Anti-Nociceptive Activity of Aqueous Licorice Root Extract on Neuropathic Pain and its Effect on some Selected Biochemical Parameters in Male Wistar Rats

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ABSTRACT

Licorice (Glycyrrhiza glabra) is a traditional medicinal, sweet and soothing herb known for its anti-inflammatory effect in painful conditions. Neuropathic pain is associated with certain set of symptoms, increased drug prescriptions and regular visits to health care providers. In the present study, we examined the effect of licorice on chronic constriction injury (CCI) of sciatic nerve induced neuropathic pain. Neuropathic pain was induced by CCI of sciatic nerve in 6-week-old male albino rats for 3weeks. Paw withdrawal thresholds were assessed on day 3, 7, 14 and 21 using von Frey test after which serum levels of cortisol, brain lactate dehydrogenase (LDH) and brain nitric oxide (BNO) were evaluated using ELISA kit.Groups treated with low dose licorice post-surgery treated (LDL), high dose licorice post-surgery treated (HDL), low dose licorice pre-surgery treated (LDLp), high dose licorice pre-surgery treated (HDLp) and imipramine demonstrated significant increase in change of paw withdrawal threshold compared with ligated control. Serum levels of Cortisol, brain LDH and BNO were significantly reduced in HDL, LDLp and HDLp treated groups when compared with ligated control. Our result shows that licorice extract demonstrates anti-nociceptive activity by reducing the serum level of cortisol, brain LDH and BNO and the effect is dose and duration dependent.

Keywords: Brain, Lactate, Dehydrogenase, Nitric, Oxide, Cortisol

INTRODUCTION

Neuropathic pain is the most common cause of chronic pain worldwide,[1] causing a lot of psychological discomfort, "physiologic stress" and physical disability challenges, which translates to high medical burden on the global economy.[2] Neuropathic pain occurs as a result of trauma to the nerve or diseased nerve conditions.[3] It is commonly present in patients with cancer, multiple sclerosis, long-standing diabetes mellitus, phantom limb pain, post-mastectomy, HIV/AIDS, spinal stenosis, stroke and old age.[4] Its classical presenting symptoms are hyperalgesia, allodynia, paresthesia and spontaneous pain.[5]

Glycyrrhiza *glabra* is native to Southern Europe and certain parts of Asia such as China and India. The root extract of Glycyrrhiza *glabra* has been used in many food products, soft drinks and snacks as a sweetener.[6] Several studies have demonstrated the pharmacological importance of Glycyrrhiza *glabra* such as anti-inflammatory,[7] anti-tumour,[8] anti-viral,[9] anti-nociceptive activities on acute pain[10] and anti-microbial[11] activities among others.

Cortisol is known to play a major role in stress response to pain perception.[12] Stress has been proved to play a pivotal role in the transition of acute to chronic pain via the dysfunction of the hypothalamus-pituitary-adrenal (HPA) axis.[13] The essence of this rise in serum cortisol level in the acute phase is to facilitate the release of energy and substrate necessary to cope with stress physiologically.[14] However, prolonged surge in serum cortisol results in conversion of its anti-inflammatory property to pro-inflammatory effect due to HPA axis dysfunction,[15]thereby exaggerating pain severity.

Likewise, lactate dehydrogenase (LDH) is a cytoplasmic cellular enzyme present in all organs of the body where it reversibly converts lactate into pyruvate, thus linking the glycolytic and oxidative metabolism energy generating pathways.[16] However, lactate is being considered as a metabolic fuel in the brain. Under normal physiologic condition, L- lactate supplies up to 10% of the brain metabolic need, which can increase up to 60% in high metabolic conditions like severe hypoglycemia, hypoxia

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and intense physical exercise.[17] It serves as a pointer of cellular integrity imbalance[18] and has been implicated in panic disorder psychiatry illness.[19] Further, nitric oxide (NO) and its associated enzymes are also involved in many physiological[20] and pathophysiological processes like pain modulation.[21]

It is apparent there are different treatments for neuropathic pain, and they have their limitations.[22] Natural substances are becoming more common in replacing established medications for managing neuropathic pain. In furtherance of our interest in neuropathic pain, we investigated the effect of licorice root extract on chronic constriction injury of the sciatic nerve to induce neuropathic pain.

MATERIALS AND METHODS

Animals

Thirty-five 6-week-old male Wistar rats weighing between 200-250g were used for the study. The rats were housed and maintained to acclimatize for 1week in standard conditions andwere given free access to rat pelleted diet and water in the Animal House, College of Medicine, Ekiti State University. The study was carried out in accordance with the standards established by the Guide for the Care and Use of Laboratory Animals.[23]

Animal groupings

The animals were randomly assigned to one of the following experimental groups (n = 5 per group), ligated and were treated accordingly.

Group I: received distilled water (10ml/kg, orally) daily; designated as non-ligated control.

Group II: received distilled water (10ml/kg, orally) daily; designated as ligated control.

Group III: received reference drug (40mg/kg, orally); designated as imipramine treated.

Group IV: post-surgery treated with low dose licorice extract (75mg/kg, orally); designated as low dose treated (LDL).

Group V: post-surgery treated with high dose licorice extract (150mg/kg, orally); designated as high dose treated (HDL).

Group VI: pre surgery treated with low dose licorice extract (75mg/kg, orally); designated low dose pre-treated (LDLp).

Group VII: post-surgery treated with high dose licorice extract (150mg/kg, orally); pre surgery; designated high dose pre-treated (HDLp).

Neuropathic pain was induced in groups II to VII. Groups I and II received no interventions. Administrations of treatment (extract and reference drug) began in groups III, IV, and V three days after surgery and continued for 18 days. Group III received 10mg/kg of Imipramine; IV and V received 75mg/kg and 150mg/kg of licorice extract respectively. Groups VI and VII received 75mg/kg and 150mg/kg respectively, for 10 days before surgery and treatment continued three days after surgery for another 18 days.

Extract preparation

Licorice root powder was purchased from Amazon and was sold by Herbs and Crops Overseas, India with batch no: LRP-2017/02. A portion of the powder (50 g) was mixed with 100ml of sterile distilled water in a flask with occasional shaking. The extract was then filtered through a muslin cloth for coarse residue and finally through Whatman No. 1 filter paper and kept in an airtight amber colored container.[24]

Induction of neuropathic pain

Chronic constriction injury (CCI) of the sciatic nerve was used to assess neuropathic pain.[25] Rats were anesthetized using sodium pentobarbital (40mg/kg) via intraperitoneal (i.p.) administration. Neuropathic pain was thereafter induced by chronic constriction (CCI) of the sciatic nerve using a suture. An incision about 3cm long was made into the skin that overlies the area between the gluteus and biceps femoris muscles, and the common sciatic nerve of the right hind paw was exposed at the mid-thigh level. The suture was tightly tied around the sciatic nerve making a diameter of approximately $^{1}/_{3}$ - ½ mm. After the surgery, the animals were allowed to recover under antibiotic cover.

Von Frey Filament Test

Von Frey Filament test was used to assess static allodynia.[26] Briefly, rats were placed in a suspended chamber that has wired mesh floor. They were allowed to acclimatize

for 20 minutes. Planter surface of left hind paws were tested using von Frey Filament Test; hair and paw withdrawal thresholds were recorded. This test was done before surgery on the rats and on days 3, 7, 14, and 21 post-surgery.

Determination of biochemical parameters

At the end of the treatment period, the rats were anaesthetized using a mixture of 25% (w/v) urethane and 1% (w/v) alpha chloralose (5ml/kg; i.p., BDH chemicals Ltd., Poole, England). Blood samples were obtained from cannulated carotid artery into heparinized centrifuge tubes. Plasma was extracted by centrifugation at 3000 rpm for 15min. Brains were quickly removed, washed in cooled 0.15M NaCl and were then homogenized in 2ml of ice-cold potassium phosphate buffer (0.1M, pH:7.4) using an improvised homogenizer. Samples were centrifuged at 5000 rpm for 15 min to obtain the supernatant. The homogenate obtained was stored at -20 degree Celsius until the time of biochemical analysis. Serum level of cortisol, brain NO and brain LDH were determined using an Enzyme Immunoassay (EIA) kit from Randox laboratory Ltd. Co (Antrim, UK).

Statistical Analysis

All data are expressed as means ± standard error of the mean (SEM) for 5 rats per group. Statistical group analysis was performed with graph pad statistical software (Graph Pad Inc., San Diego, CA, USA). Test of variance was done using ANOVA, followed by Tukey's multiple comparisons test. Statistically significant differences were accepted at $p < 0.05$.

RESULTS

Von Frey Filament Latency Test

Pain threshold of ipsilateral hind paw of animals using von Frey filaments across the groups is shown in table 1. At baseline there was no significant change in paw withdrawal threshold. On day 3, there was significant increase in paw withdrawal threshold in HDLp treated group when compared only to ligated control. By day 7, HDL, LDLp and HDLp treated groups demonstrated significant increase in threshold compared to ligated control, LDLp and HDLp treated groups also demonstrated significant increase in threshold compared with imipramine treated group. Only HDLp treated group demonstrated significant increase in threshold compared with LDL treated group. Furthermore, on day 14 and 21 HDL, LDLp and HDLp treated groups all demonstrated significant increase in threshold compared with the two control groups, imipramine and LDL treated groups.

Serum cortisol concentration

Figure 1 showed serum cortisol concentration in all the groups. There was significant increase in serum cortisol level of ligated control, imipramine treated, LDL treated, HDL treated and LDLp treated groups when compared with non-ligated control group. Moreover, LDLp and HDLp treated groups showed significant decrease in serum cortisol concentration compared with ligated control, imipramine and LDL treated groups.

Brain Lactate dehydrogenase (LDH) concentration

Figure 2 shows the effect of licorice on brain LDH concentration in all the groups. There was significant increase in brain LDH concentration in ligated control, imipramine, LDL, HDL and LDLp treated groups when compared with non-ligated control group. Moreover, HDL, LDLp and HDLp treated groups had significant reduced LDH concentration when compared with ligated control group. HDL and HDLp treated groups also demonstrated significant reduced brain LDH concentration when compared with LDL treated group. HDLp also showed a significant decrease in LDH concentration when compared to imipramine treated group.

Brain nitric oxide (BNO) concentration

Changes in BNO concentration level among the groups is shown in figure 3. Imipramine, HDL, LDLp and HDLp treated groups all demonstrated a significant decrease in BNO concentration when compared with ligated control. Moreover, BNO concentration was significantly reduced in HDLp group when compared with normal control and LDLp groups. Only ligated control group showed significant increase in concentration when compared with non-ligated control group.

Table 1: Effect of the aqueous licorice root extract administration on pain threshold in animals using von Frey test.

	(Von Frey) Force (g)				
Rat groups	**Base line**	**Day 3**	**Day 7**	**Day 14**	**Day 21**
Control	28.1±1.1	27.7± 0.4	26.7 ± 0.4	27.3 ± 0.4	25.9 ± 0.2
Control ligated	28.9 ± 0.7	12.7± 1.7 [a]	13.3 ± 1.1 [a]	14.1 ± 1.1	14.5 ± 1.0
Imip treated	27.3± 0.4	19.7 ±2.5	15.1± 0.2	27.9 ± 1.2	35.9 ± 4.4 [b]
LDL treated	27.3 ± 0.4	19.7± 2.7	24.1 ± 2.1	27.7 ± 1.3	39.7 ± 5.7 b
HDL treated	27.7± 0.4	26.9± 0.4 [b]	26.3 ± 0.2 [b]	45.3± 6.6 [a b c d]	56.1 ± 1.6 [a b c]
LDLp treated	27.7 ± 0.4	26.5 ± 0.6 [b]	35.5 ± 4.6 [b c]	51.9 ± 5.0 [a b c d]	59.9 ± 0.2 [a b c d]
HDLp treated	28.1± 0.4	32.1 ± 2.1 [b c]	40.9 ± 5.1 [a b c d e]	55.7 ± 1.3 [a b c d]	68.1 ± 7.8 [a b c d]

Data expressed are means ± SEM, n = 5. ([a]p?0.05 vs control, [b]p<0.05 vs Ligated control, [c]p<0.05 vs imipramine treated, [d]p<0.05 vs LDL treated, [e]p?0.05 vs HDL treated).

Key: Imipramine (Imip); Low dose licorice (LDL); High dose licorice (HDL); Low dose Licorice pre-treated (LDLp); High dose Licorice pre-treated (HDLp).

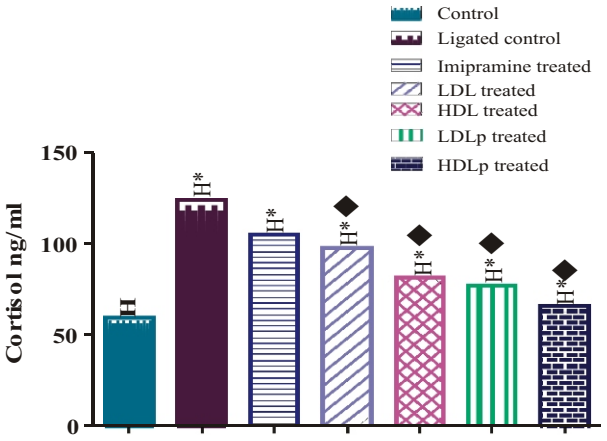

Figure 1: Effect of Licorice on serum cortisol concentration in CCI induced neuropathic pain model. *p<0.05 vs non-ligated control, [?]p<0.05 vs ligated control, [α]p<0.05 vs imipramine, [µ]p<0.05 vs Low dose licorice (LDL); High dose Licorice (HDL); Low dose Licorice pre-treated (LDLp); High dose Licorice pre-treated (HDLp).

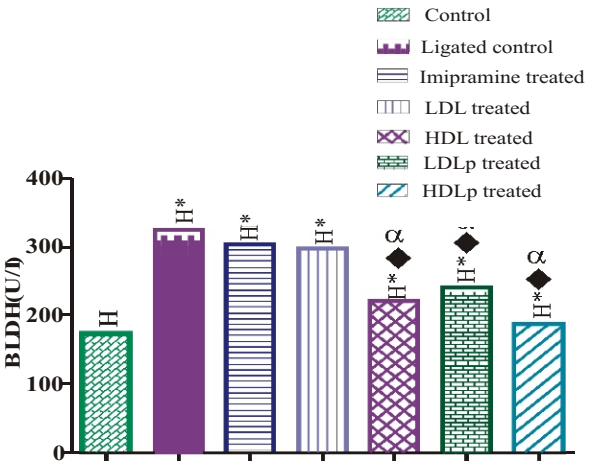

Figure 2: Effect of Licorice on serum lactate dehydrogenase concentration in CCI induced neuropathic pain model. *p<0.05 vs non-ligated control, [•]p<0.05 vs ligated control, [α]p<0.05 vs imipramine; Low dose licorice (LDL); High dose Licorice (HDL); Low dose Licorice pre-treated (LDLp); High dose Licorice pre-treated (HDLp).

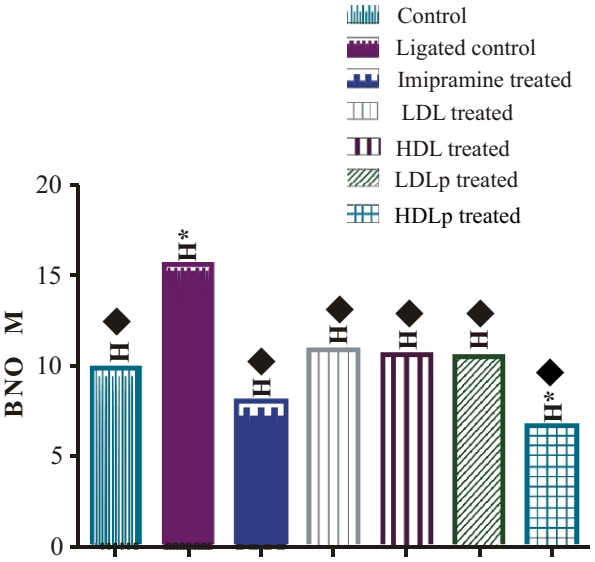

Figure 3: Effect of Licorice on brain nitric oxide concentration in CCI induced neuropathic pain model. *p<0.05 vs non-ligated control, [?]p<0.05 vs ligated control, [α]p<0.05 vs imipramine, [µ]p<0.05 vs Low dose licorice (LDL); High dose Licorice (HDL); Low does Licorice pre-treated (LDLp); High dose Licorice pre-treated (HDLp).

