels of malondialdehyde (MDA) (marker of lipid peroxidation) and the activities of superoxide dismutase (SOD) and catalase (CAT) (antioxidant enzymes) were investigated in serum of treated rats.

**Results:** The enzyme assay showed decreased levels of SOD and catalase and elevated levels of MDA in the diabetic group persistent through the period of treatment compared to control and treatment groups, which showed a gradual increase and reduction of these markers respectively (P<0.05).

**Conclusion:** This study showed that untreated diabetes mellitus is associated with oxidative stress in rats. Our findings also suggest that administration of 2ml/kgBW NGS can lower STZ-induced toxicity as shown by the up-regulation of antioxidant mechanisms, indicating apotential neuroprotective effect of the *Nigella sativa* oil in streptozotocin-induced diabetic rat.

**Keywords:** Streptozotocin, Diabetes mellitus, Nigella sativa, Oxidative stress, Prefrontal cortex.

Introduction

Diabetes mellitus is a syndrome characterized by chronically hyperglycemia and complications such as atherosclerosis, nerve damage, renal failure and infection. Recently, some evidences suggest that oxidative stress may play an important role in the etiology of diabetes and diabetic complications. In healthy individuals, oxidative damage to tissue is prevented by a system of defenses which includes antioxidant enzymes and small molecules with scavenging ability such as antioxidant vitamins. In diabetic patients, an altered balance between reactive oxygen species (ROS) production, antioxidant levels and levels of lipid peroxidation has been reported. Diabetic patients have significant defects in antioxidant defense elements, and enhanced ROS generation is one of the major determinants of diabetic complications.

Reactive oxygen species (ROS) which cause cellular damage by their oxidation ability have been implicated in the pathogenesis of diabetes mellitus. During diabetes, persistent hyperglycemia increases the...
production of ROS through glucose autoxidation\textsuperscript{10, 11}. The oxidative stress has also been associated with diabetic states in animals and humans\textsuperscript{6, 7, 12, 13}. A study, using rats with streptozotocin (STZ)-induced diabetes, showed that levels of lipid peroxidation increased, as indicated by thiobarbituric acid reactive substances (TBARS), which is an oxidative stress marker, suggesting the occurrence of oxidative stress\textsuperscript{12}.

Several factors are involved in the pathophysiology of cognitive decline in diabetes. Some of these factors are however conjectural. Factors such as hyperglycemia\textsuperscript{14}, hypoglycemia and vascular disease\textsuperscript{15}, amyloidosis and insulin resistance\textsuperscript{16} are implicated. In addition, oxidative stress (characterized by increased superoxide anion formation in the presence of chronic hyperglycemia) is involved in the pathogenesis of diabetic cognitive impairment\textsuperscript{17}.

The seed of \textit{Nigella sativa} (NSL) known as black cumin is a medicine plant, with components reported to have potent antioxidant effects, and has been used in the Middle East and Far East as a traditional medicine for a wide range of illnesses. Pharmacological properties of the seed have been reported, including anti-inflammatory, anticancer, antidiabetic, antimicrobial, antihistaminic and hypotensive effects\textsuperscript{18, 19, 20, 21}. The seeds contain more than 30\% fixed oil and 0.4–0.45\% (w/w) volatile oil, including 18.4–24\% thymoquinone (TQ) and 46\% of many monoterpenes such as \textit{r}-cymene and \textit{a}-piene\textsuperscript{22, 23, 24, 25}. Much of the biological activity of the seed has been shown to be due to thymoquinone (TQ), the active component of the essential oil. The beneficial medicinal effects of \textit{Nigella sativa} oil (NSO) and TQ have been attributed to their radical scavenging (anti-oxidative) activity\textsuperscript{26, 27} and their ability to inhibit the production of 5-lipoxygenase products during inflammation\textsuperscript{28}.

The seed oil has been shown to lower blood glucose levels in diabetic rats through either extra-pancreatic action\textsuperscript{29} or via a stimulatory effect on \( \beta \)-cell function\textsuperscript{30} with consequent increase in serum insulin level in both cases. The high antioxidant potential of TQ has been found to reduce oxidative stress and nephropathy-related toxicity, as demonstrated by decreased lipid peroxidation, and improved antioxidant enzyme status and cellular protein oxidation\textsuperscript{31}.

This study was designed to investigate the effect of \textit{N. sativa} oil on oxidative stress markers in streptozotocin-induced diabetic adult wistar rats. Also, to evaluate the oxidative stress of STZ-induced diabetes levels of malondialdehyde (MDA) and the activity of antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT).

**Materials and Methods**

This research work was conducted at the Animal holding of the Department of Anatomy, Faculty of Basic Medical Sciences, College of Health Sciences, University of Ilorin, in the year 2014.

**Procurement of oil**

The \textit{Nigella sativa} oil was purchased from a local Islamic store in Adewole area of Ilorin, Kwara State.

**Experimental Animals**

Thirty (30) normoglycemic adult wistar rats of both sexes, having fasting blood glucose levels of 70-80 mg/dl and weighing between 160g-200g with no physical abnormalities observed were used for this study. The animals were purchased from an animal holding in Ogbomoso, Oyo State. The animals were kept in mesh cages at controlled room temperature, photoperiodicity (12/12-hr light/dark cycle) and proper ventilation. Animals were fed with standard rat diet and water made available \textit{ad libitum}. All experiments were conducted following the guidelines on the care and use of laboratory animals in research and teaching by the ethical review committee of the College of Health Sciences, University of Ilorin.

**Experimental Design**

Diabetes mellitus was induced in the rats by a single intraperitoneal (IP) injection of freshly prepared STZ at dose of 70 mg/kg body weight dissolved in 0.01 M citrate buffer at pH 4.5 (32). 0.01 M citrate buffer was prepared by dissolving 2.1 g of citric acid and 2.94 g of sodium citrate in 100 ml of distilled water. The pH was adjusted to 4.5 by the addition of concentrated NaCl solution using a calibrated pH meter.

On-call plus glucometer and compatible glucometer test strips were used for the determination of blood glucose levels in over-night fasted rats seven (7) days after injection of STZ. Blood samples were obtained from the dorsal tail vein of conscious rats. Animals with fasting blood glucose FBG level of greater than...
250 mg/dl were selected for the diabetic groups (33). Hyperglycemia was allowed to stabilize for 5 days before the commencement of treatment.

The animals were grouped at random into five (5) groups (A, B, C, D and E) of six (6) animals each according to their weight ranges. Treatments involved the oral administration of the volatile oil of NSO, the oil was administered to the animals through a metallic orogastric cannula, and this was a daily procedure for 28 days consecutively.

Table 1: Grouping of the animals, Administration dose and periods

<table>
<thead>
<tr>
<th>Groups</th>
<th>Animals</th>
<th>Administered doses</th>
<th>Route</th>
<th>Period</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>6</td>
<td>Untreated diabetic-Streptozotocin 70mg/kg b.w</td>
<td>Intra peritoneal</td>
<td>Single dose</td>
</tr>
<tr>
<td>B</td>
<td>6</td>
<td>Treated diabetic-streptozotocin 70mg/kg followed by 1ml/kg b.w Nigella sativa oil</td>
<td>Oral</td>
<td>28 days</td>
</tr>
<tr>
<td>C</td>
<td>6</td>
<td>Treated diabetic-streptozotocin 70mg/kg followed by 2ml/kg b.w Nigella sativa oil</td>
<td>Oral</td>
<td>28 days</td>
</tr>
<tr>
<td>D</td>
<td>6</td>
<td>Non-diabetic 1ml/kg b.w Nigella sativa oil</td>
<td>Oral</td>
<td>28 days</td>
</tr>
<tr>
<td>E</td>
<td>6</td>
<td>Normal control-70mg/kg b.w citrate buffer</td>
<td>Intra peritoneal</td>
<td>Single dose</td>
</tr>
</tbody>
</table>

Determination of malondialdehyde

The assay method modified by Gutteridge and Wilkins (34) was adopted. Malondialdehyde, a product of lipid peroxidation when heated with 2-thiobarbituric acid (TBA) under acid conditions forms a pink coloured product, which has a maximum absorbance of 532 nm. 2 ml of 0.7% TBA and 1 ml of glacial acetic acid were added to 2 ml of homogenate. The mixture was thoroughly mixed and incubated in a water bath at 80°C for 20 minutes. It was then allowed to cool and was centrifuged at 400 rev/min. for 10 minutes. Absorbance of the supernatant was read at 532 nm against a blank wherein serum was substituted with distilled water. The results were expressed as nanomoles MDA/ml.

Determination of superoxide dismutase

A method originally described by Misra and Fridovich (35) was employed. This method involves inhibition of epinephrine autoxidation, in an alkaline medium at 480 nm in an ultraviolet spectrum. For the determination of specific activity of SOD in 1 ml of blood serum, the rate of autoxidation of epinephrine was noted at 30s intervals in all groups. Briefly, the serum was diluted to make a 1/10 dilution. 0.2 ml aliquot was supplemented with 2.5 ml of 0.05M phosphate buffer (pH 10.2) and equilibrated at room temperature. 0.3 ml of 0.3 nM adrenaline solution was then added to the reference and test solution, followed by mixing by inversion and absorbance at 480 nm was monitored every 30s over 150s. Increase in absorbance per minute was obtained and used to calculate percentage inhibition. The enzyme activity was expressed in arbitrary units considering inhibition of autoxidation as 1-unit SOD specific activity which is the amount of SOD to cause 50% inhibition of the oxidation of adrenaline to adrenochrome during 1 minute.

Determination of catalase

The assay method used was described by Beers and Sizer (36) in which the disappearance of peroxide is followed spectrophotometrically at 240 nm. One Unit decomposes one micromole of H2O2 per minute at 25°C and pH 7.0 under the specified conditions. The reagents used included;0.05 M Potassium phosphate, pH 7.0 and 0.059 M Hydrogen peroxide (30%) in 0.05 M potassium phosphate, pH 7.0. Immediately prior to use, the enzyme was diluted in 0.05 M phosphate buffer, pH 7.0 to obtain a rate of 0.03-0.07 ΔA/min. The spectrophotometer was adjusted to 240 nm and 25°C. Reagents spectrophotometer was switched on for 4-5 minutes to achieve temperature equilibration and to establish blank rate if any. Absorbance reading was taken at 240 nm for 2-3 minutes. Linear portion of the curve was calculated by ΔAΔt/min from the initial (45 second).

Statistical analysis

Data among the groups with different concentrations of the treatment agents was analyzed using Microsoft Excel and SPSS V20 by one-way analysis of variance (ANOVA) followed by "Tukey's Multiple Comparison
Data were presented as means ± SEM (standard error of mean) P value less than 0.05 (p<0.05) was considered statistically significant.

**Results**

Oxidative stress in the diabetic group was characterized by increased lipid peroxidation and/or altered non-enzymatic and enzymatic antioxidant systems. The control group of rats maintained optimal value activity of the antioxidants studied. Administration of NSO significantly decreased the elevated levels of lipid peroxidation, and also significantly increased the reduced antioxidant enzyme activities. Furthermore, *Nigella sativa* proved significantly better in restoring the altered activity of antioxidant enzymes like SOD and CAT towards their normal values in the controls. The animals treated with NSO alone showed no significant change in the levels of SOD, CAT and lipid peroxidation measured by MDA.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment groups</th>
<th>MDA (µmol/ml)</th>
<th>SOD (% inhibition/g protein)</th>
<th>CAT (U/g protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>STZ</td>
<td>6.27 ± 0.63</td>
<td>267.71 ± 20.34</td>
<td>193.20 ± 28.60</td>
</tr>
<tr>
<td>B</td>
<td>STZ+1ml/kg b.w NGS</td>
<td>5.50 ± 0.67</td>
<td>200.49 ± 20.90</td>
<td>139.68 ± 31.41</td>
</tr>
<tr>
<td>C</td>
<td>STZ+2ml/kg b.w NGS</td>
<td>4.86 ± 1.60</td>
<td>428.05 ± 20.19</td>
<td>261.60 ± 15.76</td>
</tr>
<tr>
<td>D</td>
<td>1ml/kg b.w NGS</td>
<td>3.60 ± 0.70</td>
<td>367.38 ± 18.92</td>
<td>283.80 ± 30.39</td>
</tr>
<tr>
<td>E</td>
<td>Control</td>
<td>4.40 ± 0.40</td>
<td>350.32 ± 23.40</td>
<td>318.36 ± 28.70</td>
</tr>
</tbody>
</table>

Values are mean ± SEM; n=5 in each group.

**Malondialdehyde(MDA)**

Figure 1 shows the level of serum MDA in the diabetic, treated and control rats. Significant increase in MDA concentration (6.27±0.63µM/ml MDA) occurred in the diabetic rats compared with the control (p<0.05). Reduction in MDA concentrations was significant in the 2ml/kg b.w NGS treatment group when compared to diabetic and 1ml/kg b.w NGS control (p<0.05)
CATALASE (CAT)

Figure 3 shows the level of serum catalase enzymatic activities in the diabetic, treatment and control rats. The diabetic control group and the STZ+1ml/kgBW showed significant decrease in CAT activities (193.20 ± 28.60 and 139.68 ± 31.41) respectively.

The level of CAT activity assayed in the STZ+2ml/kgBW was 261.60 ± 15.76 and there was no significant difference when compared to the 1ml/kgBW NGS (283.80 ± 30.39) and control groups (318.36 ± 28.70).

Figure 3: Effect of *Nigella sativa* on CAT activity in experimental rats

Values are mean ± SEM; n=5.

γ: p<0.05; significantly different compared to control

*: p<0.05; significantly different compared to 1ml NGS

**: p<0.05; significantly different compared to diabetic

Discussion

Oxidative stress caused by hyperglycemia leads to the activation of stress-sensitive signaling pathways, which worsen both insulin secretion and action, and promote the development of type II diabetes mellitus. Similarly, oxidative stress and damage to the tissues and blood in rats with STZ-induced diabetes enhance glucose autoxidation and may be a factor contributing to complications associated with diabetes.

Increased brain oxidative damage is reportedly associated with cognitive decline and Alzheimer’s dementia. The deleterious effects of chronic fasting hyperglycemia on cognition in the diabetic may be mediated via increased generation of free radicals. Supraphysiologic levels of blood glucose would overdrive the electron transport system, resulting in excessive production of superoxide anion. Moreover, autoxidation of the excess glucose, in the presence of transition metals, as well as non-enzymatic glycation of proteins, would generate reactive oxygen species in the diabetic. Chronic hyperglycemia can also lower the activity of antioxidant enzymes, perhaps by means of glycation.

In this study, significantly increased plasma levels of malondialdehyde (MDA) occurred in the untreated diabetic rats at 28-day post-diabetes induction. 1ml/kgBW NSO-treated diabetic rats showed similar significant increases in plasma MDA. However, in these animals, cognitive tests were not performed, and therefore, it is unclear whether the observed elevated prefrontal MDA was associated with cognitive (neurobehavioural) deficits in these animals.

In this study, activities of antioxidant enzymes (superoxide dismutase and catalase) were significantly reduced in STZ-induced diabetic rats and this result is in line with the findings reported by Jin et al. The reduction of SOD activity might be attributed to the fact that hyperglycemia activates various biochemical pathways such as glucose autoxidation and non-enzymatic glycation of proteins, which then over produce oxidants like superoxide and hydroxyl radicals as well as hydrogen peroxide. Decreased levels of CAT, which being an inducible enzyme, may be due to the decreased level of H$_2$O$_2$ generated by SOD. Low catalase activities, which have been reported in patients with schizophrenia are consistent with the hypothesis that long-term oxidative stress may contribute to the development of a variety of late-onset disorders, such as type 2 diabetes.

Conclusion

Poor glycemic control in diabetes is strongly associated with an increase in free radicals and consequent diabetic complications. Uncontrolled glucose metabolism may also be a cause of alterations in antioxidant enzymes. Among these, catalase correlates best with poor glycaemic control. Based on the findings from this study, administration of NSO may suppress STZ-induced diabetes in rats.

References


5. SzymenEy, Szymen B, Delen Y and Onat T (2001). Catalase/Superoxide Dismutase (SOD) and Catalase/Paraaxonase (PON) ratio may implicate poor glycemic control. Archives of Medical Research, 32:283-287.


Perception of cost as a barrier to cataract surgery uptake among volunteers for free cataract screening in a rural population in Edo State of Nigeria

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Abstract

Aim: To determine the role of perception of cost as one of the barriers to uptake of cataract surgery in a rural population.

Methods: One hundred and six (106) subjects who were visually impaired or blind from cataract and eligible for cataract surgery were interviewed from a total of 530 adults 40 years and above who presented for cataract screening at a cataract screening eye outreach.

Results: Of the 106 participants, 60.4% were males (M:F = 3:2). The mean age was 68 years ±12. The estimated median monthly income of participants was N5000 (approx. US $27). Poor awareness (70.8%) was the most common barrier to the uptake of cataract surgery, followed by cost (16%). Only 16 (15.1%) respondents said they could afford the full cost of surgery.

Conclusion: There is the need to intensify awareness campaigns and subsidize the cost of surgery so as to achieve one of the cardinal goals of vision 2020.

Keywords: Cataract, cost, barrier.

Introduction

Cataract is opacity of the lens of the eye. It could be age related, congenital or secondary (to metabolic disorders, trauma or primary ocular disease). Some secondary cataracts are not operable due to associated complications that would have resulted in blindness prior presentation.

Cataract remains the leading cause of blindness worldwide accounting for nearly half of the cases of blindness globally¹. In Nigeria, cataract is the commonest cause of blindness accounting for 45% of all cases of blindness in individuals above 40 years of age. At least 400,000 individuals are affected by an operable cataract in Nigeria². In Benin City, Edo state, cataract is the main cause of blindness³. It is also the commonest cause of blindness seen in patients presenting at the eye clinic of Irrua Specialist Teaching Hospital (ISTH)⁴, a tertiary hospital serving the study area.

Cataract blindness is easily reversed through good cataract surgery. The efficacy of cataract surgery in restoring lost vision is as high as 90% making it one of the most effective surgical interventions⁵. However, despite this, many people still remain blind from cataract in the developing regions of the world⁶.

Several reasons have been found to be responsible for this poor uptake. Among these reasons is cost. However, the perception of cost as a barrier varies in its significance among studies, being high in some and low in others.

This study sought to find out the major barriers to uptake of cataract surgery in particular cost in the study area.
Methodology

This was a community health center based study among 530 adults 40 years and above who presented for a free cataract screening eye outreach for the elderly were screened for cataract. Of these, 106 were found to be visually impaired and blind from cataract (presenting visual acuity in the better eye of < 6/18 – LP). An interviewer-administered questionnaire was used to obtain information on sociodemographic and socioeconomic characteristics as well as visual acuity and barriers to uptake of cataract surgery. The study was conducted in five localities in Edo State of Nigeria. The data collected was entered into SPSS version 21. Descriptive statistics was used to analyze the data. Contingency tables were generated and chi-square test was used to determine the statistical significance of observed differences in cross tables. P values < 0.05 were considered as statistically significant. Further analysis was done using binary logistic regression to determine the effect of various factors on the outcome variable which was perception of cost barrier.

Results:

A total of 160 adults of 40 years and above out of the 530 adults who were screened for cataract were visually impaired or blind from cataract.

The sociodemographic, socioeconomic characteristics and visual acuity are shown in tables I to III. The mean age of the respondents was 68 years ± 12 with the age group 70-79 years having the highest number of respondents (34%). Close to half of the respondents were widowed (48.1%). Over half of the respondents were farmers (57.5%) while the estimated median monthly income of the study participants was N5,000 (approx. US $27) with 25th and 75th percentile being N2,000 (US $11) and N10,000 (US $54) respectively.

The major reasons for not having undergone cataract surgery were poor awareness (70.8%) and perception of cost (16.0%) as shown in table IV. Cost as a barrier was significantly associated with blindness (p = 0.003). Table IV shows the prominent roles played by perception of cost and poor awareness. At moderate visual impairment level, poor awareness accounted for 72.1% of the barriers while perception of cost accounted for only 8.2%. At the level of blindness, poor awareness accounted for 62.9% while perception cost significantly increased the barrier to cataract surgical uptake by 34.3%.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Frequency(n)</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td></td>
<td></td>
</tr>
<tr>
<td>40 – 49</td>
<td>10</td>
<td>9.4%</td>
</tr>
<tr>
<td>50 – 59</td>
<td>10</td>
<td>9.4%</td>
</tr>
<tr>
<td>60 – 69</td>
<td>30</td>
<td>28.3%</td>
</tr>
<tr>
<td>70 – 79</td>
<td>36</td>
<td>34.0%</td>
</tr>
<tr>
<td>80 and above</td>
<td>20</td>
<td>18.9%</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>64</td>
<td>60.4%</td>
</tr>
<tr>
<td>Female</td>
<td>42</td>
<td>39.6%</td>
</tr>
<tr>
<td>Marital Status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Divorced/separated</td>
<td>6</td>
<td>5.7%</td>
</tr>
<tr>
<td>Married</td>
<td>45</td>
<td>42.5%</td>
</tr>
<tr>
<td>Single</td>
<td>4</td>
<td>3.8%</td>
</tr>
<tr>
<td>Widowed</td>
<td>51</td>
<td>48.1%</td>
</tr>
<tr>
<td>Total</td>
<td>106</td>
<td>100%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Occupation</th>
<th>Frequency(n)</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Civil Servant</td>
<td>3</td>
<td>3.6%</td>
</tr>
<tr>
<td>Dependent</td>
<td>20</td>
<td>18.9%</td>
</tr>
<tr>
<td>Farmer</td>
<td>61</td>
<td>57.5%</td>
</tr>
<tr>
<td>Trader</td>
<td>17</td>
<td>16.0%</td>
</tr>
<tr>
<td>Others(artisans, motorists)</td>
<td>5</td>
<td>4.7%</td>
</tr>
<tr>
<td>Monthly Income</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;N5,000</td>
<td>58</td>
<td>54.7%</td>
</tr>
<tr>
<td>&gt;N5,000-15,000</td>
<td>35</td>
<td>33.0%</td>
</tr>
<tr>
<td>&gt;N15,000-25,000</td>
<td>7</td>
<td>6.6%</td>
</tr>
<tr>
<td>&gt;N25,000-35,000</td>
<td>2</td>
<td>1.9%</td>
</tr>
<tr>
<td>&gt;N35,000-45,000</td>
<td>0</td>
<td>0.0%</td>
</tr>
<tr>
<td>&gt;N45,000</td>
<td>4</td>
<td>3.8%</td>
</tr>
<tr>
<td>Total</td>
<td>106</td>
<td>100%</td>
</tr>
</tbody>
</table>
Table III: Visual health characteristics of 106 cataract blind and visually impaired adults in a rural population of Edo State.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Frequency (n)</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Visual acuity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VI (PVA in better eye &lt;6/18 – 6/60)</td>
<td>62</td>
<td>57.5%</td>
</tr>
<tr>
<td>SVI (PVA in better eye &lt;6/60 – 3/60)</td>
<td>10</td>
<td>9.4%</td>
</tr>
<tr>
<td>Blindness (PVA in better eye &lt;3/60 - LP)</td>
<td>34</td>
<td>33.1%</td>
</tr>
<tr>
<td>Total</td>
<td>106</td>
<td>100%</td>
</tr>
</tbody>
</table>

VI= visual impairment, SVI= severe visual impairment, PVA= presenting visual acuity. (Patients with NPL were excluded from the blindness category since they were not operable).

Table IV: Barriers to cataract surgery uptake for different visual acuity levels of 106 cataract blind and visually impaired adults in a rural population of Edo State

<table>
<thead>
<tr>
<th>Visual acuity</th>
<th>VI</th>
<th>SVI</th>
<th>Blindness</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cannot afford</td>
<td>5</td>
<td>0</td>
<td>12</td>
<td>17</td>
</tr>
<tr>
<td>(8.2%)</td>
<td>(0%)</td>
<td>(34.3%)</td>
<td>(16.0%)</td>
<td></td>
</tr>
<tr>
<td>Cataract not mature</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>(3.3%)</td>
<td>(0%)</td>
<td>(0%)</td>
<td>(1.9%)</td>
<td></td>
</tr>
<tr>
<td>Destiny/God’s will</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>(0%)</td>
<td>(10%)</td>
<td>(0%)</td>
<td>(1.9%)</td>
<td></td>
</tr>
<tr>
<td>Distance too far</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>(1.6%)</td>
<td>(0%)</td>
<td>(0%)</td>
<td>(0.9%)</td>
<td></td>
</tr>
<tr>
<td>Fear</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>(3.3%)</td>
<td>(0%)</td>
<td>(2.9%)</td>
<td>(2.8%)</td>
<td></td>
</tr>
<tr>
<td>None to accompany them</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>(1.6%)</td>
<td>(0%)</td>
<td>(0%)</td>
<td>(0.9%)</td>
<td></td>
</tr>
<tr>
<td>Old age/need not felt</td>
<td>3</td>
<td>0</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>(4.9%)</td>
<td>(0%)</td>
<td>(0%)</td>
<td>(2.8%)</td>
<td></td>
</tr>
<tr>
<td>Other priorities</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>(1.6%)</td>
<td>(0%)</td>
<td>(0%)</td>
<td>(0.9%)</td>
<td></td>
</tr>
<tr>
<td>Poor awareness</td>
<td>44</td>
<td>9</td>
<td>22</td>
<td>75</td>
</tr>
<tr>
<td>(72.1%)</td>
<td>(90%)</td>
<td>(62.9%)</td>
<td>(70.8%)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>61</td>
<td>10</td>
<td>35</td>
<td>106</td>
</tr>
<tr>
<td>(100%)</td>
<td>(100%)</td>
<td>(100%)</td>
<td>(100%)</td>
<td></td>
</tr>
</tbody>
</table>

VI=visual impairment, SVI=severe visual impairment

Discussion

The most reported barrier to cataract surgery uptake in this study was poor awareness. This finding is similar to findings of Kolawole et al\(^7\) in Osun State of Nigeria. It is however in contrast to findings of Ubah et al\(^8\) in another community in Osun State who reported poor awareness as the least (8.5%) mentioned barrier. This difference may be a result of the remoteness of the study area in this study.

Cost of surgery (16%) was the second most reported barrier in this study. This is similar to findings by Yin et al\(^9\) (19.6% and 15.9%) in a two-site population based study in rural China but in contrast to Ubah et al\(^8\) (81%), Rabiu et al\(^10\) (61%) and Mehari et al\(^11\) (91.8%). This difference may be as a result of the poor awareness of the respondents in this study about cataract surgery and its cost. People who are not aware of their eye disease or of the availability of cataract surgery are not likely to seek care and because they do not seek surgical care they are unlikely to know the cost of treatment. Cost as a barrier was significantly associated with blindness (p=0.003). This is most likely due to the fact that with progressive deterioration of vision those affected were more likely to have sought eye care and in the course of such search they would have been informed about the cost of treatment which varies from one center to another.

In this study when the respondents were informed about the cost of cataract surgery at the nearest Specialist Teaching Hospital only 16 respondents (15.1%) said they could afford the cost of surgery which was ₦50,000 (approx. US $246). This is not surprising as more than half of the respondents live below the international monetary threshold under which an individual is considered to be living in poverty. As previously reported one of the ways of increasing cataract surgery uptake is to reduce the barrier of cost\(^12\). This study shows why the practice of free or highly subsidized cataract surgery needs to be encouraged as not all populations (especially the rural ones) may be able to afford the cost.

The government (both states and federal) needs to intervene in the area of subsidizing cataract surgery in the rural areas in order to reduce the burden of cataract blindness nationwide. There are some governmental agencies like the Niger Delta Development
Commission and non-governmental organizations that sponsor free cataract surgeries from time to time. However, those who really need these services are not usually able to access them because of infiltration by some patients from the urban centers even though they can afford to pay for their surgeries. There is therefore a dire need for a close monitoring of free rural cataract surgical programs in order to ensure that only those who cannot afford to pay for their cataract surgery will be the ultimate beneficiaries.

**Conclusion**

The above findings show that there is a great need for ophthalmologists close to these areas to intensify awareness about cataract and cataract surgery. However, the findings equally show that even with the elimination of poor awareness as a barrier, this may not translate to high uptake of cataract surgical services due to the barrier of cost. There is therefore a need to find ways to subsidize the cost of cataract surgery for these communities if the “Eye Care for All” initiative is to be achieved.

**References**

Susceptibility of *Staphylococcus aureus* pathogen from clinical specimens to rifampicin

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Department of Medical Microbiology, Ambrose Alli University, Ekpoma, Department of Medical Laboratory Science, Ambrose Alli University, Ekpoma, Edo State, Nigeria.

Abstract

The susceptibility of *Staphylococcus aureus* pathogen from various clinical specimen to rifampicin was investigated. A total of 100 consecutive non-repetitive clinical isolates of *Staphylococcus aureus* from various clinical specimens in the University of Benin Teaching Hospital were identified and used for this study. Of the 100 isolates used, majority were from wound swab (37%) others were from high vaginal swab (18%), ear swab (10%) and throat swab (2%). Discs susceptibility to ofloxacin, ciprofloxacin and gentamicin was performed on all isolates. Ciprofloxacin was the most active antibacterial agent (65%) in this study. All staphylococcus aureus isolates from aspirates, throat swab and eye swab were susceptible to all 3 antibacterial agents. Out of the 20 isolates that were resistant to the entire antibacterial agent, 60% were susceptible to ciprofloxacin, 100% to ofloxacin and 40% to Gentamicin after prior exposure to Rifampicin. A combination of Rifampicin and other antibacterials may be effective in the management of infection due to bacteria carrying Rifampicin curable multidrug resistant plasmids.

Key words: Rifampicin, isolates, resistant, susceptible.

Introduction

The Gram-positive extracellular staphylococci, transmitted by contact and airborne droplets, are the cause of most pyogenic infections of the skin and soft tissues, which may sometimes spread to lungs and bones\(^1\). Healthy nasal carriers of *staphylococcus aureus* are major sources of infection, especially in hospitals\(^2\). Rates of staphylococcal colonization are high among patients with intravenous drug users\(^3\); type 1 diabetes\(^4\); patients undergoing haemodialysis\(^5\); patients with acquired immunodeficiency syndrome\(^6\); and Surgery patients\(^7\).

Infections by *Staphylococcus aureus* usually result from the combination of its virulence factors which include, the ability of the organism to multiply and spread widely through the tissues, the ability to survive under harsh conditions, its cell constituents, the production of enzymes and toxins that promote tissue invasion, its potential to acquire resistance to antibacterial agents and its capacity to persist intracellularly in certain phagocytes\(^1,2\). *Staphylococcus aureus* is capable of secreting several toxins, many of which are associated with specific diseases. These include pyrogenic toxic super antigens capable of inducing toxic shock syndrome, exfoliative toxins implicated in staphylococcal scalded skin syndrome. Other toxins include alpha toxins, beta toxins, gamma toxin, delta toxin and Panton – valentine leukocidin\(^8,9,10,11\). Staphylococcus aureus produces a number of enzymes which include catalase, coagulase hyaluronidase and beta – lactamase\(^2\). Leukocytes are the primary host defense against *Staphylococcus aureus* infection\(^12\). Penicillin was originally the treatment of choice for *Staphylococcus aureus* infection but most staphylococci particularly in hospitals are now resistant, these being usually referred to as methicillin-resistant *Staphylococcus aureus* (MRSA)\(^1,2\).
Patients and Methods

Collection of specimens: A total of 100 consecutive non-repetitive clinical isolates of Staphylococcus aureus obtained from patients sent to Medical Microbiology Laboratory of University of Benin Teaching Hospital, Benin City, Nigeria were used for this study. An isolate was identified as Staphylococcus aureus, gram-positive cocci, catalase positive and coagulase positive.

