Effects of Oral Contraceptive on the Histology and Biochemistry of the Liver and Kidney of Adult Female Albino Rat

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ABSTRACT

Oral contraceptives (OCs) are the most mainstream sort of conception prevention. The pills stop ovulation and keep the ovaries from delivering eggs. The study aims to evaluate the effect of oral contraceptive on the histology and biochemistry of the Liver and Kidney using female albino rat. Twelve animals were grouped into three groups of four animals (n=3). Group I served as a control; Group II were administered oral contraceptives for seven (7) days while Group III received oral contraceptive for the period of fourteen (14) days. The animals were treated for twenty-one days (21) days, at the end of which they were anaesthetized under Chloroform, sacrificed and their blood serum was collected for biochemical assays. The Livers and Kidneys were collected for histological studies. The result showed the variation in selected Kidney function parameters of rat treated with 0.75mg of Postinor-2, a synthetic hormone. There was a significant increase ($p<0.05$) in Urea and Creatinine in the group served with OCs. Postinor-2 was observed to have a significant time-dependent impact on K, Cl and Na levels. Animals in group 2 experienced no significant increase or decreased ($p>0.05$) in the level of ALP, ALT, AST and Bilirubin but those in group 3 were significant ($p<0.05$) when compared with the control group. Histological sections reveal distorted tubules and vessels. Frequent use of OCs could cause deregulation of the extracellular fluid level, hypertensive disorder, renal and hepatic impairment.

Keywords: Oral Contraceptive, Histology, Biochemistry, Liver, Kidney, Albino Rat

INTRODUCTION

Oral contraceptives (OCs) are the most mainstream sort of conception prevention. The pills stop ovulation, keeping the ovaries from delivering eggs. They additionally thicken cervical fluid, making it harder for sperm to enter the uterus.[1] Oral conceptive may expand a lady's danger to Liver illnesses, Kidney infections, Cerebrovascular sickness and Cervical malignant growth.[2] A woman taking the pills is 1.9 times liable to die from Cerebrovascular related illnesses and 2.5 times liable to die from Cervical disease.[2] Oral contraceptives are taken by about 100 million women around the world. Studies have demonstrated that engineered chemicals utilized for oral preventatives enormously increased the danger of blood coagulation and structures in the legs and can cause injury and death if they travel to the Heart, Lungs and Brain.[3]

Leptin's consequences for body weight are interceded through impacts on hypothalamic foci that control and conduct, internal heat level and energy use. It is a protein chemical with significant impacts in directing body weight, digestion and regenerative capacity. Additionally, leptin can serve as a hunger suppressant. It lessens an excessive amount of the body fat and is more dynamic to burn off more energy. The measure of Leptin found in individual's increases as their muscle versus fat ratio increases.[4]

Combined oral contraceptives (COCs) is the most endorsed contraceptive strategy and is utilized by more than 100 million women around the world.[5] These pills contain an Estrogen part (Ethinyloestradiol, Mestranol, Oestradiol or its derivative Oestradiol valerate) and a Progestrogen (Levonorgestrel, Norethisterone, Gestodene,

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Desogestreldrospirenone, Nomegestrol, Dienogest or Cyproterone).[6] Postinor-2 is an oral preventative tablet containing the synthetic Progestogen (Levonorgestrel). The fast advancement of this preventative strategy, especially as for the decrease in the portion of Estrogen and the union of the new Progestogens has been reported.[7] As of late, new prophylactic regimens, explicitly those including consistent or extended use points toward limiting the chemical-free span between one bundle of pills and another.[8] The consolidated oral prophylactic pill is a compelling strategy that can likewise offer different advantages. Women quickly embraced the pills as they permit the solid partition of sex and multiplication and allowed them the chance to arrange for when to have kids. From that point forward the pill had evolved to guarantee great viability while limiting unfriendly impacts.[6] Women who do not use COCs have essentially lower paces of death from malignancy, Cardiovascular infection and different sicknesses.[8]

Lately, investigations have indicated that oral prophylactic pills may affect Liver and kidney functions. Excess of this medication has been demonstrated to upset ordinary Liver and Kidney morphology. As indicated by Fakhir *et al*., (2016),[9] oral preventative pills influence the biochemical boundaries of the Kidney, with raised degrees of Creatinine, Urea and Electrolytes. Oral preventative likewise affects Liver capacity. Raised degrees of Acid phosphatase, Aspartate aminotransferase and Alanine aminotransferase have been found in people on oral contraceptives.[9]

Oral Contraceptive is a medication ordinarily utilized for the prevention of pregnancy, over 200 million women overall accept oral preventative pill as methods for forestalling origination.[10] Oral preventatives are predominantly manufactured chemicals that disturb the normal hormonal cycle in a woman which may cause a lot of issues ranging from Cardiovascular illness to Kidney and Liver sicknesses. Many women because of easy access have assumed control over dosing of this medication, for the most part, because of the non-limitation and accessibility of the medication in various patent medication stores. Consequently, they may be causing destruction of their Kidney and Liver since these organs play significant roles in the metabolism and discharge of medication metabolites.[10]

Therefore, the purpose of this study is to evaluate the histological and biochemical effect of oral contraceptive on the Liver and Kidney. Information obtained from this study will be of importance in patients' guidance and counselling.

MATERIALS AND METHODS

Procurement of Oral Contraceptive

The oral contraceptive (Postinor-2) product name (0.75mg Levonorgestrel) Generic name used for the study was purchased at Ampuh Toa Pharmacy 109 Chime Avenue, New Heaven, Enugu.

Animal Housing

Twelve (24) adult albino rats of both sexes weighing between 100-145g were obtained from the animal house facility of the Veterinary medicine, University of Nigeria Nsukka, Enugu State. The animals were housed in steel wire cages and allowed to acclimatize for one week under standard conditions of temperature 25°c ± 3°c). The rats were fed with standard pellets (Guinea feed Nigeria®, PLC) and water ad libitum. All animals in this study were handled according to international guidelines for handling experimental animals.[11]

Ethical consideration

The study ethical approval was granted by College of Medicine Ethical Committee (COMREC), University of Nigeria with an approval number: 076/08/2018. The study was also conducted in compliance with policies outlined in the Guide for the Care and Use of Laboratory Animal.[11]

Experimental Design and Conduct

Animals were grouped into three groups of four animals (n=4). Group I animals served as vehicle control, Group II animals were administered oral contraceptives for the period of 7 days, while

Group III animals were administered oral contraceptive for the period of 14 days. All drugs were administered intraperitoneally. The animals were kept for twenty-one (21) days, at the end of which they were anaesthetized under Chloroform, sacrificed and their blood was collected for biochemical assays and the Liver and Kidney were fixed in 10% formalin for histological studies.

Biochemical Tests

At the end of the 21st day, the animals were weighed, blood samples were collected by retro-orbital puncture from the medial canthus of the rats, the serum was separated from each blood sample and some biochemical parameters were evaluated.[12]

Serum Alkaline Phosphatase (ALP) estimation was by the enzymatic method of Reitmen and Frankel (1957).[12] Serum Electrolytes were estimated using a machine and method designed by Champion, Pellet and Grenier called Flame Emission Spectrophotometer Method. Sodium and potassium concentration were determined using the Flame Emission Spectrophotometer, while Chlorides concentration was determined using same.[13]

Relative Organ Weight

The rats were sacrificed under Chloroform anesthesia and dissected. The Liver and Kidney of each rat weighted to determine the relative organ weight (ROW) of each organ which is calculated thus:

ROW = Absolute organ weight (g)/bodyweight of rat on sacrifice (g) x 100/1

Histological Processing

The excised Liver and Kidney were cut in slabs of about 0.5µm thick and fixed in 10% formalin. The tissues were processed with paraffin wax embedding medium using an automatic tissue processor.[14] Hematoxylin and Eosin staining was done according to Omorodion *et al.,* 2018.[14]

Microscopy and Photomicrography

Tissue sections were examined using a microscope designed by Takeshi Yamashitanamed Olympus binocular microscope with in-built lighting system. The sections were photomicrographed using a digital microscope camera (Samsung Model SS & 50) attached to an Olympus trinocular microscope.

Statistical Analysis

Results were expressed where appropriate as mean ± standard deviation (SD). Differences between valves were determined with one-way analysis of variance (ANOVA) $P < 0.05$ was considered significant.

RESULTS

Biochemical effect of oral contraceptive on serum electrolyte

The result shows the variations in selected Kidney function parameters of rats treated with 0.75mg of postinor-2 synthetic hormone. The oral contraceptive was observed to be significantly increased ($P < 0.05$) Urea and Creatinine in a matter that is dependent on period of ingestions compared to the control group Furthermore on electrolytes with Kidney Function changes as indicated by K, Cl, and Na. Postinor 2 was observed to have a specifically impact on K level, which was time dependent decrease compared to the control. Although Na was found to decrease but the result was not statistically significant.

Biochemical effect of oral contraceptive on serum ALP

The table below shows the effect of various doses of oral contraceptives on the level of ALP of normal rats. Animals in the control group have a normal concentration of ALP. Animals in group 2 experienced a slight decrease in the level of ALP but the result was not significant ($P > 0.05$) as compared with the control group. Animals in treatment groups 3 showed significant ($P < 0.05$) decrease in ALP concentration compared with the animals in the control group. Rats administered oral contraceptive for (7 days) showed a non-significant ($P > 0.05$) decrease in ALP while those rats administered with the drugs for 14 days showed a significant decrease in ALP level.

Biochemical effects of oral contraceptives on serum ALT

The table shows the effect of various doses of oral contraceptives on the level of serum Alanine aminotransferase (ALT) of normal albino rats. Animals in the control group have a normal concentration of ALT. Animals in group 2 experienced a slight increase in the level of ALT but the result was not significant ($P > 0.05$) as compared with the control group. Animals in treatment group III showed significant ($P < 0.05$) increase in ALT concentration compared with the animals in the control group.

Biochemical effect of oral contraceptives on serum AST

The table shows the effect of various doses of oral contraceptives on the level of serum Aspartate aminotransferase (AST) of normal albino rats. Animals in the control group have a normal concentration of AST.

Animals in group 2 experienced a slight increase in the level of AST but the result was not significant ($P > 0.05$) as compared with the control group. Animals in treatment group III showed significant ($P < 0.05$) increase in AST concentration compared with the animals in the control group.

Biochemical effect of oral contraceptive on serum total Bilirubin concentration

The table 2 shows the effect of various doses of oral contraceptives on the level serum total Bilirubin of normal albino rats. Animals in the control group have a normal concentration of total Bilirubin concentration. Animal in group II experienced a slight increase in the level of total Bilirubin but the result was not significant ($P > 0.05$) as compared with the control group. Animals in treatment groups 3 showed significant ($P < 0.05$) increase in total Bilirubin concentration compared with the animals in the control group.

Histological Effect of Oral Contraceptive on the Kidney of Adult Female Albino Rat

The result showed normal histology of the treated animals in comparison with the control group, as shown in the figures below. But there was a slight alteration that was not necessarily significant in the Kidney cells in group 3. The result may reflect the safety of Postinor pills, as previous studies referred to the wide use and safety of this drug. Despite the presence of biochemical changes related to the Kidney.

Table 1: Shows the biochemical effect of oral contraceptives on the Kidney of an albino rat on oral conceptive at different time intervals and was expressed in mean plus standard.

Kidney Function Parameters	Group 1 (Control)	Group 2 (OCs for 7days)	Group 3 (OCs for 14days)	*p*-value
Urea Mmol/L	6.46 + 2.040	25 + 0.05	19.76 + 1.97	$p < 0.05$
Creatinine Mmol/L	152.93 + 22.10	5746.11 + 414.60	260.79 + 29.17	$p < 0.05$

The values obtained in the above table are statistically significant at $p < 0.05$.

Table 1 shows the biochemical effect of oral contraceptives on the Kidney of adult female albino rat on oral conceptive at different time intervals. Group 1 served as the control, group 2 was administered the oral contraceptive for 7 days while Group 3 were administered the oral contraceptive for 14 days.

Table 2: Shows the variations in selected Kidney function parameters of rats treated with 0.75mg of Postinor-2 synthetic hormone.

Electrolyte	Group 1 (Control)	Group 2 (OC's for 7days)	*p*- value for 14days)	Group 3 (OC's	*p*-value
K (Mmol/L)	4.05+0.92	8.63+1.90*	0.03	13.58±1.86*	0.02
Cl (Mmol/L)	101.00±2.83	92.33±2.08	0.06	83.75±5.66	
Na (Mmol/L)	136.00±1.41	111.67±10.41	0.0 9	121.25±3.77	

Investigation on the effects of oral contraceptives on the Liver and Kidney organs of adult female albino rats were carried out. The biochemical effects were first evaluated in this work: Biochemical analysis and *histological* examination of the Liver and Kidney organs. Results obtained for the parameters assessed are shown below.

Table 3: shows the biochemical effect of oral contraceptives on the Liver of an albino rat on oral conceptive at different time intervals and was expressed in mean plus standard deviation.

LFT	Group 1 (Control)	Group 2 (OC's for 7days)	*p*-value	Group 3 (OC's for 14days)	*p*-value
ALP (n/l)	84 ± 2.96	35 ± 14.20	0.08	14+ 2.51*	0.001
ALT (/i/Z)	22 ± 3.25	25.00 + 6.69	0.79	50 ± 0.33*	0.009
AST (/r/Z)	96 ± 99	99± 1.23	0.99	118 ±2.78*	0.023
TB (mg/dl)	0.91± 0.22	1.1± 1.10	0.08	1.8 ±2.22*	0.021

The sign (*) denote significant at value less than or equal to 0.05

Keys: TB (total bilirubin), CB (Conjugated bilirubin), AST (Aspartate Transaminase), ALT (Alanine transaminase), ALP (Alkaline phosphatase.

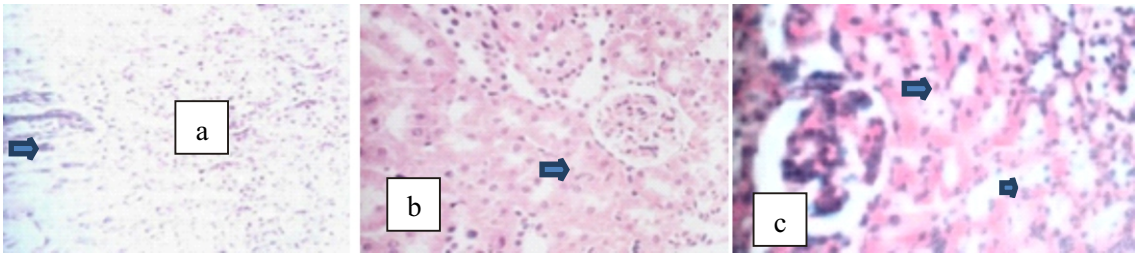

Figure 1: Photomicrograph (a)showing a section of Kidney with normal convulated tubules, urinary space, renal corpuscle and glomeruli, Photomicrograph (b) showing normal section of Kidney treated for 7 days with oral contraceptive, Photomicrograph c, showing section of Kidney treated for 14 days with disrupted tubules and vessels (arrows).

Histological effect of oral contraceptive on the Liver

The result showed normal histology treated animals in comparison with the control group, as shown in the figure below. But there was a slight alteration that was not necessarily significant in the Liver cells in Group III.

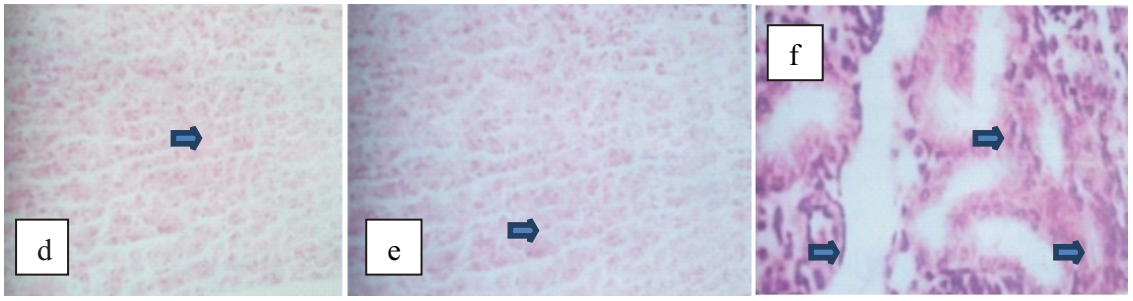

Plate 2: Photomicrograph of normal liver tissue section representing the control (d), photomicrograph of liver tissue (e)treated for 7 days showing normal histological architectures, Photomicrograph (f) treated for 14 days showing diffused vacuolation as well as periportal inflammation. There is lymphoid aggregation at the focal area, the nuclei of the hepatocyte appear hyperchromatic. Stain H&E. MAG X400.

DISCUSSION

The two most influential female sex hormones include Oestrogen and Progesterone, change in concentration across the menstrual cycle and are caused by oral contraceptive usage. In the present investigation, it was observed that oral contraceptive containing Levonorgestrel significantly increased the Creatinine output suggesting an increase in muscle metabolism. This is sequel to the fact that Creatinine is produced and excreted at a constant rate which is proportional to the body muscle mass. The mean significant increase is in line with the study of Oelker *et al*., (2005),[15] who studied oral contraceptive containing antimineralocorticoid Progesterone, but his research contradicts the study of Taneepanichskul *et al*., (2007),[16] who reported no significant change in the mean value of Creatinine following 6 cycles of oral contraceptive ingestion. Although depressed levels of Creatinine are rare and are not clinically significant, its excretion is an indication of renal impairment and are regarded as the most important marker for the diagnosis and treatment of Kidney disease and measured primarily to ascertain Kidney diseases.

From the research, findings on electrolyte with Kidney function showed that oral contraceptive containing 0.75ml of Levonorgestrel significantly increased plasma Na+ and K+ but decreased plasma Cl. This study is following several other studies Oeker *et al*., (2005).[15] The results from this study suggest that oral contraceptive usage may alter the fluid nature of extracellular fluid.