DISCUSSION

Neuropathic pain can impair quality of life when it is poorly managed. Around 7-8% of adults have pain with neuropathic characteristics and experience a distinct set of symptoms, such as burning and electric-like sensations, and pain resulting from non-painful stimulations (such as light touching), known as hyperalgesia; the symptoms persist and have a tendency to become chronic and respond less to pain medications.[27] Sleep disturbances, anxiety and depression are also

frequent and severe in patients with neuropathic pain.[28]

There are several options for drug treatment as part of an overall approach to improve the quality of life and function of patients' with neuropathic pain, with focus mainly on treating symptoms because the cause of pain can rarelybe treated.[29] In line with that, different classes of drugs with numerous therapeutic recommendations were also proposed for neuropathic pain.[30] Furthermore, pharmacological treatments for chronic neuropathic pain which are effective in <50% of patients may be associated with adverse effects that limit their clinical utility.[31]

Licorice extract constitute rich sources of novel compounds with a variety of pharmacological activities. This study shows that aqueous Licorice root extract increased pain threshold significantly in the licorice treated groups with major effect on the group pre-treated with high dose (150mg/kg) as seen in table 1. Moreover, on day 3 post-surgery, increased pain sensitivity was observed across all groups except ligated control and HDL treated groups. Furthermore, on day 7, HDL, LDLp and HDLp treated groups all demonstrated higher pain threshold compared to animals in the other groups. Again, on day 14 post-surgery, imipramine treated, LDL treated, HDL treated, LDLp and HDLp treated groups all showed appreciable increased pain threshold compared with the normal control group, whereas animals in the licorice pre-treated groups (LDLp and HDLp) demonstrated higher pain threshold compared to the LDL and HDL treated groups and Imipramine treated group.

Increased cortisol concentration is known to be associated with chronic pain which was demonstrated in this study.[32] All the animals demonstrated a significant increase in serum cortisol level where ligated control and imipramine treated groups had the highest concentration, while HDLp treated groups had the lowest. Stressful stimulus coming from acute pain is likely to elicit cortisol secretion and this is known to be commonly associated with hypercortisolism. However, the repeated secretion of cortisol following maladaptive responses to acute pain was reported to perpetuate hypocortisolism, chronic and recurrent pain.[33]

Lactate dehydrogenase (LDH) activity is known to increase in traumatized nerve.[34] Thus, results from this study showed significant increase in LDH concentration in the ligated control, imipramine treated and LDL treated groups when compared with other licorice treated groups. However, reason for increase in brain lactate dehydrogenase concentration may result from increased metabolic demand of the neurons.[35]

Nitric oxide (NO) has been reported to have a pivotal signaling role in both acute and chronic pain at the peripheral and central levels.[36] This present study showed increased BNO concentration in the ligated control group when compared with other groups. This is in consonance with a previous study[37] where increased NO was reported to contribute to various symptoms of chronic pain such as thermal hyperalgesia, mechanical hyperalgesia, and allodynia in sciatic nerve constriction model. HDLp treated groups demonstrated reduced BNO concentration as shown in this present study.

In conclusion, the anti-nociceptive activity of licorice is due to its association with reduced change in cortisol, LDH and BNO serum concentration and the effect is dose and duration dependent, thereby opening a new door in the management of neuropathic pain.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Upper Gastrointestinal Endoscopy Findings in Gusau, Zamfara State, North West Nigeria

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ABSTRACT

Upper gastrointestinal (UGI) endoscopy is indicated in patients presenting with symptoms of upper gastrointestinal disease as it provides accurate assessment of the underline disease thereby allowing for appropriate therapeutic intervention. Our aim was to report UGI endoscopic findings in a private health facility in Gusau, Zamfara State, North-West, Nigeria. A 3-year review of all patients that had upper gastrointestinal endoscopy between January 2016 and December 2018. Information was retrieved from the endoscopy register andanalyzed with SPSS 22 (IBM SPSS Statistics for Windows, Amonk, NY: IBM Inc). Three hundred and thirteen patients had endoscopy procedure. One hundred and seventy-two (55%) were males and 141(45%) were females with M: F ratio of 1.2: 1. Their mean age was 43.19±15.9 years with a range of 12 to 85 years. The commonest diagnostic indications were suspected peptic ulcer disease 138(44.1%) followed by dyspepsia 77(24.6%). Chronic gastritis was the commonest endoscopic finding, 88(28.1%) followed by gastroesophageal reflux disease. Gastric cancer and oesophageal cancer accounted for 7% and 0.3%, respectively. Forty-eight (15.3%) had normal endoscopic findings. Suspected peptic ulcer disease was the commonest indication for UGI endoscopy while gastritis was the commonest finding. High prevalence of malignancy was noted in our study and significant percentage of patients presenting for upper GI endoscopy had normal findings. Suspected clinical diagnosis performed poorly in predicting endoscopic findings.

Key-words: Spectrum, Upper, Gastrointestinal, Endoscopy, Findings, Gusau

INTRODUCTION

Upper gastrointestinal endoscopy is the diagnostic procedure of choice in patients presenting with symptoms of upper gastrointestinal disease like dyspepsia, reflux and bleeding among others.[1-3] Oesophago gastroduodenoscopy should be performed once indicated in any clinical setting to plan appropriate therapeutic intervention.[2,4]

Indications for upper gastrointestinal endoscopy are varied and range from dyspepsia to a more sinister suspicion of malignancy.[4,5] Indications for upper gastrointestinal endoscopy and the respective findings have been reported from different parts of Nigeria.[1-7] To date, data on indications and endoscopic findings in Gusau is lacking. Hence, we report the spectrum of endoscopic findings in patients referred for UGI endoscopy over a three-year period, January 2016 to December 2018 in Gusau, North-West Nigeria.

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MATERIALS AND METHODS

Study Setting

The endoscopy unit of Hilal Specialist Hospital is the only endoscopy referral centre in Zamfara State serving a population of four million people. The hospital receives referral for endoscopy from Federal Medical Center, Gusau, Ahmad Sani Yariman Bakura Specialist Hospital, Gusau, private clinics and other general hospitals in the state.

Methodology

Procedures are performed by a Consultant Gastroenterologist once a week and in some instances as an emergency procedure. All patients for upper gastrointestinal endoscopy were asked to fast overnight for at least eight to ten hours and present in the morning for elective cases while emergency cases were arranged by the Gastroenterologist. The endoscopic procedure was explained to the patients and consent for endoscopy obtained from each

patient before the procedure. Xylocaine 10% spray was administered to anaesthetize the oropharynx of patients. Patients were then placed on left lateral position on the endoscopy couch, and a plastic dental guard was held firmly in between the upper and lower incisors in the patient's mouth by the Endoscopy Nurse. A forward viewing Olympus GIF-Q140 series video (Olympus America Inc) gastroscope was gently introduced under direct vision to examine the oesophagus, stomach, and duodenum of the patients, after which the scope was gently withdrawn wholly from the mouth. Biopsy samples for histology were taken when indicated. Patients were observed after the procedure for one to two hours and subsequently, discharged.

Data collection and Analysis

The endoscopy register was reviewed and data collected include age, gender, indications for the procedure and endoscopic findings. These were coded and entered into SPSS program (version 22, Chicago, IL, USA) for analysis. Level of significance was taken as P-value less than 0.05.

Ethical Issues

Ethical approval was obtained from the Human Research Ethics Committee of the State Ministry of Health.

RESULTS

Three hundred and thirteen patients were referred for upper GI endoscopy over the study period of three years, January 2016 to December 2018. There were 172 males and 141 females with a male to female ratio of 1.2:1. The mean age of the study population was 43.19±15.9 years, the oldest was 85 years and the youngest was 12 years. The mean age of the male patients 43.01±15.51 years, was similar to that of the females 43.29±16.54 years, (*P*=0.378). Indications for upper GI endoscopy were varied with suspected peptic ulcer disease being the commonest indication followed by dyspepsia. Table 1 shows the overall indications over the study period.

Table 1: Indications for upper GI endoscopy at Hilal Specialist Hospital from January 2016 to December 2018

Indication (N=313)	n(%)
Dyspepsia	77 (24.6)
Anaemia/bleeding/Melaena	49 (15.7)
Suspected GERD	23 (7.3)
Suspected PUD	138 (44.1)
CLD Screening (Varices/PHTN)	10 (3.2)
GOO/Dysphagia	5 (1.6)
Suspected Malignancy (Oesophageal/Gastric)	11 (3.5)

GERD; gastro-oesophageal reflux disease, PUD; peptic ulcer disease, CLD; chronic liver disease, PHTN; portal hypertension, GOO; gastric outlet obstruction

Table 2: Predominant upper GI endoscopic findings

Findings (N=313)	n (%)
Oesophageal	
GERD/Reflux oesophagitis	49 (15.7)
Varices	14 (4.5)
Candidiasis	10 (3.2)
Hiatus hernia	32 (10.2)
Malignancy	1 (0.3)
Gastric findings	
Gastritis	88 (28.1)
Gastric erosions	25 (8.0)
Gastric ulcer	16 (5.1)
Malignancy	22 (7.0)
Duodenal findings	
Duodenitis	1 (0.3)
Duodenal ulcer	4 (1.3)
Normal findings	
With foamy gastric juice	18 (5.8)
Without foamy gastric juice	30 (9.6)

Major endoscopic findings are highlighted in the table above, even though overlaps of findings were common. Gastritis coexisting with gastric erosions was seen in up to 19 (6.1%) of patients. Oesophageal candidiasis coexisting with gastric cancer was seen in 7 (2.2%) and GERD coexisting with hiatus hernia was seen in 22 (7%) patients.

Although gastritis was observed more in females, there was no significant difference between age and gender in terms of endoscopic findings.

DISCUSSION

In this study, male predominance was found among those who presented for upper GI endoscopy. Similar observation was made by Danbauchi *et al.*[2] in Zaria and Jeje *et al.*[9] in Lagos, while Mustapha et al[6] reported equal male and female in Maiduguri. Perhaps male predominance in this study could be explained by the fact that the facility is a private centre and access to care may be subject to financial power which most women in this part of the country are disadvantaged.

Our patients were in the early middle age, although older than the patients' population studied by Malu *et al.* and Danbauchi *et al.* in Zaria.[1,2] They are however a decade younger compared to the study by Picardo *et al.* from Enugu in South-Eastern Nigeria.[8] There is wide variability of access to health care in public and private centres, and this variability may partly explain the different demographic characteristics of these patients.

Suspected peptic ulcer disease was the commonest indication for referral for endoscopy, followed by dyspepsia. Several studies have reported dyspepsia as a common symptom warranting referral for upper gastrointestinal endoscopy.[1-5] It would be more appropriate to report dyspepsia as presenting symptom complex rather than peptic ulcer disease, as the later is a diagnosis only established after visualizing the lesion on endoscopy. Despite this, a number of requests for evaluation of suspected lesion of the upper gastrointestinal tract come in with a presumed diagnosis of peptic ulcer disease. The commonest endoscopic finding in our study was gastritis. Gastritis has consistently appeared as a common finding on upper GI endoscopy according to several studies.[1-6] Gastritis was found in association with other findings including oesophagitis, duodenitis and gastric erosions. It is reported as gastritis when it is the predominant feature seen.

Gastroesophageal reflux disease (GERD) was found in a significant number of our patients (15.7%), which did not include the non-erosive form of the disease. The non-erosive form presents with normal endoscopic findings. The endoscopy positive GERD frequently coexisted with hiatus hernia. GERD was found in 24.1% of dyspeptic patients that underwent upper gastrointestinal endoscopy in Kano, Nigeria.[10]

Overall, peptic ulcer disease, accounted for only 6.4% of the total endoscopic findings, despite it being the commonest presumed working diagnosis/indication. Peptic ulcer disease was reported in 11.2% of patients who underwent upper gastrointestinal endoscopy in Maiduguri.[12] Based on hospitalization and death rates, the prevalence of GERD has increased while that of peptic ulcer disease has been on the decrease.[15]

This wide disparity between clinical diagnosis and endoscopic findings was also observed by Tijjani *et al.*[11] where they compared clinical versus endoscopic diagnosis of patients presenting with symptoms of gastrointestinal disease. They concluded that clinical diagnosis alone is fraught with misdiagnosis and symptom overlap. Agbakwuru *et al.*[3] also reported poor agreement between clinical and endoscopic diagnoses in patients with symptoms of upper GI disease.

Malignancy-histology confirmed; gastric cancer-7% and oesophageal cancer-0.3% were seen, representing 7.3% of the total endoscopic findings, higher than peptic ulcer disease. Picardo in Enugu reported a prevalence of 3.1% of their patients having gastric masses on endoscopy.[8] Mustapha et al reported 1.7% of their patients in Maiduguri have gastric cancer.[12] This facility is the only endoscopic centre serving the entire State and this may explain the reason behind the high prevalence of upper GI malignancy as most patients are referred there for evaluation. There is no study to date from this community that looked at the prevalence of gastrointestinal tumours that would have provided insight into earlier reports.

There were no differences observed between genders on the endoscopic findings, across the whole spectrum. Although, more females had gastritis, it's not statistically significant, $P>0.05$. Other studies have reported mixed findings, with females having more gastritis and GERD.[6,10]

A significant percentage of our patients had normal endoscopy findings. Picardo *et al.* reported 2.3% of their patients in Enugu who presented with dyspepsia had normal endoscopic findings.[8] Non-ulcer dyspepsia has been reported to be 15.4% in patients in Benin[13] which is similar to findings by other authors, such as Olokoba *et al.*[14] in Yola, who reported a prevalence of functional dyspepsia to be 6% in their series. The wide disparity seen in the prevalence of normal endoscopic findings in the setting of clinical indication for upper GI endoscopy could be explained by many factors including easy access to the procedure, affordability and availability of the procedure as well as low or high threshold for requesting the procedure by the attending physicians among others.

CONCLUSION

Presumptive diagnosis of peptic ulcer disease is the commonest indication for upper gastrointestinal endoscopy in our patients and gastritis is the most frequent endoscopic finding. Sometimes clinical diagnosis could be wrong and so, upper gastrointestinal endoscopy should be performed once indicated in patients presenting with symptoms of upper gastrointestinal disease.

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Effect of Glycohaemoglobin Adduct on Erythrocytes Osmotic Fragility in Nigerian Patients with Diabetes Mellitus

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ABSTRACT

Anemia is one of the common complications in subjects with diabetes mellitus (DM); this could be attributed to a number of causes. One of which could be due to continuous intracellular influx of glucose resulting from non-enzymatic glycosylation of haemoglobin and this may alter erythrocyte membrane architecture or cell mass. We aimed to investigate the impact of haemoglobin glycosylation on erythrocytes osmotic fragility in subjects with DM attending Murtala Muhammad Specialist Hospital, Kano.To achieve this, fasting plasma glucose level, concentration of glycated haemoglobin (HbA_1C) in subjects with DM were assayed. The erythrocyte osmotic fragility of DM was compared with controls. Seventy-five participants were enrolled including 50(66.7%) DM and 25(33.3%) control subjects Participants were both males and females within the age of 23-72 years, with (Mean ± SD = 52.46±13.98 and 40.52±10.77years for DM and controls, respectively).The fasting plasma glucose (FPG), glycated haemoglobin (HbA_1C) and erythrocyte osmotic fragility (EOF) were determined colorimetrically using glucose oxidase-peroxidase, ion exchange resin and gradient hypotonic saline methods. In this study, there was significant difference (P<0.05) of FPG and HbA_1C of DM compared to controls. HbA_1C correlates positively with FPG in diabetic subjects. There were statistically significant differences (p < 0.05) between FPG, HbA_1C and EOF levels of DM subjects compared to controls. We inferred that high EOFin DM subjects reported in our study may contribute to chronic anaemia seen in this disease.