Inoculation of Culture: The media used were MacConkey agar, nutrient agar (CM), Peptone water (CM9) and Blood agar (Oxoid). They were prepared according to manufacturer’s specification e.g Nutrient Agar (Oxoid) CM3 was prepared using 28g of the powder suspended in one litre of distilled water allowed to get soaked for 10 mins to dissolve completely and sterilized by autoclaving at 121°C for 15 minutes and cooled at 47°C. Analysis was done at the diagnostic laboratory in the Medical Laboratory science department Ambrose Alli University, Ekpoma.

Preparation of Antibacterial Discs: Antibacterial discs were prepared according to the method described by15. Gentamicin disc was prepared as follows: 1000μg/ml gentamicin solution was prepared from a 40,000μg/ml gentamicin (CN) by adding 0.1 ml of the 40,000 μg/ml gentamicin to 3.9ml of sterile water. One milliliter of the, 1,000μg/ml solutions was added to 100 sterile whatman No. 1 paper disc with a diameter of 6.25mm. Each disc has an absorbing capacity of approximately 0.01ml, which is 1/100th of a millimeter. Therefore, each disc will contain 10μg/disc. The discs were dried at 60°C overnight.

Disc Susceptibility Test: This test was performed according to the National Committee for clinical laboratory standards (NCCLS, 1993) method. An overnight culture of Staphylococcus aureus on nutrient agar was emulsified in sterile distilled water until the turbidity matches 0.5 Macfarland’s Standard (This is prepared by adding 0.5ml of 1% Barium Chloride to 99.5ml of 1% Sulphuric acid). Once matched, the bacterial count is equivalent to 10^6 CFU/ml. This is diluted 1 in 1000 to give a bacterial count of 10^5 CFU/ml. This was then used to flood the surface and excess drained off. The Discs-Ciprofloxacin (5μg), Ofloxacin (5μg) and Gentamicin (10μg) were placed on the surface of the seeded plates. The plates were incubated at 37°C overnight. After night incubation, the zones of incubation (in millimeter) were measured Incubation of Culture: The media used were MacConkey agar, nutrient agar (CM), Peptone water (CM9) and Blood agar (Oxoid). They were prepared according to manufacturer’s specification e.g Nutrient Agar (Oxoid) CM3 was prepared using 28g of the powder suspended in one litre of distilled water allowed to get soaked for 10 mins to dissolve completely and sterilized by autoclaving at 121°C for 15 minutes and cooled at 47°C. Analysis was done at the diagnostic laboratory in the Medical Laboratory science department Ambrose Alli University, Ekpoma.

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Curing Experiment: The minimum inhibitory concentration (MIC) of representative multi-resistant staphylococcus aureus to Rifampicin was determined by the broth dilution method. The tube with highest concentration of rifampicin (the next concentration after the (MIC) was subcultured on drug free nutrient plate. Disc susceptibility test was now performed on exposed and unexposed multi-resistant strains of Staphylococcus aureus. Absence of zone of inhibition on the unexposed strain and presence of zone of inhibition on rifampicin-exposed strains was taken as positive for curing and indicates plasmid-mediated resistance.

Result

A total of 100 consecutive non-repetitive clinical isolates of staphylococcus aureus were used for this study. Table 1 shows that majority of the isolates were from wound swab (37%), followed by isolates from high vaginal swab (18%) and the least was recovered...
from eye swab (1%). The susceptibility of clinical isolates of *Staphylococcus aureus* to ciprofloxacin, Gentamicin and ofloxacin is shown in table 2. Ciprofloxacin was the most active antibacterial agent (65%) in this study. All *Staphylococcus aureus* isolates from aspirates, throat swab and eye swab were susceptible to all three antibacterial agents. Of the 20 isolates that were resistant to the entire antibacterial agent, 60% were susceptible to ciprofloxacin, 100% to ofloxacin and 40% to Gentamicin after prior exposure to Rifampicin (Table 3).

Table 1: Prevalence of staphylococcus aureus in relation to clinical specimens

<table>
<thead>
<tr>
<th>Specimens</th>
<th>Number of isolates</th>
<th>Percentage %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wound swab</td>
<td>37</td>
<td>37.00</td>
</tr>
<tr>
<td>Aspirates</td>
<td>3</td>
<td>3.00</td>
</tr>
<tr>
<td>High vaginal swab</td>
<td>18</td>
<td>18.00</td>
</tr>
<tr>
<td>Eye swab</td>
<td>1</td>
<td>1.00</td>
</tr>
<tr>
<td>Urine</td>
<td>11</td>
<td>11.00</td>
</tr>
<tr>
<td>Semen</td>
<td>5</td>
<td>5.00</td>
</tr>
<tr>
<td>Catheter tip</td>
<td>5</td>
<td>5.00</td>
</tr>
<tr>
<td>Ear swab</td>
<td>10</td>
<td>10.00</td>
</tr>
<tr>
<td>Throat swab</td>
<td>2</td>
<td>2.00</td>
</tr>
<tr>
<td>Blood culture</td>
<td>3</td>
<td>3.00</td>
</tr>
<tr>
<td>Urethral swab</td>
<td>5</td>
<td>5.00</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>

Table 2: Susceptibility of clinical specimens of *staphylococcus aureus* to ciprofloxacin, ofloxacin & gentamicin.

<table>
<thead>
<tr>
<th>Antibacterials (µg/disc)</th>
<th>Specimen</th>
<th>No. tested</th>
<th>Cip (5)</th>
<th>Ofx (5)</th>
<th>CN (10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wound swab</td>
<td>18</td>
<td>19(51.35)</td>
<td>19(51.35)</td>
<td>17(45.95)</td>
<td></td>
</tr>
<tr>
<td>High vaginal swab</td>
<td>11</td>
<td>7(63.64)</td>
<td>5(45.45)</td>
<td>7(63.64)</td>
<td></td>
</tr>
<tr>
<td>Urine</td>
<td>5</td>
<td>4(80)</td>
<td>4(80)</td>
<td>4(80)</td>
<td></td>
</tr>
<tr>
<td>Catheter tip</td>
<td>10</td>
<td>3(30)</td>
<td>3(30)</td>
<td>3(30)</td>
<td></td>
</tr>
<tr>
<td>Blood culture</td>
<td>3</td>
<td>2(66.67)</td>
<td>3 (100)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eye swab</td>
<td>1</td>
<td>1(100)</td>
<td>1(100)</td>
<td>1(100)</td>
<td></td>
</tr>
<tr>
<td>Aspirates</td>
<td>3</td>
<td>3(100)</td>
<td>3(100)</td>
<td>3(100)</td>
<td></td>
</tr>
<tr>
<td>Urethral swab</td>
<td>5</td>
<td>4(80)</td>
<td>3(60)</td>
<td>1(20)</td>
<td></td>
</tr>
<tr>
<td>Throat swab</td>
<td>2</td>
<td>2(100)</td>
<td>1(50)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>65(65.00)</td>
<td>53(53.00)</td>
<td>53(53.00)</td>
<td></td>
</tr>
</tbody>
</table>

Key: Cip – Ciprofloxacin, Ofx – Ofloxacin, CN-Gentamicin

Table 3: Susceptibility of multi-resistant isolates of *staphylococcus aureus* before and after exposure to rifampicin

<table>
<thead>
<tr>
<th>Antibacterial Agents (µg/disc)</th>
<th>No tested</th>
<th>Pre (%)</th>
<th>Post (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ciprofloxacin (5)</td>
<td>20</td>
<td>0(0)</td>
<td>12(60)</td>
</tr>
<tr>
<td>Ofloxacin (5)</td>
<td>20</td>
<td>0(0)</td>
<td>20(100)</td>
</tr>
<tr>
<td>Gentamicin (10)</td>
<td>20</td>
<td>0(0)</td>
<td>8(40)</td>
</tr>
</tbody>
</table>

Discussion

*Staphylococcus aureus* has been observed to be the most predominant isolate from clinical specimens. Various antibacterial agents have been used to treat and or manage various infections by these microorganisms. However, resistance to various antimicrobial agents by *Staphylococcus aureus* is on the increase and plasmid mediated resistance has been documented.

The entire 100 isolates of *Staphylococcus aureus* were recovered from various clinical specimens, which confirms the ubiquity, adaptability and notoriety of *Staphylococcus aureus* as a pathogen. Majority of the *Staphylococcus aureus* isolates were from wound. This may be due to the fact that *Staphylococcus aureus* are normal flora of the skin and mucous membrane and can easily colonize wounds.

Ciprofloxacin was the most active antibacterial agent. Isolates from semen and ear swab were less sensitive to the antibacterial agents used, while only 1 (20%) isolate from urethral swab was sensitive to gentamicin. In a previous study in Oyo State Nigeria, all isolates of *Staphylococcus aureus* from ear swab were sensitive to getamicin. This may indicate difference in susceptibility pattern from one area to another.

In the case of the urethra, *Staphylococcus* is a normal flora that colonizes it and use of antimicrobial agents by individuals who may experience urinary or genital tract discomfort may overtime result in colonization with more resistant isolates and may explain the result observed in this study. A similar explanation may be responsible for the low sensitivity of isolates from semen.

Resistance to antimicrobial agents by microorganism could either be plasmid mediated or chromosomal
mediated. The plasmid mediated resistance is usually more important medically as it can easily be spread in a hospital setting and thus renders an antimicrobial agent ineffective. Staphylococcus aureus were susceptible to ciprofloxacin, ofloxacin and gentamicin respectively after exposure to rifampicin. The plasmid curing ability of rifampicin had earlier been noted. It has been suggested that in such combinations that rifampicin induces the loss of resistant plasmids in the staphylococcus strains, thereby conferring susceptibility to the second antibacterial agent. has also reported that rifampicin combination therapy appears to have improved treatment outcomes in cases in which there is a low organism burden, such as biofilm infections, but is less effective when effective surgery to obtain source control is not performed. Their clinical data also support rifampin combination therapy for the treatment of prosthetic joint infections due to methicillin sensitive staphylococcus aureus (MSSA) after extensive debridement. This study clearly indicates that plasmids are responsible for the majority of multi resistance observed. It appears likely that a combination of rifampicin and any of the 3 antibacterial agents used in this study may eliminate plasmid mediated resistance and improve management of infection by staphylococcus aureus.

References

Prevalence and characteristics of clinical nephropathy in type 2 diabetes mellitus patients in Ilorin, North Central Nigeria.

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Abstract

Background: The World Health Organization (WHO) estimated that about 1.7 million people are suffering from diabetes mellitus (DM) in Nigeria and this is expected to triple by 2030. With this projection, the incidence of complication like diabetic nephropathy (DN) is also expected to increase. Several previous studies on DN focused on microalbuminuria which does not always indicate nephropathy in type 2 DM. This study was to determine the prevalence and correlates of clinical nephropathy in patients with type 2 DM in University of Ilorin Teaching Hospital, Ilorin, Nigeria.

Methods: This study was a cross-sectional design. A total of 273 previously diagnosed type 2 DM patients at University of Ilorin Teaching Hospital (UITH) that met the inclusion criteria for the study were recruited. Relevant information was obtained from the subjects with pretested questionnaires. Blood and early morning urine samples were taken from all subjects for determination of urine albumin creatinine ratio (ACR), glycated haemoglobin (HbA1C), fasting plasma glucose, serum creatinine and urea. Estimated glomerular filtration rate (eGFR) was calculated with the modification of diet in renal disease (MDRD) study equation. Subjects with microalbuminuria (ACR ≥ 300mg/g) had repeat ACR estimation after at least 3 months to establish persistence.

Results: Fifty (18.3%) of the 273 subjects with type 2 DM had clinical nephropathy. Gender specific prevalence of clinical nephropathy was 15.3% and 20.6% for males and females respectively. The age of the patients, duration of DM, systolic BP, serum creatinine, fasting plasma glucose, and glycated haemoglobin (HbA1C) correlated positively with the degree of clinical nephropathy. Estimated GFR correlated negatively with clinical nephropathy (r = -0.695, p = 0.001). The predictors of clinical nephropathy were HbA1C, duration of DM and eGFR.

Conclusions: Clinical nephropathy is present in a significant proportion of type 2 DM patients in UITH, Ilorin, Nigeria. The degree of clinical nephropathy correlated with some clinical and biochemical variables which should be put into consideration in the evaluation and management of clinical nephropathy.

Introduction

Diabetes mellitus (DM) comprises of heterogeneous metabolic disorders that share the phenotype of hyperglycaemia as a result of absolute or relative insulin deficiency. Presently about 2.8% of the world population have DM, but the World Health Organization (WHO) projects an increase in the prevalence of DM worldwide, much of which is expected in developing countries chiefly due to trend towards sedentary lifestyle and poor dietary habits among other reasons. This explains why the WHO estimates that about 1.7 million people are suffering from DM in Nigeria, but expects this burden to triple by 2030. Diabetes mellitus is a leading cause of end stage renal disease (ESRD) in Europe and America. It is fast becoming a major health issue in tropical developing countries. Type 2 DM is hyperglycaemia resulting from variable degree of insulin resistance, impaired insulin secretion and increased glucose...
production. It accounts for over 90% of all cases of DM.\textsuperscript{1,2} A study in Port Harcourt, Nigeria found that the prevalence rate of type 2 DM was 7.9%, earlier national standardized prevalence was 2.2% in the 1997 non communicable disease survey.\textsuperscript{8,9}

With the increasing prevalence of type 2 DM and improvement in survival of patients, nephropathy has emerged a major health problem.\textsuperscript{4} Diabetic nephropathy (DN) is a diabetic specific complication associated with high mortality.\textsuperscript{5-7} It is the leading cause of ESRD in the western world\textsuperscript{5,6} and the third leading cause of ESRD in Ilorin, Nigeria.\textsuperscript{7}

The mechanism by which chronic hyperglycaemia leads to nephropathy involves interaction of soluble factors (growth factors, angiotensin II, endothelin), alteration in renal microcirculation and structural changes in the glomerulus and renal tubulointerstitium.\textsuperscript{5}

Albuminuria is the hallmark of DN. The early stage of diabetic nephropathy referred to as incipient nephropathy is characterized by microalbuminuria which can be defined as albumin creatinine ratio (ACR) of spot early morning urine between 30 to 300mg/g.\textsuperscript{5,10,11} Macroalbuminuria or overt/clinical proteinuria is defined as ACR of spot early morning urine specimen of >300mg/g.\textsuperscript{5,10,11} Clinical DN is persistent macroalbuminuria or dipstick positive proteinuria on at least two occasions separated by 3 to 6 months in a person with diabetes mellitus.\textsuperscript{12-15} Patients invariably develop systemic hypertension, progressive increase in proteinuria and predictable decrease in glomerular filtration rate. Most of these patients with end stage renal disease have type 2 DM.\textsuperscript{15}

While the correlates and predictors of microalbuminuria in diabetic patients have been well documented in literatures,\textsuperscript{15-20} there is paucity of information on the characteristics of clinical nephropathy in patients with diabetes mellitus from all the zones in Nigeria. Only about 20 to 40% of those with microalbuminuria will eventually progress to clinical nephropathy in type 2 DM patients.\textsuperscript{15} However, patients with clinical nephropathy will invariably develop end stage renal failure without intervention.\textsuperscript{15}

Earlier study on albuminuria in type 2 DM patients in our center did not determine the burden, and correlates of clinical DN.\textsuperscript{20} More importantly, no study has been done in this environment on clinical DN in spite of the projected increasing burden of type 2 DM particularly in developing countries.\textsuperscript{4} The risk of cardiovascular death is higher by a factor of 12 in diabetic patients with clinical diabetic nephropathy when compared with ESRD due to primary glomerular disease.\textsuperscript{15,21}

Diabetic nephropathy progresses from microalbuminuria through the stage of clinical nephropathy to ESRD with reasonable predictability in type 1 DM because of the reliable duration of illness. However, in type 2 DM, the stage of microalbuminuria could elude detection because of unreliable duration of illness and absence of symptoms. Moreover, microalbuminuria is less predictive of diabetic nephropathy and progression of disease in type 2 DM.\textsuperscript{1} Therefore, the knowledge of the burden and correlates of overt albuminuria will be useful for evaluation and management of these patients.

This study is designed to assess the prevalence and characteristics of clinical nephropathy in type 2 DM patients in UITH, Ilorin, Nigeria

**Patients and Methods**

**Study Design:** This was a Cross Sectional Descriptive study carried out from January to August 2010 at the Medical Outpatient Department (MOPD) and Medical wards of the University of Ilorin Teaching Hospital (UITH), Ilorin, Kwara state, North-central Nigeria.

**Study Subjects (Patients):** Consenting adults with type 2 DM that presented to the study location were recruited after ethical approval by the Ethical and Research Committee of UITH.

**Diagnostic Criteria for Type 2 DM:** Diagnosis was based on the clinical records that the study subjects tested positive to any of the laboratory investigation listed below on at least 2 occasions; fasting plasma glucose $\geq$ 7.0mmol/l, random plasma glucose $\geq$ 11.1mmol/l, a 2 hour postprandial plasma glucose $\geq$ 11.1mmol/l after an oral anhydrous glucose load of 75g.\textsuperscript{22} To ensure that it was only patients with type 2 DM that were recruited, all the patients who participated in this study had documented history that their diabetes was controlled in the past or presently with oral glucose lowering drugs and were not Insulin dependent.
Inclusion and exclusion criteria for patients: Type 2 DM patients were eligible for inclusion if they were on oral glucose lowering medication(s) only or if they were on combined treatment with dietary control, oral glucose lowering medication(s) and insulin. The following categories of patients were excluded: type 2 diabetic patients already on angiotensin converting enzyme inhibitors (ACEIs), or angiotensin receptor blockers (ARBs), or non-steroidal anti-inflammatory drugs (NSAID) ascertained from their clinical history and case note, patients with uncontrolled hypertension (≥160/≥100mmHg), Patients with urinary tract infection evident by symptoms and/or urinalysis finding of leukocyte and/or nitrite, patients with urinalysis evidence of haematuria or suggestive of nephritis (i.e. proteinuria with haematuria).

Methods

The subjects were interviewed and examined by the investigators and research assistants to ensure they fulfilled the set criteria. A pretested structured questionnaire was used to collect demographic and relevant clinical information. Temperature was taken with mercury thermometer put in the armpit of subjects for 3 minutes before reading to exclude those with temperature above 37.2°C. Anthropometric Measurements: Height (m) and weight (kg) were taken with Stadiometer and Seca® weighing scale respectively. Body Mass Index (BMI) was obtained by using the formula weight/height². BMI ≥ 25kg/m², but < 30kg/m² was regarded as overweight, while BMI ≥ 30kg/m² was regarded as obesity.

The waist circumference (cm) was taken with tape measure positioned horizontally at the midpoint between the costal margin and the iliac crest along mid-axillary line in an unclothed abdomen, with the subject standing and breathing normally. The hip circumference (cm) was measured with the same tape measure positioned horizontally at the widest point around the greater trochanter. Waist-to-hip ratio was obtained by dividing the waist circumference with the hip circumference. A ratio > 0.9 in women and > 1.0 in men was considered to be abnormal. Blood pressure (BP) was measured with Accosson’s Mercury Sphygmomanometer with standard cuff (25cm x 12cm) in sitting position on the right arm after at least 5 minutes of rest. Phase I Korotkoff sound was used for systolic B.P and phase V for Diastolic B.P. In patients with diabetes, systolic BP of ≥ 130 and/or diastolic BP ≥ 80mmHg if proteinuria is less than 1g/24 hr, or systolic BP ≥ 125 and/or diastolic BP ≥ 75mmHg for those with proteinuria greater than 1g/ 24 hr was considered to be suboptimal. Good BP control in patients with DN was defined as BP < 125/75mmHg (if spot urine ACR was ≥ 600mg/g), or < 130/80mmHg (if spot urine ACR was < 600mg/g).

Laboratory Investigation

All laboratory investigation except urine albumin concentration estimation was carried out at the Chemical Pathology Department of UITH. Estimation of urine albumin concentration was done by the investigators using the Immunoturbidometry method of HemoCue with urine Albumin microcuvettes and HemoCue Albumin 201 Analyser. The chemistry principle: A specific rabbit anti-human albumin antibody (polyclonal) forms an agglutinate with human albumin in the urine sample. The agglutination is enhanced by polymers. The turbidity of the agglutinates, once formed is equivalent to the degree of albuminuria. The turbidity is measured photometrically at 610nm.

Urine creatinine concentration was estimated on the same sample by Jaffé’s reaction on the same day of collection. The intra assay, inter assay, and day to day coefficient of variation were 0.4%, 1.6% and 5% respectively. Albumin creatinine ratio (ACR) in mg/g was obtained by dividing urine albumin concentration (mg/l) with urine creatinine concentration (g/l).

Dipstick (Multistix R 10SG by Bayer) was used on the same urine sample to determine presence of blood, leucocyte, glucose, ketone and nitrite. To establish persistent macroalbuminuria, a repeat ACR was estimated on first morning urine specimen of all patients with ACR >300mg/g after 3 months of initial test. The average value of the ACR was used for clinical nephropathy subgroup analysis. Fasting plasma glucose (FPG) concentration was estimated in blood sample collected inside fluoride oxalate bottles after an overnight fast at least 12 hours using glucose oxidase method. Glycatedhemoglobin (HbA1c) concentration was estimated by microchromatographic method using fortress diagnostic kit. Good glycaemic control was defined as FPG between 5 to 7.2mmol/l and HbA1c of < 7.0%. Serum creatinine and urea concentration were estimated in blood sample collected in Lithium
heparin bottle using Jaffe’s (picric) reaction and diacetylmonoxime methods respectively. Reference range for serum creatinine was 53-106µmol/L, Urea was 2.5-6.5mmol/l. Glomerular filtration rate (GFR) was estimated from serum creatinine concentration using the modification of diet in renal disease (MDRD) equation; eGFR (ml/min/1.73m²) = 186 x (Scr)-0.154 x (Age)-0.203 x (0.742 if Female) x (1.210 if Black ethnicity).

Statistical Analysis

Analysis was done using the SPSS version 16.0 computer software. The frequencies of nominal variables, the mean ± standard deviation of normally distributed numerical variable were generated. The median (minimum value – maximum value) of numerical variables that were not normally distributed were also generated. Pearson’s correlation method was used to determine associations between degree of clinical nephropathy and other variables after logarithm transformation of non-parametric variables. The independent predictors of clinical nephropathy were determined by using logistic regression analysis. Clinical nephropathy was used as the dependent variable, while other correlates were used as the predicting variables. A significance threshold in two tailed tests (p-value) was taken as less than 0.05.

Results

Sociodemographic characteristics of the study population.

Five hundred adult patients with type 2 DM were screened, of whom 280 patients satisfied the inclusion and exclusion criteria, but 273 patients (118 men, 155 women) completed the study with evaluable data. The sociodemographic characteristics of the 273 type 2 DM patients are summarized in Table 1. The study subjects were from 2 ethnic divisions, but majority were Yoruba (92.7%). One hundred and thirty seven subjects (50.2%) were uneducated and petty traders each.

Clinical and biochemical characteristics of the study population

The clinical and biochemical characteristic of the study population is summarized in Table 2. The mean age was 59.0 ± 11.1 years, mean glycated haemoglobin and eGFR were 7.41 ± 2.67% and 97.11 ± 28.29ml/min/1.73m² respectively.
The prevalence of clinical nephropathy in type 2 DM subjects
Fifty patients out of the 273 study population had clinical nephropathy giving a prevalence of 18.3%. The gender difference in the prevalence of clinical nephropathy in type 2 DM patients is demonstrated in Table 3. The prevalence of clinical nephropathy was found to be higher in females (20.6%) compared to males (15.3%). However, the difference in prevalence was not statistically significant.

Table 3: Gender difference in the prevalence of clinical nephropathy

<table>
<thead>
<tr>
<th>Clinical Nephropathy</th>
<th>Male n = 118</th>
<th>Female n = 155</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absent (% within sex)</td>
<td>100 (84.7)</td>
<td>123 (79.4)</td>
</tr>
<tr>
<td>Present (% within sex)</td>
<td>18 (15.3)</td>
<td>32 (20.6)</td>
</tr>
<tr>
<td>Total (% within sex)</td>
<td>118(100)</td>
<td>155 (100)</td>
</tr>
</tbody>
</table>

X² = 1.301, df = 1, p = 0.254

Correlates of degree of clinical diabetic nephropathy.
The degree of clinical nephropathy correlated positively with the age of the patients, current fasting plasma glucose, glycated hemoglobin, serum creatinine concentration and duration of DM, but negatively with eGFR (Table 4).

Table 4: Correlates of degree of clinical nephropathy.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Correlation Coefficient (r)</th>
<th>P value</th>
<th>Odd ratio</th>
<th>95% Confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (Years)</td>
<td>0.312</td>
<td>0.027*</td>
<td>1.020</td>
<td>0.991 – 1.049</td>
</tr>
<tr>
<td>Duration of DM (Years)</td>
<td>0.779</td>
<td>0.001*</td>
<td>1.218</td>
<td>1.152 – 1.288</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>-0.236</td>
<td>0.099</td>
<td>1.013</td>
<td>0.990 – 1.035</td>
</tr>
<tr>
<td>Waist to Hip ratio</td>
<td>0.101</td>
<td>0.484</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>0.183</td>
<td>0.204</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>0.285</td>
<td>0.045*</td>
<td>1.012</td>
<td>1.024 – 1.056</td>
</tr>
<tr>
<td>Mean arterial pressure (mmHg)</td>
<td>0.252</td>
<td>0.077</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current fasting plasma glucose (mmol/l)</td>
<td>0.392</td>
<td>0.005*</td>
<td>1.276</td>
<td>1.072 – 1.511</td>
</tr>
<tr>
<td>GlycatedHb (%)</td>
<td>0.341</td>
<td>0.015*</td>
<td>1.285</td>
<td>1.051 – 1.319</td>
</tr>
<tr>
<td>Serum creatinine (µmol/l)</td>
<td>0.750</td>
<td>0.001*</td>
<td>1.040</td>
<td>1.024 – 1.056</td>
</tr>
<tr>
<td>Serum urea (mmol/l)</td>
<td>0.229</td>
<td>0.110</td>
<td></td>
<td></td>
</tr>
<tr>
<td>eGFR (ml/min/1.73m²)</td>
<td>-0.695</td>
<td>0.001*</td>
<td>0.938</td>
<td>0.913 – 0.964</td>
</tr>
</tbody>
</table>

BP = blood pressure, Hb = haemoglobin, eGFR = estimated glomerular filtration rate
* Significant correlates based on p values less than 0.05

Predictors of Clinical Nephropathy in Type 2 DM.
Univariate binary logistic regression analysis of correlates of clinical nephropathy demonstrated that predictors of clinical nephropathy were glycatedhaemoglobin, serum creatinine, duration of DM and eGFR as demonstrated in Tables 5.

Table 5: Univariate binary logistic regression analysis of correlates of clinical nephropathy

<table>
<thead>
<tr>
<th>Variable</th>
<th>P value</th>
<th>Odd ratio</th>
<th>95% Confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (Years)</td>
<td>0.173</td>
<td>1.020</td>
<td>0.991 – 1.049</td>
</tr>
<tr>
<td>Duration of DM (Years)</td>
<td>0.001*</td>
<td>1.218</td>
<td>1.152 – 1.288</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>0.274</td>
<td>1.013</td>
<td>0.990 – 1.035</td>
</tr>
<tr>
<td>Fasting blood glucose (mmol/l)</td>
<td>0.764</td>
<td>1.011</td>
<td>0.939 – 1.089</td>
</tr>
<tr>
<td>GlycatedHb (%)</td>
<td>0.005*</td>
<td>1.178</td>
<td>1.051 – 1.319</td>
</tr>
<tr>
<td>Serum creatinine (µmol/l)</td>
<td>0.001*</td>
<td>1.040</td>
<td>1.024 – 1.056</td>
</tr>
<tr>
<td>eGFR (ml/min/1.73m²)</td>
<td>0.001*</td>
<td>0.944</td>
<td>0.927 – 0.961</td>
</tr>
</tbody>
</table>

* Significant predictors of clinical nephropathy before controlling for confounders

The independent predictors of clinical nephropathy after controlling for other variables were glycatedhemoglobin, eGFR, and duration of DM as demonstrated in table 6.

Table 6: Multivariate logistic regression analysis to determine independent predictors of Clinical nephropathy

<table>
<thead>
<tr>
<th>Variable</th>
<th>P value</th>
<th>Odd ratio</th>
<th>95% Confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycated Haemoglobin (%)</td>
<td>0.005*</td>
<td>1.276</td>
<td>1.072 – 1.511</td>
</tr>
<tr>
<td>eGFR (ml/min/1.73m²)</td>
<td>0.001*</td>
<td>0.938</td>
<td>0.913 – 0.964</td>
</tr>
<tr>
<td>Duration of DM (Years)</td>
<td>0.001*</td>
<td>1.316</td>
<td>1.198 – 1.445</td>
</tr>
</tbody>
</table>

* Significant independent predictors of clinical nephropathy after controlling for confounders

Discussion
Clinical nephropathy is an advanced stage of DN. Unlike microalbuminuria, patients with clinical diabetic nephropathy invariably develop hypertension, a progressive worsening in proteinuria, and a relentless decline in glomerular filtration rate over time without
intervention. End stage renal disease in diabetic patients is associated with poorer outcome compared to non-diabetic patients because of the comorbid conditions in the diabetic population, and majority of these patients have type 2 DM. Moreover, DN is the diabetic specific complication with highest mortality, and the risk of cardiovascular death is higher by a factor of 12 in these patients when compared to renal diseases resulting from primary glomerular diseases. Despite this dreaded complication, the prevalence of type 2 DM is increasing worldwide, particularly in the developing countries like Nigeria. This hospital-based cross-sectional study was designed to investigate the frequency of occurrence, and characteristics of clinical nephropathy in type 2 DM patients. This is against the backdrop of the facts that DN is the leading cause of ESRD in western world, the third leading cause of ESRD in Nigeria and in Ilorin, Nigeria.

Earlier study in this centre on type 2 DM patients did not investigate clinical nephropathy, but focused on microalbuminuria. Study on clinical nephropathy is important because the knowledge of its burden and characteristics could reinforce the institution of effective preventive and management plan which include; routine screening for early detection of microalbuminuria, optimal blood pressure control, glycaemic control, and the use of certain agents like angiotensin ACEIs and/or ARBs that ameliorate the structural and hemodynamic changes implicated in the pathogenesis of DN. This study will also contribute to existing literature on this subject being the first study in the whole of North-Central Nigeria on clinical diabetic nephropathy.

The prevalence of clinical nephropathy in type 2 DM patients in Ilorin.

The prevalence of clinical nephropathy as seen in patients with type 2 DM in this study was 18.3%. Although there have been several studies on diabetic nephropathy, most of these studies either focused on microalbuminuria or investigated DN as a whole. Relatively few studies specifically examined clinical nephropathy in type 2 DM patients.

Macroalbuminuria is the hallmark of clinical DN, but it does not always imply clinical DN because other factors like infection, fever, muscular exertion, heart failure and elevated blood pressure among others that could transiently increase urine albumin excretion. Persistence of macroalbuminuria in a diabetic patient over a 3 to 6 month period in the absence of other renal disease should be established before a diagnosis of clinical DN can be made. Establishing persistence of macroalbuminuria eliminates these transient causes. This study introduced a modification to the cross-sectional design by repeating the estimation of urine ACR after 3 months in all patients with macroalbuminuria and also eliminated known confounders. This approach improved the sensitivity of the diagnosis of clinical DN as there were no ethical indications for renal biopsy in these patients.

