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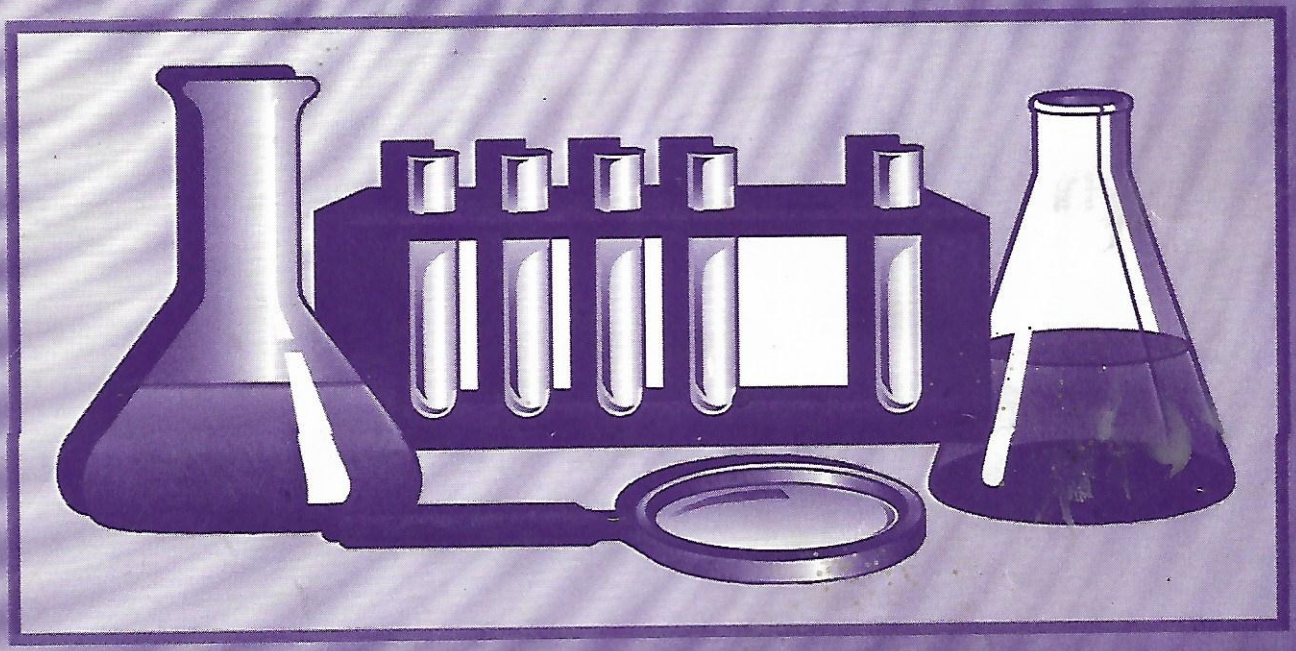

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JOURNAL OF BIOMEDICAL INVESTIGATION, JUNE 2010, VOL. 8, NO. 1

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Journal of Biomedical Investigation, 2010;8(1): 1 - 4

Original Article

SURVEY OF COMMON AEROBIC BACTERIA ASSOCIATED WITH WOUND INFECTIONS AMONG PATIENTS IN NNAMDI AZIKIWE UNIVERSITY TEACHING HOSPITAL (NAUTH), NNEWI, ANAMBRA STATE

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ABSTRACT

The aerobic organisms associated with wound infections in Nnamdi Azikiwe University Teaching Hospital, Nnewi were ascertained. Two hundred wound swab samples from patients suspected to have wound infection were obtained. These samples were processed immediately in the microbiology laboratory using Blood, MacConkey and Nutrient agar plates. The result showed that 188 (94%) of the samples yielded growth and 12 (6%) yielded no growth. Of these, 146 (77.7%) were single microbial infections while 42 (22.3)% were due to poly microbial infections. The organisms isolated in order of frequency were: *Staphylococcus aureus* 75 (32.5%), *Escherichia coli* 60 (26%), *Pseudomonas aeruginosa* 49 (21.2%), *Proteus spp* 19 (8.2%) and *Streptococcus spp* 3 (1.3%). The infection rate was least in children 0-1year. This could probably be due to the high level of hygiene observed by the mothers as well as the high circulating antibodies. Broadly speaking, the prevalence of wound infection can be reduced drastically if aseptic procedures are observed especially hand washing and sterilization as well as routine screening of healthcare providers for carriage of *Staphylococcus aureus* and those found to be carriers could be removed from the clinics/wards and relocated to offices where they would not be in contact with patients until properly treated.

Key Words: *Honey, Eusol, Cutaneous, Ulcer, Management.*

INTRODUCTION

A wound is a breach in the skin, and exposure of subcutaneous tissue following loss of skin integrity. This provides a moist, warm, nutritive environment conducive to microbial colonization and proliferation. The outcome depends on the interaction of complex host and microbial factors[1].
It is of great importance to note that the sources of micro organisms that infect wounds vary. They could come from the air, soil, water, host's body flora and hands of other patients, doctors, nurses and attendants in the ward (nosocomial infections).

When a wound becomes infected, it develops some characteristics like foul smell, discharge of pus, tenderness and pain. Wound healing is delayed and when it finally heals, it does so with secondary intention. The patient's stay in the hospital is delayed and in some unfortunate instances, the patient may die. It is therefore, pertinent to avoid wound infection through the use of proper infection control procedures.
Furthermore, the management of wound infection has become challenging to health practitioners, not only in terms of financial burden on patients but also due to the increasing antimicrobial resistance as a result of misuse of antimicrobials[2].

A number of micro-organisms have been associated with wound infection of which *Staphylococcus aureus* is the most common[2,3]. Other organisms include: *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus epidermidis*[4, 5, 6]. The type of wound, location, patient's immune status as well as other host and bacterial factors determine the outcome of wounds. Adebayo *et al*[5] conducted a study in Ile Ife, Nigeria where it was observed that out of 102 patients seen, 41 (40%) had wound infection as a result of trauma to the extremities. There is paucity of information on the aerobic bacteria associated with wound infection in Anambra State so the study was designed to ascertain that.

MATERIALS AND METHOD

The study was conducted at Nnamdi Azikiwe University Teaching Hospital, Nnewi, South Eastern Nigeria. This is a tertiary institution that serves the needs of patients from several states including Anambra, Imo, Enugu and Abia.

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SPECIMEN COLLECTION

Samples were collected from patients suspected to have wound infection, from different wards in the hospital. Before sample collection, the wound site was cleaned with sterile normal saline, and then a deep swab was taken at the base of the ulcer/wound.

METHODOLOGY

The specimens were taken to the Medical Microbiology Laboratory and processed immediately. They were inoculated onto freshly prepared agar plates (Blood, MacConkey and Nutrient). These were incubated aerobically at 37°C overnight. Isolates were identified from pure cultures.

RESULTS

The survey of common aerobic bacteria associated with wound infections among patients in NAUTH was done. A total of 200 patients suspected to have wound infections were used in the study. The analysis of organisms isolated showed that *Staphylococcus aureus* was the most common at 75 (37.5%), with *Streptococcus spp* being the least at 3(1.5%) as in Table 1.

The types of wounds and the organisms isolated from them are shown in Table 2 with surgical incision wound infection being the highest (44).

DISCUSSION

The survey of the common aerobic bacteria associated with wound infection in Nnamdi Azikiwe University Teaching Hospital showed that out of 200 samples from suspected infected wounds, 42 (22.3%) had polybacteria infection. It was observed that *Staphylococcus aureus* had the highest frequency of 75 (32.5%). *Escherichia coli* was next with 60 (26%), then *Pseudomonas aeruginosa* 49 (21.2%). This agrees with the study by some researchers [2,3,7,8] and could be due to nasal carriage of the organism by patients, medical officers and other health workers; this could result in nosocomial infections. The result however, is at variance with that of Oguachuba[9] who reported *Proteus* species as the most common isolate from wounds.

The high incidence of *Pseudomonas* in wounds could be attributed to the opportunistic nature of the organism. It has the tendency to cause infection in debilitated patients who have chronic wounds and burns [6,10].

It is not surprising that children 0-1 year recorded very low number of bacteria isolates- 3 (1.3%) with *Staphylococcus* being the most frequent, followed by *E. coli* with 1 (0.4%). This is due to the good sanitary care given to the children at this age, coupled with the high levels of maternal antibodies.

From the study, it can be concluded that *Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa* were the most common aerobic bacteria associated with wound infection in Nnamdi Azikiwe University Teaching Hospital, Nnewi, Anambra State.

RECOMMENDATIONS

The prevalence of wound infection can be reduced drastically if proper aseptic procedures are observed, especially hand washing and sterilization. Routine screening of healthcare providers for carriage of *Staphylococcus aureus* is advocated. Persons found to be carriers could be removed from the clinics/wards, and relocated to offices, in order to reduce their contact with patients until the carriers are treated.

REFERENCES

1. Bowler PG, Ducrden B L, Armstrong DG. Wound Microbiology and Associated Approaches to Wound Management. Clin Microbiol Rev. 2001;**14:** 244-69.
2. Giacometti A, Cirioni O, Schimizzi A.M, Del Prete M.S, Barchiesi F.D, Errico M.M. Patrelli E. and Scalise G. Epidermiology and Microbiology of Surgical Wound Infections. J of Clinical Microbiology. 2000. **38**:918-24.
3. Emele F.E, Izomoh M.I, and Alufohai E. Microorganisms Associated with Wound Infections in Ekpoma, Nigeria. West Afr. J. med. 1999. **18** (2): 97-100.
4. Gorecki P, Schein M, Rucinski J.C, and Wise L. Antibiotic Administration in Patients undergoing Common Surgical Procedures in a Community Teaching Hospital. The Chaos continues. World J. Surg. 1999. **23**: 429-32.
5. Adebayo O.S, Deboye O.K, and Emiola A.R.O. Wound Infection in Two Health Institutions in Ile- Ife, Nigeria: A cohort study. Biomedical letters. 2003: 52-57.
6. Agnihotrin N, Gupta V. and Joshi R.M. Aerobic Bacteria Isolates from Burn Wound

Infections and their Antibiograms: A five year study. J. Burns. 2004. 241-43.
7. Kluytmans J, Van Belkum A, and Verburgh H.A. Nasal Carriage of Staphylococcus aureus: Epidermiology Underlying Mechanisms and Associated Risks. Clin. Microbiol Rev. 1997. **10**. 505-20.
8. Mahdi S.E, Ahmed A.O and Boelens H. An Epidemiological Study of the Occurrence of Staphylococcus Aureus in Superficial Abscesses of Patients Presenting for Surgery in a Teaching Hospital in Khartoum, Sudan. FEMMS Immunol Med Microbiol. 2000.**29**:155-62.
9. Oguachuba Hubert N. Wound Infection in the Orthopaedic Traumatological, Department of Orthopaedic and Traumatological Surgery, University of Jos Teaching Hospital. Nig Med. Journal. 1987. 147-51.
10. White R.J, Cooper R, and Kingsley A. Wound Colonization and Infection: the role of tropical antimicrobials. Br J Nurs. 2001. **10**: 563-78.
11. Cruickshank R, Duguid J.P, Marimor B.P and Swaim R.H.A. The Practice of Medical Microbiology. **2**: 444-48.

Table 1: *Frequency of bacterial isolates.*

Organism	Number isolated	Percentage %
Staphylococcus aureus	75	37.5
Escherichia coli	60	30
Pseudomonas aeruginosa	49	24.5
Klebsiella spp	25	14
Proteus	19	9.5
Streptococcus	3	1.5

Table 2: *Organisms isolated from various Clinical Specimens.*

Wound	Staph aureus	E. Coli	Pseudo.	Klebsiella spp	Proteus	Strept. Spp	No growth	Total with growth
Surgical Incision	9	20	7	6	1	1	2	44
Ulcers/Blisters	12	8	6	5	4	1	2	36
Burns	3	-	5	-	3	-	-	11
Fracture/Amputation	7	7	7	5	-	-	-	26
Osteomyelitis	4	2	3	2	4	-	-	15
Bruise	2	4	2	3	-	-	3	11
Cut/Laceration	5	3	4	-	-	-	1	13
Bites/Scratches	2	2	4	-	-	-	-	8
Gunshot/Stab	6	2	-	2	-	-	1	10
Hepatosplenomyopathy/ Lymphoadenoma	2	-	-	1	-	-	-	3
Gangrene	1	-	-	-	-	-	-	1
Others	22	12	11	1	7	1	-	53
Total	75	60	49	25	19	3	9	231

Table 3: Organisms isolated from patients of various ages.

Age group (years)	S. Aureus	E. Coli	P. Aeruginosa	Klebsiella	Proteus	Strept. Spp	Total	%
0 - 1	2	1	-	-	-	-	3	1.3
2 - 12	8	4	8	1	4	1	26	11.3
13 - 18	4	3	2	1	-	-	10	4.3
>18	61	52	39	23	15	2	192	83.1
Total	75	60	49	25	19	3	231	100

Journal of Biomedical Investigation, 2010; 8(1) 5 - 8

Original Article

PREVALENCE OF HEPATITIS VIRAL CO-INFECTION IN HIV-POSITIVE PATIENTS IN NNEWI, NIGERIA

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ABSTRACT

This study was conducted to ascertain the prevalence of hepatitis B and hepatitis C viral co-infection among Human Immunodeficiency Virus (HIV)-positive patients in Nnewi South Eastern Nigeria. Two hundred (200) HIV-positive patients; (100) HIV-positive on Antiretroviral Therapy (ART) and (100) HIV-positive patients not on ART were recruited for the study. One hundred (100) apparently healthy HIV-negative individuals served as control subjects. All subjects were between the ages of 18-40 years. Hepatitis B Surface Antigen (HBsAg) and Hepatitis C Virus (HCV) tests showed that 18 (18%) of the HIV-positive patients not on ART and 20 (20%) of those on ART were co-infected with HBsAg, while 16 (16%) of the patients not on ART and 23 (23%) of those on ART were co-infected with HCV. Only 1(1%) of the control subjects was positive for HBsAg while none for HCV. The result of the present study shows that the HIV-positive patients are at a greater risk of being co-infected with either HBV or HCV. To minimize the emergence of HIV and/or HBsAg and HCV resistance, or a rise in liver enzymes should be noted and the treatment of both infections should be coordinated. Therefore, the hepatitis viral co-infection status of the HIV-positive patients on ART should be assessed occasionally as this will aid in treating and monitoring of the patients.

Key Words: *Hepatitis B, Hepatitis C, HIV, ART.*

INTRODUCTION

Co-infection with human immunodeficiency Virus (HIV) and Hepatitis B Surface Antigen (HBsAg) are common globally[1]. Both hepatitis-B Surface Antigen (HBsAg) and Human Immunodeficiency Virus (HIV) infections are common in Nigeria and are a significant cause of mortality and morbidity[2]. Reports have indicated that hepatitis will contribute significantly to morbidity and mortality in HIV infected patients because of increased use and accessibility of Highly Active Antiretroviral Therapy (HAART)[3].

Liver toxicity is a growing problem among HIV patients, particular, in those who are co-infected with hepatitis C or hepatitis B virus[4]. Multiple concurrent factors make the study of the liver function during HIV infection treated with antiretroviral combinations an emerging issue. Although the introduction of Highly Active Antiretroviral Therapy (HAART) led to sharp drop of immunodeficiency-related opportunistic infections (including hepatobiliary ones), short and long-term toxicity of each single antiretroviral agent and their combination may add its effect to the frequently underlying chronic HBsAg and/or HCV infection, and their specific antiretroviral treatment[4].

About 15 to 25 percent of people infected with only hepatitis B will develop and die of liver disease, such as cirrhosis and liver cancer. HIV-infected people are three to six times more likely to develop a chronic or long-term hepatitis B infection because of their weakened immune systems than individuals without HIV.[14] People co-infected with HIV and hepatitis B may have a more rapid progression of liver disease due to their weakened immune systems, and the use of medications that can be toxic to their HBV-infected liver[14].

Co-infection with HCV and HIV is common, occurring in 50% to 80% of individuals who acquired HIV through parenteral exposures[1].

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Chronic HBsAg infection occurs in 10% to 15% of persons infected with HIV.[5,6]. The majority of liver diseases in People with HIV (PWHIV) are caused by viruses (especially hepatitis B and hepatitis C) or opportunistic infections[7].

Viral hepatitis has similar transmission vectors as HIV and is seen often in gay men and intravenous drug users because it is blood borne[8]. HIV infection modifies the course of HBsAg infection by increasing rates of chronicity, prolonging HBsAg viraemia and increasing liver-related morbidity[1].

Therefore, the objective of this study is to determine the prevalence of HBsAg and/or HCV co-infection in HIV - positive patients in Nnewi, Nigeria and to highlight the reciprocal interactions between the HIV and HBV/HCV.

MATERIALS AND METHODS

SUBJECTS

A total of two hundred HIV positive patients were used for the study (100 HIV-positive patients not on ART, 100 HIV-positive patients on ART). The control consisted of one hundred HIV negative apparently healthy individuals drawn from among medical students, students of Medical Laboratory Science and Staff of the College of Health Sciences and Nnamdi Azikiwe University Teaching Hospital (NAUTH), Nnewi. Their ages ranged from 18-40 years. Ethical approval was obtained from the Ethical Committee of Nnamdi Azikiwe University Teaching Hospital, Nnewi before embarking on the study. Informed consent was also obtained from the patients after explaining to them the purpose of the study.

METHODS

About 3ml of blood was collected from the antecubital vein of both patients and control subjects into lithium heparin. The samples were centrifuged at 3000 revolutions per minute for 5 minutes. The sera were separated, stored and stored frozen in aliquots at -20°C (Haier Thermocool Chest Freezer, Greece) until assayed. Hepatitis B Surface Antigen (HBsAg) detection was carried out using rapid Hepatitis B surface antigens test strip[9], while detection of antibodies to Hepatitis C Virus (HCV) was done using hepatitis C virus test strip[10].

STATISTICAL METHOD

In this study, a simple percentage was used to express the number of patients co-infected with HBsAg and/or HCV.

RESULTS

The result of the screening tests for HBsAg and HCV showed that 18 patients (18%) and 16 patients (16%) of the HIV positive patients not on ART were co-infected with Hepatitis B and Hepatitis C virus respectively, whereas only 1 subject (1%) of the HIV negative control subject showed positive result for HBsAg and none for Hepatitis C. Out of the 100 patients receiving ART 20 (20%) and 23 (23%) respectively, were co-infected with hepatitis B and hepatitis C viruses.

DISCUSSION

The present study showed that the rate of co-infection with hepatitis B and hepatitis C is more common in HIV infected patients compared with the HIV negative control subjects. Co-infections with Human Immunodeficiency Virus (HIV) and Hepatitis B Virus (HBsAg) are common globally[1]. Both Hepatitis-B Virus (HBV) and Human Immunodeficiency Virus (HIV) infections are common in Nigeria and are a significant cause of mortality and morbidity[2]. Reports have indicated that hepatitis will contribute significantly to morbidity and mortality in HIV infected patients because of increased use and accessibility of Highly Active Antiretroviral Therapy (HAART)[3].

Once exposed to HBsAg, HIV-positive individuals are far more likely than others to develop chronic HBsAg infection,[11,12] and this may probably be due to immune deficiency in HIV infected patients which will eventually expose them to opportunistic infections. The finding in this study is in agreement with that of Hadler, Judson and O'Maley *et al.;* (1991)[13] who found that 21% of HIV positive men who were exposed to HBsAg developed chronic HBsAg infection compared with only 7% of the HIV negative men.

Since none of the control subjects tested positive for HCV, it might also be as a result of the suppressed immune system that enable the HIV positive subjects to be co- infected with HCV. None of the patients was co- infected with HBV and HCV at the same time. The most important risk factors for

hepatotoxicity found in this study include the co-infection with Hepatitis B Virus or Hepatitis C Virus.

In this study, we conclude that HIV infection is a predisposing factor to infection with HBsAg or HCV. Since HIV infected patients with HBsAg/HCV co-infection respond less to HAART, additional concern and care must be taken in order to minimize the complications associated with the increasing use of HAART.