Keywords: Blood, Fasting, Glucose, Non-enzymatic, Participants

INTRODUCTION

In developing countries like Nigeria, diabetes mellitus (DM) is currently becoming a common problem at a time when the burden of diabetes is rising very quickly in wealthier countries. The prevalence of diabetes mellitus is increasing exponentially throughout the world.[1] The basic pathology in DM, involves hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of body organs, especially the eyes, kidneys, nerves, heart, and blood vessels.[2]

The American Diabetes Association (ADA) and world Health Organization (WHO) recommend that the sub-classification of diabetes based on insulin dependency diabetes mellitus(IDDM) and non-IDDM (NIDDM), should now be abandoned in favor of the etiologically based classification.[3] The most important sub-categories in public health terms are type 1 and type 2 diabetes, relies primarily on the presence (type 1 diabetes) or absence (type 2 diabetes) of autoantibodies against pancreatic islet β-cell antigens and age at diagnosis.[4]

Glycosylated haemoglobin Hb1C, minor components of total haemoglobins in erythrocytes of normal adult humans, is formed by post-translational non-enzymatic glycosylation and this process occurs continuously in vivo. Normal adult haemoglobin consists predominantly of HbA (2 2), HbA2 (2 2) and HbF (2 2) (97, 2.5 and 0.5% respectively). About 6% of total HbA is termed HbA1, which in turn is made up of HbA1a1, HbA1a2, HbA1b and HbA1c.These fractions are defined by their electrophoretic and chromatographic properties, which differ slightly from those of the major component HbA0, despite the

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amino acid sequences of HbA1 and HbA0 being identical. HbA1c is the most abundant of these fractions and in health comprises approximately 5% of the total HbA fraction.[5]

Non-enzymatic glycosylation (NEG) refers to the covalent binding of carbonyl groups of reducing sugars to amino groups of proteins, lipids, and nucleic acid.[6,7] Structural and chemical investigations elucidated that glucose, in the open chain format, binds to the N-terminal to form an aldimine (Schiff base) before undergoing an Amadori rearrangement to form a more stable ketoamineform, amino-1– deoxyfructose.[8] Endogenous glycations occur mainly in the bloodstream to a small proportion of the absorbed simple sugars: glucose, fructose, and galactose. It appears that fructose has approximately ten times the glycosylation activity of glucose, the primary body fuel.[9]

Dysfunction of auto regulatory glucose concentration leads to biochemical alterations in plasma and erythrocytes in diabetes mellitus and have direct influence on the haemorheological properties of cells. Excessive availability of glucose within the cell leads to formation of HbA1c at a higher rate.[10] Persistent hyperglycemia and haemoglobin glycosylation induces changes in the red cell membrane and its cytoplasm milieu leading to alteration in the red cell deformability.[11,12]

In vitro, critical assessment of the erythrocyte deformability by incubation of normal erythrocytes in high concentration of glucose (50mmol/l), reduction in deformability was noticed.[13] In related study, Petit *et al.*[14] reported high percentage of rigid cells to be responsible for decrease in deformability in diabetes, as detected by nickel filters compared to that of polycarbonate filters. Other observations using various techniques indicate that there is a part of the cell population which is affected by the diabetes process and these erythrocytes are contributing to their decrease deformability.[15]

Until recently, it was thought that exogenous glycations and advanced glycation end-product (AGEs) were negligible contributors to inflammation and disease states, but recent work has shown that they are important as AGEs accumulate in erythrocytes.[16,17] Similar to the formation haemoglobin A1c (HbA1C), AGEs are sugar-derived substances (reducing sugars with free amino groups of proteins, lipids, and nucleic acids) produced by Maillard reaction.[18] The AGEs form at a constant but slow rate in the normal body, starting in early embryonic development, and accumulate with time. These initial reactions are reversible depending on the concentration of the reactants. A lowered glucose concentration will unhook the sugars from the amino groups to which they are attached; conversely, high glucose concentrations will have the opposite effect. In case of chronic hyperglycaemia, AGEs are actively produced and accumulate in circulating blood and various tissues.[19] A series of subsequent reactions, including successions of dehydrations, oxidation-reduction reactions, and other arrangements lead to the formation of AGEs. Several compounds, e.g., N-carboxymethyl-lysine, pentosidine, or methylglyoxal derivatives, serve as examples of well-characterized and widely studied AGEs.

In spite of the fact that, AGEs are produced endogenously by oxidative stress or hyperglycemia, they can be consumed exogenously through food. About 6% to 7% of AGEs derived from meals are known to be present in the body for a certain period of time.[20] Elevated serum AGEs were found in anaemic patient with type 2 diabetes.[21]

AGEs accelerate the expression of receptors for advanced glycation end-products (RAGEs). The constant activation of the AGE-RAGE system is presumed to create the long-term metabolic memory or legacy effect.[22] Upon the recognition of AGEs by RAGE in endothelial cells, the production of oxidative stress is accelerated in the cells, and various cytokines and growth factors are secreted.[23]

In patients with diabetes, RAGE expression is accelerated in atherosclerotic lesions in proportion to aggravation of blood sugar regulation.[24] A large body of evidence

suggests that AGEs are important pathogenetic mediators of almost all diabetes complications, conventionally grouped into macro- or microangiopathies. For instance, atherosclerosis is significantly accelerated in diabetic patients and is associated with greater risk of cardiovascular and cerebrovascular mortality. Animal and human studies have shown that AGEs play a significant role in the formation and progression of atherosclerotic lesions. Increased AGE accumulation in the diabetic vascular tissues has been associated with changes in endothelial cell, macrophage, and smooth muscle cell function.[18]

In this study, we aimed to evaluate the impact of haemoglobin glycosylation on erythrocytes osmotic fragility in subjects with diabetes mellitus.

MATERIALS AND METHODS

Study Area

The study was conducted at Murtala Muhammad Specialist HospitalKano, a secondary health care facility situated within Kano metropolis. The state is a cosmopolitan state, located in the Northwest geopolitical zone of Nigeria. Kano state adjoins with states like Bauchi, Jigawa, Kaduna and Katsina.[25,26]

Study Design

The study was prospective cross-sectional and lasted for three months (fromAugust to October, 2018).

Study subjects

The study participants were subjects with diabetes mellitus attending Murtala Muhammad Specialist Hospital Kano along with apparently healthy control subjects.

Sample collection and Processing

Five millitre(5ml)venous blood samples was collected aseptically using sterile disposable syringe from each participant, and transferred into labeled sodium fluoride anticoagulant container for fasting plasma glucose estimation and dipotassium ethylene diamine tetracetic acid (K_2EDTA) anticoagulant container for determination of HbA1c level and EOF test, respectively.

Ethical Consideration

The study was approved in accordance with institutional guidelines set forth by research ethics committee of Kano state Ministry of Health. DM and control subjects were well informed about the study per declaration of Helsinki 1975 and 2008 revised ethical principles for medical research involving human subjects and consent to participate.

Inclusion and Exclusion criteria

Outpatient adult male and female diabetes mellitus subjects who consented to participate were included into the study. While, those that did not consent and inpatients were excluded from the study.

Laboratory Analyses

Measurement of Plasma Glucose Level

Reagent: FPG was estimated by glucose oxidase - peroxidase (GOD-PAP) method, (RANDOX LABORATORIES LTD. UK, CAT. NO. GL364).

Method: Oxidase-peroxidase Method

Principle: Glucose oxidase catalyzes the oxidation of glucose to give hydrogen peroxide (H_2O_2) and glucronic acid. The enzyme peroxidase catalyzed hydrogen peroxide and the oxygen released reacts with 4- aminophenazone and phenol to give pink color.[27]

Reagents (ml)	Test	Standard	Blank
Glucose Reagent	1.0	1.0	1.0
Plasma/Standard/Distilled-Water	0.01	0.01	0.01

The preparations were mixed separately and incubated at 37°C in a water-bath for 10 minutes. The tubes were shakes occasionally to ensure adequate aeration of the samples. The absorbance of the color produced was measured with spectrophotometer at 515nm wavelength.

Calculations: The results were obtained as below

$$\text{Glocose conc.} = \frac{AT}{AS} \times \text{Conc. of Standard}$$

Where:
A_T = Absorbance of test
A_S = Absorbance of standard (mmol/l)

Measurement of Glycated Haemoglobin
Reagents: MISPA-i3HbA1c, 25T lot number: 11018001, Expiry date: Nov.2020-a product of AGAPPE Diagnostics, Switzerland GmbH was used for the quantitative determination of glycated haemoglobin.

Method: Micro Column ion exchange resin (Agappe Diagnostic, Inc).

Materials and Reagents

Reagent	Composition
Resin	25×3ml-Tubes with ion exchange resin
Lysing Reagent	10ml of lysing reagent
Resin Separators	25 nos of porous resin separators
HbA_{1C} Control	1×0.5ml

Principle: from prepared haemolysate. The $HbA_{1C}$is specifically eluted after washing away the HbA_{1C}fraction and is quantified by direct photometric reading at 415nm.

Assay Procedure:
Haemolysate preparation:
Into a chemically clean test tube, 250µl of the lysing reagent was added. 50µl of mixed-whole blood was also added. It was mixed and then left for 5 minutes at room temperature.

Test for total haemoglobin: (Thb)
Five (5ml) of deionized water was placed in to a clean test tube. 20µl haemolysate was then added and mixed well. Absorbance of the test was read at 415 nm against distilled water as blank.

Test for glycated haemoglobin: (HbA_{1C})
The resin tubes were brought to room temperature and 100µl of the haemolysate was added into each tube. The resin separator was positioned inside the tube ensuring that the rubber sleeve was approximately 3cm above the resin level. The contents were mixed by vortexing for 5 minutes. The resin was allowed to settle at specified assay temperature for 5 minutes. The resin separator was pushed down into the tube until the resin was firmly packed. The supernatant was poured directly into a cuvette and the absorbance was read against deionized water as blank at 415nm.

Calculation: The results were obtained as below

$$HbA_{1C}\text{ percentage} = \frac{HbA_{1C}\text{ Absorbance}}{\text{THB Absorbance}} \times 30 \times \text{Temperature Factor (TF)}$$

Where,
Temperature Factor: 30^0C = 0.9
The HbA_{1C} assay can also be done at 24^0C with a TF of 1.0

Measurement of Erythrocyte Osmotic Fragility:
Reagent:
Stock solution of sodium chloride osmotically equivalent to 10% was prepared as follows:

Sodium chloride (BDH)	90.0g
Disodium hydrogen phosphate	13.65g
Sodium dihydrogen phosphate	2.34g
Distilled water	1000ml

Method:

1. The stock solution was diluted 1/10 with distilled water to obtain a 1% solution
2. 12 test tubes were arranged to prepare dilutions as follows:

Test tube number	Volume of saline (ml)	Volume of distilled water (ml)	Conc. of saline (%)
1	4.50	0.50	0.90
2	3.75	1.25	0.75
3	3.25	1.75	0.65
4	3.00	2.00	0.60
5	2.75	2.25	0.55
6	2.50	2.50	0.50
7	2.25	2.75	0.45
8	2.00	3.00	0.40
9	1.75	3.25	0.35
10	1.50	3.50	0.30
11	1.00	4.00	0.20
12	0.50	4.50	0.10

The 1st tube in the series serves as blank (0% lysis) as isotonic saline (0.9%), while 12th tube contained the lowest concentration (0.1%)hypotonic saline that gave 100% lysis.

Calculation: percentage lysis was calculated as below

$$\% \text{ Lysis} = \frac{\textit{Absoebance of test}}{\textit{Absorbance of 100\% lysis tube}} \text{ X } 100$$

Statistical analysis

The unprocessed data was stored in Microsoft Excel 2010 worksheet. Statistical package for social sciences (SPSS) for Windows (version 20.0.) was used for all analyses. Unpaired Student's t-test was the inferential statistics used to compare differences between variables and expressed as mean ± standard deviation (M ± SD). A value of $p<0.05$ was considered statistically significant.

RESULTS

In this our prospective cross sectional study, which was undertaken between the months of August to October, 2018. Seventy-five participants were enrolled, including 50 (66.7%) diabetes mellitus subjects and 25 (33.3%) apparently healthy controls. The participants were both males and females within the age of 23-72 years (Mean ± SD = 48.48±14.11), Table 1.

Table 1: Distribution of Study participants by gender

Participants	Number (n)	Percentage (%)
DM subjects		
Males	25	(33)
Female	25	(33)
Control subjects		
Males	15	(20)
Females	10	(14)
Total	**75**	**(100)**

Key: n= number of subjects, %= Percentage

Table 2: Distribution of DM subjects by duration of disease

Duration (years)	Number (n)	Percentage (%)
1-5	35	70
6-10	13	26
>10	2	4
Total	**50**	**100**

Key: n= number, %= Percentage

Table 3: Distribution of FPG, HbA_{1C} and EOF of DM and Control subjects

Parameter	Subjects DM (M±SD)	Control (M±SD)	P-value
FBG (mmol/L)	7.17±2.09	4.38±1.04	0.000
HbA 1C(%)	8.67±0.71	5.97±0.91	0.000
EOF (%)	0.45±0.04	0.42±0.01	0.001

Key: FPG= Fasting plasma Glucose, HbA_{1C}= Glycated haemoglobin, EOF= Erythrocyte Osmotic fragility,M±SD= Mean±Standard Deviation

Table 4: Relationship between different parameters in Diabetic Mellitus subjects

Parameters	r-value	P-value
HbA 1Cand FPG	0.06	0.02
HbA 1Cand EO F	0.09	0.00
FPG and EOF	0.08	0.00

Key: HbA_{1C}= glycated haemoglobin, EOF= erythrocyte osmotic fragility, r-value = Pearson correlation, p-value = probability value

The distribution of DM subjects based on the duration diagnosed with disease is presented in Table 2, in the following order 1-5 years (35), 6-10 years (13), above 10 years (2), respectively. Thirty-five subjects diagnosed with the disease between 1-5 years were the highest while two that were diagnosed with the disease (>10 years) were the least. In Table 3, results of fasting plasma glucose, glycated haemoglobin and osmotic fragility of DM and control subject were presented. The values are for FPG = 7.17± 2.09mmol/l and 4.38 ±1.04mmol/l, for HbA_{1C}=8.67±0.71% and 5.97±0.91% and for EOF= 0.45±0.04 and 0.42±0.01 for DM and control groups, respectively. And results were statistically significant ($p < 0.05$) in all the three parameters.

The relationships between levels of FPG and concentration of HbA_{1C}, concentration HbA_{1C} and EOF, and FPG and EOF were r-value 0.06, 0.09, and 0.08, respectively. The results were statistically significant ($p<0.05$), Table 4.

DISCUSSION

Our study shows majority of studied subjects were type 2 DM aged 40 and above years. This is in keeping with the previous reports, where it was documented that type 2 diabetes mellitus (T2DM) to be adult onset disease.[28] The fasting plasma glucose levels of DM and control reported in this our present study was statistically significant ($p < 0.05$), this also agrees with results of several studies done elsewhere.[29-31]

We also observed that, the concentration of glycated haemoglobin was higher in DM compared to control subjects and was statistically significant ($p < 0.05$). These could be attributed to influx of plasma glucose across erythrocyte membranes and non-enzymatic post-translational glycosylation of haemoglobin. This is in consonant with reports of several studies; Arora *et al.*[32] reported increased glycosylation of both spectrin and haemoglobin in DM as compared to controls. Other studies done by Aaron *et al.*[2, 33-35] in which all reported an increased glycation of a number of proteins including haemoglobin and this increase is directly proportional to the fasting glucose levels of diabetes.

The corpuscular fragility in this study was found to be statistically significant among DM and control subjects ($p < 0.05$); this was in accord with the study conducted in Egypt, by Arora *et al.*[32] who reported percentage of red blood cells haemolysis of fourteen females and sixteen males T2DMsubjects increased compared to five healthy controls with hypo-tonicity of saline.

The EOF in fourteen females and sixteen males T2DM subjects were greater than EOF of five healthy controls. Equally, Chien-Min *et al.*[36] in a cross sectional study documented that EOF was greater in T2DM subjects compared to non-diabetic controls and EOF was positively correlated with HbA_{1C}. Harika *et al.*[35] demonstrated that erythrocyte osmotic fragility is greater in T2DM subjects compared to nondiabetic controls and erythrocyte fragility was positively correlated with increased duration of exposure of the disease for 10 years. Similarly, Arun,[37] in a study conducted in India established that erythrocyte osmotic fragility was greater in diabetic subjects when compared to non-diabetic controls and erythrocyte osmotic fragility was higher in diabetic patients with duration of exposure greater than 5 years.

Our report also indicates positive relationship between HbA_{1C} and EOF of diabetic subjects and was statistically significant ($p < 0.05$). This is in accord with the report of Padmini *et al.*[1] in a review article "Monitoring Glycosylated Haemoglobin and Osmotic Fragility with Respect to Blood Glucose Level in Type 2 Diabetes Mellitus" indicates that the diabetic patients with poor glycemic control have increased EOF compared to non-diabetic individuals. More so, according to Sharma,[38]who reported positive correlation between HbA_{1C} and EOF in diabetic patients. As the HbA_{1C} was increased, so also EOF also increases. And these can alter the haemodynamic status in diabetes patients, which can cause both macrovascular and microvascular disease.[39]

CONCLUSION

In this our present study, the increased levels of fasting plasma glucose, glycated haemoglobin and erythrocyte osmotic fragility of DM reported in this our study coupled with positive correlation between glycated haemoglobin and erythrocyte osmotic fragility may play an important role in the pathogenesis of diabetes. Though, we did not classified our subjects with related complications like renal failure, which could be a confounding factor and often predisposes patient to anaemia consequent to reduced red cell survival.

ACKNOWLEDGEMENTS

The authors acknowledged the assistance of staff of Metabolic Clinic, Murtala Muhammad Specialist Hospital, Kano. Our gratitude also goes to all participants in this study.