The closest prevalence of clinical diabetic nephropathy to this current study in previous literature was a prospective cross-sectional study in a Sudanese teaching hospital which found it to be 18.4%. The similarity in the observed prevalence of macroalbuminur in the 2 studies is likely due to the similar socio-demographic characteristics of the study subjects. This current study was however restricted to subjects with Type 2 DM, unlike the one in Sudan by Ahmed et al. The observed prevalence of clinical DN in this study is lower to the previous report by some authors in other centres in this country. Alebiosu reported that the overall frequency of occurrence of clinical DN in diabetic patients (both type 1 & 2) in OlabisiOnabanjo Teaching Hospital, Shagamu, Nigeria, was 28.4%. In Ibadan, Nigeria, Alebiosu et al in a clinical review of diabetic nephropathy commented that the prevalence of clinical DN increased from 19% in 1971 to 42.5% in 1988. An increment in the prevalence of DN in diabetic population was also reported by Afifi et al in Egypt. These observations may be explained by the fact that with the increasing prevalence of type 2 DM as projected by WHO, the prevalence of complications which include clinical nephropathy are expected to be on the increase. The relatively lower prevalence observed in this present study may be due to improvement in detection and quality of management of type 2 DM in the region, thereby forestalling concomitant increase in complication like clinical DN. There are no previous studies in Ilorin, Nigeria to determine the prevalence of clinical DN before this present study, hence, the trend in prevalence over the
years could not be ascertained. Studies by Osuntokun et al, Bella et al, and Alebiosu et al were not restricted to type 2 DM patients, this may also account for the disparity in prevalence as compared to the current study.39,43-45

In this current study, most of the subjects were on various antihypertensive drugs except ACEI and ARB. These could very much explain the relatively low prevalence of clinical nephropathy recorded, since lowering of blood pressure has been established to attenuate the progression of renal disease and reduce proteinuria.47-50 Eight patients with microscopic haematuria were excluded from this study to improve the specificity of the diagnosis of clinical nephropathy. This is potentially another reason for the relatively lower prevalence observed in this study. It has been shown with renal histologic findings that microscopic hematuria may actually be a common feature of clinical DN especially in patients with nephrotic range proteinuria.51 Although, none of the excluded patients with haematuria had nephrotic range proteinuria, it is possible that some of them may actually have clinical nephropathy.

Some other studies defined overt nephropathy (clinical nephropathy) as albuminuria ≥ 300µg/mg of creatinine or dipstick positive proteinuria in the presence of diabetic retinopathy. An example of such study is the Chennai Urban Rural Epidemiology Study (CURES 45) in which the prevalence of overt nephropathy was as low as 2.2%.52 The drawback of such definition is that, in type 2 DM, absence of retinopathy does not exclude clinical nephropathy.15

Immunologic based assay of urine albumin like Immunoturbidometry method used in this study does not detect non immune reactive albumin. This could result in the underestimation of urine albumin concentration and the actual prevalence of clinical nephropathy.15

Other reasons for the variation in the prevalence of clinical DN, macroalbuminuria, or DN in type 2 DM patients in various studies include the difference in diagnostic criteria, methods of assessment, period of study and difference in genetic susceptibility in the study populations. Regardless of the above observations, the prevalence of 18.3% reported in this current study is still alarming considering enormity of the attendant morbidity and mortality, as well as the financial implication of renal care in a developing country setting.

**Gender difference in the prevalence of clinical nephropathy in type 2 DM patients.**

The prevalence of clinical DN in the male and female subgroups of type 2 DM patients were 15.3% and 20.6% respectively, but the observed difference was not statistically significant. This may be due to higher numerical strength of female subjects in this study with male to female ratio of 1:1.3. Although, most studies on subjects with diabetes mellitus did not specifically look into gender differences in the prevalence of clinical nephropathy, previous reported male to female ratios showed male preponderance.39,45 The finding in this study may however be projected from an earlier report from the study centre on microalbuminuria in type 2 DM which reported the prevalence of microalbuminuria to be higher in females.20 If female preponderance is established by more powered future study, it could generate concerns because a case control study in Lyon, France reported increased mortality only in type 2 diabetic females with ESRD.53

**Correlates of clinical nephropathy in type 2 diabetic patients.**

The age of the subjects, duration of DM, systolic blood pressure, current fasting plasma glucose, glycated hemoglobin and serum creatinine concentration correlated positively, while eGFR correlated negatively with clinical nephropathy in this study. Out of all these correlates, the duration of DM, serum creatinine concentration and eGFR were the strongest (r = 0.779, 0.75, and 0.695 respectively). After controlling for confounders, the independent predictors of clinical nephropathy were duration of DM, eGFR, and glycated haemoglobin. It was observed that body mass index (BMI), waist-to-hip ratio, diastolic blood pressure, mean arterial pressure, and blood urea nitrogen did not correlate with clinical nephropathy. The correlation pattern seen in this study is in agreement with the established pathophysiology and clinical characteristics of DN in some earlier literatures.15,40,54,55

Although, plasma cystatin C based estimates of GFR has been shown to be more accurate than creatinine
based estimate in early stages of DN, in established DN, there is only marginal difference in these 2 estimation modalities.\textsuperscript{56} Hence, the use of MDRD formula to estimate GFR in this study is still reasonable and acceptable.\textsuperscript{15,54} The effect of antihypertensives and exclusion of subjects with poor blood pressure control in the study population could explain absence of correlation between clinical nephropathy and diastolic/mean arterial pressure. It is however difficult to explain why BMI and waist -to- hip ratio did not correlate significantly with clinical nephropathy. This contrasts with the observation of \textsuperscript{1,2,15,58} the DEMAND study, which reported that the risk of progression of urinary albumin excretion increased by 7\% for every 5cm increase in waist circumference from baseline value, and by 17\% for every one unit increase in BMI.\textsuperscript{57} The reason for the contrasting observation could be explained by the fact that the DEMAND study was more powered (1289 patients), and it was a prospective design, therefore, expected to be more sensitive at observing the association between the two variables.\textsuperscript{37}

Previous studies on DN showed varying pattern of correlates.\textsuperscript{1,2,11,15,40} The negative correlation of clinical nephropathy with eGFR is in agreement with earlier report by \textit{Alebiosu et al} in Ibadan, Nigeria.\textsuperscript{45}\textit{Dobronravov} also reported high proteinuria in type 2 DM patients with chronic renal failure (i.e. low eGFR).\textsuperscript{41} Finding in this study is in agreement of the observation in Dar es Salaam that duration of diabetes, systolic blood pressure and serum creatinine not only correlated, but actually predicted proteinuria in type 2 diabetic patients.\textsuperscript{11}In related previous literature, poor glycemic control correlated with the formation of AGEs, which in turn correlated with DM complication like nephropathy.\textsuperscript{1,15,58} Although some previous local studies reported no correlation between glycemic control and microalbuminuria,\textsuperscript{20,59} this present study found that glycemic control correlated with macroalbuminuria. This later observation may be a reflection that microalbuminuria does not necessarily indicate nephropathy in type 2 DM.\textsuperscript{12}\textit{Ballard et al} in a population based study in Rochester, Minnesota, found that elevated initial fasting blood glucose and older age at onset of diabetes were associated with the development of persistent proteinuria in type 2 DM.\textsuperscript{40} The finding of \textit{Ballard et al} by projection is in consonance with the finding in this study that age, fasting plasma glucose and glycatedhemoglobin correlated positively with clinical nephropathy. However, contrary to what was found in this present study, \textit{Ballard et al} found no association between duration of type 2 DM and incidence of persistent proteinuria after controlling for attained age.\textsuperscript{40}

Observation by \textit{Ballard et al} may be due to the prolonged asymptomatic period that characterizes type 2 DM which could distort the statistical association between the recorded duration of DM and clinical nephropathy.\textsuperscript{1,2,15} The strong correlation between duration of DM and clinical nephropathy observed in this study is however in consonance with earlier report by \textit{Nakhjavani et al} and \textit{Ranjit et al}.\textsuperscript{52,60} The later 2 studies found that increased urine albumin excretion correlated with a longer duration of diabetes in type 2 diabetic population independent of other variables.\textsuperscript{52,60}

**Conclusion**

ClinicalNephropathy is present in significant population of patients with Type 2 DM in UITH, Ilorin, North Central Nigeria, and it correlated positively with the age of the subjects, duration of DM, systolic blood pressure, current fasting plasma glucose, glycatedhemoglobin, and serum creatinine concentration. These observed correlates can help clinicians in screening, monitoring and management of patients with Type 2 DM to preventdevelopment of clinical nephropathy. A community based multi-centre study will give a more accurate picture of the burden and characteristics of clinical diabetic nephropathy in Nigeria. A more powered future research is also needed to establish gender difference in the prevalence of clinical diabetic nephropathy and any impact on morbidity and mortality of the subjects.

**References**

Gastrointestinal perforation in a child following typhoid infection: a case report.

Obi-Egbedi-Ejakpovi EB, Akhigbe OT.

Department of Radiology, Irrua Specialist Teaching Hospital, Irrua, Edo State Nigeria.

Abstract

Typhoid fever is a major health problem in developing parts of the world due to poor sanitation and children are at high risk of this infection and its associated complications. Despite the high prevalence of typhoid fever globally, Africa in particular, exact estimated of morbidity and mortality are lacking due to scarcity of published data and most case fatality estimates are extrapolations from hospital based studies that do not cover geographical regions. The increasing prevalence of typhoid fever with enteric perforation in our environment is alarming. Peritonitis follows perforation due to typhoid fever. A 5-year-old child that had intestinal perforation complicated by Typhoid fever is presented. This case presentation aims at reviewing the simple radiological modalities available in our environment that can be useful in the evaluation of suspected gastrointestinal perforation so as to reduce the mortality associated with this condition, especially amongst children.

Keywords: Intestinal perforation, Typhoid fever.

Introduction

Typhoid fever caused by *Salmonella Enteric Serovar typhi* (*Salmonella typhi*) causes an estimated 22 million cases and 216,000 deaths annually worldwide. In Nigeria, children constitute 49% of the cases of typhoid infection and majority (78%) of the patients are in the low socio-economic strata, with a male: female ratio of about 1.9:1 with a mean age of 19 years.

The most lethal complication of typhoid fever is intestinal perforation, commonly involving the ileum. The overall frequency of intestinal perforation in typhoid fever is about 4%. The typically high mortality rates from these perforations are in part due to extremely limited supportive care in hospitals in typhoid endemic areas.

Perforation of the gastrointestinal tract may result from many other causes. It could result from blunt or penetrating abdominal trauma, inflammatory conditions that penetrate the serosa or adventitia e.g. appendicitis, ulcers, infections – tuberculosis, ingested foreign bodies, iatrogenic injury, extrinsic neoplasms that invade the gastrointestinal tract or primary neoplasms that penetrate outside the wall of gastrointestinal organs.

The diagnosis of typhoid intestinal perforation begins with a careful history and physical examination. When appropriate, the clinical examination is supplemented by conventional plain abdominal radiography. Plain abdominal radiography is the initial diagnostic method of choice. Ultrasonography and CT scan are also valuable in the evaluation of intestinal perforation. Definitive diagnosis of typhoid etiology is made after laparotomy, culture, serologic and histopathologic examination of tissue specimen and in some cases at autopsy.

Case Report

Master IM, a 5-year-old boy that presented at the children’s emergency unit with complaints of fever of two weeks’ duration, nausea and vomiting of ten days’ duration, jaundice and abdominal distension of one-week duration. Patient was well until two weeks before presentation when he developed fever which was intermittent, worse in the evenings, associated with chills, no rigor, headache. Few days later nausea...
and vomiting developed, vomiting was non-projectile, contained recently ingested food. Jaundice, abdominal distension developed one week later with associated constipation.

Patient has been receiving self-medications, native herbs at home but was brought to the hospital when his condition deteriorated.

On physical examination, a chronically ill-looking child was seen. Patient was pale, jaundiced, not cyanosed, dehydrated. He had bilateral pitting pedal edema up to the knees. Pulse rate was 140 beats per minute, regular. Blood pressure was not done (no pediatric cuff). Apex beat and heart sounds were normal, no murmurs. Respiratory rate was 52 cycles per minute, breath sounds were vesicular, but reduced in the left lower lung zone.

Abdominal examination showed distended abdomen that moved slowly with respiration. Abdominal girth was 53cm. Abdominal organs were not palpable because of tenderness and abdominal guarding. Rebound tenderness was negative, percussion notes were dull, shifting dullness was positive at the flanks. Bowel sounds were reduced. Central nervous system examination was normal, there were no signs of meningeal irritation.

An impression of typhoid septicaemia with intestinal perforation was made. Laboratory investigations were done. Packed cell volume was 19%. Liver function test, full blood count, malaria parasite, electrolyte and urea done were normal. Blood culture and stool for microscopy, culture and sensitivity yielded growth of *Salmonella typhi*. Radiological investigations, (abdominal ultrasound scan and plain abdominal radiographs) were done.

Abdominal ultrasonography scan showed normal intraabdominal organs with evidence of free intraperitoneal fluid (Fig. 1), suggestive of intestinal perforation.

Plain abdominal radiograph revealed a distended abdomen with paucity of gas in the pelvis, dilated bowel loops with multiple air-fluid levels. There was also evidence of free intraperitoneal air (pneumoperitoneum) under the right diaphragm and between the anterior abdominal wall and liver (Figs. 2). Based on these radiologic features a diagnosis of intestinal perforation was made. Patient was rehydrated, transfused to packed cell volume of 30%, electrolyte imbalance corrected and placed on antibiotic therapy.

Emergency exploratory laparatomy was done and the following findings were seen: Hypotonic small intestinal motility with multiple areas of perforation in the terminal ileum and fecal stained peritoneal fluid.

Peritoneal lavage and double bowel loop enteroplasty were done and the abdominal cavity was closed in layers. Patient was taken to the intensive care unit but never recovered from anaesthesia. He was certified dead due to overwhelming sepsis.

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**Fig. 1:** Abdominal ultrasound scan showing floating bowel loops with free intraperitoneal fluid indicative of intestine perforation

**Fig. 2:** Plain abdominal radiograph (Erect view) showing multiple air fluid levels (arrows) with pneumoperitoneum (arrowhead)
Discussion

Perforation of the ileum is one of the most severe complications of typhoid fever. It is also a cause of high morbidity and mortality in endemic areas. Typhoid fever is caused by infection with Salmonella typhi. Spread of the infection is usually by carriers, often food handlers through the contamination of food, milk or water. After a few days of bacteremia, the bacilli localize mainly in the lymphoid tissue of the small intestine. The typical lesions are in the Peyer’s patches and follicles. These swell at first, then ulcerate and ultimately heal, but during this sequence, they may perforate or bleed. Abdominal pain (93.4%), fever (93.4%) and constipation (87%) are the main presenting symptoms and abdominal guarding and rigidity are the principal physical signs of typhoid ileal perforation. The patient in this case report presented with these features. Thus, the diagnosis of small bowel perforation is suspected in a case of acute abdominal pain associated with peritoneal signs (tenderness followed by rigidity).

The risk of enteric perforation in a patient with typhoid fever increases with inadequate antimicrobial therapy prior to admission, a short duration of symptoms, male sex and leucopenia.

The main role of the radiologist in the management of patients with suspicion of small bowel perforation obstruction is to help separate patients into those that need immediate surgical intervention from those that require medical therapy or delayed surgery.

Plain abdominal radiography used in the evaluation of this patient is the method of choice in the diagnosis of gastrointestinal perforation because it shows the presence of free intraperitoneal air and other associated radiologic signs. Dilatation of small bowel loops, bowel wall thickness, presence of air-fluid levels, thickness of valvulae conniventes are some associated radiographic features of small bowel perforation on plain film. Plain abdominal radiograph of our patient showed pneumoperitoneum (free air under the diaphragm, air between the anterior abdominal wall and the liver), dilated bowel loops with multiple air-fluid levels.

Recently, the modern methods of cross-sectional imaging (ultrasonography, CT scan, MRI) have become useful tools for the accurate detection and depiction of free abdominal air, especially when plain films are normal or non-specific.

Ultrasound examination is usually considered not helpful in acute abdomen because air in the intestinal lumen interferes with the evaluation of the intestinal loops. However, some authors have attested the increasing importance of sonography in acute abdominal disease in patients with perforation, with free intra-peritoneal fluid/blood. Grassi et al. recorded certain sonographic features in suspected small bowel disease and they include presence and echogenicity of extraluminal fluid, evidence or absence of peristalsis, presence of thick-walled bowel loops. Our patient had evidence of extraluminal fluid with hypoperistalsis on abdominal ultrasound scan.

CT scan has high sensitivity, demonstrating the presence of free intraperitoneal gas in more patients than conventional radiography (92% versus 74%) Catalano recorded that pneumoperitoneum can be depicted between liver surface and anterior abdominal wall, in the subhepatic region, posterior to the abdominal wall at the umbilical level, between the mesenteric folds in the pelvis and other locations. Extraluminal fluid collections, extravasation of ingested contrast media and visualisation of a discontinuity in the bowel wall are considered direct findings of intestinal perforation. The origin of the perforation can be demonstrated in about 82% of cases and its cause in 37% of cases.

Plain abdominal radiographs and abdominal ultrasound scan were the radiologic investigative modalities used in establishing a diagnosis of typhoid intestinal perforation in this patient, based on history, clinical, laboratory and radiological findings.

Definitive diagnosis of ileal perforation due to typhoid fever is based on positive laboratory culture on blood and marrow specimen, positive culture from gut biopsies, detail histopathological examination of the gastrointestinal mucosa at the site of perforation. Blood and stool specimen of our patient yielded growth of salmonella typhi thus confirming the diagnosis.

Early surgery in enteric perforation is the only accepted form of treatment after resuscitations with intravenous fluids, electrolyte replacement and broad spectrum antibiotics, nasogastric intubation/suctioning and...
urethral catheterisation has been done\textsuperscript{11}. With early diagnosis, effective resuscitation and timely intervention, this life-threatening condition is not necessarily fatal. Septic shock is however an ominous sign of poor prognosis\textsuperscript{12}. Late presentation, delay in operation, multiple perforations and drainage of copious quantities of pus and fecal materials from the peritoneal cavity increases the incidence of fecal fistula (enterocutaneous) and death from overwhelming sepsis\textsuperscript{13}. Early presentation, single perforation and moderate amounts of pus/fecal matter draining from the peritoneal cavity enhances the development of wound infection, wound dehiscence, residual intra-abdominal abscess and mechanical intestinal obstruction secondary to adhesion\textsuperscript{13}. Thus, the postoperative complication adversely affects the morbidity and mortality rates of these severely ill and debilitated patients with typhoid perforation of the ileum. However, surviving for more than ten postoperative days tends to give a better chance of recovery\textsuperscript{13}.

To improve survival in typhoid perforation, attention should be focused on early diagnosis using radiological imaging modalities, perioperative resuscitation and early intervention. The provision of potable water, adequate sanitation and active immunisation are means of eradicating this disease.

References

SECTION 2

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Laboratory measurement of haemoglobin A<sub>1c</sub>: A review

Osuji K C

Department of Chemical Pathology, Irrua Specialist Teaching Hospital, Irrua, Edo State, Nigeria.

Abstract

Glycated haemoglobin has been used to monitor the blood glucose control of diabetic patients for over thirty years. It is a very convenient test which could be carried out at any time irrespective of the patients’ prandial state and it measures glucose level over the preceding 90 to 120 days. Its utility as a relatively accurate predictor of the likelihood of the development of chronic complications of diabetes mellitus, especially as elucidated by the standard setting of the Diabetes Complications and Clinical Trial (DCCT) study, has further heightened its use and designation as a possible gold standard in the diagnosis, management and monitoring of diabetes mellitus. There are currently over a hundred different techniques of laboratory measurement of glycated hemoglobin, with many of them existing on various commercial automated and non-automated platforms; while some techniques on the other hand have not been found viable and have been discontinued. Standardization of assay and traceability of HbA<sub>1c</sub> results is another big issue which are being effectively handled by the International Federation of Clinical Chemistry (IFCC) and her affiliate societies with remarkable results. Available literature on laboratory measurement of HbA<sub>1c</sub> was sourced for using both manual and online literature search. Laboratory measured HbA<sub>1c</sub> is a fully established tool in the diagnosis, management and monitoring of patients with diabetes mellitus. It is routinely measured in most Clinical Chemistry Laboratories, employing any of the many techniques used for its measurement. Each assay method has its own advantages and disadvantages, and generated results are largely reliable, thanks to the various measures instituted to standardize its assay.

Keywords: HbA<sub>1c</sub>, Laboratory measurement

Introduction

Glycation is the non-enzymatic addition of a sugar residue to an amino group of protein.<sup>1</sup> Glycated haemoglobin is defined as haemoglobin that is irreversibly glycated at one or both N-terminal valines of the beta chains.<sup>2,3</sup> HbA<sub>1c</sub> is formed by the condensation of glucose with the N-terminal valine residue of each beta-chain of HbA, to form an unstable Schiff base called an aldimine compound or pre HbA<sub>1c</sub>. The Schiff base may either dissociate to yield glucose and haemoglobinin the presence of nomoglycaemia or, undergo an amadori rearrangement in persistent hyperglycaemic state to form a stable ketoamine HbA<sub>1c</sub>. When blood glucose levels are high, glucose molecules attach to the haemoglobin in erythrocytes. The longer hyperglycemia occurs in the blood, the more glucose that binds to haemoglobin in the red blood cells and the higher the glycated hemoglobin. Once a haemoglobin molecule is glycated, it remains that way throughout the lifespan of the erythrocyte. Buildup of glycated hemoglobin within red cells, therefore, reflects the average level of glucose to which the cell has been exposed during its cycle.<sup>1,2</sup>

The HbA<sub>1c</sub> level is proportional to the average plasma glucose concentration over the previous four weeks to three months.<sup>1,2</sup> Recent studies suggest that the major proportion of HbA<sub>1c</sub> values are weighted towards plasma glucose level in the recent 2 to 4 weeks preceding analysis. This is believed to contribute about 50% of the HbA<sub>1c</sub> value.<sup>1,4,5</sup> An observation which was supported by Siderenkov G et al<sup>6</sup> that HbA<sub>1c</sub> values improved significantly within 20 days of glucose lowering treatment intensification.

It is pertinent to note that the definition of glycated haemoglobin does not exclude haemoglobin that is additionally glycated at other sites on alpha or beta chains.<sup>3</sup> Or haemoglobin glycated with other sugar residues apart from glucose such as pyruvic acid, glucose-6-phosphate and fructose-1,6-diphosphate. However, HbA<sub>1c</sub> accounts for about 80% of total glycated haemoglobin in humans.<sup>1</sup>
Measuring HbA1c

For almost 20 years after the discovery of glycated haemoglobin, chromatography was the only available method of assay. There are currently approximately one hundred different methods of measuring glycated haemoglobin, these methods separate glycated from non-glycated haemoglobin employing differences in characteristics such as charge, e.g. Ion exchange chromatography, high performance liquid chromatography, electrophoresis and isoelectric focusing, structure, e.g. affinity chromatography and immunoassay. Also Chemical composition, e.g. photometry and spectrophotometry. Irrespective of the method used, results are expressed as a percentage of total haemoglobin. However, because most of these methods measure total glycated haemoglobin which have little or no clinical utility and are hardly reproducible even within the same laboratory; their use has been discontinued in most laboratories. A number of techniques for the measurement HbA1c have been widely accepted for use by most Laboratories worldwide, they include: High performance liquid chromatography (HPLC), boronate affinity chromatography, Iow-exchange chromatography and Immunoassay technique using antibodies.

In the United States, HbA1c testing laboratories are certified by National Glycohemoglobin Standardization Program (NGSP) to standardize their results and make them traceable to the 1993 Diabetes Control and Complications Trial (DCCT), this program was later extended to manufacturers of testing equipment for HbA1c.

Specimen collection and storage.

The Patient need not be fasting and venous blood should be collected in tubes containing EDTA, oxalate or fluoride, with sample stability depending on assay method. Whole blood may be stored at 4°C for up to one week, temperatures above this are not advisable. However, for most methods, whole blood samples stored at – 70°C or lower are stable for at least 18 months.

Removal of labile glycated Hb from red blood cells

Because the concentration of the intermediate, labile form of Hba1c (Schiff base) fluctuates rapidly in response to acute changes in plasma glucose concentrations, there is a need to remove them before analyzing for HbA1c. This is often accomplished by incubating the red blood cells in normal saline or in buffer solutions at a pH of 5 to 6 or by dialysis or ultra-filtration of hemolysates. However, most Kits for column assays contain reagents to remove this labile component.

Laboratory measurement of HbA1c

High Performance Liquid Chromatography (HPLC): Chromatography is a physical process whereby components (solutions) of a sample mixture are separated by their differential distribution between stationary and mobile phases. Planar and column are the two basic forms of chromatography. In planar chromatography, the stationary phase is coated on a sheet of paper or is bound to a solid surface, while in column chromatography the stationary phase may be pure silica, or polymer, coated onto or chemically bound to support particles. The stationary phase maybe packed into a tube or coated onto the inner surface of a tube. The mobile phase which may be gas in the case of gas chromatography, or liquid in the case of liquid chromatography, carries the sample through the stationary phase. As the mobile phase moves through the stationary phase, the solutes may reside only in the mobile phase (migration with the mobile phase), reside only in the stationary phase (nomigration) or distribute between the two phases (differential migration). When the stationary phase in liquid chromatography consists of small diameter particles 4.5µm or less, the technique is known as high performance liquid chromatography (HPLC). Generally chromatographic separations are classified based on the chemical or physical mechanisms used to separate the solutes, these include ion-exchange, absorption, partition, affinity, and size exclusion mechanisms. The basic components for HPLC are: a solvent reservoir containing the mobile phase, a pump, a column with an injector unit for sample introduction, a detector and a recorder.

The measurement of HbA1c using HPLC employs the use of cation exchange chromatography, this is based on the ionic difference between glycated and non-glycated haemoglobin and other haemoglobin fractions. Here anticoagulated blood is treated with boronate to haemolyse the red blood cells, the haemolysate is then incubated in saline at 37°C for 30 minutes to remove the Schiff base before an aliquot is
introduced into the stream of the mobile phase percolating through the column. The different fractions of HbA1c and HbA move through the column at different velocities, which are functions of ionic interactions with the stationary phase. The velocity at which each component moves, depends on its chemical nature and on the nature of the stationary phase. This also decides the elution time of each analyte. HbA1c has a very short retention time, which enables it to elute first from the column. Detection is performed at both 415 nm and 690 nm and results are quantified by integrating the area under the peak.\textsuperscript{20}

**Ion-exchange chromatograph:** This technique like HPLC, also separates hemoglobin variants based on charge differences. The cation exchange resin which are negatively charged, are packed in a disposable mini column and they have an affinity for hemoglobin which is positively charged. An aliquot of the patient’s haemolysed sample is applied to the column, a buffer is added and the eluent collected, the pH of the added buffer is selected such that glycated haemoglobins are less positively charged than HbA1, so that they bind less to the negatively charged resins and are thus eluted first. All the glycated haemoglobins (HbA1) are eluted together and are measured in a spectrophotometer. A second buffer of different ionic strength is then applied to elute the more positively charged main haemoglobin fraction, which is also read in a spectrophotometer and the glycated haemoglobin expressed as a percentage of total haemoglobin. Numerous commercial modifications to this method have been described.\textsuperscript{1} Marquat FX, et al\textsuperscript{21} in 1980, described other modifications to ion exchange chromatography, that separate other glycated haemoglobin fractions from HbA1c using different sets of buffers. In all ion exchange chromatographic methods, it is important to control the temperature of the columns and the reagents as well as maintain rigid control of pH and ionic strength.\textsuperscript{1}

**Affinity chromatography:** Here microcolumns packed with immobilized aminophenylboronic acid gels is used to selectively hold glycated haemoglobins in the columns while the non-glycated fraction which does not bind can elute. Sorbitol is then added to elute the bound glycated haemoglobin. Absorbance of both the bound and unbound fractions of haemoglobin measured at 415 nm is then used to calculate the percentage of glycated haemoglobin. The major advantages of affinity chromatography, include a lack of interference from non-glycated haemoglobins and negligible interference from the labile intermediate form of HbA1c. Other advantages of affinity chromatography are non-affectation by temperature variations and its good precision.\textsuperscript{1} It however has the disadvantage of reporting total glycated haemoglobin a flaw which most commercially available systems correct for by some acceptable form of standardization. This makes the result comparable to values obtained by methods specific for HbA1c.\textsuperscript{22}

**Immunnoassay technique:** Assay for HbA1c has been developed using antibodies raised against the Amadori product of glucose (ketoamine linkage) plus the first few (four to eight) amino acids at the N-terminal end of the β-chain of haemoglobins.\textsuperscript{23,24} A widely used assay measures HbA1c in whole blood by inhibition of latex agglutination. The agglutinator, a synthetic polymer containing multiple copies of the immunoreactive portion of HbA1c binds the anti-HbA\textsubscript{1c} monoclonal antibody that is attached to latex beads. This agglutinin produces light scattering, which is measured as an increase in absorbance. HbA1c in the patients’ sample competes for the antibody on the latex, inhibiting agglutination, thereby decreasing light scattering. Enzyme immunoassays using monoclonal antibodies are commercially available and most exhibit reasonable precision. They are also calibrated to give values comparable to HPLC.\textsuperscript{1}

**Electrophoresis/Electroendosmosis:** Menard et al,\textsuperscript{25} described a method using agar gel plates on which separation of HbA1 is achieved by electroendosmosis. The technique is relatively simple, cheap, and not temperature dependent, but reading of the plates requires careful setting of the scanning densitometer. Another method involved the use of mobile affinity electrophoresis, where glycosylation blocks the affinity of haemoglobin for the sulphate groups of dextran sulphate in electrophoresis buffers and renders HbA1 relatively immobile, it is then detected by protein staining and quantified by densitometry.\textsuperscript{26} This method is outdated and no longer in use.

**Colorimetry:** When heated with oxalic acid, ketoamine linked hexoses are hydrolyzed to 5-hydroxymethylfurfuraldehyde (HMF). The addition of 2-thiobarbituric acid yields a coloured product which can be estimated photometrically.\textsuperscript{27} However, this is no longer in use because it is tedious, time consuming, and
calls for carefully controlled reaction conditions which are often difficult to meet.

**Interpretation of results**

Assay precision is important because each 1% change in HbA1c represents approximately 35mg/dl change in average blood glucose. Laboratory results may differ depending on the analytical technique, the age of the subject, and biological variation among individuals. Results can be unreliable in many circumstances such as after blood loss, after surgery blood transfusion, anaemia or high erythrocyte turn over states, and in the presence of chronic renal or liver disease and erythropoietin treatment. In general, the reference range for HbA1c is about 20-40mmol/ml (4%-5.9%). Higher levels of HbA1c are found in people with persistently elevated blood glucose, as in diabetes mellitus. A diabetic person with good glucose control will have an HbA1c level that is close to or within the reference range for normal. The International Diabetes Federation (IDF) and the American College of Endocrinology recommend HbA1c values below 48mmol/ml (6.5%), while the American Diabetic Association (ADA) recommends HbA1c level below 53mmol/ml (7.0%) for most patients. Lower than expected levels of HbA1c can be seen in people with shortened red blood cell lifespan, such as found in patients with glucose-6-phosphate dehydrogenases deficiency (G6PD), sickle cell disease, thalassemia, hereditary spherocytosis, etc. On the other hand, higher than expected values can be seen in people with a longer red blood cell lifespan, for example in people with vitamin B12 or folate deficiency. Also situations such as hypertriglyceridemia, hyperbilirubinemia, uremia, chronic alcoholism, and chronic ingestion of salicylates have been reported to cause a false increase in HbA1c.