The finding of the study recommend that hepatitis viral co-infection status of the HIV-positive patients on ART should be assessed occasionally. It is also proposed that the testing of HIV positive patients for HBsAg / HCV may be helpful in the choice of therapy in these patients. The HIV positive patients co-infected with hepatitis B or C virus should equally be treated for the co-infection because the co-infection is one of the risk factors for developing hepatotoxicity in HIV infected patients.

REFERENCES

1. Vivian Levy and Robert M. Grant. Antiretroviral Therapy for Hepatitis B Virus-HIV-Co-infected Patients: Promises and Pitfalls. Clinical Infectious Diseases; 2006; 43:904-910.
2. Elele, O.A., Nwauche, C.A. and Erhabor, O. The Prevalence of Hepatitis B surface antigenaemia in HIV positive patients in the Niger Delta Nigeria. Niger J Med.; 2004; 13(2):175-9.
3. Nwokedi, E.E., Emokpae, M. A. and Dutse, A. I. Human immunodeficiency Virus and Hepatitis B Virus Co-infection among Patients in Kano Nigeria. Niger J Med.; 2006; 15(3):227-9.
4. Manfredi R.; HIV Infection, Antiretroviral Therapy, and Hepatic Function. Emerging Epidemiological, Pathogenetic, and Clinical Issues and their Consequences on Disease Management (Editorial) .AIDS 2003; 17(15): 2253-2256.
5. Perrillo R.P., Regenstein F.G. and Roodsman S.T.; Chronic Hepatitis B in Asymptomatic Homosexual Men with Antibody to Human Immunodeficiency Virus. Ann Intern Med. 1986; 105:382-383.
6. Zylberberg H. and Pol S. Reciprocal interaction between human immunodeficiency Virus and Hepatitis C Virus infection. Clin. Infect. Dis. 1996; 1117-1125.
7. Ian Richardson, T. Melester and David Gold Liver Disease and HIV: Gay Men's Health Crisis "Treatment Issues", 1994; vol.8, no. 5.
8. Vic Hernandez. A Publication of the Seattle Treatment Exchange Project. Liver Function and HIV-1 infection. Step Perspective 1995; 7(2).
9. Blumberg, B.S.; The discovery of Australian Antigen and its relation to Viral Hepatitis. Vitro; 1971; 7: 223.
10. Wilber, J.C.; Development and Use of Laboratory Tests for Hepatitis C Infection. A Review. J. clin. Immunoassay 1993; 16:204.
11. Eskild A., Magnus P., Petersen G., Sohlberg C., Jensen F., Kittelsen P., Skaug K.; Hepatitis B Antibodies in HIV Infected Homosexual Men are associated with more rapid progression to AIDS. AIDS. 1992; 6 (6) 571-574.
12. Botti P., Pistelli A., Gambassi F., Zorn A.M., Caramelli L., Peruzzi S. Smorlesi C., Masini E. and Mannaioni P.F.; HBV and HCV infection in i.v. drug addicts; co-infection with HIV. Archives of Virology: 1992;. Supp: 4:329-32.
13. Hadler S.C. Judson F.N., O'Malley P.M., Altan N.L., Penley K., Buchbinder S., Schable C.A., Coleman P.J., Ostrow D.N., and Francis D.P., Outcome of Hepatitis B Virus Infection in Homosexual Men and it's relation to prior Human Immunodeficiency Virus Infection. Journal of infectious disease. 1991; 163 (3): 454-459.
14. Christine M. Kuka. HBV/HIV Co-infection: What You need To Know. Hepatitis C Support Project, 2004; 1.

TABLE 1: Shows HBsAg and HCV Screening results of HIV positive patients on ART and those that are not on ART.

		HBsAg	HCV
CONTROL SUBJECTS	n =100	1(1%)	0
HIV POSITIVE PATIENTS ON ART.	n =100	20 (20%)	23 (23%)
HIV POSITIVE PATIENTS NOT ON ART.	n =100	18 (18%)	16 (16%)

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Original Article

A SURVEY OF MATERNAL MORTALITIES IN ABIA STATE, SOUTHEAST NIGERIA

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ABSTRACT

Ascertaining the burden of maternal mortality is crucial to the improvement of maternal health for any country with limited resources. A facility-based survey was carried out in Abia State, Southeast Nigeria to ascertain the burden of maternal deaths and where they occur. Health facilities in the 17 local government areas of the State were enlisted. Data on births and maternal deaths in the preceding 12 months were obtained. A total of 25,081 births and 43 maternal deaths were recorded for the study period giving MMR of 171/100,000. The public facilities had higher values of MMR (856.8/100,000) than the private (177.2/100,000). Similarly, the comprehensive Essential Obstetric Care facilities (EOC) had lower MMR than the Non-EOC. Poor documentation may be one of the reasons for the low MMR in this study. Enforcing proper documentation, reporting and investigation of maternal deaths is hereby recommended.

Key words: *Maternal mortality, Health facilities, Essential obstetric care.*

INTRODUCTION

Approximately 600,000 women die annually from complications of pregnancy, child bearing and unsafe abortions worldwide[1, 2, 3]. About 99% of these deaths occur in the developing world[1,2]. Unfortunately, most of the affected countries have difficulties ascertaining their burden of maternal mortality, as the estimates are highly susceptible to inaccuracies[2, 4,5]. The difficulties in obtaining accurate data on maternal mortalities have created the need to explore other techniques that would give more reliable data. Some advocate the use of the sisterhood method of estimating maternal mortality. However, the sisterhood method is still being refined and the extent and impact of biases have only recently received attention[6]. Others advocate community-based surveys where maternal deaths are investigated by assessing records of health facilities and augmenting it with verbal autopsies of maternal deaths in the communities[5, 7]. In the interim, the World Health Organization has recommended a number of process indicators to monitor the effect of health programmes on maternal mortality in the developing world[8]. This only goes to reveal the exasperation experienced by experts in dealing with maternal mortality data in the third world. For example, for two consecutive periods (1999 and 2003), the Nigerian Demographic and Health Survey have been unable to come out with a national value of maternal mortality rate for the country as a result of the aforementioned difficulties[9,10].

Reviews of pregnancy related deaths by nations are important public health functions[11]. Every pregnancy related death need to be reported and investigated. In countries where this is enforced, data on maternal deaths is available and it enables them to adopt comprehensive strategies to tackle maternal health problems[12,13].

A survey was carried out on health facilities in Abia State, Southeast Nigeria to ascertain maternal deaths and where they occur. This was part of the National Study on Essential Obstetric Care (EOC) in Nigeria carried out by UNFPA and the Federal Ministry of Health.

MATERIALS AND METHODS

A facility-based assessment of maternal deaths was carried out in Abia State, Southeast Nigeria to determine the magnitude of maternal deaths and where they occur in the State. This was part of a national survey of Essential Obstetric Care facilities. All the seventeen Local Government

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Areas (LGAs) in the State were enlisted into the study. The list of all health facilities in each LGA was obtained and trained interviewers conducted the interview using standard questionnaire adapted from previous studies on maternal mortalities[14,15,16]. Data on child delivery and maternal mortalities recorded in health facilities in the preceding 12-months were obtained. The types of maternal services offered in the facilities were noted and were used to categorize them into 3 groups using the following criteria:

1. Parenteral antibiotics
2. Parenteral oxytocics
3. Parenteral sedatives
4. Manual removal of placenta
5. Removal of retained products of conception
6. Assisted vaginal delivery
7. Blood transfusion
8. Caesarean section.

Facilities that do not offer anyone of the services numbered 1 to 6 were classed as Non-Essential Obstetric Care. Facilities that offer all the services numbered 1 to 6 were classed as Basic Essential Obstetric Care. Facilities that offer all the services numbered 1 to 8 were classed as Comprehensive Essential Obstetric Care.

RESULTS

A total of 370 health facilities that offer maternal services were surveyed in the 17 Local Government Areas of the State. These were made up of 215 Primary Health Centres/Maternity Homes (both public and private facilities) and 155 secondary/tertiary health care facilities. There were a total 249 (67.3%) health facilities that did not meet the criteria for Essential Obstetric Care while those that met the criteria for Basic Essential Obstetric Care and Comprehensive Essential Obstetric Care were 42 (11.4%) and 79 (21.4%) respectively. There is a fair distribution of maternal health facilities across all the local government areas of the State. However, the 6 urban LGAs of of Aba North, Aba South, Osisioma, Ugwunagbo, Umuahia North and Umuahia South had 31 of the Basic Essential Obstetric Care facilities and 61 of the Comprehensive Obstetric Care facilities while the 11 rural LGAs had only 11 Basic Essential Obstetric Care and 18 Comprehensive Essential Obstetric Care facilities, table 1.

A total of 43 maternal deaths and 25,081 births were recorded during the study period giving a maternal mortality ratio of 171 per 100,000 live births. The 6 urban local government areas had a disproportionate share of the deliveries in the State as they accounted for 73.3% (18391) of it. The urban LGAs recorded a total of 23 maternal deaths while the rural LGAs recorded 20 giving maternal mortality ratio of 125 per 100,000 for the urban LGAs and 299 per 100,000 for the rural LGAs. There is no statistical difference between the two values (x^2=0.3307, df=1, p>0.05), table 2.

Only one maternal death was recorded in all the primary care facilities in the State and that occurred in a Basic Essential Obstetric Care facility giving maternal mortality ratio of 13/100,000 for primary care facilities. So while the primary care facilities accounted for 30.5% of the births in the State, they represented only 2.3% of the maternal deaths.

Undue share of the maternal deaths were borne by the secondary public health care facilities. For while they represented only 6.5% (1634) of the births, they accounted for 32.6% of the recorded maternal deaths giving a maternal mortality ratio of 856.8 per 100,000 live births. The private secondary healthcare facilities catered for the majority of the deliveries in the State accounting for 63% (15,801) while an equivalent proportion of maternal deaths (65%; 28 deaths) were recorded by them during the same period giving MMR of 177/100,000, table 3. The private Non-EOC facilities recorded 3,956 deliveries and 14 maternal deaths giving MMR of 354/100,000 while the private Comprehensive EOC recorded 11,135 deliveries and 14 maternal deaths giving MMR of 126/100,000. It is worthy of note to observe that in both the secondary public health facilities and in the secondary private health facilities that those health facilities that were Non-Essential Obstetric Care fared poorly in their maternal mortality ratio. While the public Non-Essential Obstetric Care facilities recorded a maternal mortality ratio of 1070/100,00, the public Comprehensive Essential Obstetric Care facilities recorded a maternal mortality ratio of 829/100,000. However, there is no statistical difference between the two (x^2 =0.1104, df=1, p>0.05). Similarly, while the private Non-Essential Obstetric Care facilities recorded a maternal

mortality ratio of 354/100,000, the private Comprehensive Essential Obstetric Care facilities recorded a maternal mortality ratio of 126/100,000. The difference is statistically significant ($x^2 = 8.1659$, df=1, $p<0.05$).

DISCUSSION

The large number of primary care facilities in the State involved in the delivery of maternal services is very encouraging. This may be a direct effect of the primary healthcare programme in the country, which has resulted in the establishment of several primary healthcare centres in LGAs of the State. The large presence of health facilities in the urban areas is responsible for the relatively large numbers of deliveries and maternal deaths recorded in them. Besides, the urban dwellers are expected to make better use of orthodox health facilities than their rural counterparts. Close to 70% of the maternal health facilities in the State are Non-Essential Obstetric Care and as such, cannot cope with the major causes of maternal mortalities which are haemorrhage, prolonged obstructed labour, sepsis and pregnancy induced hypertension[5, 14,17,18]. The preponderance of such weak healthcare facilities in the country might explain the high maternal mortality ratio of Nigeria.

The total number of maternal deaths of 43 recorded in the State with MMR of 171 per 100,000 for the study period is low. This may be as a result of poor documentation of records of maternal deaths by health facilities. Health facilities in the country exhibit laissez-faire attitude towards records of mortality neither are they compelled to notify appropriate authorities of the occurrence of deaths in their facilities nor are there provisions for investigation of the deaths. Similarly, compliance to the issuance of death certificates to relations of the deceased before burial is not strictly observed. As a result, data on mortality is haphazardly maintained and their retrieval an uphill task. Instituting necessary regulations guiding the documentation, notification and reviewing of maternal deaths is necessary for proper implementation of maternal health programmes in the country[5]. Until we do this, we may never have reliable data on maternal deaths nor have yard sticks to monitor maternal health in the country and will be compelled to use process indicators to monitor the progress of maternal health programmes in the country as recommended by the World Health Organisation[8].

The low MMR of 171/100,000 recorded in this study may not completely appear strange as a previous survey have found similarly low MMR for the country. The 1993 Nigeria Demographic and Health Survey obtained MMR of 289/100,000 for the nation which was however turned down on the grounds that the value does not tally with the other development indices of the country at that time[9]. So while exploring explanations for the low MMR in this study, it might be necessary to also consider that the true MMR for the nation may not necessarily be as high as the currently accepted value of 800/100,000.

The low maternal mortality ratio in the primary care facilities may be as a result of the fact that the services at the primary health centres/maternity homes are patronised more by mothers with low risk of obstetric complications or that when pregnant mothers are perceived to be at risk of major obstetric complications, they are referred to higher levels of care. The other explanation may be poor documentation of maternal deaths at the primary care level.

A number of reasons may account for the high MMR obtained in public secondary healthcare facilities in the State. The public health facilities are more likely to comply with documentation of maternal mortality data since maintenance of health records is supposed to be part of their routine duties. Besides, they are more likely to have trained staff in their employ to carry out such responsibilities. In addition, the public health facilities are more likely to accept patients in bad state of health and are likely to receive large numbers of referrals for mothers with obstetric emergencies. Another reason could be the fact that many public referral centres are ill prepared to cope with obstetric emergencies as a national survey revealed that less than a third of the public sector referral health facilities in Nigeria met the standard for Comprehensive Essential Obstetric Care[19]. Besides, even in situations where public referral centres meet the EOC criteria for comprehensive EOC, many of them offer the services when it is too late to salvage the lives of mothers[20,21,22].

The classification of health facilities on the Essential Obstetric Care status correlated well with their performance on MMR. Both public and private health facilities that did not meet the EOC criteria fared poorly in the handling of maternal health as the Non-EOC facilities recorded higher MMR than those facilities that were classified as Comprehensive EOC. The populace generally regard most general hospitals and 'private hospitals and maternities' (as most private hospitals offering obstetric services are designated) as referral centres for obstetric emergencies without knowledge of their EOC status. As a result, they make referrals to them without knowledge of their competence. It might be necessary to properly designate all health facilities offering maternal services in the country based on their EOC status and the populace properly informed so that decisions on referral of obstetric emergencies to facilities will be based on evidence of competence.

The low MMR recorded in the private Comprehensive EOC is very encouraging. It reveals their competence in handling obstetric emergencies. No wonder such facilities had good patronage and played significant role in child deliveries accounting close to 50% of all the births in the State. Another likely reason for the low MMR in private Comprehensive EOC is the fact that the facilities may not be forthcoming with data on maternal deaths as they may feel that providing information on maternal deaths may damage the reputation of their hospitals. In the absence of strong regulation on maintenance, reporting and investigation of maternal mortality, health facilities are likely to continue to be unserious in their handling of maternal data.

Maintenance of data on maternal deaths is crucial to the implementation of maternal health programmes in the country. It is high time that necessary measures are put in place to effect proper handling of maternal records. This will provide the baseline data for planning, implementation, monitoring and evaluation of maternal health programmes. Until we do this, we are not likely to make reasonable impact in this area.

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REFERENCES

1. Kurjak A, Bekavac I. Perinatal Problems in Developing Countries: lessons learned and future challenges. J-Perinat-Med. 2001; 29(3): 179-87.
2. Ghosh MK. Maternal Mortality. A Global Perspective. J Reprod Med. 2001; 46(5): 427-33.
3. Hill K, AbouZhar C, Wardlaw T. Estimates of Maternal Mortality for 1995. Bull World Health Organ. 2001; 79(3): 182-93.
4. Robinson JJ, Wharrad H. The Relationship Between Attendance at Birth and Maternal Mortality Rates: an exploration of United Nations data sets including the ratios of physicians and nurses to population, GNP per capita and female literacy. J Adv Nurs. 2001; 34(4): 445-55.
5. Bouvier-Colle MH, Ouedraogo C, Dumont A, Vangeenderhuysen C, Salanave B, Decam C. Maternal Mortality in West Africa. Rates, causes and substandard care from a prospective survey. Acta Obstet Gynecol Scand 2001; 80(2): 113-9.
6. Smith JB, Fortney JA, Wong E, Amatya R, Coleman NA, de-Graft-Johnson J. Estimates of the Maternal Mortality Ratio in Two Districts of the Brong-Ahafo Region, Ghana. Bull World Health Organ 2001; 79(5): 400-8.
7. Olsen BE, Hinderaker SG, Bergsjo P, Lie RT, Olsen OH, Gasheka P, Kvale G. Causes and Characteristics of Maternal Deaths in Rural Northern Tanzania. Acta Obstet Gynecol Scand 2002; 81(12): 1101-9.
8. Gottlieb P, Lindmark G. WHO Indicators for Evaluation of Maternal Healthcare Services, Applicability in Least Developed Countries: a case study from Eritrea. Afr J Reprod Health 2002; 6(2): 13-22.
9. National Population Commission (Nigeria). 2000. Nigeria Demographic and Health Survey

1999. Calverton, Maryland: National Population Commission and ORC/Macro: 112-113.
10. National Population Commission (NPC) [Nigeria] and ORC Macro.2004. Nigeria Demographic and Health Survey 2003. Calverton, Maryland: National Population Commission and ORC Macro.
11. Berg CJ, Chang J, Callaghan WM, Whitehead SJ. Pregnancy Related Mortality in the United States, 1991-1997. Obstet Gynecol 2003; 101(2): 289-96.
12. Panchal S, Arria AM, Labhsetwar SA. Maternal Mortality During Hospital Admission for Delivery: a retrospective analysis using a state-maintained database. Anesth Analg 2001; 93(1): 134-41.
13. Horon IL, Cheng D. Enhanced Surveillance for Pregnancy-associated Mortalitymary Land, 1993-1998. JAMA 2001; 285(11): 1455-9.
14. Maine D, Akalin MZ, Ward VM, Kamara A. The Design and Evaluation Of Maternal Mortality Programs. Center for population and family health, School of Public Health, Columbia University, New York 1997.
15. UNICEF/WHO/UNFPA (1997). Guidelines for Monitoring the Availability and Use of Obstetric services. New York, UNICEF, 1997.
16. United Nations. The Millennium Declarations. Resolution A/RES/55/2. New York, 2000. WHO/UNFPA/UNICEF/World Bank. Reduction of Maternal Mortality: A joint statement by WHO/UNFPA/UNICEF/World Bank. Geneva, WHO, 1999.
17. Olukoya A, Ogunyemi Ma, Akitoye C O et al. Upgrading Obstetric Care At A Secondary Referral Hospital, Ogun state, Nigeria. Int J Gynecol Obstet 1997; 59 (Suppl 12) S2225-S230.
18. Orji E O, Ogunlola I O, Onwudiegwu U. Brought in Maternal Deaths in South West Nigeria. J Obstet Gynaecol 2002; 22(4): 385-8.
19. Federal Ministry of Health, Abuja. National Study on Essential Obstetric Care Facilities in Nigeria, 2003; 9-18.
20. Orji E O, Fasubaa OB, Onwudiegwu U, Dare FO, Ogunniyi SO. Decision-Intervention Interval In Ruptured Uteri in Ile-Ife, Nigeria. East Afr Med J. 2002; 79(9):496-8.
21. Okaro JM, Umezulike AC, Onah HE, Chukwuali LI, Ezugwu OF, Nweke PC. Maternal Mortality at the University of Nigeria Teaching Hospital, Enugu, before and after Kenya. Afr J Reprod Health. 2001;5(2) 90-7.
22. Olsen BE, Hinderaker SG, Bergsjo P et al. Causes and Characteristics of Maternal Deaths in Rural Northern Tanzania. Acta Obstet Gynecol Scand; 2002; 81(12):1101-9.

