DOKITA

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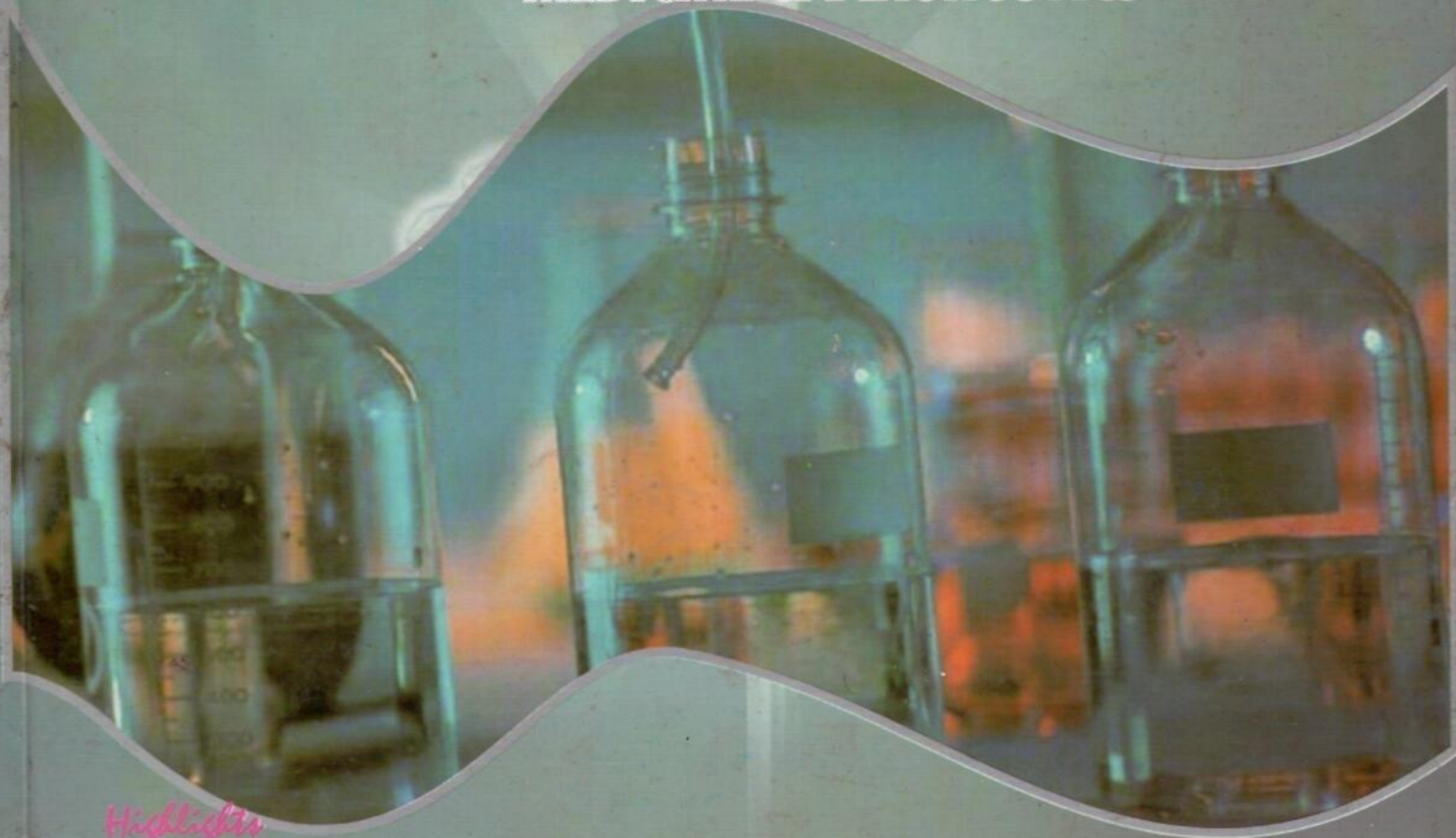
LABORATORY

MEDICINE & DIAGNOSTICS *edition*



Highlights

- Role of Papanicolaou Smear Screening in the Prevention and Control of Cervical Cancer
- Usefulness of D-dimer Assay in the Diagnosis of Venous Thromboembolism
- Antibiotic Resistance in the University College Hospital, Ibadan
- Safe Laboratory Practice and the Universal Precautions
- The use of Biomarkers in Research
  Assessing the Effectiveness of DOTS in the Treatment of TB in Igbo-Ora Community

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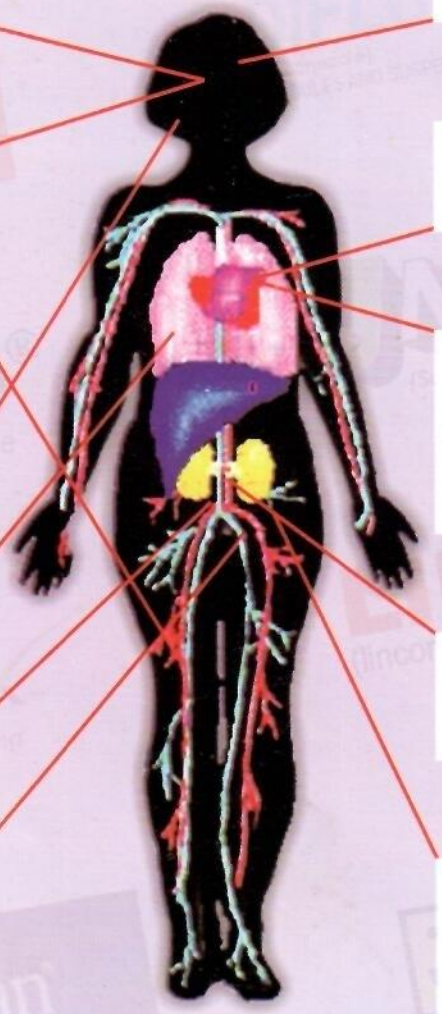

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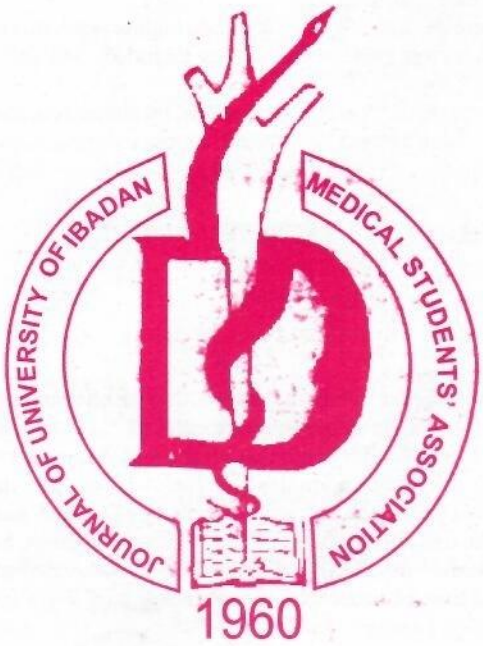
Vol. 31, Number 1 **March 2006**



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University College Hospital, Ibadan

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Original articles, reviews, case reports and other articles on any subject of medical interest are invited. Manuscripts and other communications should be sent to the Editor- in chief, **DOKITA** Editorial Board, Alexander Brown Hall, University College Hospital, Ibadan. Articles are accepted with the understanding that they are offered to this journal only and that articles and reproductions can only be made by permission of the Editorial board unless authors state before publication, that they reserve the right to themselves. Reprints can be purchased at reduced prices if authors would indicate their requirements- a minimum of fifty reprints at the same time of submission of the manuscripts.

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The requirements for submission of articles to **DOKITA** are in accordance with the "Uniform requirements for manuscripts submitted to biomedical journals" (the Vancouver style) as revised and published by the International Committee of Medical and Journal Editors in the British Medical Journal (BMJ 1991: 302:338-41). All papers should be submitted in duplicate original type script (no carbon copies). Double spacing with ample margins is desired throughout the text except for quotations. The manuscripts should have the following components each of which should begin on a new page in the following sequence:

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Below the abstract, list in alphabetical order three to eight key words for cross indexing using terms from the medical subject headings (MeSH) list of index medicus.

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References are numbered consecutively in the order in which they are first mentioned in the text. Arabic numerals in parenthesis are used. Abbreviations for journal titles should be those adopted by the *index medicus*. " Unpublished observations and "personal communications" may not be used as references but may be inserted (in parentheses) in the text. Papers accepted but not yet published may be cited with the journal designated and "in press" added. Use the style of examples given below. (Note the punctuation and spacing).

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The cover page shows laboratory flasks and a microscope reflected on the title. The concept is based on the central role the Microscope plays in Laboratory Diagnosis and the importance of the Laboratory flasks in diagnosis.

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EDITORIAL

The heights by great men reached and kept, Were not attained by sudden flight,
But they, while their companions slept, Were toiling upward in the night
Henry Wadsworth Longfellow (1807 - 1882)

DOKITA, with well over 45 years of unremitting existence remain the attainment of disciplined, diligent, and dedicated great men, whom while their companions slept, were toiling upward in the night; its publication being the primary function of the **DOKITA** Editorial Board. The journal was intended originally to serve as a medium for fledgling doctors training in this institution to whet their writing skills, as well as to publish scientific work done and original discoveries made at the University College Hospital, Ibadan as succinctly spelt out by the pioneer Editor-in-Chief, then Mr. Moses Ikechukwu Ilo, in the maiden edition of **DOKITA** where he wrote: *"whichever be the case, enough does happen in and emanate from this institution to need that reports be made, for our successors as a record of our findings, progress and ideas and to our contemporaries for record and exchange...for these, we present in the language of the Nigerian Populace,* **DOKITA**, *that through it, and in it we may arouse, terrify, teach, comfort and open our hearts to each other as brothers."*

This journal has enjoyed wide readership and carefully occupied its niche as the most relevant students' medical journal published south of the Sahara with the recognition of the World Health Organization. This recalls to mind the comment of Dr. Ian-Douglas-Wilson (Editor, LANCET Jan 1964) on **DOKITA**: "*Now that I have read the latest issue, I can only reiterate my congratulations- I don't myself know of a better student journal anywhere.*"

The 2005/2006 Board year was particularly short, and coupled with official leave of absences of Board members, the execution of the major programmes of the Board including the publication of this journal was not without hindrances. However, the toil of dedicated Board members, both day and night while their contemporaries slept, has altogether produced this must-read master-piece the ***"Laboratory Medicine & Diagnostics"*** edition. What is more, the wonderful production quality starting from the mesmerizing and scintillating cover through to the easy eye-lift prints of the overwhelming pool of knowledge all herald the substance of this edition.

The words ***"Laboratory Medicine & Diagnostics"*** will readily call to mind the various laboratory investigations that are carried out on patients for the purpose of screening, making a diagnosis, and monitoring the progress of treatment and/or progression of diseases among others. *"Diagnosis precedes treatment"* said the British Surgeon, Russel John Howard (1875 1942). This is true as exemplified by the following simple equations:

- Management = Diagnosis + Treatment;
- Diagnosis = Clinical evaluation + Laboratory evaluation

Physicians need quality laboratory testing support for the accurate diagnosis and cost-effective management of diseases. On occasion, when the clinical suspicion is strong, as in clinically overt hyperthyroidism in a young adult or with the presence of a rapidly growing thyroid mass, laboratory thyroid hormone testing simply confirms the clinical suspicion. However, some disease symptoms are subtle in presentation so that only biochemical testing or histopathologic evaluation can detect the disorder. However overt or obscure a patient's disease state may be, an open collaboration between the physicians and clinical laboratory scientists is essential for optimal, cost-effective management of the patient. Thus, the result of inaccurate diagnosis or erroneous report of laboratory tests is that of poor clinical management of patient and thus worsened progression of diseases and ultimately, death of untold number of patients. Does this justify Napoleon's assertion: *"You medical people will have more lives to answer for in the other world than even we Generals?"* May be, may be not. This illustrates how invaluable laboratory medicine has proved to be ranging from simple side-room laboratory tests to sophisticated investigations like biopsies for histological examination.

Previous editions of **DOKITA** have dealt with various aspects of medicine more than ever in the last two decades, for example the *"Oncology"* edition (1998); the *"40th Anniversary"* edition (2000); the *"Reproductive Health"* edition (2001); the *"Emergency Medicine"* edition (2003); and the *"Public Health"* edition (2005) lately. However, no edition has delved into laboratory medicine wholly; hence the Editorial Board fittingly decided to publish the *"Laboratory Medicine & Diagnostics"* edition of **DOKITA**.

The *"Laboratory Medicine & Diagnostics"* is a cornucopia of articles written by experts in various fields of laboratory medicine. Such articles as those on Gram Stain; Papanicolaou Smear Screening; D-dimer Assay; Monoclonal Antibodies; Safe Laboratory Practice; Urinalysis; and Biomedical Research Methodologies among others all reflects the wealth of knowledge contained in this publication. The winning essay of the 2006 edition of the Annual Professor J. A. Adeleye Essay Competition titled *"Health Sector Reforms in Nigeria: Meeting the Millennium Development Goals"* and the best student project in the Ibarapa Community Health posting have routinely been included. The "**DOKITA** *Extra"* section unquestionably will entertain the reader. Thus, this must-read *'magnum opus'* will prove to be educative, enlightening, and ultimately improve the reader's quality of patients' medical care.

Acknowledgment

In preparing this edition of **DOKITA** for publication, the Editorial Board received assistance from many quarters to whom we owe our gratitude. We would like to express our heartfelt appreciation to members of the Advisory Council and Editorial consultants, especially those of them who took up the appointment this Board year, for their immense contributions towards the publication of this edition. Many thanks to Professor E. E. U. Akang who took out time to edit *all* the articles published in this journal and for writing the foreword to this journal. Thanks to all consultants who proved very helpful in selecting and modifying the various journal topics.

I appreciate the invaluable input of the production manager, Mr. Ekanem Ekpenyong who bore the brunt of the production work and those of all the disciplined, dedicated, and diligent members of the Editorial Board, whose toil and labour, both day and night, have made possible the production of this edition, the capstone of all our achievements this Board year.

We as well appreciate the contributions of our various benefactors both towards the production of this journal and the execution of other programmes of the Board. Finally, my greatest appreciation goes to Almighty God who is ever faithful for his constant guidance and support throughout this Board year.

To members of the Editorial Board, I dedicate this "Psalm of Life":

Lives of great men all remind us We can make our lives sublime,
And, departing, leave behind us Footprints on the sands of time
Let us, then, be up and doing, With a heart for any fate;
Still achieving, still pursuing, Learn to labor and to wait.

Henry Wadsworth Longfellow (1807 - 1882)

Long Live **DOKITA** Editorial Board!

Salami Simpa Samuel
Editor-in-Chief,
March 2006

FOREWORD

THE ROLE OF LABORATORY MEDICINE IN CLINICAL PRACTICE AND BIOMEDICAL RESEARCH

Laboratory Medicine or Pathology is a conglomerate of medical specialties and subspecialties devoted to the study of disease, by employing laboratory techniques in order to determine the cause, pathogenesis, structural and functional changes that may be responsible for the clinical manifestations of disease, determine the cause of death and prevent evolution of disease. This process involves application of laboratory methods to tissues and fluids obtained from the human body. According to the slogan of the Royal College of Pathologists, "Pathology is the hidden science at the heart of modern medicine, vital for the diagnosis and clinical management of disease." Apart from being a crucial component in the undergraduate and postgraduate medical curriculum, laboratory medicine is the lynchpin of fundamental advances in medical knowledge and research. It is therefore apposite that an issue of **DOKITA** should be largely devoted to the subject of Laboratory Medicine.

Component disciplines of Laboratory Medicine include Haematology, Chemical Pathology, Immunology, Microbiology, Virology, Molecular genetics and Histopathology. The present issue of **DOKITA** contains thoughtfully selected papers from many of these different areas.

Dr. Fakunle and co-workers examine the usefulness of D-dimer Assay in the Diagnosis of Venous Thromboembolism (VTE). The most dreaded outcome of VTE is pulmonary embolism (PE). Six hundred and fifty thousand cases of PE occur annually in the USA and it is estimated that 42,418 cases occur annually in Nigeria. Mortality from PE is particularly high among surgical patients, as well as patients with cancer, infections and congestive cardiac failure. In view of the high morbidity and mortality from VTE, it is important that a reliable index for its early diagnosis is made available. D-dimer is one such candidate marker. This **DOKITA** review article compares enzyme-linked immunosorbent assay and newer latex agglutination methods of measuring D-dimer and points out limitations in its application to clinical diagnosis.

There is a review article on Safe Laboratory Practice and Universal Precautions written by Dr. John Anetor, a chemical pathologist. This paper identifies major hazards encountered in the clinical chemistry laboratory and highlights important universal safety precautions that will minimise occupation-related disease in the clinical laboratory. It is readily apparent that these same general principles, with minor modifications to suit the particular laboratory environment, are applicable to all clinical and research laboratories that handle human tissues or fluids for diagnostic or research purposes.

Another article by Dr. Arinola, the current Head of Chemical Pathology, University College Hospital Ibadan, reviews the Detection and Quantification Methods available in Immunology. This is a comprehensive article, which will serve not only the medical student, but also the medical doctor or laboratory scientist in the early formative phase of postgraduate research and training very well.

Dr. Adeola Raji and Professor Rasheed Bakare present a review article on the Gram stain. They discuss the historical background behind its development, describe the procedure (surely, every medical student intending to pass the Part 2 MBB/BDS examination must know this!) and outline practical clinical applications and limitations of Gram staining results.

From the Department of Pathology comes a review article by Drs. Odubanjo and Okolo on Surgical Pathology. Surgical pathology broadly concerns examination of biopsy specimens from living patients to assist definite histological diagnosis, choice of appropriate therapy, formulation of prognosis of ongoing disease processes, and monitoring outcome of therapy. As so lucidly explained by the writers of this article, the surgical pathology diagnosis is the outcome of a successful two-way communication between the surgeon and the pathologist. The medical student would do very well to cogitate upon the message of this article.

There is an article by Oyatokun and colleagues on the Role of Papanicolaou Smear Screening in the Prevention and Control of Cervical Cancer. This is a very important article, which highlights the pivotal role of cervical smear screening in the reduction of the incidence of cervical cancer, which is a persistent scourge in tropical countries such as Nigeria. There is the need for good political will, backed by increased health care funding by government, as well as adequate funding of research and treatment by national and international governmental and non-governmental agencies. In this regard, there have been a number of initiatives to alleviate the burden of cervical cancer by well-meaning agencies such as the Save Our Future Foundation of Dr. C.O Amotsuka (in collaboration with the University of Maryland Medical Centre) and more recently, the nationwide collaboration spearheaded by the MD Anderson Medical Centre in Texas.

Ajayi and co-authors examine the role of the medical practitioner in the investigation of gunshot injuries. As they state in their abstract, the spate of gunshot injuries has increased over the years, more so given the fragile socioeconomic and political clime of present day Nigeria. Gunshot injuries are being encountered increasingly in civilian medical practice. It therefore behoves every medical practitioner to be conversant with the approach to the disposition of such

medicolegal cases.

Two related articles have been contributed which at first glance may seem to be outside the mainstream of Laboratory Medicine, but which on closer perusal are enunciate principles that are fundamental to Laboratory Medicine practice and research. Dr. Adedapo has penned an article detailing what constitutes good laboratory practice, as defined by the World Health Organisation and Organisation for Economic Development, with particular reference to drug development. Another highly recommended paper is the article by Professor Adebamowo of the Department of Surgery, which discusses the role of biomarkers in research, with a slant to oncology, defining his primary area of specialisation.

Apart from the above reviewed articles, the present edition of **DOKITA** also has articles on topics as diverse as Down Syndrome and Congenital Rubella Syndrome, in addition to the usual entertaining mix of general articles. In summary, the Editorial team is to be congratulated on its presentation of erudite and highly stimulating articles that are germane and topical.

Professor E. E. U Akang,
Department of Pathology,
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SURGICAL PATHOLOGY - AN IMPORTANT DIAGNOSTIC TOOL IN CLINICAL PRACTICE

Okolo C. A* , Odubanjo M. O#

*At the time of writing, the authors * was a Consultant Pathologist and # a Senior Registrar at the Department of Pathology, University College Hospital, Ibadan.*

SUMMARY

Surgical pathology, which deals with examination of biopsy specimens for purpose of arriving at histological diagnosis, lies at the foundation of a sound clinical and surgical practice. The role of the surgical pathologist is critical in the management of many surgical and non-surgical diseases.
However, many clinicians lack adequate knowledge as to how surgical pathology can excellently complement their clinical practice; thus they fail to enlist the services of the surgical pathologist and when they do they provide inadequate clinical information and thus the quality of reports they get from the surgical pathologist fail to meet the very critical need of patient care.
A few illustrative cases have been highlighted to buttress the fact that surgical pathology is indeed an important tool in clinical practice.

Key Words:*Surgical Pathology, Surgical Pathologist, Surgical Biopsy, Clinician*

INTRODUCTION

Surgical Pathology is the arm of pathology that deals with examination of both gross and histological sections of specimens from living patients with a view to arriving at accurate diagnoses that will assist in patient management. The mainstay of surgical pathology is the examination of the specimens after fixation in formalin, embedding in paraffin and staining with Haematoxylin-Eosin.

The evolution of surgical pathology dates back to the 1870s when Carl Ruge and his associate Johann Vent of the University of Berlin introduced the surgical biopsy as an essential diagnostic tool in the surgical management of patients.[1]

THE SURGICAL PATHOLOGIST

Surgical pathology is carried out by a trained pathologist who does the gross description of the specimen, microscopic description and interpretation of the findings. General practitioners cannot assume this duty even though they are expected to have a basic knowledge of pathology.[2]

The surgical pathologist has the unique opportunity of bridging the gap between the beginning of a disease process and its end stages. He can correlate the initial stages of disease seen in specimens from living patients in the surgical pathology laboratory and make fundamental contributions not only to the management of the index patient but generally to the body of knowledge. He is able to correlate clinical findings with pathological changes and offer very useful advice on the biopsy received.[3]Microscopic evaluation can sometimes be subjective and it is therefore advisable that practising surgical pathologists do not work in isolation. Slide review and consultations with other pathologists are very important as audit tools.[4,5]Surgical pathology implies surgery but the modern surgical pathologist is closely affiliated with many branches of medicine. This includes all the surgical specialties, internal medicine, dermatology, neurology, diagnostic radiology, radiation therapy and medical oncology etc.

THE NON-PATHOLOGIST

Surgical pathology depends heavily on the input of clinicians and surgeons to facilitate correct and clinically relevant diagnosis by the pathologist. The clinician should also be fully aware of the potentials and limitations of this specialty.[3] Unfortunately many clinicians do not realize the handicap of the surgical pathologist in the absence of essential clinical data, surgical findings and type of surgery. Many clinicians also assume demographic information such as age, sex, date of collection of sample, site of biopsy and sometimes ward or clinic are not relevant to the surgical pathologist and thus they frequently omit these data in their request cards.

The request card for a surgical pathology specimen should ideally be filled by a clinician who is familiar with the case. Unfortunately, all too often this vital task is delegated to a medical student, house officer or nurse, who themselves do not have sufficient information about the patient or the surgical procedure.[3] Direct conversation between the surgeon/clinician and pathologist is always extremely helpful in surgical pathology.[4]

At the very least, an adequately filled request card containing clinical diagnosis and possibly differential diagnosis, clinical summary, surgical procedure and other specific information required, including the name and telephone number of the surgeon should be submitted

THE SURGICAL BIOPSY

The surgical biopsy as soon as it is taken should be preserved in the appropriate fixative. Formalin is the general duty fixative in this environment. The sample should be fixed in 10% formalin, which should be ideally nine times the volume of the specimen. Sadly and on a seldom note, biopsy specimen are sent to the laboratory in normal saline and sometimes in an empty container. Such samples will undergo autolysis and no

useful microscopic assessment can be made.

This writers have also encountered several clinicians in private practice who discard surgical biopsy specimens in the trash can claming the lesion to be insignificant, excised for cosmetic reasons, or clinically benign. This practice is wrong and amounts to professional negligence. All surgical biopsy specimens should be sent to the surgical pathology laboratory for evaluation, both for medico-legal reasons and for pathological diagnosis.

All surgical biopsy specimens should be properly identified and labelled e.g. for intestinal biopsies, the proximal and distal margins of resection should be properly identified, for soft tissue tumours; the margins of resection should be identified. The type of biopsy, whether incisional or excisional, and the method of obtaining it, whether Trucut, or transrectal or transurethral etc should be stated. In the event that the patient has had a previous biopsy with a histological report, it should be stated in the request card and the reference number quoted. Biopsies are also classified according to the instrument used to obtain them as cold knife, cautery, needle, or endoscopic. Of this, the one usually least suitable for microscopic interpretation is that obtained with a cautery, because this instrument chars and distorts the tissue and prevents proper staining.

THE SURGICAL PATHOLOGY REPORT

The surgical pathology report is an important medical document that should describe as thoroughly and concisely as possible all relevant gross and microscopic features of a case and interpret the significance for the clinician. Delivery of the report should be prompt and timely as delays negate the very essence of surgical pathology.

The report should be accurate and brief. To quote Richard Reed, "A competent pathologist is not simply a storage site for microscopic verbiage. It is not enough to be able to recite by heart the microscopic findings once the clinical diagnosis is established. The ability offer clinical differential diagnoses from the interpretation of microscopic findings is the mark of the mature surgical pathologist. In addition, he may record the data that are prognostically significant or offer suggestions for pertinent clinical tests. The ability to recognize cytological and histological features is simply a beginning. The ability to integrate microscopic findings into a meaningful interpretation is the distinguishing characteristic of a pathologist and is the art of pathology".

LIMITATIONS OF SURGICAL PATHOLOGY

It is important for both surgical pathologists and clinicians to realize the limitations of this specialty. This fact has been expressed by Dr. Oscar N. Rambo in an article titled "The Limitations of Histological diagnosis."

"Pathologists are physicians and human beings. They have as great a capacity for error and susceptibility to subjective distractions as other practitioners of the art of medicine due to certain nineteenth century dogmas and because the teaching of pathology used to be relegated primarily to the long forgotten pre-clinical phase, pathologists traditionally have been regarded to be more scientific than many of their colleagues. A mystic perversion of this assumption prevails among those clinicians who believe that the pathologists, given only a piece of a patients tissue has all of the other ingredients necessary to produce a statement of absolute truth at the end of his report. More dangerous to humankind is a pathologist with the same concept.

Incomplete communication between the clinician and pathologist may make diagnosis difficult or impossible. To perform intelligently, a consultant must know all the facts that have any bearing on the case. To render a diagnosis from an inherent puzzling bit of tissue with only vague knowledge of its source and no concept of the clinical problem is as foolhardy as to undertake an appendectomy based on hearsay evidence that the patient has a pain in his belly.

As an off-duty exercise, pathologists frequently like to play games with slides as 'pure unknowns'. Sometimes with their brains and microscopes, they can give a remarkably accurate reconstruction of the disease process, pronounce the exact diagnosis and flush with pride at the awed applause of those gathered around the optical altar. Sometimes they can be absolutely wrong. Showmanship has no place in life and death diagnosis....."

ILLUSTRATIVE CASES

Photomicrograph 1

H&E x 40

Case 1

26-year-old woman with right iliac fossa pain of six weeks duration with associated high-grade fever and vomiting. Pelvic ultrasound scan showed a cystic mass in the right adrenal with dilated loops of bowel indicative of an inflammatory process.

Clinical diagnosis – Right tubo-ovarian mass

Histological section showed granulomas with ova of *Schistosoma.*

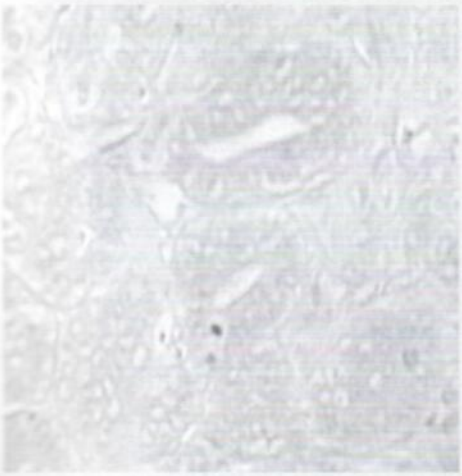
Histological diagnosis – Schistosomiasis

Photomicrograph 2

H&E x 40

Case 2
20yr old lady who was amenorrhoeic for five months and had abdominal pain of eight day duration and bleeding *per vaginam* of one week duration.
Clinical diagnosis – Hydatidiform mole
Histological section shows malignant cytotrophoblastic and syncytiotrophoblastic cells with surrounding necrosis and haemorrhage.
Histological diagnosis – Choriocarcinoma

Photomicrograph 3



H&E x 40

Case 3
Woman with right breast lump for 2month.
Clinical diagnosis – Fibroadenoma
Histological sections showed fibrocystic changes with a focal area of atypical epithelial hyperplasia.
Histological diagnosis – Fibrocystic changes with atypical epithelial hyperplasia

DISCUSSION

Case 1 An adult woman who had right iliac fossa pain, fever and vomiting. These symptoms will leave the clinician with a wide range of differential diagnoses; however, pelvic ultrasound scan further narrowed the lesion to an inflammatory lesion in the right ovarian mass.

Diagnosis after surgery was that of a right tubo-ovarian mass. However, definitive diagnosis was only made after a surgical pathologic diagnosis of schistosomiasis. Schistosomiasis is a treatable chronic granulomatous infection.[9] This diagnosis would otherwise not have been possible without a histological evaluation.

Case 2- A 20-year-old woman who after five months of amenorrhea started having abdominal pain and bleeding per vaginam. The clinical diagnosis was hydatidiform mole. However, histological evaluation gave the definitive diagnosis of choriocarcinoma.

Choriocarcinoma is a very aggressive but chemosensitive tumour and is one of those cancers that can be totally cured by chemotherapy.[10,11] However, systemic chemotherapy could not have been instituted without histological diagnosis. This shows the imminent role surgical pathology plays in diagnosis.

Case 3 A 33yr old woman with a breast lump that was suspected to be a fibroadenoma. It tuned out histologically to be fibrocystic changes with atypical epithelial hyperplasia. The presence of atypia automatically confers on this patient a fourfold increase in breast cancer risk.
Such a patient would definitely need close monitoring in years to come. This underscores the importance of surgical pathology in cancer prognostication.

CONCLUSION

This review of surgical pathology and the short cases presented were done to highlight the importance of surgical pathology as an important diagnostic tool in clinical practice. Currently, with immunohisto-chemistry, cytogenetic and molecular biology input, surgical pathology appears set to assume a more prominent role in clinical practice.

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ROLE OF PAPANICOLAOU SMEAR SCREENING IN THE PREVENTION AND CONTROL OF CERVICAL CANCER

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ABSTRACT

Edington and Maclean published the first cancer rate survey in 1965 using data from Ibadan Cancer Registry Cervical cancer was the commonest malignancy in residents of Ibadan. In Abioye's 20 year survey (1960-1980) age specific incidence of cancer in women ranged from 8.18 per 100,000 in age groups 20 to 24 at a high 373.75 per 100,000 in age groups 50 to 64 years. Patterns of cancers diagnosed in Nigerian women have been changing steadily since 1965. Cervical and breast cancer have been the most consistently diagnosed cancers in Nigerian women. The number of women diagnosed with cervical cancer continues to rise in spite of effective measures which are now available. This cancer can be controlled by adopting appropriate lifestyle and screening program[1]. It has a pre-invasive stage which takes an average of 10-15 years to develop into cancer. The pre-invasive disease can be detected and the cure rate is more than 95%[2].

INTRODUCTION

Screening is the presumptive identification of an unrecognised disease or defect by means of tests, examination, or other procedures that can be applied rapidly. The Papanicolaou cervical cytological smear test is a screening tool for the detection of pre-cancerous lesions of the cervix. It is aimed at providing pre-invasive diagnosis of lesions which when promptly treated, reduces significantly the risk of death from cervical cancer. This diagnostic cytology technique was first introduced by George Nicholas Papanicolaou (a Greek American Physician) in 1928 at Michigan, U.S.A[4].

No other single public health measure has produced so dramatic a reduction in the incidence of disease as have conventional cervical cytology smear screening programmes, particularly in developed nations. Available data from the British Columbia Screening Programme indicate a reduction from 13.5 deaths per 100,000 in 1958 to 5.8 deaths per 100,000 in 1974, following the introduction of a community wide screening programme.[5] The American Society for Cancer reported a 70% reduction in cervical cancer deaths over the forty-year interval following the widespread introduction of Papanicolaou smear screening in the United States. Currently, cervical cancer accounts for less than 3% of female cancers in the United States. An estimated 3000 new cases of cervical cancer and 1200 deaths related to cervical cancer occur annually in England and Wales. In these countries mortality from cervical cancer has been declining over the last 20 years and this decline has accelerated since the introduction of the NHS Cervical Screening Programme (NHSCSP) in 1987. Women between the ages of 20 and 64 are invited for screening every three to five years. Coverage is relatively high with approximately 84% of eligible women having been screened at some time within the last five years and about 67% at some time within the last three years. About four million women are screened each year in England and Wales (about 2.5 million following invitation and 1.5 million opportunistically)[5].

Two reviews of cervical cytology services at the University College Hospital Ibadan indicate that over 70% of routine smears are negative or show non-specific inflammatory changes.[2] Cervical intraepithelial neoplasia (CIN) was diagnosed in 8.4% to 11.8% while less than 1% had malignant smears. The mean ages were 40 (± 9) and 52 (± 6) years for patients with CIN and patients with malignant smears respectively.[2]

The need for pre-cancerous lesion identification cannot be overemphasised.

Table 1 shows the incidence and mortality rates of four common female malignancies in the world. Despite having a lower incident rate than breast cancer, it has a higher fatality rate than any one of the other cancers. A large part of this mortality rate (about 2000) is contributed by the developing countries. This is due to the late presentation of patients with cervical cancer. (Chances of curative treatment in cancers in stages III and IV are very low and only palliative care is often available.). This is totally unacceptable in a cancer that is totally preventable!

It should be noted that cervical cytology is a screening, and not a diagnostic tool. While it is helpful in the detection of significant numbers of patients who would otherwise have developed invasive cervical cancer, it must be supplemented by culposcopic examination and biopsy for confirmation.

	Incidence	Mortality
Breast Cancer	795 000	313 000
Cervical Cancer	450 000	300 000
Ovarian Cancer	165 000	101 000
Endometrial Cancer	142 000	42 000

Table 1 Annual Estimate of New Cases Globally[6].

Moreover, it should also be appreciated that there is considerable inter-observer variability in the categorisation of lesions as low-grade or high-grade squamous intraepithelial lesion (ranging from about

10-15% in a recent study by Woodhouse *et al.*, 1999).[6] The false negative rates for abnormal smears range from 0.5 to 5.4%[12]. Despite the utility of Papanicolaou smear screening, the estimated true sensitivity of the conventional Papanicolaou test is of the order of 50-60% in the routine screening setting,[8] with a range of 29-80% in different studies. For example, recent data from International Academy of Cytology Task Force shows that the undetected abnormality rate is around 10-35% of abnormal Pap smear slides.[7] This means that one out of every five or one out of every four abnormalities is not detected. Other more comprehensive studies show results between 20 and 30%. This corresponds to sensitivity rates of 70% to 80%.

BASICS OF CERVICAL CANCER

Cervical cancer begins in the lining of the cervix. Cancer of the cervix forms slowly. First, some normal cells change to pre-cancer and then to cancer cells. These changes can take a number of years, although sometimes it happens more quickly. For most women, precancerous changes go away without any treatment. For others, these changes need to be treated to keep them from changing into cancer. Unlike many cancers that cause pain, noticeable lumps, or other early symptoms, cervical cancer have no telltale symptoms until it is so advanced that it is usually unresponsive to treatment. Symptoms may even be absent at that point, although they often include abnormal vaginal bleeding, such as following intercourse or douching, between menstrual periods, or after menopause. Only in its late stages does cervical cancer cause pain in the lower abdominal or back regions.

But because the cervix, or neck of the uterus, can be easily accessed through the vagina, doctors can test for cervical cancer as well as for precancerous changes in the cervix. Most cervical cancers grow slowly over several years and often are preceded by abnormal cells. Cervical cancer can often be prevented by the removal of these abnormal cells.

PREVENTION OF CERVICAL CANCER

Most cervical cancers may be prevented in two ways:

1. Preventing Pre-cancers: Human Papilloma Virus (HPV), a sexually transmitted disease, is the most significant risk factor for developing cervical cancer. There are various strains of this virus but high risk HPV 16, 18 45 and 46 have been particularly implicated in the causation of cervical cancer. Other intermediate and low risk strains of HPV include HPV 31, 33, 35 and 6, 11, 41, 45 respectively.[9] This disease, in many cases, may be prevented by avoiding multiple sexual partners; avoiding partners who have had multiple sexual partners; and by young women delaying their first sexual experience until they are older. In addition, using a condom during sexual intercourse may provide some protection from infection by HPV. Risk also can be reduced by not smoking and by eating a healthy, balanced diet. Important research is focusing on developing vaccines, including one that would treat cervical cancer caused by HPV and another that would help boost women's immunity to HPV.

2. Detecting Pre-cancers and infection by HPV and treating appropriately: This is done by having regular screening test for the pre-invasive lesions. Pap smear test is usually the ideal. It must be emphasised that pre cancerous lesions often has no clinical symptoms and signs thus underlying the need for regular Pap smear test to detect this lesions and nip them in the bud.

RISK FACTORS OF CERVICAL CANCER

Cervical cancer is most commonly seen in women in the third to sixth decades of life. The cancer is more common among Negroes than Caucasians. Other factors are associated with an increased risk of having the disease.

These include:

- Having sex at an early age
- Having many sexual partners, or having sex with men who have had many partners
- Infection with HPV
- Infection with HIV, the virus that causes AIDS
- Cigarette smoking

Various factors have been attributed to this. They include:

1. **Ignorance:** A lot of women in these countries are still not aware of the menace of this cancer and the availability of screening. This is largely attributed to the level of education in these countries as a lot of the citizens most especially the females are either illiterates or semi literates. Accessibility to information is also a major cause for concern as information dissemination in these areas is also poor.

2. **Lackadaisical Attitude:** The educated elite in these countries who are well aware of the dangers of the disease also show some form of disinterest in taking this screening test. This could be attributed to the poor preventive culture in these countries as most are under

- Being poor (this may be because these women may not have ready access to regular Pap smear tests)
- A diet that is low in fruits and vegetables

SPECIMEN COLLECTION FOR PAP SMEAR TEST

The Cusco's speculum is used to visualize the cervix a sample of cells is taken from in and around the cervix with a wooden scraper, cotton swab, or small cervical brush. The specimen is smeared on a glass slide, preserved with alcohol, and then sent to a laboratory. There cytologists, specially trained in identifying abnormal cells, scrutinize the cervical cells under the microscope for any abnormal features associated with cancerous or precancerous cervical cells. These features include dark or irregularly shaped cell nuclei, or small or deformed cells. Women showing moderate to severe abnormalities are referred for diagnostic tests, women whose Pap smear are classified borderline are advised to re-attend at a reduced screening interval.

For effective Papanicoloau smear examination the following must be achieved:

- Patient must present herself for the test
- Clinician must take adequate representative sample
- Cytologist must be competent
- Patient must comply with follow-up and treatment

GUIDELINES FOR TAKING PAPANICOLAOU SMEAR

- All women aged 18 and above should go for regular screening
- Every woman exposed to any of the risk factors of cervical cancer should be screened
- Screening should be done every 3 to 5years

CLASSIFICATION OF CERVICAL SMEAR CYTOLOGY

Different classification of smear cytology has however been proposed. The current classification system that is widely acceptable globally however is the Bethseda System. The Bethseda 2001 Cervical Cytology classification is in 2 groups vis:

Group A: Epithelial Cell Abnormalities: Squamous Cell. This has the following classification,

1. Atypical squamous cells of undetermined significance (ASC-US)
2. Atypical Squamous Cell, cannot exclude HSIL (ASC-H)
3. Low-Grade Squamous Intraepithelial Lesion (LSIL) encompassing HPV / mild dysplasia / CIN1 encompassing moderate and severe dysplasia, CIS / CIN2 and CIN3
4. High Grade Squamous Intraepithelial Lesion (HSIL)
5. HSIL with features suspicious for invasion
6. Squamous Cell Carcinoma

Group B: Epithelial Cell Abnormalities in the glandular Cell. This has the following classifications,[11]

1. Atypical Glandular Cells (AGC)
   -Endocervical cells
   -Endometrial cells
   - Glandular cells
2. Atypical Glandular Cells (AGC)
   - Endocervical cells which favour neoplasia.
   - Glandular cells which favour neoplasia.
3. Endocervical Carcinoma in situ.
4. Adenocarcinoma
   -Endocervical
   -Endometrial
   -Extra uterine

WHY TAKE THE PAPANICOLAOU SMEAR SCEENING TEST?

- It has resulted in an over 70% decrease in mortality of cervical cancer in the past 50 years.
- It is valid for identifying preclinical lesions of the cervix.
- It is easy to take. No pain or side effects.
- It is relatively cost effective.
- It has sensitivity as high as 99% (11-99%)[9]
- Its specificity could be as high as 97% (14-97%)[9]
- False negative is between 5 to 55%.
- Early detection of the lesion gives time for treatment.

WHAT TO DO AFTER CERVICAL CANCER SCREENING

Once a Pap smear test shows signs of malignancy, then culposcopy is done to view the cervix under low magnification. Culposcopy, which was introduced by Hans Hinselman in 1925[10], is a stereoscopic binocular microscopic technique that allows inspection of the illuminated cervix under magnifications intermediate between naked eyes and lower power of microscope.

The aim of the culposcopy is to localize the source of the abnormal cells and to evaluate the extent of the lesion prior to treatment. The various parameters studied at culposcopic assessment include vascular pattern (such as punctuate end vessels, mosaic pattern as well as atypical vessels), the degree of ace to white epithelium after application of 3-5% acetic acid, the surface pattern and the border characteristics. If the transformation zone is fully visualized at culposcopy, biopsy of the worst atypical epithelium is undertaken. If the whole of the transformation zone is not visualized, then culposcopy is deemed to be unsatisfactory and recourse to a cone biopsy is recommended. The endocervix is then assessed by endocervical curettage using the endocervical brush. The treatment plan is then based on the four tests: the Papanicoloau smear, the culposcopic findings, the pathological diagnosis of the biopsy and the endocervical curettage.

The diagnostic evaluation of patients with suspected cervical cancer depends on the presumed stage of the disease at the time of evaluation. All patients should undergo examination under anaesthesia (EUA), staging and biopsy, to determine the extent of the disease and have a histological diagnosis. The International Federation of Gynaecology and Obstetrics (FIGO) system of staging for cervical carcinoma is based on clinical, not surgical data. Laboratory tests as well as radiological tests are performed for patients with invasive cervical cancer to assess them before EUA, help in staging and determination of the extent of the disease.

CONSTRAINTS OF THE PAP SMEAR SCREENING TEST

Despite the availability of screening, the gynaecology wards of most hospitals in the developing world are still congested with cases of advanced Cervical Cancer. Various factors have been attributed to this. They include,

1. **Ignorance**

A lot of women in these countries are still not aware of the menace of this cancer and the availability of

Screening. This is largely attributed to the level of education in these countries as a lot of the citizens most especially the females are either illiterates or semi literates. Accessibility to information is also a major cause for concern as information dissemination in these areas is also poor.

2. **Lackadaisical Attitude**

The educated elite in these countries who are well aware of the dangers of the disease also show some form of disinterest in taking this screening test. This could be attributed to the poor preventive culture in these countries as most are under the illusion that they cannot have the disease. Another reason why most women do not go for the test is the level of exposure involved. Most women in this environment do not subscribe to such intimacy in the name of screening.

3. **Poverty**

The economic situations in these countries has made it difficult for the government to provide free screening services and when it is heavily subsidized the citizenry most of whom are in the lower socio-economic class cannot afford the test.

4. **Lack of adequate screening centres and manpower**

Pap smear screening is not readily available in every hospital in these areas. Rural dwellers therefore do not have ready access to these screening programmes. In places where Pap smear screening is available, there is usually not enough manpower. The few cytologists available are usually overwhelmed with many requests and so results are delayed.

5. **Inadequate equipment**

Equipment used for collecting samples may not be available in developing countries.

CONCLUSION

Cervical cancer can be prevented, detected, and treated successfully. If all women who are age 18 and over or are sexually active had a Pap test on a regular basis, the survival rate for cervical cancer would be better than 95%. The burden and bereavement due to this cancer will then be a thing of the past. Every effort should then be geared towards educating and encouraging women to visit Pap smear screening centres regularly while also making screening centres, manpower and equipments needed for the screening readily available to all.

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THE ROLE OF THE MEDICAL PRACTITIONER IN THE INVESTIGATION OF GUNSHOT INJURIES

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ABSTRACT

The spate of gunshot injuries has increased over the years, from being as a result of wars to homicidal attack in the civilian setting. Thorough investigations have to be carried out on the victim, dead or alive, so as to rule out various possibilities knowing the type of gun and projectiles used and even the circumstances surrounding the injury. This article highlights all these areas in order to facilitate the reader's understanding of gunshots and associated injuries.

INTRODUCTION

Injuries from gunshots are usually investigated by forensic pathologists and forensic scientists who have to be detailed in their findings so as to maximally assist investigating police officers ascertain the circumstances surrounding shooting incidents.

TYPES OF GUNS

A gun is any firearm designed to be fired from the arm; either fired from the shoulder in case of rifles or from the hip as seen in sub-machine guns. Broadly speaking, firearms are generally classified into two broad groups, namely smooth bore weapons (shotguns) and rifled weapons (most handguns, rifles, submachine guns and machine guns).

This categorisation is based on the presence (rifled weapons) or absence (smooth bore weapons) of spiral grooves and lands on the inner surface of the barrel of the firearm. As a general rule, smooth bore weapons discharge projectiles at relatively lower velocity and over a shorter range than rifled weapons.

SMOOTH-BORE WEAPON/ SHOTGUN

Smooth bore weapons consist of one or more metal barrels of relatively wide diameter, which are smooth on the inner surface. They fire a variable number of spherical lead shot (pellets) which emerge from the end (muzzle), from where they gradually diverge in the form of a long, narrow cone. Exceptionally, a shotgun may fire a few large projectiles of even a single slug, but these are rarely met with in forensic practice, the usual load of pellets totalling scores or hundreds.

RIFLED WEAPON

Hand-guns, rifles, air-rifles, and military weapons differ from shotguns in that they fire one projectile at a time through a thicker barrel that has spiral grooves machined on its inner surface. Hand-guns, which cause over 50% of all homicides in the United States, comprise the revolver and the automatic pistol. Rifles are long-barrelled guns that may be single-shot, bolt-loaded or self-loading, and used for hunting, target shooting, or designed for military purpose.

Both shotgun and rifled weapon ammunition have a common purpose when denoted to produce large volume of hot gas under pressure that expel the bullet or shot from the barrel.[1]

Gunshot and blast wounds are increasingly seen in civilian practice. Military and civilian wounds however differ in several key respects. Military wounds are naturally associated with greater tissue trauma and are often heavily contaminated and generally are associated with delays in treatment.

THE MECHANICS OF GUN INJURIES

With the exception of deceleration injuries, all forms of mechanical trauma, whether due to punching, stabbing or kicking is caused by the transfer of energy from an external moving object to the tissues. Nowhere is this more obvious than in shooting. For damage to occur, some or all of the kinetic energy of the missile has to be absorbed by the target tissues, where it is dissipated as heat, noise, and mechanical disruption.

When a missile passes completely through soft tissue, it may retain much of its original kinetic energy and fail to transfer any appreciable amount to the tissue, which may remain relatively intact apart from the immediate bullet track. If the latter is in a limb muscle, there may be no serious effects if major blood vessels are not involved, though the same track in brain, lung, or heart may prove fatal.

To ensure transfer of energy to the tissue, some missiles are especially designed or modified to slow up or stop within the body. Explosive-tipped bullets, such as those used in the assassination attempt on Ronald Reagan, a past American president, are not designed to cause damage by the tiny donation, but drastically to deform the missile to cause maximum deceleration.