**Assay standardization**

Glycated haemoglobin (GHb) has been accepted as the gold standard for the assessment of chronic hyperglycaemia for nearly three decades. However because clinical laboratories measure GHb with diverse assays that use multiple methods and quantify different components, standardization of results from the same laboratory and even different laboratories became imperative especially in the face of bizarre intra assay, inter assay and inter laboratory variations of results. The Diabetes Control and Complications Trial (DCCT) study group recognizing these problems centralized the measurement of HbA1c from the onset of its study. Also, the American Association for Clinical Chemistry (AACC) in anticipation of the DCCT results, established A1c standardization workgroup to bring consistency to A1c and to facilitate the traceability of results back to DCCT. Such that these results could be directly related to the risk or progression of diabetes complications. After the DCCT study the National Glycohaemoglobin Standardization Programme (NGSP) was set up, to calibrate GHb results to DCCT equivalent values. Employing a network of reference laboratories, the NGSP interacts with manufacturers of GHb methods instruments to help them calibrate their methods and trace the values to the DCCT. Furthermore, the IFCC saw a need to develop a reference method that would be very specific for HbA1c and that could lead to worldwide standardization based on metrologically sound international measurement system. Not only did IFCC succeed in developing such an assay, the reference method has been approved by all their member countries and a global network of reference laboratories has been established. However because a mass spectrometer, a very expensive, largely unaffordable instrument was used by the IFCC, this method is not available for routine measurement of HbA1c, but is available as a calibrator of instruments used routinely.

**Conclusion**

HbA1c is used to monitor glycaemic control all over the world: It provides an index of diabetic control that effectively integrates blood glucose concentrations over several weeks. Its method of analysis varies. However, virtually all commercially available techniques available today are standardized and traceable to the DCCT study, thus making it an indispensable tool in the diagnosis, control and monitoring of diabetes mellitus.

**References**

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The dilemma of expired drugs: To use or not to use

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Abstract

There is considerable uncertainty concerning over-the-counter and prescription drugs that past the expiry dates: should they be used or should they be discarded? Many face this dilemma at one point or another. Contrary to common belief, literature search shows that there is little scientific evidence that expired drugs are toxic. The aim of this paper is to proffer current scientific information on expired drugs, discuss factors that negatively affect them and the factors to consider whether to use an expired medication or not. The expiration date does not indicate a point when a medication loses potency and is no longer effective or becomes harmful. It only indicates the date the manufacturer guarantees the full potency and safety of the drug. Medications react to the environment in which they are stored and decompose over a period because of inappropriate exposure to sunlight, oxygen, moisture, and extreme temperatures. Hence some drugs such as antibiotics may not be effective when used to treat serious infections and so lead to complications and antibiotic resistance. To decide whether to use an expired drug or not, one must consider the dosage formulation, packaging, storage conditions, length of time between manufacture and final use, the appearance of the drug and nature of illness. Though adjudged to be reasonably safe and efficacious, common sense suggests that if one’s life depends on a drug, one must jettison the expired product because it is “better to be safe than to be sorry”.

Keywords: Drug expiration dates, drug degradation, drug safety, drug toxicity, antibiotic resistance.

Introduction

Many medications are very expensive and so people hate to waste them. To avoid a costly visit to the Doctor for a new prescription, many patients, and the public face the dilemma of whether to consume expired drugs or not. When healthcare professionals and pharmaceutical companies are asked if drugs can be used past their expiration dates, they usually will not sanction such use and may not even comment on the effectiveness or safety of using products beyond their expiring dates because of legal restrictions and liability concerns.

Drug expiring dates started in 1979 when a law passed in the United States of America (USA) required drug manufacturers to place expiring dates on most medical labels – prescription drugs, over-the-counter (OTC) drugs and dietary (herbal) supplements. The expiry date is the final day the manufacturer guarantees the full potency and safety of a medication. This date is estimated using testing under good manufacturing practices as determined by health authorities such as the Food and Drug Administration (FDA) in USA and National Food and Drug Administration and Control (NAFDAC) in Nigeria. Once the original content is opened, either by the patient or healthcare professional, that expiry date and content can no longer be relied upon. However, the actual shelf life of the drug may be much longer, as stability studies have shown.

At the pharmacy, “beyond use dates” are put on prescription bottle labels given to patients. The dates often say “Do not use after..” or “Discard after..”, typically one year from the date on the stock bottle. It is true the effectiveness of a drug decreases over time but much of the original potency remains even a decade after the expiry date. The best evidence resides on the Shelf Life Extension Programme (SLEP) undertaken...
by the FDA for the Department of Defence. The purpose of SLEP was to determine the actual shelf life of stock-piled military medications for future use and to save dollars for Government. The results suggested that many drug products may have extended shelf lives beyond their expiry dates. However, it is difficult for any patient or healthcare professional to know which product could have an extended shelf life. Again, drug lots tested in SLEP were kept in their original package and in optimum storage conditions.

In our previous communication, it was reported that in Africa and Nigeria, improper drug custody and poor storage which lead to drug degradation by heat and humidity during distribution and display, engender antibiotic resistance through the prescription of poor quality substandard drugs. Also, Ogunshe and Adinmonye1 investigated the effect of expiry dates on in vitro bacteriostatic potentials of oral paediatric antibiotics, and showed that apart from less efficacy, administration of expired antibiotics can lead to increased antibiotic resistance and treatment failure as well as adverse drug reactions.

Factors that negatively affect Drugs-Sunlight (heat), oxygen, humidity (moisture), long-term storage

These factors accelerate drug degradation. Therefore, proper storage is important to preserve medicines and to prevent them from reacting with the environment and getting degraded over time. Bathroom cupboards, car boxes and medical cabinets in kitchens are not ideal to store drugs because of humidity and heat. Some drugs like insulin must be kept in optimum temperature in the fridge.

Drug formulation: Drugs in liquid form (solutions, suspensions) are not as stable as solid dosage forms (tablets, powder, capsule). But in one report, four expired samples of atropine solution (three were up to 5 years past expiration and one was > 50 years past expiration) were all found to contain significant amounts of the original drug. Drugs prepared by addition of a solvent before dispensing or administration (suspensions of antibiotics for oral use or lyophilized drugs in vials for parenteral use) tend to be unstable.

Factors to consider in deciding to use an expired drug

Contrary to common belief, there is little scientific evidence that all expired drugs are toxic. In other words, there are very few reports of toxicity from degradation products of expired drugs. According to the American Medical Association Journal, The Medical Letter, the only report of human toxicity that may have been caused by chemical or physical degradation products is renal tubular necrosis that was associated with the use of expired tetracycline. And since then tetracycline products have been changed to eliminate the problem. However, since drugs past expiry dates is not a well-researched subject, the following points should be carefully considered before deciding to use such drugs:

- Dosage formulation: Liquids are generally more unstable, especially after a year.
- Package type: Drug should have been stored in a container-closed system.
- Storage Conditions: Medications retain their potency and efficacy longer if they were kept in unopened or tightly-closed containers and stored in dry cool places away from light.
- If the length of time between expiry date and final use is too long, then the drug may not be used.
- Appearance of medication: Drugs in solution that have become cloudy or discoloured or show signs of precipitation, particularly injectables, should not be used. Suspensions are particularly prone to freezing. Ophthalmic drugs that are cloudy or show precipitation, should be discarded. If tablets are brittle or breaking apart; tablets or capsules have loss of sheen; tablets or capsules are soft or smelling, they should not be ingested.
- In general, don’t take any medicine that appears suspicious in any way.
- Drugs that should never be used after the expiry dates, because they degrade and lose potency quickly or have narrow therapeutic index (little changes in the pharmacological activity of these drugs can result in severe consequences for patients), include anticonvulsants such as phenytoin and Phenobarbital, warfarin, theophylline, digoxin, procainamide, paraldehyde, nitroglycerin, oral contraceptives, epinephrine, insulin, thyroid preparations, eye drops and long-
expired antibiotics because they can contribute to increased antibiotic resistance.5-19.

Conclusion

Many uncertainty surrounds the use of expired drugs. Reports of toxicity from degradation products of expired drugs are scarce. In dire need where no suitable alternative is available, some outdated drugs may be found effective. How much potency and efficacy they may retain depends on the drug, formulation, the lot, preservatives (if any), the packaging and storage conditions.

Since it is not easy to ascertain which expired drug is viable, wisdom suggests that in severe illness when life does depend on the drug, the expired drug should be jettisoned. But if life does not depend on the drug such as in the case of minor ailments like common cold, constipation, headache, etc. The expired medication can be used after consideration of the drug extenuating factors. Some drugs, because of easy degradation and loss of potency or narrow therapeutic index, should never be used after their expiry dates.

In developing countries in the tropics such as Nigeria where drugs may be adulterated from the source and storage facilities are far from ideal in a drug-unfriendly climate, the use of expired drugs can be less effective and risky, particularly the use of sub potent antibiotics that can fail to treat infections and lead to complications and antibiotic resistance. Education of the public, the patients and healthcare providers about the nature and risks of expired drugs is imperative.

References:

Head and Neck masses in children at a Teaching Hospital in Nigeria, a review of 148 cases

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Abstract

Introduction: Head and neck masses in children are a common cause of significant worry for parents. These masses vary in size and occur at any part of the head and neck. Most of them are benign. Aim: To evaluate the common causes of head and neck masses in our centre. Patients and methods: This was a retrospective study of 148 cases of children with head and neck masses. Results: Cystic hygroma and thyroglossal duct cyst were the commonest causes accounting for 13.5% each. Six percent of the cases were malignant excised lymphomas. Sixty-six percent of the cases had excision while sclera therapy and chemotherapy were used in the treatment of the others. Patients with cystic hygroma presented at a younger age. Conclusion: Head and neck masses are common in children. Most of the cases are benign. The patients with these masses present early.

Keywords: Head and Neck, Masses, Biopsy

Introduction

Head and Neck masses are common clinical concerns in infants, teenagers and adolescents. The causes of head and neck masses in children include a variety of inflammatory, congenital, and malignant conditions. These masses could be cystic or solid and they require systematic evaluation and management. A biopsy of the masses may be required to obtain a final diagnosis. Majority of head and neck masses in children are benign. Although many of the malignant masses in children are found in the head and neck region they account for only a small portion of the neck masses.

Thyroglossal duct cyst and cystic hygroma were the most common masses seen in this study accounting for a total of 27%.

Simple surgical excision is the treatment for most of the cases as only a few may require other modalities of treatment.

Complications may include recurrence and wound infection. Mortality is generally low.

This paper reviews the common head and neck masses seen in a teaching hospital in Nigeria

Patients and Methods

This was a retrospective study of paediatric patients coming into the paediatric surgery unit of our teaching hospital from 2008 to 2012, a 5 year period. The case notes of these patients were retrieved from the records department and relevant data were extracted.

The information obtained included the sex, age, address, pattern of presentation, treatment obtained and histology results. The data obtained was subjected to simple statistical analysis and SPSS 20 version.

Result

The data obtained over the 5 year period (2008-2012) showed that 148 patients presented with masses affecting the head and neck region of the body. Multiple lymph node swellings were noticed in some
patients with lymph node swellings.

There was a total of 80 males (54%) and 68 females (48%) with a male to female ratio of 1.2:1.

Figure 1: Sex distribution

The age range at presentation was 4 days to 15 years with a mean age of 2.4 years.

Table 1: Age range and number of cases

<table>
<thead>
<tr>
<th>Age group</th>
<th>Total</th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td>NEOANATE</td>
<td>16</td>
<td>10</td>
<td>6</td>
</tr>
<tr>
<td>INFANT</td>
<td>36</td>
<td>17</td>
<td>19</td>
</tr>
<tr>
<td>2-5yrs</td>
<td>65</td>
<td>37</td>
<td>28</td>
</tr>
<tr>
<td>6-10yrs</td>
<td>21</td>
<td>11</td>
<td>10</td>
</tr>
<tr>
<td>11-15yrs</td>
<td>10</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>TOTAL</td>
<td>148</td>
<td>80</td>
<td>68</td>
</tr>
</tbody>
</table>

The spectrum of cases ranges in size from 2cm to 16cm in their widest diameter.

They were of different shapes from oval, spherical to irregular in shape. Their surfaces were smooth to rough. The masses were located on the face, scalp pre and post-auricular areas as well as the posterior and anterior triangles of the neck.

Table 2: Distribution of cases

<table>
<thead>
<tr>
<th>S/n</th>
<th>Cases</th>
<th>No of patients</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Cystic Hygroma</td>
<td>20</td>
<td>13.5</td>
</tr>
<tr>
<td>2</td>
<td>Thyroglossal Cyst</td>
<td>20</td>
<td>13.5</td>
</tr>
<tr>
<td>3</td>
<td>Lymph node swelling</td>
<td>16</td>
<td>10.8</td>
</tr>
<tr>
<td>4</td>
<td>Branchial cyst</td>
<td>12</td>
<td>8.1</td>
</tr>
<tr>
<td>5</td>
<td>Lipoma</td>
<td>13</td>
<td>8.7</td>
</tr>
<tr>
<td>6</td>
<td>Haemangioma</td>
<td>14</td>
<td>9.5</td>
</tr>
<tr>
<td>7</td>
<td>Dermoid cyst</td>
<td>19</td>
<td>12.8</td>
</tr>
<tr>
<td>8</td>
<td>Parotid mass</td>
<td>11</td>
<td>7.4</td>
</tr>
<tr>
<td>9</td>
<td>Jaw Burkitts</td>
<td>11</td>
<td>7.4</td>
</tr>
<tr>
<td>10</td>
<td>Fibroma</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>11</td>
<td>Papilloma</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>TOTAL</td>
<td></td>
<td>148</td>
<td>100</td>
</tr>
</tbody>
</table>

The patients with cystic hygroma presented as follows

Figure 2: Cystic hygromapresentation

The data obtained also showed that 52 of the masses were in the head while 92 were in the neck and the remaining 4 affected both the head and neck.

Table 3: Pattern of presentation of cystic hygroma

<table>
<thead>
<tr>
<th>M</th>
<th>F</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-7days</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>8-15days</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>15-21days</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>22-28days</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>&gt;28days</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>TOTAL</td>
<td>14</td>
<td>6</td>
</tr>
</tbody>
</table>

Figure 3: Site of swelling

Of the 92 located in the neck region 62 were in the anterior triangle, 26 in the posterior triangle, and 4 involved both the anterior and posterior triangles of the neck.

106 patients presented with painless masses while 42 had pain of varying intensities. The tenderness was thought to be from inflammatory conditions, sometimes from secondary infection of the masses resulting from non-orthodox interventions.
Excision biopsy was done for 97(65.5%) of the cases while others forms of treatment included sclerotherapy, chemotherapy, and watchful waiting for cases of haemangioma.

**Discussion**

Head and neck masses in children are a diverse group of lesions. The differential diagnosis of this condition includes a variety of congenital, inflammatory, and neoplastic causes. Most cases are benign. Malignancies are less frequently encountered with lymphomas and rhabdomyosarcoma being common neck neoplasm seen in children. An asymptomatic mass is the most common presentation of head and neck malignancies in children. Painless mass was seen in 71.6% in this study and was the commonest form of presentation.

Masses of the head and neck in children could be cystic, solid or of mixed consistencies depending on the causes.

In this study, we observed that cystic hygroma and thyroglossal duct cyst were the commonest masses seen, each accounting for 13.5%. Tsutsumi et al reported also that thyroglossal duct cyst was the commonest mass encountered in their study. Thyroglossal duct cyst is a benign condition in which there is accumulation of fluid along the thyroglossal duct. The definitive treatment is excision of the cyst with the duct.

Nineteen (12.8%) patients presented with dermoid cyst, which are benign lesions involving the layers of the skin. Six of the dermoid cysts were in the post-auricular area of the left ear, 7 on the right ear and 7 were angular dermoids.

Six (12.5%) patients had swelling of the lymph nodes of the neck. Lymph nodes swelling in children could be inflammatory or malignant. Neck masses due to inflammatory lymphadenitis are common in children because of the frequency of upper respiratory tract infection. Two (33.3%) of the lymph node swelling in this study were lymphomas and malignant, the other 4 (66.6%) were inflammatory.

The other conditions seen were haemangiomas, branchial cysts, lipoma, a lymphoma, fibroma and papillomas.

History and physical examination are the most important parts of evaluation of neck masses in children. Radiologic investigations like ultrasound scan, magnetic resonance imaging are helpful in characterizing the masses. Confirmatory diagnosis is usually by tissue biopsy where indicated. Masses in the head and neck are amenable to diagnosis by fine needle aspiration cytology. The procedure is generally well tolerated in children, but there could be significant diagnostic difficulties.

Malignant tumors of the head and neck in children are uncommon. 3(6.2%) of the patients in this study had malignant lymphomas

**Conclusion**

The results from this study show that most head and neck swellings in children are benign with few malignant cases especially lymphomas. This is in keeping with similar results from multicentre studies. More researches will however be needed in this area of study.

**References**

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Prevalence of electrolytes and azotemic indices imbalance in patients with chronic kidney disease

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Federal University of Technology, Minna, Nigeria.

Abstract

Aim: Multiple electrolytes and nitrogenous compounds are reported to be altered in patients with chronic kidney disease hence the patience requires dialysis or transplantation to stay alive. This work was aimed to determine the prevalence of post-dialysis electrolytes and nitrogenous compounds imbalance in patients with chronic kidney disease. A total of 276 subjects were recruited into two groups. A study group of 138 known chronic kidney disease patients and a control group of 138 apparently healthy persons.

Methods: Ion selective electrode was used for the analysis of electrolytes while standard methods were used to analyze calcium, phosphate, urea, creatinine, and uric acid in plasma.

Results: The result showed an increase in the mean concentration of post-dialysis sodium compared to the pre-dialysis. It revealed imbalance in the post-dialysis of electrolytes and nitrogenous compounds. Sodium had an imbalance of 69.6%, potassium imbalance of 8.7%, chloride imbalance of 4.3%, calcium imbalance of 30.4, phosphate an imbalance of 10.9% while urea had an imbalance of 37% and creatinine an imbalance of 87%. It also revealed a decrease in the mean concentration of post-dialysis potassium level compared to the pre-dialysis.

Conclusion: Our findings showed that though hemodialysis seems to restore electrolytes and nitrogenous compounds levels, a significant number of chronic kidney disease patients present with post-dialysis imbalance. Therefore, we strongly wish to recommend the need for multiple-dialysis to be done on chronic kidney disease patients and if possible instant transplantation if the fund is available.

Key words: Electrolytes, Azotemic indices, Chronic Kidney Disease, Dialysis, Imbalance.

Introduction

Chronic kidney disease (CKD) is a major public health problem associated with a significant burden of morbidity, mortality and increased health care costs. The rapid rise of common risk factors such as diabetes, hypertension and obesity especially among the poor, will result in even greater and more profound burdens that developing nations are not equipped to handle. Nugent RA et al also suggested a desperate need for public awareness and advocacy towards life style changes and disease management, increased training, and availability of medical practitioners and for expanded surveillance and screening systems for chronic diseases. Early detection of chronic diseases such as CKD, which are relatively asymptomatic, has a reasonable potential to have a positive impact on the outcome of the disease and reduce the high cost associated with renal replacement therapies. Currently, screening for CKD is accepted practice only in patients with hypertension or diabetes. Accurate assessment of kidney function also known as glomerular filtration rate (GFR), is an important part of CKD screening as it facilitates earliest diagnosis, staging, proper dosing of medications and ultimately planning for renal replacement therapy. Kande E et al also stated that by earlier referral to nephrology teams, a potential opportunity to attenuate CKD progression and manage complications is created.

There are strong evidences that apart from the measurement of GFR for the diagnosis of CKD, serum electrolytes and azotemic indices as well as serum
phosphate and even single measurements of serum uric acid could be of help for early diagnosis of CKD. Forley RN et al. reported that multiple electrolytes are altered in patients with CKD. Porth also stated that phosphates and calcium levels are altered in CKD patients. This study is therefore, aimed at the determination of electrolytes and azotemic indices in patients with CKD, to evaluate their mean concentrations in pre and post-dialysis patients with a view to determine whether there are imbalances in post-dialysis levels of these parameters.

**Materials and Methods**

The study, a cross-sectional survey was carried out at National Hospital Abuja, Jos University Teaching Hospital, and Federal Medical Centre Gombe.

**Sample Population**

This comprised of control group of apparently healthy individuals and a known case group of patients with CKD in each of the areas covered.

**Sample Size**

A total of 276 subjects were recruited which were made up of 92 subjects from each of the areas; representing 138 control of apparently healthy individuals and 138 of established known cases of CKD patients on dialysis while the 92 subjects from each of the areas were made up of 46 control group and 46 of the known CKD patients.

**Sample Collection**

Blood samples (about 5cm³) were collected from the selected subjects using a 5cm³ sterile disposable syringe and needle. Each blood sample was transferred into separate lithium heparin tubes and mixed gently. At the end of each day’s collection, the heparinized blood samples were centrifuged using Mega centrifuge 1.0 Heraus instrument of German made. The instrument was set at 5,000 rpm for 5 minutes, followed by subsequent separation of each of the plasma samples into plain tubes and labeled appropriately. Plasma samples were analyzed on the same day of collection. The pre-dialysis samples were collected before dialysis commenced and immediately after dialysis, the post-dialysis samples were taken. The control samples were collected from apparently healthy individuals and were treated as the study group.

**Sample Analysis**

Sodium, potassium and chloride were analyzed by ion selective electrode method as developed by Levy in 1981. Ion-selective electrodes make possible the potentiometric measurement of specific ions when incorporated in an electrode. When an ion-specific membrane separates two solutions that differ in concentration of that ion, a potential is developed across the membrane, the size of the potential depends on the difference in the ionic concentration. The light then, measure sodium, potassium and chloride using ion selective electrode-technology. The flow through sodium electrode contains glass tubes specially formulated to be sensitive to sodium ions, the flow through potassium electrode employs a plastic tube incorporating valinomycin as the selective element and the flow through chloride electrode include a plastic tube specially formulated to be selective to chloride.

Calcium was determined by the method of chelation with O-cresolphthelein complexone as established by Lorenz in 1932. The dye O-cresolphthelein complexone, binds calcium tightly in an alkaline solution to form a highly coloured complex whose absorbance was measured at 578nm. Inorganic phosphate was measured by the method of Fiske and Subbarow in 1925. Serum proteins are precipitated by trichloroactic acid and the phosphate is converted to a phosphomolybbdate (MOi™) complex by addition of sodium molybdate. The addition of p-Methylaminophenol reduces the MOi™ in the complex to yield an intensely blue-coloured phosphomolybdate complex (MO™) whose absorbance was measured at 700nm. Creatinine was determined by modified Jaffe-kinetic method as developed by Moss et al.in 1975. At alkaline pH, creatinine reacts with picric acid to produce a coloured compound of alkaiine picrate solution which was measured at 520nm. Urea was measured by Bertholot’s method in 1859. Urea in plasma was hydrolyzed by urease into ammonia and carbon dioxide. The ammonia generated then reacts with alkaline hypochlorite and sodium salicylate in the presence of nitroprusside to yield a blue chromophore whose absorbance was measured colorimetrically. While uric acid was analyzed by uricase method as developed by Duncan et al. in 1982. In the presence of peroxidase, hydrogen peroxide reacts oxidatively with 3,5-dichloro-2-hydroxybenzenesulfonic acid and 4-amino phenazone to form a red dye. Potassium
ferricyanide was included in the reagent to oxidize ascorbate. The colour formed was measured spectrophotometrically.

Data Analysis: All data collected were fed into the computer and analyzed using Epinfo version 3.5.1, 2008. Means, standard deviations and proportions were determined as applicable. The proportions were compared using $Z$-score while a probability value (P value) less than 0.05 was taken as statistically significant.

Results:

Table 1 shows the comparison of mean concentrations of electrolytes and azotemic indices in pre-dialysis, post-dialysis, and control group. There is increase in the mean concentration in post-dialysis sodium and decrease in the mean concentration of post-dialysis potassium when compared with pre-dialysis. The mean concentration of post-dialysis potassium is significant ($p<0.003$) when compared with control group. Also there is significant different ($p<0.0001$) in the levels of urea, creatinine and uric acid in post-dialysis compared to control group.

Also there is significant different ($p<0.0001$) in the levels of urea, creatinine and uric acid in post-dialysis compared to control group.

Table 2: Prevalence of Electrolytes and Azotemic Indices Imbalance in Patients with Chronic Kidney Disease.

<table>
<thead>
<tr>
<th>Analytes</th>
<th>Study Group</th>
<th>Control</th>
<th>Cutoff Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium</td>
<td>96 (69.6%)</td>
<td>6 (4.3%)</td>
<td>&lt; 137</td>
</tr>
<tr>
<td>Potassium</td>
<td>12 (8.7%)</td>
<td>3 (2.2%)</td>
<td>&gt; 2.8</td>
</tr>
<tr>
<td>Chloride</td>
<td>6 (4.3%)</td>
<td>3 (2.2%)</td>
<td>&lt; 94</td>
</tr>
<tr>
<td>Calcium</td>
<td>42 (30.4%)</td>
<td>3 (2.2%)</td>
<td>&gt; 1.7</td>
</tr>
<tr>
<td>Phosphate</td>
<td>15 (10.9%)</td>
<td>0 (0%)</td>
<td>&gt; 0.6</td>
</tr>
<tr>
<td>Urea</td>
<td>51 (37%)</td>
<td>3 (2.2%)</td>
<td>&gt; 8</td>
</tr>
<tr>
<td>Creatinine</td>
<td>120 (87%)</td>
<td>6 (4.3%)</td>
<td>&gt; 102</td>
</tr>
<tr>
<td>Uric Acid</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>&gt; 8</td>
</tr>
</tbody>
</table>

Discussion

CKD is a well-recognized global epidemic with consequences on patient’s morbidity, mortality, and health care resources. Comparative evaluation of pre and post-dialysis study showed that the mean concentration of post-dialysis sodium was higher than in pre-dialysis, the same goes for calcium and chloride while the post-dialysis mean concentrations of potassium and phosphate were reduced when compared to the pre-dialysis mean concentration. These observations agreed with the work of Stein IH et al$^{25}$ and Michael IB et al$^{26}$ who in 2006 and 2005 explained that in CKD, due to the failure of the renal tubes, sodium absorption is hindered causing much of it to be excreted in urine but the reverse is the case in post-dialysis. The same phenomenon also applied for chloride. Also the significant difference ($p<0.0001$) observed in potassium levels in post-dialysis can be explained by sodium-potassium ATPase ion pump. Hence as the post-dialysis sodium was increasing, its potassium level was decreasing, to maintain homeostasis (26). The variations equally recorded in the mean concentrations of calcium and phosphate in post-dialysis were consistent with the
findings of Metheny and Porth who in 2002 both stated that serum phosphate and calcium levels are altered in reciprocal manner in CKD. In the comparison done for pre and post-dialysis mean concentrations of uraemia, creatinine and uric acid, the statistical difference (p < 0.0001) observed is in line with the findings of Sharon and Judith who in 2006 recorded that in CKD, the glomerular filtration is drastically reduced and so these nitrogenous compounds accumulate. The comparisons of mean concentrations of electrolytes and azotemic indices of post-dialysis and control group which showed no statistical difference in the levels of sodium, chloride, calcium and phosphate reestablished the fact that dialysis on CKD patients really returned the electrolytes to normal. However, the fact that the mean concentrations of nitrogenous compounds showed significant differences (p<0.0001) (i.e. urea, creatinine and uric acid) implies that these nitrogenous compounds do not return to normal after dialysis. The prevalence of imbalances in the studied population, which showed 96 of patients (69.6%) with sodium imbalance, 12 patients (8.7%) for potassium, 42 patients (30.4%) for calcium, 15 patients (10.9%) for phosphate, 51 patients (37%) for urea and 120 patients (87%) for creatinine revealed the fact that a significant number of CKD patients present with post-dialysis imbalance meaning that the levels of electrolytes and nitrogenous compounds did not fully or really returned to normal after single dialysis. More so, the fact that some degree of imbalance was recorded in the control population for sodium-6(4.3%), potassium-3 (2.2%), calcium-3 (2.2%), urea-3 (2.2%) and creatinine-6 (4.3%) also revealed that not all the control samples were normal.

Conclusion

Increasing evident showed that some of the major adverse outcomes of CKD can be prevented or delayed by early detection and management. Hence the need to routinely determine the electrolytes and nitrogenous compounds in individuals. Also, individuals should subject themselves to these kidney function tests quarterly a year. Equally, from our findings, though dialysis seems to restore electrolytes in CKD patients, however, in view of the imbalances observed in the post-dialysis levels of electrolytes and azotemic indices, we wish to strongly suggest that multiple-dialysis should be made a common approach in the management of CKD patients to enhance proper homeostasis.

References:

Protective activities of *Nigella sativa* oil instreptozotocin-induced diabetic prefrontal cortex of Wistar rats

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**Abstract**

**Background:** There is increasing evidence that cognitive impairments are associated with poorly-managed diabetes mellitus, with supporting structural and molecular data emerging. *Nigella sativa* has been reported to possess potent antioxidant, hepatoprotective, antiparasitic, anticancer, antimicrobial, analgesic, and anti-inflammatory effects.

**Objectives:** We investigated the effects of *Nigella sativa* (black seed) oil on the morphology of the prefrontal cortex (PFC) and the blood glucose levels of streptozotocin (STZ)-induced diabetes in rats.

**Methods:** Thirty adult Wistar rats (weighing 160-200 g) were used. Rats were divided at random into five groups (control (buffered saline); untreated STZ-diabetic (70 mg/kg B.W., IP); treated STZ-diabetic with *Nigella Sativa* oil (NGS) (1 ml/kg B.W, oral); treated STZ-diabetic with NGS (2 ml/kg B.W, oral); and NGS only (1 ml/kg B.W, oral)). On the 28th day of treatment, rats were fasted overnight, anesthetized with ketamine, and sacrificed. Brain tissues were fixed with 4% paraformaldehyde. Blood glucose levels were assessed and paraffinized sections of the PFC were stained with the haematoxylin and eosin, cresyl fast violet and luxol fast blue staining methods.

**Results:** General body weight of rats was reduced and blood glucose level was significantly increased in the diabetic group compared with the control and NGS treated groups (P< 0.05). Histological examination showed comparatively small pyramidal cells, with shrunken nuclei and reduced population in the PFC of rats in the diabetic group, compared to animals in control and NGS treated groups. Diabetic rat’s PFC neurons are also characterized by loss of axonal myelin sheath, poor Nissl demonstration and nuclear DNA morphology in the soma. However, relatively normal appearance of these proteins in the PFC of control rats and NGS treated groups was observed.

**Conclusion:** Our study showed that untreated diabetes mellitus is associated with cellular and molecular degeneration in the PFC of Wistar rats, and that 2ml/kg B.W of NGS conferred neuroprotective properties against neuronal degeneration in streptozotocin-induced diabetes in rats.

**Keywords:** Streptozotocin, Diabetes mellitus, *Nigella sativa*, Oxidative stress, Prefrontal cortex.

**Introduction**

*Diabetes Mellitus* (DM) is one of the most important and prevalent chronic diseases. It currently affects 250 million people worldwide, with 6 million new cases reported each year. This prevalence rises with age from 12% in people aged 65 to 70 to 15% in people over age 80. DM is a systemic disease that can damage any organ in the body. Complications include pathologic changes involving both small and large vessels, cranial and peripheral nerves, skin, and eyes and these may lead to hypertension, renal failure, vision loss, autonomic and peripheral neuropathy, peripheral vascular disease, myocardial infarction, and cerebrovascular disease, including stroke.

In recent years, significant interest has been demonstrated in the effect of diabetes on the brain. Along with cerebrovascular disease, diabetes is implicated in the development of other neurological comorbidities. Less addressed and not as well recognized complications of DM are cognitive dysfunction and dementia. Like diabetes, cognitive dysfunction represents another serious problem and is rising in prevalence worldwide, especially among the elderly. DM has been implicated as risk factor for dementia not only of vascular type but also Alzheimer’s disease (AD). Patients with type 1 *Diabetes Mellitus* (T1DM)
and type 2 Diabetes Mellitus (T2DM) have been found to present cognitive deficits, associated with reduced performance on multiple domains of cognitive function. Many studies suggest that the risk of cognitive decline and neurodegeneration is increased not only in DM, but also in patients with pre-diabetes and Metabolic Syndrome.