Table 1: Classification of health facilities according to Essential Obstetric Care status in Local Government Areas in Abia State.

LGAs	Types of health facilities			
	Non-Essential Obstetric Care services	Basic Essential Obstetric Care services	Comprehensive Essential Obstetric Care services	Total
Aba North	3	4	15	22
Aba South	30	16	22	68
Arochukwu	14	1	1	16
Bende	43	6	1	50
Ikwuano	15	0	0	15
Isiala Ngwa North	16	0	4	20
Isiala Ngwa South	10	0	2	12
Isuikwuato	29	0	2	31
Ohafia	15	0	2	17
Obingwa	7	1	2	10
Osisioma	9	6	9	24
Ugwunagbo	6	4	1	11
Ukwa East	5	1	2	8
Ukwa West	7	1	1	9
Umuahia North	9	0	11	20
Umuahia South	10	1	3	14
Umunneochi	21	1	1	23
Total	**249**	**42**	**79**	**370**

Table 2: Distribution of births and maternal deaths in Local Government Areas in Abia State.

LGAs	Number of births	Number of maternal deaths
Aba North	2204	0
Aba South	10037	11
Arochukwu	449	1
Bende	947	0
Ikwuano	623	0
Isiala Ngwa North	213	4
Isiala Ngwa South	557	0
Isuikwuato	754	1
Ohafia	852	11
Obingwa	1085	2
Osisioma	1955	2
Ugwunagbo	460	0
Ukwa East	188	1
Ukwa West	276	0
Umuahia North	2980	10
Umuahia South	755	0
Umunneochi	746	0
Total	**25,08 1**	**43**

Table 3: Distribution of births and maternal deaths according to types of health facilities

Type of health facilities			Births	Maternal deaths	Maternal mortality Rate
Primary care facilities:	Primary Health Centers and Maternity Homes (Both public and private)	Non-Essential Obstetric Care facilities	5,894	0	0
		Basic Essential Obstetric Care facilities	1,752	1	57.1/100,000
			7,646	**1**	**13.1/100,000**
Secondary and tertiary care facilities	Public care facilities	Non-Essential Obstetric Care facilities	187	2	1069.5/100,000
		Comprehensive Essential Obstetric Care facilities	1,447	12	829.3/100,000
			1634	**14**	**856.8/100,000**
	Private care facilities	NOn-Essential Obstetric Care facilities	3956	14	353.9/100,000
		Basic Essential Obstetric Care facilities	710	0	0
		Comprehensive Essential Obstetric Care facilities	11,135	14	125.7/100,000
			15,801	**28**	**177.2/100,000**
Total			**25,081**	**43**	**171.4/100,000**

METHOD

Method for HIV screening was as previously described by Onyenekwe et al [7]. The Bromocresol Green (BCG) method was used to determine serum Albumin concentration, while the Haemoglobin concentration was determined by Cyanomethaemoglobin method.

Detection of UIBC and TIBC: The UIBC and TIBC were determined using the ferrozine method. The procedure for the detection of UIBC and TIBC was as described by the manufacturer of the iron/TIBC reagent (TECO Diagnostic, Anaheim, USA). In brief, the test tubes for the detection were pre-treated with HCL and rinsed several times with de-ionised water after which the test tubes were appropriately labelled as participants, standard and blank tubes. Into the respective tubes was added 2.0ml of unsaturated iron binding capacity buffer reagent. While 1.0ml of iron free water was added to the blank tube, 0.5ml of iron standard solution plus 0.5ml of iron free water were added to standard tube and 0.5ml of the respective participants samples plus 0.5ml iron standard solution were added to the appropriately labelled participant's tubes. The reagent blank was used to zero the spectrophometer at 560nm wavelength. Then the absorbance of the participant's test samples was read and recorded as A_1 reading. Then 0.5ml of iron colour reagent was added to the content of all the tubes respectively; and the tubes were placed in a heating bath at 37°C for 10 minutes. The reagent blank was again used to zero the spectrophometer at 560nm before a second reading was taken as A_2 for the respective participants tests and standard. The under listed formula was used to calculate UIBC.

UIBC (ug/dl) = **Conc. of STD - (A_2 test - A_1 test)/ (A_2 S-A_1 S) x Conc. of STD/ 1**

TIBC ug/dl = serum iron concentration + UIBC

Detection of serum iron: The serum iron was determined photometrically using Iron/TIBC reagent (TECO diagnostics, Anaheim, USA). The principle of the test is based on the dissociation of iron from the transferrin complex by the addition of acidic buffer containing hydroxylamine. The procedure was as described by the manufacturer of the kit. In brief; the test tubes were pre-treated with HCL and rinsed severally with de-ionised water. Into the appropriately labelled participant's tubes, standard tube and blank tube were added 2.5ml iron buffer reagents. This was followed by addition of 500ul of participant's sample to respective tubes. Similarly, 500ul of iron free water was added to the blank tube while 500ul of iron standard was added to the standard tube. The blank solution was used to zero the spectrophotomer at 560nm. The initial absorbance of the standard and respective participants tests was read A_1. Thereafter, 0.55ml of iron colour reagent was added to all the tubes. The contents of the tubes were mixed properly and then placed in the water-bath at 37°C for 10 minutes after which the blank solution was used to zero the spectrophotometer 560nm and the absorbance of all the tubes was read as A_2. Serum iron concentration was calculated from the formula:

Serum iron (ug/dl): $[A_2T - A_1T / A_2S - A_1S]$ *x conc. of STD*

STATISTICAL ANALYSIS

The variables were expressed as mean ±SD. The student-t test was used to determine significant difference in mean. Significant level was considered as $P<0.05$.

RESULTS

The mean ±SD serum iron concentration (ug/dl), TIBC (ug/dl) and Albumin (g/l) were significantly lower in Asymptomatic HIV subjects compared with control participants ($P<0.01$ in each case). However, the mean ±SD UIBC (ug/dl) and Haemoglobin (g/dl) were not significantly different from both groups. See table 1.

Sex differentiation was only observed in both control and HIV participants with haemoglobin. No other parameter showed sex differentiation. See table 2 and table 3.

DISCUSSION

A significant drop in serum concentrations of iron in HIV infected subjects was observed in the present study. This could be an indication that they may be susceptibility to anaemia. Studies have reported high prevalence of anaemia in sub-Saharan Africa that was linked to under-nutrition [1,2]. Anaemia has serious impact on the quality of lives of HIV/AIDS patients.

The reduced serum iron and TIBC in HIV infected subjects in the present study, suggest anaemia of chronic disease. In most Health Institutions in Nigeria, Haemoglobin level and the packed cell volume are the most commonly used makers' for detection of iron status and iron deficiency anaemia. However, In the present study we did not observe any significant difference in Haemoglobin level between the HIV infected subjects and control subjects. If in the present study we had relied on Haemoglobin level alone to determine iron status in HIV infected subjects, we would have missed the diagnosis of anaemia.

Studies have reported that two or more serum iron makers should be used to determine the presence of anaemia [8,9,10]. The use of Haemoglobin with other parameters as TIBC or UIBC and percent transferrin saturation has been suggested. The use of two or more serum iron makers could also help to identify the presence of excess free iron and iron overload. The mean serum albumin concentration was lower in HIV positive subjects. The reduced serum albumin level in HIV infected participants could be due to suppression of its synthesis in the liver. It could also be due to the impact of HIV infection on their host ability to utilize nutrients intake. Serum albumin levels have been shown to be useful in predicting mortality due to HIV infection [7,11,12,13].

The study concludes that HIV infection predisposes to anaemia of chronic disease. Therefore, we suggest that blood iron status be monitored in HIV infected subjects for early detection and treatment of anaemia in order to reduce the morbidity and mortality associated with anaemia. Furthermore, the strength of Haemoglobin as an indicator of anaemia is weak and may lead to missed diagnosis of iron status.

REFERENCES

1. Eley B.S, Sive A.A, Shuttleworth M, Hussey G.D. (2002), A Prospective, Cross-sectional Study of anaemia and Peripheral Iron Status in Antiretroviral Naïve, HIV-1 Infected Children in Cape Town, South Africa. BMC Infectious Diseases, 2(3). Http:www.biomed central. Com/1471-2334/2/3.
2. Tottin D., Ndugiva C. Mmiro F, Perry RT Jackson JR, Semba RD. 2002, Iron Deficiency Anaemia is Highly Prevalent among Human Immunodeficiency Virus Infected and Uninfected Infants in Uganda. J Nutr. 132(3): 423-9.
3. Kupka R, Msamanga G.I, Mugusi F, Petraro P, Hunter D.J, Fawzi W.W. (2007), Iron Status is an important cause of Anemia in HIV-Infected Tanzanian women but is not related to accelerated HIV Disease Progression. J. Nutr. 137:2317-2323.
4. Dikshit B, Wanchu A, Sachdeva R.K, Sharma A, Das R. (2009), Profiles of Haematological Abnormalities of Indian HIV Infected Individuals. BMC Blood Disorders, 9:5. http: www.biomedcentral.com/1471-2326/9/5.
5. Cohen C, Karstaedt A, Frean J, Thomas J, Govender N, prentice E, Dini L, Galpin J, Crewe-Brown H.(2005), Increased Prevalence of Severe Malaria in HIV- Infected Adults in South Africa. Clin. Infect. Dis. 41(11):1631-1637.
6. Onyenekwe C.C, Ukibe N, Meludu S.C, Ilika A, Aboh N, Ofiaeli. N, Ezeani M, Onochie A. (2007), Prevalence of Malaria as Co-Infection in HIV-Infected Individuals in a Malaria Endemic Area of Southeastern Nigeria. J. Vector Borne Dis. 44:250-254.
7. Onyenekwe C.C, Ukibe N, Meludu S.C, Ifeayi M, ezeani M, Onochie A, Ofiaeli N, Aboh A, Ilika A. (2008), Possible Biochemical Impact of Malaria Infection on subjects with HIV Co-Infection in Anambra State, Nigeria. J. Vector Borne Disease, 45; 151-156.
8. Gambino R, Desvarieux E, Orth M et. al (1997), The Relationship between Chemically Measured Total Iron-binding Concentrations and Immunologically Measured Transferrin Concentrations in Human Serum. Clin. Chem..43(12): 2408-2412.
9. Kasvosve I, Delanghe J. (2002), Total Iron Binding Capacity And Transferrin Concentration in the Assessment of Iron Status. Clin. Chem. Lab. Med. 40(10):1014-1018.
10. Yamanishi H, Iyama S, Yamaguchi Y, kanakura Y, Iwatani Y. (2003), Total Iron Binding Capacity Calculated from Serum Transferrin Concentration or Serum Iron Concentration and Unsaturated Iron-Binding

capacity. Clin. Chem. 49 (1):175-178.
11. Onwuliri V.A (2004), Total Bilirubin, Albumin, Electrolyte and Anion Gap in HIV positive patients in Nigeria. J. Med. Sci., 4(3): 214-220.
12. Joseph G., Feldman, P.H, David N. (2000), Serum Albumin as a Predictor of Survival in HIV Infected Women in the Women Interagency HIV Study. AIDS,14:(7); 863-870.
13. Olawumi H.O and Olatunji P.O.(2006), The Value of Serum Albumin In Pre-treatment assessment and Monitoring of Therapy in HIV/AIDS Patients. HIV Med,7:357-355.

TABLE 1 : Mean ± SD Serum Concentrations of Iron ((µg/dl) UIBC (µg/dl), TIBC (µg/dl) Albumin (g/l) and haemoglobin (µg/dl) in HIV seropositive group and control group.

Parameters	HIV (n=76)	Control (n=30)	P-value
Serum iron (µg/dl)	88.51± 45.62	210.38± 78.94	(P<0.01)
UBIC (µg/dl)	204.59± 84.57	243.89± 112.53	(P>0.05)
TIBC (µg/dl)	293.36±76.91	444.26 ±122.46	(P<0.01)
Albumin (g/l)	32.86 ± 4.98	37.60± 3.32	(P<0.01)
Hb (g/dl)	11.3±2.0	11.5±1.1	(P>0.1)

Key: Hb = Haemoglobin
HIV = asymptomatic HIV with and without malaria co-infection

Table 2: Sex Distribution ofMean ±SD Serum Concentrations of Iron ((µg/dl) UIBC (µg/dl), TIBC (µg/dl), Albumin (g/l) and haemoglobin (µg/dl) in HIV Seropositive Group

Sex	Male (n=36)	Female (n=40)	P-value
Serum iron	96.17±49.83	81.61±40.87	(P>0.1)
UIBC	199.29±79.40	209.36±89.71	(P>0.1)
TIBC	296.00±68.75	290.00±84.39	(P>0.1)
Albumin	33.88±3.71	31.94±5.78	(P>0.1)
Hb	11.8±1.7	10.8±1.9	(P<0.05)

Table 3: Sex Distribution ofMean ±SD Serum Concentrations of Iron ((µg/dl) UIBC (µg/dl), TIBC (µg/dl), Albumin (g/l) and haemoglobin (µg/dl) in Control Group

Sex	Male (n=15)	Female (n=15)	P-value
Serum iron	233.05±83.16	187.71±69.95	(P>0.1)
UIBC	220.22±110.22	267.55±113.49	(P>0.1)
TIBC	433.27±137.88	455.26±108.89	(P>0.1)
Albumin	37.65±4.18	37.54±2.187	(P>0.1)
Hb	12.1±1.0	10.9±0.9	(P<010)

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Original Article

BLOOD GLUCOSE CONTROL AND BODY WEIGHT OF EXPERIMENTAL DIABETIC RATS CO-TREATED WITH MICRO NUTRIENTS

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ABSTRACT

Disturbance in blood glucose and fuel metabolism are hallmarks in diabetes mellitus, and antioxidants are believed to play a role in the control of this disturbances. In the current work, antioxidant such as manganese (10mg/kg/body weight), copper (2mg/kg/body weight) and zinc (15mg/kg/body weight) were supplemented in alloxan-induced diabetic rats for a period of 4 weeks. Initial and final Fasting Blood Glucose (FBG) of the rats was 77.29±8.65mg/dl and 75.00±7.22mg/dl in controls, 408.14±49.44mg/dl and 107.00±11.07mg/dl in diabetics treated not supplemented, and 448.14±43.18mg/dl and 83.14±5.45mg/dl in the diabetic treated and supplemented respectively. There was statistically significant difference in the final FBG concentration of supplemented and unsupplemented groups ($p<0.05$). Initial and final body weight was 155.14±4.25g and 157.57±4.16g in controls, 147.14±7.91g and 143.43±8.70g in diabetics treated not supplemented, and 159.86±13.15g and 184.71±11.50g in the diabetic treated and supplemented respectively. There was statistically significantly difference in the final body weight of supplemented and unsupplemented rats ($p<0.05$). In conclusion, supplementation with anticxidant micronutrients might improve blood glucose control and improve body wastages usually experienced in diabetics.

Key words: *Diabetes, Micro nutrients, Blood Glucose, Body Weight.*

INTRODUCTION

Requirement for micro nutrients is defined as an intake level which meets specific criteria for adequacy, thereby minimizing the risk of nutrient deficit, which is usually determined and measured through subclinical conditions, identified by specific biochemical makers. Biochemical assays have been the most relevant indices of measuring subclinical conditions relevant to vitamins and minerals intake[1]. In patient with diabetic mellitus, decreased levels of antioxidant micro nutrient have been reported[2]. It is logical therefore, that this decrease might negatively influenced the activities of major antioxidant defense enzymes in the body, which require these micro nutrients as their cofactors and co-enzymes, with resulting elevation of markers of lipid peroxidation. Dallatu *et al*[3] reported supplemented with antioxidant micronutrients, had improved the activities of some *denovo* antioxidant defence enzymes in alloxan-induced diabetic rats. Diabetics experienced a wide range abnormal fuel metabolism secondary to relative or complete absence of insulin. This trigger counter reactions and activities in which body fats and protein are mobilized to counter the effect of pseudohypoglycaemia. These result into polyphagia, polydipsia and polyuria, with attending nutrient lost and body wastages. Stephen[4] reported that dietary supplements can promote healthy blood glucose, healthy blood cholesterol, healthy immune system, and healthy digestive function and play a useful adjunctive role in the control of colorie intake. The purpose of the current research is therefore, to study the effect of supplementation with antioxidant micronutrient, on blood glucose homeostasis and body weight of alloxan-induced diabetic rats.

MATERIALS AND METHODS

Experimental Animals: Male albino wistar rats (120-180 g) were purchased from Animal House, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria. The animals were housed for a

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period of one week under similar conditions in standard cages at 25±2°C, with 12-hour light/dark cycle. The animals were maintained on poultry feed (Vital Feeds, Jos) *ad libitum*.

Chemicals: All the reagents used for the study were of analytical grade. Alloxan was purchased from Sigma Aldrich Chemical Co. (U.K), kits for the assay of serum glucose was purchase from Randox Laboratories. Micronutrients and normal saline were purchased from a reputable pharmacy in Zaria Town, Kaduna State, Nigeria.

Induction of Diabetes: Experimental diabetes was induced by a single intraperitonial injection of freshly dissolved Alloxan (150 mg/kg b.w) in normal saline maintained at 37°C, to rats fasted for 12 hours. Control rats received a similar volume of normal saline alone. After 72 hours of alloxan injection, the animals were fasted overnight and their fasting blood glucose were estimated using a commercial glucose kit. Only rats that had fasting blood glucose level of 126 mg/dl (> 7.00 mmol/l) and partial destruction of pancreas tested with positive response to metformin were included in the study.

Experimental Design: The rats were divided into 3 groups of 7 rats each:- Group 1.: (Control); Group 2. Diabetic + metformin (250 mg/kgbw) (D.T.N.S); Group 3. Diabetic + metformin (250 mg/kgbw) + copper (2 mg/kgbw) + manganese (10 mg/kgbw) + zinc (15mg/kgbw) (D.T.S.M). The supplementation lasted for one month and after the last day; the animals were fasted overnight and anaesthesized by dropping each in a transparent plastic jar saturated with chloroform vapour. Incision was made on the abdomen. Blood sample was collected through cardiac puncture and divided into plain and EDTA-containing centrifuge tubes. Humane procedure was adopted throughout the experiment.

Measurement of Biochemical Analytes: Blood glucose concentration was assayed as described by the method of Trinder[5]. 10µl of serum sample was mixed with 1ml of glucose oxidase reagent, and incubated as 37°C for 30 minutes. Absorbance was taken at 505nm. Digital bench weight balance was used to measure the weights.

Statistical Analysis: All data were expressed as the Mean ± Standard Error of Mean (S.E.M). Data were analyzed using Analysis of Variance (ANOVA) InStat3 Software. Differences in mean were considered to be significant from $p<0.05$.

RESULTS

The results of the current study were presented in Tables 1 and 2. Initial and final fasting blood glucose was 77.29±8.65mg/dl and 75.00±7.22mg/dl in control, 408.14±39.44mg/dl and 107.00±11.07mg/dl in diabetic treated non supplemented, and 448.14±43.18mg/dl and 84.14±5.45mg/dl in the diabetic group treated and supplemented. There was statistically significance difference in the final blood glucose level supplemented between result and unsupplemented groups ($p<0.05$). The initial and final body weight of the experimental animals was 155.14±4.25g and 157.57±4.169 in the controls, 147.14±7.91g and 143.43±8.70g in the diabetic group treated not supplemented, and 159.86±13.15g and 180.71±11.50g in the diabetics treated and supplemented. There was statistically significance difference between the final body weight of the supplemented and unsupplemented groups ($p<0.05$).

DISCUSSION

Dietary pattern changes overtime and these changes are dependent on such factors like agricultural practices, cultural and socioeconomic considerations. Medically, certain disease conditions necessitate alterations in life style, food intake and in certain circumstances, the need for supplementation to meet the basic health requirements as dictated by a particular disease condition[1].