In most of the shooting cases seen by forensic pathologists, death would have occurred rapidly, but where it is delayed, secondary damage from infarction, local necrosis of muscle, and organs and infection must always be borne in mind. High velocity weapons, in particular, can cause vascular damage at a distance from the site of direct impact, with resultant stretching and thrombosis, leading in turn to ischaemic necrosis.[2]

INCIDENCE OF GUNSHOT INJURIES

Firearms are a causative factor in much of violence-related morbidity and mortality, including suicide

Interventions focus on stricter gun control. Multisectoral collaboration is needed in this regard. A record review was undertaken of 10,860 medicolegal autopsies performed between 1993 and 2004 at Umtata General Hospital in South Africa. Between 1993 and 2004, 10,860 autopsies were performed on patients who died as a result of trauma and other causes at this hospital. The average number of gunshot related-deaths during this period was 48.4 per 100,000 of the population per year. The rate increased from 27 per 100,000 in 1993 to 42 per 100,000 in 2004. Firearm-related deaths accounted for 29% of all traumatic deaths, and males (82%) outnumbered females 4.6:1, although there was an increasing incidence among females. About 50% of these deaths were in the 21-40 year age group.[3]

In 2005, a retrospective review of 76 patients with gunshot wounds was undertaken to evaluate the pattern and outcome of civilian gunshot injuries by Seleye-Fubara in Port-Harcourt, in eastern Nigeria. The extremities were the most commonly affected site (51.5% of all gunshot wounds). Gunshot injuries were most common among young males in the third decade of life, and armed robbery was the cause of gunshot trauma in 69.7% of cases.[4]

A study was carried out to report the pattern of unusual accidental missile head injuries from the use of the locally-manufactured Dane gun, which presented at the Obafemi Awolowo University Teaching Hospitals Complex, Ile-Ife, Nigeria by Komolafe et al. Missile injury to the head was noted to be increasing in frequency. Firearm-related death was also found to be on the increase, similar to what currently obtains in developed countries, where gunshot-related deaths are the leading cause of fatality, particularly among young adult males. Accidental injuries from stray bullets are fairly common; however, self-inflicted injuries are usually due to the improper handling of firearms, particularly by novices, suicide attempts and faulty technology of locally made firearms.[5]

In a retrospective study of patients managed at the Lagos University Teaching Hospital for peripheral arterial injuries from January 1995 to April 2003, it was concluded that gun shot injuries from armed robbery attack was the commonest cause of peripheral arterial injuries in this environment during the period of study.[6]

INVESTIGATING GUNSHOT DEATHS

Injuries arising from these varied weapons vary in type and intensity. Apart from identification of the weapon, the range of fire, direction and number of gunshot wounds are important in elucidation of the circumstances surrounding the incident. Attention should be paid to the following details.

Scene: Handle the body as little as possible thus avoiding artefacts and loss of trace evidence. A paper bag should be worn on the hands to preserve trace evidence. Finally transport the body in clear plastic sheeting or a body bag to preserve trace evidence and avoid contamination.

Autopsy: Get an X-ray done prior to removing the clothing. Figure 1 shows an x-ray showing a bullet in the liver at the level of the 10 right intercostal space. Next recover primer residues from hands by 10% nitric acid- moistened swab or adhesive tape. Examine hands for trace evidence, soot and propellant grains, and blood spatter. Examine and remove the clothing without cutting. Examine the body, photograph wounds if appropriate, correlate with clothing. Use dissecting microscope to examine clothing defects and wounds for soot and propellant. Clean the body photograph and describe the wounds. Trace the wound tracks and recover the projectiles. Finally complete the dissection.

SHOTGUN INJURIES

Injuries resulting from the two different types of guns give the distinguishing factors in them. Where the muzzle is placed tightly against the surface of the body, the consequent wound will be single and circular, of a size approximately equal to the bore of the weapon. The skin forms a seal around the muzzle, preventing much escape of hot gas and soot, so that soiling and burning is minimal or absent. However for a distant range shot, there will be no burning or smoke staining, rarely will there be powder tattooing, and the presence of the wads will be variable. Exit wounds are uncommon in the trunk as the energy possessed by each pellet is small because of its tiny size and the relatively low muzzle velocity of the weapon.

INJURIES FROM RIFLED WEAPONS

They vary greatly according to the velocity of the projectile. Unlike shotguns, there is only one missile in each discharge, though an automatic weapon may well cause multiple wounds in close succession which impact upon the same area of the body.

CONCLUSION

Gunshot injuries from assault, homicide, or deliberate self harm occur on a daily basis, but the expertise by way of adequate numbers of qualified trained medical, scientific and law investigative personnel available to investigate the causes of these injuries are still grossly deficient in developing countries such as Nigeria. It is however important that every medical practitioner should have a basic knowledge of the different patterns of gunshot injury. With adequate knowledge, the physician can assist law enforcement agencies in resolving the circumstances surrounding individual cases.

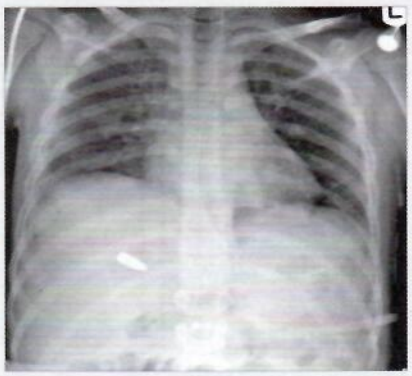

Figure 1- Bullet lodged in the region of the liver at the level of the right 10th intercostal space

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LOW MOLECULAR WEIGHT HEPARIN

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ABSTRACT

Low Molecular Weight Heparins (LMWHs) are used in the prevention and treatment of thromboembolic disorders. They possess a unique pentasaccharide sequence enabling them to bind to anti-thrombin III and subsequently inhibit factors IIa and Xa. The bioavailability and long half-life avails the ease of administration as well as predictable dose response effect and lower risk of bleeding. These characteristics make LMWHs the anticoagulant of choice in most patients. The clinical uses of LMWH are rapidly expanding and this is due to its efficacy and the seeming lack of complications. LMWH provides the ease of treatment or prevention of several difficult thromboembolic disorders such as pulmonary embolism, deep vein thrombosis and acute coronary syndromes. It is therefore preferable to unfractionated standard heparin in most cases especially when patients can afford the use of LMWH.

Key words: *LMWH, clinical uses, advantages, side-effects*

INTRODUCTION

The first description of heparin is clouded in controversy. Jay McLean, a 26yr old second year medical student at Johns Hopkins University in Baltimore, described an anticoagulant phospholipid in an extract of liver. He called it Cephalin. The name heparin was used because of its origin in the liver. Its discovery was first published in journals between 1918 and 1920 by Howell and Holt who became renowned for its discovery. Low molecular weight heparins (LMWHs) are anticoagulants that are derived from enzymatic or chemical depolymerization of heparin followed by gel separation according to molecular weight.

Commercial LMWH is derived from bovine lung or porcine intestinal mucosa. There are several distinct LMWH preparations Enoxaparin (Clexane), dalteparin, ardeparin, nadroparin and tinzaparin. Despite similar mean molecular weights, they have different anticoagulant properties and different recommended dosage regimens and should therefore not be regarded as equivalent compounds. LMWHs have been widely evaluated in a variety of clinical settings and its use has become widely acceptable in the prevention and treatment of thromboembolic disorders.

CHEMICAL STRUCTURE

Heparin is a heterogeneous mixture of sulfated mucopolysaccharides. The anti-thrombin binding region of commercial heparin is composed of chains of repeat disaccharide units of D-glucosamine and uronic acid linked by 1- 4 interglycosidic bond. The key structural unit of heparin is a unique pentasaccharide sequence which consists of three D-glucosamine and two uronic acid residues. Four sulfate groups on the D-glucosamines are found to be critical for retaining high anticoagulant activity. Removal of the unique 3-O-sulfate group results in complete loss of the anticoagulant activity. The mean molecular weight distribution of LMWH is 4,000 to 6,000 daltons (range 1,000 to 10,000 daltons) which is approximately one-third that of standard heparin. The majority of LMWH molecules are less than 18 saccharide units long. These differences in size and structure account for the distinct pharmacological actions of LMWHs.

MECHANISM OF ACTION

Heparins exhibit most of their anti-thrombotic effects by inactivating two important factors in the coagulation cascade: factor Xa and factor IIa (thrombin). LMWH molecules have a unique pentasaccharide sequence enabling them to bind to anti-thrombin III and subsequently inhibit factors IIa and Xa. LMWHs with at least 18 saccharide units are capable of forming a ternary complex with anti-thrombin III and factor IIa. Since only about a third of LMWH molecules are large enough to form this ternary structure, they have less effect on factor IIa but retain their anti-factor Xa activity. LMWH bind to lysine and arginine sites on anti-thrombin causing a conformational change in the anti-thrombin molecule, which greatly enhances its anticoagulant activity by exposing its active site for more rapid interaction with proteases.

Once the anti-thrombin heparin complex is formed, heparin is released intact for renewed binding to more anti-thrombin. Factor IXa, XIa and XIIa are also inhibited but the inhibition rate is low. LMWHs also stimulate the release of tissue factor pathway inhibitor (TFPI) from the endothelium. TFPI inactivates factor Xa and factor VIIa independent of pentasaccharide binding. Another mechanism of action is the inactivation of factor IIa by heparin cofactor II.

PHARMACOKINETICS

LMWH has increased bioavailability over standard heparin, resulting from their decreased affinity for

circulating plasma proteins such as vitronectin, fibronectin, platelet factor 4, vWF and histidine rich glycoprotein and other acute phase reactants. LMWHs do not bind to endothelial cells and macrophages, which are felt, in part, to contribute to their longer plasma half-lives. The biological half-life of LMWHs is not dose-dependent (Table 1).

CLINICAL USES

Prevention of Deep Venous Thrombosis (DVT): The use of LMWHs was first investigated for the prevention of DVT in patients undergoing orthopedic surgery. More predictable anticoagulant responses and lower risk of bleeding made LMWH a viable prophylactic option for surgical patients. LMWHs can be used safely and effectively for the prevention of DVT in patients undergoing not only orthopedic surgery, but also general, abdominal, thoracic, or gynecologic surgery and in multiple trauma or severe medical conditions. The risk of thrombo-embolic events is probably underestimated in such patients because clinical diagnosis of DVT, pulmonary embolism and other thrombotic events is often difficult as patients may present with non-specific symptoms.

Risk factors for venous thrombosis include:

1. Age >40 years
2. Prolonged immobility or paralysis
3. Stroke
4. Previous venous thromboembolism
5. Cancer and its treatment
6. Cardiac disease, e.g. MI and congestive heart failure
7. Major surgery, especially in the abdomen, pelvis, or lower extremities
8. Trauma, especially fractures of the pelvis, hip, or leg
9. Obesity
10. Varicose veins
11. Indwelling central venous catheters
12. Inflammatory bowel disease
13. Nephrotic syndrome
14. Pregnancy or estrogen use
15. Infection
16. Primary proliferative polycythaemia
17. SLE
18. Economy class syndrome

All hospitalized patients should be assessed for risk of venous thromboembolism and can be grouped into low-risk, moderate-risk and high-risk groups. The focus should be on early mobilization and mechanical interventions (such as the use of graduated compression stockings) for these patients.

Treatment of Deep Venous Thrombosis: The primary goals of treatment of DVT and pulmonary embolism are the prevention of additional thrombus formation and its complications, restoration of blood flow, and preservation of vessel function. LMWH has been used to treat DVT in some countries on out-patient basis where access to communication with the treatment centre is rapidly achieved and ambulance services are readily available should the patient require further evaluation.

Prevention of complications of acute coronary syndromes: LMWH is at least as effective as UFH in treating patients with non-Q-wave MI or unstable angina. The goal of treating unstable angina is to prevent further growth of the thrombus and blockage of artery. In treating acute MI, the goal is to open up the affected artery as soon as possible.

Percutaneous coronary intervention (PCI): Although LMWH may be superior to conventional heparin for patients undergoing PCI or for prevention of stent thrombosis after high risk PCI, it has not been demonstrated to be advantageous. Patients who have received LMWH pre-operatively may require additional conventional heparin prior to the procedure. However the incomplete neutralization of LMWH with protamine may be disadvantageous due to the long half-life of LMWH.

Thrombotic disorders during pregnancy: It is increasingly being used in pregnancy because it does not cross the placental barrier. A case of placental haemorrhage, detachment and fetal death was reported with use of enoxaparin. Dalteparin has been safely used. It may be used in lactating mothers. Although heparin is excreted into human breast milk,the heparin polysaccharide chain is degraded by the gastric acid in the stomach.

Mechanical prosthetic heart valves: LMWH is increasingly being used in patients with mechanical heart valves in whom wafarin needs to be temporarily discontinued for surgery or dental procedures.

LMWHs are currently being investigated for use in the following conditions:

- Acute peripheral arterial occlusion
- Angioplasty
- Thrombolysis
- Treatment of acute ischaemic stroke

ADMINISTRATION, DOSAGE, MONITORING

LMWH are usually administered subcutaneously (SC) although they may also be given intravenously. The SC route is the preferred because of its longer half-life and safety. Intramuscular route should be avoided because of the risk of haematoma formation. The site should be varied daily. Lab monitoring is usually not required for LMWHs due to their superior bioavailability, more predictable dose response effect, and minimal effect on IIa. insufficiency (since LMWHs are eliminated through the kidneys) and patients who are markedly obese or underweight. LMWHs do not prolong the APTT, hence monitoring of the anticoagulant response in these patients can be achieved by monitoring anti-Xa

TABLE 1:COMPARISM OF THE PROPERTIES OF UFH AND LMWH

Property	Unfractionated Heparin(UFH)	Low Molecular Weight Heparin
Mean molecular weight (range)	15(4 – 30) kD	4.5 (2 – 10)kD
Saccharide units (mean)	40 – 50	13 – 22
Anti –Xa:Anti –IIa	1:1	2:1 – 4:1
Inhibited by PF4	Yes	No
Antithrombotic effect via anti IIa	Yes	Yes
Inhibits platelet function	Yes	No
Bioavalability (at low doses)	50%	100%
Elimination	Hepatic and renal	Renal
Half-life of Anti-Xa: IV and SC	1hr and 2hrs	2hrs and 4hrs
Monitoring required	Yes	No
Frequency of HIT	High	Very low
Haemorrhagic complications	High	Very low
Ostoeporosis (long term use)	High	Low

Modified from Postgraduate Haematology 4th edition. Hoffbrand, Lewis, Tuddenham. 2001. Chapter 31, page 667. Table 31.7.

levels. The anti-activated factor X assay is a chromogenic assay that is based on the ability of heparin to inhibit the activity of activated factor X in the reagent. Limitations of this method are the role of other mechanisms in the anti- thrombotic effect and poor standardization of aassays and calibration. Therapeutic range is 0.35 – 0.7 IU/ ml by Anti – Factor Xa or 0.2 – 0.7u / ml by protamine titration. The improved bioavailability and longer half-life of LMWHs allow them to be dosed conveniently once or twice daily.

Renal Impairment: LMWHs are eliminated through the kidneys. It is prudent in patients with renal insufficiency to adjust the dosage accordingly. Data are scarce regarding dose modifications in patients with serum creatinine of greater than 2 mg/dL. LMWHs might be best administered at reduced doses or increased intervals and monitored with anti-Xa levels. If anti- Xa is not readily available reduced doses of UFH should be given and the APTT monitored accordingly.

Obese Patients: LMWH has been dosed on the basis of actual body weight. Data on pharmacokinetics and dosing guidelines in obese patients are scarce. In patients weighing more than 130 kg, consider using a modification toward ideal body weight (IBW). IBW for men = (2.3 kg X [inches > 5 feet]), IBW for women = 45 kg + (2.3kg X [inches > 5 feet]). If actual body weight (ABW) < IBW, enoxaparin dosing weight = ABW. If ABW > IBW, enoxaparin dosing weight = IBW + 0.3 (ABW – IBW).

Disadvantages

- The cost of LMWHs (the cost of a 10 day treatment at 40mg SC daily is N12,000 in Nigeria)
- Incomplete reversal with protamine in case of overdose and toxicity. It is important to note that when less than 18 saccharide heparin chains, heparin is progressively more resistant to neutralization by protamine.

Special precautions

- Threatened abortion
- Use pre/post op when regional anaesthesia is considered or used
- Advanced renal disease
- Acute septic endocarditis
- Severe hypertension
- Active tuberculosis

Contraindications

- Hypersensitivity to the drug
- Thrombocytopaenia
- Haemophiliacs
- Intracranial haemorrhage
- Ulcerative lesions of the GIT
- Neurosurgical and Opthalmic procedures
- Pregnancy unless clearly indicated

Dosage and Administration of Enoxaparin			
Indication	**Dosage**	**Initial Dose Administration**	**Average Duration /Recommendation**
Abdominal surgery	40 mg once/d	2 h before surgery	7 to 10 d
Hip replacement surgery	30 mg every 12 h	12 to 24 h after surgery	7 to 10 d
	40 mg once/d	12 (±3) h before surgery	Continue prophylaxis for 3 weeks
Knee replacement surgery	30 mg every 12 h	12 to 24 h after surgery	7 to 10 d
Medical patients during acute illness	40 mg once/d	–	6 to 11 d
Unstable angina and non-Q-wave MI	1 mg/kg every 12 h WITH aspirin 100-325 mg once/d	–	2 to 8 d
Treatment of DVT with or without pulmonary embolism	Inpatient treatment* 1 mg/kg every 12 h WITH warfarin† **OR** 1.5mg/kg once/d WITH warfarin† Outpatient treatment‡1 mg/kg every 12h WITH warfarin†	–	7 d

*Patients with DVT with pulmonary embolism or patients with DVT without pulmonary embolism who are not candidates for outpatient treatment.
†Initiate warfarin therapy when appropriate (usually within 72 h of enoxaparin initiation).
‡Patients with DVT without pulmonary embolism who can be treated at home.

ADVERSE DRUG REACTIONS

Bleeding: Hemorrhage is the most frequent and significant side effect of heparin. LMWHs have less inhibition of platelet function, less interaction between platelets and endothelial walls. There is reduced bleeding in LMWHs with higher anti-Xa: anti-IIa ratios. Patients should be educated to promptly report any symptoms of bleeding. If bleeding is minor, withholding LMWH is usually adequate. If bleeding is major, LMWHs effect can be at least partially reversed with protamine sulfate, a basic protein that neutralizes heparin's anti-IIa activity. It should be administered slowly over 10 minutes as it may cause hypotension. The total dose should not exceed 50 mg.

Heparin-Induced Thrombocytopenia (HIT): HIT is a clinicopathological diagnosis. There are two forms of thrombocytopenia associated with heparin use. The early form is benign and is reversible despite continued heparin use. The severe form is typically does not occur until day 5 of heparin therapy. It is an autoimmune and idiosyncratic reaction. The immune system forms heparin platelet factor 4-dependent IgG antibodies that bind with platelet antigens. This immune complex results in thrombocytopenia and/or paradoxical thrombosis. If a patient has been exposed to heparin within the last three months, HIT can occur within 24 hours. LMWH has been said to be less immunogenic. Platelet activation assays or antigen assays using ELISA to detect antibodies against heparin/PF4 complexes may also be used. If the platelet count falls more than 30% - 50% from baseline or if the absolute platelet count falls below 100,000/mm^3, LMWH should be stopped pending laboratory confirmation of HIT. If the patient requires continuing anticoagulation, recombinant hirudin or Orgaran should be considered.

Lipid Effects: Heparins exert lipolytic activity on lipase enzymes including lipoprotein lipase. Since the duration of therapy is usually a few days, these lipid effects are not clinically important.

Osteoporosis: Heparins augment PTH-stimulated bone resorption and stimulate osteoclasts. Increased molecular size and the degree of sulfation are major determinants of heparin's ability to promote bone resorption. Spinal fractures occur less frequently in humans on long-term LMWH compared to UFH. LMWHs seem to result in lower risk of heparin-induced osteoporosis.

Skin Lesions: Skin lesions associated with heparin use include erythematous papules, skin necrosis, and urticaria. Reactions such as asthma, tachycardia, tachypnoea, conjunctivitis, rhinitis, angioedema, and shock are less common.

Hypoaldosteronism: Long-term administration of heparin may rarely cause hypoaldosteronism due to the inhibition of aldosterone synthesis.

Neurological Impairment: Neurological impairment could result from administration of Dalteparin, Enoxaparin and Tinzaparin such as numbness, paraesthesia, leg weakness, paralysis and bowel or bladder dysfunction.

Elevation of liver enzymes: Elevation of the liver transaminases ALT and AST has been reported but has not been connected to any long-term effect on liver function.

USEFULNESS OF D-DIMER ASSAY IN THE DIAGNOSIS OF VENOUS THROMBOEMBOLISM

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ABSTRACT

Deep Vein Thrombosis (DVT) and Pulmonary embolism (PE) are the most common manifestations of venous thromboembolism (VTE). The mortality rate of this disorder in Caucasians is about 15%, which underscores the importance of early diagnosis and prevention. However, there is dearth of knowledge on the incidence of VTE in Nigeria. In the diagnosis of a suspected VTE, a pretest probability (PTP) assessment should be done. This can be followed up by the use of invasive and non-invasive imaging procedures or a simple D-dimer estimation depending on the PTP score.

D-dimer is a fibrin degradation product and serves as an indirect indicator of thrombotic activity. There are two general methods of measuring D-dimer. The enzyme linked immunosorbent assay (ELISA) method and the latex agglutination testing. ELISA technique is superior though more costly than the rapid latex agglutination test. Newly developed latex agglutination methods are now available with negative predictive value of 93 – 100% approaching ELISA methods. D-dimer assays have the potential to be the only screening test necessary to exclude VTE. The assay can be 100% sensitive in excluding DVT or PE but will not confirm the presence of VTE as numerous other diseases and procedures can increase the levels of D-dimer.

Keywords - *D-dimer assay, DVT, PE, Venous thromboembolism,*

INTRODUCTION

The most common manifestations of Venous Thromboembolism (VTE) are deep vein thrombosis (DVT) and pulmonary embolism (PE).[1,2,3] The mortality rate for these disorders is about 15%, and death is often linked with missed or delayed diagnosis.

Over 2 million people in the United States are diagnosed with venous thromboembolism annually, and contributes an estimated number of 60,000 – 200,000 deaths annually. There is a paucity of information on venous thromboembolic phenomenon amongst the black race especially in Nigeria, where there has been little documentation on this relatively silent but highly treatable condition[12].

Deep vein thrombosis (DVT) and pulmonary embolism (PE) are difficult to diagnose because patients typically present with vague symptoms, such as limb pain and swelling for DVT or breathing difficulty for PE. Approximately 75% of patients presenting to the emergency department with suspected VTE are found not to have DVT with objective tests and many institutions have developed diagnostic algorithms designed to rapidly and economically rule out VTE without increasing patient risk. The first step in most cases of suspected VTE is performance of a pretest probability (PTP) assessment. Using a set of risk factors and symptoms, a PTP score can be determined and the patient's risk defined as low, moderate, or high.

Diagnosis of VTE is difficult and may involve the use of invasive and time-consuming imaging procedures, such as venography, compression ultrasound (CUS), pulmonary angiography, spiral computed tomography, and lung scan. Venography and pulmonary angiography are considered the "gold standards" in the diagnosis of DVT and PE respectively. Both of these procedures include injection of contrast dye followed by imaging of the blood vessels. They are time-consuming, expensive, and have associated risks of fibrinolysis, which leads to dissolution of the clot and release of soluble fragments, including D-dimers, into the plasma. Therefore, the presence of D-dimers indicates degradation of fibrin specifically and serves as an indirect indicator of thrombotic activity.

D-Dimer Assays

Since the investigation of venous thromboembolism can be tedious, invasive, and expensive, there has been an increased focus on D-dimer assays, which are rapid, noninvasive, and inexpensive. Fibrin is the main component of thrombus formation, and degradation of cross-linked fibrin results in fibrin degradation products including D-dimers. D-dimers are commonly found in the circulation when venous thromboembolism is present. However, this finding lacks specificity, since D-dimers are also found in other disease states, including cancer, congestive heart failure, and inflammatory conditions [5]. There are two general methods of measuring D-dimers. The original enzyme-linked immunosorbent assay (ELISA) methods have been well studied and in general have a 95% negative predictive power. Limitations associated with classic ELISA methods include cost and inability to perform the test rapidly [7]. Recently, rapid ELISA assays have been developed (VIDAS DD [Biomerieux, France] and Instant IA DD [Stago, Asniere, France]). The VIDAS DD has been extensively studied and has a sensitivity of 94% to 100% and a negative predictive

value of 92% to 100% [7,8].

The other method of measuring D-dimer fragments is latex agglutination testing, which is both rapid and economical. However, the latex testing methods are subjective and can be difficult to read. Most of the latex methods have inadequate sensitivity and negative predictive value for clinical use [9]. Recently, new latex tests have been introduced that have sensitivities and negative predictive values approaching classic ELISA methods (SimpleRed [Agen Diagnostics Limited, Australia] and Tinaquant [Boehringer, Mannheim, Germany]) [7,8,9]. The SimpleRed assay has a sensitivity of 89% to 100% and a negative predictive value of 95% to 100% [7,9]. The Tinaquant assay has a sensitivity of 99% and a negative predictive value of 93% [8]. Evidence supporting use of D-dimers in clinical practice is rapidly emerging. Perrier and colleagues [10] evaluated the role of an ELISA D-dimer assay in 198 patients suspected of having a pulmonary embolism. Using a cutoff value of less than 500 g/L as normal, these investigators reported that of the 198 patients with a normal D-dimer value, only one patient had a pulmonary embolism and one was lost to follow-up. The negative predictive values of the D-timer test in this study were 99% (196 of 198 patients).

Elevated D-dimer fragments are too nonspecific for diagnosis of venous thromboembolism by themselves. With negative predictive values close to 100%, certain D-dimer assays have the potential to be the only screening test necessary to rule out venous thromboembolism. However, many authors and experts have been reluctant to propose such a diagnostic strategy. The American Thoracic Society's clinical practice guidelines on acute venous thromboembolism reaffirm the emerging value of D-dimers assays but currently do not endorse widespread use [6]. At present, assay performance varies widely, and different assays cannot be clinically applied interchangeably.

If D-dimer assays are to be used in a diagnostic strategy, the details of the assay should be known, including type (latex or ELISA), operating characteristics (sensitivity and negative predictive value), and outcomes of clinical studies supporting the particular assay. Until further studies emerge, testing for D-dimers should be restricted to patients in whom clinical suspicion of venous thromboembolism is low or moderate.

ESTABLISH CLINICAL GUIDELINES

Once the quantitative D-dimer has been validated with an established cut-off, the assay can be used as a negative predictor of VTE. Guidelines for the use of D-dimer should be developed with input from the clinical staff to develop criteria to be used as a negative predictor. Two major points should be incorporated into the procedure for the use of D-dimer assay.[11]

1. Use the D-dimer with caution on inpatients since numerous disease processes and invasive procedures can elevate D-dimer levels in the absence of VTE.
2. Do not use the D-dimer assay in patients on anticoagulant therapy (heparin or coumarin). Anticoagulants can decrease D-dimers and possibly generate a falsely low value, below the established cut- off.

SUMMARY

The determination of quantitative D-dimer levels is both a diagnostic and a cost-saving tool to rule out VTE. The assay can be used to eliminate those individuals without VTE, but with low or moderate clinical suspicion. After appropriate validation, the assay can be 100% sensitive in ruling out DVT or PE, but will not confirm the presence of VTE as numerous other diseases and procedures can increase D – Dimer level.

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CURRENT TRENDS IN THE DIAGNOSIS OF ACUTE LEUKAEMIAS

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ABSTRACT

The diagnosis and classification of acute myeloid (AML) and acute Lymphoblastic (ALL) Leukaemia was based almost exclusively on well-defined morphologic criteria and cytochemical stains. Although most cases can be diagnosed by these methods, there is only modest correlation between morphologic categories and treatment responsiveness and prognosis. Recently, efforts have been made in the direction of new technology such as immunophenotyping, cytogenetics and molecular analysis which have almost redefined diagnosis, classification, and patient management. The refinements in diagnosis that they provide have set the stage for more specifically directed treatment regimens.

Keywords: *Acute Leukaemias, Immunophenotyping, Cytogenetics*

INTRODUCTION

Acute leukaemias represent a group of neoplasms of immature lymphocytes, platelets, red blood cells.[1] The immature precursors of lymphocytes could be precursor B (pre B) or precursor T (pre T) lymphocytes also known as lymphoblast while those of premature forms of platelets, red blood cells and polymorphonuclear cells are known as myeloblasts. They have also been described as the presence of over 30% of blasts in the bone marrow at clinical presentation.

Various factors have been identified that predispose to acute leukaemias which include exposures to high dose external low energy transfer radiation, chronic benzene exposure and chemotherapeutic agents; a number of predisposing diseases which include Down's syndrome, Fanconi's anaemia, Bloom's syndrome, AIDS. Other proposed causes include neonatal administration of Vitamin k, maternal alcohol consumption during pregnancy and increased consumption of dietary nitrates.

Acute Lymphoblastic Leukaemia is the common form of leukaemia in children, its incidence highest at 3-7 years, while AML is the commoner form of leukaemia in adults and is increasingly common with advancing age.

HISTORY

The earliest report of leukaemia is generally credited to Velpeau, writing in 1827,[2] although it was not until 1845 that Virchow,[3] Bennet,[4] and Cragie,[5] in separate reports, recognized this condition as a distinct entity. In 1847, Virchow coined the term *leukaemia*, applying it to two distinct types of the disease, splenic and lymphatic, that could be distinguished from each other on the bases of splenomegaly and enlarged lymph nodes as well as the morphologic similarities of the leukaemic cells to those normally residing in the spleen and lymph glands.[4] By 1913, leukaemia could be described as acute or chronic, and as lymphatic or

PRESENTATION

The various leukaemias have various modes of presentation which are secondary to bone marrow failure and organ infiltration. Patients also present with anaemia evidenced by easy fatiguability, tiredness, shortness of breath, increased susceptibility to infections, bleeding from the skin, gums and other parts of the body, lymph node enlargement, hepatomegaly and splenomegaly.

The differentiation between ALL and AML poses a challenge as presentations are similar. However, morphologically, with myeloid blasts showing some evidence of differentiation into granulocytes and monocytes and ALL exhibiting no form of differentiation, this may help but this only is not reliable. Hence special tests are needed to confirm the diagnosis and subdivide the leukaemias. The myeloperoxidase and Sudan black B stains are the most commonly used and the most valuable.

CLASSIFICATION

Acute leukaemias following classification into Acute Myeloid and Acute Lymphoblastic Leukaemias have various subtypes and can be classified using morphologic features of blood films using polychromatic stains and histochemical reactions, monoclonal antibodies against surface markers (immunologic), cytogenetics, and presence of specific chromosomal translocations (molecular genetic methods). The French- American–British (FAB) group in 1976 sub classified these acute leukaemias using morphology. (See "Leukaemias" **DOKITA**-Oncology Edition, Vol. 25 No.1 1998, 56-66.), however with advances in technology, acute leukaemias cannot only be diagnosed, but can be better classified based on treatment responses and prognosis as there appears to be little correlation with the morphology and response to treatment and prognosis.

There is a poor correlation between morphologic and immunologic phenotyping of AML as would be

expected, since the former method is more subjective,given to observer variation, and is based on qualitative factors, whereas the latter method, which characterizes surface molecular features, is more accurate and reproducible. The correlation is improved only somewhat if morphology and histochemistry are coupled.[8] The use of these investigative techniques would be discussed shortly. However it should be of note that other ancillary investigations such as full blood count, lumbar puncture, chest radiographs remain invaluable in management. Even markers for drug resistance such as *MDR* expression has been proposed if only to separate the more responsive from the less responsive AML.

INVESTIGATIVE TECHNIQUES

The expansions of therapeutic options and improvement in remission induction and disease-free survival for both AML and ALL have stimulated emphasis on defining good and poor treatment response groups. This is most effectively accomplished by a multifaceted approach to diagnosis and classification using Bone Marrow aspiration/ Trephine Biopsy, immunophenotyping, cytogenetics, and molecular analysis in addition to the traditional methods. Information obtained from immunophenotyping, cytogenetics, and molecular analysis has substantially advanced our understanding of the biology of acute leukaemias and other hematological malignancies as a whole.

Bone Marrow Aspiration/ Trephine Biopsy

Bone Marrow is an integral part of the diagnosis of acute leukaemias. As many as 16% of patients lack blasts in the peripheral blood film at the time of diagnosis hence this further emphasizes the mandatory role of careful bone marrow aspiration in establishing the diagnosis of acute leukaemias. Secondly, the morphology of leukaemic cells in the peripheral blood film may differ from that in the marrow –as seen in M_4/M_5 abnormalities have relationships to prognosis[11,12,13,14,15,16,17]. Trephine Biopsy is indicated in tightly packed marrow and touch preparation of biopsied tissue can be used for cytological diagnosis. Multiple bone marrow aspirations are sometimes obtain diagnostic or representative tissue.[9]

Immunophenotyping

Immunophenotyping is useful in the identification of AML and ALL. Immunophenotyping has become a standard diagnostic procedure in evaluation of acute leukaemias. The immunophenotype should be assessed for diagnosis in all acute leukemia and in every case of ALL because of the important treatment and prognostic information that itprovides. (see Table I and II)

Multiparametric flow cytometry is the preferred method for immunophenotyping in acute leukaemias. There is an abundance of monoclonal and polyclonal antibodies available to assess myeloid and lymphoid lineage-associated antigens by cytometry, and blood and bone marrow aspirate specimens lend themselves particularly well to flow cytometric analysis because the cells are naturally in a fluid suspension.

Multicolor flow cytometry allows for the characterization of up to four different antigens on a single cell. This permits precise immunophenotypic characterization of leukaemia cells.

Immunohistochemical staining can be used to immunophenotype leukaemias when a specimen is not submitted for flow cytometry or only bone marrow trephine biopsies are available for examination. An array of antibodies to myeloid- and lymphoid-associated antigens is also available for immunohistochemical stains. The lineage of hematopoietic cells is defined both by the antigens expressed, and the absence of expression of antigens associated with a different lineage. Leukemia cells, however, may aberrantly express some antigens of another lineage or lack expression of an expected antigen.[10] It is therefore important to use panels that include sufficient numbers of antibodies to assess a spectrum of both myeloid and lymphoid antigens later. The choice of antibody panels varies among laboratories. In childhood ALL, immunophenotype is a major factor in determining the chemotherapy protocol. B-cell precursor ALLs have a more favorable prognosis than the other groups; however, within the B-cell precursor category, there are subsets with a poor prognosis.[11] Most of the favorable and unfavorable prognostic groups of B-cell precursor ALL can be

TABLE I: Immunologic Phenotypes of AML

	USUALLY POSITIVE	USUALLY NEGATIVE
Myeloblastic	CD 11, CD 13, CD 15, CD 33, CD 117, HLA-DR	CD 14, CD 10 (cALLa), CD 20
Myelomonocytic	CD 11, CD 13, CD14, CD 15, CD 32, CD 33, HLA-DR	CD 10, CD 20
Erythroblastic	Glycophorin, spectrin, ABH antigens, carbonic anhydrase I, HLA-DR	CD 10, CD 20
Promyelocytic	CD 11, CD 13, CD 15, CD 33	CD 14, HLA-DR, CD 10, CD 20
Monocytic	CD 11, CD 13, CD 14, CD 33, HLA-DR	CD 10, CD 20
Megakaryoblastic	CD 34, CD 41, CD 42, CD 61, von Willebrand factor	CD 10, CD 20

Table from Marshall A. Lichtman and Jane L. Liesveld: Acute Myelogeneuous Leukemia. In Williams Hematology 6th Edition by McGraw-Hill Medical Publishing Division New York 2001 . Table 93:2, 1049

TABLE 2: Presenting features according to immunologic subtype

SUBTYPE	TYPICAL MARKERS	FREQUENCY CHILDHOOD, %	ADULT, %	ASSOCIATED FEATURES
B-cell precursor	CD 19$^+$, CD 22$^+$, CD 79a$^+$, cIg$^+$, sIgμ, HLA-DR$^+$			
Pre-pre-B	CD 10$^-$	5	11	Infant or adult age group, high leukoctye count, initial CNS leukemia, pseudodiploidy, MLL rearrangement, unfavorable prognosis
Early pre-B	CD 10$^+$	63	52	Favorable age group (1 to 9 years), low leukocyte count, hyperdiploidy >50chromosomes
Pre-B	CD 10$^\pm$, cIg$^+$	16	9	High leukocyte count, black race, pseudodiploidy
B-cell	CD 19$^+$, CD 22$^+$, Cd79a$^+$, cIg$^+$, sIgμ^+, sIgk$^+$ or sIgλ^+	3	4	Male predominance, initial CNS leukemia abdominal masses, often-renal involvement.
T-lineage	CD 7$^+$, cCD 3$^+$			
T-cell	CD 2$^+$, CD1$^\pm$, CD 4$^\pm$, CD 8$^\pm$, HLA-DR$^-$, TdT$^\pm$	12	18	Male predominance, hyperleukocytosis, extramedullary disease
Pre-T	Cd2-, CD1-, CD4-, CD8-, HLA-DR+, TdT+	1	6	Male predominance, hyperleukocytosis, extramedullary disease, unfavorable prognosis.

Abbreviations: cCD3, cytoplasmic CD3; cIg, cytoplasmic immunoglobin; sIg, surface immunoglobulin; TdT, terminal deoxynucleotidyl transferase
Table from Chung- Hon Pui: Acute Lympphoblastic Leukemia. In Williams Hematology 6th Edition by McGraw-Hill Medical Publishing division, New York 2001. Table 97 :1148

identified by their cytogenetics, karyotype or molecular features.[12]

Cytogenetics

Clonal cytogenetic abnormalities are identified in 60–80% of cases of AML and about 80% of cases of ALL[13,14,15]. Both numerical and structural abnormalities are frequently encountered and these cytogenetic-abnormalities have relationships to prognosis.[11,12,13,14,15,16,17] Bone marrow cytogenetic findings are a major independent indicator of prognosis for both AML and ALL, and they define treatment groups. They are essential in the assessment of patients with acute leukemia and should be performed in every case.

Acute Lymphoblastic Leukaemia

Hyperdiploidy with >50 chromosomes is the most common cytogenetic abnormality (25 percent of childhood i.e. 2-10 yrs and 6 to 7 percent of adult cases) in B-cell precursor ALL[12]. There often is an extra copy of chromosome(s) 4 and/or 10, which seems to impart a particularly favorable prognosis. ALLs with hyperdiploidy with >50 chromosomes in children are highly sensitive to antimetabolite drugs and have a complete remission rate approaching 100% with about 80% long-term disease-free survival[12]. On the other hand, hypodiploidy is associated with an exceptionally poor prognosis[18,19,20]. Structural abnormalities in childhood B-cell precursor ALL are more often associated with an intermediate or poor prognosis. One exception is the 12:21 translocation [t(12;21)(p12;q22)],which is observed in about 20–25% of cases with good prognosis[21].

Cases of ALL with a t(12:21) are always B-cell precursor type but are distinct from the hyperdiploidy with >50 chromosomes group. They are highly sensitive to antimetabolite drugs and have a high rate of complete remission and presumably a high incidence of long-term disease-free survival. Patients with B-cell precursor ALL with a 9;22 translocation [t(9/22)(q24;q11)] or abnormalities involving chromosome 11q23, most often a t(4;11)(q21;q23), have an unfavorable prognosis[15,22,23,24,25,26]. In childhood B-cell precursor ALL, the likelihood of long-term disease-free survival or relapse and the decision for low-risk or more aggressive chemotherapy or a bone marrow transplant are commonly dictated by cytogenetic findings which help in determining type of treatment and prognosis.[27]

The correlations of specific cytogenetic findings with presenting clinical features and blast cell phenotypes indicate the prognostic significance of chromosomal abnormalities in patients with ALL.

Acute Myeloblastic Leukaemia

In AMLs, cytogenetic findings are also clinically important. (see table IV) This is particularly true of cases with the 15;17 translocation [t(15;17)(q22;q21) which usually has distinct clinical and morphologic features.[14,16] The translocation involves the *PML* gene on chromosome 15 and the retinoic acid receptor *(RAR)* gene on chromosome 17.[26] The fusion messenger RNA product that results inhibits maturation of the affected cells, leading to a proliferation of large numbers of atypical promyelocytes. Treatment with all-*trans*-retinoic acid (ATRA) can overcome the maturation blockage in most cases and lead to temporary complete remission of the disease.[26, 27, 28] Treatment with standard chemotherapy with or after ATRA therapy is required to sustain remission. Other types of AML do not respond to ATRA therapy.Several of these have been found to have either good or bad prognostic significance. Prognostic factors in Acute Myeloid Leukaemia are listed in table V.

Table III: Clinical and biologic features associated with the more common genetic subtypes

SUBTYPE	ASSOCIATED FEATURES	ESTIMATED EVENT-FREE SURVIVAL% CHILDREN	ADULTS
Hyperdioloidy >50 chonosomes	Predominant B-cell precursor phenotype; low leukocyte count; favorable age group (1 9 yrs) and prognosis in chilgren	80 90 at 5 yr	30-50 at 5yr
Hypodiploidy <45 chonosomes	Predominant B-cell precursor phenotype; increased leukocyte count; poor prognosis	30 at 3 yr	10 20 at 3yr
ELY6-CBFA2 fusion	CD $13^{\pm}$/$CD33^{\pm}$ B-cell phenotype; pseudodiploidy; age 1 9 yr; favorable prognosis	85 90 at 5 yr	Unknown
t(1:19)(q23:p13.3) with E2A-PBX1 fusion	CD 10^{+}/CD $20^{\pm}$/CD 34^{-} pre-B phenotype; pseudodiploidy; increased leukocytes count; black race; CNS leukemia; intermediate prognosis	70 80 at 5 yr	20-40 at 3yr
t(9:22)(q34:q11)	Prodominant B-cell precursor phenotype; older age; increased leukocyte count; dismal outcome in adults and in children with poor early responses to induction or leukocyte counts >25 x 10^{9}/liter	20 40 at 5 yr	<10 at 3 yr
t(4:11)(q21:q23)	CD 10^{-}/CD $15^{\pm}$/$CD33^{\pm}$/CD $65^{\pm}$ B-cell precursor phenotype; infant and older adult age groups; hyperleukocytosis; CNS leukemia; dismal outcome	10 35 at 5 yr	10-20 at 3yr
t(8:14)(q24:q32.3)	B-cell phenotype; L3 morphology; male predominance; bulky extramedullary disease; favorable prognosis with short-term intensive chemotherapy including high-dose methotrexate, cytarabine, and cyclophosphamide	75 85 at 5 yr	50-55 at 4yr
t(1:14)(p34:q11)	CD 10^{-} T-cell phenotype; male predominance; hyperleukocytosis, intermediate prognosis	65 75 at 5 yr	50 at 3 yr
dic(9:12)(p11-12:?p12)	CD 10^{+} B-cell precursor phenotype; male predominance; young age (<25 yr); low leukocyte count; excellent prognosis	80 90 at 5 yr	Unknown

Table from Chung- Hon Pui: Acute Lympphoblastic Leukemia. In Williams Hematology 6th Edition by McGraw-Hill Medical Publishing division, New York 2001. Table 97-5 :1149

Table IV: Clinical correlates of frequent cytogenetic abnormalities observed in

CHROMOSOME ANNORMALITY	GENES AFFECTED	CLINICAL CORRELATION
Loss or gain of chromosome Deletions of part or all of chromosomes 5 or 7	Not defined	Frequent in patients with acute myelogenous leukemia occurring de novo and in patients with history of chemical, drug, or radiation exposure and/or previous hemotologic disease.
Trisomy 8	Not defined	Very common abnormality in acute myeloblastic leukemia. Poor prognosis, often a secondary change.
Translocations t (8:21) (q22:q22)	AMLI (CBF*a*) - ETO	Present in about 12% of patients with AML; associated with loss of Y in males or of X in females in over half the cases. Present in about 40% of myelomonocytic phenotype. High frequency of granulocytic sarcomas.
t(15:17) (q31:q22)	PML- RAR*a*	Represents about 7% of cases of AML. Translocation involving chromosome 17, t(15:17), t(11:17), or t(5:17) are present in most cases of promyelocytic leukemia
t(9:11); p(22:q23)	ALL1 (MLL) AF 9	Present in about 7% of cases of AML. Associated with monocytic leukemia. 11q23 translocations common in infants, carries poor prognosis, rearranges ALL1 (MLL) gene. There are many partners (~ 20) for 11q23 translocation. Present in ~ 60% of infants AML cases.
t(9:22) (q34:q22)	BCR ABL	Present in about 3% of patients with AML
Inversions Inv (16)	CBFB MYH11	Present in about 12% of cases of AML; associated with increase marrow cosinophils; better response to therapy

Table from Marshall A. Lichtman and Jane L. Liesveld: Acute Myelogeuous Leukemia. In Williams Hematology 6th Edition by McGraw-Hill Medical Publishing Division, New York 2001. Table 93-3: 1051

Molecular Analysis

Molecular analysis in the diagnosis of acute leukaemias is useful. This may be used to establish clonality or to identify molecular translocations producing fusion gene products.[16,21,29,30,31] Molecular studiesare also powerful tools for the identification of minimal residual disease and early relapse.[32, 33] Techniques for molecular analysis of leukaemias include: Southern blot, Polymerase Chain Reaction, and Fluorescence In-Situ Hybridization. In some cases, molecular translocations

Table V

Good Prognosis	Poor Prognosis
Leukaemic cells which contain t(8;21), t(15;17), inv (16), trisomy 21 Absence of exaggerated dysmyelopoiesis Residual normal metaphases admixed with clonal cytogenetic abnormalities High telomerase activity levels High levels of caspase 3	Older age Unfavorable karyotypes Multidrug resistance phenotype Prior clonal hemopathy Higher white cell count Very low platelet count High lactic dehydrogenase Another medical disorder Low serum albumin or prealbumin Need for intubation Autonomous clonal growth of leukemic blast cells High Bcl-2 expression High Mcl-2 expression Low expression of retinoblastoma gene High levels of WAF/Cipl protein High CD34 expression GATA -1 expression Neural cell adhesion molecule (CD56) expression Elevated soluble L-selectin Higher expression of interleukin-1 gene. Low FMS expression Expression of the thrombopoietin receptor (c-MPL) mRNA.

are present when karyotypic changes are not evident. An example of this is the *TEL-AML1* fusion gene resulting from the t(12;21)(p12;q22) translocation. This chromosomal translocation generally is cryptic and can only be identified by molecular analysis, i.e., PCR or FISH[31]. This is occasionally the case with other well-established translocations in acute leukemia in which the involved chromosome segments are too small fordetection by karyotype or because the translocation is complex and involves several chromosomes. It is important, therefore, to perform molecular analysis when the presence of a fusion gene that would impact treatment decisions is suspected.[30]

In some cases, PCR or FISH studies may be performed in search of a specific fusion gene without first doing cytogenetic karyotyping. Molecular analysis should be used to supplement cytogenetics when a specific question is being addressed. Molecular studies may be performed in the absence of parallel cytogenetic analysis when there is a focused purpose for the study, such as identification of minimal residual disease.[34]

CONCLUSION

Technologies will continue to advance in diagnosis of haematological malignancies and in particular acute leukaemias. Defining the appropriate clinical indications for the new techniques, understanding their limitations, and integrating them with existing standard diagnostic methods are all vitally important in realizing their full potential. Though in our environment, most of these methods highlighted are novel or at best only taught in their theories, the embracing of them and the wide opportunities available they provide, will impact haematology as we know it practiced.

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THE GRAM STAIN: HISTORY, PROCEDURE AND APPLICATIONS

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ABSTRACT

The Gram stain is the best-known and most widely used bacteriological staining method. It is almost always the first test performed for the identification of bacteria in clinical microbiology. Hans Christian Joachim Gram discovered it accidentally in 1884 and since then it has not lost its value up till the present day. Although a German pathologist modified Gram original procedure some years later, the original concept is still pertinent. It is an example of a differential stain exploiting the differences in the peptidoglycan layer of the cell wall of microorganisms, leading to the broad division of microorganisms into gram positive and gram negative. Gram stain could be used to aid presumptive diagnosis and guide choice of antibiotics. However it has its limitations especially when dealing with microorganisms that lack a cell wall. The history, procedure and application of Gram's stain are discussed in this reveview.

Key words: *Gram stain; peptidoglycan; differential staining*

INTRODUCTION

Do you recall when Maria in sound of Music was teaching the Von Trapp children how to sing? She said that "... the very first notes just happens to be d... re... mi...". Well, if Maria were to be a microbiologist she would probably teach them that an important staining technique that has not lost its importance over the years is the Gram stain and it just happens to be so. The staining technique is referred to as the Gram Stain although Gram did not describe a stain but a method in which he used stains and solutions devised by others. The Gram reaction is a stable characteristic of bacteria and a function of their peptidoglycan cell wall [1].

The Gram stain is the "best known and most widely used bacteriological staining method. It is almost always the first test performed for the identification of bacteria in clinical microbiology" [2]. There have been various modifications of the original technique, including those described by Jensen, Lillie and Preston and Morrell . The initial stain in the technique is a para-rosaniline dye such as methyl violet crystal violet or Gentian Violet, the last being a mixture of the two primary staining dyes [3]. The crucial step in the staining procedure is the decolourization step. If not properly done, it could cause confusion, since over-decolourization would make a gram-positive organism appear gram negative and *vice versa*.