Thus, understanding the interaction between oral contraceptive and fluid regulatory system is crucial. Female sex hormone has been reported to influence Sodium and water distribution and thus, fluid compartment volumes and dynamics and may not be unrelated to the hypersensitive effect of oral contraceptive usage.[15] This mechanism behind the effect of oral contraceptive used in this study may be explained by the fluid retention potentials by activating the renin angiotensin aldosterone system, enhances vasodilation, capillary permeability and lower the operating setpoint of plasma osmolality by Oestrogen.[15] Progesterone has been known to counter the effect of Oestrogen by competing with mineralocorticoid receptor as Aldosterone which may cause natriuresis.

From the study, it can be deduced that prolonged use of oral contraceptive affects Liver function parameters. There was no significant increase in the level of serum ALT, Bilirubin and AST in the animal group treated for 7day, but there was a significant increase in the level of serum ALT, Bilirubin and AST.

In the animal group treated for 14 days, there was a marked increase in the lever of AST. This might be as a result of the oral contraceptive on the Heart. AST is also a marker of cardiac muscle damage. There was no significant decrease in the level of ALP in the animal group treated for 7days as compared with the level of ALP in the animal group treated with an oral contraceptive for 14 days.[15]

The animal group treated with 0.75mg of Levonorgestrel for 7days did not experience any form of kidney or Liver damage. This might be suggestive of the safety of the drug for women's consumption. The animal group that was treated with the drug for 14 days experienced slight alteration in the morphology of the Liver and Kidney but the result was not significant as compared with the control group.

CONCLUSION

Frequent use of oral contraceptive could cause deregulation of the extracellular fluid, can lead to hypertensive disorder and renal impairment. Also, frequent use of oral contraceptive could lead to Liver impairment.

RECOMMENDATION

More research should be carried out on the effect of oral contraceptive on the heart. More awareness sessions on health for mothers who are going on oral contraceptive should be implemented in school, audio-vision systems, and local organization. Deeper and screening research studies about

the effect of oral contraceptives intake on some other anabolic steroid hormones such as those of the thyroid could be addressed and conducted with a higher level of funding the from Ministry of Health [MOH].

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Knowledge of Symptoms, Signs and Risk Factors of Prostate Cancer Among Male Senior Staff of University of Uyo and Teaching Hospital

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ABSTRACT

This study is aimed at assessing the level of knowledge of symptoms, signs and risk factors of Prostate cancer among male senior staff of University of Uyo and Teaching Hospital. Data from this descriptive cross sectional study were collected through a self-administered questionnaire to consenting male senior staff of University of Uyo and University of Uyo Teaching Hospital over a period of one (1) month in 2021. There was a total of 182 respondents. The mean age was 50.5 years with the majority of respondents being within 51- 60 years age group (50.0%). Majority of the respondents were University Lecturers (61.5%). A total of 70 respondents were knowledgeable of the warning symptoms and signs of Prostate cancer (38.5%). Staff of Teaching hospital were more knowledgeable about the warning symptoms and signs of Prostate cancer than staff of the University of Uyo (21.5% vs 17.0%). Nocturia was the leading worrisome symptom of change in urinary pattern acknowledged by the respondents (42.9%). Acute urinary retention was the leading recognized symptomatic complication (50.0%). Over thirty percent (31.3%) of respondents were knowledgeable of the risk factors for Prostate cancer with a larger proportion of respondents correctly acknowledging a red meat enriched diet as the main contributory factor (24.6%). University staff were more knowledgeable about risk factors relative to those in the Teaching hospital (52.6% vs 47.4%). In conclusion knowledge about Prostate cancer warning signs and risk factors was poor.

Keywords: Knowledge, Adult males, Warning symptoms, Risk factors, Prostate cancer

INTRODUCTION

Although prostate cancer is the second leading cause of cancer-related mortality worldwide, after lung cancer,[1-7] it remains the most common cancer among adult males in Sub-Saharan Africa including Nigeria.[3,4,5,8,10]

Poor awareness of cancer symptoms and risk factors resulting in delayed presentation as well as low availability of screening programs and limited access to healthcare services contribute to cancer-related deaths in developing countries.[1,5] Prostate cancer screening via digital rectal examination, serum Prostate specific antigen concentration, and transrectal ultrasound scan (TRUS) with biopsy, has been shown to reduce prostate disease-related mortality. These screening methods detect some potentially curable asymptomatic localized prostate cancers before development of complications including metastasis, and thus enables early diagnosis.[1-5,9,11,12]

Risk factors contributing to the development of Prostate cancer include age, race, family history of Prostatic and Breast cancer, cigarette smoking, alcohol consumption, consumption of red meat and diets rich in fat and poor in fibres.[2,13,14] Furthermore, it has been shown that the risk of Prostate cancer is higher in black men than in white men worldwide; thus underscoring the supportive evidence of an increase in predisposing genetic mutations among black men influenced by environmental factors such as diet and migration.[4,14]

Genetic abnormalities including 8q24, 17q, novel loci on chromosomes 3,6,7,10,11,19 and X as well as hormones including Testosterone and Dihydrotestosterone have been shown to have a contributory role in the aetiopathogenesis of Prostate cancer.[14-17] Thus, effective public health interventions may be required to

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address knowledge deficits on warning signs and risk factors of Prostate cancer and emphasize the importance of seeking medical attention for ongoing symptoms.

The aim of this study was to examine the knowledge of male senior staff of University of Uyo and University of Uyo Teaching Hospital, Uyo, Nigeria with regard to symptoms, signs and risk factors of Prostate cancer.

MATERIALS AND METHODS

Survey Design and Administration

This descriptive, cross sectional study was conducted from August 18 through September 17, 2021. Respondents were consenting male senior staff of University of Uyo and her Teaching Hospital, Uyo, Akwa Ibom State and were recruited via a variety of methods, including online interactive systems such as WhatsApp and Facebook, postal mail, and telephone advertising and invitations. A total of 200 respondents were recruited by systematic random sampling and the sample size was estimated using the formula: $n = z^2pq/d^2$ (where: n = desired sample size when population > 10,000, z = level of significance at 95% CI (=1.96), p = proportion of the study population who are aware of Prostate cancer and screening from similar previous study = 0.22 [11], q = 1p = 0.78 and d = degree of accuracy desired, usually set at 0.05). Sample size (n) = $z^2pq/d^2 = (1.96)^2 \times (0.22) \times (0.78)/(0.05)^2 = 3.84 \times 0.22 \times 0.78/0.0025 = 0.6589/0.0025 = 264$. The minimum sample size required for this study was reduced from 264 to 200 for convenience as well as a necessity to reduce the impact of spread of 3rd to 4th waves of COVID-19 Pandemic.

Survey items included respondent demographic information, and knowledge questions about symptoms, signs and risk factors of Prostate cancer. Demographic information included age, marital status, ethnicity, religion, socio-economic factors, occupation, and education. Age was measured as categories of age group 31-40 years, 41-50 years, 51-60 years, and 61-70 years. Highest education obtained by respondents was measured as Doctor of Philosophy (PhD), Master Degree (M.Sc), Bachelor Degree, Higher National Diploma (NHD), Ordinary National Diploma (OND) and Secondary School Certificate Examinations.

Knowledge questions of warning symptoms and signs of Prostate cancer were assessed by asking respondents to identify warning symptoms and signs suggestive of urinary pattern change, urinary obstruction and complications ("How knowledgeable are you about prostate cancer?")

Identification of risk factors of Prostate cancer was assessed by knowledge questions: respondents were asked to judge whether the following 9 items were risk factors of Prostate cancer: consumption of red meat enriched diet, consumption of white meat enriched diet, consumption of diary product enriched diets, consumption of vegetable impoverished diet, consumption of fruit impoverished diet, prolonged alcohol consumption, smoking, increased sexual activity and family history of Prostate cancer and other cancers. Responses included 'Yes', 'No' or 'Not sure/Do not know', with 'Yes' indicating that they identified the items to be risk factors.

Inclusion criteria for the study population were consenting male senior staff of University of Uyo and Teaching Hospital who were older than 30 years. Non-consenting, oncologic patients on treatment for Prostate cancer and individuals younger than 30 years were excluded from the study. In addition, we categorized the respondents into two major groups viz: University and Teaching hospital staff to limit bias as health workers may likely be knowledgeable about this disease.

Ethical Consideration

Ethical approval to conduct the study was obtained from the Health Research and Ethics Committee of the University of Uyo Teaching Hospital. Written informed consent was obtained from all respondents before embarking on the study. Brief education on the purpose and nature of the study was given to all respondents. All respondents were assigned a unique code to ensure

confidentiality. Only the lead researcher had access to the information linking the identity of the study respondents to the study codes to ensure anonymity. This allowed for easy identification and prevention of risk of stigmatization. Respondents were reliably informed that information provided shall be strictly kept secret and he is at liberty to withdraw from the study at any time without any negative consequence to them. The study was fully supported by TETfund Institutional Research based funds.

Statistical Analysis

Statistical Analysis was performed using Statistical Packages for the Social Sciences (SPSS) statistical software for Windows, version 20 (IBM). Descriptive analyses with calculated measures of central tendency and variation were computed, along with frequency tables for categorical variables as well as presentation of data in figures were carried out. All knowledge variables were treated as outcomes, applied and p-values < 0.05 were considered significant.

RESULTS

Socio-demographic characteristics

There was a total of 182 respondents with a resultant response rate of 91.0%. The mean age was 50.5 ± 0.45 years with the majority of respondents being within 51-60 years age group (50.0%). Respondents aged 41 to 50 years were the 2nd most commonly seen in this study (n = 70, 38.5%). Respondents aged 50 years and above were slightly over-represented being 53.8% compared to respondents aged below 50 years old accounting for 46.2% (Figure 1). Majority of the respondents were University Lecturers (*n* = 112, 61.5%) with PhD being the predominant terminal qualification (57.7%). Teaching hospital staff accounted for the remaining 38.5% of total respondents. Clinical Health Professionals and Non-clinical Health professionals were responsible for 23.1% and 15.4% of respondents respectively (Figures 2 & 3). Christianity was the most preponderant religion (96.2%). Islam and Eckankar accounted for 2.7% and 1.1% of other religions respectively.

Knowledge of Respondents on the warning symptoms and signs of prostate cancer

A total of 70 respondents were knowledgeable of the warning symptoms and signs of Prostate cancer (38.5%), whereas over half of respondents (57.7%) were found to lack knowledge about warning symptoms and signs of Prostate cancer (n = 105). Among the Teaching hospital staff, 21.5 of respondents were knowledgeable of the warning symptoms and signs of Prostate cancer whereas 17.0% of University staff were knowledgeable of the warning symptoms and signs of Prostate cancer.

Over 20 percent of respondents expressed good knowledge of symptoms of urinary pattern change (n = 42, 23.1%) whereas majority of respondents were not knowledgeable of symptoms of change in urinary pattern (73.1%). Nocturia was the leading worrisome symptom of change in urinary pattern acknowledged by the respondents (42.9%) with respondents from the Teaching hospital having a higher knowledge of nocturia and urinary frequency (28.6% vs 21.4%) compared to those in University of Uyo (14.4% vs 11.9%). However, staff of University of Uyo had a higher knowledge of urinary urgency relative to those in the Teaching hospital. (4.8 % vs 0.0%). (Table 1).

Regarding warning obstructive urinary symptoms and signs of Prostate cancer, fifty-six (30.8%) respondents recognized obstructive symptoms with most respondents listing aggregate of poor stream, intermittency and feeling of incomplete bladder emptying (28.6%). Respondents from the University of Uyo were relatively more knowledgeable about obstructive symptoms than those in Teaching Hospital (55.3% vs 44.7%) (Table 2).

Overwhelming majority of respondents claimed that they have never heard about any suggestive symptomatic complications of Prostate cancer (76.9%). Forty-two respondents were knowledgeable about suggestive symptomatic complications of Prostate cancer (23.1%). Of these, 21 respondents recognized acute urinary

retention (50.0%) as the predominant worrisome suggestive symptomatic complication while low back pain was acknowledged by 15 respondents (35.7%). Only 6 respondents acknowledged weight loss as recognized suggestive symptomatic complication (14.3%).

Among respondents acknowledging symptomatic complications, 76.2% were aware of co-morbid conditions while 23.8% of respondents did not identify any co-morbid condition. Of those who identified suggestive symptomatic complications and co-morbid conditions, Hypertension was the leading co-morbid condition (59.4 %); this was followed by Diabetes mellitus (34.4%). The least identifiable co-morbid condition was Cerebrovascular accident; recognized by 2 respondents (6.2%).

Knowledge of respondents on the risk factors of prostate cancer

Among respondents, 68.7% were not knowledgeable about risk factors of prostate cancer while 31.3% of respondents were knowledgeable of the risk factors for Prostate cancer. University staff were relatively more knowledgeable about the risk factors of rostate cancer compared to those of Teaching hospital (52.6% vs 47.4%). Majority of respondents correctly acknowledged red meat enriched diet as the main contributory factor (n = 14; 24.6%) with respondents from the University of Uyo exhibiting higher knowledge than those in Teaching Hospital regarding identification of red meat enriched diet (15.8% vs 8.8%). Nine respondents identified white meat enriched diet as the 2nd leading risk factor (15.8%). Smoking and vegetable impoverished diet were also recognized in 14.0% and 10.5% respectively (Table 3).

Table 1: Good Knowledge of warning symptoms of change in urinary pattern suggestive of Prostate cancer by workplace of the respondents.

Warning Symptoms and Signs of Urinary Pattern Change	Staff of University of Uyo n (%)	Staff of University of Uyo Teaching Hospital n (%)	Total number of Respondents n (%)
Nocturia	6(14.3)	12(28.6)	18(42.9)
Urinary frequency	5(11.9)	9(21.4)	14(33.3)
Urinary urgency	2(4.8)	0(0.0)	2(4.8)
Frequency and nocturia	4(9.5)	4(9.5)	8(19.0)
Total	17(40.5)	25(59.5)	42(100.0)

Table 2: Good Knowledge of warning obstructive symptoms suggestive of prostate cancer by work place among the respondents.

Warning Obstructive Urinary Symptoms	University of Uyo n (%)	University of Uyo Teaching Hospital n (%)	Total number of Respondents n (%)
Poor stream only	4(7.1)	2(3.6)	6(10.8)
Urinary intermittent only	2(3.6)	4(7.1)	6(10.8)
Feeling of incomplete voiding	4(7.1)	3(5.4)	7(12.5)
Poor stream + incomplete voiding	5(8.9)	2(3.6)	7(12.5)
Poor stream + incompletely voiding+ intermittency	9(16.1)	7(12.5)	16(28.6)
Poor stream + Straining + Feeling of incomplete bladder emptying	4(7.1)	3(5.4)	7(12.5)
Poor stream + Intermittency + Urgency + Feeling of incomplete bladder emptying	3(5.4)	4(7.1)	7(12.5)
Total	31(55.3)	25(44.7)	56(100.0)

Table 3: Good knowledge of Risk factors of Prostate cancer by place of work of the respondents.

Risk factors	University of Uyo n (%)	Univer sity of Uyo Teaching Hospital n (%)	Total number of Respondents n (%)
Red meat enriched diet	9(15.8)	5(8.8)	14(24.6)
White meat enriched diet	5(8.8)	4(7.0)	9(15.8)
Cigarette smoking	4(7.0)	4(7.0)	8(14.0)
Vegetable impoverished diet	2(3.5)	4(7.0)	6(10.5)
Family history of cancer	2(3.5)	3(5.3)	5(8.8)
Fruit impoverished diets	3(5.3)	2(3.5)	5(8.8)
Alcohol consumption	2(3.5)	3(5.3)	5(8.8)
Dairy products (Cow milk) enrich diet	2(3.5)	1(1.7)	3(5.3)
Sexual intercourse	1(1.7)	1(1.7)	2(3.4)
Total	30(52.6)	27(47.4)	57(100.0%)

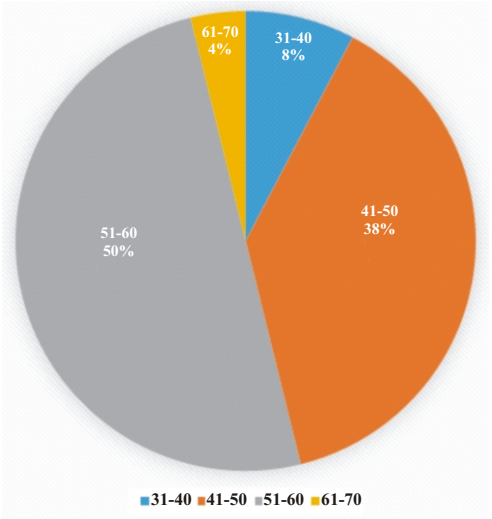

Figure 1: Age Distribution of Respondents (years

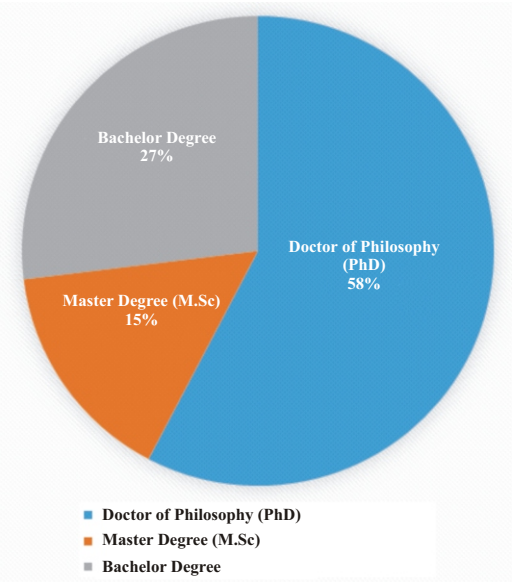

Figure 2: Academic Qualifications of Respondents

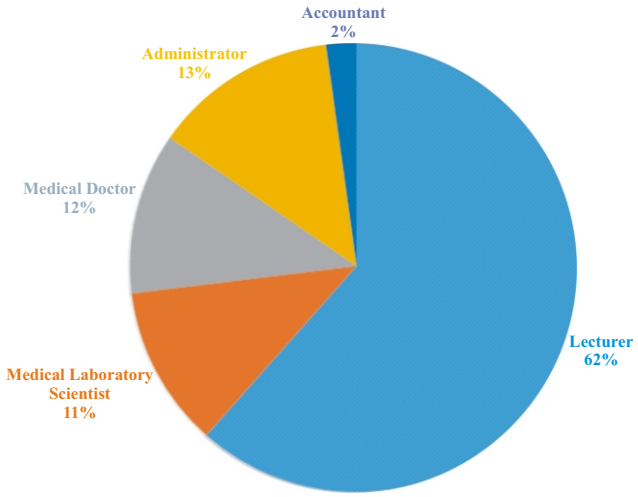

Figure 3: Occupation of Respondents

DISCUSSION

This study was carried out to explore knowledge about symptoms and risk factors of Prostate cancer among male staff in the tertiary health and educational institutions in our locality. Literature review however showed paucity of publications in this context.[18] The response rate for our study was 91.0%. This is however, slightly lower than the response rates of 93. 5% and 100.0% reported in Enugu, South East Nigeria and Brong-Ahafo, Ghana by by Adibe *et al* and Yeboah-Asiamah *et al*. respectively.[18,19] This fairly impressive response rate in our study may be adduced to a desire to know more

about their health status as some of the respondents work in health care institution or mere coincidence. In addition, the two tertiary institutions where respondents were drawn from, accommodate individuals of upper socio-economic class owing to their level of education and location within the capital city of Uyo which is the seat of socio-economic, political and administrative power of Akwa Ibom State.