In the present study, the effect of diabetes mellitus on blood glucose control and body wastage is highlighted, supplementation with antioxidant micronutrients, have positively influenced the blood glucose regulation and improved the body weight of the supplemented subjects. This is in agreement with the finding of Song *et al*[6] who reported a decrease in food and water intake, and subsequent reduction in body weight of streptozotocin-induced diabetic rats. Adeneye, *et al*[7] reported an improved lowering of blood glucose in alloxan-induced diabetic rats, treated with

metformin and supplemented with vitamin C. Mark and Ely[8] reported that appropriate micronutrient supplementation can improve glucose tolerance and reduce auto-oxidation.

El-Beshbishy[9] reported an increase in the body weight of experimental rats, after supplementation with extract of green tea, believed to be rich in antioxidant micronutrients. Lacey *et al*[10] reported that, ROS cause damage to lipid membrane, intracellular protein and DNA, and are believed to be efficient inducers of apoptosis. Jakus[11] reported that consequences of oxidative stress are damage to DNA, lipids, proteins, disruption of cellular homeostasis and accumulation of damaged molecules.

Trace elements are part of, and interact with enzymes and hormones that regulate the metabolism of large amount of substrate[12]. As such, deficiency must affect their metabolism including glucose. Oxidative damage to cell component is reported to be diverse, and the attack is non-specific[13]. This could be the basis of body wastage and reduction in weight observed in the current work.

It is generally recognized that, certain group of patients, including diabetics, are at risk of free radical initiated damage, and supplementation with antioxidant micronutrients might therefore, modify their antioxidant defenses, and minimize the potential danger associated with the result. We therefore, recommended the inclusion of antioxidant nutrients in the treatment of diabetics.

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REFERENCES

1. FAO/WHO. Vitamins and Minerals Requirement in Human Nutrition: Report of a Joint FAO/WHO Expert Consultation. Bankok, Thailand. 2000.
2. Adewumi, M.T., Njoku, C.H., Saidu, Y., Abubakar, M.K., Shehu, R.A and Bilbis, L.S. Serum Chromium, Copper and Manganase Levels of Diabetic Subjects in Katsina, Nigeria. *Asian Journal of Biochemistry*. 2007; 2(4): 284 288.
3. Dallatu, M.K., Anaja, P.O., Bilbis, L.S., Mojiminiyi, F.B.O. Antioxidant Micronutrients Potentials in Strengthening the Antioxidant Defence in Alloxan-Induced Diabetic Rats. *Nigeria Journal of Pharmaceutical Services*. 2009; 8(1): 89-94.
4. Stephen, H. Diabetes and Syndrome X. http://www.powersurge.com. 2005.
5. Trinder, P. Annals of Biochemistry: In. Cheesbrough, M. (1992), Medical Laboratory Manual for Tropical Countries, ELBS, Cambridge. 1969; Vol. 1 (2nd ed.): 527-545.
6. Song, M.K., Roselthal, M.J., Song., A.M., Uyemura, K., Yang, H., Ament, M.E., Yanaguchi., D.T., and Comford, E.M. Body Weight Reduction in Rats by Oral Treatment with Zinc Plus Cyclo- (His-Pro). *Br. J. Pharmacol*. 2009; 158(2): 442-50.
7. Adeleye, A.A., Amole, O.O., Agbaje, E.O., Izegbu, M.C and Elias, S.O. Effect of Chronic Co-administration of Metformin and Vitamin C on Plasma Glucose and Lipid Concentrations. *Afr. J. Med. Sci. 2007;* 36(2): 149-54.
8. Mark, F.C and Ely, J.R. Rationale for Micronutrients Supplementation in Diabetes. Medical Hypothesis. 1984; 13: 139-151.
9. El-Beshbishy, H.A. Hepatoprotective Effect of Green Tea Extract against Tamoxiten-Induced Liver Injury in Rats. *Asian Journal of Biochemistry and Molecular Biology*. 2005; **38(5)**: 563-570.
10. Lasey, C.J., Murphy, M.E., Sanderson, M.J., Monteiro, E.F., Vail, A. and Schorah, C. Antioxidant Micronutrients and HIV Infection. *International Journal of STD and AIDs*. 1996; **7**: 485-9.
11. Jakus, V. The Role of Free Radicals, Oxidative Stress and Antioxidant Systems in Diabetic Vascular Disease. *Bratisl Lek Listy*. 2000; 101(10): 541-51.
12. Isah, H.S. Essential Clinical Biochemistry. First ed., Tanaza Publishing Company, Zaria, pp. 2007; 145-147.
13. Zhu, Y.I. and Hassi T.D. Iron Depletion without Anemia and Physical Performance in Young Women. *American Journal of Clinical Nutrition*. 1997; **66**: 334-341.

Table 1 **Initial and Final Fasting Blood Glucose (mg/dl) [#] of Alloxan Induced Diabetic Rats Supplemented with Antioxidant Micronutrients**

Group	Initial FBC	Final FBC
Control (n=7)	77.29±8.65	75.00±7.22
Diabetic treated only (n=7)	408.14±39.44[*]	107.00±11.07[*]
Diabetic treated supplemented (n=7)	448.14±43.18[*]	83.14±5.45[**]

±± Values are Mean, ± Standard Error of Mean of Alloxan -Induced Diabetic Rats Supplemented with Antioxidant Minerals for 28 days. [*] Values Differ Significantly From Controls. [**] Values Differ Significantly From Unsupplemented.

Table 2 **Initial and Final Body Weight (g)[#] of Alloxan Induced Diabetic Rats Supplemented with Antioxidant Micronutrients**

Group	Initial FBC	Final FBC
Control (n=7)	155.14±4.25	157.57±4.16
Diabetic treated only (n=7)	147.14±7.91	143.43±8.70[*]
Diabetic treated supplemented (n=7)	159.86±13.15	180.71±11.50[**]

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Original Article

KNOWLEDGE OF DISEASE CAUSATION AND NUTRITIONAL ISSUES AMONG PATIENTS WITH DIABETES MELLITUS

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ABSTRACT

This is a prospective study to assess the awareness of insulin aetiology of diabetes mellitus among diabetics and also their knowledge of the role of various local food items in its management. Consecutive diabetes mellitus patients seen at the Guinness Eye Centre, Onitsha over a - 2 month period were interviewed with an interviewer-administered structured questionnaire on the cause of diabetes mellitus and food items that worsen or ameliorate the disease.

Of the 46 adults diabetics (M:F=1:1) interviewed only 2 patients (4.3%) knew that diabetes was due to insulin deficiency; 35 patients (76.1%) thought that diabetes was due to consuming starchy food. Forty-three patients (93.5%) believed that diabetes could be improved by eating beans or cocoyam or breadfruit; 29 (63.6%) felt that high fat diet was good for diabetes, while 40 (87%) were not sure of the role of leafy vegetable diet in the management of diabetes. It is concluded that both the awareness of disease aetiology and nutritional knowledge of diabetics among this cohort are poor. Health education of diabetics is necessary to improve patients' understanding of the causes of disease and its dietary management.

Key Words: *Diabetes Mellitus; Etiologic Knowledge; Nutritional Care.*

INTRODUCTION

Diabetes Mellitus is a metabolic disease caused by a relative or absolute lack of insulin. It is associated with hyperglycemia and disturbance of lipid metabolism. A multi-system disorder, it affects the eyes, central nervous system, autonomic nervous system, musculo-skeletal system and renal system[1].

Diabetes occurs throughout the world. The World Health Organization (WHO), a few years ago observed that an epidemic of diabetes mellitus was occurring worldwide and warned that communities in developing countries and those living within the disadvantaged areas of industrialized countries were at greatest risk[2]. It has been projected that by the year 2010 the population of diabetics worldwide would double[3]. Recent studies also noted an increase in the incidence of diabetes mellitus and hyperglycemia among Nigerians[4] and diabetics constitute more than 10% of patients seen by general practitioners in Anambra State, Nigeria[5].

Treatment of diabetes mellitus involves the use of drugs and appropriate dietary regime. Like all chronic diseases, successful long-term management of diabetes mellitus depends on the patient's cooperation and compliance with expert medical advice. Compliance with therapy is enhanced if the patient has a good understanding of the disease process. This report is on a prospective study of some Nigerians with diabetes mellitus seen at the Guinness Eye Centre, Onitsha. It aimed at assessing their knowledge of the aetiology of diabetes mellitus and the role of diet in its management.

MATERIALS AND METHODS

Consecutive patients with diabetes mellitus seen at Guinness Eye Centre, Onitsha between February and July, 2000 were the subjects of this study. Each patient was interviewed with a structured questionnaire containing 6 key questions on cause(s) of diabetes; the role of diet and the type(s) of food (carbohydrates, fat, legumes and leafy vegetables) useful in its management. Correct answers to 4 or more questions showed that the patient had a good idea about the issues pertaining to the case of the diabetes mellitus. Statistical analysis was with the confidence intervals at an alpha level of 0.05.

RESULTS

Forty-six patients made up of 21 male and 25 female diabetics were interviewed. The age range was 43-72 years; mean (SD) 60.3 (6.5) years. Three patients (6.5%) were type 1 diabetics, while 43 (93.5%) had type 2 diabetes mellitus.

Twenty-five (54.3%) attained post-secondary education; 16 (34.7%) had secondary school education; 3 (6.5%) had primary school education and 2 (4.3%) were illiterates. All the patients had symptoms of ocular disease, and diabetic retinopathy was present in 61% of the patients.

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As shown in Table 1, while 35 patients (76.1%) thought that diabetes mellitus was due to eating carbohydrate diet, only 2 patients (4.3%) knew that it was caused by lack of insulin. On the other hand, 28 patients (60.9%) said diabetes was not due to lack of insulin. These were statistically significant ($P>0.05$).

Table 2 shows that only 8 patients (17.4%) knew that diabetics could eat small quantities of the common Nigerian carbohydrates staple diet, while 29 (63.6%) felt that high fat diet was good for diabetics; forty-three patients (93.5%) believed that eating beans or cocoyam or breadfruit improves diabetes, while 40 patients (87.0%) were not sure of the role of leafy vegetable diet in the management of diabetes. Only 7 patients (15.2%) gave correct answers to at least 4 out of the 6 key questions and were deemed to have good idea about diabetes mellitus. These findings were also significant ($P<0.05$).

DISCUSSION

Diabetes mellitus is a chronic disease requiring the afflicted to be on life-long treatment and dietary advice. Reports from multi-centre clinical trials showed that sustained good control of blood sugar lowers both ocular (retinopathy) and renal complications of diabetes mellitus[6,7]. Compliance with medical advice and treatment is enhanced if the affected patient understands the issues involved in diabetes mellitus. Such understanding will expectedly motivate the patient adherence to the correct treatment regime.

Of concern to both patients and health workers in Nigeria is the place of local food staple in the dietary management of diabetes mellitus. Food recipe for diabetics found in standard textbooks often dwell on non-Nigerian diet[8]. The diabetic food exchange formula being used in developed countries is difficult to use since the Nigerian local food staple cannot be easily measured with this method[8,9]. There is paucity of data on what would constitute the proper dietary management of the Nigerian diabetic with the commonly available foodstuffs. Ohwovoriole and Johnson[10] in a study of the type of food useful for diabetics in Nigeria showed that boiled beans, `dodo', rice, yam, and `eba' (all Nigerians dietary staple) eaten with local sauce like `egusi' or `beef stew', evoke glycemic response with `eba-egusi' mixture showing the highest response and boiled beans showing the least response. Oli, Ikeakor and Onwuamaeze[11] in another study documented that roasted yam and cocoyam evoked a higher glycemic response than boiled yam, cocoyam, plantain, rice, garri (eba) and beans. This suggests that method of preparing the good (cooking) can influence the blood sugar response.

In most hospitals and clinics in Nigeria, dietary management of diabetes mellitus usually consists of low carbohydrate and high protein diets with patients being advised to consume a lot of beans and little starchy food; attention is scarcely paid to dietary fat intake[8]. It is thus, not surprising that most patients in this study do not know the implication of high fat diet on the prognosis of diabetes mellitus. But lipid metabolism is also deranged in diabetes mellitus[1].

The results of this study show that only 15.2% of the patients have good idea of the causes and dietary issues as they relate to the care of diabetics. This finding is surprising and statistically significant. All the patients in the cohort had been previously diagnosed diabetics by general practitioners or physicians and they only consulted the ophthalmologist on account of visual symptoms. The presence of diabetic retinopathy in 61% of the cohort points to the long duration of the disease in the patients. Most of the patients studied had good formal education with 89% having at least secondary school education. Yet a poor knowledge of issues relating to the care of diabetes mellitus prevails among them. A higher ignorance rate is therefore expected in the general population.

As can be inferred from the results of this study, formal education even up to tertiary level, does not automatically confer the individual with good knowledge of disease processes; its natural history and the scientifically proven modes of care. Smith[8] had opined that many diabetics in Nigeria not only lack knowledge of the disease and the role of diet in its management but their problems are compounded by misinformation they receive especially from members of the public and some care-givers. The results of the present study support that opinion. In an environment where the juju or demonic concept of disease still prevails, it requires a lot of effort to convince patients that their problems are not due to the machinations of the enemy.

It is therefore recommended that regular and repetitive health education be given to all diabetic and indeed the general public on the cause of diabetes mellitus, and its management. Information on the role of diet; the types and quantity of the local diet useful in the management of diabetes should also be made available to health workers. The trained dieticians apart from determining the actual food portions based on the caloric requirements prescribed by the physicians should also be actively involved in the dietary education of the diabetes mellitus patients in the out-patient clinic.

REFERENCES

1. American Academy of Ophthalmology. Basic and Clinical Science Course. Section 1: Update on General Medicine. San Francisco: *American Academy of Ophthalmology* 1998:155-168.
2. King H, Rewers M. Diabetes in adults is now a third world problem. *Bull WHO* 1991;69(9):643-648.
3. Amos AF, McCarty DJ, Zimmet P. The rising global burden of diabetes and its complications: estimates and projections to the year 2010. *Diabetes Med* 1997; 14 (suppl 5):S1-85.
4. Ohwovoriole AE, Kuti JA, Kabiawu SIO. Casual blood glucose levels and prevalence of undiscovered diabetes mellitus in a Nigerian diabetic population. *Diabetes Res Clin Pract* 1988;4:153-158.
5. Nwosu SNN. Medical practitioners and eye care of diabetic patients. *Niger Med J.* 2000; 38(2):42-45.
6. The Diabetes Control and Complication Trial Research Group. The effect of intensive treatment of diabetes on the development and development of long term complications in insulin-dependent diabetes mellitus. *N. Engl. J Med* 1993;329:977-986.
7. UK Prospective Diabetes Study Group. Tight blood pressure control and risk of macrovascular and microvascular complications in type 2 diabetes. UKPDS 38. BMJ 1998;317-703-713.
8. Smith IF. Current trend in dietary management of diabetes mellitus. *Nigerian Journal of Nutritional Science* 5(2):79-86.
9. Otuyelu F. Diabetic diet for the Nigerian. *Nigerian Medical Practitioner* 1982;3:48-51.
10. Ohwovoriole AE, Johnson TO. Which food for the Nigerian diabetic? *Nigerian Journal of Nutritional Science* 1984;5(1):59-62.
11. Oli JM, Ikeakor IP, Onwuamaeze IC. Glucose response to some Nigerian staple foods. *Proc. Nutr. Soc Nig.* 1983;14:8.

TABLE 1: PATIENT'S VIEW ON CAUSES OF DIABETES MELLITUS

	Yes (%)	No (%)	Don't know (%)	95% Confidence limits
DM* caused by insulin lack	2 (4.3)	28 (60.9)	16 (34.8)	11.63-18.97
DM caused by carbohydrate diet e.g. rice, yam or garri	35 (76.1)	7 (15.2)	4 (8.7)	10.36-20.24
***P* value**				**<0.05**

*DM = Diabetes Mellitus

TABLE 2: PATIENT'S VIEW ON DIETARY CARE OF DIABETES MELLITUS

	Yes (%)	No (%)	Don't know (%)	95% Confidence limits
Diabetes improved by beans or yam or breadfruit diet	43 (93.5)	0 (0.0)	3 (6.5)	9.91-20.69
High fat diet is good for diabetics	29 (63.6)	17 (36.4)	0 (0.0)	11.09-19.51
Predominantly leafy vegetable diet is good for diabetics	7 (15.6)	20 (44.6)	19 (41.3)	10.92-19.68
Diabetics can eat little rice, yam, or garri	8 (17.4)	21 (45.7)	17 (37.0)	11.48-19.12
***P* value**				**<0.05**

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Original Article

HEPATOCELLULAR INDICES OF DIABETIC RATS SUPPLEMENTED WITH ANTIOXIDANT MICRO NUTRIENTS

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ABSTRACT

Complications associated with diabetes mellitus can be moderated by supplementation with antioxidant micro nutrients. In the present study, manganese, copper and zinc were supplemented in alloxan-induced diabetic rats for a period of 4 weeks. The mean serum activity (ui) of AST, ALT and ALP were 19.14±1.56, 36.43±1.74 and 48.29±2.64 in the supplemented group against 25.0±0.95, 37.57±1.70 and 54.43±1.72 observed in the unsupplemented group respectively. Mean serum levels of total protein (g/dl) and albumin (g/dl) in the supplemented group were 6.34±1.38 and 3.98±1.53 against 6.33±1.51 and 3.50±0.76 observed in the unsupplemented group respectively. Superoxide dismutase, glutathione peroxidase and catalase activities in the supplemented group was 1.69±0.59U/mg protein, 44.86±4.81U/mg protein and 57.14±6.56U/mg protein respectively, against 1.61±0.50U/mg protein, 36.86±5.00U/mg protein and 52.14±5.84U/mg protein recorded in the unsupplemented group respectively. The concentration of malondialdehyde in the supplemented group was 1.91±0.36nmol/ml as against 2.39±0.39nmol/ml seen in the unsupplemented group. The results suggest that antioxidant micronutrients supplementation might be beneficial in strengthening the antioxidant defense enzymes with resultant possible decrease in lipid peroxidation and improved hepatic indices of alloxan-induced diabetic rats.

Keywords: *Diabetics, Liver and Antioxidant defences.*

INTRODUCTION

Diabetes mellitus is a serious metabolic disorder with micro and macro vascular complication that result in significant morbidity and mortality. In Nigeria the prevalence of diabetes is about 2.7%. In 2005 it is estimated that the country lost 400 million dollars in national income due to diabetes and cardiovascular diseases, and it was projected that the loss will increase to 8 billion in coming decades[1]. As diabetes complications mostly affect individuals in their economically productive years, the disease has enormous socio-economic impact. It is estimated that societal cost associated with diabetes mellitus exceeds 20 billion dollars per year[2]. Diabetic human and experiment animal models exhibit high oxidative stress due to persistent and chronic hyperglycemia. This might deplete the activity of antioxidant defence system, and thus promote *denovo* free radicals generation[3]. Lipid peroxidation is a free radical mediated propagation of oxidative insult to polyunsaturated fatty acids, and termination only occurs through enzymatic antioxidant means or by free radical scavenging micronutrients[4]. The aim of this research is to study the effect of supplementing some selected antioxidant micronutrients, on antioxidant defence enzymes lipid peroxidation, and hepatic functions of alloxan-induced diabetic rats.

MATERIALS AND METHODS

Experimental Animals

Male albino wistar rats (120180g) were purchased from Animal House, Faculty of Pharmaceutical sciences, Ahmadu Bello University, Zaria. The animals were housed under similar conditions in standard cages environmentally controlled room at 25 ± 2°C, with light/dark cycle. The animals were fed with laboratory chaw (commercial feeds) and water *ad libitum*.

Chemicals

Alloxan was purchased from Sigma Aldrich Chemical Co. (UK), kits for the assay of

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malondialdehyde (MDA) and Catalase (CAT), Superoxide dismutase (SOD) and glutathione peroxidase (GPX) were purchased from North-West Life Science Specialties, Vancouver Canada. All reagents used for the study were of analytical grade.

