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INDUCIBLE MACROLIDE RESISTANCE PHENOTYPES AND GENOTYPES IN CLINICAL ISOLATES OF *Staphylococcus aureus* FROM NNAMDI AZIKIWE UNIVERSITY TEACHING HOSPITAL, NNEWI, SOUTH EAST, NIGERIA

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ABSTRACT

Background: The widespread use of macrolide, lincosamide, and streptogramins-B (MLS-B) to treat Staphylococcal infections has caused an increase in resistance to these types of antibiotics.
Aim: The aim of this study is to identify macrolide resistant phenotypes and detect *erm* genes associated with macrolide resistance in *Staphylococcus aureus*.
Methodology: A total of 304 Gram positive cocci isolated from different clinical samples received at the Medical Microbiology Laboratory of Nnamdi Azikiwe University Teaching Hospital (NAUTH), Nnewi were used for this study. Oxoid Staphytect agglutination test kits were used to confirm 185 of these isolates as *Staphylococcus aureus*. Antibiotic sensitivity testing was done on the 185 verified *S. aureus* isolates while the D-test was used to check for macrolide resistance. Twenty (20) of the D-test positive isolates were tested for the presence of *erm* A, B, and C genes using a multiplex PCR method.
Results: Results showed that the occurrence of inducible MLS-B phenotype was 23 out of 185 (12.4%) while 46 out of 185 (24.9%) of the isolates displayed the constitutive MLS-B phenotype. Out of the 20 resistant isolates tested for the presence of resistance genes, 8 (40%) tested positive for *erm* C, while none possessed either *erm* A or *erm* B genes. All 8 of the *erm* C positive isolates were resistant to methicillin (MRSA). The iMLS-B phenotypes were more frequently observed in isolates that tested positive for *erm* C compared to the cMLS-β phenotype.

Conclusion: This study stresses on the need to be aware that iMLSB and cMLSB phenotypes exist among clinical isolates of *S. aureus* and that resistant genes are found in some of these isolates. Such isolates should be sought for during routine laboratory investigations in order to avoid possible treatment failure.
Keywords: *Staphylococcus aureus*; macrolides; resistance; erm genes

Introduction

Staphylococcus aureus is a major cause of infections acquired in healthcare settings and is a prevalent bacterium that causes many types of infections in people of all ages[1]. It is a very effective bacteria that often colonizes the skin and mucosa of people and animals especially in the nose, on the skin, or both areas, causing infections when given the opportunity. Outbreaks of infections can occur with community outbreaks often linked to inadequate hygiene and the spread of germs from person to person through objects. Hospital outbreaks caused by one type of *S. aureus* strain typically affects patients who have had surgery or other invasive treatments.

Macrolides, like erythromycin, azithromycin, and clarithromycin; lincosamides such as clindamycin, and streptogramin-β like quinupristin are classes of antibiotics called MLS-β. They affect the 23S rRNA of the big 50S ribosomal subunit, which stops protein production[2]. The MLS-β group of antibiotics is one of the few remaining options to treat skin and soft tissue infections caused by both methicillin susceptible *S. aureus* (MSSA) and methicillin resistant *S. aureus* (MRSA). The widespread use of macrolide, lincosamide, and streptogramins-β (MLS-β) antibiotics in treating staphylococcal infections has caused a rise in resistance to these antibiotic families. Staphylococci mostly develop resistance to these antibiotics by efflux pumps[3], target change and drug inactivation[4].

Methicillin resistant *S. aureus* are clinically important pathogens because of their ability to resist all beta-lactam and other antibacterial agents thereby limiting treatment options for MRSA infections[5]. As MRSA are associated with myriads of infections in healthcare facilities as well as in the community, knowledge of the local antibiotic resistance patterns and carriage of virulence genes by these strains can enhance better treatment outcomes, and the control and/or prevention of infections[6,7].

It has been observed that in Nigeria, testing for antibiotic susceptibility of isolates usually relies on phenotypic testing and is hindered by the absence of evaluation of the molecular mechanism behind the resistance to the drugs. In addition, recent research have used PCR to detect *erm* genes to identify and confirm macrolide resistance in *S. aureus*, however; information on the genetic factors of macrolide resistance in *S. aureus* in Nigeria is somewhat limited[7,8,9]. So, using molecular screening to find the genetic cause of *S. aureus* resistance to macrolides will be very useful in reducing and implementing adequate control measures against infections caused by *S. aureus* bacteria. This study therefore sought for macrolide resistance in *S. aureus* isolated from clinical samples from Nnamdi Azikiwe University Teaching Hospital (NAUTH), Nnewi and also focused on detecting the presence of the erm genes responsible for target site modification in the bacteria. The result is expected to aid clinicians decide on the right therapy choices for most infections caused by *S. aureus*.