CONFLICT OF INTEREST: none

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Intestinal Parasites among Patients Attending Federal Medical Centre and Specialist Hospital, Yola, Adamawa State, Nigeria

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ABSTRACT

Intestinal parasites are identified as a cause of morbidity and mortality throughout the world particularly in the developing countries. This research was conducted to determine the prevalence of intestinal parasites and the risk factors for specific and multiple infections among patients attending Federal Medical Centre and Specialist Hospital, Yola, Adamawa State, Nigeria. The unsanitary environment and indiscriminate defaecation in Yola communities and environs is common. People commonly move bare-footed; and consumption of contaminated foods and water which leads to increase in diarrhoeal cases among inhabitants. Four hundred and twenty-three (423) stool samples were randomly collected and analyzed in the laboratory using saline wet mount procedure and formal ether concentration technique. Of the 423, 103(24.3%) had intestinal parasites with a total of seven parasite species were observed. The frequency of occurrence of intestinal parasites from the stools examined indicates that Entamoeba histolytica was the most predominant 32(7.6%), and Ascaris lumbricoides was least predominant 8(1.9%). Co-infection with Hymenolepis nana and Gardia lamblia was also observed 2(0.5%). Prevalence of intestinal parasites was higher in males 71(16.8%) than in females 32(7.6%); while according to age groups, it was higher among 11-20 years old 36(8.6%) and lowest among 41 and above years old -10(2.4%). There was statistically significant difference between intestinal parasitic infection and gender (P<0.05). However, there was no statistically significant difference between intestinal parasitic infection and age (P>0.05). This finding provides data for understanding the epidemiological status of the human gastrointestinal parasites which would be useful in the effective formulation and control of the parasitic diseases.

Keywords: Prevalence, Intestinal, Parasites, Co-infection, Yola

INTRODUCTION

Gastro-intestinal parasites are a major cause of morbidity and mortality throughout the world particularly in the developing countries.[1] They are one of the most common infections in humans especially in tropical and sub-tropical countries.[2] Intestinal parasitic diseases remain a serious public health problem in many developing countries especially due to faecal contamination of water and food.[1]

Several outbreaks of diarrhoeal disease caused by *Cyclospora cayetanensis* have been reported during the last decade. Spread of these protozoan parasites in developing countries mostly occurs through faecal contamination as a result of poor sewage and poor quality of water.[3] Food and water-borne outbreaks of these protozoan parasites have occurred, and the infectious cyst form of the parasites is relatively resistant to chlorine. Other species of protozoan parasites can also be found in the human gut, but they are not pathogenic, except *Microsporidia* sp.[4]

According to United States Centre for Disease Control, the intestinal parasites are hard to completely eliminate from the environment, but the risk of human infection can be decreased. Infected persons and persons at risk should carefully wash their hands after they have any contact with faeces. Careful hand washing is important, especially for caregivers of diapered infants in day-care centres, where diarrhoea is common and carriers of *Giardia* organisms are numerous.[5]

Eating uncooked foods that may have been grown, washed, or prepared with contaminated water should be avoided. Breastfeeding appears to protect infants from *Giardia lamblia* infection. Breast milk contains detectable titres of secretory IgA,

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which is protective for infants, especially in developing countries. Furthermore, infected infants who were exclusively breast-fed had fewer clinical manifestations than those who were not exclusively breast-fed.[6]

According to McGowan *et al*.,[1] the degree of harm caused by intestinal parasitic infection to health of individual and communities depends on the parasites species, the nature of interactions between the parasites species and concurrent infection, the nutritional and socioeconomic factors of the population.

Lustigman *et al*.[7], stated that majority of the intestinal parasitic infections are attributed to Soil Transmitted Helminths (STH) and *Schistosoma* species. It is estimated that more than a billion people in the world are infected with schistosomes and STHs, most of whom suffer from associated severe morbidity. Human schistosomiasis is of considerable public health importance and mainly affects individuals living in developing countries where water resources allow development of snails and poor sanitation facilitates infection.[7] Furthermore, the construction of dams for the purpose of irrigation and hydroelectric power has created new areas of transmission, which intensified community level infection by *S.mansoni* in children living in Africa.[8] Helminth infections is based on regular anti-helminthic treatment, improved water supply and sanitation and health education.[9] In developing countries, however, control measures are difficult to implement due to clean water, sanitation and education problems. As a result, intestinal helminths infection remains a significant health problem in developing regions.

In Nigeria, parasitic infection is endemic and commonly reported among individuals in various communities.[10] Poverty and social exclusion, and the lack of political and financial priority given to building the capacity of vulnerable families to care for and protect their own children in Yola are driving factors to the prevalence of the intestinal parasitic infestations. Furthermore, the frequency of infections may be related to the inadequacy of sanitation and indiscriminate defaecation in the environment. Moreover, many people commonly move bare-footed; and consumption of contaminated foods and water leading to increase in diarrhoeal cases among the inhabitants. Against this background, Patients attending Federal Medical Centre and Specialist Hospital, Yola, Adamawa State, Nigeria, were chosen for this study on the prevalence of intestinal parasitic infection among people in Yola and the state at large.

MATERIALS AND METHODS

Study Area

This study was conducted at Federal Medical Centre (FMC) and Specialist Hospital (SH), Yola, both in Yola metropolis. Yola lies between Latitude 9°19'60.00"N and Longitude 12°29'59.99"E. Adamawa is one of the largest States in Nigeria and occupies an area of about 36,917 square kilometres and a projected population of 4,464,609.[11] The state is well drained by many rivers, most of which are seasonal. The main river is the River Benue with major tributaries which have flat sandy beds and steep rocky incised valley sides with an undulating terrain which provide for suitable fishing, irrigational farming and cattle rearing.[12]

Ethical Issues

Approval and permission for this study was granted by Adamawa State Ministry of Health and Federal Medical Centre Ethical Committee on Health Research.

Methodology

Study design

A descriptive cross-sectional hospital-based study was carried out from June to August, 2018 in FMC and SH, Yola. The two Hospitals serve the population of the study area and its environs. The hospitals also provide inpatient and outpatient services.

Four hundred and twenty-three (423) patients were selected for this study between June and August, 2018 using the formula ($n= [(Z\ score)^2 x\ SD(1-SD)]/e^2$) proposed for studies that can only be used for large populations or unknown population sizes.[13]

Parasitological Methods

Faecal specimen collection

Four hundred and twenty-three (423) stool samples were collected using wide mouthed, sterilized and leak-proof containers.[14] The containers were labelled with identification number and given to each participant who were advised to take into consideration the environmental measures to prevent degeneration and deterioration of amoebic/flagellate trophozoites and cysts, respectively. They were administered along with a questionnaire to know their level of understanding on intestinal parasites and to obtain their socio-demographic information such as age, gender, occupation and educational level followed by locations and the hospital attended.

Macroscopic faecal sample examination

Stool samples were examined macroscopically for colour, odour, consistency, presence of mucus, blood, adult worms and segments of intestinal helminthes.[15]

Microscopic faecal sample examination

Eggs, larvae, ova of helminthes and intestinal protozoa were detected through microscopic examination of the stool samples. For this study, saline wet mount and formal-ether concentration technique methods were used.[15]

Saline wet mount procedure

With the aid of a dropper, a drop of physiological saline (0.9%) was placed on a clean grease free slide. Using applicator stick, a small portion of stool was picked and drop on the slide containing saline followed by mixing until a homogenous mixture was obtained. A cover slip was then placed and viewed microscopically at x10 and x40 objective lenses as described by Cheesebrough.[14]

Formol-ether concentration technique

Faecal sample were also assayed by use of the formal-ether concentration technique as described by Cheessebrough.[14] The resultant sediment was then examined for parasitic ova under x10 and x40 objective. Parasitic ova encountered were subsequently identified on the basis of morphological characteristics with reference to standard keys.

Data Analysis

Data obtained from the study was analysed using Statistical Package for Social Sciences (SPSS) Version 22.0. The statistical associations of potential risk factors and prevalence of intestinal parasitic infection against age, gender and socio-economic status was assessed by analysis of variance (ANOVA) and Chi-square. The confidence level for all the tests were set at 95%.

RESULTS

Of the 423 samples screened, 103(24.3%) were positive for various parasitic ova. A total of seven parasite species comprising of three protozoa and four helminths were observed. *Entamoeba hystolytica* was the most predominant, occurring in 32(7.5%) followed by *Gardia lamblia* which occurred in 23(5.4%), *w*hile *Strongyloides stercoralis* was least prevalent parasite found in 5(1.1%) stool samples as shown in Table 1. Majority of the positive cases were of single infection, but 2(0.4%) were cases of multiple infection involving *Hymenolepis nana* and *Gardia lamblia*.

The prevalence of intestinal parasites infection was higher among the 11-20 years old 36(8.5%), followed by the ≤ 1-10-years old age group 35(8.2%), 21-30 and 41 and above years old 11(2.6%), respectively. While the 31-40 year old age group had the least 10(2.3%) ($P<0.05$) (Table 2). Intestinal parasite infection was higher in males 71(16.7%) than females 32(7.5%) ($P<0.05$) (Table 3). Of the 423 of patients examined, 344(81.2%) showed no symptoms, 79(18.6%) complained of at least one symptom with intestinal parasitic infection.

Among patients who complained of four symptoms, the 41 and above year old had the highest 3(0.7%), followed by the 31-40 year old 2(0.4%), while the 11-20 and the 21-30 year old age groups, had 1(0.2%), respectively. For those with three symptoms, the 11-20 year old had the highest 20(4.7%), followed by <1-10 year old 11(2.6%), 21-30

year old 7(1.5%). The least being among the 31-40 year and 40 above year old 6(1.3%). The <1-10 year old had the highest frequency among those who reported 2 symptoms 9(2.1%), followed by the 11-20 4(0.9%), 31-40 2(0.4%) and the least among 41 and above 1(0.2%). Among patients who complained of one symptom, the 11-20 year old had the highest 3(0.7%), followed by the <1-10 year old 2(0.4%), while the least was among the 41 and above year old age group 1(0.2%) (P>0.05) (Table 4).

Males exhibited highest symptoms 5(1.1%) of infections with intestinal parasites when compared to females 2(0.4%) among those with four symptoms. Also, those with three symptoms, males had a higher prevalence 32(7.5%) when compared to females 18(4.2%). Results also showed that among patients with only two symptoms, males had 11(2.6%) while females had 5(1.1%). Males also had higher frequency among those with one symptom 4(0.9%), while females had 2(0.4%) (Table 5) (P>0.05).

Patients from rural areas exhibited higher symptoms 5(1.1%) than urban dwellers 2(0.4%) of those who presented with four symptoms. Among those with three symptoms, rural dwellers had a higher prevalence 34(8.0%) when compared to those from urban areas 16(3.7%). Those patients who complained of only two symptoms showed that those from rural areas 11(2.6%) were highest. Patients from rural areas also had higher frequency among those with one symptom 4(0.9%), while those from urban had 2(0.4%) (Table 6) (P>0.05).

Table 1: Prevalence of Intestinal Parasites among Patients attending Federal Medical Centre and Specialist Hospital, Yola

Parasite Species	**No. Positive**	**Prevalence (%)**
Entamoeba hystolytica	32	7.6
Gardia lamblia	23	5.4
Ascaris lumbricoides	8	1.8
Hymenolepis nana	9	2.1
H/nana + G.lamblia	2	0.5
Hookworm	14	3.3
Taenia	10	2.4
Strongyloides stercoralis	5	1.2
Total	**103**	**24.3**

Table 2: Prevalence of Intestinal Parasites in Relation to Age of Patients

Age (year)	**No. Examined**	**No. Positive**	**Prevalence (%)**
≤1-10	119	35	8.2
11-20	162	36	8.5
21-30	60	11	2.6
31-40	43	10	2.3
41-Above	39	11	2.6
Total	**423**	**103**	**24.2**

Table 3: Prevalence of Intestinal Parasites infection in Relation to Gender of Patients

Gender	No. Examined	No. Positive	Prevalence (%)
Female	185	32	17.3
Male	238	71	29.8
Total	**423**	**103**	**47.1**

Table 4: Prevalence of Intestinal Parasites in Relation to Symptoms and Age of Patients

Age (year)	No. Examined	One Symptom(%)	Two Symptoms(%)	Three Symptoms(%)	Four Symptoms(%)
≤1 - 10	119	2(0.4)	9(2.1)	11(2.6)	0(0.0)
11-20	162	3(0.7)	4(0.9)	20(4.7)	1(0.2)
21-30	60	0(0.0)	0(0.0)	7(1.5)	1(0.2)
31-40	43	0(0.0)	2(0.4)	6(1.3)	2(0.4)
41-Above	39	1(0.2)	1(0.2)	6(1.3)	3(0.7)
Total	**423**	**6(1.3)**	**16(3.7)**	**50(11.7)**	**7(1.5)**

Table 5: Prevalence of Intestinal Parasites Infection in Relation to Symptoms and Gender of Patients

Gender	No. Examined	One Symptom(%)	Two Symptom(%)	Three Symptom(%)	Four Symptom(%)
Female	185	2(0.4)	5(1.1)	18(4.2)	2(0.4)
Male	238	4(0.9)	11(2.6)	32(7.5)	5(1.1)
Total	**423**	**6(1.3)**	**16(3.7)**	**50(11.7)**	**7(1.5)**

Table 6: Prevalence of Intestinal Parasites Infections in Relation to Symptoms and Location

Location	No. Examined	One Symptom(%)	Two Symptom(%)	Three Symptom(%)	Four Symptom(%)
Rural	200	4(0.9)	11(2.6)	34(8.0)	5(1.1)
Urban	223	2(0.4)	5(1.1)	16(3.7)	2(0.4)
Total	**423**	**6(1.3)**	**16(3.7)**	**50(11.7)**	**7(1.5)**

DISCUSSION

Intestinal parasite are important threats to healthy living of humans in developing countries.[16] The degree of each factor and the prevalence of infections vary from one region to the other.[1,7] Knowledge of intestinal parasitic infection is crucial for planning of efficient intervention programs. The present study assessed the prevalence of intestinal parasites among patients attending FMC and SH, Yola, Adamawa state, Nigeria.

The intestinal parasites detected include *Entamoeba histolytica, Gardia*

lamblia, Hookworm, *Taenia*,*Hymenolepis nana, Ascaris lumbricoides* and *Strongyloides stercoralis*. Co-infection with *Hymenolepis nana* and *Gardia lamblia* was also observed. These intestinal parasites have been reported in various parts of Nigeria.[17] Our study recorded that *Entamoeba hystolytica* was the most predominant (7.5%). This finding agrees with previous studies done elsewhere in Nigeria which reported higher prevalence values. Ajero, *et al.*[17] reported that *E. Histolytica,* an indicator organism of faecal contamination, is frequently present in street foods, and street food may cause outbreak of amoebiasis, cholera, typhoid and hepatitis A.[17] Human amoebiasis is a disease caused by the protozoan, amoeba of the genera *Entamoeba*, of which *E. histolytica* is the most medically important. Infection occurs when man ingests foodstuffs, vegetables or drink water contaminated by cysts of *E.histolytica.*[17]

Gardia lamblia 5.4% is the second predominant intestinal parasites found in this study. This is in agreement with Heidari and Rokni,[18] that reported higher prevalence (23.9%) among children in urban slums of Karachi, Pakistan. Giardia lamblia is one of the most common pathogenic intestinal protozoan worldwide transmitted by the ingestion of cysts in contaminated water, food, or by the faecal-oral route.[19]

Strongyloides stercoralis (1.1%) is the least predominant intestinal parasite in this study. This also agreed with the findings of Egwari *et al*.[20] in Lagos, Southwest Nigeria, who found no *S. stercoralis* in their study of sachet water. Ajero *et al*.[17] in Lagos, Nigeria, also noted that *Strongyloides stercolaris* and other enteric pathogens formed a significant part of the isolates on the outside sachet surfaces of samples collected from cooling receptacles (pail, basin, wheel barrow, and refrigerator).

Result also showed that people within the 11-20-year-old age group had the highest prevalence of intestinal parasites, closely followed by the ≤1-10-year-old age group (Table 2). A similar trend was reported by Jombo,[21] in which the bulk of parasitic infestation occurred among the 8-15-year-old age group in a rural settlement of Northern Nigeria. Again the prevalence of intestinal parasites decreased with age in this study. This inverse relationship between the age and the prevalence of intestinal parasites might be due to higher level of awareness and good hygienic practice in the older age groups. Contrary to this study, some workers in Nigeria and overseas had reported higher prevalence among the elderly. Wariso and Ibe,[22] reported 46.0% prevalence of intestinal parasite in some parts of Port Harcourt, Nigeria. In this study, in-patients and out-patients of FMC and SH, Yola with signs and symptoms of intestinal parasitic infections were used for the study. It is also important to note that patients may have been treated at the primary healthcare levels before being referred to FMC and SH, Yola. This may explain the lower prevalence observed in our study.

This study also found out that males had a marginally higher prevalence (16.7%) compared to their female counterparts (7.5%), although there was statistically significant difference ($p<0.05$) between the enteric parasitosis and gender. This suggested that intestinal parasitic diseases were independent of gender in the study area. This is contrary to the report by Gimba and Dawam, [23] that stated that the prevalence of intestinal parasites in children attending Gwagwalada township clinic, Abuja-Nigeria was higher in females (30.0%) than males (25.7%). This difference was statistically significant ($P<0.05$) in the association between prevalence of intestinal parasitic diseases in the two gender. Our finding also agrees with that of Anosike *et al*.[24] in a survey of intestinal parasite among students of post-primary institutions in Imo State, Nigeria, who reported that parasitic infections were significantly higher in males than females. Okonko *et al*.[4] reported that gastrointestinal parasite infection from 2002 to 2004 were significantly higher in males than females though the difference was not statistically significant ($P>0.05$). The high prevalence of intestinal parasites in men from our study may be related to occupation-related activities such as farming, fishing, and travel that put male at high risk of intestinal parasitosis than their female

counterparts. Again, females prefer treating themselves at the onset of every infection. They also visit health facilities more often than males who would rather prefer enduring illnesses as a demonstration of their masculinity.