Several factors are involved in the pathophysiology of cognitive decline in diabetes. Some of these factors are however conjectural. Factors such as hyperglycaemia, hypoglycaemia and vascular disease, amyloidosis and insulin resistance were implicated. In addition, oxidative stress (characterized by increased superoxide anion formation in the presence of chronic hyperglycaemia) is involved in the pathogenesis of diabetic cognitive impairment.

The prefrontal cortex is the anterior part of the frontal lobes of the brain, lying in front of the motor and premotor areas. This brain region has been implicated in planning complex cognitive behaviour, personality expression, decision making, and moderating social behaviour. The basic activity of the prefrontal cortex is considered to be orchestration of thoughts and actions in accordance with internal goal that is cognitive function.

The most typical psychological term for the functions of the prefrontal cortex area is executive functions which relates to abilities to relate conflicting thoughts, determine good and bad, same, and different, future consequences of current activities, working towards a defined goal, prediction of outcomes, expectation based on outcomes and social control.

Streptozotocin (STZ), a glucosamine–nitrosourea compound derived from Streptomyces achromogenes that is used clinically as a chemotherapeutic agent in the treatment of pancreatic β cell carcinoma and to induce experimental diabetes. Streptozotocin (STZ) is used to induce diabetes in experimental animals through its toxic effects on pancreatic β-cells and as a potential inducer of oxidative stress. STZ can induce diabetic state in 2 ways, depending on the dose. It is used in medical research to produce animal models for Type 1 diabetes with a high single dose as well as Type 2 diabetes with multiple low doses. The selectivity for β cells is associated with preferential accumulation of the chemical in β cells after entry through the GLUT2 glucose transporter receptor: chemical structural similarity with glucose allows STZ to bind to this receptor.

Nigella sativa, commonly known as black seed, belongs to the botanical family Ranunculaceae. It has been used in countries bordering the Mediterranean Sea, Pakistan, India and Iran, as a natural remedy for over 2000 years. Nigella sativa oil contains an abundance of conjugated linoleic (18:2) acid, thymoquinone, niggelone (dithymoquinone), melanthin, nigilline, damascenine, and tannins. Black seed components display a remarkable array of biochemical, immunological, and pharmacological actions, including bronchodilatory, anti-inflammatory, antibacterial, hypoglycaemic and immunopotentiating effects. N. sativa extract has been shown to possess immunopotentiating, anti-oxidant, anti-tumoral, and anti-diabetic properties. The oil of N. sativa exhibits analgesic, anti-diabetic and anti-inflammatory effects in rats. Most of these properties have been attributed mainly to the quinone constituents of N. sativa, of which thymoquinone is the main active ingredient of the volatile oil isolated from the black seed. Many studies have also examined the anti-diabetic effect of N. sativa in diabetic animal models.

However, the neuroprotective potentials of NGS against prefrontal cortex neuronal degeneration resulting from streptozotocin-induced diabetes are yet to be elucidated. In this connection, our study was designed to investigate the anti-diabetic effect of Nigella sativa (black seed) oil on streptozotocin-induced diabetic prefrontal cortical deficit in adult Wistar rats.

Materials and Methods

This research work was conducted at the Animal holding of the Department of Anatomy, Faculty of Basic Medical Sciences, College of Health Sciences, University of Ilorin, in the year 2014.

Procurement of NGS Oil

The Nigella sativa oil was purchased from a local Islamic store in Adewole area of Ilorin, Kwara State.

Experimental Animals

Thirty (30) normoglycemic adult Wistar rats (males and females), having fasting blood glucose levels of 70-80 mg/dl and weighing between 160g-200g with no observable physical abnormalities were used for this study. The rats were purchased from an animal holding.
in Ogbomoso, Oyo State. Rats were kept in mesh cages at controlled room temperature, photoperiodicity (12/12-hr light/dark cycle) and proper ventilation. Animals were fed with standard rat diet and water made available ad libitum. All experiments were conducted following the guidelines on the care and use of laboratory animals in research and teaching by the ethical review committee of the College of Health Sciences, University of Ilorin.

**Induction of Experimental Diabetes**

Diabetes mellitus was induced in the rats by single intraperitoneal (IP) injection of freshly prepared STZ at dose of 70 mg/kg body weight dissolved in 0.01 M citrate buffer at pH 4.5. 0.01 M citrate buffer was prepared by dissolving 2.1g of citric acid and 2.94g of sodium citrate in 100ml of distilled water. The pH was adjusted to 4.5 by the addition of concentrated NaCl solution using a calibrated pH meter.

On-call plus glucometer and compatible glucometer test strips were used for the determination of blood glucose levels in over-night fasted rats seven (7) days after injection of STZ. Blood samples were obtained from the dorsal tail vein of conscious rats. Animals with fasting blood glucose FBG level of greater than 250 mg/dl were selected for the diabetic groups. Hyperglycemia was allowed to stabilize for 5 days before the commencement of treatment.

**Animal Grouping and Treatments**

The animals were grouped at random into five (5) groups (A, B, C, D and E) of six (6) animals each. Treatments involved oral administration of the volatile oil of *Nigella sativa*, the oil was administered to the animals through a metallic orogastric cannula daily for 28 days. The various groupings of rats and the correspondent treatments are listed in Table 1 below.

Thereafter, the fasting blood glucose (FBG) level of all animals in each experimental group was measured 24 hours after days fourteen (14) and twenty eight (28) of treatment. Body weight (BW) of rats was also measured weekly.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Animal</th>
<th>Administered doses</th>
<th>Route</th>
<th>Period</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>6</td>
<td>Untreated diabetic-Streptozotocin 70mg/kg b.w</td>
<td>Intra peritoneal</td>
<td>Single dose</td>
</tr>
<tr>
<td>B</td>
<td>6</td>
<td>Treated diabetic-streptozotocin 70mg/kg followed by 1ml/kg b.w <em>Nigella sativa</em> oil</td>
<td>Oral</td>
<td>28 days</td>
</tr>
<tr>
<td>C</td>
<td>6</td>
<td>Treated diabetic-streptozotocin 70mg/kg followed by 2ml/kg b.w <em>Nigella sativa</em> oil</td>
<td>Oral</td>
<td>28 days</td>
</tr>
<tr>
<td>D</td>
<td>6</td>
<td>Non-diabetic-1ml/kgb.w<em>Nigella sativa</em>oil</td>
<td>Oral</td>
<td>28 days</td>
</tr>
<tr>
<td>E</td>
<td>6</td>
<td>Normal control-70mg/kgb.w citrate buffer</td>
<td>Intra peritoneal</td>
<td>Single dose</td>
</tr>
</tbody>
</table>

**Histological studies**

Rats were weighed, fasting blood glucose levels were determined and they were euthanised with ketamine 24 hours after the last administration. Subsequently, the rats were transcardially perfused with 4% paraformaldehyde. The animals were decapitated, the brains removed and the frontal cortices excised and separated. The PFC were then carefully excised and fixed in 4% paraformaldehyde for routine paraffin wax embedding. Sections of wax-embedded PFC were then taken with a rotary microtome and processed for Haematoxylin and Eosin (H&E), Cresyl fast violet (CFV), Luxol fast blue and bielschowsky staining techniques as described by Bancroft and Stevens and Pearse.

**Statistical Analysis**

Data among the groups with different concentrations of the treatment agents was analyzed using Microsoft Excel and SPSS V20 by one-way analysis of variance (ANOVA) followed by "Tukey’s Multiple Comparison Test". Data were presented as means ± SEM (standard error of mean), P value less than 0.05 (p<0.05) was considered statistically significant.
Results

Effect of NGS Oil on Body Weight

The effect of *Nigella sativa* oil NGS on body weight in STZ- induced diabetes rats are summarized in Table 2. There was a progressive increase in body weight in the control while a progressive decrease in body weight was observed in the STZ- diabetic group throughout the duration of the experiment, whereas the body weight in the STZ+NGS groups showed a progressive decrease and then an increase. Administration of 2ml/kg b.w. NGS attenuated the weight loss induced by STZ injection in rats. The body weight changes of the diabetic group are 173.20 ± 8.66 to 163.20 ± 8.96 at the beginning and at the end of the experiment. The STZ+2ml/kg b.w NGS group showed an initial decrease in weight from 194.40 ± 4.20 at the beginning of the experiment to 188.00 ± 4.34 after two weeks of NGS treatment to 201.60 ± 4.87 at the end of the experiment.

Table 2: Effect of *Nigella sativa* oil on body weight of experimental animals

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment Groups</th>
<th>Body weight week 1*</th>
<th>Body weight week 3#</th>
<th>Body weight week 5a</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>STZ</td>
<td>173.20 ± 8.66</td>
<td>168.80 ± 8.87</td>
<td>163.20 ± 8.96</td>
</tr>
<tr>
<td>B</td>
<td>STZ+1ml/kg b.w NGs</td>
<td>162.80 ± 4.32</td>
<td>160.40 ± 4.17</td>
<td>162.40 ± 4.87</td>
</tr>
<tr>
<td>C</td>
<td>STZ+2ml/kg b.w NGs</td>
<td>188.00 ± 4.34</td>
<td>194.80 ± 6.47</td>
<td>201.60 ± 8.75</td>
</tr>
<tr>
<td>D</td>
<td>1ml/kg b.w NGs</td>
<td>168.80 ± 8.82</td>
<td>169.20 ± 7.74</td>
<td>169.20 ± 7.20</td>
</tr>
<tr>
<td>E</td>
<td>Control</td>
<td>178.40 ± 3.97</td>
<td>181.20 ± 4.76</td>
<td>213.60 ± 2.93</td>
</tr>
</tbody>
</table>

Values are mean ±SEM; n=5 in each group.
*: Mean body weight before commencement of NGS treatment
#: Mean body weight 14 days after commencement of NGS treatment
a: Mean body weight 28 days after commencement of NGS treatment

Effect of NGS Oil on Blood Glucose

The effect of *Nigella sativa* oil on blood glucose levels in rats with STZ-induced diabetes is summarized in Table 3. Table 3 shows the change in blood glucose levels of control, diabetic and treated rats during the experimental period. After STZ and STZ+NGS treatment, the blood glucose levels of the STZ-diabetic and STZ+1ml/kg b.w. NGS groups increased significantly but reduced in the 2ml/kg b.w. NGS group. The means ± SEM of blood glucose concentrations after STZ injection and before administration of NGS (in treatment groups) were 405.00 ± 49.02, 308.88 ± 8.99, 310.32 ± 39.17, 82.44 ± 4.60, 59.76 ± 2.57 mg/dl in groups A- E. After 14 days of commencement of NGS treatment, the means ± SEM of blood glucose concentration for groups A to E were 485.28 ± 39.23, 264.24 ± 16.77, 240.84 ± 38.48, 65.52 ± 3.49, 70.20 ± 2.54.

The effect of STZ 14 days after injection was a gradually increasing blood glucose level, from 485.28 ± 39.23, to 519.84 ± 50.87, 28 days after injection. The mean ± SEM of blood glucose concentration level in STZ+1ml/kg b.w NGS group decreased from 308.88 ± 8.99 to 264.24 ± 16.77 mg/dl 14 days after NGS treatment and then increased to 542.52 ± 10.48 mg/dl 28 days after NGS treatment. The mean ± SEM of blood glucose concentration level in STZ+2ml/kg b.w NGS group reduced from 310.32 ± 39.17 to 240.84 ± 38.48 mg/dl 14 days after NGS treatment and further reduced to 183.24 ± 30.28 mg/dl 28 days after NGS treatment. These results suggest that 2ml/kg b.w. NGS treatment is an effective dose for assessing anti-hyperglycemic potential of *Nigella sativa* oil in diabetic rats.

Table 3: Effect of *Nigella sativa* oil on blood glucose of experimental animals

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment groups</th>
<th>Blood glucose week 1*</th>
<th>Blood glucose week 3#</th>
<th>Blood glucose week 5a</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>STZ</td>
<td>405.00 ± 49.02</td>
<td>485.28 ± 39.23</td>
<td>519.84 ± 50.87</td>
</tr>
<tr>
<td>B</td>
<td>STZ+1ml/kg b.w NGs</td>
<td>308.88 ± 38.99</td>
<td>264.24 ± 16.77</td>
<td>542.52 ± 10.48</td>
</tr>
<tr>
<td>C</td>
<td>STZ+2ml/kg b.w NGs</td>
<td>310.32 ± 39.17</td>
<td>240.84 ± 38.48</td>
<td>183.24 ± 30.38</td>
</tr>
<tr>
<td>D</td>
<td>1ml/kg b.w NGs</td>
<td>82.44 ± 4.60</td>
<td>65.52 ± 3.49</td>
<td>81.72 ± 5.01</td>
</tr>
<tr>
<td>E</td>
<td>Control</td>
<td>59.76 ± 2.57</td>
<td>70.20 ± 2.54</td>
<td>98.64 ± 5.98</td>
</tr>
</tbody>
</table>

Values are mean ±SEM; n=5 in each group.
*: Mean blood glucose level before commencement of NGS treatment
#: Mean body weight 14 days after commencement of NGS treatment
a: Mean body weight 28 days after commencement of NGS treatment

Effect of NGS Oil on Degenerative Changes in Prefrontal Cortical Histology

Histological study through the PFC of rats revealed neurodegeneration characterized by enlarged/swollen neurons, vacuolation of neuronal cell bodies, presence of apoptotic cells, nissl substance deficit, degeneration of myelin sheath, presence of neurotic plaques and neurofibrillary tangles in the diabetic rats while the control rats revealed normal histological architecture.
The untreated diabetic group had reduced number of lightly stained neuronal cell bodies, and degenerated neurons with numerous neuronal vacuolation and apoptotic cells. The STZ + 2ml/kgBW NGS group showed numerous deeply stained neuronal cell bodies indicative of an ameliorative effect of NGS treatment. Light microscopy evaluation using Haematoxylin and Eosin stains of the prefrontal cortex of control rats (Group E) showed normal PFC structure (Figure 1E).

In contrast, sections of the PFC from untreated diabetic rats (Group A) evinced pyramidal cells with comparatively small, shrunken and fewer pyramidal cells, which were degenerated (hydropic degeneration) and necrotized (Figure 1A). NGS treatment at a dose of 2 ml/kgBW protected the great deal of the pyramidal cells of the prefrontal cortex. Also, light hydropic degeneration and necrosis were seen in the 1ml/kgBW pyramidal cells (Figure 1B). NGS treatment at dose of 1 ml/kgBW did not show significant difference compared to control rats (Figure 1D).

**Distribution of Nissil Bodies and Myelin Morphology in Prefrontal Cortical Degeneration**

Nissl staining outcomes from cresyl fast violet staining were similar in all the groups except the untreated diabetic group. In the latter, pyramidal cells showed weak affinity for Nissl stain, and the prefrontal cortex showed generalized poor Nissl-staining characteristic when compared to the control. NGS treatment at dose of 2 ml/kgBW partially recovered the Nissl substance content of the PFC of rats in that group (Figure 2C). Treatment with NGS at dose of 1 ml/kg b.w. did not show remarkable change in Nissl substance content of the prefrontal cortex (Figure 2B) compared to diabetic animals.

Similarly, Luxol Fast Blue LFB technique showed poorly stained myelinated axons in the prefrontal cortex of non-treated diabetic rats (Figure 3A). Axonal integrity was however preserved in the non-diabetic control and normoglycaemic NGS-treated diabetic rats (Figure 3C &D).

**Figure 1 (A-E):** Photomicrograph of prefrontal cortex showing general architecture (H&E 400).

**Figure 2 (A-E):** Photomicrograph of prefrontal cortex showing nissl substances (CFV×400)
for nissl stain and well preserved structure of the prefrontal cortex.

![Image of prefrontal cortex showing myelin sheath and neuronal bodies](Figure 3 A-E: Photomicrograph of prefrontal cortex showing Myelin sheath (LFB×400).)

PC- Pyramidal cells, NS- Nissl substances, V- Vacuolation, AC- Apoptotic cell, MS- Myelin Sheath. The prefrontal cortex of diabetic animals (A) shows severe distortion of myelin sheath and poorly stained myelinated axons. Group B animals also showed distortion of myelin sheath. Groups C, D, and E showed preserved axonal integrity and presence of deeply stained myelin sheath in the normal control group.

**Neuronal and Axonal Morphology in Diabetic Neuropathy and NGS Therapy**

Bielschowsky silver stain is a marker for axons and neurons and Bielschowsky silver (Figure 4A) expression were significantly decreased in PFC sections of the untreated diabetic rats. The Bielschowsky stain technique showed numerous neuritic plaques and neurofibrillary tangles of axons in the PFC of diabetic rats. It also shows neuronal damage which was significantly increased in the diabetic rats compared to control and this damaged was reduced in the 2ml/kgBW NGS treatment group.

![Image of prefrontal cortex showing neuronal bodies and projections](Figure 4 A-E: Photomicrograph of prefrontal cortex showing Neuronal bodies and projections (BIEL×100).)

Numerous neuritic plaques, neurofibrillar tangles and neuronal vacuolations are seen in the prefrontal cortex of the diabetic group and group B animals. The prefrontal cortex of groups C, D and E shows normal neuronal organization and cellular structure of neurons and their projections.

**Discussion**

Medicinal plants have been a major source of therapeutic agents since ancient times to cure human diseases. Despite the major advances in modern medicine, the development of new drugs from natural products is still considered important. Since 1980, the World Health Organization has been encouraging countries to identify and exploit traditional medicine and phytotherapy. Diabetes mellitus is a chronic, systemic, metabolic disease defined by hyperglycemia and characterized by alterations in the metabolism of carbohydrate, protein, and lipid. These insidious diseases have been linked with many neurodegenerative disorders. Several studies report appreciable hypoglycaemic effects of NGS and its active ingredients. The hypoglycaemic effect of NGSOil has been suggested to be due to its ability to decrease hepatic gluconeogenesis, preserve pancreatic β-cell integrity, induce lipid peroxidation and increase antioxidant defence system activity.
In this study, a significant weight loss was observed in the diabetic group while 2ml/kgBW NGS - treated rats exhibited increase in body weight in comparison to diabetic group but was lower than in the normal controls. This effect on body weight was not observed at lower doses of NGS treatment. This finding is in agreement with Kanter et al.\textsuperscript{29} that reported that NGS markedly improved body weight gain in STZ-induced diabetes in rats. A possible explanation for this might be that NGS reduces hyperglycemia, and therefore protein wasting due to inaccessibility of carbohydrate does not occur.\textsuperscript{29}

Severe hypoglycemia reproducibly causes brain damage in animals. In the setting of other causes of brain damage (i.e., ischemia, stroke, traumatic brain injury), it has been observed that the presence of diabetes and hyperglycemia leads to more extensive neuronal damage \cite{31}. In the present study, the NGS volatile oil at dose of 2ml/kgBW revealed a significant hypoglycemic effect in STZ-induced diabetes in rats by diminishing the fasting blood glucose levels. The fasting blood glucose lowering effect of that dose was further increased after 28 days of treatment. In addition, results showed that the anti-hypoglycemic effect of NGS oil is time-and dose-dependent. This finding agrees with Fararh et al.\textsuperscript{32}

In the present study, the lowering effects of black seed oil on blood glucose levels were correspondent with the previous trials. Some studies have been conducted on the characterization of bioactives and mechanisms mediating its anti-hyperglycemic action. Alsaif\textsuperscript{33} reported that the blood glucose lowering effect of black seed oil was due to improved insulin insensitivity in diabetic rats. Another study proposed that its hypoglycemic effect is due to improved extra-pancreatic actions of insulin rather than bystimulated insulin increase \cite{34}. Furthermore, Abdelmeguid et al.\textsuperscript{35} reported that the anti-hyperglycemic effect of black seed oil and its active component thymoquinone could be due to reduction of oxidative stress, thus preserving pancreatic β-cell integrity lead to insulin levels increase. Also, the black seed oil contains many bioactive constituents such as thymoquinone, pcyrene, dithymoquinone and thymohydroquinone\textsuperscript{36}.

Morphologic studies of the brain in diabetic human and animals showed structural impairment of both the white and grey matter. In a study by Novak et al.\textsuperscript{37}, in human subjects, the diabetic brain showed atrophic changes in the grey matter of the frontal, temporal and parietal lobes. This agrees with the findings of Manschot et al.\textsuperscript{38} which showed brain atrophy in diabetic subjects using magnetic resonance imaging (MRI) technique. In this study, sections of the prefrontal cortex were prepared from control, untreated diabetic and treated diabetic Wistar rats and the effects of STZ-induced diabetes on the prefrontal cortex were histologically examined in order to describe any observed changes.

A variety of histological changes were observed in the PFC of diabetic rats when compared with the control (Figures 1-4). Findings from this study have shown that untreated diabetes in rats resulted in neuronal vacuolation and necrosis of PFC neurons, leading to apoptosis (Figures 1A). Since the PFC plays key roles in executive functions, memory, attention, thought, language, and consciousness, then the neurodegeneration observed in the histological demonstration of the PFC of diabetic Wistar rats could impair such functions.

Histologic study of the brain of the untreated diabetic rats showed structural impairment characterized by neuronal degeneration, vascular complications, loss of axonal myelin sheath and poor Nissl staining outcome and this finding suggests loss of Nissl substance and nuclear DNA in the soma of the diabetic brain. Although neuro-behavioural and cognitive tests were not conducted in this study, the observed morphologic impairment could provoke cognitive and other behavioural deficits of the brain in the untreated diabetic rats. In a study by Serbedzhia et al.\textsuperscript{39} DNA loss in brain neurons was reported in streptozotocin-induced diabetes in rats and this corroborates poorly-stained nuclei of prefrontal cortical neurons shown by the CFV staining technique in this study (Figure 2A).

Furthermore, the effect of unmyelinated axons in the morphologic impairment induced by untreated diabetes is observed in the present study. Loss of myelin sheath of axons was demonstrated by the luxol fast blue (LFB) staining technique (Figure 3A). Observations from the longitudinal sections of the PFC in this study showed that staining of nerve fibers was reduced in diabetic rats as compared with the control and NGS-treated rats (Figures 3A,3C and 3E). In this study, axonal atrophy, and myelin vacuolation is evident in the axonal morphology of diabetic rats. Furthermore,
disruption, vacuolation, and shrinkage of myelin are evident in diabetic rats and this is corroborated by Xiaohonget al.40. Bielschowsky silver stain (Figure 4A) expression in diabetic rats showed significantly increased neuritic plaque and neurofibrillar tangles. This is in line with the findings of Perl41, which reported that histologic features of Alzheimer’s diseases and other related disorders like diabetes include neurofibrillary tangles, amyloid angiopathy, and granulovacular degeneration.

Conclusion

Data from this study showed that untreated diabetes mellitus induces neuronal cell loss. Poor glycaemic control in diabetes is strongly associated with an increase in neurotoxicity and consequent neuro-degeneration. Uncontrolled glucose metabolism may also be a cause of alterations in neuronal morphology and functions. Furthermore, results from this study indicate the neuro-protective potentials of NGS oil (2ml/kgBW). Specifically, NGS oil has therapeutic benefits on cortical deficits of neuronal bodies, nissl substances, axonal projections, and myelin sheath. We recommend further exploration of NGS as a therapeutic source in the treatment and management of diabetes mellitus and neurodegeneration.

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Abstract

Safe drinking water is still inaccessible for populations in the developing countries and about half of this population suffer from one or more of the diseases associated with safe water supply. In Africa, the most common and deadly pollutants in drinking water are biological contaminants caused by microbial and viral pathogens as well as chemical contaminants which result from industrial wastes and their use for agriculture. The World Health Organization (WHO) has been in the forefront of promoting the use of risk assessment coupled with risk management for the control of safe water. Risk assessment involves hazard identification, exposure assessment, dose-response analysis and risk characterization and, these can only be achieved with the availability of relevant data. Unfortunately, however, there are little or no data in this respect to work with in Africa. The WHO has highlighted the use of quantitative risk assessment as a valuable and effective tool for setting health-based targets and for validation of water safety plans. This paper reviews the various contaminants that are common in drinking water and water disinfection processes that may be utilized to achieve the desired quality of drinking water. It also summarizes the risk assessment process of drinking water.

Introduction

The need for clean and safe drinking water supply for the public has been identified as far back as over 2000 years, when the early Romans identified that human activities and waste matter were a major source of water pollution. Thus, the maintenance of drinking water quality has been a major quest throughout the development of modern civilization. In 1854, while analyzing a cholera epidemic in London John Snow showed that specific diseases were associated with drinking water that looked and tasted clean. He observed that many of the people who died of cholera in the summer epidemic had a common factor: they all obtained their drinking water through the Broad street well. He had the pump handle removed and the epidemic faded away. Two decades later, Louis Pasteur discovered in the 1870s that bacteria were major causes of disease, that could be distributed in drinking water, and that removing the bacteria would protect population from important diseases such as cholera. In the late nineteenth century, filtration of drinking water using sand was introduced to clarify the water and this decreased bacterial contamination. This step decreased the incidence of cholera, but it soon became obvious that this was not adequate. The incidence of water borne diseases such as cholera and typhoid was found to correlate with the source of the drinking water supply. In major cities, even after filtration was introduced, removal of bacteria by chemicals began to be evaluated. In Africa, about half the population suffers from one or more of the six main diseases associated with poor water supply and sanitation. These include diarrhea, caused by a number of microbial and viral pathogens in food and water; trachomatis leading to blindness, Ascaris, an infection of the small intestine caused by Ascaris lumbricoides, a large roundworm Dracunculisis, (more commonly known as guinea-worm disease) is a crippling parasitic disease caused by Dracunculus medinensis, a long thread-like worm. Schistosomiasis, an acute and chronic parasitic disease caused by blood flukes infestation, leading to disability, morbidity and sometimes death. In addition,
the primary cause of stunted growth in millions of children in the Africa is poor nutrition, and this due in part to frequent bouts of diarrhea. The latter inhibits the ability of the body to absorb nutrition for a much longer period than the duration of the actual diarrheal episodes. Thus, children who survive the risk of dying from diarrheal diseases are at risk of stunted growth which arises from malnutrition1.

A very large group of chemicals resulting from industrial waste and wastes that are dumped by the developed countries on Africa water coast can find their way into drinking water through surface water sources. It may be difficult to determine which chemicals are of most concern. In general, the chemicals of concern are those that are found in concentrations above or close to acceptable concentrations in drinking-water. Consequently, the water sector has begun moving towards the use of risk assessment coupled with risk management as a more effective tool for the control of water safety5,6.

In Africa, particularly in Nigeria and most part of East Africa, treatment of drinking water with chemicals started in the mid-1980s7. In 2005, the National Council on Water Resources (NCWR) in Nigeria recognized the need to urgently establish acceptable Nigerian Standard for Drinking Water Quality. This was because it was recognized that the “Nigerian Industrial Standard for Potable Water” developed by the Standards Organization of Nigeria and the “National Guidelines and Standards for Water Quality in Nigeria” developed by Federal Ministry of Environment did not receive a wide acceptance by all stakeholders in the country. This could have been because, in Nigeria, responsibility for water supply is shared between three levels of government federal, state and local. The federal government oversees water resources management; state governments have the primary responsibility for urban water supply; and local governments together with communities are responsible for rural water supply.

Portable drinking water is defined as one having acceptable quality in terms of its physical, chemical, and bacteriological parameters so that it can be safely used for drinking and cooking. Drinking-water standards are country-specific and are generally based on WHO Guidelines for Drinking-water Quality4. WHO has recommended maximum acceptable values for a number of contaminants in drinking water. Guidelines were set for acceptable concentrations of (a) bacteria, viruses, and parasites; (b) chemicals of health significance, including specific inorganic and organic constituents, pesticides, disinfectants, and disinfection by-products; (c) radioactive constituents; and (d) substances in drinking water that may give rise to complaints from consumers. The final section of the WHO guidelines deals with protection and improvement of water quality; including selection of water sources, treatment methods, and distribution methods, in the document, WHO cautioned that the guideline values were just recommendations and not mandatory limits. To define limits, it will be necessary to consider the guideline values in the context of local and national, environmental, social, economic, and cultural conditions4.

Biological Contamination

In Africa, the most common and deadly contaminants in drinking water are of biological origin. WHO had stated that infectious diseases caused by pathogenic bacteria, viruses, and protozoa or by parasites are the most common and widespread health risk associated with drinking water. The World Bank estimated more than 3 million children below age 5 die annually from diarrheal diseases contracted through contaminated drinking water in the developing world8. The magnitude of the morbidity and mortality due to waterborne diarrheal diseases unquestionably makes these diseases the planet's biggest environmental health threat to populations. Safe drinking water quality, good sanitation and proper disposal of human and animal excrements are important factors in public health for reducing diarrheal diseases. Studies show that public education in hygienic practices can also reduce public health risk in Africa. In situations of poor sanitation and poor-quality drinking water, the beneficial impact of improving only the sanitation will be larger than that of improving only the quality of drinking water9. Furthermore, the quantity of water used for personal and domestic hygiene has been shown to be more important than the quality of drinking water in its impact on the incidence of diarrhea10. This increase in use reflects more frequent bathing and thorough washing of hands, more careful washing of food, and greater general domestic cleanliness10.

Waterborne infectious diseases are transmitted primarily through contamination of water sources with
excreta of humans and animals who are either active cases or carriers of diseases. Use of such water for drinking or cooking, contact with it during washing or bathing, or even inhalation of its fine droplets as aerosols, may result in infection. The minimum infectious dose (the smallest number of ingested pathogens necessary to cause disease) for the average healthy adult varies widely for various microorganisms. This dose ranges from just a few organisms for *Salmonellatypphi* (to produce typhoid), several hundred organisms for *Shigella flexneri* (to cause dysentery), several million cells of *Salmonella* serotype needed to cause gastroenteritis, to as many as a hundred million cells of *Vibrio cholerae* needed to produce cholera. The minimum infectious dose also varies with the age, health, nutritional and immunological status of the exposed individuals. According to WHO, individuals at greatest risk of waterborne diseases are infants, young children, people who are debilitated or those living under non-sanitary conditions.

The size of the minimum infectious dose does not directly translate into ease of prevention of the relevant disease (since the concentration of the pathogens in water is variable). However, this points to the reasonableness of the approach to minimize disease risk by defining a maximum allowable concentration of an indicator organism in drinking water.

### Chemical Contaminants

Hazardous and toxic chemicals are usually man-made materials that are not used or disposed of properly. Point sources of chemical pollution include industrial discharges and oil spills. The Oil Pollution fact sheet includes more detailed information about oil spills, as well as other sources of oil pollution. Non-point sources of chemical pollution include runoffs from paved roads and pesticide. Besides, household cleaners, dyes, paints, and solvents are also toxic, and can accumulate when poured down drains or flushed down the toilet. In fact, one drop of used motor oil can pollute 25 litres of water! In addition, individuals who use pesticides on their gardens and lawns tend to use ten times more pesticide per acre than a farmer would! Guideline TDI values are set for cyanide, nitrate, and nitrite. Among these, widespread and significant naturally-occurring waterborne toxicants are arsenic and fluoride (with guideline maximum concentrations of 10 µg/l, and 1.5 mg/l). Field concentrations in drinking water in severe problem areas reach a few mg/l to tens of mg/l causing arsenic poisoning (and cancer) and crippling skeletal fluorosis. These two chemicals alone have effect on about a hundred million persons in developing countries. For comparison, the fluoride concentration in municipally fluoridated tap water in the US is about 1 mg/l. Among organic contaminants, WHO guidelines addressed several toxicants that increasingly find their way into drinking water supplies in the developing countries. This has become imperative with the spread of modern agro-business practices, such as systemic and contact pesticides, ascaricides, nematocides, insecticides, pre- and post-emergence herbicides, soil fumigants and weedicides. Chemical dyestuff and processing industries with improper disposal of by-products, intermediates, solvents as well as plasticizers and stabilizers in manufacturing synthetic materials are also potential sources. On this list are chlorinated alkanes, chlorinated ethenes, aromatic hydrocarbons, chlorinated benzenes, and 36 specific pesticides.