HISTORY

Hans Christian Joachim Gram was a Danish pharmacologist and pathologist born in Copenhagen on September 13th 1853 and died on November 14th 1938 at the age of 85 years [4].

Gram developed an interest in medicine and in 1878 he obtained his MD from the University of Copenhagen. The work that earned him international recognition was his method for staining bacteria. In 1884, while he was working with Karl Friedlander in Berlin and trying to distinguish between two pneumonia causing organisms: *Streptococcus* pneumoniae and *Klebsiella pneumoniae*, he observed that certain stains were preferentially taken up and retained by bacterial cells [5].

In the original method, Gram in the first step dried a fluid smear on a glass slide over a burner flame and poured gentian violet (a mixture of crystal violet and methyl violet) solution over it. After a water wash, he added Lugol's solution (Potasium triiodide solution) which acted as a mordant to fix the dye. Then he poured ethanol over the slide to wash away the dye. Certain bacteria retained the colour (Gram positive) while the other species bleached (Gram negative) [4].

Some years later a German Pathologist Carl Weigert (1845-1904) added a final step of staining with safraning. Gram himself never used counter staining for Gram negative organisms [4].

Gram's method was published in Friedlander's journal" Fortschritte der Medizin". In this publication, Gram described the technique as follows: "After having been dehydrated in alcohol, the preparations are immersed in the aniline-Gentian violet solution for 1 to 3 minutes, the preparations are then placed in an aqueous solution of iodine-potassium iodide directly or after a rapid rinsing in alcohol. They are allowed to remain there for 1 to 3 minutes during which time the colour of the preparations are then completely decolorized with absolute alcohol. Further clearing is achieved with clove oil [3]. Bacteria are stained intense blue while the background tissues are light yellow" [3].

There have been various modifications of the initial method: Lillie's modification, Jensen's modification and Preston and Morrell's modification (1962). In Lillie's modification only acetone is used as a decolurizer during the decolourization step [1].

THE PROCEDURE [6]

1. Make a thin smear of the material for study. Allow to air dry.
2. Fix the material to the slide by passing the slide three to four times through the flame of a Bunsen Burner so that the material to be gram stained.
3. Place the smear on a staining rack and overlay the surface with crystal violet solution.
4. After 1 minute of exposure to the crystal violet stain, wash thoroughly with distilled water or buffer.
5. Overlay the smear with Gram's iodine solution for 1 minute. Wash with water.
6. Hold the smear between the thumb and forefinger and flood the surface with a few drops of the acetone-alcohol decolorizer, until the violet colour washes off. This usually takes 10 seconds or less.
7. Wash with running water and again place the smear on the staining rack. Overlay the surface with safranin counter stain for 1 minute. Wash with running water.
8. Place the smear in an upright position in a staining rack, allowing the excess water to drain off and the smear to dry.
9. Examine the stained smear under the 100X (oil) immersion objective of the microscope. Gram positive bacterial stain dark blue; Gram negative bacterial appear pink-red.

HOW THE STAIN WORKS [7]

During the process of Gram staining a crystal violet-iodine complex is formed when iodine is added to the smear. Gram positive bacteria have thick peptidoglycan walls, dehydrated by alcohol. This closes pores in the bacterial cell wall preventing escape of the complex. Gram negative bacteria have a thin peptidoglycan layer and an outer lipopolysaccharide membrane which is readily penetrated by alcohol. The crystal violet-iodine A counter stain is added to make them visible under the microscope.

APPLICATIONS OF THE GRAM STAIN

The Gram stain is an important therapeutic tool. Gram positive and Gram negative organisms have different susceptibilities to a myriad of antimicrobials, thus Gram's stain may be used to guide initial therapy until definitive identification of the bacteria is available [2].

The detection of specific micro organisms may also serve as a guide for selecting appropriate culture media in the laboratory [6].

It can assist in presumptive diagnosis for example a smear showing Gram negative intracellular dioplococci give a presumptive diagnosis of gonoccoccal urethritis [2].

Direct Gram stains of specimens submitted for culture are often invaluable aids in the interpretation of Show organisms under the microscope but there may be no organism isolated on culture. This discrepancy may suggest that the organisms are fastidious and its growth is not supported by the medium/media used. It could be that the organisms are labile/fragile such that they do not survive the transport period from the ward to the laboratory. For example when dealing with anaerobes slight exposure to the atmospheric oxygen is enough to inactive them [2].

It could also be that the organisms seen under the microscope are dead or dying organisms. This is especially so in specimens collected from patients who have been commenced on antibiotics prior to sample collection. In these cases direct visualization with the Gram stain may provide the only clue to the nature variety and relative proportions of infecting organisms. Gram stain may also serve as a valuable quality control tool in the laboratory [6].

LIMITATIONS

The staining technique is not effective in the demonstration of organisms that lack a cell wall such as *Mycoplasma spp* [6]. A typical Gram reactions may be obtained in very young, old, dead, or degenerate cultures, hence cultures should be Gram stained when the logarithmic phase of growth [6]. *Mycobacterium sp* cannot be effectively Gram stained due to the presence of a thick waxy coat that prevents the entry of the stain [6].The number of microorganisms required is relatively high. Visualization with the Gram stain requires greater than 10^4 organisms per milliliter [2].

CONCLUSION

Although other more sensitive and specific techniques exists in the laboratory for the diagnosis of microbial infections such as polymerase chain reaction (PCR) and serology, the Gram stain remains of great medical importance. Gram negative bacterial tend to be more resistant to antibiotics due to the presence of their outer membrane.

Opportunistic infections of gram negative bacteria are of particular concern in hospitals. Gram positive bacteria are more resistant to digestion by enzymes such as pepsin and trypsin. In the light of these differences it is important to know what variety of bacteria is being dealt with before a specific method of treatment is chosen.

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DIAGNOSIS OF TUBERCULOSIS- A CRITIC OF CONVENTIONAL METHODS WITH NEWER TECHNIQUES

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CLINICAL SCENARIO

A 62 year old woman presents at medical out - patient clinic with complaints of cough productive of sputum and weight loss of 3 kg over the past 3 months. About 3 years ago she underwent left sided mastectomy for breast cancer and is otherwise well. A sputum sample sent to the laboratory tested positive for acid fast bacilli. The physician explains the result to the patient who wants to know if she has TUBERCULOSIS. Should treatment commence or not?

INTRODUCTION

Tuberculosis is of great public health concern globally, with its impact most felt in developing countries of Asia and Africa where 95% of cases and 98% of deaths are attributed to the disease. It remains the commonest cause of death in people living with HIV/AIDS, with about 40% synchronous infection rate in Nigeria[1].

Due to the slow metabolism of the causative agent, *Mycobacterium tuberculosis*, the isolation, identification and drug susceptibility testing requires several weeks. New techniques have improved specificity, turnaround time and cost effectiveness. Although these methods yield results within hours from sample collection, the clinical significance of such positive result requires rigorous evaluation in most cases[2]. hence the importance of a more reliable, sensitive and rapid diagnostic test to facilitate early diagnosis of cases and prompt initiation of therapy for a tuberculosis control programme cannot be overemphasized.

In diagnostic laboratory methods for tuberculosis, the results obtained are used for the detection and confirmation of the disease, follow up on treatment, and a guide to treatment in cases of relapse and treatment failure. These results are also used to monitor the progress of tuberculosis control, elimination strategies and outbreaks, which allows identification of breaches in tuberculosis control measures[3-6].

In this article, there will be a discussion on the conventional old methods and the newer techniques for detecting *Mycobacterium tuberculosis*. This implies examining not only the test characteristics such as sensitivity and specificity, advantages and disadvantages but also other factors such as technical requirements and cost effectiveness.

MICROBIOLOGY OF THE ORGANISM

In tissue, tubercle bacilli are thin straight rods that are non-spore forming. Once stained by basic dyes they cannot be decolourized by acid or alcohol, hence are called "acid fast bacilli". Mycobacteria are obligate aerobes and derive energy from the oxidation of many simple carbon compounds, hence increased carbondioxide tension enhances its growth. Its growth rate is much slower than that of most bacteria with a doubling time of about 18 hours.

Constituents of tubercle bacilli are mainly found in their cell walls and this can induce delayed hypersensitivity reaction and some resistance to infection . Mycobacteria are rich in lipids. These include mycolic acids (long-chain fatty acids) , waxes, and phosphatides. Muramyl dipeptide complexed with mycolic acids can cause granuloma formation; phospholipids induce caseation necrosis. These are sometimes responsible for acid fastness. Analysis of lipids by gas chromatography reveals patterns that aid in classification of different species.

Its protein constituents elicit the tuberculin reaction when bound to wax fraction can upon injection, induce tuberculin sensitivity and formation of a variety of antibodies . The tubercle bacilli also contain a variety of polysaccharides , these can induce the immediate type of hypersensitivity and serve as antigens in reactions with sera of infected persons .

The production and development of lesions and their progression are determined by the number of mycobacteria in the inoculum , their subsequent multiplication and the host resistance to hypersensitivity[7].

EVALUATION OF DIAGNOSTIC METHODS

Diagnostic laboratory techniques for tuberculosis utilizes both conventional old methods and newer techniques but some of these have shortcomings while others have not yet been evaluated sufficiently to decide on when and where they should be used[8].

There are various characteristics that are used to assess a diagnostic test. These include - False positive, False negative, Specificity and Sensitivity of the test.

Sensitivity testing is the proportion of individuals with a positive test result for the disease that the test is intended to reveal while specificity is the proportion of individuals with negative test results for the disease that the test is intended to reveal.

A false positive tests erroneously assigns an individual to a specific diagnostic or reference group , due to insufficiently exact methods of testing while false negative tests excludes an individual from a specific diagnostic or reference group, due to insufficiently exact methods of testing.

Sensitivity and specificity are the basic characteristics , that are inherent to the technique, but independent of the population in which it is used.

Predictive values do take into consideration not only technique but also the population in which it is used via "prevalence" parameter . Prevalence indicates the frequency of the disease and will be different for different populations . With high prevalence , sensitivity needs to be high to reach a good negative predictive value (NPV) . With low prevalence , when the disease is rare , specificity needs to be very high , otherwise the positive predictive value (PPV) of a test will be poor[8].

The performance of various laboratory methods for detecting *Mycobacterium tuberculosis* is largely dependent on the numbers of tubercle bacilli or their products present in the samples . Acid fast bacilli microscopy needs minimally five thousand (5000) acid fast bacilli per milliliter of sputum to yield a consistently positive result, as this is the case for most patients who present because of symptoms .This also shows that a distinct difference exists in sensitivity between excellent and poor acid fast bacilli microscopy[8].

DIAGNOSTIC LABORATORY METHODS

In the review of conventional laboratory diagnostic tools , the examination of clinical specimens suspected of containing mycobacteria involves several diagnostic tools :

1. Microscopic examination of sputum for acid fast bacilli.
2. Culture of mycobacteria using Lowenstein - Jensen medium.
3. Radiological diagnosis of pulmonary tuberculosis.
4. Mantoux testing.

Newer techniques include:

1. Modified culture - Radiometric liquid culture system (BACTEC 460TB).
2. Use of salubrics TK medium.
3. Polymerase chain reaction-based genetic test.
4. Serology - Antigen detection.
5. Chromatography.
6 Phage system - Luciferase reporter mycobacterio-phages.
7. Fast Plaque Kits by Biotec Laboratories.
8. Molecular techniques.

MICROSCOPY FOR ACID FAST BACILLI

Sputum microscopy as a diagnostic tool in tuberculosis has been in use for the past 100 years and it was adopted by WHO as a component of DOTS strategy for tuberculosis diagnosis 20 years ago[3-6].

Its use as a preliminary diagnosis of tuberculosis is of great importance especially in the detection of active infectious cases . This method of diagnosis is insensitive as it requires One hundred thousand acid fast bacilli per high power field (HPF) to be positive . It requires multiple visits to the clinic and is technically burdensome as it requires equipment that is difficult to maintain in field settings. It also yields results that depends on studious attention of a trained and motivated microscopist . It performs poorly in HIV co - infection. It is inadequate in paediatrics and extrapulmonary tuberculosis. Hence simpler , more sensitive and more patient friendly diagnostic tools are urgently needed[3-6].

CULTURE OF MYCOBACTERIA

Culture remains the gold standard in mycobacteriology because of its high sensitivity as well as specificity. Sensitivity reaches a minimum of about one hundred (100) bacilli per milliliter of sputum . In high - prevalence countries this may correspond to 80 - 90 % of patients presenting because of symptoms. Sensitivity for cultures seems to be lower in AIDS patients with advanced immune deficiency , where fewer tubercle bacilli are present in sputum. Sensitivity will also be affected by technical factors such as centrifugation and decontamination efficiency[8].

In terms of specificity , culture has a big advantage by allowing differentiation between *Mycobacterium tuberculosis* and other mycobacteria but this is less important in tuberculous high burden countries . On the other hand, specificity may suffer with careless techniques[8].

The main disadvantage of culture is not the need for more sophisticated resources and more qualified technicians or higher cost, but the slowness[8]. This is certainly so with the classical egg - or agar-based media-Lowenstein - Jensen medium for which acid fast bacilli positive specimens are then cultured and incubated for 6 weeks . Positive isolates are further confirmed by re-staining with Ziehl - Neelsen stains and biochemical tests[7]. The diagnostic delay of culture together with the presence of advanced disease in many cases at presentation and the high index of suspicion among clinicians in countries with high prevalence of tuberculosis all work to reduce the usefulness of culture[8].

RADIOLOGICAL DIAGNOSIS OF PULMONARY TUBERCULOSIS

It is commonly used amongst physicians everywhere in the world. The main advantage is its speed

simplicity and ease of use when equipment is available and possibly because the physician sees it as his tool which he understands better. Radiological diagnosis of pulmonary tuberculosis has a high sensitivity and it can detect the disease in patients who do not excrete any bacilli ,these are a small fraction of cases . The main disadvantage of radiological diagnosis arises from its lack of specificity. Experience has shown that its generalized use will lead to 50 - 100 % of over diagnosis . This means a lot of waste of resources with attention being focused on old , inactive cases with persisting radiological lesions rather than on the active transmitters of the disease , hence in the control strategy , radiological diagnosis for pulmonary tuberculosis has been reserved for the second line , after acid fast bacilli - smears have been consistently negative[8].

MANTOUX TESTING

This is used to detect latent infection and it is not specific for tuberculosis because it shares a large number of antigens with Bacille Calmette Guerin (BCG) and environmental Mycobacteria and it is plagued by errors due to readers , interpretation and the need for return patients visits[9-16] . Mantoux testing is not thus recommended for routine tuberculosis diagnosis , except in the case of children. It will then deliver only one of the arguments leading to a diagnosis when considered together with other arguments[8].

MODIFIED CULTURE

Radiometric Liquid Culture System (BACTEC 460 TB).

These are commonly used in mycobacteriological laboratories in developed countries . The technique is specific for mycobacteria growth , where ^{14}C labeled palmitic acid in 7H12 medium is used . It detects the presence of mycobacteria based on their metabolism rather than their visible growth . A comparison of BACTEC radiometric method with the conventional culture undertaken at the Tuberculosis Research Center(TRC) Chennai, showed that the rate of isolation of positive culture was significantly faster with the BACTEC method, with 80% of the positives being obtained by 7 days and 96 % by 14 days , hence mycobacteria in clinical samples can be detected in half the time compared to conventional culture methods [17] . The difficulty working with radioactive materials , necessity of expensive equipment for detection of radioactive gas and cost of materials seriously limit its use in poor resource countries[9-16].

Salubrics TK Medium

This was developed by Tuberculosis Diagnostic Initiative (TDI) based at World Health Organization Headquarters . It is used as an alternative to Lowenstein - Jensen medium. It is a rapid culture medium. Its original red colour turns to yellow by mycobacterial growth before the colonies become visible . It is not radioactive, hence does not create a radioactive waste problem. It differentiates contamination by turning to green when many other species of bacteria or fungi grow in the same culture medium. Its protective cap makes inoculations and subcultures easy and safe . The speed of the test result , its simplicity and its discriminating capacity improves the relevance of culture and makes it ideal for use[9-16].

POLYMERASE CHAIN REACTION - BASED GENETIC TEST

Detection of compounds or products of bacteria is based on the multiplication of their genetic material chromosomal DNA , or ribosomal RNA. Specificity of the test will thus depend on the use of correct primers using sequences typical for mycobacteria tuberculosis[8]. When compared to conventional culture , it has a sensitivity , specificity and positive predictability of 83.5,99 and 94.2% respectively[18].

The main advantage of polymerase chain reaction - based genetic test is their speed .In principle only 1-2 days are needed . While its main disadvantage is their extremely high cost[8].

SEROLOGY - ANTIGEN DETECTION

Detection of antigen , antibody , immune complexes or even immune - detection of complete bacterial cells , have been described with many variations . The simplicity and ease of these methods are attractive.

The main problem seem to be the antibody response to various tuberculous antigen which is highly variable between patients , resulting in problems of sensitivity as well as specificity especially with easy -to-use formats such as latex - agglutination or immune - chromatographic (dot) tests[8].

Typically, sensitivity is comparable to that of acid fast bacilli - microscopy and clearly better also in smear positive cases . Also specificity remains well under that of microscopy. Better results are obtained with more complicated techniques such as sandwich Enzyme-Linked Immunosorbent Assay (ELISA), but they are not useful in routine practice[8] . This method is expensive and requires trained personnel and often have difficulty in differentiating between mycobacteria tuberculosis and non - tuberculous mycobacteria[19] . However , there is not yet a serological test that is good enough , especially not to detect the cases where microscopy remains deficient such as in HIV positives, children and cases of extra - pulmonary tuberculosis[8].

CHROMATOGRAPHY

Several methods have been described using high - performance liquid chromatography (HPLC) or gas - liquid chromatography (GLC) . Detection targets

substances from the cell wall such as mycolic acids or metabolic products such as tuberculostearic acid. Results have been extremely good in terms of sensitivity and specificity as well as speed[8].

Nevertheless, these techniques seem to be used rarely in routine practice because of the expense of the equipment, which has no other applications in the bacteriology laboratory[8].

PHAGE SYSTEM

Luciferase Reporter Mycobacteriophages And Fast Plaque Kits By Biotec Laboratory

Usage of mycobacteriophages for diagnosis of tuberculosis offer a phenotype-based result in short time and low cost. Luciferase reporter mycobacteriophages are phages harboring the fflux gene, which produces visible light when expressed in the presence of luciferin (enzyme substrate) and cellular ATP. Luciferase reporter mycobacteriophages are able to infect, replicate and express their genome (including the fflux gene) within viable mycobacterial cells. If a luciferase reporter mycobacteriophages infected clinical sample releases light after addition of luciferin, the presence of viable M. tuberculosis is detected[20].

These detect live mycobacteria in clinical samples or in young liquid cultures using phages that infect and replicate mycobacteria cells as indicators. The major problem with fast plaque kits is the high cost of the kits[9-16].

MOLECULAR TECHNIQUES

This determines the presence of mycobacteria tuberculosis in clinical specimens by detecting specific nucleic acid sequences after being amplified. Nucleic acid amplification assays (NAA) have been found to be more sensitive than smear microscopy but less sensitive than culture. Even though commercially available nucleic acid amplification assay (NAA) kits are simple and reliable to use. Its cost, degree of technical support and quality control requirements limit their use in poor countries[9-16].

The expectation that molecular techniques would surpass conventional methods for diagnosis of tuberculosis has not been realized. The genetic basis of resistance must be understood before achieving such a goal. However, the clinician now has a variety of new tools to improve the diagnosis of tuberculosis. Most of them still require detailed and systematic evaluation using standard techniques as references before their widespread application in clinical settings. Most of these techniques require trained personnel and specialized equipment, hindering their application in field conditions, but they can be used in reference laboratories as part of the tuberculosis control programs[2].

The diagnosis of tuberculosis should be given urgent attention to in high prevalence areas like Nigeria. However, the impediments to the diagnosis of tuberculosis in Nigeria include the widespread use of BCG vaccinations, which may affect the specificity of immunologic tests and the high prevalence of HIV/AIDS infection[9-16].

In tuberculosis diagnostics, the implementation of new tools should be facilitated by upgrading laboratory services. The first diagnostic upgrade that most tuberculosis laboratories should implement is the modification of microscopy. It should be simple enough for use by unskilled workers with less than 3 hours of training and should yield conclusive results in less than 2 hours while the patient is still in the clinic. Also, it should require little or no interpretations and function well in HIV patients.

Furthermore, a replacement for culture is required to augument microscopy for the evaluation of complex patients and this must be fast, simple to perform and inexpensive to be implemented at the peripheral level[9-16].

Laboratory workers, especially technologists and technicians, should be motivated and encouraged. They should be sent to refresher courses to update their knowledge and skills. These are enormous tasks, which require the efforts of donor agencies and private sector participation. International collaborations are strongly needed to address the substantial gap that exists in tuberculosis research between poor resource countries and the developed world[1].

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ANTIBIOTIC RESISTANCE IN THE UNIVERSITY COLLEGE HOSPITAL (UCH) IBADAN, NIGERIA

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ABSTRACT

In the last few decades the incidence of antibiotic resistance to first-line drugs is on the rise. Many hospital, clinics and laboratories all over the world, continue to report new strains of a resistant bacteria. Multi-drug resistant strains have been termed superbugs. The most resistant strains include Staphylococcus aureus, Enterococci and other gram-negative organisms such as Salmonella, Shigella, Pseudomonas, E-coli., Klebsiella and Serratia. They leave modern medicine unable to treat common infectious diseases. This review seeks to highlight the "superbugs" in the UCH and the pattern of antibiotic resistance and sensitivity as we know has dramatically changed in the past few years. Identified amongst the main causes of resistance include self medication, poor compliance and inappropriate dosing.

INTRODUCTION

Antibiotics (Greek anti; "against"; bios, life") is any chemical compound used to kill or inhibit the growth of infectious organisms, particularly bacteria and fungi[1].

Originally, the term 'Antibiotic' referred only to organic compounds produced by bacteria or moulds that are toxic to other micro-organisms, but now includes synthetic and semi-synthetic as well as organic compounds acting in that capacity.

HISTORY

The principle of using organic compounds to fight infection has been known since ancient times, though the antibiotic mechanism was not scientifically understood till the 20th century. The first observation was made in the 19th century by Louis Pasteur, a French chemist who through several experiments demonstrated that many common diseases were caused by bacteria and discovered that certain saprophytic bacteria can kill anthrax germs. The German doctor and chemist Paul Ehrlich in the first decade of the 20th century conducted experiments which led to the development, in 1909, of Salvarsan, an arsenic-containing synthetic compound which exhibited effective action against spirochaetes, the syphilis-causing bacteria. This remained the only effective treatment for syphilis until penicillin was discovered in 1940s.

Penicillin, probably the best known antibiotic, the prototype of antibiotics and a derivative of the mold *Penicillium notatum,* was discovered accidentally in 1925 by Alexander Fleming while studying the bacterium *Staphylococcus aureus.* He showed its effectiveness in laboratory cultures against many disease-producing bacteria. This discovery marked the beginning of the development of antibacterial compounds produced by living organisms. Penicillin was first used on human beings by Howard Florey and Ernst Chain in 1940 and by 1946, it was already commercially available and its chemotherapeutic value was put to use on a very large scale during the World war II.

The first antibiotic to be used in the treatment of human disease was tyrothricin isolated by René Jules Dubos, an American bacteriologist. It was however too toxic for general use. Other antibiotics produced by a group of soil bacteria called Actinomycetes have proved more successful. One of these, streptomycin discovered in 1944 by American biologist Selman Waksman and his associates is effective against many diseases including several against which penicillin is useless especially tuberculosis . Since antibiotics came into general use in the 1950s, they have transformed the patterns of disease and death. Many diseases that once headed mortality tables e.g Tuberculosis, pneumonia, septicaemia, now hold lower positions and surgical procedures have improved due to execution of complex procedures without a prohibitively high risk of infection.[1]

However, this break through development of effective antibacterial drugs is accompanied by a phenomenon called resistance, which seems to be a way by which the organisms "mock" attempts at effectively exterminating their harmful effects.

Resistance to many of the various types of antibiotics have developed over the years with antibiotic use. Some of the mechanisms of resistance have been exposed and steps also taken to curb and/or evade these mechanisms.

TYPES OF ANTIBIOTICS

Penicillins: Are the oldest group. They are bactericidal inhibiting formation of the cell wall by mechanical action. There are four (4) subtypes:

* Penicillin G types (narrow spectrum)
* Ampicillin and its relatives e.g Amoxicillin
* Penicillinase resistant
* Antipseudomonal penicillin

Cephalosporins: They interfere with synthesis of the bacterial cell wall and so are bactericidal with a lower

risk factor than penicillins though more expensive.

Aminoglycosides: Streptomycin is the oldest and second most commonly administered antibiotic (after penicillin). These aminoglycosides are narrow spectrum and act by inhibiting bacterial protein synthesis.

Tetracyclines: These are bacteriostatic, inhibiting bacterial protein synthesis. They are broad spectrum.

Macrolides: Are bacteriostatic binding with bacterial ribosomes to inhibit protein synthesis e.g. Erythromycin.

Sulphonamides: Are synthetic bacteriostatic, broad spectrum antibiotics.

These classes/types of antibiotics have been used effectively in the treatment of acute and chronic infection but a threat to their usage has emerged in form of resistance which has been developed by many organisms through various mechanisms which include inactivation of the antibiotic and mutation in the bacterial enzyme affected by the drug. All of these can be transferred genetically by the bacterium to its progeny, or transmitted by means of plasmids from one bacterium to another.

The problem of resistance has been exacerbated by the indiscriminate and inappropriate use of antibiotics as prophylaxis, in the treatment of common cold and other viral infections, and also by its use in poultry and livestock.

ANTIBIOTIC RESISTANCE

Antibiotic resistance may be defined as the capacity of bacteria to withstand antibiotics previously toxic to them; achieved by spontaneous mutation or through selective pressure after exposure to the antibiotic in question[2].

This phenomenon of resistance is not unexpected, it being an evolutionary principle that organisms adapt generally to changes in their environment. This also imposes serious constraints on the options available for the medical treatment of many bacterial infections[3].

Antibiotic resistance in bacteria spreads at three levels:

- Transfer of bacteria between people
- Transfer of resistance genes between bacteria (usually on plasmids).
- Transfer of resistance between genetic elements within bacteria or transposons.

ORIGINS OF RESISTANCE

NON GENETIC ORIGIN

Since active replication of bacteria is required for most antibacterial drug actions, organisms that are metabolically inactive i.e. slow-multiplying e.g. Mycobacterium, may be phenotypically resistant to drugs[4].

Bacteria may also lose the specific target structure for a drug after replicating for several generations and thus be resistant e.g. Penicillin-susceptible organisms may change to cell-wall deficient forms during penicillin administration and thus be resistant to cell wall-inhibitor drugs for many generations.

Bacteria may also infect at sites where certain antibacterials are excluded or not active e.g. Gentamicin, an aminoglycoside is not effective on *Salmonella* because they are intracellular and aminoglycosides do not cross the cell membrane to enter cells.

GENETIC ORIGIN

Chromosome resistance

This develops as a result of spontaneous mutations in a locus that controls susceptibility to a given antibiotic. On administration, the antibiotic "selects" the non-resistant organism and eliminates them favouring the growth of resistant mutants e.g. chromosomal mutants are most commonly resistant by virtue of a change in a structural receptor for a drug. The P-12 protein in the 30S subunit of bacterial ribosome serves as a receptor for streptomycin attachment. Mutation in the gene involving that structural protein results in streptomycin resistance.

Extra chromosome resistance

Many species of bacteria contain, in addition to the chromosome, extra chromosomal genetic elements called plasmids that exist free in the cytoplasm. Plasmids carry genes for resistance to one and often several – antimicrobial drugs. Much of the drug resistance encountered in clinical medicine is plasmid determined. These plasmids often control the formation of enzymes capable of destroying the antimicrobial agents. For example, they carry genes for the formation of β-lactamases which determine resistance to penicillins and cephalosporins. They code for enzymes that acetylate, adenylate or phosphorylate various aminoglycosides and for enzymes that determine the active transport of Tetracyclines across the cell membrane.[4]

Transfer of resistance genes within the bacterium

Some stretches of DNA can be fairly readily transferred (transposed) from one plasmid to another and from plasmid to chromosome or vice versa. Transposons – segments of DNA- can be transferred independently of the normal mechanism of homologous genetic recombination. Then transposons may contain one or more resistance genes and thus may transfer resistance from the chromosome to plasmids.

Transfer between bacteria

There are three mechanisms for gene transfer between bacterial of same species and of different species. They are:

(a) **Conjugation**: Cell-to-cell contact during which chromosomal or extra chromosome DNA is transferred from one bacterium to another. It is the main mechanism for spread of resistance.

(b) **Transduction**: Plasmid DNA is enclosed in

bacterial virus (or phage) and transferred to another bacterium of the same species. It is relatively ineffective but clinically important in transmission of resistance genes between strains of *staphylococci* and between strains of *streptococci*.

(c) **Transformation**: A bacteria can take in naked DNA from its environment and incorporate it into its genome through the crossover mechanism. However this happens when the incoming DNA comes from a same-strain cell or closely related strain. This however is not of importance in the clinical problem of resistance.

BIOCHEMICAL MECHANISM OF RESISTANCE

Production of an Enzyme that inactivates or destroys the active drug:

Examples:

(1) *Staphylococci* resistant to penicillin produce a B-lactamase that destroys the drug.

(2) Gram negative bacteria resistant to amino-glycosides produce adenylating, phosphorylating or acetylating enzymes that destroy the drug. Chloramphenicol resistance also acts via this mechanism, which is plasmid-borne.

Alteration of Drug-Sensitive Site

Examples: Erythromycin-resistant organisms have an altered receptor on the 50S subunit, resulting from methylation of a 23S ribosomal RNA. An altered DNA-dependent RNA polymerase determined by a chromosomal mutation is the basis for resistance to Rifampicin.

Decreased drug accumulation in the Bacterium

Example: The plasmid-mediated resistance to tetracycline in both Gram positive and negative. The resistance genes code for inducible proteins in the bacterial membrane which promote energy-dependent efflux of the tetracyclines. This also occurs with Erythromycin in *S. aureus* and to fluoroquinolones.

Development of a pathway that bypasses the reaction inhibited by the antibiotic.

Example: Resistance to Trimethoprim is the result to plasmid-mediated synthesis of a dihydrofolate reductase with low or zero affinity for trimethoprim. This is transformed by transduction and may be spread by transposons.

MULTIDRUG RESISTANCE

Many pathogenic bacteria e.g some strains of *Staphylococci* and *Enterococci* are resistant to virtually all current antibiotics, resistance being transferred by transposons and/or plasmids. These organisms can cause serious and virtually untreatable nosocomial infections[5].

The most disturbing development of resistance has been in *Staphylococci* many strains of which are now resistant to almost **all** currently available antibiotics. Through extra efforts are currently underway in many countries to find new antibiotics effective against the rapidly growing ranks of multi resistant bacteria, and several now potentially effective compounds are in the pipeline, it seems nature has endowed microorganism with fiendishly effective adaptive mechanisms for dealing with our pharmaceutical attack and have so far been effortlessly keeping pace with our attempts to deal with them.

FACTORS ENCOURAGING THE SPREAD OF RESISTANCE

The increasing prevalence of antimicrobial resistance is due to many interlinked factors most of which are directly related to antimicrobial misuse.

Patient related factors: are mainly related to inappropriate antibiotic use. In many developing countries there is usually **self-medication** with unnecessary drugs of which many are counterfeit. Many over-the-counter drugs used for treatment usually contain inadequate amounts of the active drug.

Also, patient **compliance** is usually poor because they usually interrupt treatment once symptoms begin to disappear, or do not take a full course of their drugs due to financial constraints, thereby, providing an environment for mutants to emerge and multiply.

Poor infection control practices, such as hand washing and changing gloves before and after contact with patient help increase cross-infection. Intensive and prolonged antimicrobial use, coupled with the use of antibiotic for prophylaxis also contributes to increasing prevalence.

Also widespread use of anti-microbials for disease control and growth promotion in animals has been paralleled by an increase in resistance in zoonoses.

THE UCH IBADAN EXPERIENCE ON ANTIBIOTIC RESISTANCE

The picture has not been any different in Nigeria (and Ibadan in Particular) from what obtains worldwide. However some variance exists owing to peculiar and abounding reasons which might occur way down in the scale of general reasons for why, but rank topmost in our environment.

In a study of pathogenic agents in discharging ears in University College Hospital, Ibadan between March 1995 and February 1997 by Oni A.A. et al., 79.9% were monomicrobial and 20.3% polymicrobial. The most frequent agents were *Pseudomonas spp, Staphylococcus aureus, Klebsiella spp. and Proteus spp*. A similar result was obtained when the same team carried out the same study in children in Ibadan Nigeria [6]. *Pseudomonas Staphylococcus, Proteus and Klebsiella* were identified as principal organisms. This later result was similar to that of Cooker et al in Lagos and Jack L. M. in Zaire.[8] It was shown that the *Pseudomonas spp*. showed 100% resistance to Tetracycline 75% to Ampicillin and 50% to Cotrimoxazole. *Staphylococcus aureus* showed 78% resistance to Tetracycline, 80% to Ampicillin, 35%

resistance to Cotrimoxazole and 50% resistance to Amoxicillin. Thus Amoxicillin, Ampicillin, Tetracycline, Cotrimoxazole and Streptomycin are not valuable and more or less useless in the treatment of ear infections in Ibadan. It is worthy to note that these drugs are easily procured without prescription in our environment, a practice which undoubtedly encourages development of resistance as explained earlier. However, the cephalosporins- ceftazidime, ceftriaxone and cefuroxime, and azithromycin and gentamycin showed good sensitivity against two-thirds of the strains whilst quinolones- Ofloxacin and Ciprofloxacin had the best activity. Though a more expensive alternative, the cephalosporins, gentamycin and erythromycin should be used as first line antibiotic therapy for discharging ears and the quinolones be reserved drugs. These should be combined with Metronidazole to effectively combat anaerobes.[6]

The antimicrobial susceptibility of the commonly encountered organisms in Ibadan changes frequently, a phenomenon long predicted by Montefiore and Okubanjo in 1970[10] and reaffirmed by Alausa and Montefiore in 1978.[11] Surveillance screening of *Neisseria gonorrhoea* isolates in Ibadan for Penicillinase-producing *Neisseria gonorrhoea* strains (PPNG strains) showed no PPNG in 1977 [17,18,19] hence almost 100% of circulating strains were non PPNG. However it has been established that strains of PPNG are now circulating freely in Nigeria.[17,18,19,20,21] In early 1979, Osoba et al.[22] found the prevalence rate of PPNG in Ibadan to be 2.7%. There has since been a steady from this to 50% by the end of 1981[22] and to about 70-80% by 1989 [21]. By early 1996, Bakare et al. [23] showed the prevalence rate of PPNG to be 92.2%. This of course went higher and is presently above 98.6% [24]. This situation poses a great threat to the usefulness of Penicillin and Ampicillin as the drugs of choice in gonococcal therapy due to the production of B-lactamase by the PPNG.

Worthy of note is the role of Gram-negative organisms in the aetiology of severe infections. Most of the bacteria are resistant to many antibiotics. This was confirmed by R.A. Bakare et al in 1999 in a study on in-vitro activity of Perfloxacin and other antibiotics against gram negative bacteria in Ibadan [12]. Of the 48 strains that were examined 15 were resistant to Gentamycin, 10-19 to Augmentin, 11 to Ceftazidime, 7 to Perfloxacin and 2 to Ofloxacin.

Alausa and Montefiore also reported that Gram negative organisms were responsible for 69% of all bacterial infection at UCH and *Klebsiella* was the most commonly encountered in the group. Most of the Gram-negative bacilli especially *Klebsiella species and Pseudomonas spp.* are intrinsically resistant to most antibiotics, a situation which favours their continued existence in hospital environment [13,14]. However, the majority of Gram-negative bacilli isolated from clinical specimen were highly susceptible to all quinolones. Ciprofloxacin was the most active while Perfloxacin was not as sensitive though significantly active [15]. However, some strains of *Klebsiella spp.* and *Pseudomonas aeruginosa* were resistant to the quinolones. A few years before, Olugbemi et al. in 1994 documented 100% sensitivity to ciprofloxacin in Lagos [13].

Surgical wound infection is a good index of nosocomial infections. In Ibadan, 30.3% of surgical wound infection has been found to be caused by *Staphylococcus aureus*.[25] The percentage of *Staphylococcus aureus* that is resistant to Methicillin has been on the increase in Ibadan. From 1% in 1972, it has increased to 27% in 1995.[26] Studies on the Methicillin resistant *Staphylococcus aureus* (MRSA) strains in Ibadan reveals that all the MRSA strains were sensitive to Vancomycin, Ofloxacin and Ciprofloxacin, 80% were sensitive to Gentamycin, Cotrimoxazole and Fusidic acid and none was sensitive to Penicillin.[27] Thus for MRSA isolates in Ibadan, Vancomycin, Ofloxacin and Ciprofloxacin offers the best effective treatment for wound infections.

Though Ciprofloxacin, Ofloxacin and Perfloxacin are potentially valuable antibiotics for severe infections in our environment, it is expensive and wouldn't replace the polypharmacy attitude which will invariably lead to another development of resistance which will of course be tougher and more resilient to antibiotic intervention.

The life time of an antimicrobial agent can be drastically shortened, if resistance develops among initially susceptible bacteria[16]. Unfortunately, the development of resistance is often inadequately assessed especially in the developing countries, so that the potential for resistance to shorten the lifespan of antimicrobials is often unknown. There is thus an emphasized need to analyze closely the emergence of resistance associated with the use of any antibiotic which will reduce the financial burden of patients and their relations in the purchase of "inactive" antimicrobial agents.

CONCLUSION

Microbes will always evolve to adapt to changes in their environment to continue their species. This is fundamental but the increased failure of antibiotics in recent few decades is alarming, more precariously so as we humans by our practices are more or less inadvertently helping the microbes thrive within our system by developing resistance to most of the emerging first-line antibiotics.

The phenomenon of resistance is one which we indeed need to look into, devise ways of delaying or preventing its occurrence and constitute practices to discourage it. Practices like discouraging self-medication and education of the public on hazards of indiscriminate and non-prescribed use of antibiotics, patient compliance to treatment in completing dosage when placed on antibiotic treatment, simple practices among health workers like hand-washing, changing of gloves after one patient before the next, etc. would go a long way in preventing nosocomial infections. If not, we

face the irony of modern medicine being unable to treat common infectious diseases.

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A REVIEW OF DETECTION AND QUANTIFICATION METHODS IN IMMUNOLOGY

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INTRODUCTION

Immunology is an inter-disciplinary science that occupies a prime position in life science. It is of great importance to basic and applied research. Immunological techniques are required for a wide variety of investigative work in diagnosis and research. There have been rapid advances in immunological methodology in tune with the status of modern immunology as a highly evolved molecular and cellular science. Immunological researches and investigations are required in fields such as microbiology, biochemistry, molecular biology, endocrinology, allergy and hypersensitivity clinics, in surgery for histocompatibility matches needed for organ transplantation, nuclear medicine and cancer research. Immunological methods are also used to detect pathogens in agricultural and environmental samples.

Accurate diagnosis depends on the affinity and specificity of the antibody preparation used, and high affinity antibodies are essential for the detection of very small amounts of pathogen. Modern immunological techniques includes enzymes immunoassays, Radioimmunoassays, immunomicroscopic methods (Immunofluorescnce assays, Fluorescence activated cell sorters, Immunogold labeling, Immunoaffinity seperation) and Emerging technologies (Multi-analyte assays, signal transduction assays). Others are Immunodiffusion (Single Radial Diffusion, Double Radial Diffusion); Electrophoresis and Immunoelectrophoresis; Agglutination techniques; Complement assays (Complement fixation tests, Haemolytic assay); Nitroblue Tetrazolium Test; Neutrophil microbicidal assay; Cell motility tests and Histocompatibility Testing (Lymphocytotoxicity Test, Mixed-lymphocyte reactions and Restriction Length Fragment Polymorphism). These methods are used in the diagnosis and prognosis of infectious diseases, autoimmune diseases, allergy, etc.

It is not possible to discuss the gamut of immunological assays in a paper of this kind, so a selective review, illustrated with some examples drawn from clinical and environmental diagnostic assays, is presented. Some of the methods are currently used, or may be usefully applied in the futrure for the detection of pathogens.

PURPOSE OF THIS REVIEW

Clinicians use an ever-increasing arsenal of molecular and cellular tests to diagnose infected individuals. Primarily, these measures include detection of specific antibodies or proteins from the patient (host), or detection and identification of proteins from the infecting organism.

Diagnostic tests must be sensitive and highly specific to avoid inaccurate results. A variety of tests utilize qualitative and/or quantitative results, and clinicians should know the molecular bases of these diagnostic methods in order to establish:

I. Appropriate use of diagnostic methods
II. Proper interpretation of results
III. Reasonable extrapolation of findings
IV. Determination of subsequent testing

This laboratory exercise will provide the scientific foundation to understand, interpret, and utilize several of the essential applications used in immunologic diagnoses. The methods discussed include the following:

I. ELISA (Enzyme-Linked Immunosorbent Assay)
II. RIA (Radio-immuno-assay)
III. Western Blot (also known as Immunoblot)
IV. Immunofluorescence Microscopy
V. Flow cytometry/ FACS (Fluorescent Activated Cell Sorting)

REVIEW OF ANTIGEN-ANTIBODY IN

The fundamental concept upon which all of these diagnostic methods rest is the principle of antigen-antibody interactions. Antigens are substances capable of inducing a specific immune response; antigens are treated as none-self by the immune system. Antibodies are molecules that bind antigens and are produced by cells. Diagnostic tests employ both monoclonal antibodies and polyclonal antibodies. In a preparation of a monoclonal antibody, all of the molecules have the same antigen-binding site, and therefore recognize a single epitope. However, the antibody response made in any given individual is polyclonal, and involves many different antibody molecules that are directed against multiple epitopes. Antibodies that are used in clinical applications are produced by a variety of species (i.e. goat, rabbit, horse, monkey, chicken and human).

The antigen-antibody interaction is reversible and is mediated by several kinds of non-covalent bonds (hydrogen bonds, electrostatic, hydrophobic, Van der Waals forces). The strength (affinity) of the interaction increases with the proximity of interacting groups. The complementarity of shape in the combining site is also important for strong binding.

ASSAY METHODS

Many immunoassays employ antibodies that react with components located on the surface of the pathogen e.g. capsid protein, or cell wall or flagellar antigens. Such assays will determine the presence (and quantity) of these antigens but not the viability of the pathogen. The use of antibodies specific for other antigens involved in the infection process such as sporozoite antigens, or for viruses, a combination of serology and nucleic acid-based methods (to detect genome and capsid proteins in the same sample), are strategies that may give a positive correlation with viability. Also, immunological methods may be combined with other tests (e.g. culture, dye permeability stains, nucleic acid- based tests) to confirm the identity and viability of positive samples.

A selective review of immunological diagnostic assays is presented herein. These include enzyme immunoassays, immunochromatography, immunomicroscopy, affinity separation and certain emerging technologies. Also discussed in this article are reliable methods that have been variously modified (immunodiffusion methods and strip electrophoresis).

(I) Enzyme immunoassays (EIA)

EIA methods combine the specificity of antibody molecules with the amplification of antibody-antigen interactions by enzyme catalysis, and therefore can detect very small amounts of pathogen. There are many different EIA methods, and both qualitative and quantitative results can be obtained. The assays can be readily standardized, and optimised to minimize variation both between and within assays[1]. Most well constructed assays can be done by individuals with little previous knowledge of training.

Assay Formats

Many assays are done in the wells of microtitre plates in which the reactants are immobilized. There are a wide variety of formats. Antigen in the sample may or may not be bound by a specific antibody immobilized on the surface (coating antibody). Direct assays employ specific antibodies conjugated to enzymes, whereas in indirect assays the antigen specific detecting antibody is in turn detected by an anti-immunoglobulin enzyme conjugate, of which a wide range is commercially available. Many EIA formats utilize the well-known strong interaction between avidin (or streptavidin) and biotin. Biotin is a small molecule and is commercially available in many derivatised forms suitable for labeling antibodies and other ligands. Biotinylated antibodies can be detected by using a streptavidin-enzyme conjugate. An advantage of this approach is that the same conjugate can be used to assay different antigens.

Tests done using membranes (nitrocellulose, polyvinylidene difluoride, nylon) to immobilize reactants often employ insoluble substrates where the products of the reaction precipitate around the site of enzyme activity. A nylon filter EIA was used to detect potyvirus in water samples[3].

Detection limits

Apart from the specificity and affinity of the antibodies used, the limits of detection of EIA depend on factors such as the specific activity of the enzyme employed, and the substrate[1]. The direct Double Antibody Sandwich Enzyme-Linked Immunosorbent assay (DAS-ELISA) is used widely for virus detection. The advantage of this assay is that the virus particles are concentrated from extracts by the coating antibody, and potentially inhibitory components of extracts are removed by rinsing before addition of detecting antibody and enzyme substrates. The limit of detection of most plant viruses in tests on host tissue extracts is between 1-10 ng/ml when using the chromogenic substrate *p*-nitrolphenyl phosphate (NPP). The product of hydrolysis of NPP by alkaline phosphatase (AP) turns yellow in alkaline solution and strongly absorbs light at 405 nm. Substantially lower limits of detection (<100x) can be achieved by using fluorogenic or chemiluminescent substrates because much smaller amounts of these reaction products can be measured compared with absorptiometry. However, the reaction products of tests utilizing fluorogenic and chemiluminescent substrates are not visible to the eye, they are measured by specialist instruments (spectrofluorometer or luminometer respectively) or for membrane based chemiluminescent tests, by exposure to X-ray film. A 24-hour test for members of the family *Enterobacteriaceae* that combines sample filtration and enrichment on selective medium followed by a DAS-ELISA (with fluorogenic substrate) was specific for *Enterobacteriaceae.* In tests on drinking water, a 98% correlation was obtained between these tests and commonly used micro-biological tests[4].

Increased sensitivity can also be obtained by using an enzyme amplification technique. In this method the product of the enzyme reaction is amplified by the addition of two further enzymes, which participate in a cyclic reaction, producing a coloured end product that can be measured spectrophotometrically[5]. This method was used to increase detection sensitivity of barley yellow dwarf virus in plants and individual aphids[6].

Automation and quantification

Microplate assays are readily automated. It is possible to test large numbers (1000s) of samples using specially designed workstations, automatic microplate washing and sample dispensers, and a microplate reader with computer assisted data analysis. An estimate of virus concentration in the samples is readily obtained by constructing a standard curve using absorbance values obtained from titrating a control antigen preparation. Such curves are typically sigmoidal with values reaching a plateau at higher antigen concentrations, and the linear portion of the curve is used to estimate concentration.

Competitive EIA

In addition to the above designs, competitive EIAs have

also been designed where the antigen in the sample competes with antigen coupled to enzyme for antibody binding. In these assays, a decrease in absorbance values indicates the presence of target antigen. They are principally used for small molecules that have few antibody binding sites. For example, a competitive assay has been developed to detect the herbicide atrazine ($L_{um}IA^{TM}$, Cambridge Sensors Ltd). These assays incorporate a chemiluminescent substrate and can detect atrazine in water samples without pre-concentration. The results can be quantified to a lower limit of 0.01 micrograms/litre.

Rapid 'on-site' assays

Many EIA have been devised for rapid 'on-site' assays, which are incorporated into self-contained kits so that no extra equipment is needed. These tests usually involve membrane immobilization of reactants, and are constructed so that the reactants are applied to the membrane surface and flow through the membrane into an absorbent layer. This format is fast because there is no need to allow time for diffusion as in microplate assays. The assays are designed for use by non-specialists, and some quantitation is possible using a handheld reflectometer[7]. In tests to detect *Cryptosporidium* oocysts in faecal samples, the results of a 15 min EIA (ProSpecTR) showed good correlation with those of an acid-fast stain procedure[8].

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(2) Immunomicroscopy Methods

Immunofluorescence assays (IFA).

In direct IFA, the sample is fixed onto a microscope slide, then a drop of pathogen specific antibody labeled with a suitable fluorochrome e.g. fluorescein isothiocyanate (FITC) is applied, incubated, and then unbound conjugate is rinsed off, and the slide is examined under an epifluorescent microscope. If antibody has bound, the sample will fluoresce green. An indirect IFA can also be done where the pathogen specific antibody is detected by a second anti-immunoglobulin antibody labeled with FITC. IFA methods are laboratory based, and require an epifluorescent microscope equipped with appropriate excitation and emission filters, and capable of magnifications of 100x, 400x, and 1000x. Also, relatively few samples can be examined per day, and the analysis of results is subjective and requires experienced personnel.