The prevalence of intestinal parasite among patients in relation to symptoms, and associated sociodemographic and environmental factors has been demonstrated. In this study, 18.6% had symptoms of nausea, vomiting, abdominal pain and/or diarrhoea, due to infections with intestinal parasites. With regard to age, elderly patients (41 and above year old age group) were reported to be the highest with all symptoms compare to other age groups (P>0.05). This agrees with the findings of Almeida *et al*.[25] who reported high prevalence of intestinal parasites among the elderly with many signs and symptoms, while frequency of lower symptoms was reported in children ≤ 1–10-year-old age group. The high prevalence of parasitic infection among elderly can be explained by immunological deficiencies that occur with aging, increasing susceptibility to such diseases. Increased difficulty in performing self-care, which hampers personal hygiene and feeding and causes a possible disconnect of elderly people from their health. It is worth mentioning that many elderly people perform activities that require contact with the soil, such as gardening and yard cleaning, which facilitate contamination by increasing the risk of exposure to parasites.[26]

The prevalence of infection with intestinal parasites among patients in relation to symptoms and gender in our study showed that males have more symptoms than their female counterparts. This agrees with the findings of Baldo *et al*.[27] who stated that infection rates for intestinal parasites were higher in males than females. This is due to the fact that males possess more symptoms and high risk of infections due to their occupational activities in relation to soil contact.

The prevalence of intestinal parasite infections among patients in relation to symptoms and location shows that patients from rural settlements exhibited high symptoms (1.1%) more than urban dwellers (0.4%). This study is in line with the report of Damen *et al*.[28] who reported high symptoms and prevalence of intestinal parasitism among the Almajiris in the rural North Eastern Nigeria. This can be attributed to unplanned urbanization, which results in poor sanitary and hygienic conditions and contamination of drinking water with faecal matter.[29]

The higher prevalence of *Entaemoeba histolytica*, *Gardia lamblia, Ascaris lumbricoides* and other intestinal parasites detected in this study was a reflection of the poor environmental sanitation and very poor personal hygiene and unclean habits practiced by endemic villagers compounded by public ignorance and illiteracy.

CONCLUSION

Intestinal parasites are prevalent among the study population. Data obtained from this study provides information on the various parasitic diseases associated with gastro-intestinal tract of people in the study area. The 24.2% prevalence of intestinal parasites from this study is a pointer to the fact that one quarter of the gastrointestinal diseases in humans in the study area might be associated with enteric parasites. There is a need for regular awareness programs on sanitary and good hygiene among people living in the study area. Public enlightenment and emphasis on personal hygiene and cleaner environment may be necessary in the prevention and control of parasitic infections among people living in the endemic areas. Preventive measures and surveillance systems should be emphasized. Hence, while treating for intestinal parasites, it is advisable to use broad spectrum or multi-agent drug combinations because of the poly-parasitism susceptibility.

ACKNOWLEDGEMENTS

The participation of all patients is greatly acknowledged. We also wish to express our thanks to the management of Federal Medical Centre, and Specialist Hospitals, Yola for granting permission to undertake this study. Likewise, the assistance of staff of the laboratory at both hospitals, is appreciated.

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Evaluation of Appropriate Completion of Request Forms Submitted to Chemical Pathology Laboratory of a University Teaching Hospital in South-South Nigeria

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ABSTRACT

Ordering of laboratory tests by physicians is a major component of the pre-analytical phase of the laboratory testing process (LTP). The most common medium of ordering for a biochemical test in the chemical pathology laboratory is by completing a laboratory request form (LRF). The main objective of this study was to review and evaluate the appropriate completion of LRFs submitted for biochemical analyses in the Chemical Pathology Laboratory of University of Calabar Teaching Hospital. The study involved a cross-sectional review of all completed LRFs submitted for biochemical tests in the chemical pathology laboratory of UCTH within a period of six (6) months. The appropriate completion of individual information (variables) contained in the request forms was examined for 1,630 patients. The data obtained were recorded using a Microsoft Excel spreadsheet prior to statistical analysis. Of the 1,630 LRFs reviewed and evaluated, none of them was completed adequately and accurately. Patient's name was filled in 99.8% of the forms followed in decreasing order by gender of patient (99.2%), examination required (99.0%) patient's age (97.5), laboratory number (96.8%) and specimen type (96.4%). The least commonly completed information were time of specimen collection (1.2%) and time of receipt of specimen (0.4%). Though the percentage completions of a good number of the items on the LRFs were appreciably high, not a single LRF had a 100% completion. This is a huge cause for concern because, a poorly completed LRF is a recipe for wrong interpretative comments, misleading diagnosis and inappropriate management of the patient.

Keywords: Laboratory, Tests, Request, Forms, Chemical, Pathology

INTRODUCTION

The laboratory testing process (LTP) involves all the procedures, techniques and actions that take place from the time a test request is made by the physician to the time the fully reported result gets back to the requesting physician.[1] The LTP consists of the pre-analytical, analytical, and post-analytical phases. Each of these processes may be affected by errors that are capable of hampering the quality and clinical utility of the laboratory test result.[2,3] A major component of the pre-analytical phase of the LTP is testing ordering which essentially, involves the request for specific type and number of tests by the requesting physician.[4] Physicians make laboratory test request for screening, diagnosis, monitoring, and follow-up management of patient.[5,6] The most common medium of ordering for a laboratory test is by completing a laboratory request form.[7-10] The request form bears important information concerning the patient's demographics, clinical history, provisional diagnosis, type of specimen needed, timing of specimen collection, type and number of test parameters to be tested for.[8,9] Also, the request form contains information about patient's location in the hospital, identity of the managing consultant and the requesting (corresponding) physician, etc.

The request form serves as a medium of communication and interface between the physician and the clinical laboratory. Above all, it has confidential information about the patient and as such is an important medico-legal document that should be handled and treated with the utmost confidence in respect of patient's autonomy. The above-mentioned attributes of the laboratory request form call for painstaking accurate and adequate completion of request forms by concerned medical professionals.[10] Inadequate information in the laboratory request form has been observed as a major source of preanalytical error in the clinical

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laboratory.[11,13] It also hampers the addition of interpretative comments by the laboratory physicians.[14-16]

Studies have shown that most request forms sent to the clinical laboratory are poorly completed and lack relevant details that will enable interpretation of test results.[17-22] In our contemporary clinical practice environment, there are few data concerning the impact of inadequate completion of request forms on the laboratory and clinical utility of test requests. Based on this, this present study is set out to review and evaluate all request forms submitted for biochemical analysis in the chemical pathology laboratory of the University of Calabar Teaching Hospital, within a six months period.

MATERIALS AND METHOD

Study location

The study was conducted at the main laboratory of the department of chemical pathology of the University of Calabar Teaching Hospital, Calabar, Cross-River State, Nigeria. The University of Calabar Teaching Hospital is a tertiary health institution located within the Calabar municipality of Cross-River State in the South-South geopolitical zone of Nigeria. It is a 334-bed hospital that serves as a referral centre to mainly Cross-River State and the neighbouring Akwa Ibom State.

Study Design

This study was a retrospective study of all completed laboratory request forms that were submitted for biochemical tests in the chemical pathology laboratory within 6 months period, from 1st January, 2018 to 30th June, 2018.

Data Collection

Data collection involved the retrieval and review of completed laboratory request forms submitted for biochemical analysis in the chemical pathology laboratory within the study period. The appropriate completion of individual information (variables) contained in the request form was examined for each submitted request form. The data were recorded using a Microsoft Excel spreadsheet before statistical analysis.

Data Analysis

The data were analyzed using SPSS version 20. The analysis involved the computation of frequencies of completed and non-completed variables in the reviewed laboratory request forms. The frequency distribution was graphically represented using an annotated bar-chart.

Ethical Consideration

The approval for this study was obtained from the joint Institutional review board of the University of Calabar and the University of Calabar Teaching Hospital. We ensured patients confidentiality during the data collection. No personal patient identification characteristic such as name, hospital number or laboratory number was recorded. The reviewed request forms were only numbered serially for ease of identification.

RESULTS

A total of 1,630 laboratory request forms were evaluated out of which none of the request forms was filled completely. Patient name was filled in 99.8% of the forms, followed by sex of patient (99.2%), examination required (99.0%), age of patient (97.5%), laboratory number (96.8%) and type of specimen (96.4%). Patient's location/ward was only filled in 91.7% of the forms while consultant's name appeared in 81.3%. The time specimen is received was the least commonly filled information (0.4%), followed by time specimen was collected (1.2%). Other information on the laboratory request form and the proportions of forms with such variables filled or otherwise, are shown in table 1 and figure 1.

Table 1: Patient-related characteristics in laboratory request forms

Variable	Complete n(%)	Not complete n(%)
Patients full name	1626(99.8)	4(0.2)
Age	1590(97.5)	40(2.5)
Sex	1617(99.2)	13(0.8)
Tribe	666(40.9)	964(59.1)
Nationality	972(59.6)	658(40.4)
Religion	821(50.4)	809(49.6)
Patients location/ward	1494(91.7)	136(8.3)
Hospital number	1074(65.9)	556(34.1)
Doctors name	1174(72.0)	456(28.0)
Doctors signature	1270(77.9)	360(22.1)
Consultants name	1326(81.3)	304(18.7)
Clinical information	858(52.6)	772(47.4)
Provisional diagnosis	1190(73.0)	440(27.0)
Laboratory number	1578(96.8)	52(3.2)
Examination required	1613(99.0)	17(1.0)
Request date	182(11.2)	1448(88.8)
Time of specimen collection	20(1.2)	1610(98.8)
Date specimen is received	1025(62.9)	605(37.1)
Time specimen is received	7(0.4)	1623(99.6)
Specimen type	1572(96.4)	58(3.6)

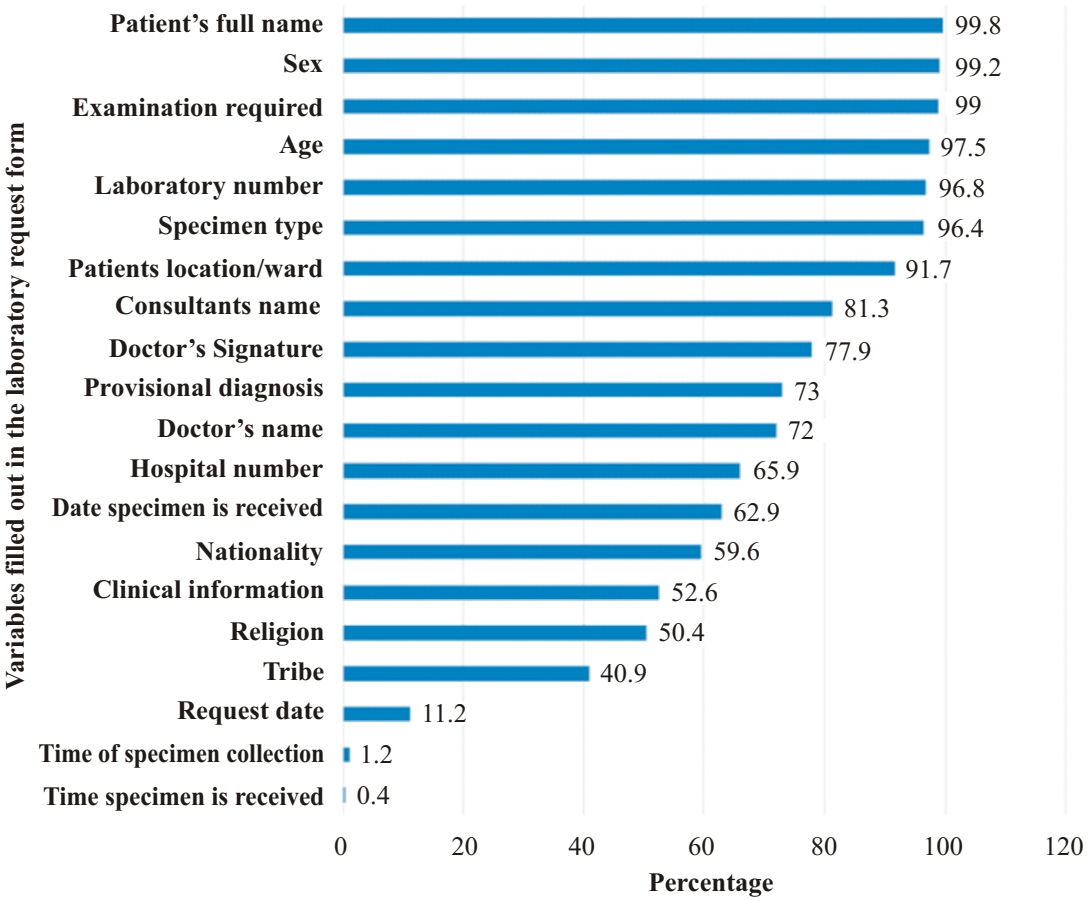

Figure 1: Patient-related characteristics illustrated graphically

DISCUSSION

This study was a retrospective review of 1,630 laboratory request forms (LRFs) submitted for biochemical analyses in a tertiary hospital chemical pathology laboratory. The study evaluated the level of completion of various items contained in the LRFs. The LRF serves as a medium of bilateral communication between the requesting clinician and the clinical laboratory. In most cases, the requesting physician does not necessarily need to have direct contact with the laboratory but communicates with the laboratory via information contained in the LRF. Consequently, incomplete, deficient or inaccurate information in the LRF may hamper the communication between the requesting physician and the clinical laboratory. This constitutes a major source of pre-analytical laboratory error.

Our study showed that patient's full name was written in 99.8% of the LRFs while 0.2% was incomplete. This is in contrast to several similar studies that reported 100% completion of patient's full name. For instance, Jegede *et al.*[9] Ikponwen *et al.*[23] Adegoke *et al*[17], Alagoe and Udoye[24] as well as Olayemi and Asiamah-Broni[20] in their respective studies reported 100% completion of patient's full names. However, our result is similar to the findings of Oyedeji *et al.* [21] who reported that patient's names were completed only in 99.0% of cases. It is not surprising that most similar studies reported a 100% completion. This is because a complete omission or incomplete writing of patient's full name in a LRF constitutes a monumental and unacceptable pre-analytical error. Patient's full name is the major item on the LRF for identification of the patient. Hence, absent, incomplete or illegible patient's full name on a LRF is bound to attract outright rejection of the laboratory tests request and this should be promptly brought to the attention of the requesting physician.

In a relatively high proportion of the reviewed LRFs, age (97.5%) was correctly identified. This is similar to the proportions reported by other studies including, Jegede *et al.* (98.8%),[9] Adegoke et al (86.4%),[17] Alagoa and Udoye (88.5%).[24] However, other studies reported rather low values including Ikponmwen *et al.*(58.02%),[23] Olayemi and Asiamah-Broni (74.4%)[20] and Oyedeji *et al.* (68.0%).[21] Writing the age of the patient in a request form is a very important exercise that needs not be omitted or treated with levity. Often, some requesting physician does not bother to ask of the real age of the patient and are in the habit of filling out space for age with just "adult". This negates the principle of good laboratory practice and should be utterly discouraged. Knowing the true age of a patient is very essential. Not only that the occurrence of some biochemical/metabolic diseases varies with age, but some reference intervals that are used for the interpretation of laboratory test results are also age-specific. Thus, to the laboratory physician, the knowledge of the real age of the patient is valuable for the sake of interpretative commenting on patients results.[14] The social issue associated with the writing of the actual age of the patient on the request form especially for the womenfolk, is not an excuse to wrongly write the age of the patient as "adult". For this reason, it is more appropriate to write the date of birth of the patient instead of the numerical age in years.[8] Again, writing the date of birth also serves as a specific means of identification for the patient. This is because more than one patient may have the same age but they are very unlikely to have the same date of birth.[8]

This study showed that the space for gender was appropriately completed in 99.2% of forms studied. This is similar to the values reported by Jegede *et al.* (97.4%),[9] Ikponmwen *et al.* (96.8%),[23] Adegoke *et al.* (99.8%),[17] Alagoa and Udoye (97.0%),[24] and Oyedeji *et al.* (90.3%). However, the study by Olayemi and Asaiamah-Broni[20] reported a percentage completion rate of 67.3% for the space that is meant for gender of the patient. Like age, gender also influences the occurrence and aetiology of certain disease conditions as well as the reference values of some biochemical analytes. For instance serum concentrations of creatinine, creatinine kinase and sex hormones all vary with gender in the adult population. Thus, the establishment of gender-dependent normative values is appropriate for these analytes.