Worldwide, there are numerous lists of hazardous substances or priority pollutants, such as those from the European Water Framework Directive 2000/60/EEC\(^1\) the Convention for the Protection of the Marine Environment of the North- East Atlantic (OSPAR Convention)\(^2\) and the list of persistent organic pollutants (POPs) drafted by the Stockholm Convention\(^3\). The pollutants on these lists were selected because of their high toxicity to the environment and to aquatic organisms. The selection of the most dangerous pollutants out of the hundreds or thousands that could be found has been carried out using prioritization models based on expected environmental concentrations, toxicity, persistence, or bioaccumulation. The different lists have similar purposes; for instance, they aim to reduce or stop the emission of certain substances into the drinking water through the environment, and to also reduce or stop the production of certain compounds. At first sight, the pollutants covered by these lists were not highly relevant to aquifer recharge, because their selection was based on their toxicity to the environment and aquatic organisms rather than to humans. Doses for
acceptable intake are not necessarily the same for humans as for aquatic organisms. However, a reduction in the emission of hazardous and priority pollutants will be beneficial for public health in general by reducing the probability that these pollutants will end up in drinking-water sources.

Pharmaceuticals
Pharmaceuticals and personal care products (often abbreviated PPCPs), including medications, lotions, and soaps, are being found in increasing concentrations in lakes and rivers. Scientists have discovered that many PPCPs act as hormone disrupters, which means that the synthetic hormones in the products interfere with the natural hormones in animals, especially fish that live in the water. There has not been enough research and data to determine the extent of disruption caused by PPCPs on humans, but there is evidence to suggest that these chemicals may be partially responsible for an increase in cancer and birth defects. Several pharmaceuticals and their metabolites have been found in raw wastewater, in surface water and even in drinking-water.14,15

Guidelines for Drinking Water Quality
There is no minimum value for the tolerable level of pathogenic contamination of drinking water. WHO recommends E. coli (or as an alternative, thermo-tolerant coliforms) as the indicator organism of choice for bacterial contamination of drinking water. thermo-tolerant coliforms are also recommended as the indicator of choice in assessing the efficiency of water treatment in removing enteric pathogens and fecal bacteria from water intended for drinking.16 WHO has recommended that E. coli or thermo-tolerant coliforms must not be detectable in any 100-ml sample. In practical terms, as evinced by WHO’s example of performance targets for water treatment plants, this implies that the maximum loading of thermo-tolerant coliform bacteria in the water intended for drinking must be less than 1 organism per 100 ml. This is consistent with the maximum contaminant level goal (of E. coli and thermo-tolerant coliforms) of zero organisms per 100 ml, and a maximum contaminant level of less than 1 organism per 100 ml, expressed in USEPA’s current Final Rules for bacterial quality under the National Primary Drinking Water Regulations (US EPA,1998). Both WHO and USEPA recommend regular sampling of treated water supplies, and that not more than 5% of the samples in any 12-month period should test positive for E. coli or thermo-tolerant coliforms. In providing this guideline, WHO is cognizant of the very large difference between the reality that obtains in the rural developing world and the guidelines. WHO knows that in the great majority of rural water supplies in the developing countries, fecal contamination is widespread. Under these conditions, the national surveillance agency should set medium-term targets for the progressive improvement of water supplies, as recommended in Volume 3 of Guidelines for Drinking Water Quality. It is a fact that, the World Bank is even more explicit on this point. While commenting on the issue of desirability of residual disinfection imparted to drinking water by residual chlorine on the one hand, and the urgency of getting clean, safe water to households (even without residual protection) on the other hand, contamination of water in the home is considered to be relatively unimportant. This is a fact that is contrary to the general expectation. What matters however, is whether the water coming out of the tap or pump is contaminated. In most developing countries the imperative is to get from “bad” quality (say, more than 1000 fecal coliforms per 100 ml) to “moderate” quality (less than 10 fecal coliforms per 100 ml), not necessarily to meet the stringent quality standards of industrial countries.17

The issue of viral contaminant in water samples is significantly more complex, costly, and time consuming than bacterial analysis as even a single virus particle is sufficient, in principle, to cause disease. Furthermore, information regarding the virological, epidemiological, and risk analysis dimensions of viral contamination of water supplies, information is grossly inadequate to enable issuing virological criteria for drinking water. Hence, WHO does not directly recommend a minimum viral guideline for the quality of drinking water. Instead, it recommends various treatment methods for different raw water sources according to their degree of detectable fecal contamination, so as to produce drinking water with negligible viral risk. These methods comprise appropriate combinations of disinfection, filtration, settling, and pre-disinfection or storage. In addition, WHO does not set guideline values for pathogenic protozoa, helminthes, and free-living organisms in drinking water, other than that
these agents should not be present in drinking water, because one or very few organisms can induce infection in humans.

**Water Treatment Process**

In Nigeria source waters, surface or ground waters, can contain a range of contaminants that may make the water unsafe to drink or aesthetically unacceptable (eg, bad taste, odour or appearance). Such contaminants include: particles, microbiological contaminants, naturally occurring chemical substances and chemical substances derived from human activities. Of these, the two for which treatment is most important are particles and microbiological contaminants. Treatment for these contaminants is particularly important for surface waters and shallow groundwater that are affected by events above ground. Deep groundwater, or groundwater from confined aquifers are expected to be of much better quality than surface or shallow groundwater, and in some instances are untreated.

Particle removal is the first of the main treatment steps, and this usually consists of a series of processes. The last of these is filtration which is preceded by steps designed to improve filter performance. Particle removal is important because this process remove the larger microbiological contaminants (protozoa, such as *Giardia* and *Cryptosporidium*), some of which are resistant to chlorine, as well as the non-living material that contributes to the cloudiness (turbidity) of the water. Particle removal is also important for the efficacy of the disinfection step. The disinfection step must take place after the particles have been removed, and the water is as ‘clean’ as possible. Micro-organisms in most cases, adsorb to particles in the water. Once adsorbed, they are shielded to a degree from the effects of the disinfectants. Therefore, particle load in the water must be removed before disinfection, so as to ensure adequate inactivation of the organisms and to also remove micro-organisms already adsorbed to the particles [18]. The main treatment processes are not primarily intended to remove large number of chemical contaminants. For instance, where contaminants in the source water cannot be removed satisfactorily by the main treatment processes, additional treatment processes should be incorporated into the treatment sequence. Additional treatment processes that do not have a direct role in removing contaminants may also be required to adjust the water chemistry e.g., adjustment of the pH. In the section below we will try to describe treatment processes that is common in most countries in Africa particularly Nigeria; their functions will also be explained. It should be noted that, the principles of operation of a particular process are independent of the treatment plant, even though the physical design and implementation of the process could vary with the treatment plant.

**Pre-treatments:** Water treatment plants are expected to reliably produce safe drinking water, if the conditions under which they operate remain constant. A source water of changing quality is difficult to treat. Treatment plants drawing water from underground or from a lake or reservoir will usually have source water that undergoes little changes. Rivers and streams, however, are subject to rain events, and treatment plants abstracting water from these types of source can be exposed to rapidly changing source water quality. One of the functions of pre-treatment processes is to provide a ‘buffer’ against changes in source water quality, so that quality changes and the rate of change are reduced. Pre-treatment processes may also be used to modify water chemistry and possibly the contaminants themselves, to improve their removal by later treatment processes. Where treatment plants experience biological growths in parts of their system, such as the clarifier tanks, pre-treatment may also be used to control these growths.

**Sedimentation basins:** Sedimentation basins reduce the load of sediment in the water reaching the main treatment processes, and reduce the magnitude of changes in water quality. This is done by providing a large impounded area in which the water flow is reduced, which gives time for particles to settle out under gravity. During rain events, they provide a buffer against rapid changes in the quality of water entering the treatment plant. Insoluble chemical contaminants may also be partially removed by the settling process. Levels of turbidity and natural organic matter (NOM), and to some extent microbiological contamination, in river or stream water can be reduced by abstracting the water indirectly from the source through an infiltration gallery[19]. By burying open-jointed or slotted pipes in the bed of a river, stream or lake, water percolates through the gravels and sands of the bed and into the pipes where it is diverted to a collection well on the
bank and pumped out for the water supply. This crudely filters the water as it passes through the media of the riverbed so that a fraction of the particles, and contaminants that may adsorb to the riverbed media, are removed. This form of pretreatment achieves little removal of *Cryptosporidium*.

**Pre-oxidation:** Pre-oxidation may be carried out using oxidizing chemicals such as chlorine, ozone or potassium permanganate. It is typically used to modify NOM (the substances that give some waters a yellow-brown colour) to improve its removal during the coagulation/flocculation step. This procedure may also be used to oxidize soluble iron or manganese (usually in ground waters) and sometimes arsenic, to precipitate them for removal by particle removal processes. This process may also control unwanted biological growths in other parts of the treatment plant. A drawback of pre-oxidation is that it tends to increase disinfection by-product (DBP) formation. To minimize DBP formation, it is usually preferable to remove as much NOM as possible before chemical disinfectants are added to the water. This may require avoiding the use of chlorine or ozone when the NOM concentration in the water is results in unacceptable levels of DBPs. Pre-oxidation can destroy some cyanotoxins (toxins produced by cyanobacteria: bluegreen algae).

**Aeration:** Aeration of source water can introduce oxygen into the water to oxidize contaminants, such as iron or manganese, to an insoluble form so that they can be removed as particles (as with pre-oxidation above). Passing air through the water will also assist in removing gases (e.g. hydrogen sulphide, carbon dioxide) or volatile contaminants (e.g. vinyl chloride, trichloroethene). The removal of contaminants by aeration is also known as air-stripping.

**Copper Sulphate treatment:** Copper sulphate is an algaecide, and is sometimes used to control algal blooms in static source waters, such as reservoirs. This approach to controlling algae can result in enhanced taste and odour problems and elevated toxin levels in the water. When the algae die, the cells break up, release toxins and compounds, that cause odour and change the taste of the water. Bloom development is better controlled by minimizing factors that encourage algal growth, such as nutrient concentration.

**Coagulation/flocculation:** This is the first step in the main treatment train of a full conventional treatment system, and prepares the water for particle removal by subsequent processes. A coagulant, usually an aluminium (e.g. alum) or iron salt or more recently *moringa* cake is added to the water. This encourages small particles in the water to stick together to form larger particles, which are more readily removed from the water by the processes that follow. The addition of the coagulant also results in the formation of ‘flocs’ (particles) of insoluble metal hydroxides. The flocs further assist in contaminant removal by providing surfaces for adsorbing contaminants, and trapping contaminants as floc formation occurs. Particle removal is often the chief purpose of the coagulation/flocculation process, but by adjusting the coagulation conditions, NOM can also be removed. This helps to control the formation of DBPs following the disinfection process. The coagulation/flocculation process, combined with the other processes discussed below, can remove metals, bacteria, and protozoa, although to varying degrees that depends on the metal and the type of micro-organism. Its role in removing *Cryptosporidium* is important because chlorine cannot inactivate this protozoan under water treatment conditions. This process also partially removes some non-metals and pesticides.

**Clarification:** Clarification follows the coagulation/flocculation step and provides more time for the particles to stick together and settle out of the water, thereby reducing the sediment load that must be removed by the filters. A process called direct filtration is used in some supplies if the turbidity (particle content) of the source water is low. In this process, the clarification step is omitted and coagulant is dosed directly before the filters. This reduces the turbidity by increasing the efficiency with which particles stick to the sand grains within the filter. In some treatment plants, *DAF* (dissolved air flotation) is used instead of clarification. DAF works by floating the larger particles out of the top of the clarifier rather than letting them settle to the bottom.

**Filtration:** Filters of one type or another are the final process by which particles are removed from the water. When the source water is highly turbid, coagulation/flocculation and clarification steps usually precede the filters, but for some low-turbidity source
waters or in small water supplies with limited resources, the filter may be the only particle removal process. No matter which type of filter is used, particles and other contaminants become trapped by the filtering medium. The amount of trapped ‘dirt’ will eventually reach a point at which the filter cannot satisfactorily operate and the filter must be cleaned. How this is done depends on the type of filtration, but in all cases, treatment plant operators aim to maximize the period the filter used before cleaning is required. This is because the cleaning process reduces the efficiency of the operation.

Sand filters are widely used for particle removal, but usually in combination with other processes. Although called ‘sand’ filters, they often contain two types of sand overlaid by a layer of small coal particles. They strain out particles that are too big to pass through the spaces between the sand grains, and allow smaller particles to travel down into the sand where they are removed by adsorbing to the sand grains. Rapid sand filters and other granular filtration systems (e.g. granular activated carbon) are cleaned by a process termed ‘backwashing’.

Disinfection: There are three methods of disinfecting presently in use in drinking water supplies in Africa

Chlorination: Chlorination is the most widely used disinfecting method in the world. It inactivates bacteria, viruses, and the protozoan. It will not, however, inactivate Cryptosporidium rapidly enough for use in water treatment. An important advantage that chlorine has over the other two main disinfectants is that it remains present long enough in the water to provide a disinfectant residual after treatment16. This is important for the maintenance of a safe water supply. In the event of low levels of contamination entering the distribution zone, the chlorine provides a degree of protection against microbiological contaminants. The efficacy of chlorine as a disinfectant, is determined by the pH; higher acidity (that is, lower pH) enhances disinfection. As well as being a good disinfectant, chlorine is a moderately strong oxidizing chemical and is therefore also used for oxidizing contaminants20, during treatment.

Ozonation: Ozone is a more powerful oxidizing agent than chlorine and a stronger disinfectant, and it can be used in both roles during water treatment. It can rapidly inactivate Cryptosporidium and therefore provides a satisfactory barrier to this organism, as well as to viruses and bacteria. It is a very reactive gas and even in ‘clean’ water it decomposes rapidly hence it does not provide a disinfectant residual after treatment.

Activated carbon adsorption: Activated carbon contains a very high surface area per unit weight that can absorb contaminants. Activated carbon adsorption can remove a wide range of contaminants from water, particularly trace organic contaminants including industry solvents and pesticides. Activated carbon is primarily used to remove taste and odour forming compounds found in minute quantities in the water. Some supplies may also introduce activated carbon treatment to deal with the cyanotoxins produced by blooms of blue-green algae. Algae and some bacteria are the usual sources of tastes and odour in drinking water. The growth of these organisms, and therefore the concentrations of the taste and odour compounds or toxins they produce, depend on several factors – some of which are influenced by the season. These factors include nutrient concentrations (nitrogen and phosphorus) which may be influenced by catchment activities, temperature, light intensity, and oxygen concentration in the water. Algal blooms are more likely to develop in the dry season where water is warm and greater sunlight assists with photosynthesis.

Combinations of treatment processes: Empirically, there is usually more than one treatment process in operation in a treatment plant. For some contaminants this will result in more than one process contributing to the removal or inactivation of a contaminant. For example, bacteria are removed to some degree, by particle removal processes and by disinfection. For other contaminants only one process may reduce the contaminant’s concentration, e.g. Cryptosporidium is only removed by particle removal processes. There are also some instances where a combination of two or more processes is required to achieve removal of a contaminant and removal of the contaminant fails if one of the processes is omitted. An example is the removal of soluble
iron or soluble manganese by precipitation. Aeration, chlorine, or ozone oxidizes the soluble metal to form an insoluble form of the metal. Particle removal processes then remove this. Omission of either the oxidation step, or the particle removal process, results in the iron or manganese being present in the finished water. The combination of coagulation/flocculation/clarification/filtration is commonly used in the treatment of surface waters. Treatment plants using direct filtration, in which the clarification step is dropped from this combination, will achieve a lower removal of particulates and protozoa than can be achieved using the full combination. The maximum turbidity level in the raw water that can be satisfactorily treated is also lower when direct filtration is used.

Use of water quality standards for health risk assessment
Generally, the presence of pathogens in drinking-water is regulated by testing for microbial indicators, to ensure that they are absent. Microbial indicators are used to search for contamination of the water by faecal matter, indicating the possible (or highly probable) presence of pathogens. Indicators are used because it is easier to search for indicators of faecal pollution than to attempt to test for a wide range of pathogens in the water. A good indicator of faecal pollution should fulfill the following requirements [4]: be present universally and in large numbers in the faeces of humans and warm-blooded animals; be readily detectable by simple methods, should not grow in natural waters, and should have similar properties to pathogens in terms of its persistence in water and its removal by water treatment. The ideal indicator, meeting all these conditions, does not exist. Also, a major shortcoming in the context of health risks is that some pathogens are more resistant to disinfection than the indicator organisms, and thus may be present even though no indicator organisms are found. For example, water that has been disinfected will not necessarily be free of enteroviruses and the cysts of some parasites (e.g. Cryptosporidium and Giardia). This means that it is possible for water to comply with water-quality standards, yet contain pathogens and be unsafe. This issue is important in the context of reuse of domestic wastewater, knowing that this water is a major source of faecal pathogens (coliforms).

Use of water quantitative standards for health risk assessment: There are four different steps in a quantitative health risk assessment. The procedure was initially set up by National Research Council in 1998 for evaluating the health risk of specific chemicals, but was recommended by WHO to also be used for microbial contaminants. The four steps include:

(a) Hazard identification, which involves definition of the human health effects associated with any particular hazard.
(b) Dose–response assessment- involves characterization of the relationship between the dose administered and the incidence of the health effect.
(c) Exposure assessment, involves determination of the size and nature of the population exposed and the route, amount, and duration of the exposure.
(d) Risk characterization or integration of steps (a)–(c), to estimate the magnitude of the public health problem.

Quantitative microbial health risk assessment: Implementing a quantitative microbial risk assessment requires the analysis of each of the steps mentioned. For every step, data should be collected, and problems and assumptions clarified.

Hazard identification for microbial contaminant: In a microbial risk assessment, hazard identification involves identifying pathogenic organisms that can be transmitted by treated recycled wastewater. The list of potential waterborne pathogens contains dozens of bacteria, viruses, and protozoa. These organisms can be harmful directly (by causing infection) or indirectly (e.g. by releasing toxins). Because of the large number of potential pathogens, certain organisms must be selected for inclusion in the risk assessment. This selection is not straightforward. From a health point of view, it is best to calculate health risk based on the pathogens with the highest impact on public health, such as those known to cause epidemics or those with a very low infective dose. From a technological viewpoint, it is best to select organisms with high persistence (survival outside the host, in the
environment), and with the highest resistance to
destruction or inactivation.
For a quantitative microbial risk assessment to be
feasible, there is a minimum amount of data that must
be available on the selected organisms, to allow the
risk to be calculated. The data required are: infective
dose, concentrations in raw wastewater and the
percentage of the organism removed by different water
treatment techniques. A number of pathogens, mainly
the newly discovered ones, cannot be included in this
type of assessment because the required data are
lacking. This is the case for the 10 organisms included
in the US EPA’s CCL6 and the so-called “emerging
pathogens”, such as the noroviruses (previously known
as Norwalk-like viruses or caliciviruses). It is believed
that only a few virus particles can cause infection, but
data on infective dose are unavailable up until now.
Therefore, the noroviruses cannot be included in the
assessment. Other organisms of concern in relation to
reuse are the opportunistic pathogens. These organisms
are not pathogenic for healthy individuals, but they can
easily infect individuals with impaired immunity, such
as the elderly or infants.

Dose–response assessment for microbial
contaminants: The dose–response assessment step of
a quantitative microbial risk assessment is aimed at
determining the relationship between the ingested dose
and the effect on health. In the case of pathogens, the
infectivity characterizes this relationship. A threshold
concentration for pathogens, under which no infection
occurs, does not exist. Infection is a phenomenon that
must be expressed as a risk and the infective dose
always must be a range, because of the variation in
sensitivity of the population. In a large population
group, the possibility exists that the ingestion of a
single pathogenic organism can infect certain
individuals. There are three ways to characterize
infectivity:

(a) The ID50 or the infective dose is the dose that
causes infection in 50% of the persons exposed to
the pathogen;
(b) The Pinf (1.0) is the probability of infection
following the exposure to a single organism;
(c) The most complete form for characterizing
infectivity is with dose/response curves, giving
the probability of infection as a function of the
dose.

The values for infectivity are determined by exposing
human volunteers to different doses of the examined
pathogen, observing the effect and recording the
infections. The criteria used for infectivity differ from
one study to another. The following criteria have been
used to determine that a person is infected:

(1) Certain symptoms of illness are observed, with
the symptoms to be defined case-by-case;
(2) Antibodies are found in the blood or an increase
in antibodies is observed;
(3) The pathogens are found in the stools, under a
form to be defined, (e.g. cysts, oocysts or eggs). The
infectivity measurement depends on the
way the infection is defined. A person can have,
for example, antibodies in the blood without
showing clear symptoms of infection. The
magnitude of infective doses, expressed as the
ID50, is in the range of 1–200 oocysts for
Cryptosporidium, 10–100 cysts for Giardia, 1–
10 plaque-forming units for rotavirus and 104–
107 colony-forming units for Salmonella typhi.
Generally, viruses are the most infective agents.
The large variation (up to a factor of 1000)
found on the value of the infective dose for an
organism is also an illustration of the fact that
the determination of infectivity is not straight
forward.

Exposure assessment for microbial contaminants:
For microbial hazards, exposure is assessed by
estimating the amount and the duration of exposure to
the pathogens. Pathogenic organisms are found in
wastewater when disease carriers are present in the
community. The duration of the exposure can therefore
be assumed to be in the order of days to weeks. An
individual’s exposure is calculated from the
concentration of the pathogens in the water and the
volume of water consumed by the individual.
Concentrations of pathogens are variable, but are at
their highest during disease outbreaks. The volume of
water consumed is generally estimated as being 2 litres
per person per day. For microbial risk assessment,
some sources only take the amount of un-boiled water
into account, estimated as being 0.25 litres per person
per day4. In the case of reuse for applications other than
drinking-water, such as household water (e.g. for toilet
flushing, garden watering and cleaning), the microbial
risk is calculated in the same way as for drinking-
water. The difference is in the water volume, which in

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this case is the water ingested accidentally. This volume is based on estimations.

Risk characterization for microbial contaminants: Data on the amount of pathogens to which individuals are exposed and the infective dose are used to calculate the risk of infection, which is then compared with the ‘acceptable’ risk of infection. Generally, a yearly infection risk of 10–4 (i.e. 1 person in 10,000) is considered acceptable. Microbial risk calculations are carried out daily. Because of the inherent variations, published values for pathogen concentrations in wastewater cover a wide range. Risk calculations should be carried out with the highest reliable concentrations found, even if these concentrations are temporary.

Conclusion

In 2011 African government voted in the United Nations in favor of a resolution making water and sanitation a human right. However, it has not passed legislation to enshrine the human right to water and sanitation in national law. The country is not on track to reach the Millennium Development Goal for water and sanitation. Availability of copious and safe water for domestic use and adequate sanitation to dispose of waste is fundamental to the development process in the continent with benefits, such as labor productivity, spread across all sectors.

References:

12. OSPAR. The convention for the protection of the marine environment of the north-east Atlantic (OSPAR Convention) 2002; http://www.ospar.org
Susceptibility pattern of gram negative bacilli to imipenem isolated from patients attending Sobi specialist hospital, Ilorin

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Abstract

This study has evaluated the susceptibility pattern of some Gram negative bacilli isolates to Imipenem by minimum inhibitory concentration (MIC) using the E-test method. Two hundred and sixty (260) Gram negative bacilli from different clinical samples were obtained for this study; analyzed and identified using differential tests for identification of pathogenic bacteria species. Antibiotic disc susceptibility testing for imipenem was carried out using disc diffusion method and interpreted according to CLSI guidelines. The identified isolates include, Klebsiella oxytoca 113(43.5%), Klebsiella pneumoniae 52(20.0%), Pseudomonas aeruginosa 43(16.5%), Escherichia coli 24(9.2%), and Salmonella typhii 22(8.5%). One hundred (100.0%) percent susceptibility to imipenem was Proteus mirabili; and Salmonella typhi, while Klebsiella pneumoniae and Pseudomonas aeruginosa had 80% and 72.1% in this order while the least susceptibility rate of 59.3% was recorded for Klebsiella oxytoca. All isolates were highly susceptible to Imipenem, an indication that Imipenem is still potent against common clinical isolates of Gram negative bacteria in Ilorin.

Key words, Imipenem, Gram negative isolates, Antimicrobial susceptibility, E-test.

Introduction

Carbapenems are β-lactam drugs that are structurally different from the penicillins and the cephalosporins. They have a methylene group in their ring in place of sulfur. Typical examples of Carbapenems are Imipenem, Meropenem, Ertapenems and Doripenems. They are the most potent agents for the treatment of Extended Spectrum Beta Lactamase infections and other multidrug drug resistant Gram negative bacteria (Overturf 2010, Igbinoba et al., 2012).

The Gram negative bacilli (example, Klebsiella species) are a large diverse family which are free living and part of the indigenous flora of humans and animals where a few are adapted strictly to living in humans. They grow rapidly under aerobic and anaerobic conditions and are metabolically active (Forage and Lin, 1982). They are by far the most common cause of urinary tract infections (UTIs) and a limited number of species are also important etiologic agents of diarrhoea or spread in to the blood stream causing gram negative endotoxin shock with dreadful complications (Ryan, 2004).

Imipenem has the widest spectrum of activity of the beta-lactam drugs, it has an excellent bactericidal activity against many gram-negative (example, Klebsiella species), gram- positive (Streptococcus species) and anaerobic bacteria (example, Clostridium species). (Balfour et al., 1996). Carbapenems are usually the last line drug for the treatment of infections caused by resistant Gram-negative bacilli. It is effective against more than 95% of strains of

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commonly encountered gram-negative pathogens, with several exceptions (Andrew et al., 2009), in most medical centres; approximately 15% of *Pseudomonas aeruginosa* are resistant; as nearly all strains of *Xanthomonas malthopia* (Brink, 2004). Gram negative bacteria that are resistant to the Carbapenems are due to their inherent production of Carbapenems hydrolysing enzymes, which have been increasingly reported in some parts of the world. (Chakrabody et al., 2010; Saderi et al., 2008; Walsh et al., 2005). Nonetheless, the prevalence of bacterial resistance to the Carbapenems is gradually on the increase (Bratu, 2005); and the consequence of the wide use of Carbapenems resulted in the emergence of the first Carbapenemase producing *Enterobacteriaceae* in 1993 (Shamsudin, 2013). Carbapenemase producing gram-negative bacteria have been reported in many African countries. The first case of Carbapenem resistance in South Africa was seen in August 2011 and was reported from a hospital in Johannesburg (Govind et al., 2013).

Multiple carbapenemase genes were detected in strains of *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* in Tanzania (Martha et al., 2014). In Nigeria Ejikeugwu et al. (2013), reported some few cases of extended spectrum beta lactamase producing strains of *Klebsiella pneumoniae* and *Escherichia coli* and all *E. coli* producing Extended Spectrum Beta Lactamase were found to be susceptible to Imipenem and Carbapenem, while some of the *Klebsiella pneumoniae* were susceptible to Imipenem. There is paucity of knowledge with regards to the susceptibility pattern of gram-negative bacilli to Imipenem and Meropenem in Nigeria. Therefore, this study aims at investigating and documenting the susceptibility pattern of gram-negative bacilli to Imipenems.

**Material and Methods**

This study was carried out at the Medical Microbiology Laboratory of the Sobi Specialist Hospital Ilorin which is a secondary health centre between August 2013 to January 2014. Ethical clearance was obtained from the ethical committee of the State Ministry of Health, Kwara State. This study was a cross sectional laboratory based analysis of the susceptibility pattern of isolates to Imipenems.

Two Hundred and sixty (260) gram-negative bacilli were isolated from clinical samples of patients who presented in the hospital with various signs and symptoms suggestive of urinary tract infections, Otitis media, pneumonia, bacterial conjunctivitis, and wound sepsis. Information regarding patient’s name, occupation, age, sex, ward, or clinic type of specimen were taken from documents and recorded.

All Gram negative-bacilli isolates recovered from clinical samples were characterized using a combination of colonial morphology on culture media and gram staining reaction, and all isolates were biochemically analysed.

The antibiotic susceptibility study was carried out using the modified Kirby- Bauer method (Cheesbrough, 2006) and the determination of minimum inhibitory concentration was carried out using Imipenem E. test strip in accordance with the manufacturer instruction.

**Data Analysis.**

Data generated in this study were analyzed with Statistical package for social sciences (SPSS) software version 20 (SPSS Inc., Chicago III) and results were presented in tables and figures as applicable. Significant level was set at P 0.05 and categorical data compared using chi-square($X^2$). Continuous variables were described by means and mode.

**Result**

Table 1 shows the distribution of the isolates of the study. Two hundred and sixty isolates were recovered during this study out of which *Klebsiella oxytoca* was (43.5%), *Pseudomonas aeruginosa* (16.5%), while *Salmonella* species was (8.5%), *E. coli* accounted for(9.2%), and *Proteus mirabilis* (2.3%).

**Table 1: Distribution of Isolates.**

<table>
<thead>
<tr>
<th>Isolates</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Klebsiella oxytoca</em></td>
<td>113</td>
<td>43.5</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>43</td>
<td>16.5</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>24</td>
<td>9.2</td>
</tr>
<tr>
<td><em>Proteus mirabilis</em></td>
<td>6</td>
<td>2.3</td>
</tr>
<tr>
<td><em>Salmonella typhi</em></td>
<td>22</td>
<td>8.5</td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae</em></td>
<td>52</td>
<td>20.0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>260</strong></td>
<td><strong>100.0</strong></td>
</tr>
</tbody>
</table>
The susceptibility pattern of the isolates is shown in Table 2. One hundred per cent (100.0%) susceptibility was observed in *E. coli*, *Proteus mirabilis* and *Salmonella typhi* isolates, followed by *Klebsiella oxytoca* and *Klebsiella pneumoniae* (78.8%) respectively. However, the difference in susceptibility pattern was not significant (P>0.05).

Table 2: Susceptibility pattern of the isolates to Imipenem Disc

<table>
<thead>
<tr>
<th>Isolate</th>
<th>N</th>
<th>Susceptible</th>
<th>Intermediate</th>
<th>Resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Klebsiella oxytoca</em></td>
<td>113</td>
<td>78.8%</td>
<td>2.7%</td>
<td>18.6%</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>43</td>
<td>86.0%</td>
<td>7.0%</td>
<td>7.0%</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>24</td>
<td>100.0%</td>
<td>0.0%</td>
<td>0.0%</td>
</tr>
<tr>
<td><em>Proteus mirabilis</em></td>
<td>6</td>
<td>100.0%</td>
<td>0.0%</td>
<td>0.0%</td>
</tr>
<tr>
<td><em>Salmonella typhi</em></td>
<td>22</td>
<td>100.0%</td>
<td>0.0%</td>
<td>0.0%</td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae</em></td>
<td>52</td>
<td>78.8%</td>
<td>5.8%</td>
<td>15.4%</td>
</tr>
<tr>
<td>Total</td>
<td>260</td>
<td>84.2%</td>
<td>3.5%</td>
<td>12.3%</td>
</tr>
</tbody>
</table>

χ² = 17.889  P-value = 0.085

Table 3 shows susceptibility pattern of the isolates to E-test. The highest resistance was observed among *Klebsiella oxytoca* closely followed by *Klebsiella pneumoniae*. But the susceptibility pattern remains statistically significant.