Induction of Diabetes

Experimental diabetes was induced by a single intra-peritonial injection of freshly dissolved alloxan (150mg/kg) in normal saline maintained at 37°C, to rats fasted for 12 hours. Control rats received a similar volume of normal saline alone. After 72 hours of alloxan injection, the animals were fasted overnight and their fasting blood glucoses were estimated using a glucometer. Only rats with fasting blood glucose level greater than 126mg/dl (7.0 mmol/l) were included in the study.

Experimental Design

The rats were divided into 3 groups of 7 rats in each group as follows:- Group i: Normal control ii: Diabetic + metformin (250 mg/kgbw); Group iii: Diabetic + metformin (250 mg/kgbw) + copper (2mg/kgbw) + manganese (10mg/kgbw) + zinc (15mg/kgbw). The supplementation lasted for one month and after which the animals were fasted over night and sacrificed under anaesthesae. Blood sample was collected after the animal has been sacrificed and divided into plain and EDTA containing centrifuge tubes. Humane procedure was adopted throughout the experiment.

Measurement of Biochemical Analytes

Blood glucose was assayed using glucose oxidase method[5]. MDA level was assayed based on MDA reaction with thiobarbituric acid (TBA) and activities expressed as nmol/ml[6]. CAT activities were measured using H_2O_2 as substrate, and the unit is expressed as U/mg protein[7]. GPX activities were measured by NADPH oxidation, and the activities are expressed as U/mg protein[8]. SOD activity was assayed using the auto-oxidation of hematoxylin and the unit of activity is expressed as U/mg protein[9].

Total protein and albumin was determined using Biuret method[10] and Bromocresol Green (BCG) binding method[11] respectively. Serum AST and ALT activities were determined by the method of Reitman-Frankel[10]. Serum ALP activities was determined by the Nitrophenol method[10].

Statistical Analysis: All data were expressed as mean ± standard error of mean (SEM). Data was subjected to Instat3 Statistical Test. Differences in mean were considered to be significant when $p<0.05$.

RESULTS

Summary of the results is shown in table 1 and 2. The lowest mean serum MDA concentration was obtained in the control group (1.83±0.16nmol/ml). Diabetic rats treated only had mean MDA concentration of 2.39±0.15nmol/ml, while diabetic rats treated and supplemented had mean MDA concentration of 1.9±0.14nmol/ml.

The mean value of MDA in the unsupplemented diabetic rats was significantly higher ($p<0.05$) than similar value in the control group. However, no statistically significant different was observed between the control and the diabetic treated and supplemented groups ($p>0.05$).

Serum SOD activities was 2.0±0.17U/mg protein in the control group, 1.61±0.18U/mg protein in the diabetic treated group and 1.67±0.22U/mg protein in the diabetic treated and supplemented group.

The difference between the groups however, was not significant statistically ($p>0.05$). The GPX activities were 46.43±3.25U/mg protein in the control group, 36.86±3.91U/mg protein in diabetic treated group and 44.86±1.82U/mg/protein in the treated and supplemented group. There is statistically significant difference between the control and the unsupplemented group ($p>0.05$). However, no statistically significance difference exists between the control and the supplemented group ($p>0.05$).

The CAT activities were 59.86±1.08U/mg protein in the control group 39.86±3.04U/mg protein in the treated group and 57.14±2.48U/mg protein in the treated and supplemented group. There is statistically significance difference between the control and unsupplemented group ($p<0.001$) and between the supplemented and unsupplemented group ($p<0.001$).

Serum activities of AST were 17.71±0.89U/I in control group 25.00±0.95U/I in the unsupplemented group and 19.14±1.57U/I in the supplemented group. There was a statistically significant difference between the control and the unsupplemented group, as well as between the supplemented and the unsupplemented group. The ALT activities were 17.71±0.89U/I in the control, 37.57±1.70U/I in the unsupplemented group. There was statistically significance difference between the control and unsupplemented group. So also between supplemented and control group.

ALP activities was 38.43±2.17U/I in the control, 52.43±1.72U/I in the unsupplemented group and the activities of 48.29±2.04U/I was measured in the supplemented group. There was statistically significant difference between ALP activities seen in the control and unsupplemented groups ($p<0.001$). There was also statistically significant difference between supplemented and control group ($p<0.001$).

Mean serum total protein value of 6.90±0.18g/dl was detected in the control group, 6.33±0.15g/dl in the unsupplemented group, and a concentration of 6.34±0.14g/dl was found in the supplemented group. Serum albumin level of 3.70±0.20g/dl was found in the control, 3.50±0.76g/dl in the unsupplemented group and 3.98±0.15g/dl in the supplemented group. The difference between total protein and albumin levels, observed in all the groups are statistically not significant ($p>0.05$).

DISCUSSION

All the enzymes studied showed decreased activities in the supplemented group, when compared to the activities recorded in the unsupplemented group. The lipid components of cellular membrane are the principal target of free radical activities which might result in compromised protective functions, with subsequent leakage of cellular contents[12].

Elevated levels of antioxidant enzymes observed in the supplemented group, when compared with those seen in the unsupplemented group, might be responsible for the observed protective effects. Antioxidant enzymes works in synergy to quench the destructive tendencies of free radicals generated in the body, thereby sparing the body cells against lipid peroxidation[13].

Our findings also show decreased concentration of malondialdehyde in the supplemented group of diabetic rats, as against the concentration seen in the unsupplemented group of rats. Malondialdehyde is widely accepted as a marker of lipid peroxidation and itself, is capable of reacting with lipid component of the cells thereby causing pathology[14]. Therefore, the present study showed the ability of the micronutrient antioxidants supplements to reduce possible lipid peroxidation activity in the supplemented diabetic rats.

Administration of antioxidants might actually stimulate cell survival through strengthening of the antioxidant defense system. The principal aim of the current communication is the consideration of antioxidants micronutrients as adjunct therapy in the management of patients with diabetes mellitus.

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REFERENCES

1. The World Health Organization (WHO). Preventing Chronic Diseases. 2006.
2. American Diabetes Association (ADA). Clinical Practice Recommendation. *Diabetes Care*.1998; **21**: 55-56.
3. Baynes, J.W. Role of Oxidative Stress in Development of Complications in Diabetes. *Diabetes*. 1991; **40**: 405-12.
5. Cheesbrough, M. Medical Laboratory Manual for Tropical Countries, ELBS, Cambridge. **Vol. 1 (2nd ed.)**: 1992; 527-545.
6. Botsogluo, N.A. Rapid, Sensitive and Specific Thiobarbituric Acid Method for Measuring Lipid Peroxidation in Animals Tissues, Food and Feeds Stuff Samples. *J. Agric. Food Chem*.1994; **42**: 193-197.
7. Beers, R.F. Jr. and ISzer, I.W. "A Spectrophotometric Method for Measuring the Breakdown of Hydrogen Peroxide by Catalase. J. Biol. Chem. 1952; **195**: 133-140.
8. Paglia, D.E. and Valentine, W.N. Studies on the Quantitative and Qualitative Charectoisation of Esythrocyte Glutathione Peroxidase. *J. Lad. Clin. Med*. 1967; **70**: 158-169.

9. Martin, J.P Jr., Dailey, M. and Sugerman, E. Negative and Positive Assays of Superoxide Dismatase Based on Hematoxylin Autoxidation. *Arch. Biochem. Biophys.* 1987; **255**: 329-336.
10. Cheesbrough, M. Medical Laboratory Manual for Tropical Countries Vol. 1 (2nd Edition) ELSB, Cambridge: 1991; 465-567, 495-526.
11. Spencer, K. and Price, C.P. Measurement of bromocresol green. Annals of Clinical Biochemistry. 1977; **14**: 105-115.
12. Freeman, B.A. and Crapo, J.D. Biology of Disease: Free Radicals and Tissue Injury. *Laboratory Investigations.* 1982; **47**: 412-426.
13. Pryor, W.A. Vitamin E and Heart Disease: Basic Science to Clinical Intervention Trials. *Free Radic. Biol. Med.* 2000; **28**: 141-164.
14. Marian, V., Dieter, L. and Jan M. Free Radicals and Antioxidants in Normal Physiological Functions and Human Diseases. *Int. J. Biochem. Cell. Biol.* 2006; **39**:44-84.

Table 1 **Mean Serum Concentration of Certain Liver Analyses of Alloxan -Induced Diabetic Rats After 4 Weeks of Antioxidant Micronutrient Supplementation**

Group	AST (U/l)	ALT (U/l)	ALP (U/l)	T. Prot. (g/dl)	ALB (g/dl)
Control (n=7)	17.71±0.89	18.74±0.89	38.43±2.17	6.90±0.18	3.70±0.20
Diabetic Treated only (n=7)	25.00±0.95*	37.57±1.70±	52.43±1.72±	6.3.3a±0.15	3.50±0.76
Diabetic treated and supplemented (n=7)	19.14±57**	36.43±1.74*	48.29±2.64*	6.34±0.14	3.98±0.15

* = Values differ significantly from control
** = Values differ significantly from unsupplemented

Table 2 **Mean Concentration of Serum Malandoaldehyde (MDA), Superoxide Dismutase (SOD), Glutathione Peroxidase (GPX) and Catalase (CAT) in control, Diabetic Treated and Diabetic Treated and Diabetic Treated and Supplemented Whistar Rats.**

Group	MDA (nmol/ml)	SOD (U/mg Prot.)	GPX (U/mg Prot.)	CAT (U/mg Prot.)
Control (n=7)	1.83±0.16	2.0±0.17	46.43±4.25	59.86±1.08
Diabetic Treated only (n=7)	2.37±0.159*	1.61±0.18	36.86±3.91*	39.86±3.03*
Diabetic treated and supplemented (n=7)	1.91±0.14	1.67±0.22	44.86±1.82	57.14±2.48**

* = Values differ significantly from control
** = Values differ significantly from unsupplemented

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Original Article

A COMPARISON OF PURE NATURAL HONEY AND EDINBURGH UNIVERSITY SOLUTION (EUSOL) IN THE MANAGEMENT OF CUTANEOUS ULCERS

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Abstract

Honey is an ancient remedy for the treatment of infected wounds. This study compared the effects of Pure Natural Honey and Edinburgh University Solution (EUSOL) in the management of cutaneous ulcers. Sixteen participants with cutaneous wounds/ulcers were studied to assess the efficacy of pure natural honey as wound dressing in comparison with Edinburgh University Solution (EUSOL). The participants were grouped into; Honey group (n=15) and EUSOL group (n=12). On daily basis, the wound sites were cleaned with saline and the base of the ulcer swabbed for microbiology culture and sensitivity studies. Thereafter, the patients' wounds were dressed with sterile gauze soaked in honey or Eusol as the case may be. The result showed that among the honey group, 80% of the ulcers were rendered sterile within one week and 90% healing was achieved within six weeks while among the EUSOL group the ulcers became sterile within one week, but only 33.3% of the ulcers healed within six weeks. Reduction of tissue oedema was seen much earlier and was more remarkable with ulcers on honey dressing. Honey is tissue-friendly. The antiseptic and wound healing properties when considered along with its low cost and high availability make it a good wound dressing agent.

Key words: *Honey, Eusol, Cutaneous, Ulcer, Management.*

INTRODUCTION

Honey was used to treat ulcers as long as 4000 years before bacteria was discovered to be the cause of infection[1-3]. In 50AD, Dioscorides described honey as being "good for all rotten and hollow ulcers"[4]. It has also been reported to have inhibitory effect to about 60 species of bacteria including aerobes and anaerobes, gram positive and gram negative bacteria, fungi and dermatophytes[5,6]. The antibacterial property of honey was first recognized in 1892 by Van Ketel[7]. It may inhibit bacteria growth due to a number of different reasons: high sugar concentration (reduced water activity), low pH, hydrogen peroxide generation, proteinaceous compounds or other unidentified components present in honey may all provide antimicrobial activity[8]. Honey absorbs the oedema on the margin of wounds and removes the foul smell from septic wounds[2,9]. The deodorizing effect of honey may be by its provision of glucose, which is metabolized to lactic acid by the infecting bacteria, rather than ammonia produced from protein, which is malodorous. More so, the hydrogen peroxide produced in honey would not allow the growth of anaerobes which causes foul smell in wounds[10]. The ability to modulate production and quenching of free radicals may contribute to the demonstrated ability of some types of honeys to help in resolving the state of inflammation typifying chronic wounds[11]. Eusol is an orthodox wound antiseptic and dressing. It is a homogenous mixture of boric acid and chlorinated lime in water. In solution, they are active as hypoborus acid and hypochlorous acid. Eusol has been shown to remove sloughs from necrotic wounds and ulcers. It also sterilizes infected wounds and ulcers and has been known to inhibit micro-organisms[12]. However, hypochlorite and hypoborite solutions have not been shown to directly encourage or enhance the regeneration or growth of tissues. Much of free radical species like oxygen radicals are generated by hypochlorites[13]. These free radicals are not

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tissue friendly. The increase in antibiotic resistant microbial strains has led to the re-evaluation of the therapeutic use of ancient remedies like honey. The study was done to compare wound healing properties of honey and Eusol.

MATERIALS AND METHOD

Study population: 27 participants with cutaneous wounds or ulcers from three hospitals-Dortem Specialist Hospital, Maple Hospital and Family Health Hospital and Maternity (all in Lagos-Nigeria) were enrolled for the study after obtaining their informed consent. The age, sex, and medical/surgical history were noted. The study population was grouped into Honey group (n=15): these participants were placed on honey dressing for their wounds and EUSOL group (n=12): these participants were placed on Eusol dressing. The surfaces of the wounds or ulcers to be studied were observed carefully during the first visit. The wounds were then cleaned thoroughly with saline and the base of the ulcer swabbed with sterile swab stick and sent to the microbiology laboratory immediately for culture. The ulcers were thereafter measured along their greatest length and breadth in centimeters. The ulcers were then cleaned very well using saline and dressed with sterile gauze soaked in honey or Eusol depending on the grouping. The wound dressing was done daily but as healing progressed, it was changed to alternate day dressing. The size of the ulcers was measured weekly and recorded as in the beginning of the study. Swabs were collected weekly for culture until the wounds became sterile.

ETHICAL ISSUES

Participation in the study was optional and participants were free to withdraw at any point in the study if they so chose. The study did not add any additional cost or health risk to the patient.

INCLUSION AND EXCLUSION CRITERIA

Participants with cutaneous ulcers/wounds with no evidence of systemic involvement (e.g. fever) and who were not currently on any antimicrobial agent were included. Participants that took antibiotics or any drug that may negatively or positively influence wound healing were removed from the study.

RESULTS

Twenty seven patients were enrolled in the study but only sixteen completed the study. Eleven patients fell out of the study because they defaulted by either taking antibiotics, or using alternative dressings like dermazine and cicatrin. Only one patient actually absconded. All the wounds dressed with either honey or Eusol were debrided and sloughs disappeared within 3 days of dressing. The wounds and ulcers became clean, deodorized and healthy in both groups. In the honey group, the inflammatory oedema on the wound margin subsided remarkably by the 2nd day and completely disappeared by the 4th day. For the Eusol group, the inflammatory oedema disappeared in 5 to 7 days.

In 8 participants (80%) whose wounds were treated with honey, the wounds became sterile within 7 days, but by the end of the 2nd week the remaining 20% became sterile while the wounds placed on Eusol were sterile within 7 days of treatment.

Ninety percent of the wounds treated with honey healed within six weeks as against 33.3% wound healing achieved at 6 weeks with Eusol. The pattern of wound size reduction is shown in Tables 1 and 2. Wounds treated with Eusol bled profusely during dressings while the reverse was the case with honey and it is worthy of note that there were no adverse reactions. The organisms isolated from the wound swabs are as follows: *Pseudomonas* was the most frequent, followed by *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella aerogenes*, *Proteus mirabilis* and *Enterococcus faecalis*

DISCUSSION

The study has reconfirmed the multifaceted approach of pure natural honey in enhancing wound healing. Honey, because of its different constituents, has advantageous physiochemical, biochemical and biological processes. Some honeys have additional phytochemical antibacterial components.

Efem[9] and Atimono et al[2] claim that honey dressing debrides wounds and removes necrotic tissues. This was well demonstrated in this study. Eusol was also seen to do the same in the same range of about 3 to 4 days. Pure natural honey and Eusol were equi-effective and very useful in wound

toileting as shown in the study. This is clear from the inference drawn from the study, where heavily infected and necrotic wounds and ulcers of different aetiologies (diabetic ulcers, sickle cell melleolar ulcer, bed-sores, ragged wounds and burn wounds), were managed with honey and Eusol.

Dressing wounds with honey controlled the inflammatory process as evidenced by marked reduction or disappearance of the tissue oedema around the wounds within a few days of treatment much faster than Eusol and also produced healthy granulation tissues earlier. Okeniyi et al[14] in a study that compared the healing of incised abscess wounds with honey and Eusol dressing observed that the wounds dressed with honey became sterile earlier, produced healthy granulation tissue faster and healed faster than those on Eusol dressing. In some other studies, honey was used in the management of burns, and it was found to produce fewer incidents of contractures[15, 16, 17]. Pure natural honey exhibited good antiseptic and antibacterial properties. It was effective in clearing the mixed bacterial load infecting the wounds within seven days.

The clearing of infection observed when honey is applied to a wound may reflect more than just antibacterial properties. Research shows that the proliferation of peripheral blood B-lymphocytes and T-lymphocytes in cell culture is stimulated by honey at concentrations as low as 0.1%, and phagocytes are activated by honey at concentrations as low as 0.1%. Honey (at concentrations of 1%) also stimulates monocytes in cell culture to release cytokines, tumor necrosis factor-alpha (TNF-α), interleukin-1 (IL) and IL-6, which activate the immune response to infection [19, 20].

It is worthy of note that in the study, only oneS of the wounds dressed with honey bled during change of dressing while the wounds treated with Eusol were found to bleed readily during dressings. Honey is tissue-friendly; its antiseptic and wound healing properties, when considered along with its low cost and high availability, make it a good wound dressing agent.

REFERENCES

1. Molan PC. Why honey is effective medicine: its use in modern medicine. Bee world. 1999.6(4): 148-158.
2. Atimomo C.E, Ohwovoriole A.E, and Anyiwo C.E. Wound and Healing Antibacterial Ability of Natural Honey: A pilot study. Nigerian Medical Practitioner. 1990. 20(2): 23-24.
3. Hall M. Minor burns and hand burns: comparing treatment methods. Prof. Nurse.1997. 12 (7): 489-9.
4. Gunther RT. The Greek Herbal of Dioscorides. New York: Hafner, 1934 (reprinted 1959).
5. Molan PC. The antibacterial activity of honey. 1. The nature of the antibacterial activity. Bee World 1992; 73 (1): 5-28.
6. Brady NF, Molan PC, Harfoot C.G. The sensitivity of dermatophytes to the antimicrobial activity of manuka honey and other honey. Pharm Sci 1997; 2-3.
7. Dustmann JH. Antibacterial effect of honey. Apiacta 1979; 14(1): 7-11.
8. Mundo AA, Padilla-Zakour OI, Worobo RW. Growth inhibition of food borne pathogens and food spoilage organisms by select raw honeys. Int J. Food Microbiol 2004 97; 1-8.
9. Efem SEE. Clinical observations on wound healing properties of honey. Br J. Surgery 1988; 76 (7): 679-81.
10. Molan PC, Betts JA. Clinical usage of honey as a wound dressing: an update. J. Wound Care 2004: 13 (9): 353-6.
11. Henriques A, Jackson S, Cooper R and Neil B. Free radical production and quenching in honeys with wound healing potentials. J. Antimicrobial Chemotherapy. 2006. 58, 773-77.
12. Ankra-Badu GA. Sickle cell leg ulcers in Ghana. East African Medical Journal: 1992. 69 (7): 366-9.
13. Godwin DC. Free radicals produced during the oxidation of hydrazines by hypochlorous acid. Chem Res Toxicol .1996. 9 (8): 1333-9.
14. Okeniyi JAO, Olubanjo OO, Ogunlesi TA, et al. Comparison of healing of incised abscess wounds with honey and EUSOL dressing. J Altern Complement Med 2005; 11 (3): 511-13.