In the index study, a larger proportion (50.0%) were in the 51-60 years age group relatively confirming with a mean age of 50.5 years. This result conflicts with findings of similar studies in Nsukka, Enugu State, South Eastern Nigeria and Brong-Ahafo, Ghana where majority of respondents were in the 31-40 years (32.1%) and 45-50 years age group (68.1%) respectively.[18,19] From the foregoing, it is obvious that predominant age group of respondents may vary from region to region and there is a likely possibility that knowledge on Prostate cancer may be influenced by age and other factors.

Only a minority of respondents (38.5%) were knowledgeable about the warning symptoms and signs of Prostate cancer with University staff being less knowledgeable about the warning symptoms and signs of rostate cancer than those staff of the Teaching hospital (17.0% vs 21.5%). This finding is however lower than a good knowledge score of 58.7% reported in Enugu, South Eastern Nigeria. It is may not be too surprising that despite the fact that larger proportion of respondents were University Lecturers with PhD (57.7%), poor knowledge score for symptoms and signs of Prostate cancer in our study underscores the need to unravel more contributing factors for the poor knowledge of symptoms and signs of Prostate cancer in this socio-economic class. They may include ignorance about symptoms and risk factors, cultural belief, ineffective health insurance scheme, and non-availability of awareness programme on prevention, prompt diagnosis and treatment of cancer.

Nocturia was the leading worrisome symptom of change in urinary pattern acknowledged by the respondents (42.9%) with respondents from the Teaching hospital having a higher knowledge of nocturia (28.6%) compared to those in University of Uyo (14.4%). This finding may be explained by the fact that knowledge of staff working in the Teaching hospital are likely to be influenced by the pre-employment academic training on health related subjects as well as cognitive experience acquired on the job as a health worker. Nevertheless, knowledge on Prostate cancer may be acquired by the two major categories of staff through mass media, printing media, internet and health talks in churches and other social events.

Regarding warning obstructive urinary symptoms and signs of Prostate cancer, 56(30.8%) of respondents recognized obstructive symptoms with respondents from the University of Uyo being relatively more knowledgeable about obstructive symptom than those in Teaching Hospital (55.3% vs 44.7%). This finding may be coincidental or uphold the view that University Lecturers are likely to be well exposed to e-learning beyond areas of sub-specialization. Our finding is also corroborated by a similar study in Ghana where staff of the University of Nigeria were reported to have appreciable knowledge regarding Prostate cancer.[18]

Minority of respondents were knowledgeable about suggestive symptomatic complications of Prostate cancer (23.1%) with acute urinary retention being the leading recognized symptomatic complication (50.0%). From the foregoing, it is obvious that poor knowledge of suggestive symptoms of urinary pattern changes (23.1%), urinary obstruction (30.8%) and symptomatic complication (23.1%) was prevalent in the index study. This compares relatively with findings from other studies.[2,13] In addition, aggressive awareness campaign for Prostate cancer should be instituted at all levels of health care system, public and corporate places including churches, mosques, schools, markets.

Among respondents, 68.7% were not knowledgeable about risk factors of Prostatic cancer whereas 31.3% of respondents were knowledgeable about risk factors of Prostatic cancer. Red meat enriched diet was the

leading risk factor recognized in the index study (24.6%). Similar to other studies, many contributory factors to development of Prostate cancer have been identified. These include red meat enriched diet, white meat enriched diet, smoking, vegetable impoverished diet, family history of Prostate cancer, fruit impoverished diet, prolonged alcohol consumption, diary product enriched diets, and increased sexual activity. The exact mechanisms by which these risk factors culminate in the development of Prostate cancer is however controversial, thus, further research in this area may be necessary.[2,4,13-17]

Even though, aforementioned risk factors are recognized in varying proportions in our study, the general knowledge of risk factors of Prostate cancer is still low in the index study (31.3%) which compares relatively with findings of 24.6% and 26.3% of respondents having a good knowledge of risk factors of Prostate cancer in a study conducted by Fidelis *et al.* and Olarewaju *et al.* respectively.[3,7] Furthermore, the results of these studies were in accordance with other reports collaborating a good knowledge of poor diet, environmental conditions, aging, race and genetics as recognized risk factors of Prostate cancer by some respondents.[2,13,14]

It is worthy of note that few studies have demonstrated that the risk factor of Prostate cancer is higher in black men than in white men worldwide; this finding could probably be explained by increased number of predisposing genetic mutations in black men influenced by environmental factors such as diets and migration.[4,14] Furthermore, some studies have shown that low knowledge about the disease including symptoms, signs and risk factors of Prostate cancer as well as Prostate cancer screening methods play an important role in cancer screening uptake.[3-7] From this, it infers that contributing factors such as age, ethnicity, religion, education level, income and cultural beliefs play a key role in influencing the knowledge of populace on risk factors of Prostate cancer[10], which suggests that there is an immense need to create awareness on timely, decisive and reportable identification of risk factors of Prostate cancer and correct almost all misconceptions associated with poor healthcare-seeking attitude of populace.[3,5,10] This is also supported by our result showing that University staff were relatively more knowledgeable about the risk factors of Prostate cancer compared to those of Teaching hospital (52.6% vs 47.4%).

CONCLUSION

This study indicates that knowledge about warning symptoms and signs as well as risk factors of Prostate cancer is poor among respondents. Respondents from the Teaching hospital were more knowledgeable about the warning symptoms and signs of Prostate cancer, whereas respondents from the University were relatively more knowledgeable about the risk factors of Prostate cancer. In this regard, there is an immense need to conduct awareness campaign related to warning signs and risk factors avoidance and modification as well as early Prostate cancer detection, as drivers of prevention and improved outcomes among the vulnerable public.

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Prevalence of Bacterial Vaginosis among Sexually Active Females in a Tertiary Institution in Benin Metropolis, Nigeria

***Moses-Otutu IM, Ngemegwai CC**

ABSTRACT

Bacterial vaginosis is the most prevalent cause of abnormal vaginal discharge in women between the ages of 15 and 44years. Bacterial vaginosis, if untreated can progress to pelvic inflammatory disease, preterm labour and ultimately infertility. The aim of this study was to determine the risk factors and their association with the prevalence of bacterial vaginosis among sexually active females in a tertiary institution in Benin metropolis. This study recruited 113 randomly selected sexually active females in a tertiary institution in Benin metropolis. The age range was 16years to 37years. After informed consent, sterile swab sticks were used to collect high vaginal swab and immediately sent for laboratory analysis. Nugent scoring method was applied for bacterial vaginosis. Results obtained were statistically analyzed. A total prevalence of 47.8% bacterial vaginosis was obtained among sexually active females in a tertiary institution in Benin metropolis. The highest prevalence of bacterial vaginosis was among age 21-25years (35.2%), 16-20years (29.6%) while 31-35 years had the least prevalence (3.7%). Bacterial vaginosis varied significantly with tribes, Bini recorded the highest prevalence (38.9%), Esan (22.2%), others (14.8%), Etsako (13.0%) and Yoruba's recording the least prevalence (3.7%). Marital status and frequency of sex did not significantly influence the prevalence of bacterial vaginosis (P=1.000; P=0.065) among sexually active females in a tertiary institution in Benin metropolis. Candida albicans, a fungus was also found (45.1%) among the study population. Public enlightenment on Bacterial vaginosis, its symptoms and effects, cessation of douching and smoking and limiting the number of sex partners is advised.

Keywords: Bacterial vaginosis, Microscopy, Nugent Scoring, Asymptomatic, C*andida *albicans

INTRODUCTION

Bacterial vaginosis (BV) is described as a shift in the balance of the vaginal microflora characterized by an increase in the vaginal pH, a reduction in lactobacilli (hydrogen peroxide producing species) and an increase in the number and type of facultative anaerobic bacteria.[1, 2] This shift in vaginal microflora causes bacterial overgrowth resulting in the common symptoms of vaginal discharge and vaginal odour as experienced by individuals.[3] The first bacterium identified to represent BV infection was G. vaginalis, and is still the known primary pathogen associated with BV diagnosis. BV has been linked with an increased risk of acquiring sexually transmitted infections (STIs) such as: human immunodeficiency virus (HIV), gonorrhea, chlamydia and herpes simplex virus.[4] Moreso, BV infection can lead to pelvic inflammatory disease, preterm birth, post-hysterectomy and postpartum vaginal infections.[5] BV is also one of the genital infections common among women of reproductive age, but its major etiology and if it is sexually transmitted are unknown.[6]

The known risk factors that increases the risk of developing BV include: sexual activity, mostly unprotected sexual intercourse, an increased number of sexual partners, women who have sex with women, Africans or African American descent, douching and having an intrauterine device insitu.[7] BV has also been associated with sexual behavior related characteristics such as young age at coitarche, life time number of sex partners, a recent history of multiple sex partners and a recent history of a new sex partner. These inconsistencies make it difficult to define what genuinely represents high-risk sexual behaviour to the acquisition of BV.[8] Moreover, some risk factors identified may be proxy variables to the true risk of

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exposure; that is a new sex partner might be predictive to frequency of sexual intercourse. However, whether BV pathogenesis does actually involve sexual transmission of pathogenic micro-organisms from men to women is still unknown.[9] Also identified to confer an increased risk of BV acquisition is heterosexuality among women, non-coital sexual behaviours, including receptive oral sex, receptive anal sex, and non-penetrative digito-genital contact.[10] Higher prevalence rates of G. vaginalis have been found in sexually abused girls as compared to non-abused girls in most studies.[8] Aside G. vaginalis carriage, BV in children is rare.[11] According to studies, once beyond the menarche, BV also occurs among sexually inexperienced adolescents and virginal women but at lower rates on average as compared to sexually active reproductive aged-women.[12] Up to one third of non-sexually active adolescents harboured G. vaginalis, which was significantly less than in sexually active adolescents (60%).[13, 8] Several studies support that BV incidence is increased by sexual activity but clearly also contradict exclusive heterosexual transmission. As such, preventive strategies of BV involves targeting the risk factors or behaviours of BV.

BV can occur in any age group and globally is most prevalent cause of abnormal vaginal discharge in women of childbearing age (15 years to 44 years).[14, 2, 9, 4] Although the prevalence of BV differs widely from country to country within the same region and even within similar population groups, it has been estimated to be in the range of 8% to 75%.[15] Clinical signs and symptoms of BV are: an increased grey-white vaginal discharge with consistency similar to milk and with a strong odour of fish. The vaginal discharge has a PH > 4.5 and is usually more noticeable after sexual intercourse and menstruation. The more severely affected individuals experience an offensive fishy-smelling discharge that frequently recurs around the time of menstruation.[9] BV is associated with an increased risk from several pathological states including post-surgery infections which arise after hysterectomy, post abortal pelvic inflammatory disease and plasma cell endometritis.[16] Facultative anaerobes and anaerobic bacteria (such as G. vaginalis, Mycoplasma hominis, Ureaplasma urealiticum and Mobiluncus) are also found besides Lactobacillus in the vaginal microbiota of healthy women.[7]

In addition to the physical risks, qualitative studies have shown that women who suffer from BV, particularly recurrent BV, experience a decrease in their quality of life (QOL). The disruptions in QOL includes: embarrassment about any perceived odour, decreased self-esteem, interruption of intimate relationships, social isolation and decrease in work productivity.[17] This epidemiological study can help in understanding the contribution of sexual activity to the development of BV, recurrence of BV after initial treatment and is also important at reducing the burden of the disease in infected females.

MATERIALS AND METHODS

Study Area and Population

This study was carried out among sexually active females in a tertiary institution in Benin metropolis, the capital of Edo State. Edo State is in the South-South geo-political zone of Nigeria and lies between latitudes 6.1^0N and 7.3^0N and longitude 5.0^0E and 6.5^0E. It has a total land area of 19,281.93Km^2. The State is bounded by Delta State to the South, Kogi State to the North, Ondo State to the West and the River Niger along the Eastern border. Benin City is located at 6.3^0N latitude and 5.6^0E longitude.

The sample population consisted of sexually active females in a tertiary institution in Benin metropolis that was randomly selected.

Study Design

A total of 113 sexually active females in a tertiary institution in Benin metropolis were randomly selected for this study. This study referred to sexually active females as females who are already exposed to sexual

activities irrespective of the age, time and duration of exposure; not necessarily those that practice commercial sex. The participants gave their verbal consent after a thorough explanation of the rationale for the study and vagina swabs were then collected by trained medical personnel from each participant. After the samples were collected, they were sent to the laboratory for microbiological assessment.

Ethical approval

Permission was sought and obtained from the Ministry of Health, Edo State for ethical approval to carry out this research in Benin City metropolis. In addition, each of the participants was given a written consent form to fill to indicate their willingness to participate in the study.

Sample Collection

Two vaginal swabs were collected aseptically by trained medical personnel. A sterile speculum was used to dilate the cervix, with the speculum in situ, the tip of a rayon-tipped applicator swab stick was passed through the speculum to the posterior fornix of the vagina, then the swab was rotated for 10-15 seconds in the posterior fornix ensuring to swab any discharge present. Each swab stick was then placed inside the transport tube (one tube containing normal saline while the other tube was dry) and the tubes were closed tightly. The two vaginal swabs were transferred without delay to the microbiology laboratory for analysis.

Sample processing

Vagina Wet Mount

The swab stick in normal saline was observed by wet mount microscopy to view the presence of Clue cells, pus cells and *Trichomonas vaginalis.* For yeast, germ tube test was carried out and *Candida albicans* was identified by its ability to produce germ tubes when incubated in serum at 37^0C for 2 hours.

Microscopic Examination

For the diagnosis of bacterial vaginosis, the dry swab stick was used to make a smear which was heat-fixed, Gram-stained and examined under oil immersion objective. Each slide was examined for yeast cells, clue cells, pus cells and normal flora. The Gram stained slide was then scored and graded as per the standardized quantitative morphological classification method developed by Nugent and colleagues.[18] This assign a score between 0 and 10 based on the following various bacterial morphotypes: large Gram-positive rods (Lactobacillus morphotypes), small Gram-variable rods (G. vaginalis morphotypes), small Gram-negative rods (Bacteroides spp. morphotypes), curved Gram-variable rods (Mobiluncus spp. morphotypes), and Gram-positive cocci. Each morphotype was quantitated from 1 to 4+ about the number of morphotypes per oil immersion field as represented in the table below and the score of each morphocyte added together to get the Nugent score.

Nugent Scoring Table

Score	Lactobacillus morphotype per field	Garderella morphotype per field	Curved bacteria (Mobiluncus) per field
0	>30	0	0
1	5-30	<1	1-5
2	1-4	1-4	>5
3	<1	5-30	
4	0	>30	

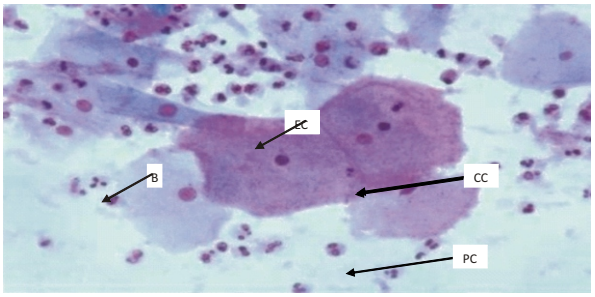

Figure 1: Bacterial vaginosis seen on a light microscope after Gram staining using oil immersion objectives (×100 objective)

KEY: B- Bacteria, EC- Epithelial Cell, CC- Clue Cell, PC- Pus Cell

Statistical Analysis

The categorical variables obtained from laboratory investigations in this study were tabulated, encoded and statistically analyzed using Statistical Package for Social Sciences (SPSS version 16) program. Test of significance was done using Chi-square and the levels of significance were accepted at $p<0.05$.

RESULTS

Prevalence of Bacterial Vaginosis among Sexually Active Females in a Tertiary Institution in Benin Metropolis, Nigeria

Based on Nugent scoring method, a total prevalence of 47.8% BV was obtained among sexually active females in a tertiary institution in Benin metropolis, Nigeria (Table 1). Age of participants did not significantly influence the prevalence of BV, as the prevalence of BV was higher among age groups 21-25 years (35.2%), 16-20years age group (29.6%), age group 26-30 years (24.1%), >35years (7.4%) while the least prevalence of 3.7% was recorded among 31-35years age group (Table 2).

Tribe significantly influenced the prevalence of BV among sexually active females in a tertiary institution in Benin metropolis, Nigeria (P=0.028). As prevalence of bacterial vaginosis was higher among the Binis (38.9%), Esan tribe (22.2%), other tribes (14.8%), Etsako (13.0%), Akoko-Edo (7.4%) with Yoruba tribe recording the least prevalence (3.7%) (Table 2).

Table 1: Prevalence of Bacterial Vaginosis among Sexually Active Females in a Tertiary Institution in Benin Metropolis, Nigeria.