Fluorescence activated cell sorter

Cells stained with FITC-antibody conjugate can be identified and separated from other components of a suspension in an instrument known as fluorescence activated cell sorter (FACS). The suspension is passed through a laser beam and cells are separated on the basis of fluorescence intensity. The sorted cells are then examined microsopically. This technique has been used to identify *Cryptosporidium* oocysts in water samples[9]. Prior separation of oocysts from contaminating materials in the sample enable the oocysts present to be seen more readily. However, the FACS instrument is expensive.

Immunogold labeling

Viruses are readily captured on antibody-coated electron microscope grids, and antibodies labeled with colloidal gold are used to identify and localize epitopes on the particles[10]. Also, antibody-gold conjugates (or protein A-gold preparations) have been used to localize *Cryptosporidium* antigens in tissue sections[11]. However, these methods are laboratory based and require access to electron microscopes and other expensive equipment and skilled staff.

(3) Immunoaffinity separation

Detection of pathogens in environmental samples is complicated by the need to concentrate large volumes (e.g. 100 – 1000 litres) for pathogen detection. Concentration is done by methods such as filtration, flocculation or centrifugation. As a result of concentrating such large volumes, samples usually contain other material such as algae, minerals and compounds that inhibit some detection methods. Separation of pathogens from these contaminants can be achieved by immunoaffinity methods such as antibody coated magnetic beads (4.5 μm diameter). For example, hepatitis A virus (HAV) was successfully detected by a nucleic acid-based method (reverse transcription and polymerase chain reaction; RT-PCR) after separation by magnetic beads coated with anti-HAV rabbit polyclonal antibodies[12]. Similarly, group A rotaviruses were detected by RT-PCR after concentration on magnetic beads coated with group-specific Mabs against the VP6 inner capsid protein. Immunocapture RT-PCR combines antibody and nucleic acid based methods, and therefore may give a better indication of virus viability than either method alone, since it shows that both the virus genome and the protective coat protein are present in the same sample.

Magnetic beads coated with a Mab preparation specific for bacterial flagellae were also used to isolate Pseudomonads from samples of unconcentrated lake water seeded with bacterial cultures[14].

(4) Emerging Technologies

Multi-analyte assays.

Some of these new kinds of assays rely on techniques developed in the microelectronics industry, and if these can be applied successfully; will represent a major change in immunoassay technology. Tests for many substances (analytes) can be done at the same time in such micro-assays, and they require much smaller reagent and sample volumes, yet sensitivity is not compromised. For example, a microspot assay for thyroid stimulating hormone that employs fluorescent antibodies had a detection limit of c. 8 x 10 molecules/ml[15].

The explosives TNT (2, 4, 6-trinitrotoluene) and RDX (hexahydro-1, 3, 5-triazine) have been detected in

groundwater samples by displacement flow immunoassays. In the assay, microcapillary flow cells are connected in parallel, the capillary tubes are coated with specific antibody and fluorophore-labelled antigen is bound to the coating antibody. The labeled antigen is displaced by target antigen in the sample, and measured by portable spectrofluorometers connected downstream from the capillary tubes. The limits of sensitivity were 0.1-0.5 ng/ml [16].

Signal transduction assays

The development of immunosensors to convert the antibody-antigen interaction into an electrical signal that can be readily measured is advancing steadily and recent work suggests that these systems may have an impact in environmental monitoring[17]. Advantages include rapid test results and minimum manipulation of samples and reagents.

(5) Immunodiffusion [18,19]

The purpose of all immunodiffusion techniques is to detect the reaction of antigen and antibody by the precipitation reaction. Although the formation of antigen-antibody complexes in a semisolid medium such as agar is dependent on buffer, electrolytes, pH, and temperature, the most important determinants of the reaction are the relative concentrations of antigen and antibody. Maximal precipitation forms in the area of equivalence, with decreasing amounts in the zones of antigen excess or antibody excess. Thus, formation of precipitation lines in any immunodiffusion system is highly dependent on relative concentrations of antigen and antibody. The prozone phenomenon refers to suboptimal precipitation, which occurs in the region of antibody excess. Thus, dilutions of antisera must be reacted with fixed amounts of antigen to obtain maximum precipitin lines. The prozone phenomenon is a cause of misinterpretation of immunoelectrophoresis patterns in the diagnosis of hyperproteinemias when large amounts of antibodies are present.

Immunoprecipitation is the simplest and most direct means of demonstrating antigen-antibody reactions. The application of immunoprecipitation to the study of bacterial antigens launched the field of serology in the first part of the 20th Century. In 1946, Oudin described a system of single diffusion of antigen and antibody in agar-filled tubes. This important advance was soon followed by Ouchterlony's classic description of double diffusion in agar layered on slides. This method is still in use today and has many applications in the detection and analysis of precipitating antigen-antibody systems.

Immunodiffusion reactions may be classified as single or double. In single immunodiffusion, either the antigen or antibody remains fixed, and the other reactant is allowed to move and complex with it. In double immunodiffusion, both reactants are free to move toward each other and precipitate. Movement in either form of immunodiffusion may be linear or radial. Specific examples are discussed in the remainder of this section.

Immunodiffusion has a clinical application in the quantitative and qualitative analysis of serum proteins. Quantitative analysis of serum proteins is often done by more sensitive and automated methods such as nephelometry, ELISA, or RIA. Single radial diffusion in agar has largely been supplanted by these methods, which do not rely on immunoprecipitation or diffusion.

Applications of immunodiffusion

Serum immunoglobulin levels are dependent on a variety of developmental, genetic, and environmental factors. These include ethnic background, age, sex, history of allergies or recurrent infections, and geographic factors (e.g., endemic infestation with parasites results in elevated IgE levels). The patient's age is especially important in the interpretation of immunoglobulin levels. Normal human infants are born with very low levels of serum immunoglobulins that they have synthesized; the entire IgG portion of cord serum has been transferred transplacentally from the mother. If an infection occurs in utero, cord IgM and IgA are elevated. After birth, maternal IgG decays, resulting in a falling serum IgG level. This trend is reversed with the onset of significant autologous IgG synthesis. There is a gradual and progressive increase in IgG, IgA and IgM levels until late adolescence, when nearly normal adult levels are achieved. Furthermore, it is clear that there is a great deal of variability in immunoglobulin levels in the healthy population.

In routine practice, only IgG, IgA, IgM, and IgE levels are ordinarily measured. Abnormalities of serum IgD concentration have not clearly been associated with specific disease states. In fact, this immunoglobulin is the major B cell receptor for antigens and plays only a minor role as a circulating antibody. IgE levels, on the other hand, are useful in differential diagnosis of allergic, parasitic and rare immunodeficiency states. Measurement of serum IgE levels required sensitive methods such as RIA or enzyme-linked immunoassay. Measurement of serum IgG levels is particularly valuable in diagnosis and in monitoring immunoglobulin replacement in hypogamma-globulinemic patients. Individual changes in serum immunoglobulins gave been recorded in many diseases.

(6) Electrophoresis and Immunoelectrophoresis [20,21]

Analysis of the heterogeneity in human serum proteins can be readily accomplished by electrophoresis. In this technique, both electrophoresis and double immunodiffusion are performed on the same agar-coated slide. Immunoelectrophoresis has become an important tool for clinical paraprotein analysis as well as a standard method for immunochemical analysis of a wide variety of proteins.More recently, immunofixation electrophoresis and electroimmunodiffusion methods have been introduced. Immuno-

-electrophoresis combines electrophoretic separation, diffusion, and immune precipitation of proteins. Both identification and approximate quantitation can thereby be accomplished for individual proteins present in serum, urine, or other biologic fluid. In this technique, a glass slide is covered with molten agar or agarose in an alkaline buffer solution. An antigen well and antibody trough is cut with a template-cutting device. The serum sample (antigen) is placed in the antigen well and is separated in an electrical field with a potential difference of approximately 3.3 V/cum for 30-60 minutes. Antiserum is then placed in the trough, and both serum and antibodies are allowed to diffuse for 18-24 hours. The resulting precipitation lines may then be photographed or the slide washed, dried, and stained for a permanent record.

Applications of electrophoresis

Zone electrophoresis is useful in the diagnosis of human paraprotein disorders such as multiple myeloma and Waldenstrom's macroglobulienmia. In these disorders, an electrophoretically restricted protein spike usually occurs in the gamma-globulin region of the electrophoretogram. Since in zone electrophoresis the trailing edge of immunoglobulins extends into the β region, spikes in these regions are also consistent with paraproteinemic disorders involving immunoglobulins.

A marked decrease in serum globulin concentration such as occurs in hypogammaglobulinemia can sometimes be detected with the technique. Reduction in IgA or IgM to very low levels cannot be detected by this method, since they represent such a relatively small fraction of total seruim immunoglobulins. Free light chains are readily detectable in urine when present in increased amounts such as Bence Jones proteinuria of myeloma. Zone electrophoresis in agarose gels has also been useful in the diagnosis of certain central nervous system diseases with alterations in cerebrospinal fluid proteins.

Oligoclonal bands in cerebrospinal fluid with restricted electrophoretic mobility have been detected in about 90% of clinical definite cases of multiple sclerosis. Agarose electrophoresis gel in conjunction with measurement of cerebrospinal fluid IgG/albumin ratios makes possible a fairly high degree of specificity for diagnosis of multiple sclerosis.

Abnormalities in serum proteins other than immunoglobulins may also be detected by serum protein electrophoresis. Hypoproteinemia involving all serum fractions occurs during excessive protein loss, usually in the gastrointestinal tract. Reduction in albumin alone commonly occurs in many diseases of the liver, kidneys, or gastrointestinal tract or with severe burns. $\acute{a}$-globulin decrease may indicate $\acute{a}$-antitrypsin deficiency, and an increase reflects acute phase reactions occurring in many inflammatory and neoplasic disorders. Increase in $\acute{a}$- 2-globulins usually reflects the nephritic syndrome or hemolysis with increased hemoglobin-haptoglobin in the serum. Because of its relative insensitivity, zone electrophoresis is almost always a presumptive screening test for serum protein abnormalities. Specific quantitative biochemical or immunologic tests must be performed to definitively identify the particular protein.

Applications of Immunoelectrophoresis

In the laboratory diagnosis of paraproteinemias, the results of zone electrophoresis and immunolelectrophoresis should be combined. The presence of a sharp increase or spike in the -globulin region on zone electrophoresis strongly suggests the presence of a monoclonal paraprotein. However, it is necessary to perform immunoelectrophoresis to determine the exact H chain class and L chain type of the paraprotein. Immunoelectrophoresis distinguishes polyclonal from monoclonal increases in globulin. Additionally, decreased or absent immunoglobulins observed in various immune deficiency disorders can be analyzed with this technique. However, a further quantitative analysis such as single radial diffusion, nephelometry or radioimmunoassay should be performed for measurement of immunoglobulin levels.

Immunoelectrophoresis can be used to identify L chains in the urine of patients with plasma cell dyscrasias or autoimmune disorders. Thus,with specific anti-k and anti-λ antisera, the monoclonal nature of Bence Jones protein in myeloma can be confirmed. Antisera to "free light chains" (k or λ) obtained from urine of myeloma patients may occasionally reveal antigenic determinants not present on chains "bound" to chains. In H chain diseases, fragments of the immunoglobulin H chain are present in increased amounts in the serum. Immunoelectrophoresis is also helpful in identifying increased amounts of proteins present in the cerebrospinal fluid in patients with various neurologic diseases.

CONCLUSION

Over the past 2 decades, immunologic laboratory methods have gradually become increasingly more refined and simplified. Because of their inherent specificity and sensitivity, these methods have now achieved a central role in the modern clinical laboratory tests employing immunologic principles. The methods of laboratory diagnosis have been uncritically applied to clinical situations. A better understanding of the methods used in the immunology laboratory should provide the student and practitioner of medicine with a useful guide for correct application and interpretation of this body of knowledge.

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SAFE LABORATORY PRACTICE AND THE UNIVERSAL PRECAUTIONS

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ABSTRACT

Dearth of information exists regarding safe laboratory practice and precautions in this environment. Safety practice, although an important and fundamental responsibility, is too often the least considered and most poorly conducted. This attitude is difficult to understand, but probably arises because safety practices are poorly recognized and neglected in laboratory medicine programmes. Teachers and heads as well as managers of laboratories are indifferent to the problem of safety and personnel in clinical laboratories find safety precautions inconvenient. Only fear of constacting hepatitis and infection with HIV seem to exist. This negligence is probably sustained because serious accidents and injuries are relatively rare, but when they do occur they may be fatal or cause serious damage to life and limb. The recent fire incident at the Nnamdi Azikiwe University College of Medicine, Nnewi that claimed the lives of a number of Medical Students during practicals is still fresh in our minds. Though dramatic hazardous situations are relative rarities but daily oblivion to safety precautions may contribute to reduced life span, accelerated aging, increased susceptibility to infection owing to chemical induced immune paresis and even greater susceptibility to carcinogens . Some of these disorders may unfortunately rear their heads either in retirement or near end of career when access to medical facilities are either non existent or very marginal. The laboratories in this environment constitute greater risk to hazards owing to poor facilities and obsolete equipment.In spite of enacting acts to make work place safer, no clinical laboratory will be completely safe unless the individuals working or studying there become informed of the dangers and their remedies. They must establish good habits and adhere to the universal precautions at all times. Prophylaxis against hepatitis is available. These steps if well articulated and followed makes the ostensibly hazardous laboratory a safe environment to practice, preserve our health and render valuable service to those put in our charge.

INTRODUCTION

The ultimate objective of physicians and other health professionals is a life of dedication to the care of the patient trying to cure, alleviate or safeguard his life and health while improving what ever can be improved. But to realize this objective, the practitioner or student has to keep himself alive and healthy. Similarly the mission of the hospital laboratory is to collaborate with the physician in the delivery of patient care by providing timely and accurate services. To achieve this the practitioner needs to be alive and healthy also. This calls for safe laboratory practice and precautions.

Safe laboratory practices or laboratory safety programmes are plans minimizing or eliminating hazards in the clinical laboratories. Specifically they are plans for preventing sickness and injury to personnel and damage or destruction of physical assets. Owing to its many health, economic, legal and environmental implications such programmes deserve careful attention by all practitioners and students in laboratory medicine.

Safety practices, although an important and fundamental responsibility, are too often the least considered and most poorly conducted. This dangerous behaviour is difficult to understand, but probably arises because safety practices are poorly recognized and neglected in laboratory medicine programmes. Teachers and heads as well as managers of laboratories are indifferent to the problem of safety, and personnel in clinical laboratories find safety procedure inconvenient. This negligence is probably sustain because serious accidents or injuries in laboratories rare, but when they do occur they may be fatal or cau serious damage to life and limb. The fire incident fro a faulty gas cylinder that occurred during a practi class that claimed the lives of about seven (7) medi students at the Nnamdi Azikiwe University Medi School in Nnewi, Anambra state about 5years ag still fresh in our minds.(Aboh Perso Communications). Though largely unrecognized, th laboratories in our environmentconstitute gre hazard than often believed owing to poor infrastructu lack of running water, obsolete facilities, unavailab first aid, unreliable electricity supply and a lot improvisations, which are not risk proof but a immediately handy. Though dramatic hazardo situations are relative rarities but daily oblivion safety precautions may contribute to reduced life s accelerated aging, increased infection owing chemical induced immune paresis and even gre susceptibility to carcinogens and co-carcinog These disorders may unfortunately rear their h either in retirement or near retirement when acce medical facilities are either non existent or marginal.

In addition, pension may not be available. T occult laboratory hazards should always be born mind and should stimulate us to imbibe the princip

cautious laboratory behaviour.

This review article examines laboratory hazards, safety practices and the universal precautions to mitigate the harm that may be encountered during laboratory practice

The main possible dangers in a clinical pathology laboratory are indicated below:

- Fire
- Infection
- Contact with corrosive chemicals
- Exposure to toxic fumes
- Cuts or puncture from broken glass ware or other sharp objects
- Exposure to carcinogenic compounds
- Possible exposure to low-level radiation

TYPES OF HAZARDS AND SOURCES OF HAZARDS

Hazards arise from three main basic causes:

1) From dangerous chemicals
2) Infected specimens
3) Faulty apparatus.

The likelihood these will have harmful effects is enhanced by:

i. Carelessness
ii. Untidiness
iii. Poor hygiene by the individuals.

The main consequences of these are personal trauma or infection and damage to the laboratory by fire[3].

HAZARDS FROM DANGEROUS CHEMICALS

The main group of harmful chemicals consists of the following

i. Corrosives
ii. Organic solvents
iii. Poisons
iv. Carcinogens.

Injury from chemicals arises from

(i) **Direct contact:**

(a) With the skin, for example when pouring reagents or from breakage of containers
(b) With lips or mouth when pipetting
(c) Toxic effects of substances absorbed on the lungs, gastrointestinal tract or skin or other tissues/ organs such as the bone marrow, liver or kidney.

Special attention should be paid to certain groups of particularly dangerous substances. There is inadequate information about the effects of many organic chemicals, for example the aromatic compounds that have been associated with cancer[4], so care should always be exercised when handling such compounds. Spillage should always be mopped immediately with special chemical sponges.

(ii) **Toxic chemicals**

Soluble poisons such as cyanide barbiturates and several others are best locked up in dangerous drug poison chest. Cyanide antidote kit should also be provided.

(iii) Corrosive chemicals

- Nitric acid (HNO_3)
- Sulphuric acid (H_2So_4)
- Hydrochloric acid (HCl.)
- Sodium hydroxide (NaOH)
- Potassium hydroxide (KOH)

Chemical burns of the skin may be caused by strong solutions or solids of the above chemicals.

NEVER MOUTH PIPETTE ANY CORROSIVE CHEMICAL – burette or a measuring cylinder should be used instead.

(iv) Organic Solvents

These are used in the clinical laboratory particularly in the Chemical Pathology Laboratory.

Some common ones include:

- Benzene
- Ether
- Chloroform
- Chloromethane
- Carbontetrachloride (CCl_4) and other halogenated hydrocarbons
- Ethyl alcohol and other alcohols.

Benzene is toxic to the bone marrow. Carbontetrachloride and other halogenated hydrocarbons are toxic to the liver. These effects may be cummulative.

A useful precaution in dealing with organic solvents is to limit the amount allowed to escape into the atmosphere in the laboratory and possibly select less toxic alternatives and use them under fume hood or a fume cupboard.

(v) Heavy Metals

Heavy metals, such as mercury (Hg), may also be encountered in the laboratory. When Hg is exposed at ordinary temperatures, it releases vapour into the atmosphere.

Under poor ventilation large amounts of droplets left may be inhaled. Mercury is nephotoxic and methylated compounds of mercury through biotransformation may cause neurological disorders[5]. Mercury must never be drained into the sink liquid metal with characteristic phase transition should be stored in thick-walled stoppered jar.

(vi) Carcinogens

Generally, it is enjoined that serious efforts must be made to minimize exposure to carcinogens[4,6]. In the laboratory attention has increasingly been drawn to certain aromatic amines, azo dyes and N-nitroso compounds of which benzidene, o-tolidine and o-dianisidine are the commoner ones and are now prohibited.

The precaution against carcinogenic chemicals involves keeping them in well stoppered containers (usually bottles). They should be labelled carcinogenic and avoid any contact with skin. In accidental spillage flush with copious amount of cold water for several minutes. When handling, rubber or plastic gloves must

be worne which are afterwards well washed under cold running tap water.

(vii) Explosive Radioactive And Inflammable Materials.

Explosion may occur in the laboratory while using some chemicals which are mainly perchloric acid and sodium azide.

Perchloric acid should be used in fume cupboards. Investigators should wear protective glasses or face shields. Picric acid should not also be washed down copper pipes. Sodium azide, a common preservative for reagents should also not be washed down laboratory sinks.

Ether is a highly inflammable organic solvent that has a very low boiling point.

Amount of flammable solvent in the working laboratory should be reduced.

(viii) Fire

Fire is an ever present hazard that should be borne in mind always either from electrical faults, gas cylinders, heating blocks and inflammable solvents.

If a fire breaks out, prompt, correct action will often limit the damage. Seconds may be very critical here. All laboratories should be equipped with automatic fire warnings and periodic fire drills by all laboratory investigators and students should be carried out.

These should be coordinated by a safety official Extinguishers:

- Fire hose
- Sand
- Chemical extinguishers generating a spray of foam, CCl_4 or Co_2 intended to limit oxygen supply.

*Note that preservation of human life is more important than preservation of buildings. The Nnewi experience is a sad reminder.

DISPOSAL OF LABORATORY WASTE

The sound management of hazardous Chemical waste is a priority. Recent legislation passed by the American congress has directed the Environmental protection Agency (EPA) to develop and implement a programme to protect human health and the environment from improper hazardous waste regulatory system[3,5,6]. There is no equivalent legislation from Nigeria. Such a legislation is desirable and should be considered.

Sound management of hazardous waste at least in part implies maximum reduction of waste generation at source. When ever possible, the laboratory substitutes a non-hazardous chemical for a dangerous one. Reducing the scale of a laboratory procedure also reduces the volume of chemicals used-adoption of microtechnics. Many hazardous chemicals such as metallic mercury, photographic fixers and some solvents should be collected for purification and recycling[7].

A variety of chemicals can be sufficiently neutralized or inactivated in the laboratory to permit safe disposable in the sewer system. Wastes that cannot be recycled or rendered sewer disposal, should however, be collected, properly stored and labeled. It should be carefully transported at intervals to an official disposal Centre.

INFECTION

Perhaps the most feared hazard though not necessarily the greatest hazard in the laboratory is the possibility of infection. Medical Personnel handle blood and body fluids that may contain infective agents (viral or bacterial) from sick and seemingly well patients. The greatest danger of disease transmission arises from:

- An accidental puncture from a contaminated hypodermic needle
- Cuts or
- Skin abrasions to infective fluids that are spilled or allowed to contaminate the outer surface of containers or from aerosols that may escape when opening a vacutainer or after a centrifuge accident.

Transmission of disease through accident can be kept to a minimum by practicing the "universal precautions" recommended by the Centers for Disease Control and prevention (CDC) outlined below.

Precautions Against Hepatitis And HIV/AIDS

Currently the most feared diseases arising from infection with contaminated materials are hepatitis and Human immunodeficiency virus/Acquired immune deficiency syndrome (HIV/AIDS). Although these may be rare occurrences, they are the most likely infections to be transmitted to medical personnel by accident or carelessness.

The two types of hepatitis that are transmitted primarily by the parenteral and sexual routes are hepatitis B (HBV) (previously called serum hepatitis) and hepatitis C (earlier referred to as non-A non B). At the moment about 90% of the hepatitis transmitted by contaminated blood products at blood banks is reportedly hepatitis C in the USA. The figures for this country and other developing countries where the standards of practice are not as high are uncertain but are unlikely to be different from the situation in the United States. It is however, anticipated that the number of such instances should decrease with the present use of tests for HCV detection.

HIV/AIDS

After a long incubation period (Latency) usually about five years (5) or much longer post exposure, infection with HIV may develop into the acquired immunodeficiency syndrome (AIDS), but such development is not universal; it is variable.

Human immunodeficiency virus infection may be defined as the detection of antibodies against HIV in plasma or serum (sero conversion). The disease AIDS on the other hand is applied on to those HIV-positive persons who exhibit a compromised immune system.

demonstrated by the presence of opportunistic infections or certain cancers such as kaposi sarcoma or who have a low number and ratio of the central immune regulatory cells T (CD4) cells. Owing to the currently incurable state of this disease and the expensive nature involved in its control and attendant morbidity and mortality every caution to avoid infection is well worth the while.

The routes for accidental transmission of HIV and the viruses of hepatitis B or C are identical. The infective viral particle must enter the body through punctures, cuts or abrasions or through mucous membrane. Thus a useful simple prevention step is to cover all cuts and abrasions. No matter the lenght of your carrer or experience, there is no guaranteed immunity!

The viruses are not transmitted by casual contact. HBV or HCV are for more infective than HIV because the average patient with hepatitis may carry as many one million more viral particles per mililitre of blood than the usual patient with HIV infection[2].

Infection with HIV, as measured by seroconversion, occurred in less than 1% of workers accidentally stuck with a needle containing blood from a subject with HIV[2]. The risk of HBV infection is much greater, but a vaccine is available and its use is recommended for all fresh clinical students pre exposure and indeed all medical personnel. This is particularly important in that an infection with HBV (or HCV) may progress to primary liver cell carcinoma (PLCC) and this has been observed in this College.

ASPECTS OF A GENERAL SAFETY PROGRAMME

All personnel (including medical students) must be convinced of the need for cautious laboratory behaviour. Cautious laboratory behaviour should receive enough emphasis so that it becomes a way of life not only in the clinical laboratory environment but in the hospital environment of which the laboratory is a subset.

It should always be borne in mind that transgressions cause a dangerous and unnecessary risk to life and limb.

Development of a meaningful and effective safety programme requires a concerted effort to identify the sources of hazard and the categories of laboratory activities. This brings about a meaningful and manageable programme.

Consistent observation indicate that:

- Most laboratory acquired sickness and injury result from overcrowding
- Poorly maintained equipment
- Careless house keeping
- Thoughtlessness
- Inexperience (neophytes)[1]

It must always be remembered that the reduction of risks within the hospital laboratory is a serious professional responsibility. This helps to curtail manifest and occult hazards[3]. The latter of which may result in immune paresis, reduced life span, greater susceptibility to cancer and accelerated aging following associated oxidatative damage etc. as earlier indicated.

Some aspects of a general safety programme are as indicated below:

- Prehospital contact physical examinations with laboratory and radiological
- Investigations that establish fitness for laboratory practice with periodic repetition of above
- Orientation of new students/entrants to the departments' attitudes and policies for assuring safe laboratory conduct (Be cautious of laboratory behaviour)
- Policies governing eating, drinking smoking and safe attire within the laboratory
- Students should be aware of the coordinated efforts of the hospital for assuring isolation of communicable diseases, control of nosocomial infections and plans for dealing with fire disaster
- Instruction for collecting and handling of specimens should be strictly adhered to
- Regular hand washing especially after handling specimens or reagents
- Instructions for cleaning/ or disposal of specimen collection equipment should be observed (gas burners, hot plates, water baths, drying ovens etc).
- Instruction on use of sand buckets, fire extinguishers, fire blankets should be observed.
- FIRST AID – Students should be able to identify and use emergency showers and eye baths.

GENERAL PRECAUTIONS

Cautions should be exercised at all times. Knowledge of transmission route is essential.

Common sense, high personnel morale standard of hygiene and general good house keeping should be encouraged.

- No eating, drinking, smoking except in designated areas. Wash hands frequently discourage frequent hand to mouth habits
- No mouth pipetting
- Do not lick labels, biros, pencils, wear laboratory coats and fasten buttons all chemicals must be labeled possibly with appropriate warning signs/symbols
- Know the warning signs/symbols
- Keep working benches tidy mop spills promptly.

Aspects of Infection Control/High Risk Procedures

- It is impossible to identify all carriers; a system of universal precautions is thus desirable,

Identifying high risk procedures rather than high risk individuals (patients)

- Use of recommended precautions should protect against inoculation risks, such as sharp injuries and splashes on to mucous membrane.
- All practitioners and trainees have a responsibility to their patients, their colleagues and themselves to maintain good working practices, general personal health and hygiene is also important.

Sharps Container

- Sharps container should be in regular supply in all clinical pathology laboratories.
- Discard sharps only into approved sharps containers not to be filled beyond three-quarter capacity (3/4 full)
- Never leave needles and sharps lying around for other staff or students to clear away
- Needles should not be resheated, bent or broken
(if needed use a safe, one-handed technique) – needle destroyer devices are also available.

SPILAGES OF BLOOD AND BODY FLUIDS

- Deal with spillages immediately, covering with chlorine releasing granules e.g. hatz-tab or house hold bleach (JIK) diluted 1 in, 10 – pour on to paper towels laid on the spillage (chlorine tablets may also be used); ensure good ventilation of room and well demarcated with hazard tape to prevent others from tripping on the spillage.
- Used gloves, plastic aprons and paper towels should be discarded into yellow bags for incineration.
- Broken glass present in spillage MUST NOT be picked up with hands even with gloves – a scoop made of plastic or cardboard must be used post disinfection lasting about 30 minutes.
- Make up bleach, freshly daily – disintegrate rapidly.
- Make available yellow plastic bags for incineration
- Wear protective clothing. Traditional lab. Coat keep clean; use gloves during procedures on occasions you may double glove

Waste disposal

- Wastes generated during clinical laboratory procedures should be placed in yellow incineration bags.

Inoculation accidents

- Report all inoculation accidents to an occupational health service (public health service). Same for splash of body fluid into mucous membranes.

THE UNIVERSAL PRECAUTIONS

The now widely recognized universal precautions largely arose from the recommendation to the National Committee for Clinical Laboratory Standards (NCCLS) and the united states Department of Labor by the Centers for Disease Control and Prevention, Atlanta, Georgia. They are essentially a synthesis of earlier precautions. They have been adopted Worldwide with some modifications depending on local circumstances.

A summary of the pertinent ones is as follows:

1. Treat all laboratory specimens as infectious. Make no exception and do not rely on warning labels to designate contaminated specimens.
2. Use a protective barrier such as gloves, when handling blood and body fluids. Wear gown or laboratory coat and change when contaminated. Disposable laboratory coats may occasionally be desirable.
3. Avoid handling of hypodermic needles. Dispose of used needles in a rigid container.
4. Avoid aerosols or droplets when opening a vacuum tube containing blood. Point tube away from yourself and open slowly, preferably in a hood.
5. Use Pasteur pipettes or other devices for transferring fluid samples. Do not pour from one tube to another because a drop or two may contaminate the outer surface of the tube.
6. Never pipette by mouth.
7. Minimize spills and spatters. If they should occur absorbs the liquid with disposable absorbent material, clean with detergent and disinfect with hypochlorite solution (commonly domestic bleach).
8. Wash hands after contamination, after removing gloves and always before eating.
9. Dispose of samples properly when no longer needed for possible re-analysis. Samples should be autoclaved before discarding.
10. Place warning signs on all known biohazards. Additional safety information is available from a number of her sources[9-11].

All practitioners or students of pathology or laboratory medicine are exposed to potential hazards. Risks can be minimized by eliminating dangerous situations when possible, establishing clean and safe working habits, taking proper precautions at all times and maintaining awareness of good safety practices[12].

FIRST AID/ PROPHYLAXIS

Simple first aid facilities such as bandage, showers, eye baths and safety chart must be available in all clinical Pathology Laboratories. In the event of needle stick squeeze blood out immediately. And report to the public health department.

In acid or alkaline solution accidents rinse immediately with water and put under running water for a while and report to the Laboratory Safety Officer and if need be a Physician.

Programme of Inoculation is available.

GUIDELINES POST EXPOSURE PROPHYLAXIS FOR HIV

As HIV is understandably the currenthy most feared laboratory hazard the following actions which may also be modified for hepatitis should be taken immediately upon possible exposure.

POST EXPOSURE PROPHYLAXIS GUIDELINES

The following actions should be taken immediately upon possible exposure to HIV.

1. Treatment of exposure site.
2. Timing of Post-HIV exposure prophylaxis

POST EXPOSURE PROPHYLAXIS GUIDELINES

The following actions should be taken immediately upon possible exposure to HIV.

1. **Treatment of exposure site.**
   Wounds and skin sites:
   Wash with soap and water
   Mucous membranes:
   Flush with water

2. **Timing of Post-HIV exposure prophylaxis**
   initiation
   if therapy is necessary, prophylaxis should be initiated promptly, preferably within 1 to 2 hours post-exposure.

3. **Assessment of exposure risk**

Low risk exposure is:

*exposure to a small volume of blood or blood contaminated fluids from symptomatic HIV-positive patients with a low viral
* an injury with a solid needle
*any superficial injury or mucocutaneous exposure

High risk exposure is:

*Exposure to a large volume of blood or potentially infections fluids
*exposure to blood or blood contaminated fluids from a patient with a high viral titre i.e in the AIDS phase or early seroconversion phase of HIV
*injury with a hollow bore needle
*deep and extensive injury
*drug resistance in source patient.

4.Post-HIV exposure prophylaxis

Risk Category	Antiretroviral prophylaxis	Duration
Low Risk	RETROVIR 200mg 8-hourly EPIVIR 150mg 12 hourly	↓ 28 days
High Risk	RETROVIR 200mg 8-hourly EPIVIR 150mg 12- hourly Indinavir 800mg 8 hourly	↑

5.Recommended drug toxicity and HIV serology testing after exposure

Time period from exposure	Recommended tests
Baseline	Full blood count (FBC) Liver & renal function tests HIV serology
Two weeks	Full blood count, Liver & renal function tests
Six weeks	HIV serology
Three Months	HIV serology
Six months	HIV serology

CONCLUSION

Inspite of enacting of acts to make work places safer, no clinical laboratory will be completely safe unless the individuals working or studying there become informed of the dangers and their remedies. They must establish good habits and take proper precautions at all times. Prophylaxis with immunization against hepatitis is also available. These steps if well articulated and followed makes the ostensibly hazardous laboratory a safe environment to practice, preserve our health and render valuable service to those put in our charge.

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THE ROLE OF URINALYSIS IN THE DIAGNOSIS OF DISEASES

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SUMMARY

For centuries medical practitioners have used examination of urine as an aid to diagnosis of a range of diseases. Though very fundamental it tends to be very often trivialized, thus the need for a reminder of some of the procedures and clinical implications of findings. Some of the current methods of examination are still traditional such as observing the appearance and odour of the specimen and making a microscopic examination of the urinary sediment.

The major advances have been in providing dipsticks or strips for the semi-quantization of a collection of constituents and in measuring urine osmolality or specific gravity and indications of total solute concentrations and the concentrating abilities of the tubules. The visual and microscopic examination may yield useful clinical information and must not be neglected because it is not completely quantitative.

A routine urinalysis consists of an examination of morning urine specimen (first morning urine) for colour, odour, specific gravity or osmolality; the performance of some qualitative or semi-quantitative tests for pH, protein, glucose or reducing substances, blood, bilirubin, urobilinogen and nitrite as well as a microscopic examination of the urine sediment. This simple, cheap and technically unsophisticated procedure provides invaluable information about the overall health of the patient and must never be trivialized.

Keywords: *Biochemical characteristics, Blood, Diagnosis, Kidneys, Microscopy, Urinalysis*

INTRODUCTION

Urinalysis (analysis of urine) is a routine investigation on many patients presenting in the clinic or at the time of hospital admission and is often repeated to evaluate the response to/progress of treatment. It is one of the earliest procedures adopted by and one of the most useful investigative tools in the hands of the physician as an indicator of health or disease, especially in conditions of renal, metabolic or endocrine disorders.[1]

When the kidneys are diseased, there is a derangement of the normal homeostatic mechanism of the body. Thus, substances which are normally excreted are retained and those which are normally retained or excreted in small amounts are excreted in large quantities; structural elements such as blood cells, epithelial cells of the urinary tract, and casts may also appear in the urine in certain abnormalities of kidney function, and all of these could be detected by routine urinalysis.

Routine urinalysis has proved invaluable in the early detection of certain disorders including chronic diseases of the kidneys through urinary screening programmes.[2] In the clinical setting, the evaluation of patients involves obtaining a comprehensive history, physical examination and carrying out indicated diagnostic procedures. Routine urinalysis will ensure that serious conditions which may be asymptomatic are not undetected and that only necessary laboratory investigations are performed.[3]

Urinalysis involves the observation of the physicochemical characteristics of urine; chemical examination; microscopic examination; and bacterial culturing. The conventional methods for detection of substances in urine have largely been replaced by commercial reagent strips, for example albustix, multistix etc. However, conventional methods are more reliable with high sensitivity and specificity and use commonly available reagents.

In this article, an attempt was made to provide the reader with the pivotal role routine urinalysis plays in the early detection and/or diagnosis of diseases by reviewing selected laboratory procedures useful in routine urine analysis and their clinical importance as well as providing concise technical details where appropriate.

PHYSICAL CHARACTERISTICS OF URINE

Colour

The normal urine colour ranges from crystal clear to amber or even darker depending on the hydration status of the body. A deviation from what is regarded as normal depends on the substance(s) present in the urine. For example in haematuria, urine colour ranges from red to brown and this is dependent on the amount of blood in and the pH of the urine.[4] However, in microscopic haematuria there is no discolouration of urine and the presence of blood in the urine is detected only by chemical testing but isolated microscopic haematuria is only rarely a sign of significant kidney or urinary tract disease.[3] (See below)

Although several substances can produce discolouration of urine, this is of limited value in the diagnosis of renal diseases[3] because of the non-specificity of this parameter except in haematuria.

However, certain urine colour changes are known to be associated with well recognized substances. (Table 1)

Odour: Normal urine has an ammoniacal odour. Deviations from this may give the clinician a clue to an existing underlying disease process in the patient. An infected urine may have a fishy or pungent smell, while a sweet or fruity odour is due to ketones, the commonest cause being diabetes mellitus. Some inborn errors of metabolism confer a characteristic smell to the urine.[5] These are maple-syrup urine disease ('maple syrup' odour), phenylketonuria ('musty' or 'mousy' odour), isovaleric acidaemia ('sweaty feet' odour), hypermethioninaemia or hypermethioninuria ('rancid butter' or 'fishy' odour).

Turbidity: The first part of a urinalysis is direct visual observation. Normal, freshly voided urine is usually clear or transparent. High concentrations of leucocytes, erythrocytes, epithelial cells, certain crystals or bacteria give the urine a cloudy appearance, as seen in urinary tract infections or glomerulonephritides. Urine samples with alkaline pH also appear cloudy as a result of their high contents of carbonates and phosphates. The presence of high amounts of uric acid in the urine may give it a pinkish turbidity.

Chyluria is another cause of turbid urine. The urine characteristically appears milky as a result of a mixture of fat, white blood cells, red blood cells and fibrin. Chyluria is due to a fistulous connection between the Lymphatic and urinary system caused by the obstruction of the lymphatic system by *Filaria bancrofti*, which is endemic in several developing countries. Very rarely, chyluria may have non-parasitic causes, such as trauma, tuberculosis, neoplasms, and pregnancy.[5]

Specific gravity: Urine specific gravity is a measure of the weight of dissolved particles in urine.[6] It is intended to assess the concentrating and diluting ability of the renal tubules. This is subject to standardized conditions of fluid restriction, increased intake or renal tubular pathology.

There are several methods of determining the specific gravity namely:

a. Use of a well calibrated urinometer

b. Use of a dipstick

The dipstick method utilizes a colour indicator which could be bromothymol blue and changes colour based on the concentrations of ions in the urine. The normal reference value for specific gravity is 1.005 – 1.030 but usually remains between 1.010 and 1.025.

Causes of low specific gravity values

Specific gravity values of less than 1.005 may be seen in diseases like diabetes insipidus, glomerulonephritis, pyelonephritis, and excessive fluid loss e.g. sweating, vomiting, diarrhoea. These disease conditions reflect loss in the effective concentrating ability of the renal tubules.

Table 1: Substances responsible for change in Urine Colour

Substance	Colour	Comment	Differential Diagnosis
Blood	Red - Brown	Depends on Amount and pH	Bleeding diathesis, Glomerulonephritis, Polycystic kidney disease, Renal Tumours, UTIs, Bladder Carcinoma, Schistosomiasis etc
Haemoglobin	Red	Urine usually darker than in haematuria	Glucose-6-Phosphate dehydrogenase deficieny (G6PD)
Myoglobin	Dark red		Severe crush injuries, Venom of Hydrophidae
Drugs	Red, Brown, Black, Green, or Blue	Rifampicin, Phenindione, Nitrofurantoin all produce red colouration; Methylene blue produces blue discolouration	
Bilirubin	Dark brown		Acute hepatitis, Obstructive Jaundice
Urobilin	Yellow	This is especially prominent in maximally concentrated urine	
Urates	Brick-dust	In Acidic urine	
Phosphates	Milky	Precipitation of phosphates in alkaline urine	
Pus cells	Cloudy urine	Also produced by precipitated amorphous salts in alkaline urine	Urinary tract infections (UTIs)

Causes of Elevated Specific gravity Values

Specific gravity values greater than 1.030 may be seen in conditions like diabetes mellitus, hepatic disease, adrenal insufficiency, and congestive cardiac failure. For every 1% glucose present in urine there is a 0.004g/ml rise in the specific gravity while for every 1% protein, there is a 0.003g/ml rise in specific gravity. In some conditions, the specific gravity is low and fixed (isosthenuria) at approximately 1.010 e.g. chronic renal disease. This is due to the loss of both concentrating and diluting abilities of the kidneys.

pH: The pH is a measure of the acidity or alkalinity of urine. It has a wide range (4.5- 8.0) with post-prandial variations. Litmus indicator paper may be used to estimate the pH of a urine sample. The indicators are bromothymol blue and methyl red.

A decrease in pH (< 4.5) indicates increased acidity of urine and is seen in renal tubular acidosis, uraemia, chronic renal failure, diabetic ketoacidosis, gout, fever, and phenacetin intake. On the other hand, an increase in pH (> 8.0) indicates an increased alkalinity of urine and is seen in bacterial infections especially those caused by *Pseudomonas* and *Proteus sp* and vegetarian diet.

BIOCHEMICAL CHARACTERISTICS OF URINE

Test for Urine Protein

In healthy individuals, less than 150mg of protein is excreted in urine per day. Normally, the protein concentration in urine is below 10mg/dl and is not detectable by the usual urinalysis methods. The presence of detectable amounts of protein in urine occurs when there is increased level of serum protein (normal or abnormal) e.g. Bence Jones Protein; altered glomerular permeability e.g Nephritic/Nephrotic syndrome; increased tubular secretion (Tamm-Horsfall proteinuria); and decreased re-absorption of normal filtered proteins by the renal tubules.[4]

Methods of detecting proteinuria

1. **The Dip-stick method**[4]: The dip-sick is especially useful in detecting albumin in the urine in the side-room laboratory or even in the consulting room. The sensitivity is such that it can detect albumin levels of as low as 5-20mg/dl, however it is less sensitive to globulins and mucoproteins. There may be a false negative result in Bence Jones proteinuria.

2. **The Boiling test**[5]**:** Heating of urine (of pH 5 achieved by the addition of 10% acetic acid in the presence of a pH indicator paper) over flame leads to the coagulation of proteins resulting in a cloudy precipitate when examined against a dark background. However, since boiling results in the release of carbon dioxide thus rendering the urine more alkaline, cloudiness can also result from the formation of phosphate precipitates. Nonetheless, these could be differentiated by adding more acid where the precipitate disappears if it was due to phosphates or persists if it was due to proteins. False-positive results can however occur in ingestion of tolbutamide or large doses of penicillin.

3. **The Sulphosalicylic Acid test**[7]**:** The presence of protein in urine results in the formation of a cloudy precipitate when 20% Sulphosalicylic acid is added drop wise to about 5 ml of urine in a test tube. False-positive results may be due to ingestion of tolbutamide, sulphonamides, para-aminosalicylic acid or large doses of penicillin or if the urine contains elevated levels of uric acid; presence of radiographic contrast medium.

Test for Urinary Sugar

Glucose is not normally present in urine except in renal glycosuria which in itself is uncommon. The presence of glucose in urine therefore heralds the presence an abnormality of glucose metabolism commonly diabetes mellitus. Others include thyrotoxicosis, Cushing's syndrome, growth hormone excess and phaechromocytoma.

Methods of detecting Urinary Sugar

1. **Benedict's test**[7]**:** When about 8 drops of urine is added to 5 ml of Benedict's reagent, boiled for 2 minutes and allowed to cool, the formation of a precipitate varying from light green turbidity to a brick-red precipitate indicate the presence of a reducing substance e.g. glucose, fructose, lactate, ascorbic acid, phenacetin etc.

Test for Urinary Ketones

Ketone bodies (3-Hydroxybutyrate, Acetoacetate, and Acetone) which are products of fat metabolism by the liver are normally excreted in the urine and the presence of an excess amount is referred to as Ketonuria. It results from uncontrolled diabetes mellitus, starvation, pregnancy or any other condition in which fats are consumed as body fuel instead of carbohydrates.[8]

Methods of detecting Urinary Ketones

1. **Rothera's test**[7]: A sample of urine about 10 ml contained in a test tube is saturated with excess amount of ammonium sulphate crystals; with the addition of 3 drops of strong, freshly prepared sodium nitroprusside solution and 2 ml of strong ammonia solution, a resultant deep permanganate colour indicates the presence of ketone bodies in the urine sample.

2. **Gerhardt's test**[7]: This test could be used in estimating the level of ketone bodies present in the urine since it is only positive in the presence of considerable amount of acetoacetate. In the presence of a small amount of acetoacetate, the urine is positive to Rothera's test and negative to Gerhardt's test. However, if both are positive, it indicates that the patient is severely ill and requires urgent therapy.

Procedure: To 5 ml of urine in a test tube, 10% ferric chloride is added drop wise until the ferric phosphate

precipitate usually formed dissolves with the addition of more ferric chloride solution. A resultant brownish-red solution indicates the presence of acetoacetate.

Salicylates (e.g. aspirin), phenothiazines, phenol and some other drugs give a similar reaction to ferric chloride. However, boiling the urine sample for about 5 minutes before adding ferric chloride destroy the acetoacetate while the other substances are unaffected.

Test for Urinary Bilirubin

Bilirubinuria is seen in jaundice due to hepatic and post-hepatic (typically obstructive) lesions, where the bilirubinaemia is typically of the conjugated type. Simple tests for detecting bilirubin in urine include the Ictotest, which makes use of a specially designed tablet that changes to bluish purple when two drops of urine containing bilirubin are added to it and the Fouchet test, which utilizes sulphuric acid and 10% barium chloride, a blue or green colouration indicates the presence of bilirubin in the urine sample.

Test for Urobilinogen

The urobilinogen concentration in the urine increases in conditions associated with haemolysis (pre-hepatic jaundice) and hepatocellular disease. A simple laboratory method of confirming its presence in excess in urine is by adding 1ml of Ehrlich's reagent to 1ml of freshly voided urine followed by adding 4 ml and 3 ml of a supersaturated solution of sodium acetate and chloroform respectively, shaking the mixture vigorously at each stage. Urobilinogen forms a purplish red aldehyde soluble in the chloroform layer. The intensity of the colour is related to the amount of urobilinogen present in the urine. Urobilinogen is normally present in trace amount. Persistent absence in urine may suggest cholestasis.

Nowadays, dipsticks for detecting bilirubin and urobilinogen are commercially available. They are detected based on the reaction between bilirubin and a diazonium salt in an acid medium or by the reaction with *p*-diethylamino-benzaldehyde in an acid buffer solution (urobilinogen).Urobilinogen may also be absent in antibiotic abuse.

Test for Bile salts

The presence of bile salts in the urine generally indicates an obstructive cause of jaundice. They can be detected by gently sprinkling fine flowers of sulphur on the surface of urine in a test tube, (Hay's test) allowing this to stand undisturbed for about 2 minutes. Presence of large amounts of bile salts is shown by the sulphur particles sinking to the bottom of the tube.

Test for Blood

Haematuria is the presence of abnormal numbers of red blood cells in the urine due to glomerular damage, urinary tract tumours, renal trauma, urinary tract stones, renal infarction, and urinary tract infections etc. Theoretically, no red cells should be found but some find their way into the urine even in healthy individuals. However, if one or more erythrocytes can be found in every high power field and if contamination can be excluded, then the urine sample is probably abnormal.

Haematuria can be detected macroscopically or microscopically. However, a chemical test for it is the occult blood test which uses a peroxidase occult kit. A colour change to blue or bluish green of a special test paper in the presence of the tablet and a drop of urine and water resdpectively, indicates the presence of blood in the sample.

Test for Nitrite

Nitrite is not a usual finding in urine. it is an indication of bacterial infection. its presence is determined using indicators such as tetrahydro[h]quinolin-3-ol;sulfanilic acid.

False positives are seen in urine samples containing dye e.g beetroot. This test is positive in urinary tract infection caused by bacteria containing nitrate reductase (E. coli, salmonella, Citrobacter, Proteus and Klebsiella).