The column for tribe/ethnicity was only completed in 40.9% of submitted request

forms. This is rather low. Surprisingly, majority of previous studies did not lay much emphasis on the writing of the patient's tribe as part of the quality specification parameters in the LRFs. Like age and gender, ethnicity is a major determining factor in the plasma levels of some biochemical substances. Thus the establishment of ethnic-specific references values may be appropriate for some of these analytes.

Proper patient identification is one of the major pre-analytical quality indicators contained in the LRF. Besides patient's full name, other items that are used to identify the patients include patient's hospital number, laboratory number and patient's location/ward. Our study showed that the patient's hospital number was only completely written in 65.9% of the forms. This is rather low when compared to the values obtained in previous studies including those of Jegede *et al.* (94.3%),[9] Ikponmwen *et al.* (79.45%)[23] and Adegoke *et al.* (95.6%).[17] Our findings are however similar to that of Alagoa and Udoye[24] who reported 66.0% in their study. Clinicians should be made to appreciate the importance of writing the hospital number of the patient in the request form. This is not only for the sake of the patient's identification; it is also for the certification of the authenticity of the laboratory request form and the results therein. Contrary to the value obtained for the hospital number, 96.8% of the forms we reviewed had their laboratory number written. This is similar to those of Jegede *et al.* (99.9%)[9] and Adegoke et al (95.6%).[17] Writing the laboratory number is very important for the clinical laboratory. It enhances easy identification, documentation, and retrieval of the patients results whether a manual or an electronic laboratory information system is in operation in the laboratory.

This study showed that 91.7% of the LRFs had their patients location/ward rightly indicated. This is similar to the findings of Jegede *et al.* (99.6%),[9] Ikponmwen *et al.* (90.23%),[23] Adegoke *et al.* (99.7%)[17] and Alagoa and Udoye (90.4%).[24] In contrast, Olayemi and Asiamah-Broni[20] reported 52.2% in their study. Knowing the patient location is essential for easy communication between the clinical units and the laboratory. It also enhances the dispatch of completed and reported laboratory tests results.

The identity and signature of the requesting physician as well as the consultant under whose care the patients are also important information for the clinical laboratory. In our study, requesting physician's name, his/her signature, and the consultant's name were appropriately completed in 72.0%, 77.9% and 81.3% of the reviewed request forms respectively. These are similar to the corresponding values reported by Jegede *et al.*[9] viz; requesting physicians full name (88.6%), and consultants name (75.0%). However, Jegede and colleague recorded a relatively low value (39.2%) for the requesting physician's signature.[9] Other similar studies and their corresponding values include: Ikponwen *et al.* (67.8%, 67.8% and 93.52%),[23] Alagoa and Udoye (84.5%, 72.9% and 74.7%)[24] and Adegoke et al (95.7%, 95.7% and 96.6%).[17]

In this study, clinical information/details and provisional diagnosis were written in 52.6% and 73.0% of reviewed LRFs respectively. Our values are only similar to that reported by Oyedeji et al (65.9%)[21] but relatively low compared with other studies such as Jegede *et al.* (80.9%),[9] Ikponmwen *et al.* (93.52%),[23] Adegoke et al (93.2% and 92.2%),[17] Alagoa and Udoye (83.5%),[24] Olayemi and Asiamah-Broni (77.3%).[20] The importance of writing the clinical details and/or the provisional (working) diagnosis can never be overemphasized. Though some clinicians erroneously believe that giving of such information is not necessary, they are valuable items for the laboratory physicians interpretative comments as well as a possible suggestion for further biochemical investigations that may help to unravel the diagnosis if necessary.[14]

In the majority of the LRFs we reviewed, records for time-related items were very poor. Essentially, the request date, time of specimen collection, and time of receipt of the specimen were recorded only in 11.2%, 1.2% and 0.4% respectively. This is surprisingly similar to the values reported by

other studies. Adegoke *et al*.[17] in their study, reported the date of specimen collection and time of specimen collection to be 36.5% and 10.3% respectively. For most biochemical analyses, the date of specimen collection and time of specimen collection are very important. This stems from the fact that certain analytes vary in their plasma levels with the time of the day. Also, for analytes that are under the influence of cyclical variations, knowledge of the time of specimen collection will be of great help in applying the appropriate normative values for interpretative commenting. Also, some biochemical analytes are volatile and can be affected by environmental factors such as light or ambient temperature. Thus, the time lag between specimen collection and analysis needs to be taken into cognizance to avoid interpreting wrong results consequent upon a delayed analysis.

CONCLUSION

Our study has shown that out of 1,630 LRFs that we reviewed, none was completely and adequately filled out. The most-affected were time-related items such as the date of the time of specimen collection and time of receipt of the specimen by the laboratory. Accurate completion of the laboratory request form is an essential pre-analytical component of the laboratory quality assurance system. Thus, a poorly completed request form is a potential source of pre-analytical laboratory error. To produce a test result that is reliable and qualitative, both the clinicians and clinical laboratorians must place a high premium on the necessity of a thoroughly completed request form. For the pathologist or laboratory physician to add relevant interpretative comments on laboratory test results there is a need for the provision of adequate information concerning the patients that are being investigated. A poorly completed request form is a recipe for wrong interpretative comments, misleading diagnosis, and inappropriate management of the patient.

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Correlation of Hemolysin Production and Multi-Drug resistant Phenotype among Methicillin-Resistant Staphylococci Species

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ABSTRACT

Methicillin-resistant Staphylococci are shown to cause various forms of infections in human with fatal consequences on the health and economy of the patient. This study is aimed at determining the prevalence and antibiotic resistance pattern of methicillin resistant Staphylococci in relation to hemolysin production. A total of 100 clinical samples were screened for Staphylococci spp by standard method. Isolates obtained were tested for methicillin-resistance and susceptibility to other antibiotics by standard procedure. A total of 30 Staphylococcus spp comprising 18 S. aureus and 12 Coagulase-Negative Staphylococci (CoNS) where obtained. Twenty-seven (90%) of the Staphylococci spp were methicillin resistant comprising 17(94.4%) MRSA and 10(83.3%) MRCoNS. Thirteen (13) resistance profiles were exhibited by MRSA out of which 9 were MDR, while 9 resistance profiles were shown by MRCoNS out of which 5 were MDR. There isa significant correlation in antibiotic resistant phenotype between MRSA and MRCoNS (P=0.01). The result also revealed that 5 and 12 MRSA were -hemolytic and -hemolytic respectively, whereas 2 and 8 MRCoNS were -hemolytic and -hemolytic respectively. The high prevalence rate of methicillin resistance coupled with high multi-drug resistance phenotype among Staphylococcus spp in the study area is alarming. There is therefore need for periodic or regular surveillance of MRSA and MRCoNS infections not only in the hospital settings but also in the communities.

Keywords: Methicillin, Resistance, Hemolysin, Staphylococci

INTRODUCTION

Staphylococci are Gram-positive, non-motile, non-spore forming, facultatively anaerobic, spherical bacteria with inherent ability to breakdown carbohydrates, producing peculiar characteristic colour (white to deep yellow) on culture media. Species are classified as coagulase positive (e.g. *Staphylococcusaureus)* and coagulase-negative staphylococci (e.g. *Staphylococcus epidermidis*and *Staphylococcus-saprophyticus*.). Fermentation of mannitol and deoxyribonuclease (DNase), catalase and coagulase enzymes is often used for their identification[1]. The ability of *Staphylococcus* spp to caused infection is usually associated with the presence of some virulence factors such as hemolysins, toxic-shock syndrome toxin, coagulase, enterotoxins, exfoliatins, Panton-Valentine leukocidin, protein A and capsular polysaccharide.[2]The high incidence of antibiotic resistance among *Staphylococci spp*in humans has been associated with increased cost of healthcare, coupled with a huge burden of disease among different populations. Among the antibioticresistant strains of *S. aureus,* increased attention has been devoted to the methicillin-resistant *S. aureus* (MRSA) due to its significance in clinical environments globally.[3] Resistance to this class of antibiotics is usually mediated by mecA gene which encodes for the production of penicillinbinding protein 2A (PBP-2A) mediating resistance to all penicillins including methicillin and resulting in reduced affinity for binding betalactam antibiotics.[4]Apart from resistance to methicillin or its derivatives, MRSA also exhibits multi-drug resistant (MDR) phenotype[4] and a low-level resistance to vancomycin;[5] this is a major call for concern. This is possible because the mecA gene complex is said to contain insertion sites for plasmids and transposons that facilitate the acquisition of resistance to other antibiotics.[6] Consequently, studies have shown that methicillin-resistant Staphylococci are often

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associated with infections which hardly respond to therapy.[7] They are also implicated in sepsis, endocarditis and neonatal meningitis.[8]

Risk factors for the acquisition of hospital-acquired methicillin-resistant Staphylococci include prolong usage or abuse of antibiotics, prolonged hospitalization, parenteralfeeding, nasal carriage of MRSA, direct or indirect contact with an infected individuals or materials, regular exposure to clinical specimens without adequate preventive measures, crowded and unhygienic living conditions, compromised immune system and underlying chronic illness amongst others.[9] A study showed that the economy and health loss dueto methicillin-resistant Staphylococci infection is far greater than the one caused by methicillin-sensitive *Staphylococcus spp.*[10] In Africa, the frequency of MRSA is low and changes from place to place.[11] Prevalence rates between 10-49% has been reported in some Africa countries.[9]

Studies have shown that the pathogenicity of Staphylococci spp is enhanced by a variety of virulence factors, one of which is hemolysin. Staphylococci spp is reported to produce different types of hemolysin which include , and - hemolysin.[12]

Gamma haemolysin cannot haemolyse human or rabbit red blood cells, unlike - and -haemolysins.[13] Surprisingly, some studies have associated hemolysin production with increased virulence and antibacterial resistance.[13, 14]

This study therefore reports the prevalence and susceptibility pattern of methicillin-resistant Staphylococci and their resistance pattern in relation to hemolysin production.

MATERIALS AND METHODS

Study area

Mubi metropolis comprises of two local government areas; Mubi North and Mubi South lies between latitudes 10° 05' and 10° 30'N of the equator and between longitude 13° 12'E and 13° 19'E of the Greenwich meridian. The area shares boundaries with Maiha, Hong, Michika Local Government Areas (LGA) in Nigeria and the Cameroun Republic to the south west and east respectively. Majorly, Mubi is made up of the following ethnic groups; Gude, Njanyi, Kilba, Fali, Higgi and Margi.[15]

Sample population

A total of 100 clinical samples were collected randomly from patients attending Mubi general hospital and New life medical clinic from May 2017 to August 2017. The clinical samples include urine (68), high vaginal swab (14), wound swab (7), sputum (1), semen (1), ear swab (1) and stool (8).

Ethical consideration

Verbal inform consent was obtained from both hospital managements and patients.

Identification of *S. aureus*

Standard procedure was used to identify Staphylococci isolates. This was based on their ability to grow and utilised Mannitol in Mannitol salt agar (MSA) medium, morphological characteristics, reaction to Gram staining, coagulase and catalase tests.[16]

Hemolysin production:

Hemolysin production was detected using blood agar. All bacterial isolates were grown on blood agar (Nutrient agar supplemented with sheep erythrocyte) and incubated at 37°C for 24 hours. The organisms were classified as either , or -hemolytic. Detectionof clear zone around the colonies was taken as -hemolysis (complete lysis of RBC). The presence of a halo (greenish colouration) around the bacteria growth was taken as -hemolysis (partial hemolysis), while -haemolysis was recorded when there was normal growth without changes in the culture medium (no hemolysis).

Antibiotics Susceptibility Testing

Susceptibility of the isolates to antibiotics was carried out by modified Kirby-Bauer disc diffusion method based on CLSI procedure and guidelines for reading zone of inhibition interpretative table.[17] The antibiotic discs used include; perfloxacin(10ìg), gentamycin (10 g), ampiclox (30 g), cefuroxime (20 g), amoxicillin (30 g), ceftriaxone (25 g),

ciprofloxacin (10 g), streptomycin (30 g), cotrimoxazole (30 g) and erythromycin (10 g).

Phenotypic Detection of Methicillin-Resistant *Staphylococcus* spp

Methicillin-resistant *Staphylococcus* spp isolated from clinical samples were detected phenotypically using 1 g oxacillin disc CT0159B (Oxoid, UK). Approximately 0.1ml of 0.5 McFarland standard *Staphylococcus* spp was inoculated onto Mueller-Hinton Agar (MHA) plates. Commercially available oxacillin disc was placed on the plate of Mueller-Hinton agar and incubated aerobically at 37°C for 24 hrs. Zone of inhibition ≤12mm was interpreted as methicillin-resistant, while inhibitory zone ≥13mm was interpreted as methicillin-sensitive.[12]

Statistical analyses:

Bivariate correlation was used to determine the association in antibiotic resistance between MRSA and MRCoNS and also between -hemolytic and -hemolytic MRCoNS. More so, Non-Parametric Mann-Whitney statistics was used to determine the level of significance in MDR phenotype between -hemolytic and -hemolytic methicillin-resistant Staphylococci spp. All statistical analyses were carried out using the SPSS 17.0 Windows based program. Significant difference and Non-significant difference was defined when p=0.05 and p>0.05 respectively.

RESULTS

The result showed that 30 staphylococcal spp were isolated from the 100 clinical samples screened; of these, 18(60%) were *Staphylococcus aureus* while 12(40%) were coagulase-negative staphylococci (CoNS). *S.aureus* was mostly isolated from urine (38.9%) followed by HVS (33.3%) and wound swab (27.8%). Whereas CoNS were isolated mostly from urine (75%) and HVS (25%) (Table 1).

Table 2 showed the prevalence of methicillin resistance among the isolated staphylococci spp. A total of 27(90%) Staphylococci spp were observed to be methicillin-resistant. This included17(94.4%) MRSA and 10(83.3%) MRCoNS. There is a significant correlation in antibiotic resistance phenotype between MRSA and MRCoNS (P=0.01).

The hemolysin production and multi-drug resistant (MDR) phenotype among methicillin-resistant staphylococci spp. is shown in Table 3. Thirteen (13) resistance profiles were exhibited by MRSA of which 9 are MDR. In the same vein, 9 resistance profiles were shown by MRCoNS out of which 5 are MDR. The result also revealed that 5 and 12 MRSA are -hemolytic and -hemolytic respectively. More so, 2 and 8 MRCoNS are -hemolytic and -hemolytic respectively. The MDR phenotype is significantly higher in -hemolytic than -hemolytic methicillin-resistant staphylococci spp. (P=0.001).

Antibiotic resistance pattern in -hemolytic MRCoNS is significantly higher than -haemolytic MRCoNS (P=0.030). However, the antibiotic resistance pattern in -haemolytic MRSA is significantly higher than that of -hemolytic MRSA (P=0.006).