Bacterial Vaginosis	Frequency	Percent
Positive	54	47.8
Negative	59	52.2
Total	113	100.0

Table 2: Sociodermographic Parameters and Frequency of Bacterial Vaginosis among Sexually Active Females in a Tertiary Institution in Benin Metropolis, Nigeria.

Variable	No examined (%)	No. positive (%)	X^2	P- value
Age (years)				
16-20	28(24.8)	16(29.6)	3.416	0.491
21-25	48(42.5)	19(35.2)		
26-30	24(21.2)	13(24.1)		
31- 35	06(5.3)	02(3.7)		
>35	07(6.2)	04(7.4)		
Tribe				
Yoruba	04(3.5)	02(3.7)	12.516	0.028
Bini	38(33.6)	21(38.9)		
Esan	17(15.0)	12(22.2)		
Etsako	11(9.7)	07(13.0)		
Akoko-Edo	12(10.6)	04(7.4)		
Other	31(27.4)	08(14.8)		
Total	113(100)	54(100)		

Effect of some Associated Risk Factors on the Prevalence of Bacterial Vaginosis among Sexually Active Females in a Tertiary Institution in Benin Metropolis, Nigeria

Marital status as a risk factor was not significantly associated with the prevalence of Bacterial vaginosis among sexually active females in a tertiary institution in Benin metropolis, Nigeria (OR=1.077, 95% CI = 0.338, 3.431, P=1.000). Bacterial vaginosis was however higher among single participants (89.0%) when compared to their married counterpart (11.0%) (Table 3).

Frequency of sex as a risk factor did not significantly influence the prevalence of bacterial vaginosis among sexually active females in a tertiary institution in Benin metropolis, Nigeria (P=0.065). However, females having sexual intercourse few times a month had a higher percentage prevalence of bacterial vaginosis (48.1%) followed by those having sexual intercourse few times a week (37.0%), those rarely having sexual intercourse recording 11.1% while the least prevalence was recorded among females having daily frequent sexual intercourse (3.7%) (Figure 1).

Table 3: Marital Status as a Risk Factor for the Prevalence of Bacterial Vaginosis among Sexually Active Females in a Tertiary Institution in Benin Metropolis, Nigeria.

Marital status	No examined (%)	No. positive (%)	OR	95% CI	P-value
Single	100(88.5)	48(89)	1.077	0.338 -3.431	1.000
Married	13(15.5)	06(11)			
Total	113(100)	54(100)			

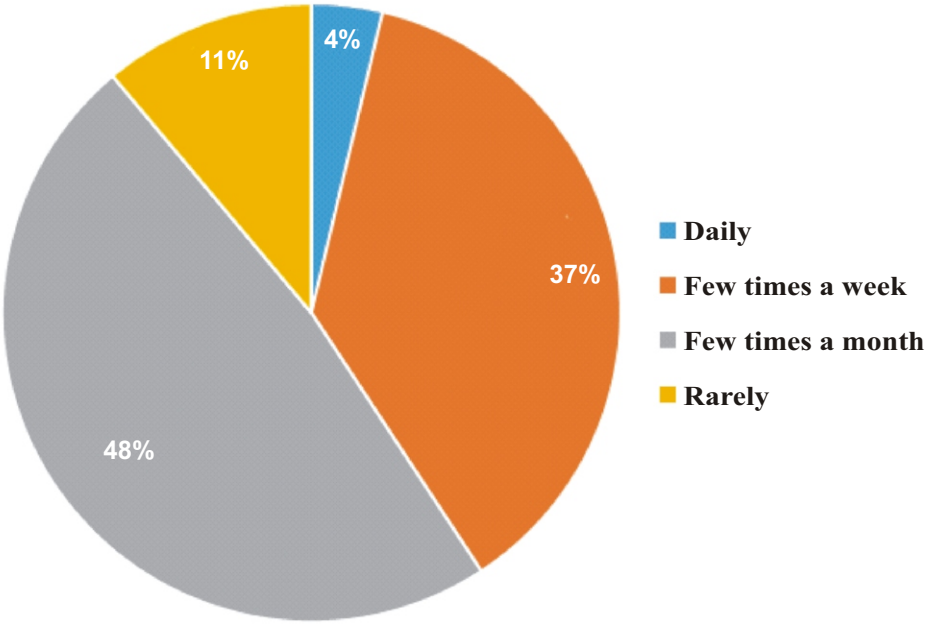

Figure 2: Prevalence of BV with Frequency of Sex among Sexually Active Females in a Tertiary Institution in Benin Metropolis, Nigeria.

DISCUSSION

The prevalence of BV has been shown from previous studies to differ widely from country to country within the same region and even within similar population groups. This study reported a total prevalence of 47.8% BV among sexually active females in a tertiary institution in Benin metropolis, Nigeria. This value though relatively high lies within the reported prevalence range of 8% to 75% of BV from previous studies obtained globally[15] Similar studies on BV among sexually active women in USA and Cameroon recorded a prevalence of 29.2%[21] and 38%[19,20]

respectively. The differences in prevalence may be due to geographical distribution, race, ethnicity and method of analysis.

This study recorded the highest prevalence of BV among age's 21-25years (35.2%), age's 16-20years (29.6%) and age's 26-30years (24.1%) while 31-35years age group had the least prevalence (3.7%). Age had no statistical significant relationship with prevalence of BV among our study population (p=0.491). A study in Cameroon also recorded the highest prevalence of BV among age group 20-25 years (48.1%) with a statistically significant relationship between age and prevalence of BV among sexually active women.[20] Also observed from another study is an association among 17-21-year-old females with oral sex and non-penetrative digito-genital contact and the occurrence of BV.[27]The reasons behind our results may be because more of the risk factors for acquisition of BV such as young age at coitarche, douching, non coital sex behavior, receptive anal and oral sex and non-penetrative digito genital contact[10, 22] occurs mostly at ages 16-25years due to teenage and youthful exuberance. Moreso, once beyond the menarche, BV also occurs among sexually inexperienced adolescents and virginal women but at lower rates on average as compared to sexually active reproductive aged-women.[12]

BV prevalence varied significantly with the tribes: Bini's had the highest prevalence (38.9%), Esan (22.2%), other tribes (14.8%), Etsako (13.0%), Akoko-Edo (7.4%), with the least prevalence among the Yoruba's (3.7%). Race and ethnicity are known risk factors for acquiring bacterial vaginosis among sexually active black women.[23] BV is known to affects blacks differently and predisposes them to the acquisition of sexually transmitted diseases, human immunodeficiency virus, preterm labour, late miscarriage and other gynecologic infections.[21,23]

This study also recorded no statistically significant relationship between marital status and prevalence of BV (p=1.000), though single women had higher prevalence (89%) when compared to their married counterparts. Single sexually active women are more prone to BV because while the married females tend to stick to their spouse as the only sex partner, the single women practice more of the risk factors for acquiring BV such as: having multiple sexual partners[24, 25] having a new sex partner as well as douching which can upset the balance of bacteria in the vagina and predispose them to bacterial vaginosis.[26]

Frequency of sex as a risk factor did not significantly influence the prevalence of BV among sexually active females in our study (P=0.065).The highest prevalence of BV was seen among females who engage in sexual intercourse a few times a month (48.1%), females who had sexual intercourse a few times a week (37.0%), those who rarely had sexual intercourse recording 11.1% while those having sexual intercourse daily recording the least prevalence (3.7%). However, there was a statistical significant relationship between the prevalence of BV and the sexuality of respondents in another study.[21] Though frequency of sex is a critical factor in the acquisition of BV, differences in our studies may be because higher prevalence rates of G. vaginalis have been found in sexually abused girls as compared to non-abused girls in most studies.[8] BV also occurs among sexually inexperienced adolescents and virginal women but at lower rates on average as compared to sexually active reproductive aged-women.[12] Several studies support that BV incidence is increased by penetrative sexual activity, non penetrative digito-genital contact, oral sex but clearly also contradict exclusive heterosexual transmission. Moreso, unprotected receptive anal sex before vaginal intercourse also predisposes females to BV prevalence.[27,28]

T.vaginalis was not isolated in the wet mount during the course of this study. A previous study among Nigerian students recorded 25% prevalence of BV from cultural study.[9,27] T.vaginalis is among the most prevalent cause of sexually transmitted infections globally, this makes its examination mostly done as part of the recommendation in the investigation of vaginal discharge. The wet mount microscopy is the most cost effective

diagnostic test for BV but lack of sensitivity contributes to the under diagnosis. This is because delay in transport and evaporation of moisture from the specimen reduces motility and consequently diagnostic sensitivity which might be the case in our study.

Candida albicans, a fungus was isolated among the study participants. The lactobacillus normally present in the female vagina creates an environment that does not encourage yeast overgrowth but disturbances in its delicate balance can lead to the development of yeast infection. Moreso, Candida albicans though not a sexually transmitted infection, can spread through oral-genital contact, during sexual intercourse and is also associated with history of recent masturbation with saliva by the participant as well as the sexual partner.[29,30,8]

CONCLUSION

This study recorded a high prevalence of BV among sexually active females in a Tertiary Institution in Benin Metropolis, Nigeria. The risk factors studied had no significant relationship with the acquisition of BV among the study population.

RECOMMENDATIONS

Public enlightenment on the existence of BV, its symptoms and effect, instructing women to stop douching, cessation of smoking and limiting the number of sex partners is advised.

ACKNOWLEDGEMENTS

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CONFLICTS OF INTEREST

The authors declare that there are no conflicting interests.

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Diet Induced Acidosis is Associated with Lower Circulating Adiponectin Levels regardless of Body Weight Status

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ABSTRACT

Low circulating Adiponectin level have been observed to significantly increase the risk of developing type 2 Diabetes mellitus, cardiovascular diseases and several malignancies. A number of potential determinants of the circulating Adiponectin levels have been investigated in order to develop newer preventive strategies for such diseases. The possible role of diet induced acidosis in decreasing the circulating level of Adiponectin in healthy population has been proposed. However, findings from previous studies are not consistent. It is likely that the association is affected by genetic and environmental factors that differ among different populations. We investigated the impact of diet induced acidosis on the circulating level of Adiponectin in a group of apparently healthy adult Nigerians. Food frequency questionnaire and the Nigerian Food Composition Table were used for the assessment of dietary intake. Acid forming potential of our local diets were estimated as Potential Renal Acid Load (PRAL) scores. Plasma total Adiponectin was measured. Across the quartiles of the PRAL scores, there was a statistically significant trend with higher intake of dietary acid associated with significant decreased circulating Adiponectin level (p for trend < 0.05). Study participants in the highest quartile of the PRAL scores have a statistically significant lower Adiponectin level compared with participants in the lowest quartile (12.6 ± 2.2µg/mL vs. 6.1 ± 2.5µg/mL, p < 0.05). We conclude that among the subjects in this study lower intake of dietary acid is associated with significant increase in circulating Adiponectin level.

Keywords: Dietary acid load, Adiponectin, Adults, Nigeria

INTRODUCTION

Adiponectin is an adipocytokine that is predominantly produced by adipocytes and plays a significant role in metabolic and cardiovascular homeostasis through its insulin-sensitizing actions and anti-inflammatory and antiatherogenic properties.[1,2] More recently, it has been observed that, among the general population, lower circulating levels of Adiponectin can substantially increase the risk of developing type 2 Diabetes mellitus, cardiovascular diseases and several malignancies.[3-10] This has led to studies on the investigation of potential determinants of the circulating Adiponectin levels in order to develop newer preventive approaches for diseases such as type 2 Diabetes mellitus and cardiovascular diseases, and a number of potential determinants of circulating Adiponectin level have been investigated.[11]

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Report from an experimental study suggests that cellular acidosis down-regulates Adiponectin gene expression and therefore decreases circulating Adiponectin level; and a number of reports from interventional studies, mainly done among Caucasians, suggest that consumption of foods with high acid forming potential, such as processed foods, is associated with a decrease in circulating level of Adiponectin, while consumption of diets with base forming potential result in increased Adiponectin circulating level.[12-26] However, some reports from other investigators do not confirm these findings.[20,27,28] There is a recent increase in the prevalence of habitual consumption of high acid forming diets in our setting, and the effect of that on the circulating level of Adiponectin, in the general population, has not been investigated.[29]

Therefore, using the potential renal acid load (PRAL) score to estimate the acid-forming potential of our local diets, we tested whether diet-induced acidosis is associated with low circulating level of Adiponectin among a group of apparently healthy adult Nigerians.

MATERIALS AND METHODS

Study Participants

We conducted this cross-sectional analytical hospital-based study among two hundred and thirty-two (232) apparently healthy adult Nigerians (128 males and 104 females) aged above 18 years. The subjects were recruited into the study at the State Specialist Hospital, Gombe, Nigeria, from among individuals who presented to the hospital for routine medical check-up, pre-marital screening and blood donors. All study subjects are Nigerians of African descent and living in Gombe State. Subjects with diagnosed diabetes mellitus or any other illness, smokers, alcoholics, pregnant and lactating women, under-reporters or over-reporters of dietary intake ($\leq$ 800kcal/day or > 4,200kcal/day respectively) and individuals on special diets were all excluded from the study.

This study was approved by the Ethics Committee of the Gombe State Ministry of Health, Gombe, and the study procedures adhered to the recommendations of the Declaration of Helsinki. All the study subjects gave informed consent.

Data collection

The study subjects were evaluated in the morning following a10-12 hours overnight fast. History and physical examination were carried out on each of the study subjects at the time of blood sample collection. Data on age, sex and dietary intake were noted and recorded. During physical examination, blood pressure measurements were obtained with each subject in a sitting position using a mercury Sphygmomanometer. Systolic and diastolic blood pressures were measured and recorded. Body weight and height were measured with subjects standing erect and not wearing shoes or headgear. A wall-mounted measuring tape was used for the measurement of body height and a weighing scale for the body weight measurements. Body mass index (BMI) was calculated as weight in kilogram (kg) divided by height in meter squares (m^2).

Dietary assessment

During the interview a food frequency questionnaire was used to assess the intake of food items over the preceding week. To determine the frequency of consumption, nine response options were given, from rarely (less than once in month) to more than once a day. And to determine portion size, a standard portion size for each food item was specified according to local household measures, and each participant was asked to specify his/her portion size as half the standard, the standard or one and half times the standard portion size.

The average daily intake of each of the food items was determined by multiplying the frequency of daily consumption and the standard portion size by the participant portion size. Dietary intakes of energy and selected nutrients were estimated from the Nigerian Food Composition Table.[30]

Estimation of Dietary Acid Load

The dietary acid intake was estimated using the Potential Renal Acid Load (PRAL) score;

The PRAL score was calculated using the following equation:

PRAL (mEq/day) = 0.4888 × Dietary Protein (g/day) + 0.0366 × Dietary Phosphorus (mg/day) - 0.0205 × Dietary Potassium (mg/day) - 0.0125 × Dietary Calcium (mg/day) - 0.0263 × Dietary Magnesium (mg/day).[31]

Laboratory analysis

Overnight fasting venous blood samples were collected in to lithium heparin bottles for the quantification of Adiponectin and Glucose. Blood samples were immediately centrifuged for 15 minutes for separation of plasma, which was stored in aliquots at -20^{0}C until analysis. Fasting plasma Glucose was measured using the Glucose oxidase method (Agappe Diagnostics Limited, India) and plasma total Adiponectin was measured using an Enzyme-Linked Immunosorbent Assay (ELISA) kit (Bioassay Technology

Laboratory BT Lab. China) which utilizes an antibody specific for human Adiponectin coated on the walls of the micro wells. The Adiponectin present in the plasma samples bound to the wells, by the immobilized antibody, on adding the samples into the wells. Subsequent addition of biotinylated anti-human Adiponectin antibody and HRP-conjugated streptavidin into the wells, together with a substrate (TMB) resulted in the development colored solution whose intensity is directly proportional to the concentration of the Adiponectin in the samples. Stop Solution (Sulphuric acid) changes the color from blue to yellow, and the intensity of the color was measured at 450nm. Standard curve of Adiponectin determination was plotted and the Adiponectin level in each sample was determined from the curve.

All laboratory analyses were done at the Chemical Pathology laboratory of Gombe State University/Federal Teaching Hospital, Gombe.

Statistical analysis

Statistical analysis of the generated data was done using the Statistical package for social sciences (SPSS) version 20.0. Kolmogorow-Smirnov test was used to test for normality of distribution of data and logarithmic transformation was used to improve the normality of distribution of skewed data. Quantitative variables were presented using proportions and measures of central tendency and dispersion. The mean values of quantitative variables were compared across the PRAL quartiles categories using ANOVA test. Partial correlation analyses were used to determine relationship between dietary acid load and plasma Adiponectin and to adjust for confounders. All p-values were two-sided and considered significant if less than 0.05.

RESULTS

Demographic, dietary and biochemical parameters of the study subjects are shown in Table 1. The mean age of the study subjects, which were predominantly males (55.2%), was 34.0 ± 5.4 years and the mean BMI was 24.5 ± 4.4kg/m^2. There were no significant differences in the age and BMI levels between male and female subjects (34.1 ± 5.6 vs. 34.0 ± 4.1 years, $p > 0.05$) and (24.8 ± 4.3 vs. 24.1 ± 4.4kg/m^2, $p > 0.05$) respectively. The mean energy intake of the study subjects was 2245 ± 423kcal/day. There were no statistically significant differences in the intake of energy, protein, Calcium, Phosphorus, Potassium and Magnesium among the male and female subjects (p-values for all > 0.05).

The relationship between dietary acid load and plasma level of Adiponectin in the study subjects was examined (Figures 1 and 2.) A significant inverse relationship, independent of age and BMI, was found between PRAL scores and plasma Adiponectin levels ($r = -0.56$, $p < 0.05$) in all the study subjects (Figure 1). When the study subjects were categorized into males and females, the inverse relationships between PRAL scores and plasma Adiponectin levels was observed to be stronger in male subjects. The subjects were categorized in to four quartiles according to their median dietary acid intake (Figure 2). Study subjects consuming higher levels of dietary acid in the fourth quartile were observed to have a significantly lower level of plasma Adiponectin level compared with the study subjects consuming lower levels of dietary acid (12.6 ± 2.2µg/mL vs. 6.1 ± 2.5µg/mL).

Table 1: Demographic and Biochemical Parameters of the Study Subjects.