Materials and Methods

Three hundred and four (304) Gram positive coccal-shaped bacteria isolated from different clinical samples received at the Medical Microbiology Laboratory of NAUTH were used for this study. All the isolates were presumptively re-identified using standard microbiology techniques, including colony morphology on growth media, Gram staining, catalase, coagulase and DNAse tests. Of these, 185 were confirmed to be *S. aureus* using the Oxoid Staphytect agglutination test kits[10].

The Kirby–Bauer disk diffusion method was used to evaluate the antimicrobial susceptibility profiles of the confirmed *S. aureus* isolates, using a standardized single antibiotic disk on Oxoid Mueller–Hinton agar (MHA) as described by[11.12.] Various commercially available antibiotic discs (Oxoid Ltd Basingstoke) were used to determine susceptibilities of the isolates to the test antibiotics[10]. The diameter of the zone of inhibition for each antibiotic produced by the *S. aureus* isolates was measured in millimetres (mm), and this was considered as either sensitive or resistant to the test antibiotics based on the documented breakpoint guidelines of the CLSI standard interpretive criteria[13].

Methicillin resistance was evaluated using 30µg of cefoxitin. Isolates that had an inhibition diameter of ≥ 22mm were reported as methicillin susceptible *S. aureus* (MSSA) while those with zone diameters of ≤ 21mm were reported as methicillin resistant *S. aureus* (MRSA). This was done by measuring the inhibition zone diameter around the cefoxitin disc.

Double disk diffusion test (D-test) was used to screen for macrolide resistant phenotypes[14]. Isolates that were resistant to erythromycin but were susceptible to clindamycin with flattening or blunting of the inhibition zone around the clindamycin disc in a D-shaped form (D-Test positive) were reported as inducible-MLS-β (iMLS-β) phenotype. Also, MS phenotype was reported for isolates that showed resistance to erythromycin with no flattening or blunting of the inhibition zone around the clindamycin disc in a D-shaped form (D-Test negative). D-test positive isolates were screened for the presence of *erm* A, *erm* B and *erm* C genes using multiplex Polymerase Chain Reaction (PCR) technique.

Data collected was computed and statistical analysis was carried out using Statistical Package for Social Sciences version 22.0 (SPSS Chicago, USA). Frequency distribution tables and figures were used to show results.

Results

Among the 185 confirmed *S. aureus* isolates, 167 (90.3%) were found to be resistant to erythromycin, 165 (89. %) were resistant to cefoxitin and the least number of resistant isolates were to linezolide 48 (25.9%) (Table 1). Forty-four isolates from the 167 erythromycin resistant isolates were susceptible to clindamycin (26.3%). The D-test conducted on these 44 erythromycin resistant, clindamycin susceptible isolates revealed that 23 (52.3%) isolates were positive for the D-Test (Table 2).

In all, inducible macrolide resistance (inducible MLS-B phenotype) was demonstrated by 23 out of 185 (12.4%) *S. aureus* isolates, while 46 out of 185 (24.9%) *S. aureus* isolates were resistant to both erythromycin, clindamycin and linezolide (constitutive MLS-B phenotype). Also, 2 (1.1%) isolates were resistant to erythromycin and linezolide (MS-B phenotype) while 8 (4.3%) isolates were sensitive to erythromycin but resistant to clindamycin (L-phenotype) (Table 3).

Among the 165 MRSA isolates, 46 (27.9%) had constitutive MLS-B (cMLS-B)

phenotype while 38 (23.0%) showed inducible MLS-B (iMLS-B) phenotype. In addition, 6 (30%) of the 20 MSSA isolates showed iMLS-B phenotype while the cMLS-B phenotype was absent in MSSA isolates (Table 3). The resistance profile of the 165 Methicillin resistant *Stapylococcus aureus* (MRSA) isolates are shown in Figure 1.

The findings from the molecular screening for macrolide resistance genes in Figure 2 indicate that out of the 20 D-test positive *S. aureus* isolates that were screened for macrolide resistance genes, 8 (40%) isolates were positive for *erm C* gene. Of the 20 isolates also, none had the *erm A* or *erm B* genes (Figure 2). Also, all of the 8 *erm C* positive isolates were resistant to cefoxitin (MRSA).