Table 1: Frequency of Staphylococci spp from clinical Samples

SN	Specimen	Frequency	*S. aureus*(%)	CoNS (%)
1.	Urine	68	7(38.9)	9(75.0)
2.	High Vagina swab	14	6(33.3)	3(25.0)
3.	Wound swab	7	5(27.8)	-
4.	Sputum	1	-	-
5.	Semen	1	-	-
6.	Ear swab	1	-	-
7.	Stool	8	-	-
	Total	100	18(60)	12(40)

Legend: CoNS: coagulase negative staphylococci

Table 2: Prevalence of methicillin resistance among Staphylococci spp

Sex	*S.aureus*			CoNS		
	No. tested	MRSA (%)	MSSA (%)	No. Tested	MRCoNS (%)	MSCoNS (%)
Male	5	5(100)	0	3	3(100)	0
Female	13	12(92.3)	1(7.7)	9	7(78)	2
Total	18	17(94.4)	1(5.6)	12	10(83.3)	2

Table 3: Resistance profile of Methicillin Resistance Staphylococci spp based on hemolysin production

Organisms	No. of resistance profile	Resistance Profile	No. of isolates -hemolytic	-hemolytic	No. of antibiotics
MRSA	1	pef, cn, apx, cxm, am, cro, cip, s, sxt, e	2	2	10
	2	pef, cn, apx, cxm, am, cro, s, sxt, e	0	1	9
	3	pef, cn, apx, cxm, am, cro, cip, sxt, e	0	1	9
	4	pef, cn, apx, am, cro, cip, s, sxt, e	1	0	9
	5	pef, cn, apx, cxm, am, cro, sxt, e	1	1	8
	6	pef, apx, cxm, am, cro, sxt, e	0	1	7
	7	apx, cxm, am,sxt	1	0	4
	8	apx, cn, am, sxt	0	1	4
	9	am, sxt, e	0	1	3
	10	am, cip, s	0	1	3
	11	apx, am	0	1	2
	12	am, sxt	0	1	2
	13	Am	0	1	1
		Total	**5**	**12**	
MRCoNS	1	pef, cn, apx, cxm, am, cro, cip, s, sxt, e	0	2	10
	2	pef, cn, apx, cxm, am, cip, s, sxt, e	0	1	9
	3	pef, cn, apx, cxm, am, cro, cro, e	0	1	8
	4	pef, cn, apx, am, s, sxt, e	0	1	7
	5	apx, cxm, am, cro, s	0	1	5
	6	cn, am, cro	1	0	3
	7	cn, sxt, e	1	0	3
	8	cn, apx, am	0	1	3
	9	Am	0	1	1
		Total	2	8	

Legend: pef =perfloxacin, cn=gentamycin, apx=ampiclox, cxm=cefuroxime, am=amoxicillin, cro=ceftriaxone, cip=ciprofloxacin, s=streptomycin, sxt=cotrimoxazole, e=erythromycin

Table 4: Resistance pattern of Methicillin Resistance Staphylococci sp

Antibiotic	MRSA (%)[a] N= 17	MSSA N = 1	MRCoNS (%)[a] N= 10	MSCoNS N = 2
Perfloxacin	10(59)	-	5(50)	1(50)
Gentamycin	10(59)	-	8(80)	1(50)
Ampiclox	13(76)	-	7(70)	1(50)
Cefuroxime	9(53)	-	5(50)	-
Amoxicillin	17(100)	1(100)	9(90)	2(100)
Ceftriaxone	11(65)	-	5(50)	-
Ciprofloxacin	7(41)	-	3(30)	-
Streptomycin	7(41)	-	5(50)	-
Cotrimoxazole	14(82)	-	6(60)	-
Erythromycin	11(65)	-	6(60)	1(50)

[a]= correlation is significant (P=0.01)

Table 5: Resistance pattern of Methicillin Resistance Staphylococci sp based on Hemolysin production

Antibiotic	MRSA (%)		MRCoNS (%)	
	-hemolytic[a] (n=5)	-hemolytic[b] (n=12)	-hemolytic[c] (n=2)	-hemolytic[d] (n=8)
Perfloxacin	4(80)	6(50)	0	5(63)
Gentamycin	4(80)	6(50)	2(100)	6(75)
Ampiclox	5(100)	8(67)	0	7(88)
Cefuroxime	3(60)	6(50)	0	5(63)
Amoxicillin	5(100)	12(100)	1(50)	8(100)
Ceftriaxone	5(100)	6(50)	1(50)	4(50)
Ciprofloxacin	3(60)	4(33)	0	3(38)
Streptomycin	3(60)	4(33)	0	5(63)
Cotrimoxazole	5(100)	9(75)	1(50)	5(63)
Erythromycin	4(80)	7(58)	1(50)	5(63)

a and b (statistics is significant, (P=0.006), c and d (statistic is significant, P=0.030).

DISCUSSION

The finding that Staphylococci spp is mostly isolated from urine as shown in this study is in conformity with previous studies.[18] In contrast, others opined that wound swab harbours more Staphylococci spp than other clinical samples.[19,20]

Quite a number of recent studies have shown that *S.aureus* is the causative agent of many infections in Nigeria involving the bloodstream, ear, skin and lower respiratory tract including many other infections which are difficult to treat.[4,21] On the other hand, Coagulase-negative Staphylococci (CoNS) which were previously regarded as either contaminants[22] or normal flora are now recognized as a major cause of significant clinical infections. They are associated with infections in the immune compromised host, bacteremia, wound-related infections, intravascular catheter-related infections and a variety of post-operative infections.[23]

Data from previous studies have shown that methicillin resistance among *Staphylococcus* spp is on the increase and constitute health challenges not only in Africa but also in Europe, America and Asia.[24] Due to the ability of Staphylococci to mutate over time; methicillin-resistant *Staphylococcus* spp will continue to constitute nuisance both in the hospital and community settings. The high rate of resistance to methicillin (Oxacillin) by *S. aureus* and CoNS isolates from clinical samples in this study area with no previous reasonable report of MRSA and MRCoNS prevalence, is alarming but not unexpected because it has been reported that MRSA prevalence is ever increasing.[25] This is similar toearlier reports from Ota, Ogun state in which 94-100% *S. aureus* isolated were reported to be methicillin-resistant.[26] The high prevalence rate shown in this study is also comparable to report in Lagos where 85% of *S. aureus* were methicillin resistant and multi-drug resistant.[27] Another study in Benin-city demonstrated that 79% of *S. aureus* isolates were methicillin resistant.[19] Similarly, MRCoNS prevalence rates of 82.4 % and 83.3%[28] were reported in conformity with the findings of this study.

High MRCoNS prevalence rate comparable to this study were previously reported as 87%[23] and 90%.[29] Contrary to the findings of this study, several previous studies in Nigeria have reported a lower prevalence rate of MRSA. These include69% prevalence rate reported from Zaria,[6] 47.8% prevalence rate reported from Osogbo,[30] 38.5%,[31] 35.7%,[12] 33.3%,[24] 30.4%,[20] and 11%[32] prevalence rates reported from Enugu, Uyo, Jimeta-Yola, Ibadan and Benin-city respectively. Similarly, the low prevalence

rate of 33.3%[12] for MRCoNS was also reported in Uyo, Nigeria. It is evident in this study that the proportion of *S.aureus* resistant to methicillin (94.4%) were more than that of *CoNS (83.3%) as shown by their prevalence rate*. This is contrary to the previous finding from Iran[10] *which revealed that CoNS are more resistant to methicillin than S.aureus.* Although the antibiotic resistant pattern of MRSA in developing countries is not uniform but varies from one country to the other, various reports have indicated that MRSA exhibits extreme resistance to other antibiotics in addition to methicillin.[33] Previous studies have also shown that methicillin-resistant *S.aureus* (MRSA) of clinical origin exhibits multi-drug resistant (MDR) phenotype.[18,27] This is in agreement with the findings of this study which demonstrated high MDR phenotype among MRSA and MRCoNS. Relatively high MDR MRSA has also been reported in some African countries.[32]High MDR phenotype in this study indicates the presence of strong selective pressure from antibiotics use in this community. Also, therapy associated with MDR strains are usually problematic and incurred huge financial drain on the hospital resources.[34] MDR was considered when an organism was non-susceptible to at least one agent in three or more antimicrobial categories or class.[35] The study further showed that MRSA and MRCoNS have high resistance to amoxicillin, ampiclox, ceftriaxone and cefuroxime which are -lactam antibiotics. This is comparable to an earlier report from Ogun State,[27] Delta State[36] and Ibadan[20] where it was reported that MRSA were also resistant to all antibiotics of -lactam class.[6] This may be due to the *mec*A gene, by a unique mobile genetic element, staphylococcal cassette chromosome *mec* (SCC*mec*) integrated into the *S.aureus* chromosome.[37]This observation can be correlated to commonly used and unauthorized prescription of these antibiotics. Also, exposure of isolates to these drugs enhances the development of high level of resistance.

The study demonstrated that both MRSA and MRCoNs are also resistant to classes of antibiotics other than the -lactam antibiotics which includes; perfloxacin, gentamycin, erythromycin, cotrimoxazole and streptomycin in varying proportions ranging from 30%-82%. This is commensurable to previous reports on MRSA and MRCoNS.[26] Contrary to the findings, a previous study in Zaria,[6] Delta[36] and Ibadan Nigeria[20] demonstrated a higher level of MRSA susceptibility to gentamycin and the fluoroquinolones. Thus, the existence of MRSA resistant to classes of antibiotics other than â-lactam antibiotics, in addition to penicillin and cephalosporin may limit the chances for recommending these drugs for therapy in the study area.

The pathogenicity of both *S. aureus* and Coagulase negative *Staphylococci* comes from their produced stock of virulence factors that enhance host's tissues invasion, their spread within the tissues and inhibition of phagocyte engulfment.[38] One of such virulence factors is hemolysin. Detection of and -hemolysin among MRSA and MRCoNS as shown in this study was reported previously[12] and detection of and -hemolysin among *S.aureus* and CoNS has been reported by various authors.[39] But -hemolysin was also reported among both MRSA and MRCoNS[12]unlike this study. Another study revealed that CoNS did not produce -hemolysin[40]which was contrary to the findings of this study. Most of the methicillin-resistant staphylococcal spp are rather -hemolytic (non-hemolytic) than -hemolytic contrary to previous report.[12] In agreement with findings of this study, a number of other studies have associated hemolysin production to antibacterial resistance.[12,13] In this study, lack of hemolysin (-hemolysis) contributed immensely to the antibiotic resistance pattern of MRCoNS, while production of hemolysin (-hemolysis) contributed immensely to the antibiotic resistance pattern of MRSA. This is in agreement with the report of Martinez-Martinez *et al*.[13] with respect to MRCoNS. According to them, antibiotic resistance (especially quinolones) was more on non-hemolytic *E.coli* than haemolytic *E.coli*. Contrary to the finding of this study however, a study from Edo state Nigeria showed that

there was no significant association between haemolysin productions and resistance to the antibacterial agents used in their study.[40] The reasons for this discrepancy are rather obscure but might not be unconnected with the sample size of each genera and the type of genera used. In the studies of Martinez-Martinez *et al.*[13] and Drews *et al.*[14] only one genera of *Escherichia* with a large number of strains was used while various genera of bacteria were used in the study of Egbe and Enabulele.[40] None of the studies used methicillin-resistant *Staphylococcus* spp as employed in this study.

CONCLUSION

The widespread dissemination of MDR phenotype among MRSA and MRCoNs may increase morbidity and mortality and complicate diagnosis. They may also decrease the effectiveness of drugs, increased hospital stay including the cost of chemotherapy and also limit the chances of recommending these drugs for empirical treatment. Therefore, this study underscores the need for periodic surveillance of MRSA infections especially in areas or among people that are at risk. There should be reasonable dispensing of antibiotics based on swift and dependable laboratory test. By and large, there is need to develop and enforce programs, policies and strategies that will underscore the menace of irrational or misuse of antibiotics in both hospital and community settings.

CONFLICT OF INTEREST

Authors have declared that there is no conflict of interest

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Comparison between Mean Prostate Specific Antigen Density in menwith Benign Prostatic Hyperplasia (BPH) Following Biopsy and Conventional Value of 0.15ng/ml/cc.

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ABSTRACT

Prostate specific antigen density (PSAD) is a volume corrected prostate specific antigen (PSA). It has been a very useful tool in discriminating between benign prostatic hyperplasia (BPH) and prostate cancer especially in the gray zone of the PSA (4-10ng/ml). The guideline for the diagnosis and treatment of prostate malignancy prescribes 0.15ng/ml per volume of prostate tissue as the cut-off value to enhance diagnostic suspicion of prostate cancer. This study was aimed at comparing the mean value of PSAD in our cohort of patients with the conventional value. Seventy-one patients with histological diagnosis of BPH were evaluated between January 2016 and December 2017. Their clinical information wascollated including bio-data, important findings on history and physical examination, imaging studies and prostate biopsy results. Data were analyzed using the statistical package for the social sciences (SPSS) version 20.0. The patients were aged between 50 and 85 years with a mean age of 64.79±8.09 years. Mean PSA was 4.95±3.24ng/ml, while mean prostate volume and PSAD were 73.20±57.82mls and 0.078±0.55ng/ml/ccrespectively. Using one samplet-test for data analysis, there was significant difference in means (.0716);P-value was set at 0.05. There was a marked difference in means between the PSAD values which was also statistically significant.

Keywords: Benign Prostatic, Hyperplasia, Prostate, Antigen, Density

INTRODUCTION

Mean Prostate Specific Antigen Density (PSAD) isa volume corrected PSA. It is defined as serum PSA divided by the volume of the prostate[1] and it is said to enhance the specificity of Prostate cancer diagnosis especially in the gray zone of the PSA (4-10ng/ml). PSAD complements PSA and digital rectal examination (DRE) in the diagnosis and treatment of Prostate cancer. Many cut-off values for PSAD have been documented in the literature to help discriminate between patients with BPH and Prostate cancer, but Tauro *et al*.[2] Lujan *et al*. [3] and Udeh *et al.* [4] proposed a mean cut-off value of 0.15ng/ml/cc of prostate tissue to minimize false positive results.Despite increased sensitivity and specificity recorded at this cut-off value, Sasaki *et al.*[5] and Lujan et al[3]advocated a shift to 0.18ng/ml/cc since the former value missed 8 cases of cancer and 43% of patients underwent unnecessary prostate biopsies in their study.[5] However, Sarkar *et al*.[6] did not miss any cancer case at the same cut-off value. All these efforts are geared towards achieving 100% sensitivity and an acceptable high specificity to prevent missed cases of cancer and unnecessary prostate biopsy.

PSA is a tumour marker for the diagnosis and treatment of patients with Prostate cancer. Although with acceptable sensitivity, it lacks adequate specificity especially when used as a screening test.[8] It is organ specific but not cancer specific and can be elevated in benign conditions like BPH and Prostatitis. Its value can also be raised after DRE and prostate biopsy. PSA is secreted by the ductal epithelial cells of the prostate and thought to be dependent on prostate volume and the number of epithelial cells in the prostate.[9] Since benign conditions can also produce clinically significant PSA (i.e. above the reference range of 0-4ng/ml) and indicate a need to rule out a malignant lesion, the concept of PSAD had evolved to checkmate unnecessary biopsies based on a conventionally applied guideline for diagnosis and treatment of Prostate cancer.[10]Although this concept is faced with a lot of controversies, some authors have adopted it with close monitoring of their patients in combination with other tools such

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as PSA velocity and free to total PSA ratio to optimize accurate diagnosis while minimizing unnecessary biopsies with its attendant complications. The purpose of this study was to compare the mean PSAD in our cohort of BPH patients with the guideline cut-off value.

MATERIALS AND METHOD

This study was conducted retrospectively in the histopathology laboratory for prostate biopsy results and the health records department for patient's case notes between January 2016 to December 2017. They were 71 patients that met the inclusion criteria. Exclusion criteria included patients diagnosed with Prostate cancer, bladder cancer, or urinary tract infection; or patients who had prior urethral instrumentation, prostate biopsy and patients with incomplete clinical information. Information retrieved included findings in the history and physical examination and relevant investigation results such as full blood count, fasting blood sugar, renal function test, PSA and prostate scan with trans-rectal ultrasound scan guided prostate biopsy. Ten (10) to twelve (12) cores of tissues were taken and sent for histopathological analysis. Data collected were entered into a structured proforma. Statistical analysis was done using SPSS (Statistical Package for Social Sciences) version 20.0software. Frequency of variables were determined. Continuous variables were summarized using means and standard deviations. One sample t-test was used to obtain the difference between the means; P-value was set at <0.05.

RESULTS

71 patients with histological evidence of BPH aged between 50 and 85 years were studied. Mean age was 64.79±8.09 years while mean PSA was 4.95±3.24ng/ml. Mean PSAD was 0.078±0.055ng/ml/cc and mean prostate volume was 73.20 ± 57.82mls. Majority of the patients were in their 7th decade of life (43.7%). Retired civil servants were more in number (38.0%). Patients with PSAD <0.15 were 86.0% while those >0.15 were 14.0%. Using one samplet-test statistics, there was a statistically significant difference between mean PSAD recorded in our study and the conventional cut-off value (P-value <0.05) with a mean difference of 0.0716. Prostate volume correlated with PSA, r(71) = 0.40; P<0.05 and inversely with PSAD; r(71) = -0.31 P<0.05

Table 1:
(i) Frequency Table for Age

Age (years)	Frequency (n)	Percent (%)	Cumulative Percent (%)
50-59	18	25.4	25.4
60-69	31	43.7	69.0
70-79	19	26.8	95.8
80-89	3	4.2	100.0
Total	71	100.0	

(ii) Frequency Table for occupation

Occupation	Frequency (n)	Percent (%)	Cumulative Percent (%)
Farming	4	5.6	5.6
Trading	13	18.3	23.9
Business	4	5.6	29.6
Civil Servant	15	21.1	50.7
Retired Civil Servant	27	38.0	88.7
Clergy	8	11.3	100.0
Total	71	100.0	

(iii) Frequency Table for PSAD

Value	Frequency (n)	Percent (%)	Cumulative Percent (%)
<0.15	61	86.0	86.0
>0.15	10	14.0	100.0
Total	71	100.0	

Table 2: Descriptive Statistics for Variables

Variable	Mean	Standard Deviation
Age	64.79	8.092
PSA	4.95	3.247
PSAD	.078	0.055
PV	73.20	57.820

Table 3: Independent t-test

		Test value = 0.15		
Number (n)	Mean	Std. Deviation	Mean Difference	P-value
71	0.078	0.0556	-0.0716	0.000

Table 4: Correlations between Variables

			r	P-value
PV	versus	PSA	0.410	.000*
PV	versus	PSAD	-0.313	.008*
PSA	versus	PSAD	0.565	.000*

*Correlation is significant at P<.05.