URINE MICROSCOPY

Casts

Casts are cylindrical structures of mucoprotein in which cellular elements, proteins or fat droplets may be entrapped. There are different kinds of casts namely:

a) **Hyaline casts:** Hyaline casts are relatively transparent renal cysts which are composed of proteinaceous material derived from disintegration of cells.[10] They consist of Tamm-Horsfall protein. They are present in normal urine and can be increased in febrile illnesses or following exercise.

b) **Granular casts:** These are relatively dark, dense urinary casts of coarsely or finely particulate cellular debris and other proteinaceous material. It is also called waxy cast. It is seen in chronic proliferative or membranous glomerulonephritis, diabetic nephropathy, and amyloidosis.

c) **Fatty casts:** These casts consist of fat globules and are sometimes described as oval fat bodies. They can be seen in nephrotic syndrome and Fabry's disease.

d) **Epithelial cell casts:** These consist of epithelial cells and their remnants. They are seen in acute tubular necrosis and acute glomerulonephritis.

e) **Red cell cast:** This cast is composed of a matrix containing red cells in various stages of degeneration and visibility. It is usually seen in acute nephritis, proliferative glomerulonephritis, vasculitis, and malignant hypertension.

f) **White cell cast:** This cast is composed of polymorphonuclear leucocytes. It indicates interstitial disease and is seen in proliferative glomerulonephritis and pyelonephritis.

g) **Mixed cell cast:** This cast contains hyaline casts

and various cells such as red, white and tubular Cells. It is seen in proliferative glomerulonephritis and lupus nephritis.

h) **Waxy and broad cast:** This cast consists of homogenous proteinaceous material that has a high refractive index in contrast to the low refractive index of hyaline cast. It is seen in advanced chronic renal failure.

i) **Bacteria cast:** This cast is composed of bacteria and is seen in bacterial pyelonephritis

Cells

a) **White blood cells**: In a normal uncentrifuged midstream urine specimen, the number of white cells seen is less than 3cells/ml, 3-10 cells is considered of doubtful significance. A value > 10 cells is abnormal and indicative of urinary tract infection or other inflammatory disease. A normal centrifuged midstream urine or catheter urine specimen should not contain more than 5 white blood cells per high power field and a value greater than this indicates pyuria.

b) **Red blood cells:** There are usually only a few red cells in urine. If however, the number increases, it may be due to several causes like trauma; renal or ureteric calculi; infection e.g. schistosomiasis; haematologic condition e.g. HbS haemoglobinopathy, paroxysmal nocturnal haemoglobinuria; neoplasms e.g. bladder cancer; and drugs e.g. aminoglycosides.

Bacteria: Bacteria can be identified using microscopy and this may indicate urinary tract infection if contamination is excluded by examining a clean catch.

CONCLUSION

The visual and microscopic examination of urine using basic procedures may yield useful clinical information and must not be neglected because it is not completely quantitative. Routine urinalysis may serve as a window into the patient through which vital information about health such as the presence of renal, metabolic and genetic diseases. It is also very useful because of its cost-effectiveness.

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SYNOPSIS OF GOOD LABORATORY PRACTICE (GLP) IN DRUG DEVELOPMENT

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ABSTRACT

For drug research and development to result in successful registration of effective and safe drug products, component studies must comply with quality standards. This will ascertain the quality, reliability and integrity of data and ultimately the protection of public health. Various stages from discovery, non-clinical studies, clinical studies and post-approval surveillance are involved in the development and life cycle of a new drug product. Internationally accepted rules to be followed in the respective stages of drug development are Good Manufacturing Practice[1], Good Laboratory Practice[2] and Good Clinical Practice[3]. Good Laboratory Practice focuses on quality standards in non-clinical testing to ensure the safety of potential products.

INTRODUCTION

Good laboratory practice(GLP) first evolved in the United States of America in the 1970s when malpractice and fraud were observed in non-clinical testing of drugs by some pharmaceutical companies and contract research organization. In order to ensure the validity of preclinical safety data submitted to the Food and Drug Administration (FDA) towards new drug application (NDA), some mandatory requirements were put in place. This is the concept of good laboratory practice. The FDA has revised these regulations severally but the basics and focus which is safety in non-clinical studies remains unchanged. Non-clinical studies usually involve animals or in-vitro analysis.

Good Laboratory Practice is defined by the Organization for Economic Cooperation and Development (OECD) Principles as: A quality system concerned with the organizational process and the conditions under which non-clinical health and environmental safety studies are planned, performed, monitored, recorded, archived and reported[4]. Premium is placed on the quality, reliability and integrity of studies. This in turn promotes the generation and the reporting of verifiable conclusions since the traceability of data is mandatory. Good laboratory practice involves the application of technically valid and approved standard operating procedures thus preventing many sources of systematic errors. The scientific yield of studies adhering to GLP is optimal with diminished incidence of incomplete or inconclusive studies.

What then are the fundamentals of GLP?

These can be broadly classified under the following:- Resources, Rules, Characterization, Documentation and Quality Assurance of the preclinical study.

All aspects of the studies from the resources; human and material, through to the conduct, documentation, reports and archiving of results are important from the point of view of good laboratory practice. The resources include all the personnel, the facilities and equipment required in the organization and conduct of the study. The responsibilities of personnel must be clearly defined. Organizational chart and job description facilitate a quick understanding or appraisal of these as well as the way the laboratory functions and the relationship between parts. Personnel must have relevant qualifications and be adequate in number to carry out the study in a timely manner. Internal and external training of personnel is also paramount in order to maintain levels of excellence. Competence, education, training and experience of personnel must reflect in their curricula vitae.

Facilities: Buildings and equipment, where tests or studies are carried out should be of suitable size, construction, and location to meet the requirements of the study. This is to minimize disturbances that would interfere with the validity of the study. Laboratory should be built of durable materials that allow for easy cleaning. This prevents accumulation of dirt or other materials which could otherwise cause cross contamination of test items. Ventilation system must be adequate. Animal facility should be such that minimizes the environmental effects on the animals. A typical animal house should be well maintained with adequate provision of space for species, cleanliness, waste disposal, quarantine, necropsy, utilities and changing rooms. Entry into the animal rooms should be restricted.

Equipment: These must be of adequate capacity, suitability, properly calibrated and maintained to function accurately and optimally. Records of all repairs, and routine maintenance should be kept.

Conduct of the study must be as detailed in the approved protocol. The Study Director is responsible for the study. He or she has to adopt, sign and date the study protocol before the commencement of the study. The protocol then becomes binding as the standard document for the conduct of the research and no alterations can be made unless a formal amendment procedure or permission is sought. However in view of the fact that limited technical detail or description may be accommodated in the protocol, there will be a need

To describe routine procedures in written standard operating procedures (SOPs). Laboratories may also need to standardize certain techniques to facilitate comparison of results. Procedures need to be flexible to accommodate advancement in knowledge and techniques. Use of outdated procedures or techniques should be avoided. Hence the need for regular review and modifications to reflect the actual state of the art practices in standard operating procedures. Current version of SOPs must be available at the work place.

Characterization of the test item and the test system: In evaluating the safety of pharmaceutical compound during preclinical phase, it is a prerequisite to have a detailed knowledge about the properties of the materials used during the study. These include the test item such as a compound or drug and the test system to which it is administered; an animal or *in-vitro* facility. For the test item, vehicle or medium of transport and any other reference material, it is mandatory to know the identity, purity, composition, stability and other physical and chemical profile. If the test system is an animal as is often the case, the strain, health status and biological values must be known and clearly stated.

Documentation of the records and raw data: All studies generate raw data which will either be analyzed statistically or used directly. It is important to ensure that the study report is a true and accurate reflection of these data. Records of studies must be stored for long periods of time and this must be available for prompt retrieval. Raw data must be archived in areas restricted to a limited number of people, to ensure no part of any data is lost or tampered with. There must be adequate system of log-in and log-out for both documents and the people involved.

Quality assurance: Good laboratory practice states the minimum quality assurance requirements necessary to monitor the integrity of any study to validate the experimental results. A team of personnel is charged with the responsibility of assuring compliance with the study. This is the Quality Assurance Unit. It is an independent audit and control service for the experimental conduct of the study. The team must review all phases of preclinical studies from planning, through monitoring of on-going studies, to reporting and archiving of documentation. It is mandatory to place Quality Assurance Unit statement in the report. This must indicate the date on which the study was inspected, the study phases inspected, the findings reported to the study director and the management.

IMPLEMENTATION

Implementation of good laboratory practice is multidisciplinary and is best organized in the form of a project. It is more of an organizational challenge than a financial burden. It requires collaborative efforts of personnel in research, quality assurance, maintenance, human resources and documentation. There must be an enthusiastic support by the top management.

CHALLENGES

How near to or far from GLP are we in our research and development? How much of systematic errors are a result of unawareness or complacency to the principles of GLP?

CONCLUSION AND RECOMMENDATIONS

This is to sensitize researchers and stakeholders to the importance of GLP. Efforts should be made to disseminate and ensure adherence to the principles of GLP in non-clinical research. Policy makers and management need to support and make provision for GLP in research institutions. Studies to evaluate the knowledge, attitude and practice of GLP in research institutions and pharmaceutical companies are required. Regular auditing to assess compliance and adherence to GLP should be put in place.

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PROMOTING PHARMACOVIGILANCE IN NIGERIA

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INTRODUCTION

Modern medicines have brought significant benefits to humans, offering reduction in morbidity and mortality from diseases. However, notwithstanding the many benefits, all medications are capable of producing adverse or unwanted effects.

While no studies have comprehensively assessed the burden of Adverse Drug Reactions (ADRs) on health care in Nigeria, it is likely that the problem is considerable, given certain unsafe drug practices which are common in the country. ADRs present in emergency rooms across the country. They are particularly notorious for the difficulty in making a clinical diagnosis, and are even more challenging due to the lack of toxicology laboratories in most centres in a developing country like Nigeria[1].

Pharmacovigilance gained world-wide attention following the thalidomide incident in the 1960s. Thalidomide was a drug given to pregnant women to prevent 'morning sickness'. The babies born to some of these mothers were deformed, but it took a while before the link between the deformities and the drug was made. The drug was subsequently banned and regulatory bodies around the world became aware of the fact that seemingly safe drugs could have potentially serious side effects. By continuously monitoring all drugs used in a country, it is possible to detect drugs causing unwanted ADRs and to prevent and control them[2].

DEFINITION OF TERMS[3]

Drug

A pharmaceutical product, used in or on the human body for the prevention, mitigation, diagnosis and/or treatment of disease or for the modification of physiological function.

Pharmacovigilance

The science and activities relating to the detection, assessment, understanding and prevention of adverse events (any untoward medical occurrence appearing during the use of a drug) or any other drug related problems.

Adverse Drug Reaction

A response to a medicine which is noxious, unintended and occurs at doses normally used in man for the prophylaxis, diagnosis or treatment of disease or for the modification of physiological function.

Adverse Event/Experience

Any untoward medical occurrence that may occur during treatment with a pharmaceutical product which does not necessarily have a causal relation with this treatment.

Serious Adverse Event or Reaction

An untoward medical occurrence which usually results in death, is life-threatening, requires patient hospitalization or prolongation of existing hospitalization or results in persistent or significant disability, causes a congenital anomaly, or requires intervention to prevent permanent impairment or damage.

Side Effect

Any unintended effect of a pharmaceutical product occurring at doses normally used in humans, which is related to the pharmacological properties of the drug.

ADR Case Report

A notification relating to a patient with an adverse medical event or laboratory test abnormality suspected to be induced by a medicine.

Signal

Reported information on a possible causal relationship between an adverse event and a drug; the relationship being unknown or incompletely documented previously

IMPORTANCE OF PHARMACOVIGILANCE

Pharmacovigilance aims to detect ADRs early, detect increase in known adverse reactions, and identify potential risk factors and possible mechanisms underlying adverse reactions. It also aims to estimate and communicate risks and benefits of drugs with view to promoting their safe, rational and more effective use of medicines thereby improving the care and safety of patients and the public at large[4]. It has been shown to reduce the risk and severity of ADRs. The greater the amount of knowledge available on the efficacy (or otherwise) and risks of a drug, the more safely and effectively the drug can be used. This is important in view of the fact that ADRs have been shown to be a substantial contributor to morbidity and mortality both in hospital and community settings and are preventable[5,6,7]. In some developed countries, up to 15-20% of hospital budgets are spent on dealing with drug-related complications[8]. This cost in human and financial terms justifies and necessitates pharmacovigilance activity.

METHODS OF PHARMACOVIGILANCE

Methods of pharmacovigilance include the registration of all drugs by the drug regulatory body, spontaneous reporting and epidemiological methods.

Drug Registration serves to ensure that all drugs in use in the country are approved and known to the drug regulatory authority. It also ensures that all pre-registration safety standards are met and possible side-effects known. Before a drug is registered, the regulatory body (NAFDAC in Nigeria), ensures that it has undergone pre-marketing evaluation which includes animal studies and clinical trials – in humans. However, pre-marketing evaluation may miss, or even never-report ADRs. This is due to the uniformity and relatively small number of patients who participate in the clinical trials, an average of about 5000, compared with the millions of patients who will eventually use the drug[1].

The commonest method is the use of spontaneous reporting (passive surveillance) which entails the detection of ADRs and the reporting of these to the local, regional and national pharmacovigilance teams. Spontaneous reporting usually involves the health professional filling in the details of the patient and the ADR into a specially designed form for the purpose and sending it to the pharmacovigilance body which then uses such information gathered from an area/region/country to take the necessary action. It is easier, costs less, but may under-report ADRs.

The third method is the use of epidemiological methods (active surveillance) which are more systematic and robust and take the limitations of spontaneous reporting into account. These intensive surveillance methods include case control/cohort studies, epidemiological studies, hospital based intensive monitoring systems, and record linkage systems. Epidemiological methods are more complicated and costly.

THE WHO PROGRAMME FOR INTERNATIONAL DRUG MONITORING

All reports that are received in a National Pharmacovigilance Center are forwarded to the WHO Collaborating Centre for International Drug Monitoring in Upsalla, Sweden also called the Upssala Monitoring Centre (UMC). These reports are then entered into an international ADR Report database called Vigibase. This database can be searched to retrieve all reports on, for instance, a particular drug. The UMC uses an artificial intelligence system (neural network) called the Bayesian Confidence Propagation Neural Network (BCPNN) to detect significant drug-ADR combinations (called associations) in the database. These associations are then sent to members of an international expert review panel. These experts examine the associations and use clinical experience and available information such as original ADR reports and literature to decide if a causal relationship exists between the drug and the ADR. If they think so, then a 'signal' has been generated and the information is included in a quarterly publication called SIGNAL which is distributed to national centers to alert them. The national centres, in collaboration with the drug-regulatory body, can then take necessary action for patient safety. The most common drug groups causing ADRs worldwide and the most commonly reported symptoms are shown in Tables 1 and 2 respectively[4].

PHARMACOVIGILANCE IN NIGERIA

The first attempt at pharmacovigilance was made in the country in 1981 when a member of staff of the Federal Ministry of Health was trained for one month at the WHO Collaborating Centre for International Drug Monitoring in Upssala, Sweden (a.k.a. Upssala Monitoring Centre, UMC). Efforts to set up a nationwide body were eventually successful in September 2004, when a National Pharmacovigilance programme (and Center) was launched.

Nigeria was the 74th nation in the world to join the WHO global drug monitoring programme. The National Agency for Food and Drug Administration and Control (NAFDAC) is the host body for the centre[9].

The programme involves the National Pharmacovigilance Centre (NPC), a National Advisory Committee comprising health experts, Reporting Centres at local and regional level, drug manufacturers, the media and other stakeholders[2,10]. The organizational and functional structure of the programme in Nigeria is

Rank	Drug Group	No. of Reports
1.	Non-steroidal anti-inflammatory drugs (NSAIDS)	236 793
2.	Anti-depressants	205 891
3.	Viral vaccines	153 609
4.	Bacterial Vaccines	150 523
5.	Anti-psychotics	115 110
6.	Anti-thrombotic agents	103 073
7.	Penicillins	102 373
8.	Cholesterol and triglyceride reducers	88 617
9.	.Anti-epileptics	88 233
10.	Drugs for treatment of peptic ulcers	80 978

Table 1 Most reported drug cases in the WHO database up to April 2004

Shown in Figure 1.

Rank	Adverse Reaction Term	System Organ Class	No. of Reports
1.	Rash	Skin	188 220
2.	Fever	General	144 519
3.	Pruritis	Skin	133 871
4.	Nausea	Gastrointestinal	131 386
5.	Uticaria	Skin	125 296
6.	Headache	CNS	101 513
7.	Vomiting	Gatrointestinal	104 898
8.	Dizziness	CNS	101 513
9.	Breathlessness (Dyspnoea)	Respiratory	85 611
10.	Diarrhoea	Gastrointestinal	81 902

Table 2 Most reported ADR symptoms in the WHO database up to April 2004

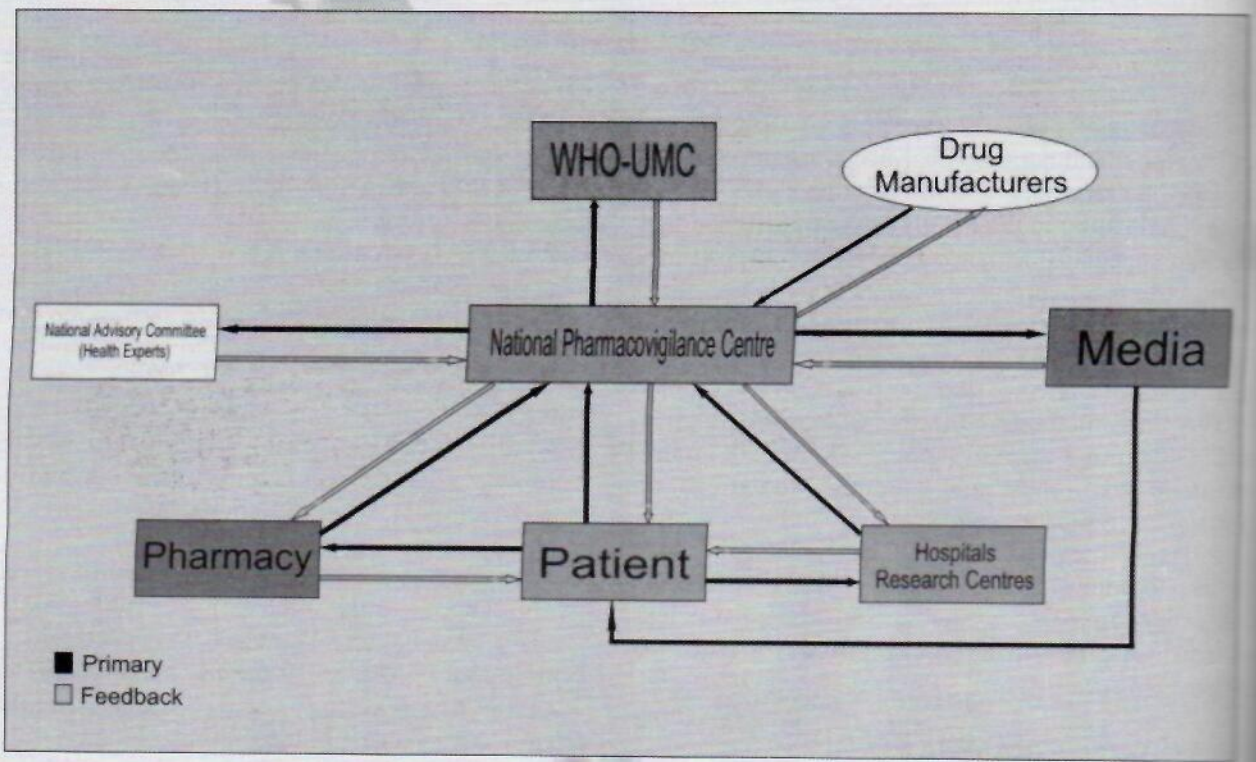

Figure 1 Structure of the Pharmacovigilance programme in Nigeria

- **Use few drugs**
- **Use drugs that you know very well**
- **Do not change from familiar drugs to unfamiliar drugs without good reasons**
- **Use textbooks, formularies and other reference materials providing information on drug reactions and interactions**
- **Take extra care when you prescribe drugs known to exhibit a wide variety of interactions and adverse reaction e.g. Hypoglycaemics, anticoagulants and ONS drugs and monitor patient carefully**
- **Beware of drug reactions with food, alcohols and House Chemicals**
- **Review all drugs used by patients regularly, taking notes of those bought without prescription**
- **Be careful when prescribing for children, elderly, pregnant and nursing patients, critically ill and patient with liver or renal disease. Monitor them carefully**
- **If patients show any signs or symptoms not clearly explained by course of their illness, think of ADR**
- **If you suspect ADR, consider stopping the drug or reducing dosage and fill the ADR reporting forms and send immediately**

Courtesy, National Pharmacovigilance centre

Figure 2 Useful tips on rational prescribing and preventing ADRs

THE ROLE OF HEALTH WORKERS IN PHARMACOVIGILANCE

Healthcare professionals are strategically placed in society to act in preventing adverse drug reactions. They are in a position to and can help to rationally prescribe drugs, tell patients about possible reactions, detect ADRs, treat promptly and report to the NPC[3]. Rational prescribing, patient monitoring, patient follow-up, reporting ADRs and communicating with other health professionals are all essential.

The rational use of drugs prevents ADRs, just as medication errors are a principal cause. Medication errors may be due to wrong diagnoses, wrong prescription, wrong dosing, poor patient selection, drug interaction, poor compliance, self medication, polypharmacy or polytherapy. Figure 2 shows a list of steps to take in preventing ADRs. Even after prescribing a drug, the onus rests on the physician to r monitor the patient and inquire as to any untoward reaction as the illiterate or ignorant patient may consider an ADR as evidence that a drug is working.

The ability to communicate with other health professionals on drug-related problems is a useful tool. Local and Regional Pharmacovigilance centers should provide a ready source of local information and feedback to doctors reporting ADRs. Special workshops to discuss the problems in a locality will be valuable.

ADR Reporting in Nigeria is currently done by all healthcare professionals. All suspected ADRs should be reported, whether serious or not. This is importantto ensure that no potentially life-threatening ADRs in other patients are missed, as a reaction which has not been known to be attributable to a drug will be promptly investigated by a reporting centre. Healthcare professionals will also benefit from good pharmacovigilance activity as it will result in improved standard of care offered patients, improved patient confidence, better treatment outcomes and improved satisfaction.

Problems with product (drug quality) should also be reported. These include expired drugs, contamination, questionable stability, faulty labeling and packaging, inactive components and therapeutic failures.

The ADR Reporting form (Appendix 1) is a yellow form which is filled in duplicate, with one copy sent off to the NPC and another filed for the record. The form enjoys pre-paid postage. The submitted reports should be timely, reliable and completely filled.

Preventing and monitoring ADRs is a multi-faceted endeavor that requires the efforts of NAFDAC, drug manufacturers, researchers, health, patients, the media, advocacy groups and other professionals. These different components of society need to work together, complementing one another.

RECOMMENDATIONS

Nigeria has taken a bold step in establishing a pharmacovigilance programme, but the programme is still limited to a few secondary and tertiary hospitals. If grassroots coverage must be achieved. All primary health care centers, state hospitals and private practices should have access to forms and should be informed on the processes of the programme.

Future healthcare professionals -students of medicine, pharmacy, nursing and allied health professions- should be taught the basics of pharmacovigilance as part of the undergraduate curriculum. This teaching will help inculcate a good reporting culture at an early stage in their professional careers. It may be included in the pharmacology lectures or given separately.

Education of the citizenry is an important part of the effort to ensure drug safety. Mass media campaigns should be undertaken to discourage habits such as self medication and inform patients to take note of untoward effects of drugs they might be taking and report to the prescribing doctor.

The use of the essential drugs list should be encouraged and efforts should be initiated to publish a national formulary which will include a list of all registered drugs for use in the country, their safe dosages and other relevant information. This will boost the practice of rational prescribing among healthcare professionals.

Although the pharmacovigilance policy states that herbals are included in the programme, it is common knowledge that many of the herbals in public consumption are unregistered, and their components and safe dosages are not known. A comprehensive effort to register and monitor consumption of herbals in Nigeria will be required, if drug safety is to be achieved.

CONCLUSION

Developing an extensive and vibrant pharmacovigilant practice in the country is a major step in advancing our current level of healthcare delivery as small steps by all healthcare professionals and other stakeholders in this direction will translate to a giant step for all Nigerians and the world in general. Inculcating a pharmacovigilance culture in healthcare's future leaders and increased public awareness are essential steps in this direction. Furthermore, the potentially fatal dangers inherent in most herbal supplements currently in circulation can only be avoided by subjecting them to scientific scrutiny and drug monitoring. A combination of these measures will definitely improve drug safety and the quality of the national healthcare delivery.

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THE USE OF BIOMARKERS IN RESEARCH

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Keywords: Biomarkers, Validation, Cancer Research

INTRODUCTION

Biomarkers are anatomic, physiologic, biochemical, genetic or molecular parameters associated with the presence and severity of specific disease states. They are indicators of events in biologic samples or systems. Biomarkers are detectable and measurable by a variety of methods including physical examination, laboratory assays and medical imaging; therefore they can serve as tools that can be used to clarify relationships, if any, between exposures of interest and outcomes. Regardless of this succinct description, the actual definition remains contentious. Some biomarkers are easy to concede, like changes in serum levels of markers such as CA125, PSA, etc. Other parameters include target inhibition, functional imaging, the time it takes for a certain percentage of patients to reach certain end-points, measurements of residual disease, immunological markers and proteomics

In the last few years, identification of biomarkers has become an important cancer research enterprise. This is partly because of the remarkable progress that has been made in the prevention and treatment of diseases, such as coronary heart disease and stroke, for which there are effective biomarkers and the challenge posed by other complex diseases for which no biomarker has been found. For example, elevated serum cholesterol and blood pressures are powerful biomarkers of coronary heart diseases and stroke. In addition, rapid advances in genetics, genomics, proteomics, modern imaging techniques and other technologies has increased our ability to measure even more variables and characteristics than before. Furthermore, improved understanding of biochemical processes, disease pathways, targets of pharmacological intervention is increasing our knowledge of opportunities for finding new biomarkers of disease or drug response. Studies that use disease as endpoints tend to be large, costly and last a long time. They are therefore inefficient hence the attraction of biomarkers as alternative study endpoints.

CLASSIFICATION OF BIOMARKERS

There are many ways to classify biomarkers. One way is in the definition. Another is:

a. **Biomarkers of exposure**. These are exogenous substances that are found within biologic systems or substances that result from interaction between exposure and endogenous components, or other events in biologic systems related to exposure
b. **Biomarkers of effect**. These are substances that reflect qualitative or quantitative changes which are predictive of potential or actual health impairment resulting from exposure.
c. **Biomarkers of susceptibility**. These are defined as indicators that the health of an organism is especially sensitive to the challenge posed by exposure.

Biomarkers can also be classified according to their location in the health continuum:

a. **Risk assessment biomarkers**. These are markers of susceptibility to disease.
b. **Screening markers**. These markers discriminate between "healthy" and "asymptomatic diseased" members of the population.
c. **Prognostic markers**. These are probabilistic markers of outcome of disease and can be used to determine the aggressiveness of therapy
d. **Stratification biomarkers**. These predict the likely response to a drug or treatment.
e. **Therapy monitoring biomarkers**. These are markers of the efficacy of treatment.

CHARACTERISTICS OF BIOMARKERS

Relevance: For a characteristic to be a biomarker, it must be relevant to the exposure or outcome under consideration. It must provide information that can not be obtained by simpler means on questions of interest and importance to public health and health care. The questions of interest must be important and not just ones that can be answered: they must be questions that should be answered. For example, biomarkers that detect evidence of exposure is not as relevant as those that detect that exposure associated with increased risk of outcome.

Validation: Given the wide array of potential biomarkers, validation has become important in order to ensure utility. Validity is related to the intrinsic character of the biomarker itself and of the laboratory method used to measure it. There are 3 aspects to this:

a. Measurement validity (Also known as Accuracy to physical scientists and Validity to social scientists): This refers closeness of the measurement to some "true" or "real" value. The philosophical and pragmatic difficulty with this definition is immediately obvious as

often we do not know what the real value of interest is. So we often compare our test with some "gold standard".

b. Internal study validity: This refers to the degree with which true inferences can be drawn from a study with regards to participants in that study.

c. External study validity: This is the extent to which the results of a study can be generalized to those people who did not participate in the study of the biomarker.

Factors that can affect the validity of biomarkers include sampling constraints; sample size required for acceptable precision (precision being defined as "variability of repetitions" in the frequentist sense; time to assay, storage methods and impact of storage methods on assay results; simplicity and potential for routine usage; speed of assay; standardization of procedure sensitivity, specificity, positive predictive value, negative predictive value, dose-response curve, inter and intra individual variability and knowledge about confounding factors (factors that must be measured in order to properly interpret the relationship between the biomarker and outcome) that can affect the marker. It is critical that biomarkers used in research be valid according to these criteria because invalid biomarkers lead to erroneous risk assessment.

CORNERSTONE FOR DEVELOPMENT OF BIOMARKERS

To test the causal association between a biomarker and an outcome of interest, there is still no alternative to the criteria set out by Bradford Hill on causality. These are:

a. Strength of the association between biomarker and outcome.

b. Specificity.

c. Temporality. This is the strongest of all the criteria. Essentially, it means that the effect must precede the outcome, but in longitudinal biomarker studies where there is a more complex pattern of events that leads to an enzyme induction which is only relevant if there is follow-up exposure, strict temporality rules may be violated.

d. Biological gradient or a dose-response curve

e. Plausibility. However this depends on current knowledge. In the evolving scenario of gene-gene, gene-environment and other interactions, this may not apply.

f. Coherence refers to consistency between observation and experimental data.

BIOMARKERS IN ONCOLOGY RESEARCH

Given the generally poor prognosis of patients who present with advanced cancers, oncologists are particularly interested in the role of biomarkers in screening and early diagnosis of cancers, so that appropriate therapeutic strategies can be applied with good prognosis. In the past decades, many biomarkers have been reported in the literature but few have made it into routine clinical use and fewer still have had success as screening tools. This situation has been blamed on several factors:

a. Poor study design and methodology of biomarker studies

b. Lack of standardization of assay methods

c. Lack of reproducibility of assays

d. Inappropriate or misleading statistical methods

e. Small sample sizes

Most biomarker research is based on case-control methods and these are often plagued by methodological bias and availability of specimens. Cases are more likely to recall events than controls and the presentation phase of the disease may have altered biomarker levels. Patients with slow growing tumors are more likely to present to hospital and live long enough to participate in research. There is likely to be bias towards easy to obtain specimens which have been adequately processed and preserved. The implication of this is that rigorous standards are needed for biomarker studies but this is often not the case. As a response to this, the National Cancer Institute of the United States and the European Organisation for Research and Treatment of Cancer First International Meeting on Cancer Diagnostics recommended the development of guidelines for reporting tumor marker studies. This recommendation led to the publication of the REMARK (Recommendations for Tumor Marker) after the success of the CONSORT guideline for reporting clinical trials and STAD statement for studies of diagnostic tests. The guideline suggests relevant information that should be provided about study design, preplanned hypothesis, patient and specimen characteristics, assay methods, and statistical analysis methods. With this guideline, it is expected that there will be more standardization and transparency in reporting biomarker studies. We will then be able to truly take advantage of the "omics" revolution and change the prognostic landscape of cancers.

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CONGENITAL RUBELLA SYNDROME

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ABSTRACT

Congenital Rubella Syndrome is a debilitating condition which can be effectively prevented by active immunization and prompt diagnosis of cases. The advent of vaccines have reduced the incidence of the infection in developed countries but a lot more awareness is needed in developing countries. Laboratory diagnosis is essential in the overall management of congenital rubella syndrome. However cost and availability of laboratory facilities for diagnosis in hospitals and related centers remains a constraint to achieving the desired goal.

INTRODUCTION

The name rubella is derived from a Latin term meaning "little red." Rubella (German measles, 3-day measles, rubeola, röteln, roetheln, third disease) generally a benign communicable exanthematous disease. It is caused by rubella virus, which is a member of the Rubivirus genus of the family Togaviridae. Rubella virus is a human-specific ribonucleic acid virus that causes mild infection in children and adults with peak incidence in the late winter and early spring[1,2] .About 15% to 24% of adults are susceptible to rubella in the United States[2]. However, the public health importance of rubella relates to the teratogenic effects when rubella infection is acquired in the early months of pregnancy. Rubella infection of the fetus can result in fetal death or in the birth of an infant with serious congenital birth defects. The congenital rubella syndrome (CRS) is an important cause of blindness, deafness, congenital heart disease, and mental retardation.

The original studies of Gregg and others relied on clinical diagnosis of rubella in the mother and congenital infection in the infant. However, with the isolation of Rubella virus in 1962, laboratory confirmation became possible. The duration of the virus excretion in acquired and congenital cases and the nature of the antibody responses were extensively studied. With these, documented, laboratory methods for the confirmation of suspected clinical cases could now be established using virus isolation and serological techniques.

BURDEN OF DISEASE

Worldwide, it is estimated that more than 100 000 infants are born with CRS each year.Cutts and Lievsky estimate that in 1996 there were 110 000 infants affected by CRS in developing countries (excluding the WHO European Region) that had not introduced rubella vaccine[3]. A separate estimate for the WHO European Region suggests some 4 000 CRS cases occur annually in countries of that Region that have not introduced rubella vaccine[3]. A recent global review of CRS sequelae analyzed data from prospective studies with laboratory-confirmed cases[4]. Among infants with CRS, 60% had hearing impairment, 45% congenital heart disease, 27% microcephaly, 25% cataract(s), 23% low birth weight (<2500 grams), 17% purpura, 19% hepatosplenomegaly, 13% mental retardation, and 10% meningoencephalitis.

PATHOPHYSIOLOGY

The usual portal of entry of rubella virus is the respiratory epithelium of the nasopharynx. The virus is transmitted via the aerosolized particles from the respiratory tract secretions of infected individuals. The virus attaches to and invades the respiratory epithelium. It then spreads hematogenously (primary viremia) to regional and distant lymphatics and replicates in the reticuloendothelial system. This is followed by a secondary viremia that occurs 6-20 days after infection. During this viremic phase, rubella virus can be recovered from different body sites including lymph nodes, urine, cerebrospinal fluid, conjunctival sac, breast milk, synovial fluid, and lungs. Viremia peaks just before the onset of rash and disappears shortly thereafter. An infected person begins to shed the virus from the nasopharynx 3-8 days after exposure for 6-14 days after onset of the rash.

Fetal infection occurs transplacentally during the maternal viremic phase, but the mechanisms by which rubella virus causes fetal damage are poorly understood. The fetal defects observed in congenital rubella syndrome are likely secondary to vasculitis resulting in tissue necrosis without inflammation. Another possible mechanism is direct viral damage of infected cells. Studies have demonstrated that cells infected with rubella in the early fetal period have reduced mitotic activity. This may be the result of chromosomal breakage or due to production of a protein that inhibits mitosis. Regardless of the mechanism, any injury affecting the fetus in the first trimester (during the phase of organogenesis) results in congenital organ defects[3].

CLINICAL FEATURES

The classic triad presentation of congenital rubella syndrome consists of the following:

Sensorineural hearing loss is the most common manifestation of congenital rubella syndrome. It occurs in approximately 58% of patients. Studies have shown that approximately 40% of patients with congenital rubella syndrome may present with deafness as the only abnormality without other manifestations. Hearing impairment may be bilateral or unilateral and may not be apparent until the second year of life.

Ocular abnormalities including cataract, infantile glaucoma, and pigmentary retinopathy occur in approximately 43% of children with congenital rubella syndrome. Both eyes are affected in 80% of patients, and the most frequent findings are cataract and rubella retinopathy. Rubella retinopathy consists of a salt-and-pepper pigmentary change or a mottled, blotchy, irregular pigmentation, usually with the greatest density in the macula. The retinopathy is benign and nonprogressive and does not interfere with vision (in contrast to the cataracts) unless choroid neovascularization develops in the macula.

Congenital heart disease including patent ductus arteriosus (PDA) and pulmonary artery stenosis is present in 50% of infants infected in the first 2 months of gestation. Cardiac defects and deafness occur in all infants infected during the first 10 weeks of pregnancy and deafness alone is noted in one third of those infected at 13-16 weeks of gestation[7].

Other findings in congenital rubella syndrome include the following: intrauterine growth retardation, prematurity, stillbirth and abortion; central nervous system abnormalities including mental retardation, behavioural disorders, encephalographic abnormalities, hypotonia, meningoencephalitis and microcephaly; jaundice, hepatitis, skin manifestations(including blueberry muffin spots that represent dermal erythropoiesis and dermatoglyphic abnormalities); bone lesions, such as radiographic lucencies; endocrine disorders, including late manifestations in congenital rubella syndrome usually occurring in the second or third decade of life (e.g, thyroid and growth hormone abnormalities, diabetes mellitus); Hematologic disorders, such as anemia and thrombocytopenic purpura; polycystic kidney and cryptochidism[7].

DIAGONSIS

Standard rubella case definitions have been adopted by WHO, including for: (1) a suspected rubella case, (2) a clinically confirmed rubella case, and (3) an epidemiologically confirmed rubella case[8-10].

- A suspected rubella case is any patient of any age in whom a health worker suspects rubella. A health worker should suspect rubella when a patient presents with fever, maculopapular rash, and one or more of the following: cervical adenopathy, suboccipital adenopathy, postauricular adeopathy, or arthralgia/arthritis.
- A laboratory-confirmed rubella case is a suspected case with a positive blood test for rubella-specific IgM. The blood specimen should be obtained within 28 days after the onset of rash.
- An epidemiologically confirmed rubella case is a patient who meets the suspected case definition and is epidemiologically linked to a laboratory-confirmed case.

Unlike acquired infection, congenital infection can result in chorionic villi excretion; this appears to be confined to infants infected during the first 4 months of pregnancy. Diagnosis can be made by isolating virus from urine, nasopharyngeal swabs or other body tissue. The period of virus excretion persists for up to 6 months after birth with congenital risk of such infants infecting susceptible contacts.

- Demonstration of Rubella specific IgM antibody in cord blood. Unlike IgG antibody, maternal IgM does not cross the placenta and its presence in cord blood thereafter indicates fetal infection. IgM antibody only begins to appear in fetal blood at 20 weeks GA even when infection occurs in pregnancy.
- Detection of Rubella specific IgG antibody between 10-12 months of age is also indicative of congenital infection since passively-acquired maternal antibody disappears by this age.
- Diagnosis of congenital Rubella after the first year of life by the detection of IgG antibody is less reliable since an increasing proportion of children will have Rubella antibody from an acquired infection.
- Lymphocyte transformation testing for cell-mediated immunity (CMI) to Rubella virus may be of help in distinguishing acquired from congenital Rubella in older children since a significant reduction in CMI has been demonstrated in children infected *in utero* during the first trimester.

LABORATORY DIAGNOSIS

Laboratory Criteria for Diagnosis: Isolation of rubella virus, or Demonstration of rubella-specific IgM antibody, or An infant's rubella antibody level that persists above and beyond the expected from passive transfer of maternal antibody (i.e., rubella titer that does not drop at the expected rate of a twofold dilution per month).

Every effort should be made to establish a laboratory diagnosis when rubella infection is suspected in pregnant women or newborn infants. Congenital rubella in infants and children is diagnosed by virus isolation or by serologic testing. In contrast to postnatal infection, viral isolation is the preferred technique in congenital rubella syndrome because rubella serology may be difficult to interpret in view of transplacental

passage of rubella-specific maternal IgG antibody. In addition, rubella-specific IgM antibody may not be detectable at the time of evaluation. Congenital rubella syndrome has also been diagnosed using placental biopsy, rubella antigen detection by monoclonal antibody, and PCR. Specimens used for viral isolation in congenital rubella include nasopharyngeal swab, urine, cerebrospinal fluid, and buffy coat of the blood. In some infants with congenital rubella syndrome, rubella virus can persist and can be isolated from the nasopharyngeal and urine cultures throughout the first year of life or later. The same serologic testing methods (ELISA, IFA, LA, HI, CF) discussed for postnatal rubella can be used to detect specific antibodies in congenital infection. Rubella-specific IgM antibody is actively produced by the fetus or neonate and may be detected in the cord blood or neonatal serum. Congenital rubella syndrome should be strongly suspected in infants older than 3 months if rubella-specific IgG antibody levels are observed and do not decline at the rate expected from passive transfer of maternal antibody (i.e., equivalent of a 2-fold decline in HI titer per month) in a compatible clinical situation. Patients with concomitant immunodeficiency, such as agammaglobulinemia or dysgammaglobulinemia, may have a false-negative serology result for rubella. Therefore, viral isolation is required to confirm the diagnosis in this group of patients[7,11].

AMNIOTIC FLUID TESTING[12]

It is uncertain whether Rubella is invariably present in the amniotic fluid of infected fetuses. The largest study to be reported was by Hayes et al, 1982.

In 10

In 3 subsequent studies

weeks for virus to grow in culture.

Nucleic acid hybridization for Rubella antigen detection, both specific and rapid, and is potentially highly sensitive if augmented by the technique of PCP.

CHORIONIC VILLI SAMPLING, CVS[12]

CVS+RNA hybridization can diagnose infection as early as 15weeks. The problem is the placenta infection without infection from early studies on abortion materials using virus isolate records.

COMBINATION[12]

CVS+Initial screen, subsequently, amniotic fluid and fetal blood taking.

FULL BLOOD COUNT[13]

- Thrombocytopaenia < 10,000 platelets/μL
- Haemolytic anaemia, neuroblastoma, reticulocytosis and erupted hyperplastic marrow and eventual decrease in Hb.

LIVER FUNCTION TEST[13]

Increased levels of direct reacting bilirubin and evidence of hepatocellular dysfunction.

VIRAL ISOLATION[13]

- Virus may be recovered from peripheral leucocytes, throat, stool, urine for months or years after birth.
- High persistent Rubella antibody titre in 2 serum
- Abnormalities in immunoglobulin concentration (variable, but induces depression of IgA and IgG levels with increased IgM)
- Defect in cellular immunity
- CSF pleocytosis and increase in protein

IMAGING STUDIES

Chest radiography is indicated for infants who develop respiratory distress or other respiratory symptoms to exclude rubella-related interstitial pneumonitis or pulmonary edema that may result from congestive heart failure in children with severe or complicated congenital heart anomalies. Radiography of the long bones may reveal radiolucencies in the metaphyses of long bones.

Echocardiography is important for patients with congenital heart defects to help diagnose the type of heart anomaly and evaluate the severity of the heart defect so that appropriate surgical plans can be made.Computed tomography (CT) scan of the head may reveal intracranial calcifications and enlargement of the ventricles. Magnetic resonance imaging (MRI) of the head may reveal cortical atrophy and white matter changes in patients with late-onset progressive panencephalitis.

HISTOLOGIC FINDINGS

The gross neuropathologic features that present during autopsy of babies who are stillborn include microcephaly and various other malformations (for example, polymicrogyria, nonhemorrhagic subependymal germinal matrix cysts). Histologically, chronic inflammatory cells are found in the meninges and surrounding the intraparenchymal blood vessels. The vessel walls also show foci of subintimal fibrosis and mineralization.

CONTROL MEASURES

School and child care

Children with postnatal rubella should be excluded from school or child care for 7 days after onset of the rash. Children with congenital rubella should be considered contagious until they are at least 1 year of age, unless nasopharyngeal and urine culture results repeatedly are negative for rubella virus. Caregivers of these infants should be made aware of the potential hazard of the infants to susceptible pregnant contacts.

Care of exposed people

When a pregnant woman is exposed to rubella, a blood specimen should be obtained as soon as possible and

tested for rubella antibody. An aliquot of frozen serum should be stored for possible repeated testing at a later time. The presence of rubella-specific IgG antibody in a properly performed test at the time of exposure indicates that the person most likely is immune. If antibody is not detectable, a second blood specimen should be obtained 2 to 3 weeks later and tested concurrently with the first specimen. If the second test result is negative, another blood specimen should be obtained 6 weeks after the exposure and also tested concurrently with the first specimen; a negative test result in both specimens indicates that infection has not occurred, and a positive test result in the second or third specimen but not the first (seroconversion) indicates recent infection.

Immune Globulin

The routine use of Immune Globulin (IG) for postexposure prophylaxis of rubella-susceptible women exposed to rubella early in pregnancy is not recommended. Administration of IG should be considered only if termination of the pregnancy is not an option. Limited data indicate that intramuscular IG in a dose of 0.55 mL/kg may decrease clinically apparent infection in an exposed susceptible person from 87% to 18%, compared with placebo. However, the absence of clinical signs in a woman who has received intramuscular IG does not guarantee that fetal infection has been prevented. Infants with congenital rubella have been born to mothers who were given IG shortly after exposure.

Vaccine

Although live-virus rubella vaccine given after exposure has not been demonstrated to prevent illness, vaccine theoretically could prevent illness if administered within 3 days of exposure. Immunization of exposed nonpregnant people may be indicated, because if the exposure did not result in infection, immunization will protect these people in the future. Immunization of a person who is incubating natural rubella or who already is immune is not associated with an increased risk of adverse effects.

Rubella vaccine

Vaccine is administered by subcutaneous injection of 0.5 mL, alone or, preferably, as a combined vaccine containing measles-mumps-rubella (MMR). Vaccine can be given simultaneously with other vaccines. Serum antibody to rubella is induced in 95% or more of the recipients after a single dose at 12 months of age or older. Clinical efficacy and challenge studies have demonstrated that 1 dose confers long-term, probably lifelong, immunity against clinical and asymptomatic infection in more than 90% of immunized people. Because of the 2-dose recommendation for measles vaccine as MMR, 2 doses of rubella vaccine now are given routinely. This provides an added safeguard against primary vaccine failures[7,12].

TREATMENT

Treatment is supportive. For asymptomatic newborns, vision and hearing screenings are required. However, for symptomatic newborns, provide careful evaluation of the eyes and ophthalmology referral for babies with corneal clouding, cataract, and retinopathy. Corneal clouding may indicate infantile glaucoma. Babies with congenital rubella syndrome who develop respiratory distress may require supportive treatment in the intensive care unit. Hepatosplenomegaly is monitored clinically; no intervention is required. Patients with hyperbilirubinemia may require phototherapy or exchange transfusions if jaundice is severe to prevent kernicterus. True hemorrhagic difficulties have not been a major problem; however, IVIG may be considered in infants who develop severe thrombocytopenia. Corticosteroids are not indicated. Infants who have a rubella-related heart abnormality should be carefully observed for signs of congestive heart failure. Echocardiography may be essential for diagnosis of heart defects. Contact isolation is required for patients with congenital rubella during hospitalizations because babies are infected at birth and usually are contagious until older than 1 year unless viral cultures have produced negative results. Surgical treatment may be required for congenital heart anomalies, including PDA, coarctation of aorta, ventricular septal defect (VSD), atrial septal defect (ASD), and pulmonary artery stenosis. Surgical treatment may be required for eye defects such as glaucoma, cataract, and retinal neovascularization[7].

PROGNOSIS

The prognosis of postnatal rubella is good with full recovery, while congenital rubella syndrome may have a poor outcome with severe multiple-organ damage.

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MONOCLONAL ANTIBODIES (MAbs): PRODUCTION AND USES IN MEDICINE

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ABSTRACT

Today there is an increasing demand for pure homologous antibodies due to their remarkable specificity. Monoclonal antibodies have become promising agents for human therapy especially as immunotherapeutic agents in cancer treatment. They are also used in various investigative techniques such as immunoflurence, Enzyme Linked Immuno-Sorbent Assay (ELISA) or Radioimmuno Assay (RIA), Immunohistochemistry etc. Kohler and Milstein in 1975 developed hydridoma technology where hybridomas (hybrid cells) were generated and cloned to produce monoclonal antibodies from the fusion of variant myeloma cells with antibody-producing cells obtained from an immunized animals (mice). One major problem with monoclonal therapy is the production of HAMA (human anti-mouse antibodies) by the human immune response against the mouse antibody generated from the hybridoma technology. However, with the successful import of genetic engineering into human MAbs development and future production of transgenic mice through transgenic technology, their uses in diagnostic imaging and medicine will be enhanced.

Key words: *Cloning, Epitope, HAT (hypoxanthine, aminopterin and thymidine), HAMA (human anti-mouse antibodies) Hybridomas, Monoclonal antibodies, Myeloma cells.*

INTRODUCTION

An antibody or immunoglobulin (Ig) is a glycoprotein made by plasma cells in response to an antigen, and can recognize and bind to the antigen that caused it's production. Antibodies are present in blood serum, tissue fluids and mucosal surfaces of vertebrates. Humans and mice have the ability to make antibodies that are able to recognize virtually any antigenic determinant (epitope) and even to discriminate between similar epitopes present on an antigen. This provides the basis for protection against disease organisms, thereby making antibodies attractive candidates to target other types of molecules found in the body such as receptors or other proteins present on the surface of normal cells and molecules present uniquely on the surface of cancer cells. Thus, the remarkable specificity of antibodies makes them promising agents for human therapy.