15. Bangroo AK, Katri R, Chauhan S. Honey dressing in pediatric burns. J Indian Assoc Pediatr Surg 2005; 10 (3): 172-5.
16. Nagra ZM, Fayyaz GQ, Asim M. Honey dressings; experience at Department of Plastic Surgery and Burns Allied Hospital Faisalabad. Prof Med J 2002; 9 (3): 246-51.
17. Molan PC. The Evidence Supporting the Use of Honey as a Wound Dressing. Int J Low Extrem Wounds. 2006; 5; 40-54.
18. Abbas T. Royal Treat- Living in the Gulf. 1997; 50-1.
19. Abuharfeil N, Al-Ovan R, Abo-Shehada M. The effect of bee honey on the proliferative activity of human B- and T-lymphocytes and the activity of phagocytes. Food Agric Immunol .1999; 11: 169-77.
20. Tonks A, Cooper RA, Price AJ, Molan PC and Jones KP. Stimulation of TNF-alpha release in monocytes by honey. Cytokine 2001; 14 (4): 240-2.

Table 1: Honey dressing group: wound size for weeks 1-6

Age Year	Sex	Ht M	Wt Kg	B.M.I Wt/Ht^2	Wound surface	Primary disease	0	1	2	3	4	5	6
26	M	1.83	78	23.29	Superficial burn Neat	Thermal burn	15.0x10.2	12.4x7.6	9.8x5.2	5.0x3.6	3.4x2.0	0	-
74	M	1.67	110	39.44	Deep infected Necrotic	Uncontrolled Diabetes mellitus	14.3x10.2	14.2x10	13.4x9.8	13x8.6	11.9x7.8	10.7x7.6	10.2x7.1
39	F	1.62	75.4	28.73	Shallow Necrotic and infected	Thermal burn	12.2x11.9	10.9x10	8.6x8.2	6.4x6.1	4.8x4.4	2.6x2.0	0
15	M	1.64	57	21-19	Ragged and Infected	Trauma	8.4x6.1	7.5x4.3	4.8x3.2	3.4x2.0	2.1x0.8	0	-
21	F	1.58	58	23.23	Deep, necrotic and infected	Bed sore	8.0x7.2	7.8x7.0	6.7x6.5	5.5x5.1	3.1x2.9	2.7x2.1	0
27	F	1.70	61	21.11	Superficial neat Wound	Trauma	6.0x45	4.7x3.8	2.1x2.0	1.0x0.8	0	-	-
44	M	1.87	105	30.03	Deep, necrotic and infected	Diabetic with abscess	5.4x4.6	4.4x3.2	3.1x2.5	2.0x1.4	0	-	-
10	M	1.24	27.8	18.08	Deep, ragged and infected	Sickle cell Malleolar ulcer	5.2x4.6	4.9x4.2	4.2x2.4	3.8x2.0	3.2x1.5	2.0x0.7	0
28	M	1.83	76.5	22.84	Deep and ragged but neat	Trauma	4.8x4.1	4.2x3.0	3.8x2.0	3.5x1.8	2.8x1.0	0	-
14	F	1.41	46	23.24	Shallow and neat	Chemical burn	4.5x3.8	3.8x2.7	2.9x2.1	1.6x1.0	0	-	-

BMI=Body Mass Index=Weight (Kg)/Height^2(M); The normal range of BMI is between 20 to 25.

Table 2: Eusol Dressing group: wound size for week 1 -6

Age Year	Sex	Ht M	Wt Kg	B.M.I Wt/Ht^2	Wound surface	Primary disease	0	1	2	3	4	5	6
17	F	1.57	61.2	24.83	Shallow and neat	Superficial burn	14.3x11.1	14x10.8	13.2x10.2	11.6x8.4	10.9x8.0	9.7x7.1	8.4x6.0
24	M	1.55	62.8	26.14	Ragged but neat	Trauma	10.6x7.2	10.2x6.7	8.6x4.8	7.9x4.1	7.3x3.8	6.2x3.2	5.1x2.0
27	F	1.61	64.0	24.69	Shallow and neat	Superficial burn	7.6x5.4	7.1x4.8	6.2x4.4	5.0x3.7	3.6x3.1	2.4x2.1	0
10	M	1.46	48.7	22.85	Shallow and neat	Trauma	5.6x5.2	5.2x4.8	4.7x4.3	4.1x3.7	3.4x2.9	2.4x2.0	0
19	M	1.68	67.2	23.81	Deep and infected	Sickle cell Malleolar ulcer	5.4x4.7	5.1x4.2	4.7x3.7	4.1x3.2	3.6x3.2	3.0x2.7	2.6x2.1
55	M	1.64	96.8	35.99	Ragged, necrotic and infected	Trauma in a diabetic	5.2x4.8	5.0x4.5	4.7x4.2	4.6x4.0	4.2x3.6	3.8x3.4	3.2x2.9

BMI=Body Mass Index=Weight/Height^2

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Original Article

DETERMINATION OF STARCH, PROTEIN AND CALCIUM CONTENTS OF YAM AND PLANTAIN VARIETIES

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Abstract

Background

Yam and plantain of several varieties are staple foods in many parts of the world especially in west and central Africa, parts of Central America and the Caribbean, the Pacific Island and South East Asia[1] Nutrients such as carbohydrate (starch), protein, minerals and vitamins are derived from them.

Since these nutrients are needed for proper functioning of the body and maintenance of good health, yet, certain disease conditions like diabetes mellitus need certain dietary restrictions and modifications as a very essential aspect of their management, determination of the nutritional contents of the different varieties of these staple foods is undoubtedly necessary.

Objective

To determine the value of the different nutrients present in each of the different species of yam and plantain. This would aid nutritional planning dietary modification in health and diseases like diabetes mellitus.

Method

The edible portions of the respective five varieties of plantain and yam were carefully mashed and analysed using known analytical methods for each given nutrient. Various percentages were determined using SPSS version 11.0.

Results

The research focused on the determination of starch, protein and calcium contents of the various varieties of yam and plantain.

On a general note, yams had lower starch contents but higher protein and calcium contents than plantains. Dioscorea cayenensis (Yellow yam; ji-ayabe) had the least starch content (9.05%), good Protein (5.60%) and the highest Calcium content (18.04 mg/ 100g).

French horn plantain had the highest starch content (34.07%), good calcium (12.02mg/100g) but the lowest protein (3.50%) contents. Discorea dumentorum (Trifoliate yam; ana) had the highest protein content (11.5%), moderate starch (13.3%) and good calcium (14.03 mg/100g) contents.

CONCLUSION

Yams are generally more nutritious than Plantains. The observations are important for nutritional planning in health and disease. Increase in production of highly nutritive varieties will lead to improvement in the economic/commercial benefits of the crops.

Key Words: *Nutrient, Quality Variety, Human.*

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INTRODUCTION

Yam is the third most important root crop after cassava and sweet potato. This is especially true in West Africa, Parts of Central America and the Caribbean, the Pacific Islands and Southeast Asia[1]. Plantain, on the other hand, is an important staple food in the humid tropical zones of Africa, Asia, Central and South America. It is undoubtedly, one of the oldest cultivated fruits in West and Central Africa[2-4] Yam and Plantain are staple foods from which nutrients such as carbohydrate (starch), Proteins, Lipids, Minerals and Vitamins are derived[5,6].

Since these nutrients (starch, protein and calcium) are important in the proper functioning of the body and maintenance of good health, the determination of the starch, protein and calcium contents of the different varieties of yam and plantain is necessary. Again, many people with diabetes mellitus are often advised by quacks and other ill informed health workers to avoid or eat certain species of yams and or plantains believing such to have negligible or no carbohydrate at all.

The aims of this research work were to determine the starch, protein and calcium contents of the different varieties of yam and plantain respectively; to determine which of the various varieties of yam and plantain that possess the greatest nutritional value. It was also to find out the varieties of yam and plantain that have tolerable energy values as well as to ascertain the economic/commercial benefits of the various species of yam and plantain. Thus, these could guide dietary recommendation for diabetic patients and patients suffering from other diseases where dietary modification may be required.

MATERIALS

The test samples used in this study were obtained from the Horticulture and Yam programmes of the National Root Crops Research Institute, Umudike, Abia State. The respective coordinating officers of the different programmes authenticated their respective botanical identities.

Laboratory and other facilities were obtained from the Central Service Laboratory of National Root Crops Research Institute Umudike, Abia State.

Five varieties of yam were used and included:

A. Dioscorea dumentorum (Trifoliate yam) Ona (una).
B. Dioscorea alata (Water yam) ji-abana.
C. Dioscorea rotundata (White yam) ji-ocha.
D. Dioscorea bulbifera (Aerial yam) Adu.
E. Dioscorea cayenensis (yellow yam) ji-ayabe.

Five varieties of plantain were used and included:

A. Horn plantain. (Aka Nkita, Ohi bere odu)
B. Musa cadaba (cooking banana). (Unere-igbo or Unere-osukwu or unere oji).
C. French horn plantain.
D. French plantain (Ojoko-oyo).
E. Musa balbisiana (False horn plantain) (Obughunu).

METHODS

Processing of Samples

The test samples were in each case peeled using a kitchen knife to obtain the edible portion. This edible portion was then cut into slices and mixed very well to accommodate all parts (head portion, middles and bottom) of the sample.

50g of each test sample was weighed out and homogenized (macerated in a blender). It was sieved to get the starch and oven dried at a temperature below 60°C to avoid gelling of the starch.

The rest of each of the samples was equally oven dried and stored in carefully labelled sample bags. Some quantity of the dried samples were ground and stored in equally labelled sample bottles. These were used for the protein and mineral tests.

Laboratory Analysis

Each of the above samples was analysed for parameters which included starch content, protein content and mineral content (calcium) of the various species or varieties.

Starch content was determined by the gravimetric method as described by Balagopalan[7] The protein content was determined by the modified Kjeldhi method as described by James[8] and chang[9] For each sample, the mineral content was determined by the dry ash extraction method of James[8], while the calcium content was determined by the standard EDTA complexiometric titration method

In each case, the samples were analysed in duplicates and the appropriate SPSS version 11.0 applied as statistical software for the determination of the various percentages and other statistical analysis.

Table 1: Starch content of Yam and Plantain Varieties (Values in % dry matter)

Sample	W^1	W^2	$W^2 - W^1$	% starch	Remarks
D. dumentorum	17.861	24.515	6.654	13.30	GOOD STARCH CONTENT
D. alata	18.405	30.635	12.23	24.46	
D. rotundata	18.541	23.625	5.084	10.17	
D. bulbifera	18.207	26.255	8.048	16.10	
D. cayenensis	16.213	20.736	4.523	9.05	
					HIGH STARCH CONTENT
Horn plantain	17.977	31.633	13.656	27.31	
Cookingbanana	18.187	29.812	11.625	23.25	
French horn plantain	17.914	34.950	17.036	34.07	
French plantain	27.321	34.700	7.379	14.76	
False horn plantain	18.096	30.382	12.286	24.57	

$$\% \text{ starch} = \frac{w^2 - w^1}{50g} \times \frac{100\%}{1}$$

Where w^1 = weight of empty container; w^2 = weight of the container and starch.

From table 1, plantain had a higher starch content between the two crops and has the following pattern for the varieties; French horn plantain > horn plantain > false plantain > cooking banana > French plantain. Yam had a lower starch content than plantain and has the following pattern for the varieties, D. alata > D. bulbifera > D. dumentorum > D. rotundata > D. Cayenesis.

From the table, French horn plantain had the highest starch content (34.07%) while D. cayenensis (yellow yam) had the lowest starch content (9.05)

Table 2: Protein Content of Yam and Plantain Varieties (value expressed in mg/100g edible portion)

Sample	2nd (cm^3) Titre	1st (cm^3) Titre	(cm^3) Titre	T-Blk	$\%N_2$	% protein
Blank	1.20	1.00	0.20	-	-	-
Dioscorea dumentorum	18.50	15.00	3.50	3.30	1.848	11.55
D. alata	20.70	18.50	2.20	2.00	1.120	7.00
D. rotundata	22.20	20.70	1.50	1.30	0.728	4.55
D. bulbifera	24.70	22.20	2.50	2.30	1.288	8.05
D. cayenensis	26.70	24.70	1.80	1.60	0.896	5.60
Horn plantain	8.10	6.70	1.40	1.20	0.672	4.20
Cookingbanana	9.40	8.10	1.30	1.10	0.616	3.85
French horn plantain	10.60	9.40	1.10	1.00	0.860	3.50
French plantain	12.00	10.60	1.40	1.20	0.672	4.20
False horn plantain	13.40	12.00	1.40	1.20	0.672	4.20

$$\% N_2 \left(\frac{100}{0.5} \times \frac{14 \times 0.02}{1000} \times \frac{100}{10}\right) T - Blk$$

$$= 0.56 (T - Blk) \qquad \% \text{ protein} = \% N_2 \times 6.25$$

Table 2, shows that yam had a higher protein content than plantain. The protein content for yam varieties had the following pattern: D. dumentorum > D. bulbifera > D. alata > D. cayenensis > D. rotundata, while plantain which has lower protein content had this pattern: Horn plantain > French plantain > False horn plantain > Cooking banana > French horn plantain.

It was observed that D. dumentorum (Ona) had the highest protein content (11.55%) while French horn plantain had the lowest protein content (3.50%).

Table 3: Calcium content of yam and plantain varieties (Values Expressed in mg/100g Edible Portion)

Sample	2nd (cm^3) Titre	1st (cm^3) Titre	(cm^3) Titre	T-Blk	Ca mg/100g
Blank	2.20	2.00	0.20	-	-
Dioscorea dumentorum	8.20	7.65	0.55	0.35	14.03
D. alata	8.70	8.20	0.50	0.30	12.02
D. rotundata	9.30	8.70	0.60	0.40	16.03
D. bulbifera	9.85	9.30	0.55	0.35	14.03
D. cayenensis	10.50	9.85	0.65	0.45	18.04
Horn plantain	5.60	5.10	0.50	0.30	12.02
Cookingbanana	6.15	5.60	0.55	0.35	14.03
French horn plantain	6.65	6.15	0.50	0.30	12.02
French plantain	7.20	6.65	0.55	0.35	14.03
False horn plantain	7.65	7.20	0.45	0.25	10.02

$$\text{Ca mg/100g} = \left(\frac{100}{5} \times 20.04 \times 0.02 \times \frac{100}{20}\right) T - Blk$$

$$= 40.08\ (T - Blk)$$

Table 3 shows that aside D. cayenensis and D. rotundum species of yam which had the highest calcium contents (18.04mg/100g and 16.03 mg/100g respectively) and False horn plantain specie with the least calcium value (10.02 mg/100g), the other varieties of plantains and yams have relatively comparable values of calcium contents.

DISCUSSION AND CONCLUSION

The result obtained showed that the starch content of the crops had the following pattern; plantain > yam while the protein content showed a reverse trend; yam > plantain. The calcium contents of the different species of the two crops appear to be fairly maintained. Table 1 showed that yam species or varieties are lower in starch content compared with plantain varieties with the yellow (ji-ayabe) variety of yam being the lowest in starch content (9.05%). The same cannot be said of the report by Oyenuga[5] in which the starch content was not determined rather the calories per 100g of the various varieties were determined. The starch content of plantain was higher than that of yam with the French horn plantain being the highest in starch content (34.07%). This relatively high starch content found in plantain in this study is, however, lower than the value found in a study by Ketiku[6] in which plantain starch content was shown to be 83.25% and 66.4% for unripe and ripe plantains respectively. This significantly high starch content of plantain shows it to be a very good energy source.

From table 2, the protein contents of yam varieties were higher than that of plantain varieties with the variety D. dumentoum (Ona) being the highest in protein content (11.55%). This is in keeping with the report by Oyenuga[5] that showed the same

variety to possess the highest protein content (11.73%) among the species he studied.

Also, from the result (see table 2), the protein content of plantain varieties appeared to be low. French horn plantain had the lowest protein content (3.50%). This is in line with the finding by Ketiku[6] who showed that plantain was low in protein content; 3.0% and 3.5% for unripe and ripe plantains respectively.

The calcium content of yam species as shown in table 3 was higher than that of plantain species, with yellow yam (ji~ayabe) being the highest in calcium content (18.04%). The calcium content of all the species of plantain studied appeared not to differ significantly. This is in line with the report by Ketiku[6] in which calcium content in plantain varieties appear to be fairly maintained.

Yellow yam (ji~ayabe) with the least starch content, good protein content and the highest calcium content is thus, regarded as the most nutritious. Yams, irrespective of the specie, had better protein and calcium contents than plantain. On the other hand, French horn plantain with the highest starch content appears to be the richest source of energy.

The findings of this study is quite informative because it has revealed that contrary to the wide belief, particularly in the South Eastern part of Nigeria, that D. alata (water yam) had the least starch content compared with other yams, it has actually been shown to possess the highest starch content. It was also generally believed that yams had higher starch content than plantain but the opposite is the finding in this study. Therefore, the advice often given to diabetic patients to eat water yam instead of other species and to eat plantain rather than yam on the assumption that they respectively had lower carbohydrate content than the others has been shown to be a very erroneous advice.

Cooking banana (Unere oji in Ibo land) which most diabetic patients eat or are often advised to eat in preference to any other species of yam or plantain had second to the least starch content among all the plantain species studied. However, its starch content (23.25%) is comparable to the starch content of the yam; D. alata, which had the highest starch content (24.46%). Therefore, D. Cayensis iji ayebe) and D. Rotunda iji ocha) with the least starch contents (9.05% and 10.17%) as well as reasonable protein (5.6% and 4.55%), and calcium (18.04mg/Kg and16.03mg/Kg) contents would be preferred for diabetic patients with; as usual, emphasis of moderation of quantity.

CONCLUSION

Yam is a good energy source, richer in protein and calcium than plantain and, thus, is more nutritious than plantain. Contrary to public view, plantain is, generally, higher in starch content than yam. On the other hand, plantain is a very good source of energy, but is low in protein and calcium compared with yam.

These observations are therefore, important for nutritional planning in health and disease conditions; for example, in diabetes mellitus, where dietary modification may be required.

The commercial consequences of these findings are also worthy of further exploration. An increase in the production of highly nutritive yellow yam and energy rich French horn plantain will lead to provision of raw materials for industries and improved economic/commercial gains of the crops.

RECOMMENDATIONS

From the findings of this research, the following recommendations could be made:

The public should be informed and encouraged to consume varieties of yam and plantain that are of good nutritional value in other to improve their dietary habits.

Patients on dietary modification, e.g. diabetic patients and others suffering from diseases that may require intake of low carbohydrate diets, should take more of yellow yam (ji-ayabe) and for those that require intake of high energy diet, French horn plantain is a good source.

Governments, agriculturists and farmers should be encouraged to increase the production of the Yellow yam and French horn plantain varieties as well as the other varieties of yam and plantain because of their nutritional and economic values.

REFERENCES

1. Lawton, J. R. S. (1967), A key to the Dioscorea Species in Nigeria. J. West Afr. Sci. Ass. 12: 3-9.
2. Ogazi, P. O. (1978), "The Traditional system of Plantain Flour Production with References to Local Literature and Literature about Peurto Rican Experience". A Report Submitted to the Director, National Horticulture Research Institute, Ibadan, Nigeria.
3. De Langhe, E. (1964), The Origin of Variation in the Plantain Banana Medel land boowhoge School, Kent, 39 (1) 45 - 50.
4. Swennen, R (1990), Limits and Morphotaxonomy, Names and Synonyms of Plantain in Africa and Elsewhere in Jaret R. C. ed. Identification of Genetic Diversity in the Genus Musa IINIBAP Montpellier. Pp 172 - 210.
5. Oyenuga, V. A (1968), Nigeria Foods and Feeding Stuffs. Their Chemistry and Nutritive Value (3rd Edition), University Press, Ibadan, Nigeria. Pp 90- 93.
6. Ketiku, A. O. (1973), Chemical Composition of Unripe (green) and Ripe Plantains (Musa paradisica) J. Sci. Food Agric 24: 703 - 709.
7. Balagopalan .C, Padmaja. G, Nanda S. D and Moorthy S. N (1988), Cassava in Food, Feed and Industry. CRC Press Inc. Florida USA. Pg. 189.
8. James C.S., (1995), The Analytical Chemistry of Foods. Chapman and Hall. New York. USA. 36-40.
9. Chang, SW. K. C. (2003), Protein Analysis: In Food Analysis (3rd Edition) Kluwer Academic/Plenum Publishers. New York. Pp 131 135.