DISCUSSION

Prostate specific antigen density is defined as serum PSA divided by the volume of the prostate.[1] It depends on the prostate volume and serum PSA value. PSAD is said to discriminate between BPH and Prostate cancer and many cut-off values have been postulated with different results. Benson *et al.*[10] documented a cut-off value of 0.15ng/ml/cc which currently serves as an international guideline in the diagnosis and treatment of prostate cancer. Benign conditions of the prostate such as BPH can result in elevated PSA mainly due to increased volume of the prostate and application of PSAD as a tool for evaluation obviates the need for biopsy since PSAD remains superior to PSA in the diagnosis of Prostate cancer.[11] The use of PSA alone in prostate cancer diagnosis often leads to over-diagnosis and unnecessary biopsy.[12]Fu-Xiang L *et al.,*[13]using the conventional PSAD cut-off value recorded a sensitivity of 86.6% and a specificity of 71.2% for diagnosis of prostate malignancy while Zlotta et al[14] reported a sensitivity and specificity of 74.3% and 65.9% respectively. Yet Sarkar *et al.*[6] had a 100% sensitivity and 78.38% specificity.These authors further experimented on a PSAD cut-off value of 0.18ng/ml/cc and got a specificity of 91.59% while maintaining 100% sensitivity. All focus is directed on eliminating false positive and negative results especially in the gray zone of the PSA. However, these studies failed to document the histology reports of those missed cancer cases to assess the clinical significance and prognosis of those patients to warrant concerns. The natural history of Prostate cancer is that of a slow growing

tumour of which may not manifest clinically throughout the lifetime of the sufferer. Moreso, age of these patients were not mentioned together with the number of biopsy cores taken which could have also affected cancer detection especially when cores were few. Besides our concerns raised above, Oesterling LE *et al.*[15] noted that PSAD varies with age and as a component of the equation, Kleer E et al[16] reported an approximately 10% error in prostate volume measurement using transrectal ultrasound scan (TRUS). Moreover, there is about a three-fold difference in the ratio of epithelium to stroma between prostates,[17] while prostate volume and as a follow up with serum PSA, varies between races.[18,19] Prostate volume has been noted to be lower in the Japanese population than in whites with more PSA per unit Prostate volume recorded in the Japanese men.[19] This also emphasizes the need to incorporate racial and ethnic differences in making decisions about PSAD cut-off values.

The mean age of our patients was 64.79 ± .09 years (Table 2) similar to another study in Nigerian men with BPH[20] and lower than a study of Italian men with the same condition.[21] The peak incidence occurred in the 7th decade of life. This was also recorded among Indian men.[20] Mean PSA was 4.95±3.24ng/ml (Table 2) which was higher than mean PSA in another study even with a similar mean age.[22] Mean PV was 73.20 ± 57.82mls (Table 2) in gross excess of the Indian study reflecting an increase in PSA as PV increases, background racial and ethnic differences notwithstanding.[22] We noted that 86.0% of patients had PSAD value <0.15ng/ml/cc while the remaining14.0% had PSAD > 0.15ng/ml/cc [Table 1(iii)]. The latter group had smaller prostates that magnified PSAD at a given PSA. Here, prostate biopsy could not completely rule out foci of malignancy, the study being retrospective, but they will be followed up with repeat DRE, PSA and repeat biopsy where indicated. Mean PSAD was 0.078 ± 0.55ng/ml/cc (Table 2). Other authors recorded values between 0.17-0.27ng/ml/cc.[23-25] Their mean PV was also lower than in our study which can account for the higher value of PSAD for a given serum PSA.[23] Our patients were noted to have a higher prostate volume thereby reducing the PSAD at a given serum PSA. Again, they were hospital based who only presented with severe symptoms and advancing age accounting for the high Prostate volume since most studies had already documented a positive correlation between Prostate volume and age.[15,23,26]

Comparing mean PSAD with the standard guideline value, there was a significant difference in means with a mean difference of -0.0716 (P-value<0.05) (Table 4). This implies that our men have larger prostates and relatively lower levels of serum PSA which therefore accounts for the lower mean PSAD value. We will need a longitudinal study to characterize and probably determine our local cut-off value to prevent misdiagnosis and most importantly deploy PSA velocity, PSA total and free ratio as adjuncts, to better discriminate between BPH and Prostate cancer prior to prostate biopsy, when indicated. Prostate volume correlated inversely with PSAD (Table 4); meaning that the higher the Prostate volume the lower the values of PSAD.

Some limitations of this study include the retrospective nature which did not allow verification of true absence of malignancy. A pre-biopsy prostate multi-parametric MRI was not done because this facility is not available at the index hospital. Being a hospital based study, we may have used patients with larger prostates than the mean in the general population. However, we consider our study to be informative enough to promote and stimulate future research in this area.

CONCLUSION

PSAD has been one of the tools that can be used to discriminate between BPH and Prostate cancer especially in the gray zone of PSA. In our study, there was a significant difference between mean PSAD and the guideline cut-off value suggesting bigger prostate volumes that produce comparatively less PSA. Further study is needed to standardize our local value since the conventional cut-off value is already influenced by contending variables such as ethnic and racial factors apart from differences in the methodology, study design as well as age and number of patients studied.

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Correlation between Prostate Volume and International Prostate Symptom Score with Quality of Life in Men with Benign Prostatic Hyperplasia in South-South, Nigeria

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ABSTRACT

Benign prostatic hyperplasia (BPH) is a disease of the ageing male. Clinically, patients present with lower urinary tract symptoms (LUTS) which are usually both storage and voiding secondary to bladder outlet obstruction. International prostate symptom score (IPSS) is used to asses symptom severity and the need for treatment. Prostate volume on the other hand, though important in evaluating the patient does not predict symptom severity. This study aimed to determine the correlation between prostate volume and IPPS with Quality of Life (Qol)in patients diagnosed with symptomatic BPH. This was a prospective study involving eighty nine patients aged 43 to 84 years seen at the Urology clinic between January to December 2018 in whom a detailed history, physical examination and transrectal ultrasound scan of the prostate was performed. The mean age of the patients was 64.02 ±9.60 years while the mean prostate volume was 64.94±42.95mls, mean IPSS was 14.47±5.28 and mean Qol was 4.55 ± 0.97. Correlation between prostate volume and IPSS was weak though statistically significant, no correlation was found between prostate volume and Qol. Prostate volume demonstrated a weak correlation with IPSS while there was no correlation with quality of life scale.

Keywords: Benign, Prostatic, Prostate, Hyperplasia, Volume, Life

INTRODUCTION

Benign prostatic hyperplasia has been known to be a common disease affecting ageing males. It is a progressive disease and patients usually present with lower urinary tract symptoms which may deteriorate over time.[1] Symptom progression has been shown to impact negatively on the health-related quality of life of the sufferers.[2] Lower urinary tract symptoms (LUTS) should be complemented with a rectal examination of the prostate for size although symptom severity is a better guide for BPH management than prostate volume.[3]

In 1992, Barry *et al.*[4] developed the American Urological Association (AUA) score and was later in 1994 adopted by the World Health Organization (WHO) as the international prostate symptom score (IPSS) meant to assess LUTS severity with the inclusion of the quality of life scale.[5] Each symptom is measured on a scale of 0-5 (0=No symptom to 5 = symptoms almost always present). Disease-specific quality of life (Qol) question assesses the level of satisfaction with the above symptoms ranging from 0 (Delighted) to 6 (Terrible). IPSS, besides assessing symptom severity is a useful tool for evaluating therapeutic outcome. Prostate volume can be crudely assessed by digital rectal examination, but a trans-rectal ultrasound scan (TRUSS) measurement is far more accurate[6] and only limited by the ability to assess the upper tracts for which trans-abdominal ultrasound scan can be used. This study aimed to evaluate thecorrelation between prostate volume and IPSS with Qolin men with symptomatic BPH.

MATERIALS AND METHOD

This study was carried out at the University of Uyo Teaching Hospital, Uyo in South-South Nigeria on men who were referred from the general out-patient clinic to urology clinic from January 2018 to December 2018 on account of LUTS. Exclusion criteria were patients diagnosed with prostate cancer, bladder cancer, Urethral stricture, history of prostate surgery and neurogenic bladder from any cause.

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Eighty-nine (89) patients who met the inclusion criteria were evaluated with detailed history taking, physical examination and relevant urological investigations. The inclusion criteria were patients who presented with LUTS, normal findings on rectal examination of the prostate and a PSA of <4.0ng/ml. The international prostate symptom score is a numerical scoring system meant to grade the severity of seven lower urinary tract symptoms based on the frequency of bother. Physical examination was detailed with a focused rectal examination to assess and characterize the nature of prostate enlargements. Relevant laboratory investigations including full blood count, renal function test, fasting blood sugar, Prostate-specific antigen (PSA), urine analysis, microscopy and culture were carried out. A trans-rectal ultrasound scan was used to measure the prostate volume using the prolate formula; AP X T X Cranio-caudal diameter x 0.52.

Statistical Analysis

Data from filled protocols was analyzed using statistical package for social sciences (SPSS) version 20. Descriptive statistics were used to find the frequency, mean and standard deviation for the variables while Pearson correlation was used to assess the relationship between prostate volume and IPSS/Qol. Statistical significance was set at $P<0.05$.

RESULTS

Eighty-nine (89) patients were evaluated with a mean age of 64.02±9.60 years ranging from 43 to 84 years. Majority of them were retired civil servants (38.2%) compared to other categories of occupation (Table 1). Mean IPSS was 14.47±5.28. Categories of symptom severity were 14.1%, 78.8%, 7.1% for severe, moderate and mild respectively. Mean Qol was 4.55±0.97. Mean prostate volume was 64.94±42.95mls while the mean PSA was 7.71±11.25ng/ml. Correlation between prostate volume and IPSS was weak but statistically significant; $r(89) = .25$, $P<.05$ and there was no correlation between prostate volume and Qol; $r(89) = .08$, $P>.05$.

Table 1: Categories of occupation of male patients in the Urology clinic referred on account of LUTS in 2018

	Frequency(n)	Percent(%)	Cumulative Percent(%)
Farming	9	10.1	10.1
Trading	10	11.2	21.3
Business	4	4.5	25.3
Civil Servant	23	25.8	51.7
Retired Civil Serv.	34	38.2	89.9
Clergy	9	10.1	100.0
Total	**89**	**100.0**	

Table 2: Descriptive statistics of parameters assessed in the index sample

	N	Mean	Standard deviation	Range
cAge	89	64.02	9.60	41.0
PSA	89	7.71	11.25	70.7
Prostate Volume	89	64.94	42.95	256.0
IPSS	89	14.47	5.28	30.0
Qol	89	4.55	0.97	4.0

Table 3: Showing Pearson correlation of Prostate volume

	Correlation	
	IPSS	QOL
Prostate Volume		
Pearson correlation	253	.088
Sig. (2- tailed)	.017*	.411
N.	89	89

*Correlation is significant at the .05 level.

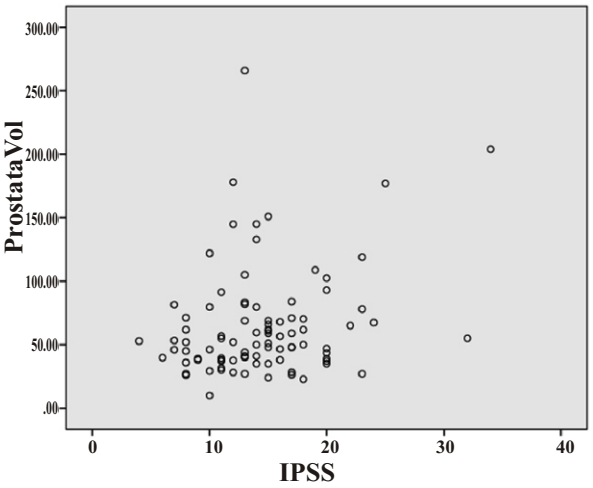

Figure 1: Scatter plot - Correlation between prostate volume and IPSS

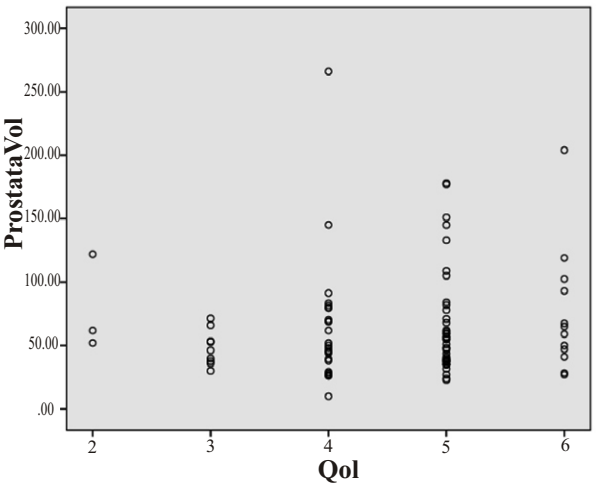

Figure 2: Scatter plot - Correlation between prostate volume and Qol.

DISCUSSION

BPH is a chronic and progressive disease[7] affecting ageing males with a reduction in health-related quality of life. The sufferers usually present with LUTS whose severity and degree of bother can be assessed with a World Health Organization (WHO) tool notably IPSS and QoL Scale. Measurement of prostate volume is an important adjunct in the management of BPH patients that may guide surgical technique and choice of medical therapy. Symptoms severity as categorized by IPSS is a useful tool to determine the need for treatment and assessment of treatment outcome.

In this study, we set out to determine the correlation between prostate volume and IPSS with Qol. Mean age of the patients was 64.02±9.60 years, majority of them were in their seventh (7^{th}) decade of life. This has been consistently reported by other authors[3,8-11] who also evaluated men with BPH symptomatology. Retired civil servants formed the bulk of the patients as compared to other categories of occupation. This is in line with the Nigerian Civil Service Rule where the retirement age is set at 60 years. But in another study[9] also documenting Nigerian men, farmers were more in number of which some of them may have retired from civil service and took to farming.

The mean value for IPSS was 14.47±5.28 and most of them (78.7%) had moderate symptoms. This same observation was recorded in other studies[8,9,12-14] across Africa and Asia. In the same continents of Africa and Asia, other studies documented predominantly severe symptom score.[15,16] This moderate to severe symptoms may be due to late presentation, further explained by lack of awareness of prostate diseases, superstition and poverty that plague some parts of Asia and Sub-Saharan Africa. Therefore, there is need for health awareness, education and poverty alleviation to solve many challenging conditions including symptoms due to BPH.

Mean prostate volume in this study was 64.94±42.95mls in gross excess of that recorded in another study in same south-south Nigeria (22.85mls). This is probably because the men in the latter group were evaluated in a screening programme who were also younger, in contradistinction to the ones that presented with symptoms and advanced age to our facility for care. A similar study in Europe and America [17] reported yet smaller prostate volumes which could be due to gross awareness and early

presentation for care typical of the Caucasians as opposed to late presentations in our environment.

Clinical BPH can be defined as prostate adenoma irrespective of size, causing varying degree of obstruction with or without symptoms .[18]McNeal[19] postulated the zones of the prostate that are involved in BPH being the transition zone (TZ) and the peri-urethral zone (PZ). Randall[20] reported that adenomatous growth from the TZ forms the lateral lobes while growth from the PZ forms the median lobe and that the degree of obstruction depends on where the growth is sited rather than the prostate size. Keong[21] confirmed that intra-prostatic protrusion (median lobe) is more important than prostate volume in causing obstruction. He added that the median lobe obstructs by distorting the Prostatic Urethra while the lateral lobes compress the urethra with lesser magnitude of obstruction.

This summary of the pathophysiology of clinical BPH may explain in part the controversial reports and results on the correlation between prostate volumes and IPSS with Qol in BPH patients. In our study, there was a weak positive correlation between prostate volume and IPSS that was statistically significant $r(89)=25$, $P<.05$, while there was no correlation between prostate volume and QOL $r(89)=08$, $P>.05$. Other studies also documented same findings,[8,9,13,15,17,22,23] yet many authors reported contrary findings of no correlation between the two variables.[3,12,16,24,25] This lack of homogeneity of reports across board suggests few facts of clinical interest to the clinician. Firstly, the prostate volume does not predict the severity of clinical symptoms and so should not be used in isolation to guide selection of patients for care (watchful waiting, medical or surgical treatments). Secondly, prostate volume alongside symptom severity and urodynamic studies (to rule out detrusor under-activity), can guide the choice of modalities of treatment (medical or surgical). Lastly, prostate volume in combination with symptom severity can guarantee choice of surgical techniques (minimal access or open).

CONCLUSION

This study finds a weak but positive correlation between prostate volume and IPSS which was also statistically significant in support of other studies done on this subject. However, there was no correlation between prostate volume and QoL. Other studies across the globe documents mixed reports. Correlation being weak, prostate volume alone should not be used to predict the degree of bladder outlet obstruction vis-a-vis severity of lower urinary tract symptoms and the degree of bother to patients.

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