Table 1: Resistance/Susceptibility profile of *S. aureus* to different tested Antibiotic classes (N=185)

Antibiotic type	Antibiotic Class	No. Susceptible (%)	No. Resistance (%)
Erythromycin	Macrolide	18 (9.7)	167 (90.3)
Clindamycin	Lincosanimide	54 (29.2)	131 (70.8)
Linozolide	Streptogramin-B	137 (74.1)	48 (25.9)
Cefoxitin	Cephalosporin	20 (10.8)	165 (89.1)
Ciprofloxacin	Fluoroquinolone	49 (26.5)	136 (73.5)
Trimetoprim/ Sulpumethoxazole	Folate Antagonist	50 (27)	135 (73)
Quinupristin/ Dalfopristin	Oxazolidinone	136 (73.5)	49 (26.5)
Gentamycin	Aminoglycoside	82 (44.3)	103 (55.7)

Table 2: Antimicrobial Susceptibility Test Result of the D-Test Positive Isolates (N = 44)

Antibiotic Class	SUSCEPTIBLE No. (%)	RESISTANT No. (%)
Erythromycin	0 (0)	44 (100)
Clindamycin	44 (100)	0 (0)
Linezolide	42 (95.5)	2 (4.5)
D-Test	23 (52.3)	21 (47.7)

Table 3: Occurrence of macrolide phenotypes in *S. aureus* isolates

Type of Macrolide phenotype	No. (%) of total isolates (N=185)	No. (%) of MRSA isolates (N=165)	No. (%) of MSSA isolates (N=20)
iMLS-β Phenotype	23 (12.4)	38 (23.0)	6 (30)
cMLS-β Phenotype	46 (24.9)	46 (27.9)	0(0)
MS-β Phenotype	2 (1.1)	0(0)	0(0)
L- Phenotype	8(4.3)	0(0)	0(0)