The need for pure homologous antibodies has increased dramatically in recent years. Currently antibodies are produced either naturally by immunization or artificially through hybridomas. The fluid that remains after the blood clots is known as the serum. When the serum is obtained from an immunized host and contains the desired antibodies, it is called antiserum. The limitations of antiserum however, as a source of antibodies have been overcome with the development of hybridoma techniques to manipulate and culture various mammalian cells that synthesise antibodies *in vitro*. Each cell and its progeny normally produce a monoclonal antibody (MAb) of a single specificity (Prescott *et al.*, 2005).

Production of these antibodies of desired specificity in quantity and with reproducible characteristics has Always been a challenge. These goals were achieved by the introduction hybridoma technology by Kohler & Milstein in 1975. These antibodies of a single specificity are all built alike because they are being manufactured by a single clone of plasma cells that can be grown indefinitely. They were called monoclonal antibodies (MAbs). Since then, MAbs have come to play an enormous role in biological research.

MAbs are now being used in many techniques such as immunofluorescence, ELISA or RIA and imunohistochemistry because undesirable cross reaction can be avoided (William, 1993).

WHAT IS AN ANTIBODY (IMMUNOGLOBULIN)?

Antibody refers to the group of related glycoproteins which are capable of specific non-covalent binding to the molecules that induce their formation. Antibodies play a central role in humoural immunity by attaching to pathogens(antigens) and then recruiting effector systems to destroy the invaders.

The five major classes of immunoglobulins (Ig) are IgG, IgM, IgD, IgE and IgA. They all have certain structural features in common. The basic unit of antibody is a complex of 4 polypeptide chains composed of two identical light chains and two heavy chains (Fig.

Some of the well-defined functions of Igs include simple neutralization by blocking functional sites on pathogens; complement activation and stabilization; receptor recognition on effector cells; and polymerization/transport to apical epithelium.

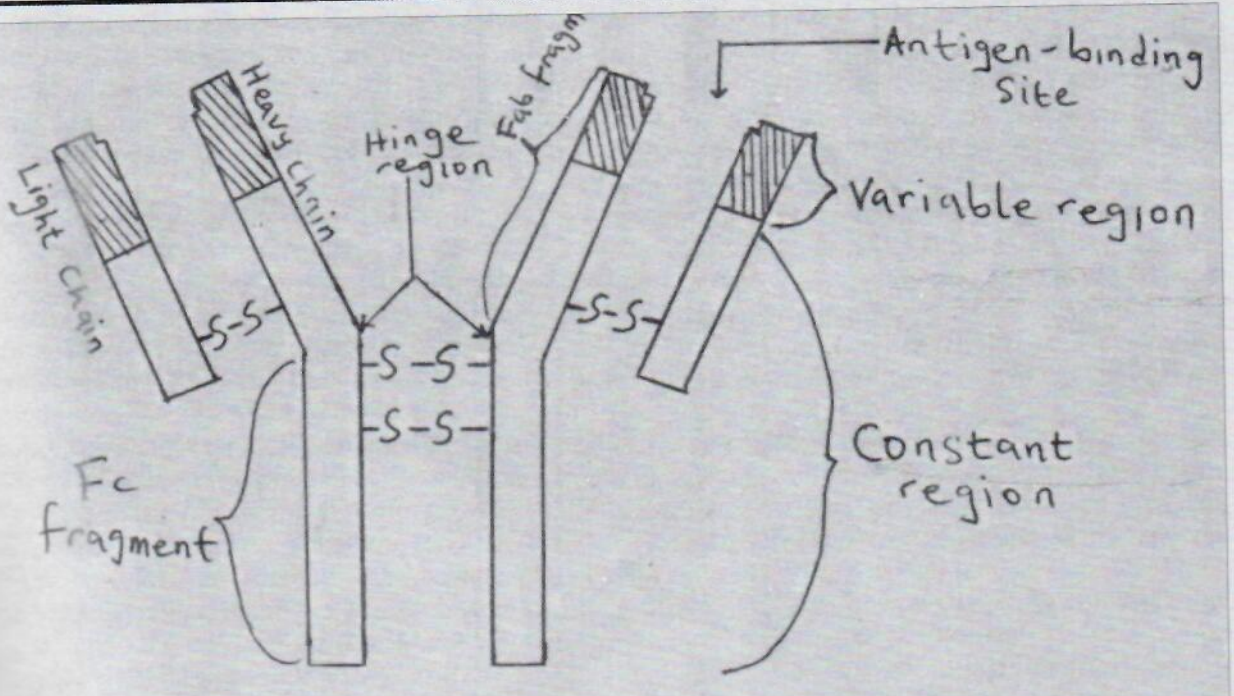
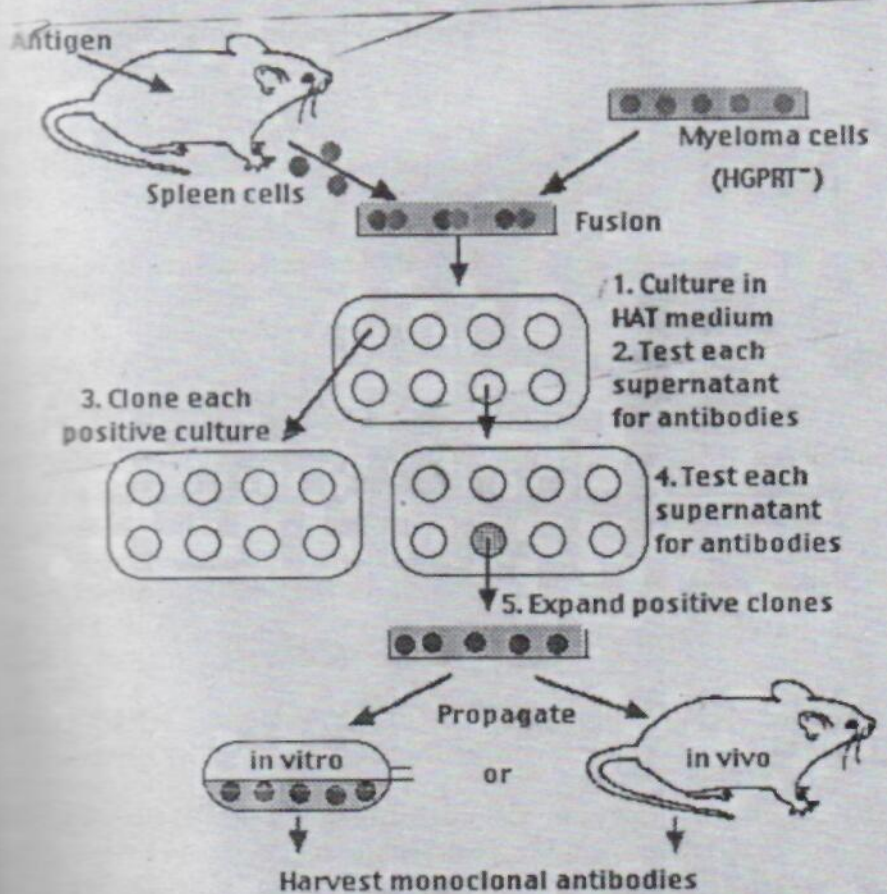


Fig 1: AN IMMUNOGLOBULIN MOLECULE



2: PRODUCTION OF MONOCLONAL ANTIBODIES (HYBRIDOMA TECHNOLOGY)

from Precott et al 2005
from Biotechnology Industry Organization 2003

PRODUCTION OF MONOCLONAL ANTIBODIES (Mabs)

The problem of production of monoclonal antibodies of desired specificity was solved in 1975 by Kohler and Milstein. They developed a technique called Hybridoma technology. This technology allows production of large quantities of pure antibodies especially using mice. The technique involves the somatic cell fusion of two cell types: antibody-producing cells from an immunized animal (mice) which by themselves die in tissue culture in a relatively short time; and myeloma cells, which contribute their immortality in tissue culture to the hybrid cell (called Hybridomas). The myeloma cells are variants carrying drug selection markers so that only those myeloma cells that have fused with spleen cells providing the missing enzyme will survive under selective conditions (Fig 2).

A brief overview of the various processes involved is given below:

Immunization of Donor Animals

The standard routes and schedules of immunization use include; antigen with complete Freund's adjuvant (CFA) subcutaneously or intraperitoneally and boosting with antigen in incomplete Freund's adjuvant or saline or skin grafting and boosting with lymphocytes intraperitoneally.

Myeloma Cell lines used as Fusion Partners

A commonly used selective marker is sensitivity to medium containing hypoxanthine, aminopterin and thymidine (HAT).

Unfused spleen cells expressing the enzyme hypoxanthine guanine phosphoribosyl transferase (HGPRT) are able to survive in HAT medium but die after a short time due to their limited life span unless immortalized by fusion with the myeloma cell. The myeloma cells, mutagenized and selected to be HGPRT-negative, are killed by HAT containing medium unless they have fused and therefore contain the enzymes of the spleen cells. Thus for several days after fusion there is extensive cell death, subsequently, the culture should contain only cells resulting from spleen-myeloma fusion (Hybrid cells).

Fusion Methods

Several different agents have been used to cause cell fusion. Early somatic cell fusion work used sendia virus (William, 1993). That approach has been replaced by use of polyethylene glycol (PEG). Both methods cause random fusion. Application of a strong electric field is also used (Lomms, *et al.*, 1984). This fuses the aggregates.

Screening Methods

Fusions have been successfully screened using RIA, ELISA or other binding assays; and assays for activation or blocking of biological effects such as cell mediated Lympholysis (CML), receptor activation, and lymphokine activity. Fusions are also screened by hybridizations to detect Igs or certain types of mRNA in the cells rather than antibody in the supernatant. Choosing a screening assay depends on the assay's ability to give clear-cut discrimination between positive and negative identification to avoid false positive results.

Post fusion processing of Hybridomas

After identification of positive cultures, hybridomas must be cloned to assure production of only one antibody, and cells must be frozen for future use. Cloning can be performed by limiting dilution or by colony selection from soft agar (subculturing). Cloning is done promptly, before possible non-producing cells in the same well including variants of the positive ones can overgrow the antibody producers.

Clones producing the desired antibody must then be expanded, and supernatant collected for antibody preparation. The hybridoma cultures can be maintained indefinitely :

(i) In vitro, i.e. in culture vessels. The yield runs from 10 - 60µg/ml.

(ii) In vivo i.e., growing in mice. Here the antibody concentration in the serum and other body fluids can

Problems Encountered in Fusion Technology

These include lot-to-lot variation in PEG and fetal calf serum, requiring batch testing. Identifying the characteristics of the myeloma cell used as fusion partner may be a problem since all cell lines drift in their properties. The problem of myeloma contamination of any of the cell lines or reagents used has been identified. All cell lines including new hybrids once established should be tested periodically for mycoplasma by commercially available kits.

Fusions in Species other than Mice

Two approaches have been taken in this direction. First, interspecies hybridization can be preformed e.g. human-mouse fusion to produce antibody to the human T cell receptor, hamster – mouse fusion and Rabbit-mouse hybridomas have been described. Second, use of fusion patner cells from the desired species. A rat myeloma line adapted for this purpose, IR983F, was described by Bazin (Bazin, 1981)

SPECIFICITY AND CROSS-REACTIONS OF MONOCLONAL ANTIBODIES

Specificity of Monoclonal Antibodies (Mabs)

Since all the molecules in a sample of Mabs have the same variable region structure, they all have the same specificity. This uniformity has the advantage that batches of Mabs do not vary in specificity as polyclonal sera often do. The homogeneity of Mabs allows refinement of specificity analysis that was not possible with polyclonal sera. A few examples are as follows: one can use Mabs to distinguish closely related antigens in cases where most antibodies in a polyclonal serum would cross-react and so absorption of serum

leave sufficient activity to define additional specificity. This ability is useful in designing clinical assays for related hormones. Also, the fine specificity analysis possible only with Mabs is the discrimination of spatial site (epitope clusters) by competitive binding.

Cross Reactions of Monoclonal Antibodies

MAbs display many cross-reactions, emphasizing that antibody cross-reactions represent real similarities among antigens, not just an effect of heterogeneity of serum antibodies. Even antigens that differ for most of their structure can share one determinant, and a MAb recognizing this site would then give 100% cross-reaction. Example is the reactivity of autoantibodies in sera with both DNA and cardiolipin (Koike, *et al.*,

APPLICATION OF MONOCLONAL ANTIBODIES (MAbs)

Monoclonal antibodies are used in a wide variety of procedures, not only in immunology but also in other sciences, physical or biological. Some of the immunology-based monoclonal antibody uses include affinity chromatography based on MAbs, which is used as a step in purification of molecular species that are difficult to purify chemically. Purification on MAb columns has been applied successfully for major histocompatibilty complex (MHC) antigens.

MAbs are also used in the study of the structure of antibody and of antigen-antibody complex by x-ray diffraction (William, 1993). The possible clinical uses of monoclonal antibodies are many. They are widely used in RIA and ELISA measurement of substances in biological fluids, from hormones to toxins, and are extremely valuable in flow cytometric assays of cell populations using antibodies specific for differentiation antigens expressed on cell surfaces. An example is that CD4+ (HIV binding site) was detected in flow cytometry with MAB 379. The use of prostate-specific membrane antigen (PSMA) specific MAbs for immunohistochemistry and imaging of tissues with prostate cancer (Cancer and Metastasis has been achieved.

THERAPEUTIC USES OF MONOCLONAL ANTIBODIES

Only one MAb, anti-CD3 (OkT3), had been approved for clinical use. Between 1995 and 2000, a number of MAbs were approved for human therapy. How many more MAbs are in the market.

Many approaches have been developed to enhance the efficacy of the MAbs in human disease therapy.

Naked – use of only one MAb. Once bound to its antigen, it triggers the normal effector mechanism of the body e.g. OKT3, Daclizumal

Conjugated – Conjugated or conjugated two or more agents e.g. mylotarg® an immunotoxin MAb

Radiolabelled – Radiolabelled MAb i.e. coupling a strong – radioactive atom such as Iodine-131 or yttrium-90 with MAb represent an effective approach to hamper tumor re-growth or kill the target.

A List of some MAbs that have been introduced into human medicine are presented below:

To suppress the immune system (especially prevention of acute rejection of transplanted tissue and organ):

i. OKT3 –
ii. Daclizumab (Zenopax®-
iii. Infliximab (Remicade®
iv. Omalizumab (Xolair®)

To kill malignant cells

i. Rituximab (Rituxan®)
ii. Herceptin –
iii. Mylotarg® - A conjugate of anti-CD 33 MAb and Calicheamicin.
iv. Lymphocide – anti-CD 22,
v. Alemtuzumab (MabCampath®)

Angiogenesis Inhibitors

i. Vitaxin
ii. Bevacizumab (Avastin®)

Others

Abciximab (Repro®) – it inhibits the clumping of platelets.

PROBLEMS WITH MONOCLONAL THERAPY

The main difficulty with monoclonal therapy is that mice antibodies are "seen" by human immune system as foreign, and the human patient mounts an immune response against them, producing HAMA (human anti-mouse antibodies). The therapeutic antibodies are quickly eliminated from the host, and also form immune complexes that cause damage to the kidneys. It is of note that MAbs raised in humans would lessen the problem, but few people would want to be immunized in an attempt to make them. To solve the problem of HAMA, two approaches have been used:

(i) Production of chimeric antibodies using genetic engineering. The antigen-binding part (variable regions) of mouse antibody is fused to the effector part (constant region) of a human antibody. Infliximab and abciximab are examples.

(ii) "Humanised" antibodies. The amino acids responsible for making the antigen binding (hyper variable) regions are inserted into a human antibody molecule replacing its own hyper variable regions. Daclizumab, rituximab vitaxin, mylotarg®, Herceptin and xolair® are example.

In both cases, the new gene is expressed in mammalian

cells grown in tissue culture (though *E. coli* cannot add the sugars that are a necessary part of these glycoproteins). (Biotechnology Industry Organization, 2003).

LOOKING AHEAD

Other ways of solving the problem of HAMA are currently being exploited. One of particular interest is to delve into the use of transgenic technology to make transgenic mice that would have had human antibody gene loci inserted into thei bodies (using the embryonic stem cell method) and also that have had their own genes for making antibodies "knocked out". This might produce mice that can be immunized with desired antigen; produces human antibodies against the antigen, and can yield cells to be used with myeloma cells to manufacture all-human MAbs.

CONCLUSION

Based on the specificity and reproducible characteristics of monoclonal antibodies, they have come to play an enormous role in biological research. Also offered as advantages are the relative ease of production and purification of large quantities of the antibody, the uniformity of antibody batches; and the ready availability of Ig mRNA and DNA from the hybrid cell. These have enhanced the development of many monoclonal antibodies for human therapy especially in the field of prevention of acute rejection of transplanted tissues and organs and treatment of cancer tumours. With the successful import of genetic engineering into human Mabs development, their uses in diagnostic imaging and medicine will further be enhanced.

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DOWN SYNDROME

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INTRODUCTION

The first adequate description of this condition , which was called mongolism at first , was given by **Langdon Down** in **1866** , while he was classifying some mental defectives on an ethic basis .

Because of certain facial features, somewhat like those of Orientals, they became known as "mongoloid idiots or imbeciles" or "mongols". However, since the resemblance of these patients to normal oriental persons is very superficial with no aetiologoical significance as originally suggested by Dr Down and because Orientals may be offended by the association, the term **Down syndrome** has been more widely used till date.

Down syndrome was the first condition found to be caused by **autosomal trisomy**. In 1959, **Jerome Leguene et al** found that cells from patients with Down syndrome contained 47 chromosomes and that an extra small acrocentric chromosome was present[1]. They designated this chromosomal abnormality **trisomy 21** although it was difficult to differentiate the two pairs of chromosomes involved by techniques available at that time .This abnormality was said to be probably due to maternal primary non- disjunction of chromosome 21 in the majority of cases.

CLINICAL FEATURES

Dr Langdon Down specifically and accurately described the features associated with trisomy 21. These have been summarized in Table 1.

RISK FACTORS

Perhaps the best known epidemiologic fact about Down syndrome is its association with **increasing maternal age**. Below is the statistics quoted for maternal age as a risk factor for the occurrence of Down syndrome.

The basis for the increasing risk of an offspring with trisomy 21 as maternal age increases is not known. However , some explanations have been proposed They include:

- The fact that at birth , the ovaries of a female child contain all the germ cells they will eveproduce (approx. 2 million oocytes at 40weeks Gestation) Each germ cell remains "suspended " in the **prophase of the first meiotic division** until the specific oocyte is ovulated .Thus an oocyte , when ovulated , is as old as the woman whose ovary produced it .The old oocyte may be especially susceptible to insults from exposure to diagnostic x-rays , viral infections and autoimmune disorders, which have been suggested to have an association with the incidence of offspring with trisomy 21.

- It has been suggested that intercourse becomes less frequent between ovulation and thus a prolongation of the interval between ovulation and fertilization is associated with prolonged meiosis and "overripeness" of the ovum. The overripeness may predispose to nondisjunction.

- There are also evidences suggesting genetic predisposition to nondisjunction in some families.

- It has been suggested that nondisjunction is not more frequent in older women but that a trisomic fetus is more likely to be live-born in an older mother than in a younger one.[2]

Although results have not been consistent, critical analysis of available data reveals no **convincing correlation** between **advancing paternal age** and **incidence of Down syndrome**.

GENETICS

In majority of cases, Down syndrome occurs when there is an extra small acrocentric G group chromosome in the 21st pair of chromosome following maternal primary nondisjunction of chromosome 21 and the abnormal oocyte undergoes fertilization . However, Down syndrome can also occur secondary to **translocation** in which case the long arm of the extra chromosome 21 is attached to another chromosome, most commonly the long arm of a D group or another G group chromosome e.g chromosome 14. This abnormality can be present at conception or can take place as a mutation during cell growth .When chromosomal translocation involving virtually the entire arms of two acrocentric chromosomes occurs, this is termed **Robertsonian Translocation**. This can also result in trisomy 21. In patients with 21-D translocation Down syndrome, the karyotype includes 46 chromosomes instead of 47 chromosomes as in the case of maternal primary nondysjunction. This state is called **mosaicism.** Out of the 23 pairs of chromosomes, the 21st pair of chromosomes are the smallest, and contain only about 250 or less genes. Out of these, some 2 to 4 dozen genes are such that if they are over expressed due to triplication, they may produce the abnormalities of Down Syndrome. These genes are not located in one place but scattered, and not all of them have been identified yet.

Due to trisomy 21, some genetic information gets over-expressed, contributing to the various developmental abnormalities associated with Down syndrome.

Facial	Upward sla nt to the eyes; marked epicanthic folds;microcephaly[head is noticeably flatter at the back] Short neck Small ears Large tongue[macroglossia] with distinctive deep furrows on the surface
Eyes	Cataract: squint: nystagmus[not always present] Brushfield spots[white flecks on the iris that do not disturb vision] N.B:Brushfield spots are present in the majority of Down Syndrome patients and are a helpful diagnostic feature
Limbs	Limbs are relatively short but bodily proportions are normal: Fingers are short a nd stubby with in -turning of the little finger in many patients: Single palmar crease[not pathognomonic]: Great toes are widely separated from the other toes[this is a specific characteristic, most obvious when looking at the soles of the feet
Muscle tone	Muscle tone is reduced, making the babies floppy
Developmental milestones and cognitive functions	There is a delay that becomes more obvious as the child matures: Mental retardation
Personality	Most patients are affectionate, happy young people who are a delight to have around. They have an inherent sense of fun which can add much to family life
Cardiovascular system	The most common anomalies are; Atrial septal defect: Ventricular septal defect: Patent ductus arteriosus
Respiratory system	Upper respiratory tract infections are common throughout infancy and childhood[due in part to the smallness of air passages and an impaired immune system]
Ears	Conductive deafness can occur as a complication of frequent ear infections
Haematological system	Acute lym phatic leukemia[ALL]has a higher incidence in Down Syndrome children
Reproduction	It is believed that all men with Down Syndrome are fertile, and the women have low fertility
Nervous system	Alzheimers disease can also occur in later life, adding to the difficulties of caring for older people with Down Syndrome

Table 1: Clinical features of Down syndrome

The NO (Nitrous oxide) gene has been definitely linked to Down syndrome, though the ones given below are strong contenders. Even within the same chromosome, the alleles may differ in their placement and expression from one person to another .Hence Down syndrome presents in a wide variety of features and marked inter-individual variation. In general ,once a couple has had an infant with Down syndrome, the first risk that they will have a second affected offspring is approximately 1%[1].However , the risk in a given case cannot be accurately estimated without chromosomal analysis since as stated above ,Down syndrome can also occur secondary to chromosomal translocation. In about **25%** of **translocation Down syndrome** individuals, the translocation is **inherited**.The carriers of this trait are usually unaware because there are no problems for the **balanced** translocation carrier. Only with the birth of a Down syndrome child or a Down syndrome fetus by miscarriage, does the couple find out one parent is a translocation carrier. However, a carrier parent can also have a chromosomally normal child or a child who is a balanced carrier like the parent.Curiously, the risk depends on which parent is the carrier .When the mother is a balanced of a t(14;21),there is about a 10% risk for another Down syndrome child to be born in each subsequent pregnancy. When the father is the carrier, the observed drop to about 3%.The reason for this difference in risks is not clear.

In about **75%** of translocation Down syndrome neither parent is a carrier, and a **mutation** in the germ cells of one parent has caused the translocation. The aetiology of the mutations is not known. In cases of **de novo** (new) **Robertsonian translocation**, the risk to a couple producing a second Down syndrome child is estimated to **2-3%.** There are rare instances of reoccurrence on record, and prenatal testing for subsequent pregnancies should be considered.

Translocation Down Syndrome occurs with about equal frequency in younger and older women, unlike the case in maternal nondisjunction Down syndrome.

There are other rare translocations leading to Down Syndrome .One is a Robertsonian translocation between two chromosomes 21, t(21;21) ; this has a 100% risk for Down Syndrome when transmitted by a carrier parent .Also rare is a non-Robertsonian translocation formed by the union of two chromosomes21 such that the translocation forms a mirror image of the normal 21.

DIAGNOSIS

The diagnosis of Down syndrome can **almost always** be made **clinically** .Chromosomal analysis is used for laboratory confirmation and more importantly, to identify the unusual mosaic or translocation karyotype that might indicate heritable Down syndrome.

Prenatal diagnosis would involve chromosome analysis of fetal tissue obtained via **amniocentesis** or **chorionic villus sampling (CVS).**

A study on Amniocentesis utilization and incidence reduction of Down syndrome in 5-county metropolitan Atlanta carried out between 1974 and 1986 by S.Karam, C.Huether, J.Priest and L.Edmonds of the University of Cincinnati, Emory University and centre for Disease control ,Atlanta ,Georgia, revealed that amniocentesis had a significantly greater effect than expected in reducing births with Down Syndrome.[1]

Amniocentesis, a sampling of the **amniotic fluid** surrounding the fetus , is routinely done at **14-16wks** of gestation. Amniocentesis testing for chromosomes disorders is **99.8%** reliable for chromosomes number and there is a risk of miscarriage **(0.004 or less)** after the procedure .

Chorionic villus sampling (CVS) can be done at 10-11 wks of gestation. Earlier testing is thought to be associated with a small risk of fetal limb. The CVS test is done earlier and is usually more rapid than amniocentesis. There is a small chance of maternal cell contamination and a **0.01** risk of miscarriage after the procedure.

Prenatal testing also involves the measurement of **maternal serum alpha fetoprotein** (MSAFP) by a blood test in the pregnant woman at **15-18 weeks** of gestation. AFP is produced in the fetal liver, and some escape into the maternal circulation. It is widely used as a screening test for a Down Syndrome fetus.MSAFP testing is based on the fact that Down Syndrome fetuses tend to be a little smaller than average, have smaller placentas, and secrete less MSAFP and other materials, which are determined in the serum of the pregnant women. Factors that affect this test include gestational weight, maternal age, diabetes and ethnicity.

Although MSAFP screening is not a definitive test, a low level suggests the risk of a Down Syndrome fetus equals to the risk of a woman aged 35(see above),and prenatal chromosome studies are suggested if the parents want this information. If MSAFP alone is tested **20%** of Down Syndrome fetuses will have low levels if MSAFP and **human Chorionic Gonadotrophin (hCG)** are determined, **50-60%** of Down Syndrome fetuses will be identified. If MSAFP, hCG and **estradiol (E2)** are tested, **60-70%** of Down Syndrome will be identified.

Some Ultrasonographers can determine suggestive findings of Down Syndrome by changes in the neck, or heart .This is not yet confirmed or in wide spread use. **Ultrasounds** can be **normal** in a Down Syndrome fetus.

The advantages/disadvantages of Prenatal diagnosis is still a subject of debate, and should be discussed with a geneticist or obstetrician experienced with the procedures.

MATERNAL AGE (IN YEARS)	Frequency of live Births of babies with Down Syndrome to normal births
15-19	1/1250
20-24	1/1400
25-29	1/1100
30-31	1/900
32	1/750
33	1/625
34	1/500
35	1/350
36	1/275
37	1/225
38	1/175
39	1/140
40	1/100
41	1/85
42	1/65
43	1/50
44	1/40
45 and older	1/25

Table 2: Maternal age as a risk factor for the occurence of down syndrome[2]

The suspected defects	Their possible culprits
Premature ageing Immune depression Senile Dementia, Alzheimers type Decreased cognitive function	Super Oxide dismutase(SOD1)
Congenital Heart defects	COL6A1
Congenital Skeletal abnormalities Susceptibility to develop leukemia	ETS2
Problems with DNA synthesis	CAF1A
Metabolic disturbance and defective repairs to DNA	Cystathione beta synthase(CBS)
Mild to moderate mental retardation	DYRK
Susceptibility to develop cataract	CRYA1
Abnormalities o f immune mechanisms including interferon, as well as other organ systems	IFNAR
Additional lists of suspects whose roles need further study	APP,GLUR5,S100B,TAM,PFKL Some others not yet identified.

Table 3.Suspected defects and the gene products that are possible culprits[2]

MANGEMENT STRATEGIES

Occupational Therapy

Occupational Therapy is one of the mainstays of managing a child with Down Syndrome .It helps to develop;

- Activities of daily living (ADLTraining), Feeding , dressing , grooming, going to the toilet ,etc
- Maintaining and improving fine and gross motor skills
- Rehabilitation therapy depending on physical and intellectual abilities and trainable skills
- Psychosocial adjustment through games and interactive projects, games, plays and other activities.

During infancy the child may have hypotonia and weakness of facial muscles , making it difficult to feed the child .Here , an occupational therapy is needed to suggest feeding techniques ,suitably modified taking into account the disabilities of the child.

Other management strategies will include Surgical corrections of congenital heart defects, and other problems e.g. cataract when necessary.

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CLINICAL UTILITY OF GASTROINTESTINAL ENDOSCOPY IN MODERN MEDICINE

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The quest and curiosity to view the milieu interieure has been a major drive for various advances and discoveries in clinical medicine. Physicians of old started with postmortem examinations and subsequently ante mortem dissection of the human body. Thus theories such as the Gallenic theory of circulation and the miasma theory of infection were dumped into the waste heap of science with the advent of Harvey's description of the circulation of the blood and the bacterial theory of infection, the former by dissection in human anatomy and subsequently the hitch of angiography.

Endoscopy which essentially means the "viewing of the inside" has contributed immensely to antemortem diagnosis of disease as well as minimally invasive and less expensive therapeutic interventions.

Today, various disciplines in clinical Medicine have embraced the use of endoscopy because of its relatively safe and minimally invasive nature both in diagnosis of disease (diagnostic endoscopy) and in treatment (therapeutic or interventional).

The major disciplines utilizing this novel approach include otorhinolaryngology, anaesthesia, cardiothoracic surgery, urology, orthopaedics, general surgery, respirology, cardiology, neurosurgery, gynaecology and gastroenterology among others.

The focus of this write up will, however, be limited to gastrointestinal endoscopy.

Gastrointestinal endoscopy started with the rigid endoscopes, which have limited utility, and difficult for the patient and the endoscopist because of the lack of flexibility of the endoscope and the tortuous anatomy of the bowel, as such only a few procedures such as oesophagoscopy and proctosigmoidoscopy were feasible.

Advances in technology especially in Japan has led to the development and perfection of flexible fibreoptic endoscopes with unlimited ability in trained hands to view the entire gastrointestinal tract as well as the pancreatic and biliary ducts.

The practice of gastroenterology has witnessed landmark advances in the recent past especially with the advent of CT-colonography, MR-colonography (virtual colonoscopy) endoscopic ultrasonography and wireless video capsule endoscopy to mention a few. All the aforementioned are still tall dreams to the gastroenterologist practicing in Nigeria. Flexible and manoeuverable endoscopes have been available since the 1960s and have replaced the rigid of several decades ago and the semi-flexible scopes of the 1930s and 40s.

The immense usefulness of gastrointestinal endoscopy is in no doubt in the diagnosis and therapy of disorders of the long and tortuous gut and its accessories. It is now an established fact, with or without videoendoscopy, that gastroenterology is the leading discipline in Medicine that provides the most vivid visual assessment of human anatomy.

The advantage of gastrointestinal endoscopy over the conventional barium meal/follow through and enema cannot be overemphasized especially with the advent of enteroscopes, which can view the entire small intestine up to the caecum. In addition to this, possibility of biopsies, photography, therapeutic interventions obviating the need for surgery and general anaesthesia as well as sending of images for exchange of information and ideas via the internet, make the procedure very enticing. In the area of training, endoscopic bionic simulators are now available for trainee endoscopists before being allowed hands-on experience. Some of the areas of utility are in:

OESOPHAGEAL DISORDERS

Carcinoma

Recent reports suggest an increasing trend in the incidence of oesophageal carcinoma[1,2]. Clinical symptoms are mainly dysphagia, odynophagia, chest pain, ptyalism, anaemia and weight loss, especially in a patient with significant alcohol and tobacco use.

Fever and chest signs may be seen with aspiration or fistulation. Availability of gastrointestinal endoscopy is becoming increasingly available in developing countries and there is a need to take advantage of this relatively safe and cheaper mode of diagnosis and treatment.

Fibreoptic oesophagoscopy affords visual evaluation, biopsy and brushing for histological characterization and exfoliative cytology, which are very helpful in diagnosis. Barium swallow is often diagnostic with the characteristic filling defect and rat-tail deformity, but it lacks the advantage histological characterization afforded by endoscopy.

A novel approach to early diagnosis is chromoendoscopy with in vivo staining of oesophageal tissue using iodine. Confirmation by histology, which yields diagnosis in over 90% of cases is however still required after chromoendoscopy.

Endoscopic ultrasonography(EUS) or endosonography using a fibreoptic endoscope equipped with an ultrasound transducer at the tip is very useful for delineating local spread of the disease. This effectively stages the disease and is useful in formulating management strategy. Palliative measures such as endoscopic placement of stents, percutaneous endoscopic gastrostomy (PEG procedure) or jejunostomy (PEJ),with feeding tube to ensure

adequate nutrition, have all been made possible by endoscopic techniques[4]. Other palliative measures include oncolytic therapy using laser beams or injections of ethanol via endoscopy. Dilation and endoscopic mucosal resection (EMR) are useful in relieving dysphagia and odynophagia and is replacing traditional oesophagectomy in many cases. The technique of EMR involves injecting 5 to 20 mL of saline beneath the affected mucosa followed by resection.

Endoscopic thermal tumor ablation with neodymium:yttrium-lithium-fluoride laser ablation or photodynamic therapy and placement of self-expanding mesh stents are other therapeutic modes applied to oesophageal carcinoma.

Gastroesophageal Reflux Disease (GORD)

It is now possible to implant some biocompatible material at the lower oesophageal sphincter (LES) to prevent reflux of gastric content into the oesophagus. Endoscopic suturing device has also been used to tighten the esophagogastric junction in an attempt to reduce reflux. The Stretta[R] device, which uses radiofrequency energy to create thermal injuries to the esophageal wall at the esophagogastric junction, could be used to induce cicatrization of the injured area, leading to decreased transient relaxation of the LES.

Varices

Endoscopic variceal banding ligation and injection of sclerosants or vasoconstrictors have been in use for a long time in contolling upper gastrointestinal haemorrhage from varices arising from various causes but principally from portal hypertensive complication of liver cirrhosis.

Achalasia

Achalasia of the cardia is often associated with distressful dysphagia, regurgitation and respiratory symptoms. Endoscopic balloon dilatation, which leads to guided rupture of the hypertensive LES, restores normal swallowing and oesophagogastric transit of ingested material.

Barrett's Oesophagus

This is a premalignant disease in which bile reflux plays an important role and usually occurs in the presence of acid. Newer endoscopic technology and techniques, including chromoendoscopy and magnification endoscopy have opened the field by providing a more accurate and "nonbiopsy" diagnosis of intestinal metaplasia and dysplasia. More novel endoscopic therapies are being developed and appear promising.

Photodynamic therapy is also being used increasingly as a method of ablating dysplastic Barrett's tissue.

Mallory-Weiss Tear

Endoscopic clips have been shown to safely and reliably control bleeding from post-emetic oesophageal laceration.

Diffuse Oesophageal Spasm

Endoscopically injected Botulinum toxin at multiple sites in the esophagus has been shown to reduce pain from diffuse esophageal spasm for several months in most patients.

Zenker Diverticulum

Placing a transparent, oblique-end hood can help simplify the endoscopic incision of the septum of a Zenker diverticulum.

Stricture

Patency could easily be restored in strictures that arise. Fibreoptic gastroscopy and histology of biopsy specimens affords the best chance for early diagnosis. Doubtful reports from Barium studies could readily be debunked or confirmed within minutes, thus saving the patient unnecessary laparotomy and the attendant anaesthetic risks in negative cases.

Other diagnostic uses of gastroscopy include gastric aspirate cytology.

GASTRIC ULCER

Source of bleeding as well as the various vascular anomalies such as Dieulafoy lesion, watermelon stomach, vascular ectasia, portal hypertensive or congestive gastropathy, cardiac varices etc could readily be confirmed.

Chromoendoscopy in combination with mounted endoscopic microscope now affords in vivo histological staining and characterization of tissue, whether malignant or benign. Thus guiding site of biopsy if necessary, and obviating the need for sending specimens to the pathologist!

Duodenal ulcer can also be diagnosed and bleeding arrested in a similar way to what obtains in gastric ulcer management.

FOREIGN BODIES

Various foreign bodies(FB)such as accidentally ingested knife, spoon and bezoars, could safely be removed without causing mucosal damage. Dangerous FBs such as razors or knives could be sheathed before removal.

POLYPS

The use of snares to trap and resect polyps with haemostatic control is one the bread and butter stuff of interventional endoscopy.

OBESITY

Laparosopically placed devices to reduce the effective capacity of the stomach is being used successfully in morbid obesity when other measures have failed.

SMALL BOWEL

Malignancy of the duodenum, ileum and jejunum are very uncommon both in Africa and in the Western

world. However occasionally primary intestinal lymphoma may be seen presenting with features of malabsorption. Small bowel carcinoma or lymphoma may also complicate celiac or Crohn's disease in regions of the world where they are found. Diagnosis requires high index of suspicion. Small bowel enema or enteroclysis used to be the only mode of diagnosis until the advent of push enteroscopy, which can be used to endoscopically visualize the entire length of the small intestine including the ceacal pole.

A new technology in endoscopy called wireless capsule endoscopy was recently developed and has been found to be very useful in the diagnosis of small bowel lesion as it affords a better mucosa detail.

This entails the swallowing of a pill-sized (capsule) device with mounted camera, which takes images as it transits the entire bowel. The captured images are recorded with a transducer worn as a belt by the patient. The capsule is subsequently expelled in the feaces and the recorded images downloaded into a computer for analysis. A major setback of capsule endoscopy is impaction of the capsule leading to intestinal obstruction[7].

COLON

Colorectal carcinoma

This is the commonest GIT tumour in Western societies and is second only to cancer of the lung as a cause of cancer death in the United States of America. It is relatively uncommon in Africa ostensibly due to high content of dietary fibre and lower incidence of colonic polyposis. In Africa, colonic cancer is commoner in the higher socioeconomic groups, who have taken more to Western diet rather than the high fibre native diet. Because it is rather a slowly growing tumour, it tends to present late in Africans where family history of disease is rarely obtainable and no organised screening procedure for people above the age of 50 years as it is done in Western society. This situation is not helped by the fact that rectal bleeding is often attributed to "pile"(locally referred to in Western Nigeria as jedi-jedi), which has a panoply of traditional remedies.

Endoscopic diagnostic procedures available include Fibreoptic videocolonoscopy or proctosigmoidoscopy with multiple biopsies and are the procedures of choice. With colonoscopy the large bowel could be viewed from the anus to the ceacal pole and to a few centimeters into the terminal ileum. Digital images could be captured and printed along the line.

Virtual colonoscopy with computed tomography (CT-colonography) or Magnetic resonance (MRI-colonography), performed by radiologists are now being frequently used in developed countries. CT- or MRI-colonography are particularly useful when there is bowel occlusion or in incomplete conventional colonoscopy. Cost, exposure to radiation (for CT) and inability to take tissue biopsies are drawbacks.

Polyps

Endoscopic diagnosis of polyps and surveillance for colorectal carcinoma in familial polyposis syndrome are essential in areas of the world where colorectal carcinoma are rampant. Endoscopic polypectomy using a snare and a coagulating probe or diathermy is an easier way of polyp removal compared with traditional polypectomy carried out during laparotomy. It is cheaper, less risky and carried out as an outpatient day-case procedure.

Vascular anomalies

Ablation of vascular malformations such as vascular ectasia, angiomas, varices and tumoral bleeding source could be done endoscopically with heater-probe diathermy or argon-plasma coagulator (APC)

PANCREAS

Pancreatic carcinoma

Endosonography has become very useful in staging and biopsying pancreatic tumours for histological diagnosis. Endoscopic retrograde cholangiopancreatography (ERCP) is more or less a routine procedure in cases of obstructive jaundice due to stones, stricture or tumours in the pancreatobiliary tree. Various baskets could be used to trap stones and placement of stents for biliary drainage has been so much perfected to the extent that gastroenterologists generally now believe that no patient with pancreatic tumour with biliary obstruction should die jaundiced! Choledochoscopes are also now available for viewing the biliary pathways and removing stones and dilating biliary strictures.

BIOPSY PROCEDURES

Liver and peritoneal biopsies are possible via peritoneoscopy or laparoscopy.

It is quite obvious that the clinical utility of gastrointestinal endoscopy is advancing and will continue to play a major role in saving patients from the knife of the general surgeons. Laparoscopic surgery is also now an expanding field with wide applications in surgical practice. Gastrointestinal endoscopy has come to stay in the forefront in the practice of gastroenterology, hepatology and pancreatology. We cannot afford to be left out in Nigeria if we are to engage current approaches in the management of gastrointestinal maladies[8].

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ASSESSING THE EFFECTIVENESS OF DIRECTLY OBSERVED THERAPY SHORT COURSE (DOTS) IN THE TREATMENT OF TUBERCULOSIS IN IGBO-ORA COMMUNITY

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Keywords: *Cure-rates, Default-rates, DOTS, Mortality-rates.Tuberculosis*

BACKGROUND

Tuberculosis is a highly contagious disease that is of global significance and is caused by *Mycobacterium tuberculosis*- a fastidious acid-fast bacillus. Like the common cold, it spreads through the air when an infected individual sneezes, coughs, talks or spits. A person need only inhale a small number of the bacilli to become infected. Left untreated, each person with active tuberculosis will infect an average of 10-15 people every year although, not all people infected with tuberculosis will come down with active disease. When a person does develop active disease, it may affect any body organ or system. Pulmonary tuberculosis is the commonest presenting form of tuberculosis occurring alone or with other forms of the disease in 70% of cases[1].

Tuberculosis kills approximately 2 million people and someone in the world is newly infected with it every second. The global epidemic is growing and becoming more dangerous as evidenced by the following statistics: Nearly 1% of the world's population is newly inffected every year. Overall one third of the world's population is currently infected; 5%-10% of people infected with tuberculosis become sick or infectious at sometime in their life.

In Eastern-Europe and Africa, deaths are increasing after almost 40years of decline. In terms of number of cases, the biggest burden of tuberculosis is in South-East Asia. Nigeria has the fifth highest tuberculosis burden in the world and the number of notified cases is on the rise[3].

In recent years, the breakdown in health services, the spread of HIV/AIDS, worsening economic conditions, increasing numbers of displaced persons and the emergence of resistant strains are contributing to the increasing impact of this disease. Indeed about two million cases occur in Sub-Saharan Africa with these numbers rising rapidly as a result of the HIV/AIDS pandemic[4]. HIV and tuberculosis form a lethal combination, each speeding the progress of the other. Someone who is HIVpositive and infected with tuberculosis is many times more likely to become sick withtuberculosis than someone infected with tuberculosis who is HIV negative. Tuberculosis is the leading cause of death among HIV positive persons accounting for 11% of AIDS-related deaths.

In Africa, HIV is the single most important factor determining the increased incidence of tuberculosis in the past 10 years[5].

In trying to combat this global problem, the World Health Organization (WHO) introduced a treatment strategy on a global scale in 1991. This WHO recommended strategy for detection and cure of tuberculosis is the Directly Observed Therapy Short-course (DOTS). DOTS combine five elements :

- Political commitment,
- Microscopy services,
- Drug services,
- Surveillance & monitoring services,
- Use of highly efficacious regimes with directly observed treatment[6].

Once patients with infectious tuberculosis that is bacilli visible on a sputum smear have been identified using microscopy services, health & community workers and trained volunteers observe patients swallowing the full course of the correct dosage of anti-tuberculosis medicines. Treatment lasts 6-8months. The most commonly used anti-tuberculosis drugs are Isoniazid, Rifampicin, Pyrazinamide, Streptomycin and Ethambutol. Sputum smear testing is repeated after two months to monitor progress. It is done again at five months and finally at the end of treatment. A recording system documents patient's progress throughout and also notes the final outcome[7].

By the end of year 2000, all twenty-two of the highest burden countries which bear 80% of the world's estimated incident cases had adopted DOTS. 55% percent of the global population had access to DOTS, double the fraction reported in 1995. The DOTS system has been adopted in Nigeria across the various levels of health care delivery with the establishment of special tuberculosis clinics. One of such clinics is located in Igbo-Ora oyo state Nigeria. The presence of International donor agencies such as Damien and the German anti-tuberculosis & Leprosy Foundations have helped greatly in funding this treatment modality[8].

PROBLEM STATEMENT

The DOTS system was introduced on a global scale in 1991 because the WHO was concerned about the poor compliance with the standard

treatment regimens leading to development of multidrug-resistant strains. This led to higher costs in treating these strains. DOTS was supposed to solve this problem as well as provide a relatively cheaper and readily accessible treatment strategy. This would lead to increased compliance rates with concomitant decrease in spread and in more effective control.

JUSTIFICATION

This study is necessary because it is the first of its kind in this environment. Similar studies have been carried out in other parts of the world like China and Peru as well as in early places like Ilorin and Ife by local researches. No study has been carried out in Igbo-Ora to compare the effectiveness and usefulness of the DOTS system in the treatment of tuberculosis since it was introduced in the early 1990s.

OBJECTIVES

The general objective was to assess the effectiveness of the DOTS system in the treatment of tuberculosis in Igbo-Ora.

Specific objectives include:

- To identify the sociodemographic characteristics of patients who presented in the Pre-DOTS and DOTS periods
- To assess and compare Pre-DOTS and DOTS patients with regards to treatment failure rate, relapse rate, cure rate and default rate
- To determine and compare mortality rates in the two periods.

This study was carried out to assess the effectiveness of DOTS in the management of patients with tuberculosis over a ten year period. The study compared the cure rate, default rate, relapse rate, treatment completion and failure and fatality rates of the pre- DOTS era (1983-1993) with DOTS era (1993 – 2003)

METHOD

This was a clinic-based retrospective comparative study done in Igbo-Ora. The target populations were patients treated for tuberculosis between 1983 and 1993 before inception of DOTS and those who were treated between 1993 – 2003 under the DOTS system.

A total of 633 patients treated during these periods were studied and the treatment outcome; treatment completed, cured, default, treatment-failure, relapse and mortality in the two periods were analyzed and compared.

RESULTS

The mean age prevalence was 32.9yrs (SD±14.3) and 31.7yrs (SD±20.8) for DOTS and pre-DOTS respectively. Pulmonary tuberculosis accounted for 96% of all cases in the area.

Out of the patients treated, 412 (65.1%) were for DOTS and 221 (34.9%) were treated prior to DOTS.

Using DOTS regime cure rate was 289 (70.1%) compared with 24(10.9%) for pre-DOTS (X^2 = 202.256;df = 1; P<0.0001). Treatment completion for DOTS was 361 (87.6%) compared with 38 (17.2%) for pre-DOTS (X^2 = 306.18; df=1; P< 0.0001). Those that defaulted for DOTS were 27 (6.6%) compared with 176 (79.6%) for pre-DOTS (X^2 = 352.68; df 1; P< 0.0001). Mortality was however observed to be higher in DOTS with 26(6.3%) compared with 4(1.80%) for pre-DOTS. The relapse and treatment failure relates were not significant.