	All (m ± SD)	Male (m ± SD)	Female (m ± SD)	*p*-value
Sample size (n)	232	128	104	-
Age (years)	34.0 ± 5.4	34.1 ± 5.6	34.0 ± 4.1	0.793
Sex ratio	128/104	-	-	-
Body Mass Index (kg/m^2)	24.5 ± 4.4	24.8 ± 4.3	24.1 ± 4.4	0.221
Systolic BP (mmHg)	118.0 ± 5.3	118.0 ± 5.1	118.0 ± 5.6	0.469
Diastolic BP (mmHg)	78.6 ± 3.9	78.8 ± 3.8	78.4 ± 3.8	0.451
Energy (kcal/day)	2245 ± 423	2282 ± 416	2200 ± 430	0.143
Protein (g/day)	89.8 ± 17.8	89.4 ± 18.3	90.4 ± 17.3	0.655
Calcium (mg/day)	526 ± 156	522 ± 157	530 ± 156	0.678
Phosphorus (mg/day)	974 ± 181	967 ± 185	979 ± 177	0.667
Potassium (mg/day)	3057 ± 716	3057 ± 697	3057 ± 742	0.994
Magnesium (mg/day)	845 ± 615	898 ± 610	780 ± 617	0.144
PRAL (mEq/day)[*]	+9.1	+9.0	+9.9	0.221[#]
Fasting PG (mmol/L)	4.3 ± 0.7	4.2 ± 0.7	4.3 ± 0.6	0.463
Adiponectin (µg/mL)	7.3 ± 3.8	7.5 ± 4.0	7.1 ± 3.7	0.393

Keys: m, mean, SD, standard deviation, BP, blood pressure, PG, plasma glucose
PRAL, potential renal acid load, [*]median values, [#]Mann- Whitney test

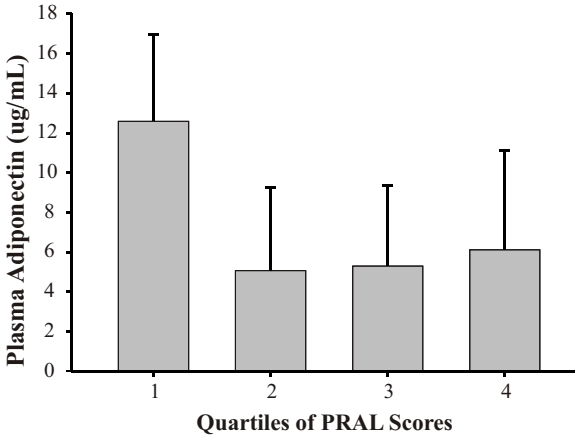

Figure 1: Plasma Adiponectin Level among the Study Subjects. Mean and Standard Deviation are shown. *PRAL, potential renal acid load.*

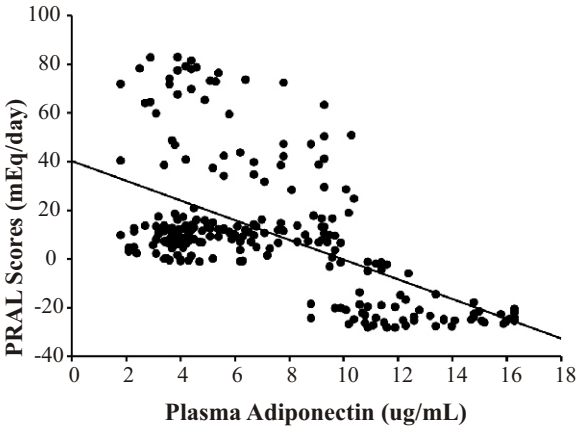

Figure 2: Correlation of Plasma Adiponectin and Dietary Acid Load Scores among the Study Subjects. *PRAL, potential renal acid load*

DISCUSSION

We examined the impact of diet induced metabolic acidosis on the circulating level of Adiponectin in a group of apparently healthy adult Nigerians. We found a significant negative association between diet-induced acidosis and circulating Adiponectin level among the study subjects independent of age, gender and body weight status.

Our results are supported by previous prospective studies carried out mainly among Caucasians, in which habitual consumption of acidifying foods (high PRAL foods), as red meat, processed meat, refined sugars and high-fat dairy products, is significantly associated with decreased circulating Adiponectin levels.[13-19]

In support of our findings, Luisi et al[24] and Spadafranca *et al*[25] reported that adherence to Mediterranean diet, which consists largely of low PRAL foods as vegetables and fruits, is associated with significant increase in circulating Adiponectin levels compared to non-adherence. Similar findings of significant increase in circulating Adiponectin levels

among individuals whose eating pattern consists mainly of alkalinizing food items were also reported by many other investigators.[20-23,26]

However, findings from some other studies do not support these. Lovrencic *et al*[27] did not find a significant correlation between following a vegetarian diet and Adiponectin circulating level in male adults.[27] Adherence to Mediterranean diet was also not significantly related to Adiponectin circulating level in female adults and adolescents of both sexes according to reports from a study by Sureda *et al*.[20] Adding to the controversy, Gunn et al[28] reported a decreased total Adiponectin circulating level in postmenopausal women following three months dietary intervention with low PRAL foods (fruits and vegetables).

Differences in sample sizes and heterogeneity of the study subjects, including differences in dietary patterns, and whether total Adiponectin or high molecular weight Adiponectin was measured in the different studies might partly explain the discrepancies in the findings from the previous studies.

Nutrient composition of food items plays an essential role in the maintenance of acid base homeostasis by supplying acid or base precursors. Diets rich in animal proteins, including red meat, eggs and high fat dairy products among others, enhance the production of acid in the body, while diets rich in vegetables and fruits increase production of alkali in the body.[32] The potential renal acid load (PRAL) score defines the capacity of any food item to generate acid or base precursors in the body.[30] Habitual consumption of diet containing high PRAL food items causes a decrease in blood pH towards the lower end of normal which, if not compensated for by the normal homeostasis or modification of diets, can induce a state of chronic low grade metabolic acidosis (diet-induced acidosis) in the body.[32]

Chronic acidosis induces cellular oxidative stress, and adipocytes that secrete Adiponectin are sensitive to oxidative stress.[33] Exposure of adipocytes to reactive oxygen species suppressed Adiponectin mRNA expression and secretion and also increased the mRNA expression of pro-inflammatory cytokines such as interleukin (IL) 6.[34] Interleukin (IL) 6 is also a negative regulatory factor of Adiponectin gene.[35,36] Thus, the higher level of interleukin (IL) 6 further suppresses the transcription of Adiponectin gene in adipose cells. Additionally, metabolic acidosis has been shown to directly inhibit the transcription of Adiponectin gene in adipocytes.[12]

LIMITATIONS

Firstly, the assessment of energy and nutrient intake was done using a self-reported food frequency questionnaire which might be affected by recall bias. Secondly, causality cannot be inferred from the findings because the design of the study is cross sectional. Although potential confounders were adjusted for, residual confounding is still possible and could weaken the strength of association.

CONCLUSION

We conclude that among apparently healthy adult individuals in this study, higher intake of dietary acid is associated with decreased circulating Adiponectin levels. This finding may require replication from studies involving larger sample size and various ethnic groups. We recommend further longitudinal studies that will investigate the impact of dietary acid reduction/restriction on the circulating Adiponectin level in our setting.

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CONFLICT OF INTEREST: Nil

ETHICS APPROVAL

The Health Research Ethics Committee of the Gombe State Ministry of Health, Gombe, Nigeria approved the study protocol.

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Delayed Wound Healing: A Focus on Bacterial Intracellular Proteases in Diabetic Wound Ulcers

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ABSTRACT

A diabetic skin ulcer (DSU) is a breach on the skin characterized by progressive destruction of the surface epithelium of the skin and a disintegrating base resulting from wounds obtained by a diabetic patient. This study was aimed at determining DSU wound colonization by bacteria and to quantitatively analyze some intracellular enzymes produced by these bacteria that could affect the wound healing process. Wound swab samples were collected from 150 subjects comprising of diabetic patients with skin ulcers as well as non-diabetics with wounds (control). The wounds were found to be colonized by an array of bacteria which included Pseudomonas aeruginosa, Staphylococcus aureus, Acinetobacter sp, Enterococcus faecalis, Proteus vulgaris, Proteus mirabilis, Klebsiella pneumoniae, Escherichia coli, Coagulase negative Staphylococcus aureus, Klebsiella oxytoca, Alcaligenes faecalis and Citrobacter freudii. These bacterial isolates produced the following proteolytic enzymes: collagenase, caseinase, hyaluronidase, alkaline protease and gelatinase on the DSU in higher amounts than the non-diabetic wounds (control). Intracellularly, S.aureus (0.199 ± 0.000) and E.coli (0.145 ± 0.006) secreted the maximum and minimum amounts of hyaluronidase respectively. For caseinase, S.aureus (0.048 ± 0.001) exhibited the highest activity and K.oxytoca (0.018 ± 0.000) showed the least activity. E.coli (18.173 ± 0.157) secreted the highest amount of gelatinase while P.aeruginosa (5.057 ± 0.496) had the least while maximum intracellular activity for alkaline protease was secreted by P.aeruginosa (0.016 ± 0.001). Bacterial wound flora was found capable of producing and secreting intracellular proteolytic enzymes which could be implicated in impairing proper wound healing.

Keywords: Diabetic Wounds, Intracellular Bacterial Proteases, Wound Healing

INTRODUCTION

Diabetic skin ulcers are the most dreadful and difficult to treat complications of diabetes mellitus. Diabetic skin ulcers evolve in about 15 percent of people with Diabetes mellitus and have become the principal proximate trigger for non-traumatic extremity amputations throughout the world. An estimated 85 percent (85%) of amputation cases are due to diabetic skin ulcers.[1] In Nigeria, prevalence of diabetic skin ulcer ranges from 0.8-11 % in urban and rural populations.[2]

A diabetic skin ulcer (DSU) is defined as a breach on the skin characterized by progressive destruction of the surface epithelium of the skin and a disintegrating base resulting from wounds obtained by a diabetic.[3] It involves various pathological complications including peripheral vascular disease, neuropathy, ulceration of wounds and infections (with or without osteomyelitis) which lead to gangrene development and may even necessitate amputation of the limb. DSUs can even result in considerable morbidity and mortality of the patient due to increased susceptibility to infection and impaired wound healing. A major hindrance in the controlling of DSU is the colonization of wounds by bacterial pathogens.[4]

Diabetes decelerates the normal functioning process of wound healing, leading to vascular and neuropathic disorders. The microbes are therefore able to overcome the host's cell barrier due to the degradation of cell epithelium resulting from the peripheral neuropathy associated with Diabetes.[5] Also, increased hyperglycemia activates advanced glycation of lipids and proteins which accumulate in the capillaries, thus resulting in decreased flow of blood to the infected wound site.[6] This impaired circulation in micro-vascular system in diabetics with wounds hinders the phagocytes' access thus enhancing

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the development of an infection.[5] Hence, increased susceptibility to infection is found in diabetic patients, rather than non-diabetic persons.

Bacteria produce proteolytic enzymes such as collagenase, gelatinase, caseinase, alkaline protease hyaluronidaseetc, which locally damage the cells of the host and aid the pathogenic bacteria in their spread.[5,7-9]These enzymes play a significant role in impairing wound healing because bacterial proteases can cause the destruction of collagen, cell membrane and muscle fibres and increase capillary permeability for the establishment of the infecting bacteria. They hydrolyze the proteins and peptides and therefore cause cell membrane degradation and disruption of various biological activities.[5] The recognition of the organisms and their biochemical parameters will help provide better treatment for the diabetic skin ulcer problem and other inadequately healing wounds.[10] Even though the diabetic skin ulcer is a multi-factorial condition, one of the most significant reasons for the non-healing nature of the wound is the destruction of tissues caused by the increased bacterial protease activity.[5]

Hence, the aim of this study was to determine the presence and effects of some intracellular proteolytic enzymes of bacterial origin on tissue damage and impaired wound healing in diabetic skin ulcers.

MATERIALS AND METHODS

A total of 150 subjects were recruited in this study. Wound ulcer swab samples were collected from diabetic skin ulcer patients as well as non-diabetics with wounds (control) in the Central Hospital Benin, St Philomena Hospital, Benin, Stella Obasanjo Hospital, Benin and the Federal Medical Centre, Asaba, Nigeria. Informed consent and ethical approval were obtained from the Institutes' Ethics Committee/ Hospitals' Management Board. The samples were cultured and bacterial isolates were obtained and were identified using standard bacteriological procedures. A quantitative study of the intracellular enzymes collagenase, caseinase, hyaluronidase, alkaline protease and gelatinase was carried out.

Isolated bacteria were separately inoculated into 10ml of nutrient broth and incubated for 24hr at 37°C. Each cultured isolate was centrifuged, and the supernatant and pellet were collected separately. The pellets were then suspended in 1ml phosphate buffer,sonicated and centrifuged and were used for the intracellular enzyme assay.

Collagenase and caseinase were assayed using the procedure.[11]A unit of the enzyme activity was described as the μM of L-leucine liberated in 5hr at culture conditions. Hyaluronidase assay was done using the methods of Tolksdorf and McCready, and Kass and Seastone.[12,13] The activity of alkaline protease was determined by the method of Meyers and Ahearn.[14] One unit of alkaline protease activity was expressed as the amount of the enzyme which released 1μmol tyrosine per ml per min.

Gelatinase activity was determined using the method described by Tran and Nagano and it was defined as the μM of leucine released per ml per min.[8]

RESULTS

The frequency of occurrence of bacterial isolates in diabetic skin ulcers and non-diabetic wounds is shown in table 1. Among the total bacterial isolates obtained, 34.78% of the isolates were Gram-positive, while 65.22% were Gram-negative. Among the Gram-positive bacteria, *Staphylococcus aureus* was the most predominant isolate (18.12%). Coagulase negative Staphylococcus (CONS) was the least isolated (2.17%). Among the Gram-negative bacteria, *Escherichia coli* was the most common isolate (17.39%) and *Acinetobacter* species was the least common bacterial isolate (2.17%). A higher percentage of the bacterial isolates also occurred in the diabetic skin ulcers than the non-diabetic wounds.

A profile of the different proteolytic enzymes produced by the bacterial isolates from diabetic skin ulcers is shown in table 2. All isolates secreted intracellular gelatinase which had 100% occurrence. Alkaline protease was the least secreted enzyme intracellularly. Intracellular enzyme

activities on the wounds were observed as follows: collagenase (81.88%), caseinase (86.23%), hyaluronidase (69.56%), alkaline protease (54.35%) and gelatinase (100%).

A comparative analysis of the intracellular proteases secreted by bacterial isolates in diabetic skin ulcers and non-diabetic wounds of studied subjects is shown on table 3. The values obtained revealed that higher quantities of the intracellular proteases were secreted by the bacterial isolates in the diabetic ulcers than those in the non-diabetic wounds.

Table 1: Frequency of occurrence of bacterial isolates in diabetic skin ulcers and non-diabetic wounds.

Bacteria	Diabetic Skin Ulcers n=138 (%)	Non-Diabetic Wounds n=18 (%)
Gram Positive		
Staphylococcus aureus	25(18.12)	4(22.22)
Enterococcus faecalis	20(14.49)	-
Coagulase negative staphylococcus (CONS)	3(2.17)	-
Gram Negative		
Escherichia coli	24(17.39)	6(33.33)
Klebsiellapneumoniae	13(9.42)	2(11.11)
Proteus mirabilis	12(8.70)	1(5.56)
Pseudomonas aeruginosa	10(7.25)	2(11.11)
Klebsiellaoxytoca	10(7.25)	-
Citrobacterfreundii	7(5.07)	-
Proteus vulgaris	7(5.07)	-
Alcaligenesfaecalis	4(2.90)	3(16.67)
*Acinetobacter*sp.	3(2.17)	-

Table 2**:** Profile of intracellular protease enzymes produced by different bacterial isolates on diabetic skin ulcers.

	Intracellular Enzymes (Mean ± SEM)				
Bacteria	**Collagenase (unit/ml/min)**	**Caseinase (unit/ml/min)**	**Hyaluronidase (mg HA digested)**	**Alkaline Protease (unit/ml/min)**	**Gelatinase (unit/ml/min)**
S.aureus	0.018±0.001	0.048±0.001	0.199±0.000	0.007±0.000	180.054±0.363
E.faecalis	0.025±0.003	0.045±0.001	0.164±0.012	-	160.279±0.016
CONS	0.016±0.006	0.026±0.012	0.183±0.005	0.006±0.001	60.096±0.065
E.coli	0.018±0.007	0.033±0.007	0.145±0.006	-	180.173±0.157
K.pneumoniae	0.017±0.001	0.039±0.008	-	0.004±0.002	135.032±0.132
P.mirabilis	-	-	-	-	143.228±0.069
P.aeruginosa	-	0.046±0.001	-	0.016±0.001	50.057±0.496
K.oxytoca	0.009±0.001	0.018±0.000	0.162±0.015	0.012±0.001	129.760±0.345
C.freundii	0.011±0.001	0.040±0.001	0.186±0.002	0.011±0.002	120.087±0.036
P.vulgaris	0.020±0.006	-	-	-	150.069±0.008
A.faecalis	0.009±0.001	0.043±0.001	0.174±0.012	0.009±0.001	122.005±0.081
***Acinetobacter*sp.**	-	0.045±0.001	0.168±0.008	0.005±0.000	130.274±0.017

Table 3: Comparative profile of intracellular enzymes secreted by bacterial isolates from diabetic skin ulcers and non-diabetic wounds.