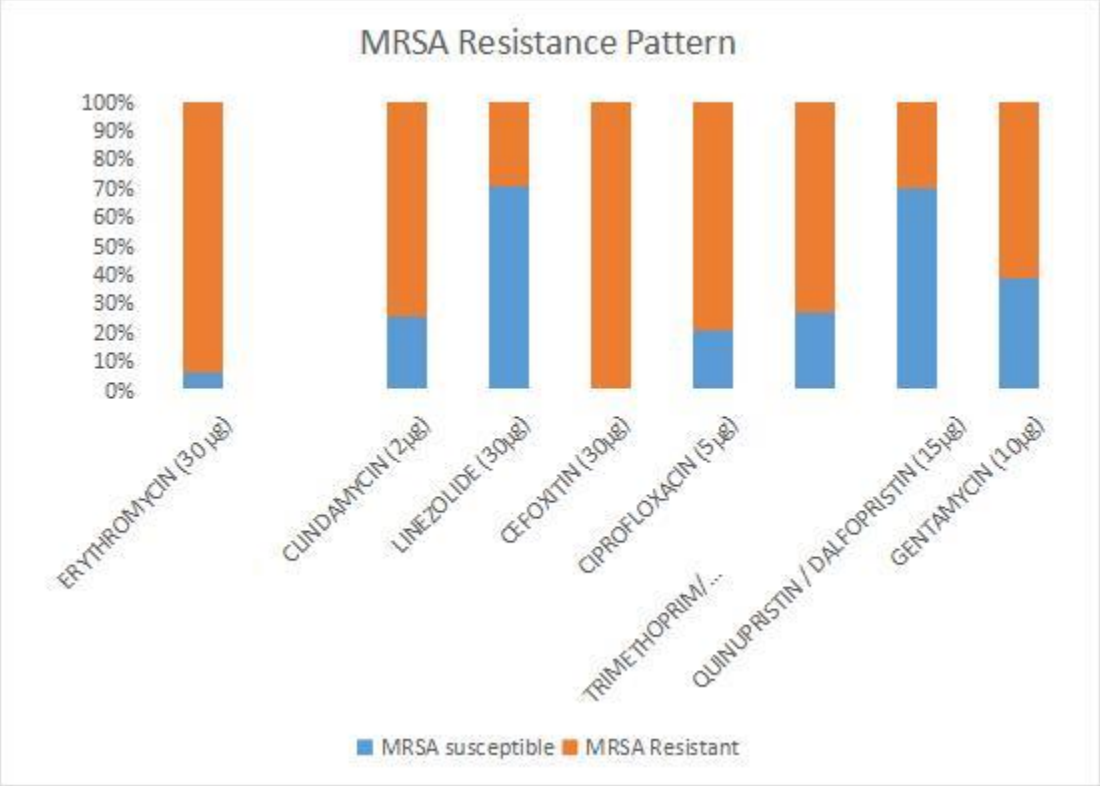

Fig 1: **Resistance Profile of MRSA**

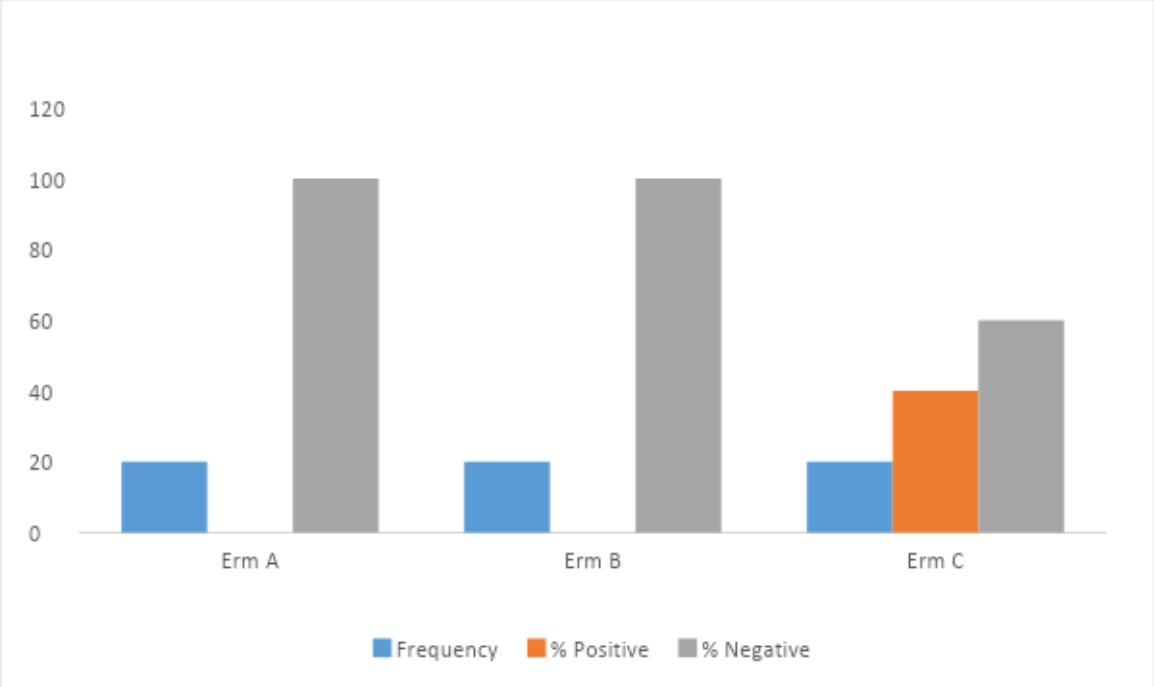

Figure 2: **Distribution of *erm* Gene in *S. aureus* Isolates**

Discussion

The results of this study shows that the overall prevalence of inducible (12.4%) and constitutive (24.9%) clindamycin resistance (iMLS-β and cMLS-β) among *S. aureus* isolates was consistent with the findings of another researcher who reported an overall prevalence of iMLS-β and cMLS-β resistance as 15.4 % and 20.5 % respectively[15]. Likewise, the high prevalence of erythromycin and clindamycin resistance obtained in this study with 90.3% and 70.8% respectively, is similar to those with overall prevalence of 74.4% and 76.9% for erythromycin and clindamycin respectively[15]. Two previous studies in Iran and Nepal recorded equally high inducible clindamycin resistance of 12.5% and 23.4% respectively[16,17]. These findings are contrary to those of other investigators who recorded an iMLS-β prevalence of 5.4 % and a cMLS-β prevalence of 6.2%[17]. These differences could be due to function of accuracy of diagnosis, study population[17], variation in geographical locations and specific characteristics of the healthcare facility.