Table 3: Susceptibility pattern of the isolates to E-test

<table>
<thead>
<tr>
<th>Isolate</th>
<th>N</th>
<th>Susceptible</th>
<th>Intermediate</th>
<th>Resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Klebsiella oxytoca</em></td>
<td>113</td>
<td>59.3%</td>
<td>31.0%</td>
<td>9.7%</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>43</td>
<td>72.1%</td>
<td>18.6%</td>
<td>9.3%</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>24</td>
<td>100.0%</td>
<td>0.0%</td>
<td>0.0%</td>
</tr>
<tr>
<td><em>Proteus mirabilis</em></td>
<td>6</td>
<td>100.0%</td>
<td>0.0%</td>
<td>0.0%</td>
</tr>
<tr>
<td><em>Salmonella typhi</em></td>
<td>22</td>
<td>100.0%</td>
<td>0.0%</td>
<td>0.0%</td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae</em></td>
<td>52</td>
<td>80.8%</td>
<td>9.6%</td>
<td>9.6%</td>
</tr>
<tr>
<td>Total</td>
<td>260</td>
<td>73.8%</td>
<td>18.5%</td>
<td>7.7%</td>
</tr>
</tbody>
</table>

χ² = 38.808  P-value = 0.000

Table 4 shows the susceptibility and the resistance patterns of the isolates to Imipenem disc in which *Klebsiella oxytoca* had the highest resistance with (18.6%) while *E. coli*, *Proteus mirabilis* and *Salmonella typhi* were each 100% susceptible closely followed by *Klebsiella pneumoniae* (78.8%) susceptible. The pattern of susceptibility was statistically significant when compared with the total number of the isolates. There was also a level of intermediary where isolates demonstrated neither susceptible nor resistance as presented as intermediary.

Table 4: Minimum Inhibitory Concentration of Imipenem for the Isolates showing MIC 50 and 90

<table>
<thead>
<tr>
<th>MIC</th>
<th>Frequency</th>
<th>Percent</th>
<th>Cumulative Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.125</td>
<td>26</td>
<td>10.0%</td>
<td>10.0%</td>
</tr>
<tr>
<td>0.190</td>
<td>22</td>
<td>8.5%</td>
<td>18.5%</td>
</tr>
<tr>
<td>0.250</td>
<td>61</td>
<td>23.5%</td>
<td>41.9%</td>
</tr>
<tr>
<td>0.380</td>
<td>20</td>
<td>7.7%</td>
<td>49.6%</td>
</tr>
<tr>
<td>0.500*</td>
<td>19</td>
<td>7.3%</td>
<td>56.9%</td>
</tr>
<tr>
<td>0.750</td>
<td>12</td>
<td>4.6%</td>
<td>61.5%</td>
</tr>
<tr>
<td>1.000</td>
<td>19</td>
<td>7.3%</td>
<td>68.8%</td>
</tr>
<tr>
<td>1.500</td>
<td>18</td>
<td>6.9%</td>
<td>75.8%</td>
</tr>
<tr>
<td>2.000</td>
<td>5</td>
<td>1.9%</td>
<td>77.7%</td>
</tr>
<tr>
<td>3.000</td>
<td>31</td>
<td>11.9%</td>
<td>89.6%</td>
</tr>
<tr>
<td>4.000**</td>
<td>6</td>
<td>2.3%</td>
<td>91.9%</td>
</tr>
<tr>
<td>32.000</td>
<td>21</td>
<td>8.1%</td>
<td>100.0%</td>
</tr>
<tr>
<td>Total</td>
<td>260</td>
<td>100.0%</td>
<td>100.0%</td>
</tr>
</tbody>
</table>

* = MIC 50, ** = MIC 90

Table 5 shows the summary of MIC of Imipenem to isolates in which *Klebsiella pneumoniae* has the highest mean MIC while *Salmonella typhi* has the lowest mean MIC.

Table 5: Summary of MIC of Imipenem to isolates

<table>
<thead>
<tr>
<th>Isolate</th>
<th>n</th>
<th>MIC 50</th>
<th>MIC 90</th>
<th>Mean</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Klebsiella oxytoca</em></td>
<td>113</td>
<td>0.5</td>
<td>3.0</td>
<td>4.1645</td>
<td>0.000</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>43</td>
<td>1.5</td>
<td>4.0</td>
<td>3.69477</td>
<td>0.004</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>24</td>
<td>0.25</td>
<td>0.38</td>
<td>0.25021</td>
<td>0.000</td>
</tr>
<tr>
<td><em>Proteus mirabilis</em></td>
<td>6</td>
<td>ND</td>
<td>ND</td>
<td>0.38000</td>
<td>0.000</td>
</tr>
<tr>
<td><em>Salmonella typhi</em></td>
<td>22</td>
<td>ND</td>
<td>ND</td>
<td>0.19000</td>
<td>0.000</td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae</em></td>
<td>52</td>
<td>0.38</td>
<td>3.0</td>
<td>3.73529</td>
<td>0.006</td>
</tr>
</tbody>
</table>

ND = Not Determine

Discussion

Resistance to antibiotic therapy is an increasing public health problem all over the world. In recent years, through the abuse and misuse of antibiotics, many bacteria have developed resistance to a variety of
antibiotics. This pattern of resistance varies from population to population and region to region. The Enterobacteriaceae are the most common group of Gram negative rods cultured in the clinical laboratory. Usually the predominant species vary depending on the region. Many researchers have reported E. coli to be the predominant species isolated, but in this study Klebsiella oxytoca was the predominant. Majority of the Klebsiella oxytoca were isolated from wound swab, pus, catheter tip and urine specimen submitted to the microbiology laboratory of the hospital.

In this study, Klebsiella oxytoca was found to be the most predominant isolate among the Gram negative bacilli isolated, and at variance with the findings of Siew et al. (2007), showing E. coli as the predominant Gram negative bacteria isolated. It was also observed that the rate of isolation of Klebsiella and Proteus in this study agrees with the findings of Maharashtra et al where these two species predominate. However, Pseudomonas isolation in our study was high (16.5%) compared with study conducted by Jose’ and Fernando (2002).

The present study provided an insight to the prevalence and the susceptibility pattern of the common gram negative pathogens isolated at the study area to Imipenem. Imipenem are usually indicated for infections due to organisms resistant to other drugs, it has good activity against the gram negative rods and the gram positive as well (Kollef, 2003).

From this study E. coli, P. mirabilis and S. typhi were found to show 100% susceptibility to Imipenem while K. oxytoca, P. aeruginosa and K. pneumoniae were found to show 18.6%, 7% and 15.4% resistance to Imipenem, using disc diffusion. This agrees with the findings of Boroumand et al., in 2007 and Shahcheraghi et al., in 2008 that Pseudomonas show 94.4% and 92% susceptibility to Imipenem. The report of (Shahcheraghi et al., 2007) which showed that E. coli was 100% susceptibility to Imipenem agrees with the finding of this study. The susceptibility pattern by disc diffusion as shown on table 4.3 agrees with the susceptibility pattern by E-test except for the significant difference observed for K. oxytoca.

It is documented that the indiscriminate use of Imipenem can lead to the selection and dissemination of antibiotic-resistant organism (Percival, 1997). But in the study area Imipenem is a very scarce antibiotic which is just been introduced into the clinics because of resistance to the commonly used conventional antibiotics such as Ampicillin and the Cephalosporins. Imipenem is an injectable which cannot be easily abused compared to the oral group of antibiotics which are easily sold over the counter. The development of resistance to an antibiotic is directly proportional to the usage of such antibiotic. Never the less Imipenem is not used alone to prevent the emergence of resistance, it is usually used in combination with Gentamycin and Cilastatin which is a peptidase inhibitor.

The value 0.5µg and 3.0µg observed as MIC 50 and MIC 90 for Klebsiella oxytoca, 1.5µg and 4.0µg observed as MIC 50 and MIC 90 for P. aeruginosa, and 0.38µg and 3.0µg observed for K. pneumoniae disagree with the report of Livermore (2001) which reported 0.25µg and 0.5µg as MIC 50 and MIC 90 for K. oxytoca, 4.0µg and 16.0µg as MIC 50 and MIC 90 for P. aeruginosa, and 0.25µg and 1.0µg as K. pneumoniae. While 0.25µg and 0.38µg observed as MIC 50 and MIC 90 for E. coli is in line with the work of Livermore (2001) which reported 0.25µg and 0.5µg as MIC 50 and MIC 90.

The MICs observed in this study were higher than those reported by Livermore et al. (2001), this may be due to the difference in the period of study. With the time lapse between the two studies there has been an increase in the use of Imipenem. This might have resulted into the selection of the resistant strains, hence the recorded 7.7% recorded in this study.

In general, from this study 0.5µg and 4.0µg is recorded as MIC 50 and MIC 90 for all the Gram negative bacterial isolates from Sobi Specialist Hospital this implies that 0.5µg is required to inhibit the growth of 50% of Gram negative bacterial isolate from Sobi Specialist Hospital while 4.0µg is required to inhibit 90% of the Gram negative bacterial isolates from Sobi Specialist Hospital.

Imipenem remains a drug of choice in the treatment of infection caused by susceptible Gram negative bacteria but the emergence of the resistant strains is threatening the efficacy of the drugs. Judicious use of this antibiotic is very essential, it should not be used as first line antibiotic but reserved; and can only be used when there is resistance to other first line
antibiotics. Imipenem has demonstrated potent antimicrobial activity against commonly isolated Gram negative pathogens including that are multidrug-resistant in this environment and can be used for life threatening infections. To prevent treatment failure, patients’ management should be based on Imipenem MICs as varying Imipenem MICs within the susceptible range predict different treatment outcomes and the use of any antibiotic should be based on susceptibility information from the laboratory.

**Limitation**

The E-test method employed for the determination of Imipenem MICs in Gram negative bacilli in this study has been reported to have good correlation with broth micro dilution method recommended by CDC as the gold standard for determination of Imipenem MICs. However, the Imipenem E-test strips contain fixed concentrations of the antibiotic in double dilution folds which does not give room for determination of Imipenem MICs in between two folds.

**References**

Ultrasonic diagnosis of orbito-ocular disorders in Irrua, Nigeria

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Abstract

Aims and Objectives: To determine the usefulness of ultrasound scan in the diagnosis of ocular disease in patients sent for ultrasonography from the Eye clinic of Irrua Specialist Teaching Hospital, Irrua.

Materials and Methods: A retrospective study of request cards and ultrasound reports of 107 patients over a 24 months period (between January 2014 and December 2015) was done. The age, sex, presenting complaints and ultrasonographic findings were evaluated.

Result: One hundred and seven patients consisting of 68 males (63.6%) and 39 females (36.4%) with a male to female ratio of 1.7:1 were seen. Age range was from 3 days to 85 years, with mean age of 42.5 years. Seventy-five patients (70.1%) had ocular pathology while 32 patients (29.9%) had normal ocular ultrasonic findings.

Conclusion: Ultrasound scan is very useful and reliable for ocular examination and beneficial to the Ophthalmologists in establishing diagnosis of some ocular disorders.

Key words: ultrasound, ocular disease, diagnosis.

Introduction

Ultrasonography is a dynamic procedure routinely performed on all patients in developed countries whose ocular fundi cannot be visualised but its use in Nigeria only started in recent years. Ultrasound scan (USS) is excellent at showing the normal anatomical structures. The cystic composition and the superficial position of the eye offer optimum conditions for adequate evaluation by ultrasonography. Ultrasound is cheap, readily available, has no radiation hazard or magnetism for metallic foreign bodies. The patient can be asked to rotate the eye in several desired directions during USS and this important aspect of real time assessment of the eyes is not available with either computerized tomography (CT) scan or Magnetic resonance imaging (MRI). Accurate and adequate spatial resolution which is available using USS is absent in both CT and MRI. Therefore, ultrasonography is the most cost effective, hazard-free, real time and practical method of imaging of the eye globe in trauma and in hazy media. Real time B-scanning is the mainstay technique, and has been adjudged to be more effective than either A-scanning or Doppler scanning in the examination for hazy ocular media and trauma. Although USS is operator dependent, in the hands of skilled practitioners, accurate identification of the different intraocular structures with differentiation of the different pathological lesions is easily achieved.

The aims of this study are to found out the pattern of ocular ultrasound findings in patients who were referred for USS from the Eye clinic of the Irrua Specialist Teaching Hospital over the period of study.

Materials and Methods

Patients who had USS for their eyes in the Irrua Specialist Teaching Hospital over a 24-month period (between January 2014, and December 2015) were evaluated. Their age, sex and clinical diagnosis were noted and analyzed. Some of these patients had opaque...
media and their fundi could not be visualized with the ophthalmoscope. Others had mature cataract, trauma to the eyes, tumors or other ocular pathological conditions and needed ultrasonic evaluation of their eyes.

The ultrasound scans were done with the patients placed in comfortable recumbent position and simple closure of the eyelids achieved. Patients were advised to maintain reasonable fixation of the eyeball. The ultrasound scans were performed by Consultant Radiologists, and Senior Registrars using curvilinear probes of capacity frequency 3.5MHz and 6.5MHz. They were adapted well to scan the eyeball and orbit. The lower frequency probe was used to achieve deeper macroscopic analysis of the eyeball with chamber differentiation.

The routine method in the department which was also adopted for this study, is direct probe contact on lubricated eyelid. Inert ultrasonic gel, a lubricant known to have no contact irritation, was used. The probe placements were horizontal and transverse scanning. Each orbit was independently scanned, after which comparative evaluation of the eyeball and intraorbital structures were made. The patients were occasionally asked to rotate the eyes in different desired directions to assess sediments or particles in the vitreous humour and to show the retro-orbital space or the lens adequately.

Results:

A total of 107 patients comprising of 68 males and 39 females with male to female ratio of 1.7:1 were seen. Their ages ranged between 3 days to 85 years with mean age of 42.5 years. Table 1 shows the age and sex distribution of the patients. The ultrasound scan findings of patients’ eyes examined is shown on table 2. Seventy-five patients (70.1%) had ocular abnormalities detected, while 32 patients (29.9%) had no ocular abnormality. Out of the patients that had ocular abnormalities, 64 of them (85.3%) had single abnormality detected, while 11 of them (14.7%) had multiple ocular abnormalities, bringing the total number of abnormalities to 97. Cataract was the most common abnormality found (24.7%), followed by vitreous haemorrhage (11.3%) and vitreous detachment (10.3%).

Table 1: Age and Sex distribution of patients referred for ultrasonography

<table>
<thead>
<tr>
<th>Age range (years)</th>
<th>Males</th>
<th>Females</th>
<th>Total</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 – 10</td>
<td>2</td>
<td>1</td>
<td>3</td>
<td>2.8</td>
</tr>
<tr>
<td>11 – 20</td>
<td>10</td>
<td>8</td>
<td>18</td>
<td>16.8</td>
</tr>
<tr>
<td>21 – 30</td>
<td>15</td>
<td>10</td>
<td>25</td>
<td>23.4</td>
</tr>
<tr>
<td>31 – 40</td>
<td>10</td>
<td>5</td>
<td>15</td>
<td>14.0</td>
</tr>
<tr>
<td>41 – 50</td>
<td>3</td>
<td>3</td>
<td>6</td>
<td>5.6</td>
</tr>
<tr>
<td>51 – 60</td>
<td>13</td>
<td>3</td>
<td>16</td>
<td>15.0</td>
</tr>
<tr>
<td>61 – 70</td>
<td>8</td>
<td>2</td>
<td>10</td>
<td>9.3</td>
</tr>
<tr>
<td>71 – 80</td>
<td>3</td>
<td>3</td>
<td>6</td>
<td>5.6</td>
</tr>
<tr>
<td>81 – 90</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>1.9</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>68</strong></td>
<td><strong>39</strong></td>
<td><strong>107</strong></td>
<td><strong>100</strong></td>
</tr>
</tbody>
</table>

Table 2: Type of ultrasound findings within the globe

<table>
<thead>
<tr>
<th>Ultrasound findings</th>
<th>Number of eyes</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>32</td>
<td>24.8</td>
</tr>
<tr>
<td>Cataract</td>
<td>24</td>
<td>18.6</td>
</tr>
<tr>
<td>Vitreous haemorrhage</td>
<td>17</td>
<td>13.2</td>
</tr>
<tr>
<td>Pseudophakia</td>
<td>11</td>
<td>8.5</td>
</tr>
<tr>
<td>Vitreous detachment</td>
<td>10</td>
<td>7.8</td>
</tr>
<tr>
<td>Retinal detachment</td>
<td>7</td>
<td>5.4</td>
</tr>
<tr>
<td>Vitreous bands</td>
<td>5</td>
<td>3.9</td>
</tr>
<tr>
<td>Aphakia</td>
<td>4</td>
<td>3.1</td>
</tr>
<tr>
<td>Enophthalmos</td>
<td>4</td>
<td>3.1</td>
</tr>
<tr>
<td>Phthisisbulbi</td>
<td>3</td>
<td>2.3</td>
</tr>
<tr>
<td>Retinoblastoma</td>
<td>2</td>
<td>1.5</td>
</tr>
<tr>
<td>Lens subluxation</td>
<td>2</td>
<td>1.5</td>
</tr>
<tr>
<td>Ethmoidalmucocele</td>
<td>2</td>
<td>1.5</td>
</tr>
<tr>
<td>Orbital mass</td>
<td>1</td>
<td>0.8</td>
</tr>
<tr>
<td>Myositis</td>
<td>1</td>
<td>0.8</td>
</tr>
<tr>
<td>Ocular displacement</td>
<td>1</td>
<td>0.8</td>
</tr>
<tr>
<td>Retrobulbar mass</td>
<td>1</td>
<td>0.8</td>
</tr>
<tr>
<td>Subpalpebral lipoma</td>
<td>1</td>
<td>0.8</td>
</tr>
<tr>
<td>Preseptal cellulitis</td>
<td>1</td>
<td>0.8</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>129</strong></td>
<td><strong>100.0</strong></td>
</tr>
</tbody>
</table>

Discussion

This study has shown the usefulness of B-scan ultrasonography in the diagnosis and management of ocular lesions in the presence of clear or opaque...
media.\textsuperscript{2,6,16} In the evaluation of hazy ocular media, B-scan gives a real time evaluation of the vitreous humor and the retina.

In this study, USS examination revealed several ocular lesions. Cataract ranked the highest and was seen in 24 eyes (18.6%). This finding is higher than cases seen in previous similar studies\textsuperscript{2,6,16}. This could be because most Ophthalmologists do not routinely request USS for cataract cases except associated retinal lesion is suspected. Vitreous haemorrhage ranked second in 17 eyes (13.2%). Vitreous haemorrhage is a common finding in blunt ocular trauma\textsuperscript{3,7,16}. Retinal detachment was present in only 7 eyes (5.4%). Some previous studies recorded higher incidence of retinal detachment.\textsuperscript{3-6} Ultrasound scan indicates the location and extent of retinal detachment and reliably differentiates rhegmatogenous retinal detachment from detachment secondary to solid tumors.\textsuperscript{3,8} Also, USS is very useful in the diagnosis of non-rhegmatogenous (secondary) retinal detachment.\textsuperscript{3,8} A previous study in Nigeria\textsuperscript{3} had reported retinal detachment as the commonest abnormality in orbito-ocular ultrasonography involving various lesions including tumors, cataract and trauma. This could be because retinal detachment is a frequent complication of several conditions including trauma, tumour, and lens dislocation. Retinal detachment cannot be easily visualized when there are opacities in the ocular media and this requires USS for differential diagnosis. Patients with retinal detachment and those with hazy ocular media usually present early because of the associated visual loss. In this study, one of the cases of retinal detachment was secondary to a retrobulbar mass lesion.

Conclusion

USS is a very useful investigative modality for the assessment of the globe, especially when the ocular media is opaque or hazy. This will be of immense help to the Ophthalmologist to intervene very early especially when there is retinal detachment. The visual prognosis of which is highly dependent on time of surgical intervention. Ocular ultrasound scan will also help the Ophthalmologists and General practitioners to refer such patients with that need to go to some specialized centres early enough for treatment thereby preventing blindness and improve quality of life.

References:

Random fascio-cutaneous medial thigh flap for scrotal reconstruction post Fournier gangrene: A pilot case series.

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Abstract

Introduction: Fournier gangrene is a perineal fasciitis. Serial debridements often lead to soft tissue defects affecting the scrotum, thigh, groin, and abdominal wall. Many procedures to cover the scrotal defect post Fournier gangrene have been described. We present our experience in using random fascio-cutaneous medial thigh flap.

Patients and methods: This is a retrospective study of ten patients who were admitted for Fournier gangrene and treated with a random fascio-cutaneous medial thigh flap. Demographic and other clinical information were retrieved from the casenotes. Cosmetic outcomes and patients’ level of satisfaction were scored from 6-month follow-up using the numerical scale.

Results: The average age of the studied patients is 48.5±11.3 years with a range of 35 to 60 years. The average length of hospital stay before scrotal reconstruction was 53.3±18.7 days. Most patients were satisfied with the cosmetic outcome.

Conclusion: Random medial thigh flap is a good option for extensive scrotal defect caused by Fournier gangrene.

Introduction

Fournier gangrene is a perineal fasciitis with high morbidity and mortality1. As a medical and surgical emergency, debridement, antibiotics and resuscitation are the initial treatment. Serial debridements may lead to soft tissue defect of the scrotum that can spread to the thigh, groin, and abdominal wall. The high mortality associated with this condition is usually due to the delay in presentation to the hospital and co-morbidity. When patients survive, the residual soft tissue defects which have to be covered may pose a reconstructive challenge.

Many procedures have been described for the reconstruction of scrotal defects caused by Fournier Gangrene1, 2, 3, 4, 5. This denotes the difficulty in the choice of the best procedure. In 1995, Boukind H et al published the surgical management of scrotal defect after infectious causes which was based on his experience working in our department6. This has imparted positively on the surgeons to use the simplest and most efficient procedures. We use procedures that have minimal complication post-surgery, allowing quick recovery and swift return home after such a serious and stressful sickness.

We present our experience in using random fascio-cutaneous medial thigh flap for extensive scrotal reconstruction after Fournier gangrene.

Patients and Methods

This is a retrospective study of 10 patients managed in the plastic and reconstructive surgery department of IBN Rochd Teaching Hospital between January 2013 and December 2015 for extensive scrotal defect secondary to Fourniers gangrene. Information retrieved from patients’ casenotes included age, history and type of co-morbidity, and duration of hospital stay. We contacted these patients to ask about their level of
satisfaction with the cosmetic appearance and the outcome generally. These information were scored using the numerical scale. All the patients recruited had extensive wound with loss of all the scrotal covering but limited to the scrotum. They were all treated with random fascio-cutaneous medial thigh flap. All the patients were followed up for a duration of between six months and two years.

**Surgical procedure**

Spinal anaesthesia was administered on the patients and they were positioned supine with the lower limbs in abduction. A pinch test was done on the medial thigh to determine the maximum defect that can be primarily closed without tension. Flap was planned after preparation of the recipient site, and orchidopexy. Depending on the size of the defect, the fascio-cutaneous medial thigh flap was dissected from caudal to cranial through the layer of the fascia (Figure 1) up to the pivotal point just a few centimetres from the groin. We ensured that the flaps’ width: length ratios were at least 1:1 but not more than 1:1.5. The dimension of the flaps varies from 8 to 10 cm in width and 12 to 15 cm in length. Haemostasis was achieved with epinephrine (1:100 000) and bipolar diathermy. Flap in-settings on the recipient site were done in two layers with penrose drain in situ. The donor sites were closed directly without drains (Figure 2).

**Result**

There were ten patients that were seen within the study period. The average age of the studied patients was 48.5±11.3 years with a range of 35 to 60 years. Forty percent (n=4) of the patients had co-morbidity. Two of these patients (20%) had non-insulin-dependent diabetes mellitus. One of the remaining two had hypertension while the other had anorectal inflammation. The average length of hospital stay before the scrotal reconstruction was 53.3±18.7 days. Only 10% (n=1) of the patients had wound breakdown from wound sepsis that resolved on daily dressing with antibiotics. All reconstructed scrotums had good cosmetic appearance based on patient’s assessment (Figure 3). Most of the patients were very satisfied with the outcome of the treatment. See Table 1.
Table I: Clinical and therapeutic characteristics of patients

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Co-morbidity</th>
<th>Donor Limb (right/left/Bilateral)</th>
<th>Length of hospital Stay (Days)</th>
<th>Flap Dimension [Width (cm)/ length(cm)]</th>
<th>Complication</th>
<th>Cosmetic aspect</th>
<th>Patient satisfaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient 1</td>
<td>35</td>
<td>Nil</td>
<td>Right</td>
<td>30</td>
<td>8/10</td>
<td>None</td>
<td>Good</td>
<td>Satisfied</td>
</tr>
<tr>
<td>Patient 2</td>
<td>48</td>
<td>Non-insulin-dependent diabetes mellitis</td>
<td>Bilateral</td>
<td>48</td>
<td>8/15</td>
<td>None</td>
<td>Excellent</td>
<td>Very satisfied</td>
</tr>
<tr>
<td>Patient 3</td>
<td>56</td>
<td>Nil</td>
<td>Right</td>
<td>75</td>
<td>7/12</td>
<td>None</td>
<td>Good</td>
<td>Very satisfied</td>
</tr>
<tr>
<td>Patient 4</td>
<td>38</td>
<td>Nil</td>
<td>Right</td>
<td>40</td>
<td>8/13</td>
<td>None</td>
<td>Good</td>
<td>Very satisfied</td>
</tr>
<tr>
<td>Patient 5</td>
<td>44</td>
<td>Nil</td>
<td>Left</td>
<td>39</td>
<td>6/10</td>
<td>None</td>
<td>Good</td>
<td>Very satisfied</td>
</tr>
<tr>
<td>Patient 6</td>
<td>60</td>
<td>Non-insulin-dependent diabetes mellitis</td>
<td>Bilateral</td>
<td>90</td>
<td>8/12</td>
<td>Yes (Partial wound breakdown 2º wound infection)</td>
<td>Good</td>
<td>Very satisfied</td>
</tr>
<tr>
<td>Patient 7</td>
<td>30</td>
<td>HTN*</td>
<td>Left</td>
<td>51</td>
<td>7/13</td>
<td>None</td>
<td>Good</td>
<td>Satisfied</td>
</tr>
<tr>
<td>Patient 8</td>
<td>62</td>
<td>Ano-rectal inflammation</td>
<td>Right</td>
<td>60</td>
<td>8/15</td>
<td>None</td>
<td>Excellent</td>
<td>Very satisfied</td>
</tr>
<tr>
<td>Patient 9</td>
<td>57</td>
<td>Nil</td>
<td>Right</td>
<td>38</td>
<td>8/14</td>
<td>None</td>
<td>Good</td>
<td>Very satisfied</td>
</tr>
<tr>
<td>Patient 10</td>
<td>55</td>
<td>Nil</td>
<td>Bilateral</td>
<td>42</td>
<td>7/14</td>
<td>None</td>
<td>Good</td>
<td>Satisfied</td>
</tr>
</tbody>
</table>

Discussion

Main goals in scrotal reconstruction are to provide a coverage that can protect the testes against trauma, maintain, and keep normal temperature for good spermatogenesis, and give a good cosmesis. These goals are not easy to achieve. That explains why so many procedures have been described in the literature. The clinical presentation should guide the choice of the appropriate procedure. In some cases, the experience of the team in the surgical department can also guide the choice of procedure.

Groin pedicle axial flap vascularized by the superficial circumflex iliac artery and innervated by the femoris cutaneous nerve of the thigh had been used for scrotal reconstruction. This flap has an advantage of preserving contractile ability of the cremasteric muscles which is needed to move the testes, thus regulating its temperature for spermatogenesis. The groin flap is suitable for the median raphe reconstruction. The disadvantage of this flap is the limited surface area that it can provide. To overcome this limitation Kwon EO et al. used the tissue expander to increase the surface area. Muscular flaps had also been used. On example is the gracillis flap which is thin and thus advantageous in scrotal reconstruction. For us, the fasciocutaneous medial thigh flap has the advantage of not sacrificing a functional muscle. Burying the testes in the medial thigh pocket is a simple technique which immobilizes the testis and does not create a real scrotum. Split thickness skin grafts have been performed by many authors this seems to be the simplest procedure with 100% take of graft in some hands. Scar contracture seen in the early wound healing may get released with time. In our department we perform skin grafting when defects are covered with good granulating tissue after a period of wound care. We are of the opinion that skin grafted scrotums are not thick enough to withstand trauma. Random fasciocutaneous thigh flap seems to be a simple solution to most of the problems enumerated above.

We have not encountered any vascular complication in our pratice. Anecdotally, some authors have argued...
that random fascio-cutaneous thigh flap provides a thicker coverage resulting in reduced mobility of the testicles. However, that is not the observation in our practice, probably because of the post-infectious fat loss. Even after two years follow up, we did not see any fat increase.

Contrary to perforator flaps and free flaps (2, 3, 5, 8, 12, 13) that will require high surgical experience, special equipment, and post-surgical special nursing care, random pattern fasciocutaneous medial thigh flap has a short learning curve in the hands of young surgeons. It is less time-consuming and has low failure rate. Donor sites are closed primarily. It provides good tissue which imports new vascular supply to the scrotal area. This is advantageous in infection control. The neo-scrotum is pliable, has good skin colour and a thickness not more than 10 mm. However, it has the disadvantage of not being able to cover defects that are beyond the scrotal boundary.

Conclusion

The fasciocutaneous random pattern medial thigh flap is a good alternative for the coverage of the scrotal defect caused by infections. This flap in our experience showed good skin colour, pliability, and trophicity for the coverage of the testis. It is simple, time-saving, of low cost, and well appreciated by patients. In conditions of post-Fournier gangrene with testicular exposure, this flap can be suitable for young surgical teams when indication.

References

Lyme disease: calling for high index of suspicion – a case series

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Abstract

Lyme disease is a zoonotic infection caused by Borrelia burgdorferi, with protean manifestation affecting several systems at once. It is characterized by a typical skin lesion named erythema chronicum migrans (ECM). Patients with this lesion may also have headache, meningeal irritation, mild encephalopathy, multiple annular secondary lesions, malar or urticarial rash, generalized lymphadenopathy and splenomegaly, migratory musculoskeletal pain, hepatitis, sore throat, non-productive cough, conjunctivitis, peri orbital edema, or testicular swelling. With progression of this condition, some patients develop frank neurologic abnormalities such as meningitis, encephalitis, cranial neuritis (including bilateral facial palsy), motor or sensory radiculoneuritis, mononeuritis multiplex, or myelitis. Cardiac involvement includes AV block, acute myopericarditis, cardiomegaly, or pancarditis. Also, migratory musculoskeletal pain in joints, tendons, bursae, muscle, or bone are common manifestations in this condition. Ticks of the genus Ixodes are the vectors for this condition. The diagnostic evaluation for Lyme disease is not easily available in India as in many other developing countries and this may explain why it is easily missed in many cases. We highlight two cases of serologically confirmed Lyme disease in two male children of different origins presenting with serial facial nerves palsy as well as multiple nerves paresis without any other cutaneous or systemic manifestation.

Introduction

Earliest medical literature on Lyme disease (borreliosis) attributed the first description to Dr Alfred Buchwald in 1883. It was however re-discovered by other physicians in 1909, 1922, 1941 and 1975. It was named after Dr Willy Burgdorferi who carried out extensive research on the organism in the year 1981. Borrelia burgdorferi is a Gram negative, anaerobic, pleomorphic, spiral-shaped, intracellular, bacterium with more than 12 months life cycle and more than 300 strains. It causes a systemic infection with manifestations caused by the interaction between the spirochete Borrelia burgdorferi and the body’s immune response to the organism. The spirochetes are inoculated into the skin by a tick bite, from ticks of the genus Ixodes. It is endemic in North America, Europe, and some parts of Asia with temperate climate.