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Review Article

A REVIEW OF CURRENT IMMUNODIAGNOSTIC TECHNIQUES IN PROTOZOOLOGY

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ABSTRACT

Immunodiagnostic techniques are based on the principles of immunochemistry. Current diagnostic methods employed in protozoology include ELISA technique, counter immunoelectrophoresis, immunoblotting technique, immuno-fluorescence Assay methods and complement fixation tests. These methods have been effectively employed in protozoology (exemplified by malaria and Trypanosomiasis). These methods have been found to be effective, sensitive and specific and are useful tools in research and fieldwork especially in manpower-constrained areas of the tropics and sub-tropics.

Key words: *Immunodiagnosis, Protozoology, Techniques.*

INTRODUCTION

The importance of parasitological diseases cannot be over-emphasized. Malaria, for instance constitutes a menace to human development, having been ranked as the world's number one cause of morbidity and mortality, followed closely by schistosomiasis. The need for appropriate diagnosis of parasitic diseases has become important to both the clinician and the parasitologist as well.

The development of immunodiagnostic methods for the appropriate identification of parasites in human specimens be it blood, stool or urine has become a sine qua non to effective clinical medical practice. This has become a great challenge to the parasitologist and the researcher at large. The development of modern immunoassays is based primarily on their increased sensitivity, combined with acceptable specificity[1].

Parasites can be identified at their various developmental stages - the eggs or ova, the larva or the adult. Immunodiagnosis employs the principle or application of antigens resulting from these parasites or the antibody, which the host organism mounts against the parasites.

The invention of the microscope in the early centuries made it possible to identify parasitic eggs or larva in human specimens, making use of their characteristic morphological structures. This however, has its limitations in field studies and surveys. With the advancement in immunology and related fields in human medicine, it has become feasible to isolate parasitic antigens and use these to provoke antibodies in hosts, the presence of which in sera can be used to identify these parasites or their components hence giving rise to the concept of immunodiagnosis.

Parasites can be considered according to their biological groups such as protozoa, helminthes, bacteria or viruses. Immunodiagnostic techniques have been found relevant in the laboratory diagnosis of each and every one of the above-mentioned groups. For the sake of this review, emphasis will be placed on protozoan parasites.

Immunodiagnostic techniques are based on the principles of immuno chemistry, which itself, is a branch of chemistry which deals with the detection of and quantitation of chemical substances by the measurement of antigen-antibody interactions[2]. In these cases, the antibody is used as the reagent to detect the chemical substance (the antigen) of interest. On the other hand, specific antigens can be employed to detect the presence of specific antibodies in the sera of humans.

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Antibodies are immunoglobulins, which are capable of binding to a variety of natural and synthetic antigens including proteins, carbohydrates nucleic acids, lipids and other chemicals. Immunoglobulins (1g) consist of five general classes designated as IgG, IgA, IgM, IgD and IgE. Of all these, IgG is the most prevalent and the most often employed.

The National Committee for Clinical Laboratory Standards (NCCLS), USA[3] define certain characteristics of anti bodies which make them appropriate for agglutination reactions and hence, their application in immunodiagnostic techniques. These include specificity, potency, labeling and stability.

Amongst the five most important parasitic diseases, which have been marked for special attention by the World Health Organization (WHO), three protozoan diseases namely, malaria, leishmaniasis and trypanosomiasis are included. Greater research is encouraged in these diseases considering the fact that their prevalence continues to be high[4]. There is a clear need for techniques that diagnose clinical infections and for use in fieldwork and surveys. Such techniques also are needed to study the epidemiology of such infections i.e. the pattern of transmission in the community, and the detection of persons who have had the infection in the past or carriers.

The method of diagnosis, which uses the immunological binding reaction between antibodies and antigens, is termed immunodiagnosis and the assays for measuring these antibodies or antigens are called immunoassays.

IMMUNO DIAGNOSTIC TECHNIQUES IN AMOEBIASIS/AMOEBIC LIVER ABSCESS

Amoebiasis is the infection caused by a specie of protozoan organism known as Entamoeba histolytica. One of the rare complications of amoebic dysentery is amoebic liver abscess and this occurs when the parasites are carried to the liver via the portal circulation. Immunodiagnostic techniques, which have been employed in the diagnosis of amoebiasis and amoebic liver abscess, are:

(a) Latex slide test

(b) Cellulose Acetate Precipitation (CAP) Test.

The Latex Slide Test: The test detects antibodies to *E. histolytica*. It is also known as the fomouze Bichio-Latex Amibe test. It takes 5 minutes to detect *E. histolytica* and is available in a 25-test kit.

Cellulose Acetate Precipitation (CAP) Test: This test method detects antibodies to *E. histolytica* antigens in serum of any patient with invasive amoebiasis. It is highly specific and useful in epidemiological studies.

IMMUNODIAGNOSTIC TECHNIQUES IN GIARDIASIS

Giardiasis is the infection by specie of protozoan organism known as *Giardia lamblia*. The organism is a flagellated protozoa which exists in two forms: a non-infectious pear shaped trophozoite (9-20mm) inhabiting the small intestine and the highly infectious cyst form which is elliptical in shape and ranges in size from 8-12 mm[5]. The trophozoites are highly labile and die quickly once outside the host body while the cyst form are more resistant and survive for days outside the host body[5]. The parasites easily contaminate water in endemic areas and people travelling to such areas can easily contract the infection[6,7,8,9].

Direct transmission of *G.lamblia* occurs in healthy carriers by food contamination[10, 11]. Stephens and colleagues[12] report that high-risk subjects include young children, immune-compromised people, and those without previous exposure. Phillips and Colleagues[13] have reported that recently, giardiasis has become a common sexually transmitted disease. Animal reservoirs are very important in transmission, and have been implicated in several cases of water contamination[14,8,15].

The most common and traditional method of diagnosis of giardiasis has been stool microscopy. However, this method requires extensive experience and the presence of intact cysts in the stool sample. Immunodiagnostic techniques have been employed in the diagnosis of giardiasis. These methods include:

(a) ELISA
(b) Counter Immunoelectrophoresis
(c) Immunoblotting technique
(d) Direct Immuno Fluorescence Assay (DFA)

ELISA: This means enzyme-linked immunosorbent assay and employs trophozoite immune rabbit serum to detect antigen of *G. lamblia*. The procedure is fairly simple to perform and exhibits increased sensitivity when compared to microscopy. Various techniques[16] are employed to examine faecal samples and accuracy of results is dependent on the skill of the technician. In 50 -70% of faecal examinations, positive results have been obtained[17,18]. Several investigators[19,20,21] have found the ELISA technique to be sensitive, specific and easy to perform.

Janoff and Colleagues[20] compared three different diagnostic techniques including ELISA and found similar results. Comparing counter immuno electrophoresis and ELISA with microscopy, these investigators noted that the methods have identical sensitivity, specificity, positive predictive value and negative predictive value. However, the false positive rate by ELISA was 24% (10 of 42) in day care centres but only 3% (1 of 32) in healthy adults as corroborated by microscopy. The authors concluded that ELISA might be more sensitive than microscopy, which is considered the reference standard, and that result may be dependent, in part, on the epidemiology of infection in the study subjects.

In a similar study as above, Azia and colleagues[1] made similar observations while Garcia and colleagues[22] in their own study found both sensitivity and specificity of the DFA method to be 100% when compared to the conventional microscopy.

Furthermore, Beth and his colleagues[23] obtained positive results with ELISA technique in 92% of samples of giardiasis subjects studied. Two percent (2%) of the subjects showed false positive results. Like the previous investigators mentioned, they also found the ELISA technique to be simple, sensitive and specific for the diagnosis of *Giardia lamblia* infections.

HAEMOFLAGELLATES

These are parasites of great public health importance characterized by the possession of flagellae. Representatives of this group include:

(a) *Trypanosomes* which causes trypanosomiasis

(b) *Leishmania* which is responsible for Leishmaniasis.

The three most important diseases of public health importance to be considered in this review are:

(a) American trypanosomiasis or chagas disease
(b) African trypanosomiasis
(c) Visceral Leishmaniasis or kala-azar.

CHAGAS DISEASE

This highly debilitating illness is caused by species of Trypanosomes known as *Trypanosoma Cruzi*. Four immunodiagnostic techniques have been employed in the diagnosis of American Trypanosomiasis or chaga's disease. These include:

1) Complement Fixation Test (CFT)
2) Indirect Fluorescent Antibody Test (IFAT)
3) Indirect Haemagglutination Test (IHAT)
4) Enzyme Linked Immunosorbent Assay (ELISA)

The complement fixation test (CFT): detects anti T-cruzi antibodies in sera of patients suffering from Chagas' disease and like other techniques, it becomes positive one month following infection and remains so even after treatment. Among the four methods mentioned above, ELISA technique is the most extensively applied and is simpler than others. Breniere and colleagues[24] evaluated the Micro Double Diffusion test (MD) in Bolivian patients with Chaga's disease and found a sensitivity of 84%. The test easily detected T. cruzi antigens in sera of the patients used in the study. It was simple to perform and said to be highly specific. In a similar study, Marco and Colleagues[25] assessed the use of recombinant antigens for the accurate immuno diagnosis of Chagas' disease and obtained better results than when a single antigen was used. In the same study, recombinant ELISA technique was found to be better than conventional ELISA and when it was compared with other techniques like haemagglutination and immunofluorescence, it was found to be the best, producing a specificity and sensitivity of 100%. The workers recommended the use of recombinant ELISA technique in blood transfusion procedures so as to prevent the transmission of Chagas disease. In a related study, Claudia and Rossi[26] evaluated the ELISA technique and compared it with Indirect Immunofluorescent technique (IIF) and found out that ELISA technique exhibited 98.6% sensitivity

and 98.7% specificity respectively as opposed to 94.5% and 96.2% recorded for indirect immunofluorescence. They concluded that the ELISA technique was better than IIF method.

Recently, Gonzalezi and colleagues[27] have concluded a research work where three different protein antigens of T. cruzi have been coupled in polystyrene based latexes and used to develop a novel immunodiagnostic tool for effective diagnosis of Chagas disease. This will soon be put to use in field surveys since it has been found to be simple, very sensitive and specific.

AFRICAN TRYPANOSOMIASIS

This is an acute/chronic protozoan infection caused by two species of trypanosomes called:

(a) *Trypanosoma brucei rhodesiense*
(b) *Trypanosoma brucei gambiense.*

Whereas T. brucei rhodesiense is responsible for the acute infection in man, T. brucei gambiense causes the chronic form of the disease. Immunodiagnostic techniques have been employed extensively in the diagnosis and treatments of Trypanosomiasis. One of the most commonly employed methods is the latex Card Agglutination Trypanosoma Test (CATT). This method is based on the measurement of antitrypanosome 1gM in Cerebrospinal Fluid (CSF). It has been found very useful in the late stages of the disease when treatment is very crucial. One of the handicaps of CATT method is false positive result due to cross-reaction resulting from the presence of IgM in CSF in other conditions such as viral meningitis, tuberculous meningitis and neurosyphilis. However, Lejon and colleagues[28] evaluated the CATT method and found it to be simple, rapid and useful in field studies. It was not found to be useful in T. brucei rhodesiense endemic areas because, it detects only a few of the parasites. The CATT method can be performed using whole blood or diluted serum (to reduce cross reactions) and blood collected on filter paper[29].

VISCERAL LEISHMANIASIS

This is a chronic debilitating condition caused by species of protozoan organisms called *Leishmania donovani*. During the course of the infection, both specific and non-specific antibodies are formed against the Leishmania parasites. Immunodiagnostic techniques have been employed to detect the specific antileishmania antibodies in sera of affected individuals. Three of such methods or techniques include:

(a) Direct Agglutination Test (DAT)
(b) Rapid Latex Agglutination Test (LAT)
(c) ELISA.

These methods have been found to be cheap, reliable and useful in field studies[30].

Cummins and colleagues[31] evaluated the DAT method and compared it to other immunodiagnostic methods. He found out that DAT was more sensitive and specific than the Indirect Fluorescent Antibody Test (IFAT) and the Counter Immunoelectrophoresis (CIE) techniques. The rapid latex agglutination test was also evaluated by Mood and El-Safi[32] and they found the method to be quicker and easier to perform and interpret than DAT. Felix de Lima and colleagues[33] have assessed the use of ELISA methods to diagnose visceral leishmaniasis in lower animals in Brazil. They found the ELISA to be sensitive and specific and suggested its use to detect early infected animals in endemic areas so as to prevent human transmission and spread of the infection.

Furthermore, Krishna and colleagues[34] have also developed a direct ELISA technique using *Leishmania donovani* promastigote antigens for the diagnosis of kala-azar. In the said study, the test was able to differentiate between Kala-azar and other diseases prevalent in Asia and has the potential to be used in developing countries with laboratories that are poorly equipped.

Toxoplasmosis: This is a very deadly disease which is considered a zoonosis. It is due to infection by coccidian parasites known as *Toxoplasma gondi.* The organism naturally infects lower animals like the pig but may incidentally infect man when the later comes into close contacts with affected animals. The most serious form of the disease occurs during pregnancies when the baby in the womb contracts the infection via the placenta leading to congenital toxoplasmosis. Congenital toxoplasmosis is associated with very severe fetal abnormalities.

Immunodiagnostic techniques have been employed

in the diagnosis of toxoplasmosis especially in pregnancy and neonates where the infection is usually severe. Rotmars and colleagues[35] have assessed an indirect ELISA and antibody capture ELISA techniques using a major serological toxoplasma antigen (M.6KD), to detect IgM antibodies in human sera, and also, comparing the result with that obtained by the Immunoblot technique. The investigators concluded that the three methods were highly sensitive but whereas the indirect ELISA gave false positive result, the immunoblot test gave false negative result. The antibody capture ELISA gave no false negative reactions. The specificity was also high.

Two techniques that have been frequently applied in the diagnosis of toxoplasmosis are:

(a) The Sabin Feldman dye test
(b) The Eiken toxoreagent latex agglutination test.

SABIN FELDMAN DYE TEST

This is the most reliable immuno-diagnostic technique in the diagnosis of toxoplasmosis. It is based on complement mediated neutralizing antibody antigen reaction, using live trophozoites of *Toxoplasma gondi* to measure the parasite specific antibody. It is highly sensitive and specific.

EIKEN TOXOREAGENT LATEX AGGLUTINATION TEST

This test is simpler to perform and gives a comparative result to that of the Sabin Feldman test. However, the disadvantage of the latex agglutination test is its difficult interpretation due to the high prevalence of antibodies in most endemic populations as a result of the presence of past and sub clinical infections. Detection of toxoplasma specific IgM is indicative of recent infections. The major limitation of the latex agglutination test is its high cost and the fact that it has to be done in a reference laboratory such as the Centre for Disease Control (CDC) laboratory. Thus, it may not be useful in most field surveys.

HAEMOSPOROZOITES

The most important disease under this category is malaria, which has been ranked number one World parasitic disease. Four species of malaria parasites are important in human infection namely:

(a) *Plasmodium falciparum*
(b) *Plasmodium malariae*
(c) *Plasmodium ovale*
(d) *Plasmodium vivax.*

Amongst all these species, P. falciparum is the most prevalent in the tropics. The diagnosis of P. falciparum malaria has been made easier and simpler by immunodiagnostic techniques. Some of the most recently developed techniques can also detect P. vivax (mixed infections).

Immunodiagnosis of malaria is based on the immunochromatographic detection of two main plasmodium antigens known as Histamine Rich Protein 2 (HRP-2) and the specific Parasite Lactate Dehydrogenase (PLDH). These antigens are produced by the malaria parasite during its developmental cycle in the red blood cells. Extensive studies[36] have been carried out on recombinant and synthetic P. falciparum antigens to assess their immunodiagnostic properties. Six P. falciparum antigens were tested for their immunodiagnostic properties in ELISA and Direct Agglutination Tests (DAT). The authors concluded that the application of molecular P. falciparum antigen to ELISA had the best diagnostic properties. The traditional method of diagnosis of malaria is the identification of the parasites in a blood smear. However, there are cases in endemic areas where blood smear results come out negative whereas, the patient is having serious features or signs and symptoms of clinical malaria. Latonio and colleagues[37] have assessed such cases and found that whereas blood smear gave a false negative report in 22.7% cases, serological test using the Immunofluorescent Assay (IFA) gave only 1.4% false negative result, thus, emphasizing or highlighting the place of immunodiagnosis in malaria infection. In the study cited above, blood smear and IFA results agreed in 59.74% of the all the cases (where both tests were negative) and disagreed in approximately 5% of results.

In another study, Latonio and colleagues[38] write that malaria simulates other infections such as Salmonellosis (7.46% cases), Leptospirosis (7.46% cases), Respiratory infections (2.98%) and Urinary tract infections (1.49%). This makes immunodiagnosis necessary especially in conditions where something has to be done fast so

as to save life. Indirect Haemagglutination Assays (IHA) have been found reliable in such cases.
Based on the two main malaria antigens; HRP-2 and PlDH, three main blood tests for malaria have been developed.
These are:
(a) HRP 2 test- parasight F.
(b) ICT malaria PF
(c) PLDH Test Optimal.

THE MALARIA ANTIGENS

HRP-2: Is produced primarily by *P. falciparum* and is released into circulation by parasitized red blood cells. The antigen persists in circulation several days after the patient has been fully treated especially in heavy infections. Studies[39] carried out in Mali have shown that about 2-3% of P. falciparum strains found in that country were naturally lacking the gene for the production of HRP-2 antigen (the HRP-2 gene). When these strains of falciparum are involved in infections, the parasight F-test will give a false negative result.

PLDH Antigen: This is a malaria parasite enzyme produced by all species and strains of malaria parasites. It is normally released into the circulation by parasitized ruptured red blood cells. It is also found in urine in small concentrations[40].
Unlike the HRP-2 antigen, PLDH disappears from the circulation once the patient has been successfully treated.

The Para Sight F-Test: This is the first immunodiagnostic test method to be developed. Studies[41, 42,43] carried out in different places in endemic areas to evaluate the test method have shown that the Para Sight F method is easy to perform sensitive and specific for malaria parasite. It is a very useful tool in field surveys.

ICT Malaria PF Test: This test method has been evaluated by several workers[44] and found to be easy to perform, sensitive and specific for P. falciparum infections. The authors showed that the ICT malaria test remained positive seven days after successful treatment of infection due to the persistence of HRP-2 antigen in the blood as noted previously. Recently, ICT combined test has been developed, making it possible to diagnose both falciparum and vivax infections simultaneously.

Optimal Test: This is the most recently developed immunochromatographic rapid malaria strip test available in the market[45]. It is capable of detecting both *P. falciparum* and *P. vivax* (mixed) infections and is useful in differentiation of malaria species due to the fact that there is "antigenic differences between PlDH isoforms[45]. It is easy to perform, sensitive and specific for malaria parasite. Since it is based on PlDH antigen which disappears after treatments, it can be used to monitor responses to drug therapy and to detect drug resistant strains of malaria. The PLDH antigen level in blood reflects the presence of viable malaria parasites in the blood.

CONCLUSION

Traditionally, diagnosis of parasitic infection has been based on microscopic examination for parasites or their eggs in human samples such as urine, blood and stool, or biopsy material. This has been okay for clinical medicine but for quick diagnosis and epidemiology, this approach is viewed critically as practically impossible. This is particularly so in situations when few or no parasites or their eggs are available in specimens for identification, such as in low intensity of infection, in early incubation period, or in late chronic stages of the infection. The development of immunodiagnosis has changed this picture.