Intracellular Enzymes	Diabetic Skin Ulcer	Non-Diabetic Wounds
COLLAGENASE (mg/ml/min)	0.009±0.001 - 0.025±0.003	0.002±0.000 - 0.009± 0.001
CASEINASE (mg/ml/min)	0.018±0.000 - 0.048±0.001	0.009±0.002 - 0.017±0.002
HYALURONIDASE (mgHA digested)	0.145±0.006 - 0.199±0.000	0.034±0.003 - 0.118±0.025
ALKALINE PROTEASE (mg/ml/min)	0.004±0.002 - 0.016±0.001	-
GELATINASE (mg/ml/min)	5.057±0.496 - 18.173±0.157	0.129±0.007 - 3.024±0.401

DISCUSSION

The array of bacteria isolated from the wounds included *Pseudomonas aeruginosa, Staphylococcus aureus, Acinetobactersp, Enterococcus faecalis, Proteus vulgaris and Proteus mirabilis, Klebsiella pneumoniae, Escherichia. coli*, Coagulase negative *Staphylococcus aureus*, *Klebsiella oxytoca, Alcaligenes faecalis* and *Citrobacter freudii*. Bacterial colonization limits the healing process of the wound. Therefore, these bacterial pathogens cause disease by establishment mechanisms, invasins production and evading the defense mechanisms of the host.

This study revealed that these bacteria produced some intracellular proteolytic enzymes. These enzymes play very significant roles in making the wounds worse. Bacterial protease cause deterioration of the cell membranes and disrupt several biological functions by hydrolyzing the proteins and peptides of the cells.[5]From the results obtained, it was observed that the enzyme intracellular collagenase was secreted by all isolated bacteria except *Proteus mirabilis, Pseudomonas aeruginosa* and *Acinetobacter sp*. The high rate at which collagenase was secreted by the bacterial isolates depicts its ability in causing uncontrolled proteolytic destruction of tissues, thus acting as a pathogenic factor in the non-healing wound. Due to the abundance of collagen in the skin, bones and other connective tissues, bacterial collagenase which tend to break down the on-site collagen can bring about uncontrolled proteolytic destruction of tissues and pose as a virulence factor in the unhealing wounds, resulting in ulceration of the skin and osteomyelitis of the bones.

A few of the organisms under consideration gave intracellular activities for hyaluronidase and this enzyme functions as a virulence factor and is responsible for the breaking down of cell membrane polysaccharides and thus, increasing the permeability of the cell wall, which enhances bacterial spread around the wound surface.[15]

The presence of the enzyme caseinase has been detected in many hospital clinical isolates such as those obtained from respiratory tract secretions, in corneal ulceration during bacterial keratitis and also in some strains. The proteolytic activity of the enzyme has also been found to be related with the pathogenesis of the producing bacteria and the development of hospital-acquired infections and also essential for the activity of haemolysin.[5] Hence, the occurrence of caseinase-producing bacteria in diabetic skin ulcer has an influence in delayed diabetic skin ulcer management.

All bacterial isolates in this study were observed to secrete gelatinase intracellularly. Bacterial gelatinase breaks down gelatin present in connective tissues and aid in the continuous spread of the microorganisms into the tissues.[5]Thus, they might play a role making diabetic skin ulcers worse.

Alkaline proteases affect the wound healing process by causing an increase in the pH at the site of the wound. Normal wound healing process readily occurs in an acidic environment between pH 4-6. The enzyme acts by elevating the wound's pH, and therefore affects many functions like

angiogenesis, release of oxygen, bacterial toxicity, protease activity etc., thus causing the wound to stay unhealed.[9] Alkaline proteases cleave protein peptide bonds and are usually stable at a higher or alkaline pH. Alkaline proteases also exhibit proteolytic activity on proteins that are involved in the defense mechanism of the host, such as the activation of complement through the classical and the lectin pathways and also the penetration of the barriers of the body, causing damage to the cells of the host. They also help the organism to evade the host's immune system by protecting them. Bacterial alkaline protease can cause these effects even in very small amounts.[5] This supports the findings of the present study because organisms positive for alkaline protease were significantly less. Although the activity of the enzyme was less, it has severe consequences on already debilitating conditions of diabetic skin ulcers hence greatly impairing the wound healing process.[5]

In comparing the diabetic skin ulcers and the non-diabetic wounds, it was observed that much higher amounts of all the proteolytic enzymes secreted (collagenase, caseinase, hyaluronidase, alkaline protease and gelatinase) were observed in diabetic skin ulcers. This indicates that the presence of these proteases can be implicated in the slow healing process of diabetic skin ulcers compared to non-diabetic wounds.

CONCLUSION

This study suggests that bacterial enzymes play a major role in tissue damage and impaired wound healing associated with diabetic skin ulcers, thus providing information to help produce better treatment options for diabetic skin ulcers.

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Serum Folate Levels in Hormonal Contraceptive Users in Kano

***Abdullahi HL, Shafiu LM**

ABSTRACT

Hormonal contraceptives (HCPs) are the major class of prescription drugs, used by a large proportion of women starting from early adolescence. Folate is a vitamin with significant roles to play in cell division and amino acid metabolism, under its function in various coenzyme-dependent reactions. This study was aimed at evaluating serum folate level in HCP users and compare these levels to non-HCP users. Sixty healthy females of reproductive age on HCP's were recruited as the test group and 30 non-HCP users were recruited as the control group. The serum Folate levels of the subjects and the controls were determined by human Folate specific ELISA Kit. The results showed a significant decrease in mean serum Folate levels of subjects compared to controls with mean and standard deviation values of 2.3747 ± 1.62129ng/ml and 6.4200 ± 3.69803ng/ml respectively (p <0.001). Thus, based on the findings of the current study, HCP use is associated with a lowered serum Folate level. Therefore, it is recommended that women continue periodic Folate supplementation during HCP use.

Keywords:Folate, folate deficiency, contraceptives, estrogen and progestin

INTRODUCTION

Contraception is the use of different devices, drug agents, sexual practices or surgical procedures to avoid conception or impregnation.[1] Contraception can be hormonal or non-hormonal based. Hormonal contraceptives (HCP's) are synthetic biochemical substances that act on the endocrine system and permit sexual union without resultant pregnancy.[2]

Hormonal based contraceptives may be combined oral contraceptives (OC), injectables, transdermal patch, vaginal ring, intrauterine devices (IUD), or implant. Among the hormonal based contraception methods, combined oral contraceptives are the most frequently used and they contain synthetic Estrogen (ethinylestradiol) and synthetic Progesterone.

Folate is a water-soluble vitamin whose coenzymes serve as donors and acceptors of a myriad of one-carbon entities required for enzymatic reactions including those involved in amino acid metabolism (e.g., homocysteineremethylation to methionine), and DNA synthesis and methylation.[3] Naturally occurring Folate is found in abundant quantity in some food sources, including green leafy vegetables, orange juice, and legumes.[(4)] Impaired Folate metabolism can dramatically alter cell division which is especially evident when cells are rapidly dividing during early embryogenesis.

There have been several reports indicating that OC use is associated with impaired status of some nutrients, including Folate and Vitamins B6 and B12, each of which plays vital roles in fetal development. Since pregnancy often occurs shortly after OC use is discontinued or when use is intermittent, it is important to know if OCs impair Vitamin status in women of reproductive age, which may negatively impact fetal development should pregnancy occur.[5]

There is controversy regarding the potential side effects associated with alteration in Folate levels in women using HCP's. Despite the widespread and increasing acceptance and use of HCPs by women especially in the Northwestern region, metabolic complications arising from these HCPs are poorly understood and not well documented in this region. Hence the need for research aimed at a better understanding of these metabolic derangements associated with HCP use.

Specific Objectives

1. To determine the serum Folate levels in healthy non HCP users (control group).

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2. To determine the serum Folate levels in HCP users (subjects) in some selected hospitals in Kano metropolis.
3. To compare the serum Folate levels between HCP and non-HCP users in some selected hospitals in Kano metropolis.
4. To compare serum Folate levels in different HCP users (subjects).

MATERIALS AND METHOD

Study Area

The study was carried out in Murtala Muhammad Specialist Hospital and Muhammad Abdullahi Wase Specialist Hospital, Kano State. Both hospitals located within Kano Metropolis had an average of 25 OC users daily.

Study Population

The study population involved a test group (women using HCP's) and a control group of apparently-healthy, pre-menopausal women who were had no history of HCP usage.

Inclusion Criteria

Only women of reproductive age using various forms of HCP's for at least six months within Kano metropolis were recruited.

Exclusion Criteria

Recent Folate supplementation
Folate-affecting medications like methotrexate
Neoplastic diseases

Ethical Approval

Ethical approval to conduct the research was obtained from Kano state Hospital Management Board, Research Ethics Committee. The participants' (subjects and controls) written consent was sought before the administration of the questionnaires.

Questionnaire Survey

A structured questionnaire was administered to all the study participants to determine the type of HCP use, the duration of use, and sociodemographic data of the participants. This was done for both the test and control groups.

Sample Size Determination

This was calculated using Rao's formula for minimum sample size estimated in health studies.

$$\text{Where, } N = \left(\frac{ts}{me}\right)^2 = \left(\frac{1.96 \times 1.88}{0.475}\right)^2 = 7.5^2 = 60$$

N= minimum sample size (no of participants) = 60
T = confidence interval on the normal distribution curve and is given as 1.96
me = 5% precision × mean used = 0.05×9.50 = 0.475
s= standard deviation of the parameter used = 1.88
The sample size was calculated to be 60 and 30 subjects were taken as control.

Sample Collection, Processing and Survey

Two (2ml) of venous blood sample was collected aseptically from all the participants and dispensed into plain vacutainer containers. The samples were allowed to clot, retracted and then centrifuged at 3000rmp for 5minutes. The serum was transferred into pre-labelled 2ml plain containers and stored at 2-8 until analysis.

Laboratory Analysis

Serum sample was assayed for Folate, using Human folate specific Sandwich ELISA assay kit.
Reagents and samples were allowed to stand at room temperature for 30minutes, the content of wash solution was diluted to 1000ml with distilled water and an extraction agent were prepared by making 1/40 (stabilizing agent/ releasing agent) dilute solution.

All samples, controls and calibrator were extracted by dispensing 0.1ml (100µl) of all samples into individual test tube, pipetting 0.05ml (50µl) of extracting agent to each test tube and dispensing 0.05ml (50µl) of neutralizing buffer following 15 minutes of incubation while shaking after every addition.

The wells were numbered with sample identification number including the six Folate calibrators (cal A, B, C, D, E and cal F), 0.05ml of extracted Folate calibrators, control and patients were dispensed into the assigned wells and 0.05ml of Folate biotin reagent were added to all the wells. The contents of the microplate were mixed, covered and incubated for 45 minutes at room temperature after which were added 0.05ml of Folate enzyme reagents to all the wells followed by 20 minutes of incubation. The microplate was washed five times using an automatic plate washer, the plate was blotted, and 0.1ml of Folate substrate reagent was added to all the wells followed by 20 minutes of incubation and the addition of 0.05ml of stop solution to each well. The absorbance in each well was read at 450nm.

Statistical Analysis

All collected data and result obtained where analyzed using SPSS version 20 software. Results were expressed as mean and standard deviation (SD). $P < 0.05$ was considered as a statistically significant difference.

RESULTS

A total of 60 HCP users were recruited as the test group and 30 females with no history of HCP use were recruited as the control group.

Table 1 shows the distribution of cases and control by the type of HCP's used.

Table 2 profiles the duration of use across the subject group

Mean Serum Folate Levels

A statistically significant reduction was observed in the mean serum Folate levels of subjects when compared to the control group (2.3747 ± 1.62129 and 6.4200 ± 3.69803 respectively) (Table 2).

Table 3 shows the comparison of serum Folate levels in different subjects using various forms of HCP's.

Table 1: Distribution of subjects based on the types of HCP's used

Contraceptive methods	Number of subjects	Percentage (%)
Oral	11	18.3
Implant	29	48.3
Injectables	14	23.3
Intrauterine devices	6	10.0
Total	60	100

Table 2: Distribution of subjects by the duration of HCP use

Duration of HCP use (months)	Number of subjects	Percentage (%)
5-60	53	88.3
61-116	2	3.3
117-172	4	6.7
173-228	1	1.7
Total	60	100

Table 3: Mean serum Folate levels of subjects and controls ng/ml

Parameters	Subjects (HCP users) Mean ± SD	Controls Mean ± SD	P-value
Folate	2.3747 ± 1.62129	6.4200 ± 3.69803	<0.001

DISCUSSION

HCP's are synthetic biochemical substances that act on the endocrine system and permit sexual union without resultant pregnancy. Folate functions as a coenzyme in the acceptance, oxidation/reduction and transfer of one-carbon units and are particularly important in amino acid metabolism and the synthesis of nucleic acids. There have been several reports indicating that HCP use is associated with impaired status of several nutrients, including Folate and Vitamins B6 each of which plays vital roles in fetal development.

Our study reports a significant decrease in Folate levels in HCP users. This is consistent with a case-control study carried out in Toronto University Canada, on the impact of HCP's on serum Folate levels that reported significantly lower serum Folate levels in HCP users.[6]It is also in accord with the pioneering work of Shojani *et al.* and another study by Sutterlin *et al.*[7,8]

However, the findings in the current study are in contrast with that of Castren and Rossi who found no significant relationship in serum Folate levels between users of OC's and non-users.[9]

There are various explanations for how HCP may deplete Folate level. Stephanie *et al.* postulated that Folate is poorly absorbed in HCP users.[10]ThepolyglutamylFolate form seems to be the most impaired. OC may impair the enzymatic cleavage by the intestinal Folate conjugate enzyme required for polyglutamyl Folate absorption.

CONCLUSION

In this current study, the serum Folate levels of subjects and controls were assessed and the results show a statistically significant decrease in mean serum Folate levels of subjects compared to the controls.

RECOMMENDATION

This study recommends that women on HCP's should consider Folic acid supplementation especially if they desire to conceive in the future.

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The Hepatic and Hematologic Toxic Effects of Pro-Vitamin A High Quality Cassava Flour Obtained by Different Processing Methods

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ABSTRACT

The hepatic and hematologic toxic effect of Pro-Vitamin A High Quality Cassava Flour (HQCF) by different processing methods was studied in male albino Wistar rats, fed for 28days on the Cassava diet containing 4.794mgCN kg^{-1} and 10% protein supplement, using spectrophotometry, enzyme assay and automated haematology analyzer. There was significant increase in the serum Glucose concentration, serum activity of Aspartate Aminotransferase and Alanine Aminotransferase of the test animals compared to the control, whereas the serum total protein concentrations showed a significant decrease between the test and control groups. The hematology studies showed no significant difference ($p > 0.05$)between the test and control groups. Toxicity study suggested that the in vivo metabolism of Pro-Vitamin A HQCF was capable of altering some biochemical parameters such as elevation of serum Glucose concentration and some enzymes such as Aspartate Aminotransferase and Alanine Aminotransferase, whereas a reduction in serum total protein concentration was observed. The findings indicated that the processing methods (most especially oven dry) have reduced the toxicity of HQCF to a minimum safe level.

Keywords: Toxicity, Concentration, Hematology, Metabolism

INTRODUCTION

Cassava (Manihot esculenta Crantz) is the common food of many people in the tropical region.[1-2]It belongs to the family of Euphorbiaceae and the root is rich in carbohydrates.[3] Different Cassava varieties vary in their starch content,[4] with older roots having less starch content than younger ones.[5] The root of Cassava is also rich in cyanogenic glucosides (Linamarin and Lotaustralin).[6,7] These cyanogenic compounds can undergo hydrolysis after ingestion to generate hydrogen cyanide (HCN) and other compounds containing cyanide. Ingestion of cyanide causes its higher levels in the liver than through inhalation. This knowledge is very useful in scientific investigations.[8]

After cyanide absorption, it is rapidly circulated throughout the body. A large proportion of cyanide in the body is protein-bound (60%). Cyanide reacts reversibly but with a high affinity with metals such as the Ferric ion (Fe3+) and Cobalt. Cyanide can also react with compounds that contain Sulfur. Tissues that contain cyanide include the heart, liver, spleen, brain, kidneys, blood, and lungs.[9] Cyanide poisoning inhibits enzymes that contain metals. The toxicity of cyanide appears in the inhibition of the enzyme, Cytochrome oxidase a_3, terminal enzyme of the respiratory chain which compromises oxidative phosphorylation leading to cytotoxic hypoxia[10]. Due to this toxicity, there is an important need in exploring the best Cassava processing method to reduce cyanide to the barest minimum.

The traditional methods of peeling and grating, dewatering and fermentation for 72 hours reduce the cyanogens in Cassava roots to a considerably safe level.[11,12] Consumption of poorly processed cassava food products can lead to devastating health disorder and even death in man.[13, 14]Studies have confirmed that peeling has shown to represent the first processing step to reduce cyanogenic contents and to lower the Cassava toxicity, as the cyanogenic glycosides (CNG) are distributed in large amounts in the roots cortex (skin layer).[15] Moreover, grating of the pulp as the second step of sample preparation, breaks compartmental barrier and creates a higher surface area enabling Linamarin to

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have contact with its hydrolytic enzyme (Linamarase), resulting in the hydrolysis and subsequent removal of the breakdown products.[16] Since some cyanogenic compounds are soluble in water, its amount is reduced by traditional detoxification methods such as dewatering.[17] Further reduction occurred due to the volatile cyanogenic compound (HCN), which evaporated into the air during different drying methods.

MATERIALS AND METHODS

Sample Collection

A variety of Pro-Vitamin A Cassava tubers (Manihot esculenta Crantz) identified as UMUCASS 36, were purchased from the Cassava programme farm of the National Root Crops Research Institute (NRCRI), Umudike, Abia State, Nigeria. The Cassava tubers were harvested after 8 months of planting.

Preparation of Pro-Vitamin A High-Quality Cassava Flour

A variety of Pro-Vitamin A Cassava tubers, named UMUCASS 36, was processed with the following procedures. The tubers were processed within 24 hours after harvest. The tubers were peeled, washed, grated, pressed, disintegrated and dried using four (4) different drying methods which include;

- Sun-dried method.
- Oven-dried method
- Solar dried method
- Tray dried method

Animal Study

Twenty-five (25) Albino rats of the Wistar strain with body weight of 120-200g were used for the experiment. The animals were purchased from the animal house of Veterinary College, Michael Okpara Federal University of Agriculture, Umudike, Abia State. All animals were kept at room temperature and were allowed to have free access to drinking water and their diets. The animals were also allowed to acclimatize to their environment and their diet for 7 days before the experiment commenced. The animals were fed throughout the experiments with Pro-Vitamin A High Quality Cassava Flour (HQCF) based diet as shown in the table below. The control diet was prepared as above with Pro-Vitamin A HQCF replaced with corn flour, which was given to the control animals.