Lyme disease is a multi-systemic inflammatory disease. If left untreated it can be devastating. The bacterium has developed exceptional survival mechanisms. The bacterium can duplicate itself rapidly and can hide from the body’s immune system by mimicking healthy cells.

Several systems of the body can be affected. The skin, lymph nodes, spleen, lungs, bones, heart are all not spared from the effect of the disease. Nervous system involvement, cranial nerve palsy especially the seventh may be commonly seen as part of other manifestations and the occurrence of this as the sole manifestation has also been documented. Involvement of the recurrent laryngeal nerve may be associated with feeding and breathing difficulty.

In India, only few regional surveys of prevalence studies have been done and it is found to be quite uncommon in the tropical climate. No similar prevalence study has been done in Nigeria on this disease condition probably due to the tropical nature of the region.

We hereby present these two cases to highlight the need for a strong index of suspicion of Lyme disease in...
children presenting with serial facial nerve palsies and with stridor.

**Case 1**

JJ was an eleven-year-old male child resident in Chennai, (South East coast of India) who presented with a week’s history of inability to close the right eye and deviation of the angle of the mouth to the left. No history of fever or associated headache, blurring of vision or double vision. There was no irritability, or stiff neck. No history of personality change or depression. There was no weakness of any of the extremities. No history of rash. There is no history of pain, numbness, or tingling sensation. No visitation to any zoological garden or forest. There was no significant past medical or antenatal history. The child was the first of two children.

Examination revealed no other abnormality except the features of right sided facial nerve palsy of lower motor neuron type. A diagnosis of right Bell’s palsy was made and the child was commenced on steroids and gastro-protective measures.

He improved significantly but re-presented six weeks later with inability to close the left eye and deviation of the angle of the mouth to the right side. No other symptom was also reported. Examination now revealed only features of left sided facial nerve palsy of lower motor neuron type. Differential Diagnoses of Lyme disease, Acute Demyelinating Encephalo-Myelitis (ADEM), and Tuberculous meningitis, were made. Investigations requested included: Lyme serology, MRI brain with contrast and complete blood count.

The brain MRI was normal, the complete blood count was also normal, the Lyme serology was abnormal with very high positivity (91.6U/mL) for serum *Borrelia burgdorferi* IgG, and high positivity (62.2U/mL) for serum *Borrelia burgdorferi* IgM.

He was commenced on Amoxycillin for four weeks following the review with the results. The child is presently stable and the palsy has resolved. (Consent for clinical picture was refused)

**Case 2**

AS, an eleven-year-old male Omani who presented with a two-month history of inability to close both eyes well as well as lack of facial expression. The child finds it impossible to puff his cheeks. No history of facial asymmetry, no significant swallowing difficulty and no history of tremors or unsteady gaits. He is a known Asthmatic on medications as required. He produced a stridulous sound when he attempted to rise from the sitting or lying posture. No cough, no wheeze. No history of fever or blurring of vision. There was no irritability, or stiff neck. No history of personality change or depression. No other symptoms referable to other systems. No history of exposure to any domestic animal or visitation to a zoo in the last three years.

He has presented at various hospitals in Oman since the onset of the illness and several investigations have been carried out and drugs including steroids have been given with no significant improvement resulting in presentation to our institution.

He was the second of 6 children in a non-consanguineous family. No similar history in the siblings.

Examination revealed a conscious child with no facial expression. The child was unable to puff his cheeks as well as unable to close both eyes well. Stridor was heard with exertion such as standing up from sitting position, or coughing.

Laryngoscopy by an ENT surgeon showed bilateral paresis of the vocal cords.

Other investigations requested included MRI brain with contrast which was normal. Repetitive nerve stimulation test for both facial nerves showed bilateral absence of facial nerve stimulation, CBC, LFT, calcium and magnesium were all normal. Lyme serology was highly positive for IgM (90.3U/mL) and negative for IgG (5.3 U/ml).

He was commenced on Amoxycillin (500mg t.d.s) for six weeks and subsequently improved on follow-up.

**Discussion**

The age of the two patients happened to be eleven years and the condition is said to be commoner amongst boys in this age range than girls. It has also been reported that about 25% of cases occur in children younger than 14 years and this has been attributed to
increased levels of outdoor activity and environmental exposure in patients in this age group rather than any intrinsic difference in susceptibility.4 Persons with increased occupational, recreational, or residential exposure to tick-infested woods or fields (the preferred habitat of ticks) in endemic areas are at increased risk of developing Lyme disease7,8,13 but none of the children had such history.

The skin is the initial target of infection by this organism as the inflammation it induces leads to the development of the characteristic rash, erythema migrans.1,2,4,6 Neither of the patients presented had history of a single cutaneous manifestation.

The typical Lyme disease has a protean manifestation affecting many systems that include skin, joints, muscle, central nervous system, eyes and the heart and the presentation could be in form of early localized disease, early disseminated disease and late or chronic Lyme disease.2,6 These children did not fit into any of the descriptions and that again could have made a diagnosis of Lyme disease unthinkable in them, thus the diagnosis of the second child was missed by many Paediatricians and Family Physicians in Oman before the presentation of the child to our institution.

The serial lower motor neuron palsy of the facial nerves one after the other was the sole clinical manifestation in the first child, while the second child had bilateral involvement of the facial nerves and recurrent laryngeal nerves. These are rare manifestation being the sole complaints in these children.

Conclusion

These cases are intended to heighten the index of suspicion of Lyme disease in a child found to have facial or other nerves palsy or paresis in the absence of any other cutaneous or systemic manifestation. Lyme disease may be more common than it is thought to be even in the tropical climate.11-12 If our indices of suspicion are higher than what is presently obtained, we may pick up quite a few cases. Also, if institutions capable of diagnosing the condition are more freely available, the real magnitude of the condition and its peculiar variation in manifestation can be better appreciated.

References

5. Praharaj AK, Jetley S, Kalghatgi AT. Seroprevalence of Borrelia burgdorferi in North Eastern India. MJAFI 2008; 64; 26-28
Hyponatraemia : A case study

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Abstract

Hyponatraemia is a diagnosis that is not commonly made. This is because of the analytic and diagnostic challenges usually encountered in water and sodium balance. However, careful assessment and skillful interpretation of results can be of immense help. We report a case of Hyponatraemia whose prompt diagnosis helped in the management of a case with positive result. The hospital notes of the patient and the literature on Hyponatraemia were reviewed. Management modalities for this case in question and for the generality of cases show the need for adequate knowledge and corrective action. It is necessary to promptly identify cases of hyponatraemia.

Keywords: Hyponatraemia, Plasma and urinary osmolality

Introduction

Hyponatraemia generally is defined as a plasma sodium level of less than 135 mEq per L (135 mmol per L). This electrolyte imbalance is encountered commonly in hospital and ambulatory settings. The results of one prevalence study in a nursing home population demonstrated that 18 percent of the residents were in a hyponatraemic state, and 53 percent had experienced at least one episode of hyponatraemia in the previous 12 months. Acute or symptomatic hyponatraemia can lead to significant rates of morbidity and mortality. Mortality rates as high as 17.9 percent have been quoted, but rates this extreme usually occur in the context of hospitalized patients. Morbidity also can result from rapid correction of hyponatremia. Because there are many causes of hyponatraemia and the treatment differs according to the cause, a logical and efficient approach to the evaluation and management of patients with hyponatraemia is imperative.

Sodium is the most abundant extracellular cation and, with its associated anions, accounts for most of the osmotic activity of the extracellular fluid (ECF). Water is an essential body constituent, and homeostatic processes are important in the maintenance of total body water within narrow limits. The distribution of water between the vascular, interstitial, and intracellular compartments is also maintained. Thin homeostasis depends on hydrostatic and osmotic forces acting across the cell membrane.

In a 70kg man, the total body water (TBW) is about 42L and contributes about 60 per cent of the total body weight, there are approximately 3000mmol of sodium mainly in the ECF as shown in Table1. Notably, water and electrolyte intake and output usually balance in urine, faeces, sweat and exhaled air.

Clinical Signs and Symptoms

Most patients with hyponatraemia are asymptomatic. Symptoms do not usually appear until the plasma sodium level drops below 120 mEq per L (120mmol per L) and usually are nonspecific (e.g., headache, lethargy, nausea). In cases of severe hyponatraemia, neurologic and gastrointestinal symptoms
predominate. The risk of seizures and coma increases as the sodium level decreases. The development of clinical signs and symptoms also depends on the rapidity with which the plasma sodium level decreases. In the event of a rapid decrease, the patient can be symptomatic even with a plasma sodium level above 120 mEq per L. Poor prognostic factors for severe hyponatraemia in hospitalized patients include the presence of symptoms, sepsis, and respiratory failure.

Table 1: Approximate osmolality contribution of solutes in plasma

<table>
<thead>
<tr>
<th>Osmolality (mmol/kg)</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium and anions</td>
<td>270</td>
</tr>
<tr>
<td>Potassium and anions</td>
<td>7</td>
</tr>
<tr>
<td>Calcium and anions</td>
<td>3</td>
</tr>
<tr>
<td>Magnesium and anions</td>
<td>1</td>
</tr>
<tr>
<td>Urea</td>
<td>5</td>
</tr>
<tr>
<td>Glucose</td>
<td>5</td>
</tr>
<tr>
<td>Protein</td>
<td>1(approx.)</td>
</tr>
<tr>
<td>Total</td>
<td>292(approx.)</td>
</tr>
</tbody>
</table>

Case Study

K.C.A, a 68-year-old man with bronchiectasis was admitted to the hospital with a three week history of a productive cough with greenish sputum. He became confused about three days before the transfer to the teaching hospital.

On examination, he was well oriented in time, place, and person but with slow mental responses. His blood pressure was normal. His hydration status was normal. There was widespread coarse crepitation in the lungs. No finger clubbing.

The following results were obtained on electrolytes and urea estimation:

Plasma | Reference range
---|---
Creatinine | 65nmol/L | 55 – 110
Urea | 24mmol/L | 25 – 70
Sodium | 123mmol/L | 135 – 145
Potassium | 4.1mmol/L | 3.5 – 5.0

1. Clinical interpretation of biochemical results. Confusion, ataxia, dysarthria, and apathy can all be attributed to cerebral cellular over-hydration secondary to hyponatraemia. However, a plasma sodium concentration of 123mmol/L is not usually associated with many symptoms. The risk of developing clinical symptoms and signs of hyponatraemia is related more to the rate of fall rather than to its actual or absolute level.

2. Causes of hyponatraemia are shown in table 2. In this case the following causes should be considered.

Table 2: Some causes of hyponatraemia

<table>
<thead>
<tr>
<th>Without plasma hypo-osmolality</th>
<th>With plasma hypo-osmolality</th>
</tr>
</thead>
<tbody>
<tr>
<td>With ECF volume depletion</td>
<td>With undetectable increase in ECF volume</td>
</tr>
<tr>
<td>With ECF volume expansion</td>
<td>With ECF volume depletion</td>
</tr>
<tr>
<td>Pseudohyponatraemia</td>
<td>Diabetes Mellitus</td>
</tr>
<tr>
<td>Diuretics</td>
<td>Syndrome of inappropriate ADH secretion(SIADH)</td>
</tr>
<tr>
<td>Renal failure</td>
<td>Cardiac failure</td>
</tr>
<tr>
<td>Salt-losing Nephritis</td>
<td>Renal failure</td>
</tr>
<tr>
<td>Adrenal insufficiency</td>
<td>Nephritic syndrome</td>
</tr>
</tbody>
</table>

Hyponatraemia with normal plasma osmolality.
Pseudohyponatraemia caused by analytic interference by high concentrations of lipid or protein. Because the ECF osmolality is normal there is no movement of water into the intracellular compartment.

Hyponatraemia with increased plasma osmolality.
Appropriate hyponatraemia due some causes, for example, to hyperglycaemia. This is a dilutional hyponatraemia due to the movement of water out of the cellular compartment along the osmotic gradient generated by extracellular hyperglycaemia. A similar situation can occur in patients with uraemia despite a high proportion of functioning glomeruli.

Hyponatraemia with decreased plasma osmolality.
Diuretics (thiazide and loop diuretics). These diuretics inhibit renal tubular sodium, and therefore water reabsorption. Consequently, ECF volume is reduced which, by stimulating antidiuretic hormone (ADH) release, increases water reabsorption and causes a dilutional hyponatraemia.

Adrenocortical hypofunction, due to primary adrenal hypofunction in which hyponatraemia is a consequence of acute ECF volume depletion. ADH release and subsequent water reabsorption; secondary adrenal hypofunction, in which aldosterone secretion is normal.
but cortisol deficiency results in dilutional hyponatraemia because of reduced renal water excretion.

**Severe primary hypothyroidism.** Associated with a dilutional hyponatraemia due to increased ADH secretion.

**Inappropriate ADH secretion.** This diagnosis should be considered after the exclusion of other causes of hyponatraemia (Figure 1). Hyponatraemia may occur in any severe illness. However, in this syndrome, ADH is secreted inappropriately, either from ectopic sites or from the posterior pituitary gland, in conditions where its secretion would be normally suppressed by the low plasma osmolality (Table 3).

3. The following additional investigations were requested:

   - Plasma osmolality and calculated osmolarity. The plasma osmolarity can be calculated using the formula,
     \[
     \text{Osmolarity} = 2[(\text{Na}^+) + (\text{K}^+)] + [\text{Urea}] + [\text{Glucose}]
     \]
     where all the units are mmol/L.

**Figure 1:** Some cause of hyponatraemia that must be excluded before a diagnosis of SIADH secretion is made.

<table>
<thead>
<tr>
<th>Any severe illness that may cause SIADH</th>
<th>Oedematous disorders</th>
<th>Endocrine disorders</th>
<th>Drugs that impair water excretion eg. diuretics, psychotropic drugs,</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Hypovolaemia</td>
<td>• Cardiac failure</td>
<td>• Hypothyroidism</td>
<td></td>
</tr>
<tr>
<td>• Renal failure</td>
<td>• Liver cirrhosis</td>
<td>• Adrenal insufficiency</td>
<td></td>
</tr>
</tbody>
</table>

**Table 3:** Some disorders which may be associated with inappropriate ADH secretion

<table>
<thead>
<tr>
<th>Neoplasia</th>
<th>Pulmonary disorders</th>
<th>Neurological disorders</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bronchogenic carcinoma</td>
<td>Pneumonia</td>
<td>Meningitis</td>
</tr>
<tr>
<td>Islet cell tumours(Pancreas)</td>
<td>Tuberculosis</td>
<td>Head injury</td>
</tr>
<tr>
<td>Lymphoma</td>
<td>Pneumothorax</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Positive pressure ventilation</td>
<td>Acute intermittent porphyria</td>
</tr>
</tbody>
</table>

If the measured plasma osmolality is normal but the calculated osmolarity is low, and if there is no unmeasured solute such as ethanol, ethyl glycol, mannitol, pseudohyponatraemia due to an increased concentration of either protein or lipid is the most likely cause.

Urinary osmolality. In ADH-induced hyponatraemia, plasma osmolality is low and urinary osmolality inappropriately high. If the kidneys are functioning properly the following diagnostic criteria must be satisfied before a diagnosis of hyponatraemia due to inappropriate ADH secretion is made:

- Absence of signs of volume depletion or oedema,
- Normal renal, adrenal, and thyroid function,
- Hyponatraemia and a low plasma osmolality,
- Inappropriately high urinary osmolality when compared with that of plasma, and
- High urinary sodium concentration.

Urinary sodium concentration: If it is less than about 10mmol/L, ECF volume depletion due to nonrenal fluid loss must be considered. It is normally greater than 20mmol/L in diuretic-induced hyponatraemia, adrenocortical insufficiency or conditions associated with inappropriate ADH secretion.

Plasma glucose concentration; The plasma sodium concentration may fall due to dilution, as the plasma glucose concentration, and therefore osmolality, rises provided that adequate fluid intake is maintained.

Thyroid function tests [plasma TSH, and free or total T₄ concentrations]; It is necessary to exclude hypothyroidism because the patient presented with some clinical features of hypothyroidism.

Later results

<table>
<thead>
<tr>
<th>Plasma</th>
<th>Reference range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creatinine</td>
<td>63µmol/l</td>
</tr>
<tr>
<td>Urea</td>
<td>23mmol/L</td>
</tr>
<tr>
<td>Sodium</td>
<td>122 mmol/L</td>
</tr>
<tr>
<td>Potassium</td>
<td>4.1 mmol/L</td>
</tr>
<tr>
<td>Glucose</td>
<td>3.8 mmol/L</td>
</tr>
<tr>
<td>Osmolality</td>
<td>260mmol/Kg</td>
</tr>
<tr>
<td>Calculated Osmolality</td>
<td>258 mmol/L</td>
</tr>
<tr>
<td>Total T₄</td>
<td>65 mmol/L</td>
</tr>
<tr>
<td>TSH</td>
<td>1.4mU/L</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Urine</th>
<th>Reference range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Osmolality</td>
<td>560mmol/Kg</td>
</tr>
<tr>
<td>Sodium</td>
<td>56 mmol/L</td>
</tr>
</tbody>
</table>
Comment on laboratory results: these results satisfy the diagnostic criteria for hyponatraemia due to inappropriate ADH secretion. The urinary osmolality is inappropriately high for the relatively low plasma osmolality. Low-normal plasma urea and creatinine concentrations indicate that the patient is well hydrated and has normal renal function. The urinary sodium concentration is appropriate for a normovolaemic subject. We ruled out adrenocortical insufficiency because of the presentation and the normal plasma potassium concentration.

Prognosis: Haemophilus influenzae was cultured from the patient’s sputum and appropriate antibiotics were given. Fluid intake was restricted to about 800ml per 24hoursium concentration. After two days he developed a negative water balance and the plasma sodium concentration and osmolality rose to within the reference range. After 7 days his mental function improved significantly.

<table>
<thead>
<tr>
<th>Plasma</th>
<th>On admission</th>
<th>Day 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creatinine</td>
<td>64</td>
<td>70</td>
</tr>
<tr>
<td>Sodium</td>
<td>122</td>
<td>137</td>
</tr>
<tr>
<td>Potassium</td>
<td>4.0</td>
<td>4.5</td>
</tr>
<tr>
<td>Osmolality</td>
<td>261</td>
<td>292</td>
</tr>
</tbody>
</table>

Comment: The management of symptomatic hyponatraemia is to treat the underlying disorder and to restrict water intake. A water diuresis can be drug-induced with drugs like demeclocycline, which causes nephrogenic diabetes insipidus by inhibiting the action of ADH on the renal collecting ducts.

The final diagnosis was inappropriate ADH secretion due to bronchopneumonia in a patient with bronchiectasis.

General Treatment

The treatment of hyponatraemia can be divided into two steps. First, the physician must decide whether immediate treatment is required. This decision is based on the presence of symptoms, the degree of hyponatraemia, whether the condition is acute (arbitrarily defined as a duration of less than 48 hours) or chronic, and the presence of any degree of hypotension. The second step is to determine the most appropriate method of correcting the hyponatraemia. Shock resulting from volume depletion should be treated with intravenous isotonic saline.

Acute severe hyponatraemia (i.e., less than 125mmol per L) usually is associated with neurologic symptoms such as seizures and should be treated urgently because of the high risk of cerebral edema and hyponatraemic encephalopathy. The initial correction rate with hypertonic saline should not exceed 1 to 2mmol per L per hour, and normo/hypernatraemia should be avoided in the first 48 hours.

In patients with chronic hyponatraemia, overzealous and rapid correction should be avoided because it can lead to central pontine myelinolysis. In central pontine myelinolysis, neurologic symptoms usually occur one to six days after correction and often are irreversible. In most cases of chronic asymptomatic hyponatraemia, removing the underlying cause of the hyponatraemia suffices. Otherwise, fluid restriction (less than 1 to 1.5 L per day) is the mainstay of treatment and the preferred mode of treatment for mild to moderate SIADH. The combination of loop diuretics with a high-sodium diet may be required to achieve an adequate response in patients with chronic SIADH.

In patients who have difficulty adhering to fluid restriction or who have persistent severe hyponatraemia despite the above measures, demeclocycline (Declomycin) in a dosage of 600 to 1,200 mg daily can be used to induce a negative free-water balance by causing nephrogenic diabetes insipidus. This medication should be used with caution in patients with hepatic or renal insufficiency.

In patients with hypervolaemic hyponatraemia, fluid and sodium restriction is the preferred treatment. Loop diuretics can be used in severe cases. Hemodialysis is an alternative in patients with renal impairment.

References

Case Report


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Abstract

Subdural empyema can originate from contiguous infectious sites. Acute sinusitis in children and adolescents and its complications are rare. They can be extremely severe and cause high morbidity and mortality. Because of their rarity, they are often not identified early, hence most patients tend to have an unfavourable outcome. Cranial Computed Tomographic diagnosis of a 14-year-old boy with left Subdural Empyema complicated by bilateral maxillary and ethmoidal sinusitis is reported.

Key words: Subdural Empyema, Sinusitis, Computed Tomography

Introduction

Subdural empyema is a focal accumulation of pus in the subdural space; between the arachnoid and dura mater1. Abscess in the subdural space usually arises as a complication of nearby infection in the paranasal sinuses, the middle ear in paediatric patients, tooth infection, meningitis or from distant spread from sites such as pulmonary infection and less commonly, it can follow a penetrating wound1,2,3,4. It can arise also as a complication of therapeutic procedures e.g. intracranial monitoring, craniotomy and after drainage of a subdural hematoma5.

Paranasal sinus infections are very common. Most cases can be treated without complications. However, rare life threatening intracranial complications can occur5,6. Generally intracranial complications progress rapidly and can cause meningismus, focal neurological disorder, loss of consciousness and occasionally death.2,4Subdural empyema represents 13 – 20% of all intracranial suppuration3. Almost 80% of subdural empyema occurs over the convexities and 12% in the interhemispheric fissure3. It is most often seen in pediatric patients, majority of whom are males with a male: female ratio of 3:1. The age between 10 – 30 years, with a mean age of 16.9 years are mostly affected5,6.

The diagnostic modality of choice for intracranial subdural empyema is Computed Tomographic (CT) scanning and Magnetic Resonance Imaging with contrast enhancement. Proper selection of antimicrobial agents with good penetration of the central nervous system after surgical drainage is critical for the management of this life threatening condition.

The aim of this case presentation is to emphasize the importance of neuroradiological investigative modalities in the diagnosis, treatment, follow up and prognosis of subdural empyema.

A Case Report

Master OI, a 14-year-old male presented at the Children’s Emergency Unit with complaints of headache, fever and vomiting of two-week duration.

The symptoms started two weeks prior to presentation with left-sided frontal headache which was
throbbing, continuous, associated with double vision, photophobia, fever, and vomiting. There was no associated neck stiffness, cough, or catarrh. The fever was intermittent, worse in the evenings, and associated with chills and rigors. There was no seizure, sore throat, or ear discharge. Vomiting occurred 3–4 times daily, and contained recently ingested food vomitus was not bloody, non–projectile and became associated with reduced appetite and generalized body weakness and pain.

At the onset of illness, the patient was admitted in two private hospitals and treated for malaria fever and typhoid fever, but there was no improvement.

At admission in ISTH, on physical examination, the patient was acutely ill-looking. He was conscious and alert, afebrile, not pale, not jaundiced, mildly dehydrated and lethargic. His neck was supple, and Kernig’s sign was negative.

He was subsequently admitted and an impression of poorly treated Malaria was made with a differential diagnosis of enteric fever. Treatment with broad spectrum antibiotics (Ceftriaxone and Metronidazole) was commenced Two days later there was purulent discharge from both eyes with altered sensorium and subsequently he became unconscious with a Glasgow coma scale score of less than 8. An impression of an intracranial space occupying lesion was made with a differential diagnosis of meningitis. Lumbar puncture yielded clear, colourless fluid. Microscopy cerebrospinal of the fluid showed red blood cells less than 5, Gram stain and culture yielded no organisms.

An emergency cranial CT scan done showed an extensive concavo-convex hypodense lesion in the left frontoparietal region extending to the temporal lobe with significant mass effect and effacement of the ipsilateral lateral ventricle (Fig. 1). Post contrast injection, there was marginal enhancement of the lesion (Fig. 2). Also noted were bilateral opaque maxillary and ethmoidal sinuses suggestive of sinusitis (Figs. 3 and 4). There were no calvarial fractures. A diagnosis of extensive subdural empyema with significant mass effect and bilateral maxillary and ethmoidal sinusitis was made.

Emergency exploratory craniotomy was done and the finding was a bulging and tense dura containing frank subdural pus with Escherichia coli odour. The adjacent cerebral cortex was normal but edematous with no intracerebral extension of pus. 70mls of frank pus was aspirated. The displaced cortex spontaneously returned as the pus was evacuated. The patient’s postoperative condition was satisfactory. The aspirate was sent for, culture and sensitivity, but yielded no growth, presumably because the patient had been on antibiotic therapy; but microscopy and Gram staining revealed pus cells and Gram-positive cocci.

Fifth day post operation, the patient developed temperature spikes, became drowsy, was slipping in and out of consciousness. His scalp was edematous. There was a fluctuant swelling along the suture lines in the region of the anterior fontanelle which were scalp abscesses from reaccumulation of the subdural abscess. These were drained but patient’s level of consciousness did not improve. The next day he became deeply unconscious (Glasgow coma scale score of <5) and died five hours later presumably from septic shock.

Discussion

Subdural empyema is a focal accumulation of pus in the subdural space; between the arachnoid and dura mater. Abscess in the subdural space usually arises as a complication of nearby infection in the paranasal sinuses, the middle ear in paediatric patients, tooth infection, meningitis or from distant spread from sites such as pulmonary infection. Less commonly, follows a penetrating wound, bullet injury. It can arise also as a complication of therapeutic procedures e.g. intracranial monitoring, craniotomy and after drainage of subdural hematoma.

Paranasal sinus infections are very common. Most cases can be treated without complications. However, rare life threatening intracranial complications can occur. Generally intracranial complications progresses rapidly and can cause meningismus, focal neurological disorder, and loss of consciousness and occasionally death.

Subdural empyema represents 13 – 20% of all intracranial suppuration. Almost 80% of subdural empyema occurs over the convexities and 12% in the interhemispheric fissure. It is most often seen in pediatric patients, majority are males with a male:

Fig. 2: Axial cranial computed tomographic scan (post – contrast) showing rim enhancement of the concavoconvex hypodense area in the left frontoparietal region (arrow).

Fig. 3: Axial cranial computed tomographic scan (bone window) showing bilateral maxillary increased densities with air-fluid levels (arrows) more marked on the right suggestive of sinusitis.

Fig. 4: Axial cranial computed tomographic scan (bone window) showing right ethmoidal increased opacity suggestive of sinusitis (arrow)
female ratio of 3:1. The ages between 10 – 30 years, with a mean age of 16.9 years are mostly affected5-6.

There are three types of subdural empyema: supratentorial, infratentorial with co-existing cerebellar abscess and infratentorial without co-existing cerebellar abscess 7,8.

The clinical presentation of subdural empyema is variable and there are no pathognomonic features9. The clinical features could be confused with those of a tumor or any other intracranial space occupying lesion7. Thus, a high clinical index of suspicion must always be maintained since symptoms may be masked by previous antibiotic therapy. Tsai et al6 documented that the three most frequently encountered clinical features include fever (79%), disturbed consciousness (58%) and seizures (54%). Our patient presented with headache, fever, and vomiting. Determination of the point of entry and source of infection is paramount to adequate treatment. The source of infection in this patient was bilateral ethmoidal and maxillary sinusitis. Thrombophlebitis often associated with paranasal sinusitis is considered to be the main route of intracranial spread of disease10.

The most common pathogens in intracranial subdural empyema are anaerobic and microaerophilic streptococci, in particular those of the Streptococcus melleri group. Staphylococcus aureus is present in a minority of cases and multiple additional organisms including Gram – negative organisms such as Escherichia coli and anaerobic organisms such as Escherichia coli and anaerobic organisms such as Bacteroides, may be present. Pseudomonas aeruginosa or Staphylococcus epidermidis may be present in cases related to a neurological procedure3. No organisms were isolated in the aspirate from our patient possibly because of previous antibiotic therapy.

Subdural empyema may cause rapid compression of the brain and represents an extreme medical and neurosurgical emergency. Thus prompt diagnosis of this disease condition is mandatory. The diagnostic procedure of choice is cranial Computed Tomography (CT) scan which was done for our patient. The Subdural empyema is seen as a spindle shaped (concavoconvex) area of reduced attenuation adjacent to the skull vault or falx, whose margins enhance markedly11. The spindle shape of the empyema on CT is determined by the confining boundaries of neovascularisation in the abscess capsule and compression of cerebral tissues on the other1. The overlying bone may largely obscure small lesions. When subdural empyemas are adjacent to the tentorium, coronal sections may be indicated to show whether the lesion is supra or infratentorial11. Serial CT scans on treatment can also be done.

MRI with gadolinium enhancement is another diagnostic procedure. Apart from identifying infectious brain lesions, it can also differentiate subdural effusions or cystic brain tumours from subdural empyema. It can be used in follow – up of cases postcraniotomy/burrhole11.

MRI is advantageous over CT scan in cases where the empyema is difficult to see due to overlying bone or in the early stages when they are relatively small3,11,12. Coronal MRI readily detects them. Subdural empyema appears as a spindle shaped (concavoconvex) more hypointense area than surrounding brain on T1-weighted images and on T2-weighted images, subdural empyema collection appears variably hyperintense with respect to normal brain11,12. Using diffusion weighted magnetic resonance imaging (DWI) and Apparent Diffusion Coefficient (ADC) maps, empyemas are hyperintense on DWI and show low signal intensity on ADC maps with an ADC value lower than that of the normal cortical gray matter11. DWI can be valuable also in distinguishing subdural empyema from effusion and in the follow-up of subdural collection11.

The method of treatment of subdural empyema is multiple burrhole, surgery evacuation of the pus and thorough irrigation of the subdural cavity with saline and antibiotic solution3,5. All infectious materials, whether purulent or granulation tissues must be removed and sent for microscopy, culture and sensitivity3. They are often multiloculated and care must be taken to make sure that all pockets are drained which can be confirmed by serial CT scans or MRI when it is available3. A single burrhole was done for our patient which may have resulted in poor drainage and reaccumulation of the subdural abscess. A repeat cranial CT scan was also not done before he died. Appropriate antibiotics like a combination of Penicillin or Cephalosporin, Metronidazole and an aminoglycoside or antibiotics that are sensitive to organisms isolated from aspirates should be continued
for 3 – 4 weeks. \(^3\)\(^9\)\(^{13}\). Our patient was on Ceftriaxone and Metronidazole, but could not survive the resultant overwhelming sepsis. However, with recent advances in diagnostic and therapeutic modalities, CT and MRI guided stereotactic aspiration of brain abscesses helps achieve all treatment goals. \(^14\) It drains the contents of the abscess, reduces mass effect, and confirms diagnosis. It is minimally invasive, carries minimal morbidity and mortality and can be performed under local anaesthesia. \(^14\)

Epileptic seizures and various neurological sequelae are common even when a subdural empyema is treated immediately. \(^2\)\(^4\). Delay in diagnosis and institution of therapy may lead to fatal outcome, thus his condition should be treated with great urgency. In this patient, the diagnosis of subdural empyema was made more than two weeks after he became symptomatic, and despite prompt intervention did not survive.

**Conclusion**

Acute bacterial sinusitis is common in the Pediatric population but intracranial complications are rare and are associated with significant morbidity and mortality. Clinical history and physical examination may not be sensitive in detecting the presence of bacterial sinusitis and its intracranial complications, thus necessitating neuro-radiological investigations.

**References**