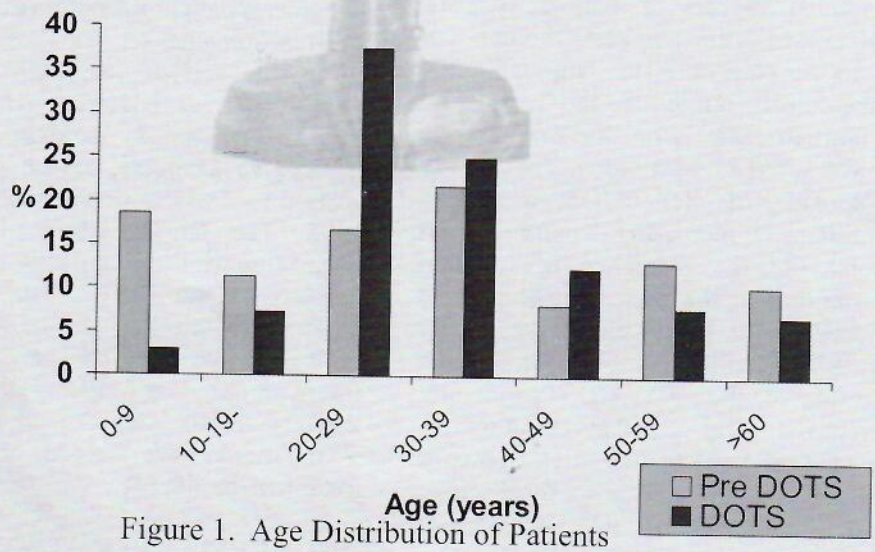

Figure 1. Age Distribution of Patients

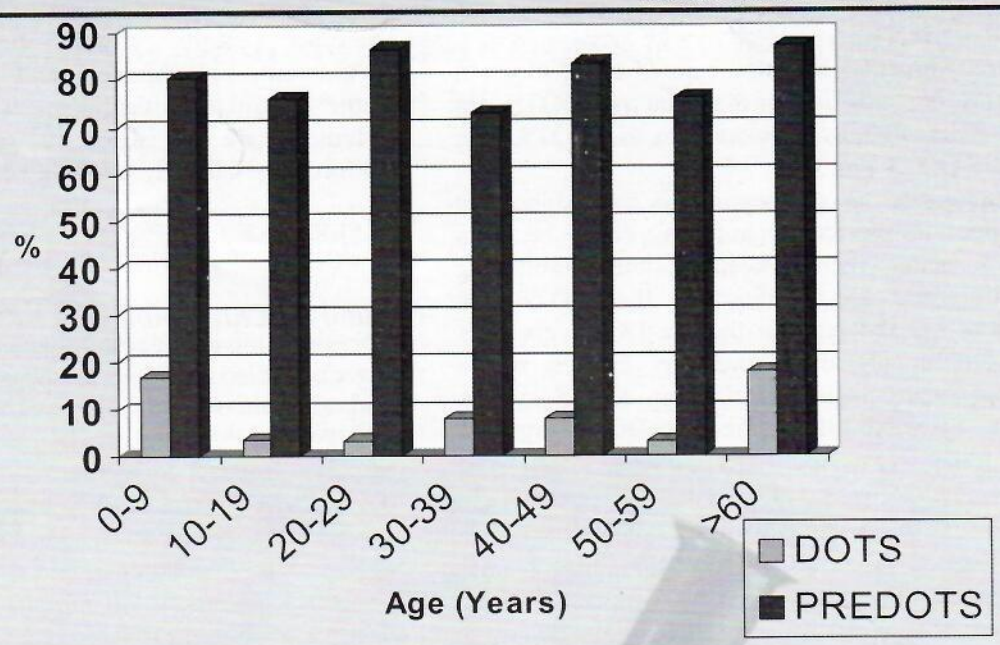

Figure 2. Default Patterns Among Age Groups In DOTS & Pre-DOTS Period

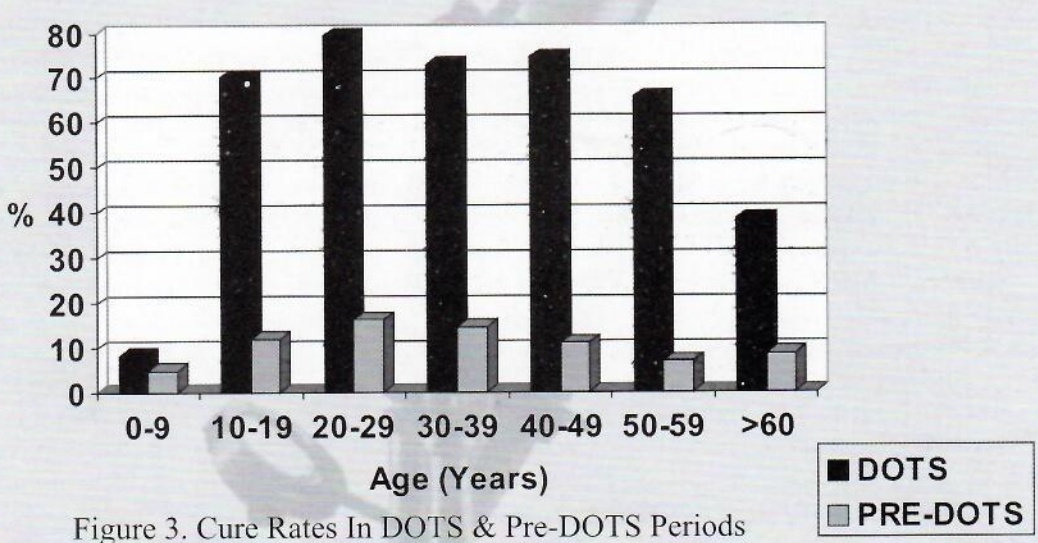

Figure 3. Cure Rates In DOTS & Pre-DOTS Periods

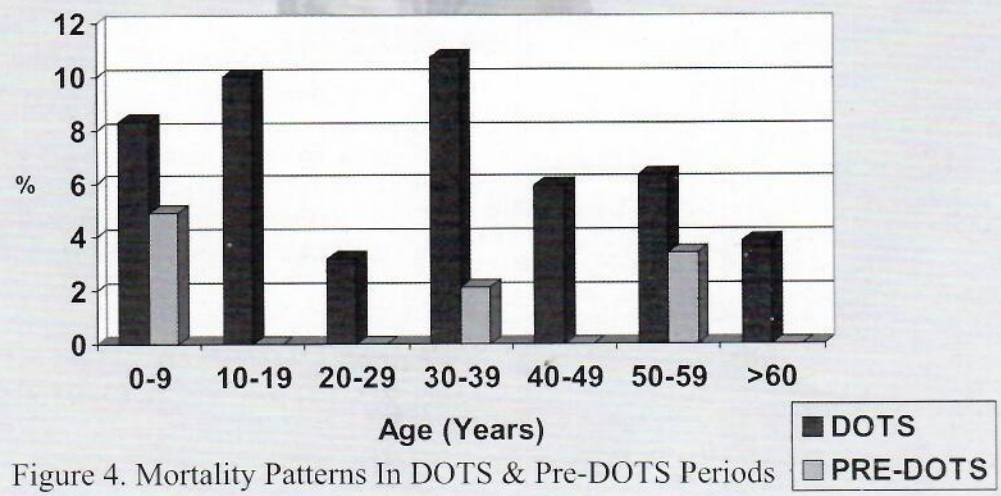

Figure 4. Mortality Patterns In DOTS & Pre-DOTS Periods

CONCLUSION

The cure/treatment completion rate of tuberculosis in the DOTS era was higher than the pre-DOTS era while the default rate was lower in the DOTS than in the pre-DOTS era.

Mortality-rate was observed to be higher in DOTS than in pre-DOTS and this could be as a result of better record keeping and monitoring system through case holding in the DOTS era which was not the case in the pre-DOTS era. This study compliments other previous studies which show that tuberculosis control using DOTS system is more effective than the pre-DOTS system. Therefore efforts should be made by the government, policymakers and other stake holders in the control of tuberculosis to implement DOTS system where it is not in operation and also reinforce the already existing ones.

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Editor-in-Chief's note

The research work was nominated as the best student project carried out by a sub-group of the 2006 graduating class of the College of Medicine, University of Ibadan.

BIOMEDICAL RESEARCH METHODOLOGIES IN THE 21ST CENTURY

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WHAT IS SCIENCE?

Science is a social institution about which there is a great deal of misunderstanding, even among those who are part of it. It is an institution, a set of methods and a set of people- a great body of knowledge that we call scientific. It constitutes part of the forces that rule our everyday lives and governs the structure of our society.

Science is about objectivity. Science has brought numerous benefits. It has tremendously increased the production of food and our life expectancy. It has put people on the moon and made it possible to sit in the comfort of our homes and watch unfolding events in the world.

FUNCTIONS OF SCIENCE

Science serves two major functions. First, it provides us with new ways of manipulating the material world by producing a set of techniques, practices and inventions by which new things are produced and by which the quality of our lives are changed.

Secondly, science explains things even when scientists are not actually changing the material mode of our existence, they are consistently explaining why things are the way they are. For example we can only think of curing cancer if we understand what causes cancer. We can only increase food production if we understand the laws of genetics and plant e.g. animal nutrition. Gilbert and Watson said that an understanding of the workings of the DNA code is the path by which complete human health will be reached.

MOLECULAR BIOLOGY AND GENETIC ENGINEERING

The advent of molecular biology and genetic engineering in the past few decades has resulted in the discovery and application of many techniques and products that have revolutionized medical research. The technology has also recently entered the industry in several areas.

Genetic engineering covers a broad area including medicine, pharmaceutical industries, brewing industries etc. It is therefore highly beneficial to mankind. It involves the exploration and manipulation of the genetic materials of cells

APPLICATIONS OF MOLECULAR BIOLOGY GENE PROBES

A gene probe is a DNA sequence which can detect its complementary DNA sequence.It is a powerful tool that can be used to determine the presence or absence of certain genes. Gene probes are used in diagnosis of infectious and non-infectious diseases by the identification of specific strains species of bacteria, parasites or viruses.

Probes can also be developed for the detection of hereditary diseases. Commercial gene probe kits are available in many parts of the developed world.

GENE THERAPY

This is used in the treatment of inherited diseases in the replacement of a defective gene with a copy of the correct gene. This will change the genetic make-up of the individual. Germline gene therapy has to do with manipulations of sperm and ova (transgenic technology) but is currently illegal in humans. Somatic cell gene therapy involves other cells in the body like the muscles, bone and nerves etc. It is aimed at correcting a genetic or non-genetic defect. An example is bone marrow therapy, several cancer treatments including introducing cells engineered to produce more TNF or IL-4 into a cancer patient.

THE POLYMERASE CHAIN REACTION (PCR)

PCR is a specific, sensitive and versatile tool introduced in 1985 by Saiki and used for the amplification of specific DNA sequences.It was the beginning of revolutionized molecular biology. The method is highly sensitive and amplifies genes even when there are only few cells. It is believed that if 1millilitre of PCR product is evenly distributed in a swimming pool, PCR will still be able to detect DNA in one millilitre of the water in the pool.It is very useful in the diagnosis of various kinds of diseases including slow growing or non-cultivatable organisms such as *Mycobacteria*, Rickettsia and leptospira as well as for infectious diseases where a rapid diagnosis is required or where very small amounts of target DNA are present. The problem with PCR is that it detects the DNA of both live and dead microorganisms, therefore inactive or past infections may also be detected. It is also an expensive procedure and requires special skill.

A major advantage of PCR is that it allows for the production of large quantities of a specific region of DNA without recourse to cloning. All that is required for the process is a knowledge of short lengths of the base pair sequence on either side of the region to be amplified.

OUTLINE OF THE PCR PROCEDURE

A small sample of DNA is placed in a tube then two oligonucleotides "primers" are added. These have matching sequences of the DNA that flank the region of

interest. A thermostable DNA polymerase is added e.g. Taq polymerase. The mixture is heated to just below 100°C which causes the DNA to dissociate into 2 single strands. The solution is allowed to cool, and the single strands bind to the oligonucleotides. The oligonuclectides acting as primers for DNA polymerase are extended to form new double stranded molecules. The cycle is repeated with the amount of DNA produced doubling each time.

USES OF PCR

(1) It can be used to detect people at risk of a particular disease e.g. diabetes mellitus, myocardial infarction, hypertension etc.
(2) Antenatal diagnoses of trisomies, haemoglobinopathies and other hereditary diseases can be detected using fetal samples.
(3) Diseases can be diagnosed e.g cancer, malaria, *Helicobacter pylori*, HIV infection, tuberculosis *etc.*
(4) Drug resistant species of certain organisms can be detected e.g. *Plasmodium* or tuberculosis.

LUCIFERASE ASSAY

This is used in the food industry for the detection of microbial contamination and also for clinical research purposes. The enzyme is found in the tail of fireflies and in the presence of ATP, visible light is released. This technique is used both for quantitative and qualitative assays and is also used to determine antibiotic susceptibility in bacteria. The gene coding for luciferase (Lux gene) is introduced into the bacteria before adding the antibiotic of interest. Light emission is then measured after incubation with a photo multiplier. If light is emitted, then the bacteria are sensitive to the antibiotic.

GENETICALLY MODIFIED ANIMALS

A significant breakthrough in medicine is the advent of gene cloning. Animals with genetically modified organs expresses human genes that can be used for transplantation in humans without severe immune reactions leading to rejection. Animals have also been genetically modified to produce certain human proteins e.g insulin, factor IX etc.

RESEARCH METHODS IN MOLECULAR MEDICINE

-Gene Expression.
-Cloning Gene Sequences.
-Nucleic Acid Hybridisation.
-Gene structure analysis.
-Southern blotting.
-Northern blotting.
-Western blotting.
-Polymerase Chain Reaction.

GENE EXPRESSION

Steps involved in the expression of eukaryotic protein coding genes.

A copy of the DNA is first copied (transcribed) into single stranded RNA by RNA polymerase II. The primary RNA transcript is then converted to mRNA which is further translated into proteins.

CLONING GENE SEQUENCES

To clone a single gene sequence from the enormous amount of genomic DNA in each human cell, the DNA is first cut into smaller fragments using restriction enzymes. The fragments are then isolated from each other and produced in large quantities by means of DNA cloning. This is achieved by a process known as ligation, or joining each of the genomic DNA fragments to a vector DNA molecule.

The most commonly used vector is the bacterial plasmid which is a naturally occurring circular DNA molecule that is found in most strains of *Escherichia coli.* The recombinant DNA molecules produced by ligation are put into *E. coli* cells by a process of transformation such that each transformed cell will contain only one recombinant DNA molecule. This will be replicated to a high copy number.

When grown on an agar plate, each transformed cell gives rise to a bacterial colony containing many copies of a single cloned human genomic DNA fragment. If this collection of cloned fragments is sufficiently large, the bacterial colonies will contain many fragments of the genome. Such a collection of cloned fragments is called genomic library.

NUCLEIC ACID HYBRIDISATION

Many analytical techniques depend on nucleic acid hybridization. Double stranded DNA is denatured by heating or treatment with an alkali. If the solution is cooled or the pH is lowered, a double stranded DNA will be formed through the process called annealing. As long as the sequences are complementary, hybrids of DNA-DNA, DNA-RNA or RNA-RNA can be formed.

Thus if a single stranded nucleic acid molecule is labeled with radioactive or non-radioactive tracer, it can be used as a probe to search for complementary nucleic acid strands to which it will hybridize. If the probe corresponds to the sequence of a gene, then it can be used to identify that particular gene or the mRNA transcribed from it in whole preparations of DNA or RNA isolated from cells and tissues.

SOUTHERN BLOTTING

The structure of a gene can be analysed by this technique. Genomic DNA is digested with restriction enzymes yielding a large number of DNA fragments which can be separated according to size by agarose gel electrophoresis. To hybridise to a gene probe, the DNA fragments are transferred from the gel to a nylon membrane which is then incubated with a single stranded labeled probe for the gene of interest under favourable conditions. Excess probe is washed off and the position of the bound probe is detected by

autoradiography with X-ray film in the case of radio-labeled probes or by colorimetry for non-radioactive probes.

NORTHERN BLOTTING

It is a modification of Southern blotting which enables detection of mRNA rather than genomic DNA. mRNA is initially isolated from tissues. Aggregated mRNA is separated on agarose gel containing formaldehyde as a denaturing agent. The mRNA is transferred to a nylon membrane as done in southern blotting and then incubated with the probe.The bands are then analysed as in Southern blotting.

WESTERN BLOTTING

It is used for detection of specific proteins. Proteins in cell extracts are size separated by SDS-PAGE and transferred via electrophoresis to a nitrocellulose membrane. The membrane is then incubated with antibodies against the protein of interest. Bound antibodies are also detected by an enzyme-linked assay. The technique gives information about the size and quantity of the molecule detected.

GENOMICS

The term genome refers to the sum total of the DNA that exists in every nucleated cell of an organism. The Human Genome Project (HGP) is an international research programme designed to construct detailed genetic and physical maps of the estimated 30.000 genes that make up the human genome. It is one of the greatest scientific developments of our time which is influencing our proper understanding of human biology and evolutionary history.

If the DNA in one cell were stretched out, the DNA will be 3 metres long. Since the cells that make up human is about 3 trillion, if all the DNA in the body is unraveled and laid out end to end, it would stretch from the earth to the moon and back 30 times. It is now known that 97% of the genome does not code for anything hence they are called junk DNA. In other words, only 3% of the body DNA code for proteins (that is make up the genes). Since it is believed that nature could not have just put such a number of DNA in the human body for no reason, it is hoped that some day, the role of these junk DNA would be unraveled.

BIOINFORMATICS

This is a relatively new field in Biomedical Research. Bioinformatics is the use of computer technology to store, analyse and interprete biological data while Data mining is the search for comparative genomic data. Based on genomic information of humans, it will soon be possible to individualise therapy including the right drug for the right person. Pathogen genomics should also provide information about the movement of infectious diseases within and between populations and anticipate the emergence of epidemics and of new or virulent forms of known infections.

BIOTECHNOLOGY

Biotechnology is the application of knowledge of living systems in order to use those systems or their component for industrial purposes. The term was first used by the Hungarian agricultural economist Kark Freky in 1919.

Biotechnology is a pragmatic combination of science and technology to making use of our knowledge of living systems for practical applications. This includes aspects in biological science, chemistry, chemical technology, engineering, pharmaceuticals, environmental treatments and agriculture. Examples include:

(1) **Animal Cloning**

A clone is a group of genetically identical animals. Animal cloning is done by injecting the nucleus from another type of cell into an enucleated egg as it was done successfully in sheep in 1996 in Scotland. Through transgenic technology it is now possible to make animal with desirable genetic characteristics.

(2) **Antibiotics**

Particularly penicillin, streptomycin and a host of others were the first products of biotechnology 1940s-1950s and are major products of fermentation industries.

(3) **Bacteriophage**

Bacteriophage is a virus that attacks bacteria. They are used as vector molecules for DNA cloning work. *E.Coli* is the most commonly used host bacterium.

(4) **Blood Products**

Biopharmaceuticals such as thrombolytics, tissue plasminogen activator (tpa), streptokinase, and eminase. Clotting agents such as factors VIII and IX are used for treating haemophilia. Erythropoietin stimulates the bone marrow to produce red cells.

(5) **Chemicals**

Chemicals produced by Biotechnology include: ethanol, acetone, butanol, citric acid, acetic acid, glutamate, lysine and some other amino acids.

(6) **Enzyme production by fermentation**

This is by extraction from large plants, animals or microorganism. The conditions monitored for optimal enzyme production are: pH, oxygen, CO_2, aeration, temperature, agitation and foaming.

(7) **Fermentation is a key part of Biotechnology**

It is the growing of microorganism under anaerobic conditions on a carbon substrate.

It requires a bioreactor design which is the container in which fermentation is to take place and substrates which is what the micro organisms are to grow on. Also, supports determine whether the organism is to be grown on solid media or in suspension.

It has also been used to enhance existing foods or generate new ones. For example, it has been used to remove lactose in milk (for lactose intolerant individuals), make rennin free cheese (for vegetarians and people allergic to rennin).

It is also used to produce a wide range of chemical components of food such as vitamins, colouring agents, modified starches, fats and lipids.

(8) **Genetic disease diagnosis**.
These are diseases caused by genes and inherited from our parents. Gene probes are used to detect genes that cause diseases and also the carrier status of individuals. It can detect genes that can manifest in the future as severe disease in infants.Several diagnostic kits are products of biotechnology

(9) **Vaccines**
Vaccines are preparations that when given to a patient, elicit an immune response that protects the patient from a disease. Usually, vaccines consist of the organism that causes the disease (attenuated or killed organisms). Recombinant DNA technology can be used to generate attenuated strains by deleting pathogenesis-causing genes, or by engineering the protective epitope from a pathogen into a safe bacterium.

BIOETHICS IN BIOTECHNOLOGY

Bioethics is the branch of ethics, philosophy and social commentary that deals with the life sciences and their impact on society

Both pro- and anti-biotechnology schools of thought are actively involved.It can be used to regulate biotechnological activities such as;

a. The validity of making animal models for human diseases e.g. transgenic models of cancer.
b. The use and abuse of information about the genetic make-up of humans.
c. Problem of ignoring a new drugs' potential side effects when there is a need to have patients benefit from it quickly.
d. The conditions under which recombinant organisms can be released to the world
e. The role of embryo and foetal research
f. The conditions for patenting life forms and pieces of living materials especially genes.
g. The use of genetic resources in biotechnology exploration

GOOD LABORATORY PRACTICE (GLP)

This is defined as a quality system concerned with the organizational process and the conditions under which non-clinical, health and environmental safety studies are planned, performed, monitored, recorded, archived and reported.

The purpose of GLP is to provide the development of quality test data and to provide a management tool to ensure a sound approach to the management including conduct, reporting and archival of laboratory studies.
GLP requires institutions to allocate roles and responsibilities in order to improve the operational management of each study and focus on the cardinal areas specified by GLP.
To be GLP compliant all the rules must be complied with in full.

RESOUCES REQUIRED FOR GLP

Organisation and personnel

It requires that the structure of the research organization to be and the responsibilities of the research personnel must be clearly defined. GLP stresses that the number of personnel must be sufficient to perform the tasks required in a timely and GLP compliant way. It attaches importance to qualification of staff and to internal and external training given to staff.

Facilities and Equipment

GLP principles emphasize the adequacy of facilities and equipment which have to be sufficient.It should be spacious enough to avoid cross contamination, overcrowding, confusion and cramped working conditions.
Proper utilities must be adequate and all equipment must be in working condition
There must be a strict programme of validation, calibration and maintenance. Records of equipment use and precise state of each equipment must be kept.

RULES GOVERNING GLP

Proper protocols must be well outlined. Standard Operating Procedures (SOP) must be clearly described and reviewed regularly and modified when necessary so that they reflect the actual state of the organisation. A good knowledge of characteristics of the materials used is important. The properties especially the identity, purity, composition, stability, impurity profile for all test materials should be standard. For animals used, it is essential to know the strain, health status and normal biological values.

HEALTH SECTOR REFORMS IN NIGERIA-MEETING THE MILLENNIUM DEVELOPMENT GOALS

Ogbodo E. I

At the time of writing, the author was a 300 level Medical Student of the College of Medicine, University of Ibadan.

INTRODUCTION

The Millennium Development Goals (MDGs) is the result of the Millennium Summit held in New York in September 2000, during which all 189 united nations member states adopted the Millennium Declaration, which contained a group of goals and targets. Some of them were later refined through the 'Roadmap towards the implementation of the UN Millennium Declaration: Report of the Secretary General to the General Assembly' and has become known as the MDGs. Currently, 19 UN member states have pledge to reach the target by the year 2015.

The MDGs are a set of economic and social objectives agreed to by the International Community as common concerns for the eradication of extreme poverty in the world. It summarizes our common and collective aspirations as citizens and responds to our natural impulse to improve the human condition. They also provide a common framework for collective action against poverty, the scourge of diseases and illiteracy.

THE MILLENNIUM DEVELOPMENT GOALS

1. Eradicate extreme poverty and hunger
2. Achieve universal primary education
3. Promote gender equality and empower women
4. Reduce child mortality
5. Improve maternal health
6. Combat HIV/AIDS, malaria and other diseases
7. Ensure environmental sustainability
8. Develop a global partnership for development

HEALTH SECTOR REFORMS IN NIGERIA: AN OVERVIEW

A reform is a set of programmes put in place by an organization, institution or country to revamp, re-engineer and re-invigorate its activities for better output.

The Health Sector reforms in Nigeria have already been approved by the Federal Executive Council (FEC). At a stakeholders' interactive workshop on 27 July 2005, the Health Minister, Professor Eyitayo Lambo reiterated the goal of the reforms which is to 'break the vicious cycle of ill-health, poverty and underdevelopment through the allocation of more funds to the health sector, revamping of the secondary health care facilities to ensure good quality referral services for the primary health facilities, and to get local governments to commit at least 40% of their total budget to the health sector, out of which only 60% should be utilized for staff emoluments, leaving 40% for running and maintaining the health facilities.'

These reforms have seven main thrusts:

a. Improving the stewardship role of Government
b. Strengthening the natural health system at the three levels of care i.e. primary, secondary and tertiary
c. Reducing the burden of major diseases like HIV/AIDS, malaria and tuberculosis, etc through the attainment of the health related MDGs
d. Improving health resources (human, finance, etc) and their management
e. Improving access to quality health services
f. Promoting private/public partnership, and
g. Increasing consumers' awareness of their rights and obligations.

Having reviewed the Health Sector Reforms in Nigeria, it is imperative to trace the origin and progress of the reforms in Nigeria.

HEALTH SECTOR REFORMS IN NIGERIA: THE JOURNEY SO FAR

The search for a comprehensive cost-effective healthcare plan began in the 60's at the inception of self-government in Nigeria. Public Health insurance was first considered an administrative policy in 1962 by the Halevi Committee and acquired legal teeth through the Lagos Health Bill.

However, it was not until 22 years later that Government, driven in part by a dwindling revenue profile and a spiraling birth rate set up a committee headed by Professor Diejomoah to advice it on the desirability of otherwise of a National Health Insurance Scheme.

The committee's positive recommendation set the ball rolling. Two committees with wider public and organized private sector participation were set up in 1985 to further study the subject while a harmonization committee was inaugurated to work out a feasible model in 1988. Several meetings of the National Council on Health, the country's broadest health policy formulating body, deliberated on the recommendations of these committees, fine-tuning contentious proposals until it finally convened the special meeting of July 2001 where the council set up an Implementation Planning Committee upon whose report the scheme finally took off.

The Federal Government's health programme has

recorded landmark achievements with substantial progress in re-vitalization of the primary healthcare (PHC) sub-sector and flag off of the National Health Insurance Scheme.

Government policy of constructing 200 model healthcare centres across the country is yielding a rich harvest. When completed, the PHC model centres would be the focal point of the community health management and eradication of major child killer diseases.

The National Programme on Immunization is recording success in the renewed efforts at immunizing all Nigerian children up to the age 5against polio and other child-killer diseases. Since after laying to rest the polio vaccine controversy, the programme has redoubled its efforts through accelerated immunization aimed at eradicating the disease by 2007.

Reforms in the health sector are being boosted by advocacy tours to the 6 geographical zones by the Health Minister. He visited the South East zone from 12 to 18 June, 2005 and the North West zone from 24 to 27 July, 2005 where he held far reaching consultations and sensitization talks with relevant stakeholders.

The FG has refurbished and equipped 8 teaching hospitals so far, while 6 more are in the line for restoration, in line with the National Health Policy, which assigns responsibility for the tertiary health care funding to it. Also, the FG is constructing one Model Primary Health Centre at each Local Government Council in the country, which in turn would be turned over to the community after completion, as well as the launching of the NHIS for the formal sector.

THE NATIONAL HEALTH INSURANCE SCHEME (NHIS)

The scheme took off in Ijah, a rural community in Niger State, where the First Lady flagged off the Rural Community Social Health Insurance and the Under-5 Children Health Programme components of the scheme. The NHIS was launched to encourage widespread and timely resort to medical consultation, health care delivery and development

The scheme comes in an attractive package of six components and contributors can access healthcare needs from approved public and private health service providers. Health Management Organizations (HMOs) which are limited liability companies will be licensed by the NHIS to facilitate the provision of healthcare benefits to contribute under the Formal Sector Social Health Insurance Programme to interface between eligible contributors, including voluntary contributors and the healthcare providers.

These outside formal employments would operate under the Urban Self-employed Social Health Insurance Programme where they are expected to belong to a social group which must be occupation based and make a monthly payment of between N120-N150 for the most common of ailments like malaria, typhoid fever, diarrhea, etc.

Participation under the Rural Community Social Insurance Programme requires similar modus operandi except that participants need not belong to the same occupational group but must belong to the same community.

THE HEALTH MILLENNIUM DEVELOPMENT GOALS: SITUATION ANALYSIS

The 2003 Nigeria Demographic and Health Survey (NDHS) provide data to track progress towards the country's attainment of health goals. Evidence form NDHS April/May 2003 findings reveal that:

- Low Immunization Coverage:
  Only 13% of children (age 12-23 months) have received the recommended course of immunization (as at when due). 27% of children have not received immunization at all.
- High Infant/Child Mortality:
  Infant mortality rate is 100 per 1000 live births. Child (under five) Mortality Rate is 201 per 1000.
- High Maternal Mortality Ratio:
  Average total fertility rate of the Nigerian woman is 5.7 children. Maternal mortality ratio ranges from 300 to 1,200 per 100,000 live births. 60% of women received antenatal care at least once from a trained health care provider. Two-thirds of births occur at home. 17% of women have no assistance during delivery and 36% are assisted by untrained persons.
- High prevalence of Malaria:
  Only 12% of households own a mosquito net, and only 2% own Insecticide Treated Nets (ITN).
- High Prevalence of HIV/AIDS:
  Only half of all Nigerians know that both condom use and remaining faithful are ways to prevent HIV transmission. Almost about 40% of men who are sexually active reported having high-risk sex. Less than half used a condom.

On December 8 2004, the FEC approved N60 billion for the MDGs health scheme, which is designed to reduce infant mortality rates in the country by two-thirds by 2015. In all, the funds were expected to:

- Reduce the under-five mortality rate by two-thirds between 1990 and 2015
- Reduce the infant mortality rate by three-quarters
- Reduce the spread of HIV/AIDS
- Reduce the incidence of malaria and other diseases
- Provide access to affordable drugs in developing countries.

Fourteen pilot states were selected on the basis of not having World Bank/ African Development Bank (ADB) funding and are shown to have a high prevalence rate in under-five mortality and in diseases like HIV/AIDS and malaria. They include:

- Niger and Kogi (North Central)
- Katsina, Zamfara and Sokoto (North West)
- Bauchi, Taraba and Adamawa (North East)
- Anambra and Imo (South East)
- Ogun and Ekiti (South West)
- Edo and Bayelsa (South South)

MEETING THE HEALTH MILLENNIUM DEVELOPMENT GOALS IN NIGERIA

In discussing the strategies for meeting the health-related MDGs, it is imperative to first review the problems that have slowed down the pace of progress in meeting these health MDGs.

Low immunization coverage:

- Lack of adequate materials, especially syringes. Only the materials provided by the Federal Government are available
- Polio eradication takes a lot of resources and time to the detriment of routine immunization
- Lack of community ownership i.e. immunization is seen as a government programme
- A weak health management information system and diseases surveillance and notification system.

High Infant/Child Mortality:

- Inadequate trained staff, e.g. midwives and unmotivated Community Health Extension Workers
- Non-affordability of cost of services
- Low level of residual education of mothers as primary health givers
- Unwholesome sale and poor knowledge of expired drugs in rural communities and large peri-urban settlements.

High Maternity Mortality:

- Delays in going to health facilities and in seeing health staff
- Delays due to supplies and delays in making necessary referrals
- Basic essential obstetric care not available in most facilities
- Doctors' and workers' refusal of rural postings

High Prevalence of Malaria:

- Bed nets not widely accepted and used
- Focus of Roll-Back-Malaria (RBM) programmed has excluded vector control and environment health
- Anti-malarial drug resistance
- Lack of effective leadership, resulting in weak policy implementations.

STRATEGIES FOR MEETING THE HEALTH MILLENNIUM DEVELOPMENT GOALS IN NIGERIA

The three directly health-related MDGs-reducing child mortality, improving maternal mortality and combating HIV/AIDS, malaria and other diseases can be directly affected by improved health services delivery and more effective public health interventions. The other five MDGs have close ties to health in that improved health outcomes both influence and are influenced by the achievement of other goals.

The attainment of the MDGs is an enormous task, requiring not only adequate funds, but also the right policies as well as effective implementation, appropriate health systems, increased demand and above all, enough health staff. There is a growing consensus that a health system approach is the best way forward to achieving the MDGs. Decisions need to be made about how services should be delivered and who should provide them. One challenge is how to integrate vertical, disease-specific approaches with horizontal, system-wide approaches and how this mix should change over time. A further challenge is how to make best use of all service providers-state and non-state within the health systems. Generating demand for services in environments where the health sector has traditionally performed very poorly is another key issue.

Health service delivery requires health workers, and the resources to train, equip and pay them. The Nigerian economy is so weak that it cannot sufficiently finance health systems and retain health workers. Even if an adequate percentage of the national budget were spent on health, a substantial health financing gap continues to exist for the delivery of a very basic package of health services to the majority of the population. Significant donor assistance is therefore needed to rebuild systems.

Development assistance is most effective when it is harmonized (through the use of common procedures for all donors) and aligned (by supporting Nigeria's own priorities and delivery systems). Development agencies can use existing resource mobilization and planning tools such as common action plans, trust funds and common assistance frameworks to minimize transaction costs in Nigeria.

It is important for donor countries to develop a longer-term vision of a pro-poor health system, even when donor and government efforts are focused on short-term measures that will keep the health system going. These short-term measures are likely to include actions that will help retain health workers and maintain trust in the health system.External donors are reluctant to fill the gap for a variety of reasons, including the current orthodoxy to support only 'good governance' countries, the risk of embezzlement and the sheer lack of trust that money spent on health in this environment will have measurable positive effects. The final resort-to start relying on user fees collected from patients to fill the gap-is counter productive.

There are a number of issues specific to Nigeria that should be mentioned. These include: the problem of much project-based training without accreditation, the forced migration and voluntary emigration of health workers, and unregulated use of incentives by various agencies.

More work is needed in a number of areas to reduce

the costs and uncertainty of working in Nigeria:

- Finding better tools for harmonization and alignment in Nigeria
- Further research into health policy formulation, including developing tool-kits for post-conflict health planning and the identification and tackling of bottlenecks
- A better evidence base for models of healthcare delivery and their applicability in Nigeria
- More work in the best mix of service providers and the trade-offs between building Ministry of Health capacity and contracting out to no-state providers
- More evidence on how to rebuild the health workforce in Nigeria
- More evidence on how best to finance the health sector in Nigeria, including how to use resources (whether government or donor) more efficiently
- Convincing donors to increase investment in health systems in Nigeria, by tackling skepticism about the impact of programmes, and identifying ways of reducing costs of engaging in Nigeria.

CONCLUSION

The success of the MDGs requires major improvements in health systems and health outcomes in Nigeria. Meeting the Health MDGs is a complex and challenging task. Inadequate funding, wrong policies as well as ineffective implementation, inappropriate health systems, and sufficient staffing all stare us in the face. Rapid progress is needed if these goals are to be met by 2015.

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Editor-in-Chief's notes:

This article was the winning essay of the 2006 edition of the **DOKITA** Editorial Board Annual Professor J.A. Adeleye National Essay competition.

Other Participants are:

- Adegbenga Ismaila. (500 level, University of Ibadan)
- Okeke Fidelis U. (400 level, University of Ibadan)
- Ogaraku Magnus C. (600 level, Ambrose Alli University, Ekpoma)
- Jatto Moses O. (400 level, Ambrose Alli University, Ekpoma)
- Hwabejire John O. (600 level, University of Ibadan)
- Adebayo Olugbenga George (300 level, University of Ibadan)
- Ogbimi Henry O. (300 level, University of Ibadan)
- Lanre Ajetumobi (600 level, Ladoke Akintola University of Technology, Ogbomoso)

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THE MEDICAL STUDENT AND HIS PATIENT

Olabumuyi A. A*, Odewumi C. O*, Olowoyo S. O*, Olapade-Olaopa E. O#

*At the time of writing, *were final year medical students, College of Medicine, and #a Senior lecturer, Department of Surgery, College of Medicine, University of Ibadan.*

SUMMARY

The medical student plays an important role in the care of patients; hence in relating with patients, he or she can not afford to let religion, tribe, race, culture, social status or other differences to affect the trust upon which this relationship is based. This article discusses the proper way a medical student should relate with patients and how to avoid prejudicing such relationship.

Keywords: *Medical Student, Patient, Doctor*

INTRODUCTION

The word patient is derived from the Latin word *patiens*, a word meaning sufferance or forbearance. This implies that the duty of any medical personnel towards a patient is to relieve suffering.

A medical student is a person undergoing formal training to become a Qualified Doctor. As a doctor holds a major role if not the chief role as regards patient care, medical students, whose intent are to become good doctors, should have good relationships with any patient they come across.

THE MEDICAL STUDENT-PATIENT RELATIONSHIP

This is the most important aspect in a medical student's training, as this determines how well or bad a doctor the student turns out to be. The relationship between a medical student and a patient should be based entirely on trust[1].

Medical students should listen to patients, respect them, be polite to them and respect their views and their privacy. Medical students should not allow their views about a person's lifestyle, culture, beliefs, race, colour, gender, sexuality, age, social status, or perceived economic worth to prejudice their interaction with patients[1].

As stated above the medical student-patient relationship is that of trust as patients disclose information that they would not necessarily disclose to other people. Hence medical students should be honest in interacting with their patients and not abuse this trust.

The medical student is a medical student not a doctor this position should be clear to the patient and the medical student hence aspects of patient care which include recommending treatment or giving medical advice should be left to a doctor[1].

The appearance of a medical student is very important as patients and other colleagues make decisions as to the character of medical students based on this. Therefore medical students should maintain appropriate standards of dress, appearance and personal hygiene so as not to cause offence to patients, teachers, or colleagues. The appearance of a medical student or practitioner should not be such as to potentially affect a patient's trust in that person's medical judgment or standing[1].

A medical student should be willing to physically examine patients (which includes touching) in order to establish a clinical diagnosis irrespective of the gender, colour, culture, beliefs, disability, or disease of the patient.

Most importantly as medical students are human beings like patients, they can become patients at any point in time hence the need for them to treat patients the way they would want to be treated if they were patients[1].

Below is a copy of the American Medical Student Association (AMSA) code of medical ethics as it stands for now and it highlights how medical students should interact with patients with respect and dignity regardless of their race, colour, social status, or religion. It also highlights how medical students should be dedicated at learning so as to be able to become good doctors and be helpful to patients.

AMSA CODE OF MEDICAL ETHICS

I. A medical student shall be dedicated to learning the art and the science of medicine, and shall pursue this course of study with compassion and respect for human dignity.

II. A medical student shall approach the study of medicine with the utmost academic integrity, deal honestly with patients and members of the health care team, and shall seek to promote these virtues in one's colleagues.

III. A medical student shall respect the directives of one's superiors and recognize a responsibility to seek changes in those requests that seem contrary to the wishes or best interests of the patient.

IV. A medical student shall respect the rights of patients, of fellow students, and of members of the health care team, and shall safeguard patient confidences within the constraints of the law.

V. A medical student shall not accept patient care responsibility, perform any action, nor allow oneself to be identified in a manner that is beyond

one's level of training or competence; one shall ask for supervision when appropriate, assistance when necessary, and never allow patients or patients' families to believe that one is anything but a medical student.

VI. A medical student shall recognize the importance of participation in activities contributing to an improved community, and shall strive to impact favorably upon one's fellow students and members of the health care team to aid in the continued refinement and improvement of the practice of medicine.

VII. A medical student shall acknowledge the importance of social, economic and psychological factors impacting upon health.

VIII. A medical student shall serve patients to the best of one's ability regardless of diagnosis, race, sex, ethnicity, national origin, sexual orientation, physical or mental disability, socioeconomic status, religion or political beliefs.

IX. A medical student shall not allow competitiveness with colleagues to adversely affect patient care.

X. A medical student shall guard one's own health and well-being; likewise, one should strive to promote wellness in one's colleagues, including assisting impaired colleagues to seek professional help, and accept such help if one is impaired. The medical student-patient relationship is one based on trust; as both have the same desire for the patient to become well, both of them have to interact in a courteous, dignifying and respectful manner so as not to be divided against their common enemy; disease and ill health.

ACKNOWLEDGEMENTS

The authors would like to thank the Department of Surgery, College of Medicine, University of Ibadan, Nigeria, for her lecturer-student tutorial groups, which encourage the sort of relationship between students and lecturers that led to the writing of this article.

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2. ***Cataract***
3. ***Kleptomania***
4. ***Embolectomy***
5. ***Lumbricosis***
6. ***Somnabulism***
7. ***Tachycardia***
8. ***Glaucoma***
9. ***Sonography***
10. ***Hepaticostomy***
11. ***Dyspepsia***
12. ***Melalgia***
13. ***Gingivitis***

DOKITA NEWS

ADMISSIONS

The Editor-in-Chief on behalf of the Board heartily congratulates the following people on their admission into the DOKITA Editorial Board:

August 2005

Miss 'Dunni Adeniyi
Mr. Gbolahan Yusuf
Mr. Patrick Braih
Mr. Opeoluwa Akinbami
Mr. Akinwunmi 'Femi
Mr. Ugochukwu Maduforo

February 2006

Mr. Bamsa Godwin
Miss Adedoyin Lanase
Mr. Owhofasa Agbedia
Mr. Tolu Oloruntoba

May 2006

Miss Oluleye Damilola
Mr. Ikekwere Joseph
Miss Olokode Ayobami
Mr. Ogungbemi Wale
Miss Onwuka Nkechi
Miss Oderinde Motunrayo

DOKITA SYMPOSIUM

The 40th annual symposium of DOKITA was held on the 14th of June 2005 under the distinguished chairmanship of Professor Jibril Aminu, Senator of the Federal Republic of Nigeria, ably represented by Professor Kikelomo Osinusi, deputy provost of the College of Medicine, University of Ibadan.

Theme: CURBING THE MENACE OF MALARIA- The New Agenda

Subthemes:

Malaria Control- The Journey so Far
Dr. Bayo Fatumbi,
National Coordinator, Roll Back Malaria Programme, World Health Organization (WHO)

Socio-economic Impact of Malaria Endemicity
Professor A. Soyibo,
Head, Department of Economics, University of Ibadan

Current Trends in the Case Management of Malaria and its Complications
Professor O. Sodeinde
Head, Department of Paediatrics, College of Medicine,
University of Ibadan.

PROFESSOR J. A. ADELEYE ESSAY COMPETITION

The annual Professor J. A. Adeleye Essay Competition held from the 9th of February, 2006 to the 5th of March, 2006.

Topic: ***"Health Sector Reforms in Nigeria- Meeting the Millennium Development Goals"***

WINNERS

1st Prize - Ogbodo Elisha
(University of Ibadan)

1st Runner-up - Adegbenga Ismail
(University of Ibadan)

2nd Runner-up - Okeke Uche
(University of Ibadan)

ANNUAL GENERAL MEETING

This held on the 29th May, 2006 during which Professor J. A. Adeleye was conferred as a honourary member of the Board.

Executives for the new board year were also elected, they are as follows;

Editor-in-chief	Mr. Ekanem Ekpenyong
Board Secretary	Miss Oderinde Ayobami
Production Manager	Mr. Ajayi Temitope
Distribution Manager	Mr. Amanor-Boadu Nana
Financial Controller	Miss Otesile Funke
Business Manager	Miss Falade Funmi
Publicity Editor	Mr. Oyatokun Gbenga
News and Quiz Editor	Miss Adeniyi Dunni

Compiled by the News & Quiz Editor,
DOKITA Editorial Board.

UNIVERSITY OF IBADAN MEDICAL STUDENTS' ASSOCIATION (UIMSA) NEWS

The UIMSA Executive Council for the year 2004/2005 was sworn in on Monday 11th July 2005 at a remarkable event graced by the College Secretary, Mrs. Elizabeth Etteh and our Staff Adviser, Prof. Onadeko.

The members of the Executive Council are:

Mr. Okoye Carl Obiora	-	President
Mr. Craig Olamide	-	Vice President
Mr. Adigun Agboola	-	General Secretary
Mr. Nkemjika Stanley	-	AssistantGeneral Sec
Mr. Popoola T. O.	-	P.R.O
Miss Ojo Oluwaseyi	-	Treasurer
Miss Ojo Opeyemi	-	Financial Secretary
Mr. Ilejimi Gideon	-	Sport Secretary
Mr. Ige-Orhionkpaibima	-	F.S. - Special Duties officer (Clinical)
Mr. Adesope Adedeji	-	Special Duties Office(Pre-clinical)

The Senate officials are:

Mr. Ekanem Ekpenyong	-	Senate Chairman
Mr. Olubodun O.	-	Deputy Chairman
Mr. Olatunde Kolade	-	Senate Registrar

The Congress Officials are:

Miss Ilori Motunrayo	-	Chancellor
Mr. Oladimeji Olanrewaju	-	Deputy Chancellor
Mr. Braih Patrick	-	Scribe

ACHIEVEMENTS

On assumption of office the Executive Council mapped out programmes for the executive year to facilitate the smooth running of the association and commitment to set goals.

The Executive year started with preparations to attend the *Nigerian Medical Students' Association (NIMSA) General Assembly.* 6 delegates represented the association together with a FAMSA delegate. The General Assembly took place at Imo State University Teaching Hospital Complex at Orlu Imo State between Wed. 17th to Sunday 21st August 2005. It was an avenue for UIMSITES to appreciate what NIMSA stands for.

The association organized a befitting ***welcome party*** for the newly admitted 100 level medical students at the Trans Amusement Park U.I. Ibadan on Friday 2nd September 2005. The event was well attended by some lecturers, the new students, senators, congressmen, other UIMSITES and UIMSA Executive Members and it turned out to be a huge success.

The association also organized the 8th Annual Dr. V.O. Awosika Memorial Symposium at the College Auditorium on Tuesday 1st November 2005. The theme for the symposium was *"Towards Eradicating Tuberculosis"* and was well attended by lecturers, students and the Awosika family.

Also started was the ***UIMSA Ventures*** at the College Building in October 2005. It comprises photocopying and spiral binding services. The association also got a donation of a refrigerator from the Coca-Cola Company.

On Saturday 11th February 2006, the association organized an ***Orientation Programme*** for the in coming 200 level medical students at the Anatomy Lecture Theatre, U.I. to get them well acquainted with the demands of medical school and adequate preparations. The programme attracted a large audience.

The association also organized an ***inter-level table tennis competition*** in November 2005. This was to stimulate UIMSITES' interest in sports.

Projects in the Pipeline

Preparations are in earnest to organize the annual Sports Fiesta/Provost Cup.

The association is also preparing for the ***Capacity Building Workshop*** which is a programme designed at preparing students as future managers and leaders.

Preparations are also in top gear for the publication of the maiden edition of the ***UIMSA Newsletter***, which will serve as a means of keeping UIMSITES up to date about UIMSA activities. Also to be published is the 2nd edition of the UIMSA magazine *"The Masquerade"*.

The ***Annual Health Week*** for this year promises to be unique and special. Different committees have been set up towards this and preparations are in top gear to give UIMSITES what they deserve.

Lastly, a big boost was added to the scheme of things in the executive year. The Awosika Family instituted a ***₦1 million Endowment Fund*** for the association to be used for the annual Dr. V.O. Awosika Memorial Symposium. This feat was borne out of the unrelentless efforts of the Executive Council.

Compiled by the Public Relations Officer,
University of Ibadan Medical Students' Association (UIMSA)

BRAIN GAME

Bakare T. A

Bakare is a 300 Level Medical student of the University of Ibadan

BRAIN GAME

T	A	B	M	O	I	N	S	O	M	N	I	A	M	N
E	M	B	O	L	E	C	T	O	M	Y	S	T	T	H
M	L	F	L	J	T	V	E	S	T	X	I	J	A	E
B	U	G	K	D	E	C	V	Z	Y	W	O	A	C	P
C	M	I	L	V	Q	U	A	P	H	O	I	M	H	A
A	B	N	S	A	Y	X	U	R	Z	N	B	N	Y	T
I	R	G	A	N	U	M	B	O	A	Q	Y	L	C	I
S	I	I	X	A	V	C	W	M	W	T	I	V	A	C
P	C	V	F	L	A	T	O	C	A	C	A	N	R	O
E	O	I	J	K	T	T	P	M	C	D	C	C	D	S
P	S	T	T	M	P	N	B	C	A	B	I	N	I	T
S	I	I	M	E	L	A	L	G	I	A	S	G	A	O
Y	S	S	L	M	O	A	N	I	E	G	M	P	S	M
D	L	K	I	J	S	O	N	O	G	R	A	P	H	Y
K	D	S	O	M	N	A	M	B	U	L	I	S	M	Q

QUESTIONS

1. Inability to sleep when sleep should normally occur
2. A disorder of impulse control characterized by morbid tendency to steal
3. Complete of partial opacity of the ocular lens
4. Removal of embolus
5. Infection with round intestinal worms
6. A disorder of 'sleep walking'
7. Rapid breathing with heart rate above 90 beats/min
8. A disease of the eye characterized by increased intraocular pressure, excavation, and atrophy of the optic nerve
9. Location, measuring, or delineation of deep structures by measuring the reflection or transmission of high frequency or ultrasound waves
10. Establishment of an opening into the hepatic duct
11. Impaired gastric function due to some disorders of the stomach
12. Pain in limbs, specifically burning pain infact extending up to the legs or even thigh
13. Inflammation of the gums as a response to bacteria plaque on adjacent teeth

*See solution on page 92

POEMS

BY THE BED SIDE

Sparkling wide sight
Bouts of cough heard
Whoops soon to follow
Emaciated fellows on the bed
Some close to hearing the cherubic voices
Calling them home
Other think how fast life ends
'It still seems mysterious', they say

Younglings of Adam proceed
Distributing these tools and materials
They need to learn from them
Many in immaculate clothings
'They are new', the grey clothings say
'This life is horrible'
Many younglings think
This sight
Is not appealing
'Healthy people don't come here'
A grey clothing whispered

These tools lay on the bed
Tired of seeing white apparels
Out of the blues, a youngling
Started on his way
Working on his tool
Others marvel
I don't know the next step
Fleshy tools lay in wait
'Doctor come and attend to me'
It's between life and death

(+ A grey clothing in this piece is a senior in medical school)

AJAYI, Temitope
400L MBBS

DECEMBER BLUES

October blues, that I've heard of
But December blues?
In my mind's eye
I see, the about to be mauled chickens
Squawking their cackled protests
I seek the essence of this season

I see the newly cut *buba and soro*
Sense the approaching madness
The magic of the fireworks
Is this the essence I seek?
The reason for it all

I see the street kids, screaming in glee,
They do a crazy jig, with frantic gestures
In anticipation, mouth a watering
Wine corks pop in candle lit dinners
Is this the essence?