All the animals were fed for 28 days, after which they were sacrificed by stunting and their blood was collected directly from the heart through cardiac puncture using a syringe and needle inside the red and purple blood sample bottles. The serum was separated from the whole blood by centrifugation for 10 minutes at 1,000 revolutions per minute. The urine samples were also collected. This was with the approval from the Animal Ethic Committees (AECs), College of Veterinary medicine, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria.

Determination of Total Cyanide Content in Pro-Vitamin A HQCF

Cyanogenic content of the samples was determined using simple picrate paper test as described by Trinder.[18]

Determination of Serum Glucose

The serum Glucose was determined using glucose oxidase/peroxidase (GOD/POD) method as described by Tietz.[19]

Determination of Serum Total Protein

The serum total protein was estimated using the Biuret method as described by Tietz.[20]

Assay of Aspartate Aminotransferase and Alanine Aminotransferase Activity

A Randox commercial Enzyme kit according to the method by Reitman and Schmidt was used.[21,22]

Determination of Hematology Parameters

The haematology parameters were obtained at once for each blood sample using an Automated Hematology Analyser (Mindray BC 2300, China).

Statistical Analysis

Analysis of variance test was carried out using the statistical package for social science (SPSS).[23] Results are presented as Mean Standard Error and significant means were separated using Duncan Multiple Test.[24]

RESULTS

The cyanogenic content of Pro Vitamin A Cassava cultivar showed significant reduction (88.7%) in the cyanogenic content of the dewatered sample with concentration of 11.088ppm compared to the fresh sample with concentration of 97.812ppm, resulting in reduction/removal of 86.724ppm of cyanogenic content. Further reduction of cyanogenic content was observed during the different drying methods. The Sun dried reduced cyanogenic content by 91.9% (89.892ppm), Oven dried by 97.4% (95.268ppm), Solar dried by 97.2% (95.040ppm), and Tray dried by 93.9% (91.872ppm), when compared to the fresh sample, as shown in table 3. The average of the cyanogenic content of the different drying methods was computed as 4.794mgCN^{-}kg^{-1} to obtain the actual cynogenic content of the sample (Pro Vitamin A HQCF).

The concentration of Glucose in the serum of rats fed with yellow Cassava diet, in table 5, shows a significant difference at $p < 0.05$ in the test groups compared to the control group. Group D (tray dried) was the highest with concentration of 80.601.435mg/dl, whereas the control group was the least with concentration of 61.40 $\pm$ 2.315mg/dl. Table 2 also shows the results of the concentration of total protein in the serum of rats fed with yellow Cassava diet. There were significant differences at $p < 0.05$ in the serum total protein concentration of group A and C and slight difference in group B and D compared to the control group. Group C (Solar dried) was the least with concentration of 6.378 $\pm$ 0.248g/dl, whereas the control group was the highest with concentration of 7.794 $\pm$ 0.101g/dl.

The results of the activity of Aspartate Aminotransferase in the serum of rats fed Pro-Vitamin A High Quality Cassava Flour (HQCF) diet shows a significant increase ($p < 0.05$) in the serum levels of Aspartate Aminotransferase activity of the animals fed cassava diet above those of the control group (Figure 1). Figure 2 shows the results of the activity of Alanine Aminotransferase in the serum of rats fed yellow Cassava diet. There was significant increase ($p < 0.05$) in the serum levels of Alanine Aminotransferase activity of the animals fed Pro-Vitamin A HQCF diet above those of the control group (Figure 2).

The haematological parameter results in the plasma of rats fed with yellow Cassava diet detect slight reduction in Red Blood Cell (RBC) count in the test groups when compared with the control group (although there is no statistically significant different at $p < 0.05$). Group A (Sun dried) has the least RBC count with 7.342 $\pm$ 0.104 x10^{12}/L, whereas the control group has the highest RBC count with 8.130 $\pm$ 0.153 x10^{12}/L. There was no significant difference at $p < 0.05$ in the White Blood Cell Count, Platelets Count, Packed Cell Volume, Haemoglobin, Mean Corpuscular Volume, Mean Corpuscular Haemoglobin and Mean Corpuscular Haemoglobin Concentration of the test groups compared to the Control group.

Table 1: Dietary Animal Feed Composition

Ingredients	Quantity (g/kg)	(%)
Pro -Vitamin A HQCF	750	75
Protein (Soya beans)	100	10
Groundnut oil	40	4
Vitamin Mixture	40	4
Banana Flavor	40	4
Minerals Mixture	30	3

Experimental Design

The twenty-five (25) rats were grouped into five (5) groups, namely: Group A, B, C, D and E. Each group is comprised of five (5) animals. The test animal groups (A, B, C and D) were fed throughout the investigation on Pro-Vitamin A HQCF based diet as shown in the table 1 below. While Group E (Control) were fed with corn flour-based diet.

Table 2: Groups and their Treatments

Groups	Treatment
A	Sun -Dried Pro - Vitamin A HQCF Based Diet
B	Oven -Dried Pro - Vitamin A HQCF Based Diet
C	Solar Dried Pro - Vitamin A HQCF Based Diet
D	Tray Dried Pro - Vitamin A HQCF Based Diet
E(Control)	Corn Flour based diet

Table 3: Cyanogenic Content of Fresh, Dewatered and Dried (Flour) Pro-Vitamin A High Quality Cassava (UMUCASS 36)

Samples	Total CN content (ppm)	CN content removed from fresh (ppm)	% of CN removed from fresh (%)
Fresh	97.812	-	-
Dewatered	11.088	86.724	88.7
Sun Dried	7.920	89.892	91.9
Oven Dried	2.544	95.268	97.4
Solar Dried	2.772	95.040	97.2
Tray Dried	5.940	91.872	93.9

Each sample(n) is collected in duplicate and the average is recorded.

Table 4: Cyanogenic content of Pro-Vitamin A HQCF.

Samples	Cyanogenic content (mgHCN equivalent per kg)
UMUCASS 36	4.794

Cyanogenic content of Pro-Vitamin A HQCF was obtained by taking the average of cyanide content of Sun dried, Oven dried, Solar dried and Tray dried samples. All samples are collected in duplicate and the average was recorded.

Table 5: Concentration of Total Protein and Glucose in the Serum of Rats Fed Pro-Vitamin A HQCF diet.

Groups	Serum total protein concentration (g/dl)	Serum glucose concentration (mg/dl)
A	$6.46 + 0.30^{b}$	$79.60 + 1.21^{a}$
B	$7.30 + 0.33^{a}$	$74.20 + 1.98^{a}$
C	$6.38 + 0.25^{b}$	$76.60 + 3.08^{a}$
D	$7.28 + 0.29^{a}$	$80.60 + 1.44^{a}$
E	$7.79 + 0.10^{a}$	$61.40 + 2.32^{b}$

Table 6: Haematological parameters of Rats Fed Pro-Vitamin A HQCF

Parameters (Control)	Group A	Group B	Group C	Group D	Group E
RBC count ($x10^{12}$/L)	7.34 ± 0.10^{b}	8.11 ± 0.18^{a}	7.96 ±0.15^{a}	7.67 ±0.16^{ab}	8.13± 0.15^{a}
WBC count ($x10^{9}$/L)	12.38 ± 1.03^{a}	14.10 ±1.58^{a}	11.60± 0.51^{a}	12.22± 0.92^{a}	11.78 ± 0.73^{a}
Platelets ($x10^{9}$/L)	462.60 ±49.06^{b}	639.40 ±70.76^{a}	511.60 ±17.44^{a}	453.20 ± 54.29^{b}	539.20± 49.71^{a}
PCV (%)	43.68± 0.32^{a}	47.10 ± 2.36^{a}	47.12±1.32^{a}	47.38±2.09^{a}	47.42±1.05^{a}
Hb conc. (g/dl)	13.02± 0.15^{a}	13.24± 0.45^{a}	13.02±0.16^{a}	13.52±0.26^{a}	13.50±0.28^{a}
MCV (fl)	59.62 ± 095^{a}	57.80± 2.22^{a}	58.38± 1.83^{a}	60.30± 1.24^{a}	59.62± 1.11^{a}
MCH (pg)	17.70 ± 0.37^{a}	16.18± 0.42^{b}	16.18± 0.53^{b}	16.78± 0.37^{ab}	16.90± 0.19^{ab}
MCHC (g/dl)	29.76 ± 0.22^{a}	28.16± 0.54^{b}	27.22± 0.70^{b}	27.66± 0.54^{b}	28.44± 0.33^{ab}

Means with different superscript along the columns are significantly different at $p < 0.05$. Values are recorded as mean ± SE.

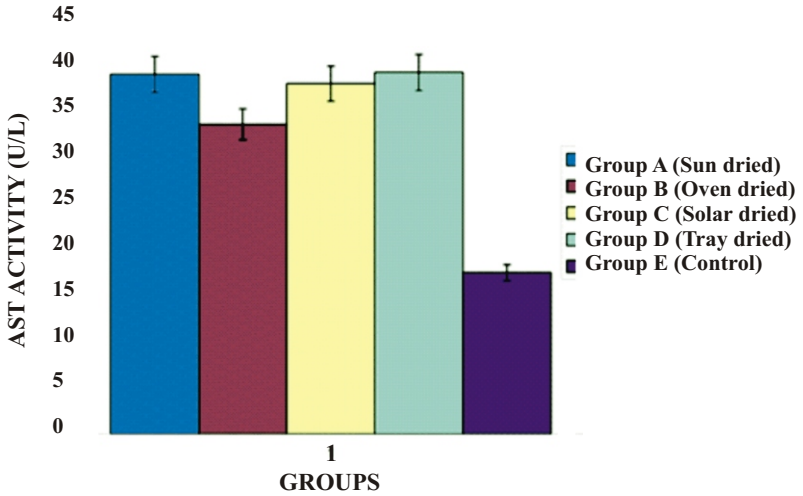

Figure 1: Activity of AST in the Serum of Rats Fed Pro-Vitamin A HQCF diet

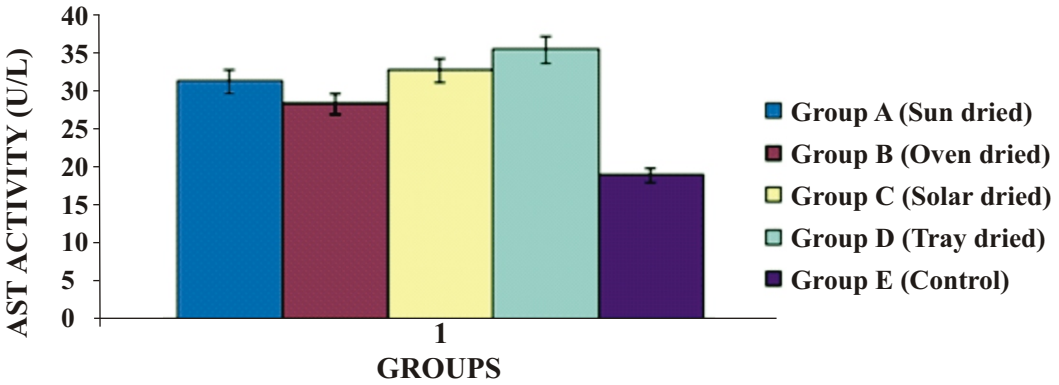

Figure 2: Activity of ALT in the Serum of Rats Fed Pro-Vitamin A HQCF diet.

DISCUSSION

Studies have confirmed that peeling represents the first processing step to reduce cyanogenic contents and to lower Cassava toxicity, as the cyanogenic glycosides (CNG) are distributed in large amounts in the root cortex (skin layer).[15] Moreover, grating of the pulp as the second step of sample preparation, breaks compartmental barrier and creates a higher surface area enabling Linamarin to have contact with its hydrolytic enzyme (Linamarase), resulting in the hydrolysis and subsequent removal of the breakdown products.[16] Since some cyanogenic compounds are soluble in water, its amount is reduced by traditional detoxification

methods such as dewatering.[17] Further reduction occurred due to the volatile cyanogenic compound, which evaporated into the air during different drying methods.

Data from this study showed that the serum Glucose concentration of rats fed Pro-Vitamin A HQCF increased significantly ($p < 0.05$) in the test Groups when compared to the Control group (Table 2). The increase suggests exposure of the test animals to dietary cyanide because studies have revealed that cyanide alters Glucose metabolism resulting in increased Glucose and lactic acid level and a decreased in the ATP/ADP ratio indicating a shift from aerobic to anaerobic metabolism. This is following work that reports Diabetes as a toxic effect produced by ingesting cassava, a cyanogenic plant, in various species.[25,26] There was a statistically significant decrease ($p < 0.05$) in the concentration of the serum total protein of the test animal groups below that of the control group (Table 5). The decrease indicates an attempt to use Sulphur-containing amino acids of their body to detoxify the cyanide ingested through diet. In the human body, cyanide is detoxified mainly by enzymatic conversion to the much less toxic thiocyanate. This detoxification requires Sulphur donors, which are provided from Sulphur-containing dietary amino acids, cysteine and methionine[27].

The activity of Alanine Aminotransferase and Aspartate Aminotransferase increase significantly in animal groups fed Pro-Vitamin A HQCF when compared to that of the Control group (Figure 1 and 2 respectively). This increase suggests that the samples are capable of causing hepatocellular injury. Alanine Aminotransferase and Aspartate Aminotransferase are important enzymes used in monitoring liver damage.[28] While Alanine Aminotransferase is cytosolic, Aspartate Aminotransferase is both cytosolic and mitochondrial. These enzymes leak out from injured hepatocytes into the blood during liver damage to the cell membrane of the hepatocytes. As a result, increase levels of enzymes are found in the serum and may be caused by a wide range of liver diseases.[13]

In the present study, the results obtained from haematology parameters, Red blood cell count, White blood cell count, Platelet, Packed cell volume, Haemoglobin, Mean Corpuscular Volume, Mean corpuscular Haemoglobin, and Mean corpuscular Haemoglobin concentration showed no statistically significant difference ($p < 0.05$) in all the test groups when compared to the control group (Table 6). This suggests that the cyanide content of Pro-Vitamin A HQCF was reduced to a tolerable level that the blood components were unhindered. All the data obtained for haematological study fell within the normal ranges according to Research Animal Resources, of 7.16-9.24 10^{12}/L for Red blood cell count, 5-8.9 10^{9}/L for White blood cell count, 37-48 % for Packed cell volume, 599-1144 10^{9}/L for Platelet count, 11-15g/dl for Haemoglobin, 67-77fl for Mean Corpuscular Volume, 11-17 pg for Mean corpuscular Haemoglobin, 27- 34g/dl for Mean corpuscular Haemoglobin concentration.[29]

CONCLUSION

In conclusion, the findings from this research work showed that the Pro-Vitamin A Cassava Cultivars was capable of altering some biochemical parameters such as elevation of serum glucose concentration and inactivity of some enzymes such as Aspartate Aminotransferase and Alanine Aminotransferase, whereas a reduction in serum protein was recorded. These effects were due to the presence of cyanide content in the roots of these Pro-Vitamin A Cassava Cultivars, in which the cyanide toxicity has been reduced to a safe barest minimum. Therefore, the oven dry method has shown the least toxic effect while the sun dry method has the most toxic effect.

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Megbelayin Affordable Trocar System (MATS): A Locally Fabricated Trocar-Cannula System for Vitreoretinal Use

Megbelayin EO

ABSTRACT

In attempts to meet human needs, necessities often form the basis of inventions. Amid scarcity of financial, material and skilled human resources, especially in underserved parts of the world, inventions and innovations are always apt. This article is a modest attempt to bridge a technology gap. It describes a less than ten dollars do-it-yourself needle trocar and cannula system for minimal invasive vitrectomy and sundry vitreoretinal procedures. The article utilized available literature and internet search for relevant information on subjects of Ophthalmic innovations. The Trocar System (Megbelayin Affordable Trocar System-MATS) is designed from a 23G hypodermic needle, 21G irrigating ophthalmic cannula, a bead, glue, iron files and sandpaper.

Keywords: Megbelayin, Affordable, Trocar, Fabricated, Vitreoretina

INTRODUCTION

Innovations continue to be the bedrock of modern societies. The focus of every innovative idea ought to be to cut costs, achieve the same or better results with minimal effort or improve on the existing quality of a product. Creating usable medical items from commonly available day-to-day materials is a viable panacea to the prohibitive cost of health services in developing countries.

Vitrectomy has evolved from a trocar-less 20G system in the 1970s to a 23G Trocar system that began in 2002.[1,2] A trocar-cannula system ensures better wound construction and management. The need to maximize the merits and minimize the demerits of earlier systems led to the introduction of 23G cannula in 2004 by Claus Eckardt.[3,4] This allows for trans-conjunctival (no peritomy) sutureless sclerotomies. It also enables rigid and efficient vitrectomy up to the vitreous base thereby saving surgical time.[5]

Unfortunately, this minute vitreoretinal item is expensive at an average price of 100 to 200 dollars (50 to 100 thousand naira) depending on the product. It is often recommended by the manufacturers to be used once and discarded. This write-up aims to report a re-usable cost-effective trocar system that competently serves its role of creating unhindered paths for intravitreal instrumentation at a cost of less than ten dollars ($10), about 5 thousand naira only.

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Cannula: A 21G Ophthalmic irrigating cannula is cut to 4mm, filed with iron files and smoothened with sand-paper. It is best cut with a machine cutter. The challenge is the occlusion of the lumen during the process of cutting as the heat generated melts the iron to cause luminal occlusion. To minimize this, before cutting with a machine cutter, the 21G cannula is "cannulated" with a 23G syringe or solid metal that could go in through the lumen of the 21G cannula. After cutting, the 23G syringe or solid metal is removed from the cut piece.

Bead: This can be obtained in an open market. It should be 3mm in diameter and 2mm in length or less so that it does not preclude a proper view of the surgical field.

Glue: Cement the bead to the upper 2mm of the cut cannula, exposing its remaining 2mm. Additional strength could be derived by creating a beveled portion of the cannula which is then bent over the bead to prevent bead removal during manipulations. The opposite end of the cannula is also beveled to aid intra-ocular penetration during sclerotomy construction.