Immunodiagnostic techniques have a wide application in parasitology. In certain parasitic diseases, some of these techniques are sensitive, specific and diagnostic whereas in a few others, they have limited value due to non-specificity and cross-reactions. Techniques like the ELISA has made the diagnosis of most parasitic diseases easier, quicker and cheaper and have been applied widely in field studies and surveys where they have proved to be indispensable. For instance, the ParaSight F-test, for malaria parasite can be used to screen so many people for P. falciparum infection in a given population in a short period of time, a task which microscopy alone will not be able to achieve in record time.

Immunodiagnositic techniques are therefore an essential tool for field workers and epidemiologists for effective screening of target populations.
With the recent technological advancement in the Western countries, it is expected that in the near

future, immunodiagnosis will form the bedrock of all laboratory diagnostic methods. These will make diagnosis easier and quicker and enable clinicians to arrive at correct clinical judgements and appropriate treatments of their patients. The World will be better for it.

REFERENCES

1. Azia, Hassan, Beck, Carolyn E, Flux, Mary F., Hudson, M Jame (2001) A comparisms study of different methods used in the detection of Giardia Lamblia Clinical Laboratory Science, Summar 2001. hhtp://findarticles.com/p/articles/mi_3890/is_200107/ai_n8956237/pg-2/.
2. Grigory J. Bulfone. Principles of Immunoclinical Techniques. Biochemistry Vol. 1. (IC) pp. 290-2207
3. The National Committee for Clinical Laboratory Standards (1993). Agglutination analyses. Antibody Characteristics, Methodology, Limitations, and Clinical Validation; Approved Guildline NCCLS document. Vol 13 (15).
4. Castelino J.B. (1986) Immunodiagnosis of Parasitic infections. Tools are evolving to help control diseases affecting over 900 million people. IAEA Bulletin, Summer 1986.
5. Erlandsen, L.S., E.A. Meyer (1984). Giardia and giardiasis: biology, pathogenesis and epidemiology. Plenum Press, New York.
6. Petersen, H. (1972). Giadiasis (Lambliasis) Scand J. Gastroenterology. 7 (Suppl 14): 1-44.
7. Brodsky, R.E., H.C. Spencer, and M.G. Schultz (1974) Giardiasis in American travelers to the Soviet Union. J. Infect. Dis. 130:319-231.
8. Crawn G.F. (1979) Waterburne out break of Giardiasis In Jakubowski, W., J. Hoff (Ed) Water born transmission of giardiasis. U.S. Environmental Protection Washington Pp. 127-1.
9. World Health Organization Scientific Working Group (1980). Parasite related diarrhoeas. Bull. WHO 58:819-830.
10. Pickering L.K., W.E. Woodward, H.L. Duporil, and P. Sullivan (1984). Occurrence of Giardia Lamblia in day care centres J. Ped. 104: 522-526.
11. White, K.E., C.W. Hedberg, L.M Edmonsen, D.B.W. Jones, M.T. Osterholm, and K.L. MacDonald (1989) An outbreak of giardiasis in a nursing home with evidence for multiple modes of transmission. J. Infect. Dis. 160: 298-304.
12. Stevens, D.P., and A. A. Mahmond (1980). Giardiasis: the rediscovery of an ancient pathogen. Curr. Clin. Trop. In Infect. Dis. 1:195-207.
13. Phillips, S.C., D. Mildvan, and D.C. Williams (1981) Sexual transmission of an enteric protozoa ad helminthes in a veneral disease clinic population. New Eng. J. Med. 305: 606.
14. Friend, D.S. (1966) The fine structure of Giardia Muris. J. Cell. Biol. 29:317-332.
15. Faubent, G.M. (1988). Evidence that giardiasis is a zoonosis. Parasit Today 4:66-89.
16. Sun. T. (1980) The diagnosis of giardiasis. Am. J. Surg. Pat. 4:265-271
17. Kamath K, R Murugasu (1974) A comparative study of four methods for detecting Giardia Lamblia in children with diarrhoeal disease and malabsorption. Gastroenterology 66:16-21.
18. Burke J.A. (1977) The Clinical and Laboratory diagnosis of giardiasis. Crit. Rev. Clin. Lab. Sci. 7:373-391.
19. Nash, T.E., D.A. Herrington and M.M. Levinc (1987). Usefulness of an enzyme linked immunoassay for detection of Giardia antigen in faeces. J. Clin. Microbiol. 27:1169-1171
20. Janoff, E.N., J.C. Croft, L.C. Pickering, T. Novotny, M.J. Blaser, C.V. Knisley and L.B. Reller (1989) Diagnosis of Giardia Lamblia infections by detection of parasite specific antigens J. Clin. Microbiol. 27: 431-435.
21. Stibbs, H. (1989). Monoclonal Antibody based enzyme immunoassay for Giardia Lamblia antigen in human stool. J. Clin. Microbiol. 27: 2582 - 2588.
22. Garcia L.S., Brewer T.C., Bruckner D.A. (1979) A comparism of the formalin-ether concentration and trichrome S methods for recovery and identification of intestinal protozoa. Am J. Med. Technol. Nov. 45(11).
23. Beth L.P Ungar, Robert H. Yoken, Theordore E. Nash and Thomas C. Quinn (1984). Enzyme-linked immunosorbent Assays for the detection of Giardian lamblia in faecal specimens. J. Infect Dis Vol 149(1): 90-97.
24. Breniere F.S., Carlier Y, Carrasco R,

Molinedo S, Lemestic J.L. Desjeux, P. Afchain D. (1987). Specific Immunodiagnosis of Chagas disease: Immunodiffusion test using a specific serum anti-Trypanosoma cruzi component 5. Trop Geogr. Med. 39(3): 281-285.

25. Marco A. Krieger, Elza Almeida, Walter Oelemann, Juan J. Lafaille, Josse Borges Pereira, Henirque Krieger et al (1992) Use of Recombinant Antigens for the accurate immunodiagnosis of chagas disease. Am.J. Trop. Med. Hyg. 46(4): 427-434.
26. Clandia D. Partel and Clandio L. Rossi (1998) A rapid quantitative ELISA for immunodiagnosis of Chagas disease. Immunol. Inv. 27(1) 89-96.
27. Gonzalezi Veronica D.G., Valeria S. Garcig Jorge R. Vega, Ivan S. Marcipa, Gregorio R, Merra et al (2010). Immunodiagnosis of Chagas disease: Synthesis of three latex-protein complexes containing different antigens of T. cruzi. Biointerfaces 77(1): 12-17.
28. Lejon et al (1997). Further evaluation of latex IgM assay for IgM quantification in cerebrospinal fluid of sleeping sickness patients. Paper presented 24th ISCTRC meeting. Maputo Maza Mbique 23/9/97 3/10/97.
29. Miezan T. et al (1991). Evaluation of Testryp CAT applied to blood samples in filter paper and to diluted blood in a focus of trypanosomiasis due to Trypanosoma brucer gambiense in Cote d'Ivoire. Bull WHO 69(5): 603-609.
30. Harith A.E. et al (1986) A simple and economically direct agglutination test for serodiagnosis and seroepiemiological studies of visceral leishmaniasis. Transact. Rog. Soc. Trop. Med. Hyg. 80:583-587
31. Cummins A.J. et al (1994) Development of a rapid Latex agglutination for the detection of visceral Leishmaniasis: Trans. Roy.Soc.Trop.Med. Hyg. 88: 300
33. Felix de Lima Valeria Maical, Luciane Biazzono, Ana Clandia Silva, Ana Paula Ferveira Lopes Correa and Maria Cecilia Rui Luvizotto (2005).
34. Krishna Mukerji, Abhijit Pal, Kshudiram Naskar, Dillip K, Ghosi et al (2005). Direct ELISA: A simple immunoassay using L. donovani promastigote for diagnosis of kala-azar. J. Clin. Lab. Analysis 5(4): 299-301.
35. Rotmans, J., W. Duchenne, C. Linscooten, N. Int'l Veld, and A Polderman (2005) Comparative study of three immunoassays for detection of immunoglobulin M. antibodies against toxoplasma gondic. J. Clin. Microbiol. Infect. Dis pp. 535 538.
36. Knobloch M, Schreiber, S. Grokho Sky, A. Scherf (1987). Specific and non-specific Immunodiagnostic properties of recombinant and synthetic P. falciparum Antigens: Eu. J. Clin. Microbiol. 547-551.
38. Latonio A. Arcilla, D; Josefina C. Alzaga, and Valorie F. Chan (1983) The value of immunodiagnosis in problematic tropical and infectious diseases encountered in the Philippines. Phil. J. Microbiol. Infect. Dis 12(2): 147-154.
39. Traore I. et al (1998). Field studies of parasight F test in a malaria endemic area: cost feasibility, sensitivity specificity, predictive value and detection of the hrp-2 gene among wide type plasmodium falciparum in Mal. Am. J. Trop. Med. Hyg (in press 1998).
41. Mbarakurwa, S; Manyame,B; and Shiff, C.J.(1997) Trial of ParaSight F test for malaria diagnosis in the primary health care system Zimbabwe. Trop. Med. Int. Health Vol. 2(6) 544-50.
42. Craig, M.H; and Sharp, B.L (1997) Comparative Evaluation of four Techniques for the Diagnosis of Plasmodium falciparum infections. Trans. Roy.Soc.Trop. Med. Hyg. 91:279-282.
43. Udechukwu, C.U and Onyali I.O (2010) Comparative analysis of different diagnostic techniques for plasmodiasis. Nig. J. Parasitol.31 (2): 108-112.
44. Singh N, Valecha, V.and Sharma, V.P (1997) Malaria diagnosis by field workers using an immuno chromatographic test. Trans. Roy. Soc. Trop. Med. Hyg. 91 pp 396-7.
45. Markler M.T. (1997). Introducing OptiMAL rapid malaria test. Flow Inc 6127 SW orbett. Portland O.R. 97201 U.S.A.

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Case Report

UNUSUAL PULMONARY PRESENTATION OF WILMS TUMOR MESTASTASIS DETECTED BY ABDOMINAL ULTRASOUND WITH REVIEW OF THE LITERATURE

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ABSTRACT
We report a case of 9 years old boy that presented at 5 years with nephroblastoma. He had an unusual metastatic deposit at the right lung base after 3 years following surgery and courses of cytotoxics. Metastasis to the lungs following Nephroblastoma is common[1] but the presentation as an elevation of the right hemidiaphragm on a chest radiograph and a right basal mass are rare. The objective of the report is to highlight the fact that elevation of the diaphragm in patients with nephroblastoma may well be hiding a metastatic deposit.

Key words: *Wilms tumor, Metastasis, Ultrasonography.*

INTRODUCTION
Wilms tumor is the most common renal malignancy of childhood under 5 years. The annual incidence in the United States of America is about 7.5 million children under 15 years of age. The annual incidence in Nigeria is not known. The tumor has been diagnosed in the new born. It is both heritable and sporadic. It is inherited as an autosomal dominant trait with incomplete penetrance[2].

Presentation is most often seen as a protuberant abdomen. Radiological evaluation usually include:-

(a) Chest radiography
(b) Ultrasonography and
(C) Computerized Tomography.

These modalities will be able to establish the presence of a renal mass and a normal functioning contralateral kidney. They are useful in demonstrating the presence or absence of pulmonary metastasis.

Nephrectomy is the primary treatment and the decision for further therapy will depend on the stage of the tumor. Relapse occurs most often within 4 months of initial diagnosis and most common site of metastasis reported include; mandible, brain, paratesticular and duodenum. In the case reported here, the authors highlight the fact that it can mimic any of the causes of elevated hemi diaphragm.

CASE REPORT
C.O. is a 9 year old boy who presented at the age of 5 years in 2003 with abdominal mass and painless haematuria following trauma to the abdomen. Clinical examination suggested a diagnosis of nephroblastoma. Routine investigations ordered included urinalysis, which essentially showed no white blood cell but a red blood cell count of 1000. There was no bacteria growth seen in the culture. Serum urea / creatinine were normal, white cell count 5.3 x 10^9 d/l and platelet of 189 x 10^9/L were recorded. Geneotype is AA.

Abdominal ultrasound revealed an enlarged right kidney, 110mm in its longest axis with a mixed echo complex mass within the mid portion and extending to the superior pole. No caliectasis was noted. The left kidney 83mm in its longest axis had a normal echo texture. Other intra abdominal organs, liver gall bladder, pancreas and the spleen were normal. An impression of wilms tumor was made and intravenous urography advised.

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At Intravenous Urography (IVU), the right kidney showed a bulge on the superior lateral aspect of the nephrogram. There were no calyces demonstrated even after 2 hours. The left kidney outline was normal and the collecting system as well as the urinary bladder outline. An impression of a right renal mass at the upper pole was made with impaired renal function.

At surgery, the right kidney was enlarged with necrotic mass at the upper pole which ruptured on mobilization. Involvement of the lymph nodes was not clear except within the mesentery. A right nephrectomy was done and a mesenteric lymph node biopsy as well. Cytotoxic therapy was instituted with cyclophosphamide, actinomycin D and vincristine. Histology of the specimen consists of the kidney, rupture at the one of it's poles with accompanying friable grayish white tissue, measuring 10cm x 9cm x 6cm.

Microscopy of the histologic section showed sheets of primitive ovoid cells with deeply eosinophillic nucleic stroma. These primitive cells were seen forming tubular structures in some areas. The lymph node tissue showed hyperplastic changes but it is spared of infiltration by these primitive malignant cells. A diagnosis of nephroblastoma was made.

After 6 courses of cytotoxics, a follow up abdominal ultrasound, showed normal organs and no lymph nodal enlargement. Follow up chest x-ray was normal but for the elevated right hemidiaphragm, that was dense as well. The carina was not well defined and mediastinum was displaced to the left. Bony ribcage was intact.

An impression of right sided pleural effusion and a possible right lobe basal mass was made. A repeat abdominal ultrasound following the above chest x-ray findings showed a huge homogenous roundish mass seen "sitting" on the dome of the right hemi diaphragm, the central portion was hypoecoic and curvilinear. There was no associated pleural effusion. The liver was normal and the intra abdominal organs as well. There was no ascities.

DISCUSSION

Wilms tumor (Nephroblastoma) is a renal tumour that develops in childhood and one of the most common malignant tumors in children under 5 years[2].

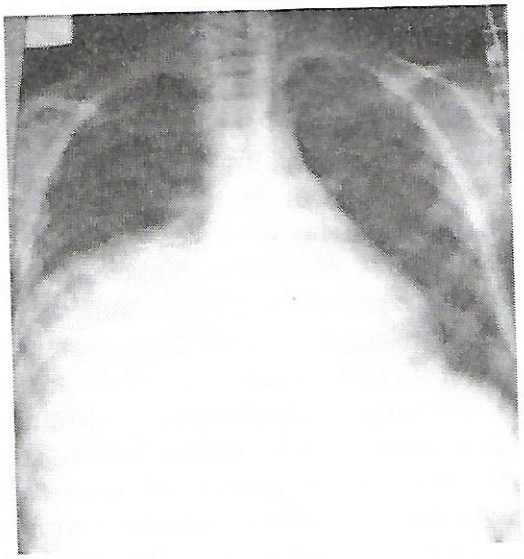
The incidence of this disease is unknown in Nigeria and under reporting is a major contributory factor. Metastasis of this tumour has been reported involving many parts of the body [3,4,5,6,7].

Tracking metastasis of this tumour needs an efficient painstaking approach and the relevant facilities. With the dearth of pathologists Nigeria, it's often difficult to collaborate this effort. In a series reported from department of pediatric surgery at the India institute Of Medical Sciences, over 11 years, 101 cases of wilms tumour were seem. Of the thirteen that had metastasic disease at onset, 24 patients presented with relapse at a latter date. Our patient did not present with metastasis at onset. Risk factor associated with relapse was found to be unfavourable histology. Lymph node involvements, age more than 6 years, diffuse spill, capsular and vascular invasion and anaploidy. There was report of spill at surgery but no lymph node involvement was noted except in the mesentery. Mainstay of the management in most centers in Nigeria involves surgery and chemotherapy but elsewhere "judicious" use of other options are in place, chemotherapy and radiotherapy to the metastasic sites, second look surgery, resection of the pulmonary metastasis and use of CIS-Platinum. Diaphragmatic elevation of no obvious cause must be further investigated in such patients with a history of nephrectomy following a diagnosis of nephoblastoma with other imaging modalities other than the usual chest radiograph. The reporting session by radiologists should not stop at the common differential diagnosis of hemidiaphragmatic elevation like hepatic tumour, amoebic liver assess, subphrenic collections, phrenic nerve plasy[7]. In centres where investigative modalities are limited to plain radiographs, ultrasound can go a long way in making a diagnosis of Wilms Tumour metastasis. It

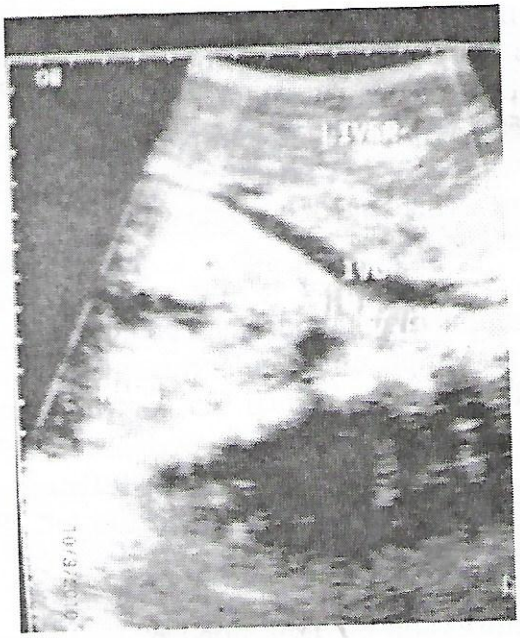
has been used to detect intracaval involvement.
In conclusion, we highlight the need to further investigate cases of wilms tumour for metastasis following resection with abdominal ultrasound. At follow up visits, this could be the first indication of metastasis especially if there is diaphragmatic elevation.

REFERENCES

1. Matsunaga E. Genetics of Wilms Tumour. Hum. Genetics, 1981; 57:231.
2. Parambil J.G, Aughenbaugh GI, Perara TC et al, Solitary Pulmonary Metastasis 20 years after Primary Resection of Wilms Tumour' Mayo Clinic Pro. 2005 links Nov; 809 (11):
3. Jia J Clen, XM, Sun Z.J, Z Hang WF Mandibular Metastases of Nephroblbrown: A rare case; Int J Maxiloo et al, Surg 2006, Dec; 35 (12) 1160-1161.
4. Lall A, Pritchard Jones K, Walker Jeral, Paediatr Wilms Tumour with Intracaval Thrombosis in the Uk Children Cancer Study Group UKW3 GROUP Surg. 2006 Feb:41(2);382-7.
5. Coutinho GF. Heitor F, Antunes PE et al, Left Atrial Extension of a Wilms Tumour; Annal Thorac Sug 2005 Sep; 80 (3): E 8-9.
6. Aydin GB, Clifci AO. Yalcin B Akacorenz et al Paratesicular Metastasis from Wilms Tumour Associated with a Hydrocoere; Peadiatric. Blood Cancer, 2006 July; 4(1)97-9.
7. Misra D, Gupta DK, Mitra DK et al; Wilms Tumour with Intracaval Involvements. Management of mestasic Wilms Tumour: An Eleven Years Experience; Indian j Cancer 1996, Mar; 33(1):1-5.
8. Martinez Ibanez, Sarchez De Tsledo J. Drdigo M et al, Med Wilms Tumor with Intracaval Involvement Peadaiator Oxcology 1996, April; 266 (4);2668-71.
9. Radiogical and Imaging Secrets, 2nd Edition LC gupta, Abritabs Gupta JAYPEE BROTHERS, Medical publishers (P) LTD B-3 EMCA House, 32/33 Ansir Road Daryanganji India, pp. 57-58.



Chest X-ray



Liver

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