I hear the resounding silence
I see the brightly shining star
Herald of good tidings
Treetops sway in a rhythm of love
The reason for it all!

buba and soro: native outfit in Nigeria

ATANDA Adejare
300 Level BDS

UNANSWERED QUESTION

Burning slowly
The candle shortens
Eyes twitching
Reading texts with his whole mind
Aspiring for the peak
But lolling in the doldrums of insufficiency
Nothing deters the determined mind
He would give his life
To reach the height
He would spend a million nights like this
Just to reach that apex
And then all goes dark
Ohhhhh
The candle burnt out
Overwhelming grief and anger
Mixed up in his befuddled mind
A wrenching scream from his soul emanates
"Success, is sufferance thy vehicle?"

BRAIH, Patrick
300 Level MBBS

POEMS

OUR GREAT TEACHER

You will always be remembered…
…A rare gem indeed!
The babes of the other women you served…
… With your loving heart;
That sweet and faultless seed!

Great teacher;
Your disposition of noble inspiration,
We will never forget your charming form,
The long black and exquisite skirt…
…Your heart-lifting crimson shoes,
The very aura of royalty did you unsurprisingly exude!

We will always consider our Grand Mother,
Who cultured us on the ethics of the 'measles'
So vast your unmatchable practice,
A priceless gift to our dignified institution,
Your beloved spirit rests in ideal peace!

ADEKUNLE O. A
2003 MBBS

UNFORSEEN DEPARTURE

The magic of summer
The warmth of sunshine
I called out to you
But a second too late
All alone to share the thrills

The heaviness of autumn
Leaves fall slowly but surely
Myself in a vacuum of pain
Why? Why leave so soon?
Whom do I share my grief with?
Silently mourning my loss
Unanswered questions I ask
Thoughts of your smiling face crowd out my pain
Your sun set a mite too early
Is it for better days to come?

Wait for me in the land beyond
The beginning unknown, the end witnessed
Hold my hand a moment more
For the warmth of your hands to linger on…
…Your untainted image to be ever

ATANDA ADEJARE
300 Level BDS

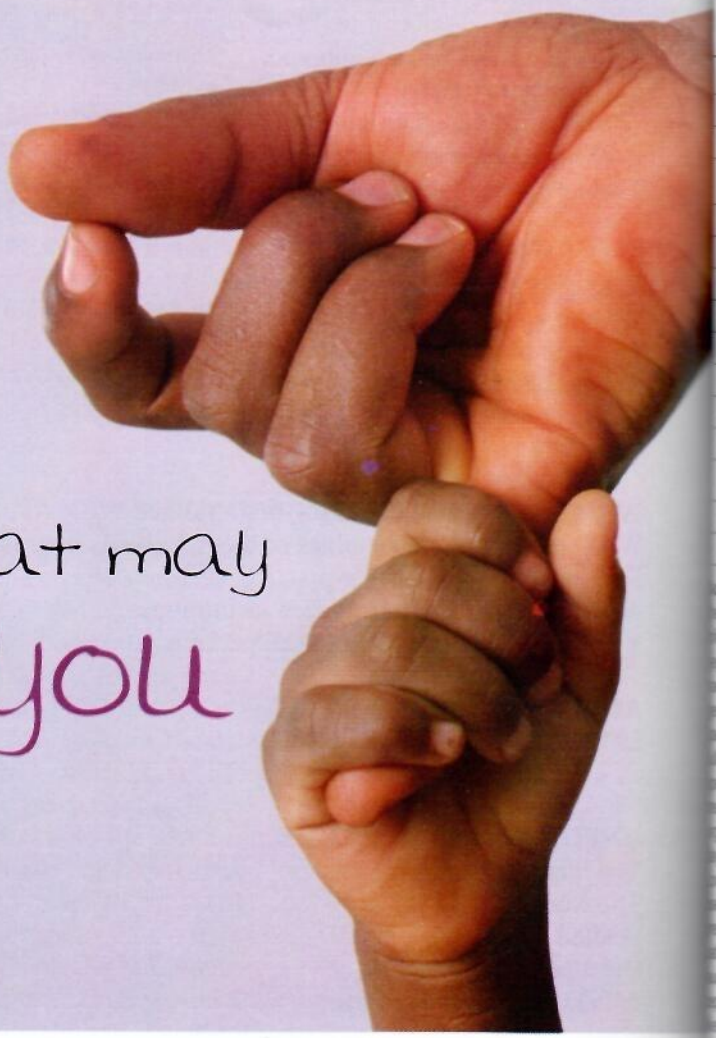
SCRUTINIZED…

As she drew near, I discerned these charms
"…An infectious aura of trouble-shooting and undisturbing equanimity,
Of course! The glory of her proficient garb gave,
It's like her proceedings had unequivocally been
Premedicated in an oblivious era.
An indescribable ambiance of you

THE BEST OF TIMES

Clipity- clap-clop-clu
Noisy bunch of coat-wearing freshers,
Ties loose
Shirts glistening
Enthusiasm high,
They know not
What lies before them,
Postings that break minds
Sights that induce sighs
Books that create worms
Distractions that ensnare like temptations,
Of all these
They are totally unaware
Believing all to be
A bed of flower petals
Until the first exams
Come rolling in
And then they see
The hand
Writing on the wall
The story of their lives
Their flowery beds
Becoming thorny planks
For them I pray
Oh Lord
The best of times

BRAIH, PATRICK
300 Level MBBS



Trust the one that may
not fail you

Augmentin has been available for clinical use in a wide range of indications for over 20 years and is now used primarily in the treatment of community acquired respiratory tract infections.[1] Augmentin is well tolerated and has a simple, convenient twice daily dosage regimen[1]

Augmentin B.D *Syrup*
amoxycillin/clavulanate

Please see Abbreviated Prescribing Information on adjacent Page

For further information contact:

gsk GlaxoSmithKline

GlaxoSmithkline, Nigeria
GlaxoSmithKline House
Km. 16, Ikorodu Road, Ojota
P.M.B. 21218, Ikeja, Lagos
Tel: 234-1-4938000, 4732824
E-mail: lekan.m.asuni@gsk.com

GlaxoSmithkline, Ghana
Coplan House, 2nd floor,
Koja Thompson Road, Adabraka,
P. O. Box CT 3067, Accra, Ghana
Tel: 021-222900, Fax 021-22074
E-mail: gsk@gh.com

Reference:
1. White R. et al; Journal of chemotherapy, 2004, 53 suppl. 51

ugmentin®

amoxycillin/clavulanate

BBREVIATED PRESCRIBING INFORMATION FOR USE IN INTERNATIONAL AREA

UGMENTIN Oral presentations

he following strengths and ratios are currently available

Strength	Ratio	Amoxicillin (mg as amoxicillin trihydrate)	Clavulanate (mg as clavulanate potassium)
Adult			
1 g	7:1	875	125
1125 mg	8:1	1000	125
Adult and Paediatric			
156.25 mg	4:1	125	31.25
187.5 mg	2:1	125	62.5
312.5 mg	4:1	250	62.5
375 mg	2:1	250	125
625 mg	4:1	500	125
15%	2:1	10%	5%
457 mg/5 ml	7:1	400	57
228.5 mg/5 ml	7:1	200	28.5
Paediatric			
62.5 mg/ml	4:1	50	12.5
112.5 mg/ml	8:1	100	12.5

ive ingredient: Amoxycillin trihydrate + clavulanate potassium. **Indications:** ***Adult formulations:*** Amoxicillin-clavulanate is indicated for short term treatment of bacterial infections at following sites when caused by amoxicillin-clavulanate-susceptible organisms: upper respiratory tract infections (including ENT), lower respiratory tract infections, genito-urinary tract ections, skin and soft tissue infections, bone and joint infections e.g. osteomyelitis where more prolonged therapy may be appropriate. Other infections e.g. septic abortions, puerperal sis, intra-abdominal sepsis, refer to full prescribing information for list of sensitive organisms. ***Paediatric formulations:*** Amoxicillin-clavulanate is indicated for short term treatment of terial infections at the following sites when caused by amoxicillin-clavulanate sensitive organisms: upper respiratory tract infections (including ENT), lower respiratory tract infections, nito-urinary tract infections, skin and soft tissue infections, refer to full prescribing information for list of sensitive organisms. **Amoxicillin-clavulanate Paediatric three times daily.** The diatric three times daily dosing regimen is also indicated for the following infections, bone and joint infections, e.g. osteomyelitis where more prolonged therapy may be appropriate, refer to prescribing information for list of sensitive organisms) ***All formulations:*** A comprehensive list of sensitive organisms is provided in the full prescribing information. Some members of these cies of bacteria produce beta lactamase, rendering them insensitive to amoxicillin alone. Infections caused by amoxicillin-susceptible organisms are amenable to amoxicillin-clavulanate tment due to its amoxicillin content. Mixed infections caused by amoxicillin susceptible organisms in conjunction with amoxicillin-clavulanate-susceptible beta-lactamase-producing anisms may therefore be treated by amoxicillin-clavulanate **Dosage and Administration:** Adults and children over 12 years - Mild to Moderate infections - One 375 mg tablet three times a OR one 625mg tablet two times a day; Severe infections - One 625 mg tablet three times a day OR two 375 mg tablets two or three times a day OR one 1g tablet two times a day; Children er dose(mg/kg/day) for three times daily use 4:1 formulations 20/5 to 40/10 and 7:1 formulations for twice daily formulations 25/3.6 to 45/6.4.Higher dose(mg/kg/day) for three times daily e 4:1 formulations 40/10 to 60/15 and for twice daily use 7:1 formulations 45/6.4 to 70/10. Refer to the full prescribing information. Reduce dosage in patients with renal impairment (see full scribing information). For patients with hepatic impairment, dose with caution and monitor hepatic function at regular intervals. **Contra-indications:** AUGMENTIN is contraindicated in ents with a history of hypersensitivity to beta-lactams, e.g. penicillins and cephalosporins. AUGMENTIN is contraindicated in patients with a previous history of AUGMENTIN-associated ndice/hepatic dysfunction **Precautions:** Before initiating therapy with AUGMENTIN careful enquiry should be made concerning previous hypersensitivty reactions to penicillins, halosporins, or other allergens. Serious and occasionally fatal hypersensitivity (anaphylactoid) reactions have been reported in patients on penicillin therapy. These reactions are more y to occur in individuals with a history of penicillin hypersensitivity (see *Contra indications*). AUGMENTIN should be avoided if infectious mononucleosis is suspected since the occurrence a morbilliform rash has been associated with this condition following the use of amoxycillin. Prolonged use may also occasionally result in overgrowth of non-susceptible organisms. ongation of prothrombin time has been reported rarely in patients receiving AUGMENTIN. In patients with renal impairment, dosage should be adjusted. In patients with reduced urine put, crystalluria has been observed very rarely, predominantly with parenteral therapy. During the administration of high doses of amoxycillin, it is advisable to maintain adequate fluid ke and urinary output in order to reduce the possibility of amoxycillin crystalluria (see Overdosage) Amoxicillin-clavulanate Suspensions/Sachets/Chewable Tablets (where applicable), ain aspartame, which is a source of phenylalanine and so should be used with caution in patients with phenylketonuria **Drug interactions:** Concomitant use of probenecid is not mmended. Probenecid decreases the renal tubular secretion of amoxycillin. Concomitant use with AUGMENTIN may result in increased and prolonged blood levels of amoxycillin but not clavulanic acid. Concomitant use of allopurinol during treatment with amoxycillin can increase the likelihood of allergic skin reactions. There are no data on the concomitant use of GMENTIN and allopurinol. In common with other broad spectrum antibiotics, AUGMENTIN may reduce the efficacy of oral contraceptives and patients should be warned accordingly. **gnancy and lactation:** In a single study in women with preterm, premature rupture of the foetal membrane (pPROM), it was reported that prophylactic treatment with AUGMENTIN may be ciated with an increased risk of necrotising enterocolitis in neonates. As with all medicines, use should be avoided in pregnancy, unless considered essential by the physician. GMENTIN may be administered during the period of lactation. With the exception of the risk of sensitisation, associated with the excretion of trace quantities in breast milk, there are no mental effects for the infant. **Adverse Reactions :** The following convention has been used for the classification of frequency :- **Infections and infestations:** Common - Mucocutaneous diasis **Blood and lymphatic system disorders:** Rare - Reversible leucopenia (including neutropenia) and thrombocytopenia. Very rare- Reversible agranulocytosis and haemolytic emia. Prolongation of bleeding time and prothrombin time (see Warnings and Precautions). **Immune system disorders** Very Rare - Angioneurotic oedema, anaphylaxis, serum ness-like syndrome, hypersensitivity vasculitis. **Nervous system disorders:** Uncommon - Dizziness, headache. Very Rare - Reversible hyperactivity and convulsions. Convulsions may r in patients with impaired renal function or in those receiving high doses. **Gastrointestinal disorders: Adults:** Very common - Diarrhoea. Common - Nausea, vomiting **Children:** mon - Diarrhoea, nausea, vomiting. All Populations Nausea is more often associated with higher oral dosages. If gastrointestinal reactions are evident, they may be reduced by taking GMENTIN at the start of a meal. Uncommon Indigestion . Very Rare - Antibiotic-associated colitis (including pseudomembranous colitis and haemorrhagic colitis). Superficial tooth colouration has been reported very rarely in children. Good oral hygiene may help to prevent tooth discolouration as it can usually be removed by brushing. **Hepatobiliary disorders:** mmon A moderate rise in AST and/or ALT has been noted in patients treated with beta-lactam class antibiotics, but the significance of these findings is unknown. Very Rare - Hepatitis cholestatic jaundice. These events have been noted with other penicillins and cephalosporins. Hepatic events have been reported predominantly in males and elderly patients and may be ciated with prolonged treatment. **Children:** These events have been very rarely reported in children. All Populations: Signs and symptoms usually occur during or shortly after treatment in some cases may not become apparent until several weeks after treatment has ceased. These are usually reversible. Hepatic events may be severe and in extremely rare umstances, deaths have been reported. These have almost always occurred in patients with serious underlying disease or taking concomitant medications known to have the potential for atic effects. **Skin and subcutaneous tissue disorders** Uncommon - Skin rash, pruritus, urticaria. Rare - Erythema multiforme. Very Rare - Stevens-Johnson syndrome, toxic epidermal rolysis, bullous exfoliative-dermatitis, acute generalised exanthemous pustulosis (AGEP) If any hypersensitivity dermatitis reaction occurs, treatment should be discontinued. **Renal and nary disorders:** Very rare - Interstitial nephritis, crystalluria (see overdosage) **Overdose: Symptoms and Signs** Gastrointestinal symptoms and disturbance of the fluid and electrolyte nces may be evident. Amoxicillin crystalluria, in some cases leading to renal failure, has been observed (*see Warnings and Precautions*).**Treatment:** GI symptoms may be treated ptomatically, with attention to the water/electrolyte balance. Amoxicillin-clavulanate can be removed from the circulation by haemodialysis.**Drug Abuse, Dependence:** Drug dependency, iction and recreational abuse have not been reported as a problem with this compound.

se read the full prescribing information prior to administration, available from: GlaxoSmithKline Abbreviated Prescribing Information prepared November 2000. Version Abbrev. 3.2 pdated April, 2005

GlaxoSmithkline, Nigeria
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Km. 16, Ikorodu Road, Ojota
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GlaxoSmithkline, Ghana
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E-mail: gsk@gh.com

AMNESIA

He can't remember.

He squints at the sun, grime stained rivulets of sweat flow through the ancient creases in his face. He carries a world with him, different specimens of an insane wilderness, this desert caked on feet that carry him further into this forest of *deja vus*.

Scrunch scrunch walk walk earth sun sky earth earth the earth thearth...step step white sky carrion vulture mutter groan step walk sand shrub grass dried leaf ashes cinder walk walk walk more sky more sand darker sand sweat sweat drip drop fall fall earth, earth... earth...

...but no one in sight. He walks on. It seems he has walked this way for eons. How long has he been here? He stands stock still on the perimeter tangoing with his consciousness, amid still shouldering carcasses, eyes torn out, thoracic baskets only half filled. The vultures eye him languidly, too bloated or confident to flee.

He drinks in every detail. He can now identify every note of his wheezing breath. Air...there's something on it sniff sniff twitch twitch, his moustache convulses, bristles and shivers off the sweat drops hanging on to hair tips. His moustache moves up and down, groping for something, like a blind man. It shudders like a rat's whiskers, animatedly searching...yes! Smell...of course. Carrion...around him but there is something more...what? His starvation kindled senses blaze, the air is a little bit too still. He now knows what is on the air. He knows fear like a friend. Sunrays prick and stab him, his vision swims derisively. Hearing a thin, shrieking call, almost imperceptibly, his world stands still. With a start, he begins to move again, he knows not where; this wilderness seems to expand with each advancing step. He feels as if he is on some fateful peristalsis into the bowels of this desert thing.

Step shuffle stumble shriek step walk walk stumble fall up eyes dart desert sky sun! More sky more cries tumble shiver limp cries tears sweat spittle grime madness rush tumble but... sand, more sand...endless

Blazing teeth strewn all around, nay, swords, shields...battleground...He remembers...yes! This place was not always like this- the men who were once men but now bulge from vulture bellies once fought here...this place was once a battleground. He remembers...who he is...almost...he once fought here...yes he remembers, his whole face contorts with the excitement of remembering, his eyes spasm uncontrollably...The errant thought swims away. These men dead...why are these men dead. Why are these men dead...why are...why...www...his thoughts turn into a wail, revulsion breaks through him as he remembers a terrible day dark as night, an amorphous black mass pouring off and cascading into the valley, swirling forward like a blizzard, blotting the vision and something...only the heart saw...the one who took these lives.

He drops onto the sand and retches emptiness, only more spittle and sweat slick off his face in elastic slivers. He gets up and begins to run again. He is running in circles. He stops. The sand...crystals, exquisite, mmm...so many suns... smells dry, crisp, salt metal iron. Blood. He brushes the sand from his palms and he notices that he is wearing metal...gauntlets, gleaming dully from under all the dust. A blade runs from each elbow to wrist, blazing defiantly, silver slices of the sun. He remembers...almost. He feels his head expand, sensations pour over him like a waterfall, raining icicles of fear on him, melting into a soup of apprehension.

EVERYTHING IS SO SHARP. He smells the grass dried and dusty...the detail of a sand grain catches his eye...tiny, gleaming with a million edges, the blazing reflection of the giant ball of heat touch-counting the capillaries in his eyes. His sparse clothing crawls around him like some cockroach in its final throes. His moustache still swivels on its end like a toad's bulbous eyes, untrusting, shuddering, restless. The sound that has followed him so far increases, the grotesque sound of a beast, dripping with reptilian hatred, the sound moving with its own life source. It enters into his head and navigates the intricate convolutions and labyrinths, snaking inside it, squeezing, clasping, choking out his life fluid to a mere trickle. The sharpness and brightness at once ebb -that's a relief, he shudders to his knees. The darkness clouding his vision clutches at him from below, his eyesight pirouettes like a bug. The sound following him is...he knows with a growing dread that he has heard it before...it is...he remembers...Almost. He drops face down in the dirt. He remembers no more.

OLORUNTOBA, Tolu
300 Level MBBS

JOKES AND CARTOONS

JOKES

Patient: Doctor, I have yellow teeth, what do I do? **Dentist**: Wear a brown tie...

Patient: How much to have this tooth pulled? **Dentist**: N3000.00.

Patient: N3000.00 for just a few minutes work???

Dentist: I can extract it very slowly if you like.

An anesthesiologist is a doctor who works in the operating room to delay your pain until such time as you get his bill.

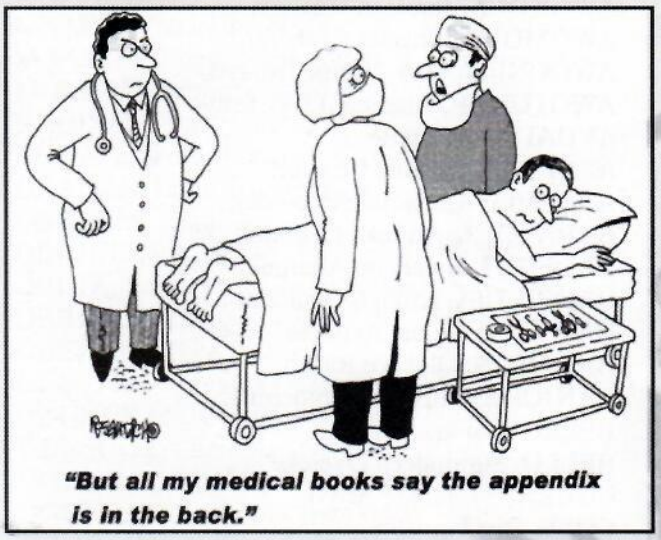
LAUGH IT OFF

This old man visits his doctor and after a thorough examination, the doctor tells him, "I have good news and bad news, what would you like to hear first?"

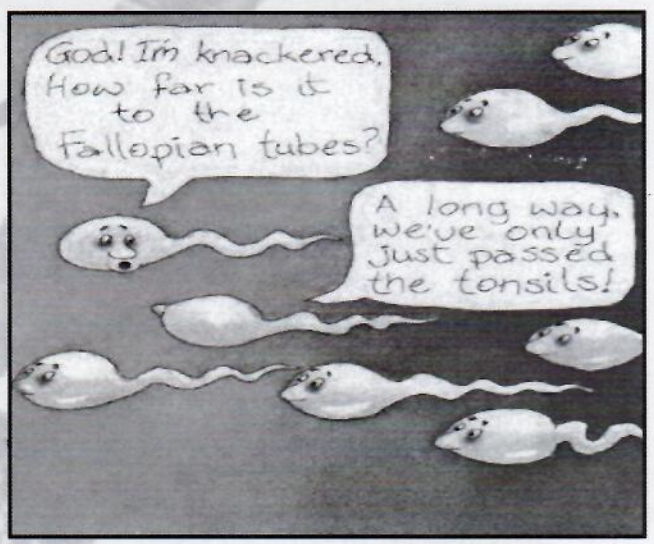
Patient: Well, give me the bad news first.
Doctor: You have cancer; I estimate that you have about two years left.

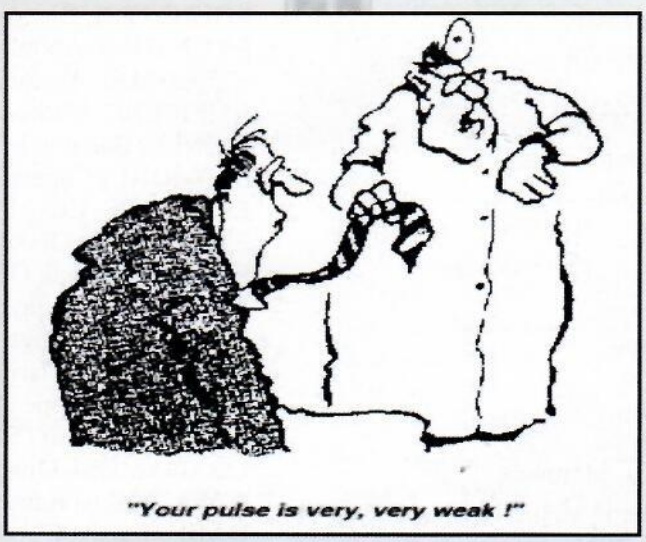
Patient; OH NO! That's awful! In two years, my life will be over! What kind of good news could you probably tell me, after this???

Doctor: You also have Alzheimer's. In about three months you are going to forget everything I told you.



"But all my medical books say the appendix is in the back."





"Your pulse is very, very weak !"

Cartoons were sourced from www.medicalcartoons.com(accessed March 8, 2006)
Compiled by AJAYI, Temitope (400 Level, MBBS)

COLLEGE OF MEDICINE UNIVERSITY OF IBADAN

BACHELOR OF MEDICINE , BACHELOR OF SURGERY DEGREE

LIST OF GRADUANDS

SEPTEMBER 2004

ABARA, Winston Eromosele
ABDULRAHEEM, Muhydeen Abiodun
ABIONA, Bolanle Funmilayo
ABIMBADE, Omowumi Funmilayo
*ABOLARINWA, Abimbola Ayodeji
ADABA, Franklin Uche
ADARAMOLA, Akindele Abiodun
ADEBALE, Khadijat Abosede
ADEBAMBO, Abimbola oLufolajimi
ADEBANJO, Adeoye Adewunmi
ADEBAYO, Tiitlayo Adebimpe
ADEBOLA, Susanna Oluwalope
ADEDIJI, Ebenezer Olumide
ADEFOLAJU, Olufunke Oladolapo
ADEGOKE, Titilade Felciia
ADEGUNSOYE, Ayodeji Olakunle
ADEJOKUN, Adebayo Emmanuel
ADEJORIN, Oluwaseyi David
ADEJUWON, Tolulope Opeyemi
ADEKUNLE, Adebola Oyebimpe
ADEMOSU, Olukemi Moyosola
ADEMOSU, Ope-Oluwa Olufunmilayo
ADENIJI, Elizabeth Adebola
ADEOSUN, Increase Ibukun
ADEPOJU, Adebayo Adesola
ADESINA, Adekonla Tolulope
ADESINA, Adenike Oluseyi
*ADESOKAN, Dorcas Idowu Odunayo
ADESUYI, Oluwatoyin Adekemi
ADETULA, Itioye Olurinde
ADEWOYE, Ifeoluwa Adenike
ADEYEMI, Olutoyin Morenike
ADIBUAH, Felicia Nkiru
ADIGUN, Adedoyin Oyeyimka
AFE, Tolani Adenike
AIKI, Olayinka Olanike
AINA, Olufemi Ayoola
AJAERE, Ijeoma Dorothy
AJAKAIYE, Ife Oluwa Atinuke
AJAYI, Aderonke Olawunmi
AJAYI, Nasirudeen Oladipupo
AJULO, Adeshina Ayodele
AJUONU, Jolly Ezidinma
AKANJI, Rukayat Adebimpe
AKINBOLAGBE, Abisola Monisola
AKINDEHIN, Oludoyinmola Omobolade
AKINODEKO, Olayinka Arike
AKINMERESE, Olawale Opeyemi
AKINPELU, Tolulope Oluwaseun
AKINTUNDE, Olugbenga Paul
AKINYELE, Abosede Grace
AKINYELE, Olusegun Adedeji
AKOSILE, Oluwaseun Adewole
AKPAIDU, Enobong
ALADE, Folasade Abiola
ALAKIJA. Oluwaseun Temitope
ALALADE, Andrew Folusho
ANENI, Ehimen Chima
ANUMBRA, Gideon Ogugua
ARCHIBONG, Arit Ayi
AREGBEYEN, Oshuare Ohinamua
*ASEMOTA, Osato Josephine
AWOKOLA, Babatunde Ishola
AWOMOLO, Olusola Ayobami
AWOONIDANLA, Kemi Titilayo
AWOTUNDE, Oladimeji Awofisayo
AYOADE, Adekunle
AYODELE. Bolanle Comfort
AYODELE, Oabamidele Ayodeji
BABALOLA, Atinuke Oladunni
BADEJO, Oluwakemi Aderonke
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BAIYEWU, Lateef Ayodele
BALOGUN, Oluseye Rasaq
BANJOKE, Olufunke Abidemi
BELLO , Amina Titilayo
BELLO, Surajudeen Oyeleke
COKER, Sordeinde Ariyo
COLE, Bisola
DANILE, Adefmi Adetokunbo
DIBOSA-OSADOLOR, Onome Joan
EFFIONG, Cynthia Imeh
EGUNYOMI, Bisayo Fadekemi
EKUNSUMI, Abimbola Ajoke
ELEGBEDE, Ayokunnumi Adufe
EMEFIENE, Mathew Onyekachukwu
ERINLE, Babatunde Babajide
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ESUTURIE , Eseoghene Ethel Catherine
ETOK, Uduak-Obong Joshua
ETUDOK, Uduak Obong Gideon
FABUNMI, Temitayo Elijah
FADIPE, Olugbenga Olufemi
FASUYI, Omofolarin Babatunji
FATUYI, Babatope Olayinka
FETUGA, Adeniyi Shamsideen Olusegun
GBADAMOSI, Olugbenga Babatunde
GIWA, Olabisi Raheemat
HABU, Laraba Ciroma
HAMILTON, Olusegun George
IBIGBAMI, Ibidolapo Fisayo
IBILAIYE, Olubunmi Olufunke

SEPTEMBER 2004 cont'd

IDOWU, Olubukola Abosede
IGE, Macrina Olusolape
IGE, Rosaleen Oluwabunmi
IHEKUNA, Chiaka Maryanne
ILESANMI, Ibitomilayo Ayoka
IMOAGENE, Ekhasomhi Albert
ISEKO, Kinsley
ISEMEDE, Sarah Olohijie
IVENSO, Jude Chiwuba
JEJE, Oluyinka Motunrayo
JEMINI, Opeyemi Laura
JIKI, Michael Ggenga
JOHNSON, Simon Mafuka
KETIKU, Adegboyega Olufemi.
LADITAN, Adenrele Oyebode
**LAMIKANRA, Olaoluwatomi Kehinde
LUFADEJU, Adetoye Adedolapo
MALLUM, Chindo Bala
MANN, Tolulope Oluwaseun
MBABA, Vivian Imaobong
MBANGA, Ndeni Njom Evang
MOMOH, Comfort Ahuoiza
NNODIM,. Aina Nyenma
NUGA, Edwina Olusola
OBADAN, Emitope Inyang
OBADEMI, Temitope
OBAJULUWA, Kolapo Kehinde
OBETA, Emmanuel Chukwuemeka
OBIHIELO, Ehiozogie Ighodalo
OBIDIKE, Chika Esther
ODULAJA, Omolara Ololade
ODUNUKAN, Olufunso William
ODUSOGA, Omotola Olufunke
OGUNDAIRO, Abidemi Olubunmi
OGUNDALO, Abisola Temitope
OGUNDIMU, Fausat Abidemi
*OGUNDOKUN, Akinjide Olurotimi
GOUNNIYAN, Dunni Ayo
OGUNNIYAN, Tope Banji
OGUNSEYINDE, Olanrewaju
OGUNYEMI, Funke Adebowale
OKETONA Olajide
OKOLI Barbara Chioma
OKON, Alero Kufre
OKONKWO Chijioke oseloka
OLADIRAN, Abibola Babatunde
OLAIYAN, Olufunmilayo Tinuola
OLANIRAN, Abimbola Ayodele
OLAOYE Olusola Abimbola
OLATUNDE, Sunday Ayodeji
OLOFIN, Ibironke Oluwaseun Ayobosede
OLOWOJOLU, Olayinka Patrick
OLOWOYO, Paul
OLUKIRAN, Olufunmilayo
OLUTOGUN, Tolulase Aderayo
ONUORAH, Michael Nnaemeka
ONYIDO, Okwuchi Ekenedilichukwu
OPAKUNLE, Olajumoke Bukola
OPAKUNLE, Oluwatosin Abimbola
ORIDUPA, Olubukola Omolola
OSIGBESAN, Olusegun Oyetola
OSUNLAJA, Tafa Oluwakayode
OTI, Olubukola Omobolanle
OWOSENI, Olubukola Oluwabusayo
OYEBOLA, Bolanle Tanwa
OYEDOKUN, Festus Adegboyega
OYENIRAN, Olubukola Oyewumi
OYETAYO, Olutosin Damilola olatunji
SALAWU, Olubukola Hadija
SHIYANBOLA, Oyewale Olakunle
SHONOWO, Oluwafunmilayo Temitope
SIBEUDU, Uchenna Nneka
SOKUNBI, Olatola Omobolanle
SUNMBOYE, Kehinde O
TAYO, Oluwayemisis Adunni
UDI, Uchenna Efeoghene
UFARUNNA, Ojonugwu Daniel
UGALAHI, Ogbenyi Mary
UMAR, Adeiza Charles
UZO, Onoyinyuchi Chinoso
WILLIAMS, Adeola Omosalawa
WILLIAMS, Akintunde Olatokunbo
WILLIAMS, Simisola Omowumi
YUSUF, Hakeem Oluwatoyin

JANUARY 2005

ABIOLA, Olaniyi Akeem
ABIOYE, Funmito Omolola
ABOLADE, Olugbenga Olayinka
ADELANA, Adeola Omolade
ADEDAPO-AISIDA, Ifedolapo Victoria
ADEBIYI, Taiwo Ademola
ADEDEJI, Abimfolu Oluhirere
ADELAKUN, Olaide Tajudeen
ADELEKE, Oyeyemi
ADENIYI, Ayodeji Adewale
ADELERE, Saheed Abayomi
ADEOYE, Moses Olusegun
ADESOJI, Omobolanle Adenike
*ADEWUMI, Adedayo Babajide
ADEWUMI, Bankole Adeyemi
ADESOKAN, Paul Oluwatimilehin
AFOLABI, Ggbolahan Abayomi
AGBARA, Nnenna Uzoamaka
AGBELEYE, Opeyemi Bukola
AIKHUELE, Egbonghon Happiness
AIKOYE, Salisu Adejo
AJAKAIYE, Michael Adebayo
AJAKAIYE, Afolake Olufunmilayo
AJAYI, Omotola Oyeyemi
AKANJI, Akinwunmi Olalekan
AKANDE, Sarah Ese
AKANLE, James Olufemi
AKINYEMI, Martina Yemisi

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JANUARY 2005 cont'd

AKINYEMI, Akinwunmi Olalekan
AKINTOLA, Akinrinola Adeleke
AKINNUOYE, Akinwumi Ayodele
AKUBUIRO, Adaoha Onyinyechukwu
ALEWI, Olanrewaju Akinlayo
AKINSOLA, Oluranti
ALLI, Weniyu Agbokhaode
AJETUNMOBI, Olawale Olaitan
ALESHINLOYE, Olabode Bamidele
ANIFALAJE, Oluwatosin Lisa
ANUMNI, Chinedu Chidiebere
ARINOLA, Ajibola Oludolapo
ARIJE, Olujide Olusesan
AROLE, Oyinola
ATINMO, Temitope Titilope
ATTA, Kabiru Ovurevu Mohammed
AROWUBUSOYE, Folake Bukola
AWOYODE, Rotimi Clement
AWOLAJA, Babatunde Stephen
BABALOLA, Olubukola Christianna
BAKARE, OriyomiOlufadeke
BAKARE, Rafiu Adetunji
BABASANYA, Babatunde Alabi
BADA, Simbat Olabisi
BASSEY, Arit Effiom
BANJO, Babatunde Afolarin
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CAREW, Azeezat Damilola
CHUKWUEME, Cynthia Nkemdilim
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DANIA, Omotayo Oluseyi
DARAMOLA, Bukola Olateju
DAVIES, Afolake Feyisola
EBE, Chijioke Adaobi
EDEWOR, Ufuoma
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KOLEBOLA, Isaac Obayemi
KOLADE, Olamide Bamidele
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LAYIOYE, Adewumi Olabimpe
MADU, Brendan Ejiofor
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MOMOH, Rasak Onozutu
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OBI, Chika Uzochukwu
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OGUNDANA, Olajide Oluseyi
OGUNLOWO, Taiwo Olusesan
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OGUNKEMI, Ayokunle Adedayo
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OJO, Oluwatoyin Oyebimpe
OJO, Omolola Marian
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OKAEME, Chukwuka
OKE, Akintokunbo Olufunlade
OKE, Olubunmi Olushola
OKOLI, Innocent Chinazom
OKORO, Lynda Odiri
OKONJO, Somawina Chiazo
OLADIGBOLU, Remilekun Abiola
OLADIPO, Bamidele Fatai
OLAGBNRO, Michael Adeola
OLAREWAJU, Michael Tolorunju
OLATUNDE, Haifiz Olasupo
OLATUBOSUN, Oladele Olaniyi
OLATUNJI, Olubukola Olubanke
OLATUNJI, Oluwayemisi Temilola
OLATUNJI, Folasade Oladoyin
OLANIYAN, Segun Isaac
OLAYINKA, Olusoji Omotayo

LIST OF GRADUANDS

JANUARY 2005 cont'd

OLAREWAJU, Oludotun Babatunde
OLEGHE, Izehi
OLAWUYI, Mathew Oluwaseun
OLAWUYI,, Kafilat Abimbola
OLUOHA, Raphael Uchechukwu
OLUGBOJI, Oluwasina Akintunde
OLUSANYA, Olumuyiwa Oladipo
OMOKANWAYE, Tolulope Simisola
OMORONYIA, Ogban Ezukwu
OMOTOMILOLA, Olatomirera Morolayo
ONADEKO, Olukemi Morenike
ONANUGA, Josephine Oluyemisi
ONAKOYA, Adeolu Adedotun
*ONASANYA, Olukunle Oludare
ONASANYA, Babatunde Adeleke
ONI, Omodaramola Oluwaseun
ONI, Abiola Jessica
ONI, Ebenezer Temitope
ONOCHIE, Chizoba Geneviere
ONYENEFA, Uba Olayemi
ONYEASO, Obinna Okechukwu
ORHORHORO,M Igberhi Omuvwie
ORIJI, Abimbola Modupe
OSINNUWA, Caroline Oluwatoyin
OSINUGA, Oluyemsis Olatokunbo
OSHODI, Omoyele Adetola
OSUNOLALE, Bamidele David
OPUKUM, Adaeze Nnenna
OSIBERU, Olusola Oludayo
OYELAMI, Folasade Oyefunmike
OYELADE, Babatunde Oladimeji
OYEWOLE, Olusegun
SAIKI, Olalekan Emmanuel
SALAMI, Kikelomo Rachael
SHITTU, Oluwe
SHOBAYO, Oluwakemi

KEY
* Former Board Members

JULY 2005

ABOBARIN, Abimbola Bukola
ABU, Abel
ADEBAYO, Saheed Olayode
ADEGBOYEGA, Adebukola Omolara
ADEJORO, Oluwakayode Olumide
ADEJUMO, Adedapo George
* ADETOSOYE, Folusade Adejumoke
ADEWARA,. Funmi Rebecca
ADEYEMI , Ademola Tope
AGOMO. Obinna Chukwuma
AGORO, Olanrewaju Titilola
AGORUA, Chinelo Paula
AHMED, Saheed Bolaji
AIYEPE, Sekinat Bolanle
AJAYI, , Olubukola Ayoola
AKINLOYE, Akintunde Oluwaseun
AKINSOLA, Adebukola Olajumoke
AKINTAYO, Adebowale Opeyemi
AKINTAYO, Akinyemi Akinsoji
AKINERINWA, Olumide
AKPATI, Ugochuku
ALLEN-TAYLOR, Adeoye
ASANGANSI, Ime Effiiong
ASHAYE, Ajibade Opeoluwa
AWOMOLO, Adetokunbo Folasade
*AYODELE, Olulade Adeola
BADRU, Mutiat Olaide
DABIRI, Tawakalit Modupeola
EBENIBO, Sotonte Eresia
EGUAGIE, Naruna Maria Nina
EGUNSOLA, Elizabeth Oluwatoyin
EHIMIYEIN, Oluwayemisi Ekata
EJENAVI, Efe
EKE, Ngozi
EWEDAIRO, Desola Shakirah
EZEAKU, Chijioke Ifeanyi
FALADE, Oluwatosin
FALOLA, Michael Idowu
FARRI, Oadimeji Feyisetan
FATOKUN, Modupe Olajumoke
JIBOYE, Tinuola Oluwasola
KAYODE- ADEDEJI, Babatunde
KOKU, Michael Oluseyi
KUSHIMO, Oyewole Adebiyi
LAWAL, Abimbola Olukayode
LAWAL, Adewale Hakeem
MBAH, Chukwudi Bernard
NNADI, Augustine Kanayo
OBUZOR, gbonna Oigozie
OGBUAGU, Lamin Chimaije
OGUNBAYO, Solabomi
OGUNBAYO, Solabomi
OGUNDIRAN, Temitope Kemi
OGUNTOYE, Oluwatosin Oluwagbenga
OJEDIRAN, Babawale
OJELABI, Oluwayemisi Adenike
OJIFINNI, Kehinde Abraham
OKAFOR, Philip Nnamdi
OKONGWU, Vincent Ekena
OKOLI, Chinenye Obiageli
*OKULATE, Tolulope Omotoyosi
OKUNOLA, Ayoola Babatunde
OLADELE, Babatunde Akintunde
OLAYINWOLA, Adedolapo Oladunni
OLONINIYI, Ibidunni
OLOPADE, Olasunmbo Omobolaji
OLOWOLAIYEMO, Mlerlumhe Ude
OLUSANMABO, Olufunmbi Olukemi
OLUSANYA, Oyekunle Adebayo
OLUSANYA, Oyekunle Adebayo
OLUYOMBO, Olluseye Babatunde
ONYEBUEKE, Hilary Kanayo
OROGBEMI, Olubunmi Olubisi

LIST OF GRADUANDS

JULY 2005 cont'd

OYEKOLE, Oyedayo Olabanjo
SHEYIN, Olusegun Ayodeji
SHOBO, Olukolade George
SHODUNKE, Ayotunde Olaposi
SOBUKUNOLA, Oluwaseun Babatunde
SOKOYA, Oluyemisi Temitope
SOLADOYE, Oyeyemi Adebola
TUME, Aondoyima Alfred
TUNWASE, Adedolapo
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NOVEMBER 2005

ABDULSALAM, Atanda, Adekunle
ADEBISI, Adewale, Olufemi
ADEDEJI, Oluwatoyin, Bola
ADEDEJI, Taofik, Adekunle
ADEFALU, Modupe Oluwagbemiga Ademola
ADEGBESAN, Folakemi Iyabo
ADEGOKE, Helen, Olubunmi
ADEGOKE, Olusola, Olasupo
ADELOYE, Adeleke Temitope
ADENEGHA, Adebanke Oluwakemi
ADEYALO, Toyin Temilola
ADEYIOLU, Adewale Temitope
AGBORGA, Charity
AIKOMO, Olusola, Oladayo
AIYELOKUN, Oluwadunni Elizabeth
AIYETAN, Paul Oluwaseyi
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AJEDE, Kehinde, Shakirueen
*AJIBOYE, Onaopemipo Benjamin
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AJUYAH, Tosan
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AKANNI, Saheed Olatunde
AKINGBALA, Omobolaji Oluseye
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*AKINOLA, Ibrahim Temitope
AKINPELU, Olusola Matthew
AKINRIMOYE, Olugbenga Oyewumi
AKINSIPE, Elizabeth Omobolanle
AKOTAIBE, Paul Ifaniyi
AKUKALIA, Roselyn Nwab
ALABI,. Christian Popoola
ALFA, David Akoji
ALIYU, Kolawole Waheed
ALUKO, Michael
AMAIFE, Fidelma Chiedu
AMANGELE, Tamaranebi Maria
AMOS, Adeniyi, Olugbenga
AMUDA, Lateef
ANJORIN, Olasunkanmi Gbenga
AREMU, Isiaka,Olatoye
ARIKAWE, Oluwakayod Peter Alfred
ARIMI, Subedat, Temitayo
ATOKI, Akinwunmi Olusegun
ATOLAGBE, Oluseun Olumide
AWE, Julius Adebayo
AWOMOLO, Morenikeji Oluwakemi
AWOYEMI, Ayodeji Lawrence
AYINDE, Olatunde Olayinka
BABATUNDE, Olaniyan Akintunde
BAIYEROJU, Babatunde
BALOGUN, Dele Dannis
BELLO, Olusola Billi
BITTO, Sewuese
BOBAI, Nathan Dawah
CHIZEA, Chike Trevor Edward
DADA, Afolake Olufunke
DADA, Olufunmilayo Opeyemi
DISU, Risikat Aramide Aina
DIXON, Yewande Alaba
DUROJAIYE, Oluwagbemiga Ade
EGUZOZIE, Emmanuel Chinedu
FADEYI, Abimbola Akintunde
*FADIPE, Babatunde
FAGBUARO, Abimbola Oyinfunmilayo
FAMODIMU, Ggboyega, Ayodeji
FAMOSAYA, Abimbola Adebola
GBADEBO, Tolulope Adedoyin
GIGI, Charles Ehianeta
GIWA-OSAGIE, Omoruyi Cadwall
HASSAN, Yetunde Yemisi
IBENEME, Chimedinma Girima
IDIRHE, Florence Urukpeoghene
IGBOANUGO, Schola Amoge
IJILADE, Oluseye Akinfolarin
* IKEAGUWUOHA, Ogadinama Adannaya
IKHILE, Emmanuel Aimebeloma
IKOKOH, Matthias Osigbemeh
ISIMAEL, Ibinabo
ISIKEPEI, Iwumekwunechukwu
JAKANDE, Olanrewaju Khadijat
JIBOYE, Justus Oluwafemi
JIMMY Escor Emmanuel
KALEJAIYE, Adedeji Olufemi
KAREEM, Saka Adeleke
KAYODE, Temitope Oluwaseun
KODE, Oluwadamilola Oluwaseun
KOLADE, Tomilola Olawunmi
* KUFONIYI, Ibukunoluwa Omololu
KUTEYI, Bunmi Vincent
MACULAY, Bisola Adebimpe
MALOMO, Grace Oluyemisi
MEGBUWAWON, Adekunle Adeniyi
MOMODU, Olufisayo Kafilat
MOROOF, Suleman Omobayowa
MUJAKPERUO, Ojititen Ojis
MUOGHALU, Nneka Maureen

LIST OF GRADUANDS

NOVEMBER 2005 cont'd

NDUKWU, Kingsley Aloysius Chinedu
NJOKU, Isaac Chukwuemeka
NWAIWU, Obioma Benjamin Ifeayi
OBATAYO, Oluwadare Adeolu
OGELEYE, Oluwadare Adeolu
OGIDIAGBA, Efe Louis
OGUNJULUGBE, Taiwo Philip
OGUNLEYE, Temitope Oduola
OGUNRINDE, Taiwo Philip
OGUNSANYA, Laniyi Penuola
OGUNSANYA. Olawande Oladapo
**OGUNSUA, Adedotun Anthony
OHIAERI, Uzodima Amarachi
OKOLI, Okwuchukwu
OKONKWO, Ekene Kingsley
OKORO, Uchechi Chinedu
OLAIYA. Olanrewaju Oluwadare
OLANIYI, Bukola Olanrewaju
OLANREWAJU, Olabisi Olaitan
OLAPOJOYE, Olatubosun Ismail
OLATOREGUN, Adedamola Olabanji
OLAWOYIN, Oluwafemi Oluwaseun
OLAYIWOLA, Ayoola Omobolanle
OLOGUNDUDU, Olatunde Olumide
OLOKEDE, Olugbenga Augustine
OKUKITIBI, Olanrewaju Ezekiel
OLUOKUN, Motunrayo Tolu
OMATIGA, Achimugu Gabriel
OMOTARA, Olufemi Abidemi
OMOTOLA, Omobowale Effiong
OMOYENI, Richard Adelana
ONASANYA, Titilayo Modupe
ONI, Ayobami Oluwayimika
ONI, Olubukola Monlsola
ONOFUA, Augustina
ONOTU, Oziome, Zainab
ONWUEGBUSI, Anthony Chukwudumeje
ONYEKA, Pauline Amoge
OPARAH, Chukwuma Corlette
OSHIN, Adebayo Oludare
OSIJIRIN, Mufutau Omotola
OSUNBUNMI, Abimbola Temitope
OSUNDIJI, Tawakalit, Abosede
OVBA, Ofejiro Sarah
OWOLABI, Babatunde Oludare
OYELUDE, Oyebimpe Olusayo
POPOOLA, Mutiu Abiodun
PUDDICOMBE, Akinwande Tolulope
SALUBI, Akporodafe Margaret
SANYAOLU, Olumide Oladimeji
SHITTU, Saliu Ajedotun
SUBUKUNOLA, Toluwalope Andrew
SODIPO, Gbolahan Nureni
SODIYA, Grace Adebimpe
SOILE, Babatunde Hakeem
SOLAJA, Kafilat Olufunke
SULAIMAN. Mufutau Abiodun
UDOEKEZIE, Ugochukwu Feliz
UNIGWE, Obinna Michael
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WAHAB, Adelani Musbau
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