Iron Files: Iron files of varying coarseness are used to reduce cannula diameter. This ensures that sclerotomy is not too large when the cannula is removed.

Sand-paper: To smoothen cannula outer body to avoid ragged sclerotomy.
Plier: Assists in gripping the cannula during cutting or filing. However, minimal force is needed to avoid luminal collapse of the cannula.
Lighter: The point the metallic part joins the green plastic part of a 21 G hypodermic is minimally heated by a flame from a lighter and a plier used to separate the stainless iron part from the green plastic part. The iron part serves as an introducer and the green plastic part serves as a component of a reducer. The introducer is passed through the reducer to help railroad 23G needle which serves as the needle-trocar. Railroading ensures the needle-trocar is not blunted as it passes through the reducer (see Figure 2).

The reducer, on the other hand, reduces the effective length of a 23G needle trocar from 25mm to 8mm. The tip of a 5ml syringe is severed and inserted into the green plastic part of a 21G needle (with an iron part removed by flame) to form a 17mm reducer (Figure 2). Without a reducer, 23G needle would be too long to serve as a trocar, with the likelihood of injuring the opposite retina during sclerotomy construction. When the reducer is placed on a 23G needle, which then serves as a trocar, the part of the needle in front of the reducer should be 8mm. This is the effective length with the potential to penetrate the globe during sclerotomy construction and it is unlikely to touch the opposite retina. Four millimeters of the 8mm is later covered by a MATS cannula before creating sclerotomy.

The final MATS parameters are tabulated below comparing it with commercially available prototypes like Alcon.

Table 1: MATS parameter juxtaposed with commercially available Alcon trocar-cannula

S/N	Parameter	MATS	Alcon
1	23G needle -trocar length	8mm (MATS reducer in situ)	8mm
2	Length of trocar not covered by cannula	4mm	4mm
3	Bead diameter	3mm	2mm
4	Bead length	2mm	2mm
5	Cannula lumen diameter	0.8mm	0.7mm
6	Outer cannu la diameter	1.1mm	1.0mm
7	Total cannula length (Bead inclusive)	4mm	4mm

MATS COMPONENTS

MATS has 4 main parts: a syringe, a 23G needle, the reducer and a cannula.
Syringe: Serves as a handle. Any size of the syringe with which the Surgeon is comfortable is suitable.
23G needle: Commercially available beveled disposable hypodermic 23G needle serves as a needle-trocar. It makes the first contact with the globe by penetrating the sclera through the pars plana. Then the MATS' cannula goes through the preformed sclera tunnel. The 23G needle trocar is gently removed from the cannula to avoid explanation of the latter.
Reducer: The metallic part of a 23G needle is 25mm of which not more than 8mm should be inserted during sclerotomy construction. To reduce the length of the needle that goes into the globe, a reducer of 17mm is fashioned as described above and preplaced on a 23G needle by a railroading technique illustrated in figure 2.
Cannula: For better ergonomics, MATS cannulae are beveled to aid scleral penetration.

MATS COUPLING

Coupling MATS can be done with ease. A 23G needle is mounted on a syringe, either 2ml or 5ml. The introducer is passed through the reducer which railroads the 23G needle through the reducer and ensures it does not become blunt (figure 2). Finally, the beaded cannula is then placed just in front of the reducer.

Megbelayin Affordable Trocar System (MATS) design

MATS is fashioned from locally sourced materials shown below in figure 1.

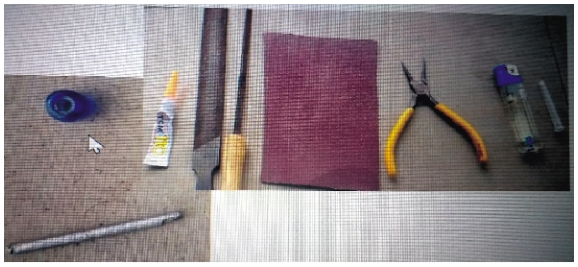

Figure 1: MATS materials from left to right: (21G-cannula, bead, super-glue, iron files, sand-paper, plier, lighter and needle cap).

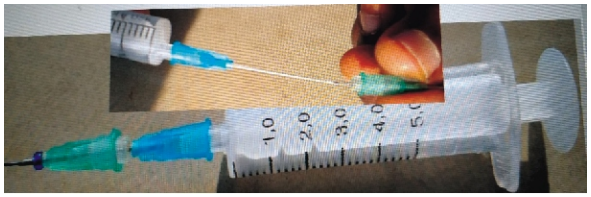

Figure 2: Top Image: rail-roading technique to minimize needle-trocar blunting. Bottom image: Fully loaded MATS consisting of a beaded cannula (deep blue), a green reducer, a sky-blue needle-trocar and a 5ml syringe.

MATS USE DURING SURGERY

Figure 3 shows MATS cannulae in a human eye and on an eye model.

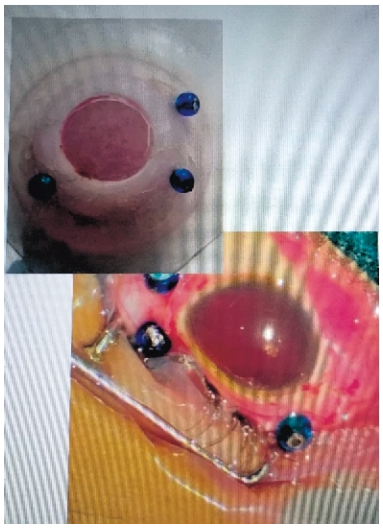

Figure 3: Top image: MATS cannula placed on an eye model. Bottom image: MATS cannula placed on a human eye.

Two-step insertion is advised with MATS. It could be through bare sclera or transconjunctival. The syringe, bearing MATS needle-trocar and cannula, is held tangentially to the sclera with the bevel parts of the needle and cannulae flat on the sclera. This is then advanced until the cannula tip is reached. The direction of insertion is now changed to about 90 degrees pointing to mid-vitreous and the trailing cannula is gently maneuvered until it is completely inserted into the preformed scleral tract created by the needle trocar. The bead serves as a stopper. A pair of Colibri forceps is used to hold the bead at any convenient position while the needle-trocar is gently removed. A pair of Colibri forceps is used to gently pull the conjunctiva in the opposite direction of intraocular penetration as a counterforce. Holding the conjunctiva also creates a sclero-conjunctival mismatch that serves as a physical barrier to microbial invasion when sclerotomy is sutureless.

Having been guided by a 23G needle-trocar, the cannula is left in situ to help ease the traffic of intraocular instrumentation. It is subsequently removed after the procedure. It is advised that the three sclerotomies be closed with 7/0 Vicryl to ensure a well-formed globe and reduce infection. However, with adequate size and proper filing, resultant sclerotomies may be minimal to warrant leaving them sutureless.

PEARLS TO MAXIMIZING MATS EFFICIENCY

Railroading 23G needle through the reducer minimizes blunting. Loading the MATS cannulae carefully under the microscope at the start of surgery minimizes needle prick. Sometimes, the reducer may not be firm on 23G needle, to avoid excessive mobility, the needle should be bent by about 10 degrees at its midpoint. Ethylene Oxide (ETO), or autoclave with MATS wrapped in several layers of gauze ensures re-usability.

CONCLUSION

MATS is a very cost-effective, reusable, technologically simple do-it-

yourself surgical tool that serves the primary purpose of easing intravitreal instrumentation during vitreoretinal procedures. It is versatile and its needle-trocar part could be used with the commercially available cannulae from Alcon and Midlabs, especially when their trocars have become blunted from repeated use.

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Gossypiboma Masquerading as Calcified Mesenteric Cyst: A Case Report

***Agbonrofo PI, Brotobor O, Odigie VI**

ABSTRACT

Retained textile material following surgery (Gossypiboma) occurs rarely, though it causes significant morbidity and mortality. It is usually under-reported for fear of litigation. The clinical course could be subacute or chronic with difficult diagnosis due to non-specific symptoms and inconclusive radiological imaging findings. This report is that of a 55year-old female who presented 91 months after total abdominal hysterectomy and bilateral salpingo-oophorectomy with an asymptomatic abdominal mass in the umbilical and suprapubic regions. An abdominal computed tomographic scan suggested a calcified mesenteric cyst. However, exploratory laparotomy revealed an abscess cavity with an embedded abdominal mop densely adherent to the small bowel and walled off by omentum. The mop was removed with resection and anastomosis of adherent bowel loops and drainage of the abscess. The postoperative period was uneventful and the patient is doing well after discharge. The diagnosis of gossypiboma should be considered in patients with non-specific symptoms following abdominal surgery. Prompt radiological imaging of the abdomen and a high index of suspicion in such patients could enhance early diagnosis and intervention resulting in reduced morbidity and mortality. Prevention of its occurrence is of utmost importance, with particular attention to thorough swab counts before and during operative procedures.

Keywords: Gossypiboma, Retained foreign body, Mesenteric cyst, Retained Surgical sponge, Textiloma, Swab count

INTRODUCTION

Retained foreign body (RFB) following operative procedure is a rare occurrence. It is of dire consequence in surgical practice as it is a significant cause of morbidity and mortality. The true prevalence of RFB is believed to be higher than reported due to perceived under-reporting for fear of litigation.[1] It accounts for about 50% of medical malpractice claims.[2] It is a source of distress to the patient (and family), operating team and management of the hospital where it occurs.

The retained foreign body may be textile (gauze, mop), instruments (whole or part), needles, pieces of plastic, etc. Retained textile constitute the majority of retained foreign bodies following operative procedures.[3] No form/type of surgery is exempt from this distressing though avoidable clinical quagmire. However, abdominal surgeries, especially obstetrics and gynaecological surgeries are the most common source of retained textile.[3] Gossypiboma (Gossypium (Latin): cotton; boma (Swahili): a place of concealment) is the term used to refer to a retained textile material following an operative procedure.[4] Synonyms for gossypiboma include gauzeoma, gauzoma, textiloma, cottonoid, cottonballoma and muslinoma.[2,5] The first reported case was by Wilson in 1884.[2] Its incidence is 1 in 100-5,000 operative procedures and 1 in 1000-1,500 abdominal operative procedures.[2] Gossypiboma is an avoidable operative complication. Thorough swab counts before and during the operative procedure would greatly reduce its occurrence. Factors that have been reported to increase the risk of occurrence of gossypiboma include poor communication within the operating team, emergency operation, change of perioperative nurses/doctors in the course of the procedure, change of operative procedure, fatigue, etc.[6,7]

Pathologically, gossypiboma causes either exudative or aseptic fibrous reaction; exudative reaction leads to abscess formation

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with or without bacterial contamination while aseptic fibrous reaction results in tissue adhesions, encapsulation and eventually foreign body granuloma.[2] The exudative variant is associated with an earlier clinical presentation while the fibrous reaction tends to result in later manifestation.[8]

Gossypiboma may be asymptomatic or symptomatic (often with non-specific symptoms). The clinical course is often subacute or chronic. Complications that may arise from an intra-abdominal gossypiboma include subacute/chronic pain, abdominal mass, intestinal obstruction, fistulation, perforation, haemorrhage.[7,9] The mop forgotten at surgery, is also frequently forgotten as a likely cause of the clinical presentation of the patient as more common abdominal pathologies (e.g. adhesions, neoplasia) are considered to be the likely aetiology. The aim of reporting this clinical rarity in this 55-year-old female (91 months post-surgery) is to highlight gossypiboma as a potential differential diagnosis of non-specific symptoms, such as an abdominal mass, in patients who had abdominal surgery previously, and to emphasise the importance of preventing its occurrence.

CASE PRESENTATION

The patient is a 55-year-old female trader who presented 3 months earlier at the surgical emergency room with a complaint of vomiting of one-day duration. Her last bowel movement was 4 hours before the presentation. She had no abdominal pains at presentation.

She had a total abdominal hysterectomy with bilateral Salpingo-oophorectomy (TAH+BSO) for symptomatic uterine fibroids, 91 months before the index presentation. The postoperative period following her TAH+BSO was uneventful. Histological diagnosis of the resected uterine lesion was Adenomyoma.

Abdominal examination revealed an oval-shaped mass occupying the umbilical and suprapubic regions, approximately 18cm by 16cm, firm, non-tender, mobile. Her attention was first drawn to the presence of the abdominal mass by the examining doctor.

An ultrasonographic scan of the abdomen showed a poorly defined heterogeneous mesenteric mass measuring about 8.5cm by 6.2cm overlying the abdominal aorta and underlying the umbilical region.

Abdominal Computed tomographic (CT) scan showed a large well defined heterogeneous mass predominantly hypodense in the mid-abdomen with a near-complete calcified rim and no significant enhancement pattern (Figure 1). All other intra-abdominal organs appeared normal. The considerations from the CT scan were (1) calcified mesenteric cyst (2) calcified hydatid cyst.

She subsequently had an exploratory laparotomy (94 months after TAH+BSO) with operative findings of 500mls of thick pus in the lower central abdominal cavity walled off by omentum and bowel loops with a mop within it; the mop was densely adherent to small bowel loops, which were adherent to the abdominal wall underlying the site of previous operative scar (Figure 2). At laparotomy, the abscess was drained, the mop was removed, and resection and anastomosis of the small bowel to which the mop was densely adherent was done (Figure 3). The postoperative period was uneventful. She was subsequently discharged to surgical outpatient clinic and is presently doing well on follow-up.

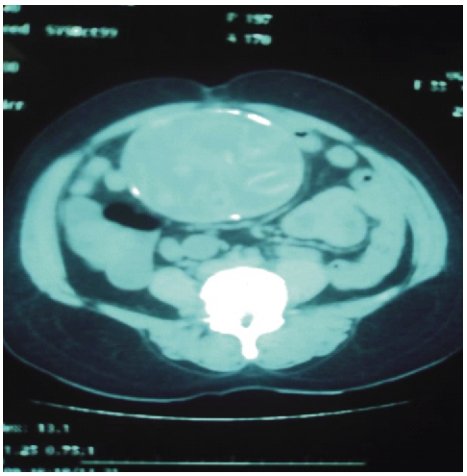

Figure 1: Computed tomographic scan of the abdomen showing a cystic lesion.

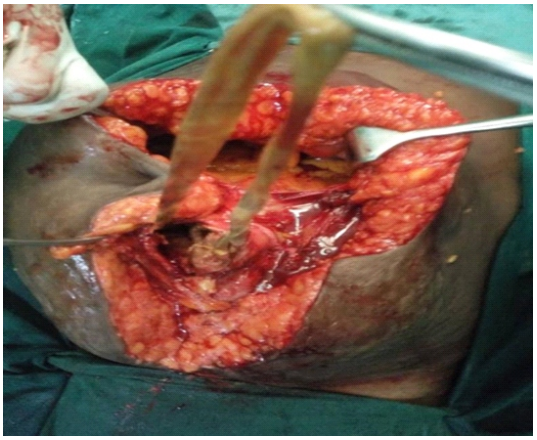

Figure 2: Abdominal mop in situ.

Figure 3: Mop retrieved from the abdominal cavity.

DISCUSSION

Gossypiboma occurs rarely after surgery but it is of dire consequence to all involved when it does. Under-reporting is considered to be rife, as it is believed to dent the operating team's image and damage its reputation.[1] Risk factors for Gossypiboma include emergency surgery, unexpected change in operative procedure, change in surgical team/nursing staff, high body mass index, high instrument count, large-volume blood loss and female gender.[6]

The diagnosis of Gossypiboma is difficult because the clinical symptoms are non-specific and the imaging findings are mostly inconclusive, as occurred in this case.[9] It may present as an acute pathology, non-specific chronic abdominal pain following a clinically silent period or it may be an incidental finding at the surgery for an unrelated pathology. Gossypiboma is more commonly found in females as in the case reported with a mean age at diagnosis of 49 years.[10] The index diagnosis was made at an age of 55 years. The female gender is associated with a higher prevalence of Gossypiboma because the predominant operative procedures associated with this pathology are gynaecological.[11]

The time from surgery to diagnosis of Gossypiboma has a wide variation from days to many years. The index diagnosis was made after almost 8 years following the initial surgery. The patient was relatively asymptomatic hence the long-time-interval (91 months) from the initial surgery to the identification of a clinical feature of the Gossypiboma (abdominal mass). Sozutek *et al.* reported a case of Gossypiboma in a 22-year-old lady, 14 days after caesarean section while Rajkovic *et al.* reported a case of Gossypiboma in a 66-year-old man, 40 years after laparotomy for knife stab injury.[12,13] It may simulate other intra-abdominal pathologies such as mesenteric cyst, hydatid cyst, tumour, etc. Both mesenteric cyst and hydatid cyst were considered differentials in the index case.

Gossypiboma is more common following abdominal surgeries especially

obstetrics and gynaecological surgeries as in the index case (which occurred following a total abdominal hysterectomy and bilateral salpingo-oophorectomy).[11]

The incidence of gossypiboma may be reduced by a thorough swab count, effective communication among members of the operative team, a thorough search for swabs by the surgeon even when counts have been declared complete by the perioperative nurse, change of swabs when fully soaked, attachment of long haemostats to the tails of mops (where feasible) when used in cavities and early re-exploration in case of a missing swab.

The main treatment option is the open surgical removal of the retained foreign body.[4] Resection of densely adherent tissues/organs may be done, as in the case presented.[5,12] Endoscopic removal may be feasible in cases of gossypiboma that have migrated into a lumen.[14] Gossypiboma could also be removed via a laparoscopic approach.[14] Spontaneous migration with passage of the foreign body to the exterior through a natural orifice may occur.[4]

CONCLUSION

The prevention of gossypiboma occurrence is of utmost importance, with particular attention to swab counts before and during operative procedure**.** The saying that 'prevention is better than cure' is apt to describe the burden of gossypiboma. Gossypiboma should be included as a differential diagnosis in cases of non-specific abdominal symptoms in patients with previous history of abdominal surgeries. Prompt radiological imaging of the abdomen and a high index of suspicion in such patients could enhance early diagnosis and intervention resulting in reduced morbidity and mortality.

CONFLICT OF INTEREST

The authors declare no conflict of interest

ETHICAL CONSIDERATION

Informed consent was obtained from the patient for this report and confidentiality was maintained in keeping with the World Medical Association Declaration of Helsinki**.**

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