Very high prevalences for erythromycin and cefoxitin resistance of 90.3% and 89.2% respectively were recorded in the present study, as was similarly reported by[9,18.]. Resistance to macrolides, lincosamides, and streptogramin-B (MLS-B) in most strains of *Staphylococcus aureus* correlates with resistance to methicillin[19]. This report corresponds with the findings of our study where 27.9% of the MRSA isolates exhibited cMLS-B resistance while 23.0% had iMLS-B resistance. These findings suggest that methicillin resistance in *S.*

aureus equally leads to resistance to other antibiotics, particularly macrolides. Macrolide-resistant methicillin resistant *Staphylococcus aureus* (MR-MRSA) strains pose a serious health issues because they are mostly implicated in difficult-to-treat infections that usually result in higher treatment cost and longer hospital stays[19].

The 40% prevalence recorded for *erm* C and non-availability of other *erm* genes indicates that *erm* C is the most dominant resistant gene in this part of the world. *Erm* C has been reported to be the most dominant macrolide resistance gene in Africa and Asia[20]. Other macrolide resistance genes, *erm* A and *erm* B, have been reported to be most dominant in Tunisia, Denmark and United Kingdom[16]. Most studies in Nigeria seek to determine the prevalence of inducible clindamycin resistance in clinical isolates of *S. aureus***,** hence there is limited data on the genetic factors of macrolide resistance in clinical isolates of *S. aureus* in this region.

The findings from this study show that iMLS-B phenotypes were more frequently observed among *erm* C positive isolates, compared to the cMLS-B phenotype. This could be due to the transfer of resistance genes between isolates through horizontal gene transfer, as macrolide resistance genes are usually found on mobile genetic elements[21]. Additionally, all the *erm* C positive isolates were resistant to cefoxitin, indicating that MRSA has a resistance to multiple drugs.

The high prevalence of MLS-B resistant genes found in isolates from this study may be due to the transfer of resistant genes among isolates. The non-detection of cMLS-B and iMLS-B phenotypes amongst *S. aureus* isolates as well as inability to look out for other resistance phenotypes from clinical samples in hospital laboratories in Nigeria could result in treatment failure in our hospitals. It is of utmost importance for Nigerian hospitals to be on the lookout for inducible-clindamycin resistance (iMLS-B) and constitutive-clindamycin resistance (cMLS-B) phenotypes amongst *S. aureus* isolates from clinical samples owing to the clinical importance of antibiotics in the MLS-B family.

Conclusion

The increasing frequency of therapeutic failures of clindamycin used for treating *S. aureus* infections especially those that were susceptible to it but actually resistant to erythromycin, necessitates the need for clinical laboratories to include screening for iMLS-B and cMLS-B in their routine work. Therefore, the use of correct diagnostic methods and the right antibiotic for treating macrolide-resistant methicillin-resistant *S. aureus* (MR-MRSA) isolates will not only reduce mortality in patients but also preserve the few remaining alternatives for future use.

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DOMINANT BODY SOMATOTYPE AND GENDER DIFFERENCES IN HAND GRIP STRENGTH OF YOUNG ADULTS IN A NIGERIAN UNIVERSITY

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Abstract

Background: Hand Grip Strength (HGS) is a measure of the grasping power of an individual and a known indicator of physical capability in males and female who evidently have different body compositions, and may be used to evaluate patient recovery progress throughout injury treatment and rehabilitation.
Aim/ Objective: To determine the influence of dominant body somatotype and gender on hand grip strength of young Adults in a Nigerian University.
Material/Methods: This was an ex-post facto research which was carried out among 162 undergraduates in Southern Nigeria. An electronic handheld dynamometer was used to evaluate the handgrip strength while the Heath-Carter Instruction Manual was used to determine the anthropometric dominant body somatotype. Data collected was summarized using descriptive statistics of frequency, percentages, mean and standard deviation, and analyzed using inferential statistics of Pearson's Product Moment Correlation, Two-way and one-way ANOVA at an alpha level of 0.05.
Result: Endomorphy was more predominant in the population than mesomorphy and ectomorphy (48.1%, 25.3% and 26.5%). A significant effect was found in dominant body somatotype on the left and right hand grip strength ($t = 11.959$, $p = 0.001$ and $t = 9.817$, $p = 0.001$) with mesomorphy having the strongest effect on HGS, Furthermore, differences between genders and dominant body somatotypes in the left and right HGS was not significant statistically ($F_2 = 0.821$, $p = 0.442$) and ($F_2 = 0.553$, $p = 0.576$) but there was a significant main effect for dominant somatotype ($F_1 = 149.188$, $p = 0.001$) and ($F_1 = 135.552$, $p = 0.001$). Mesomorphic males were seen to have greater HGS. Result also revealed significant correlations between height and weight and HGS of both left and right hands ($r = 0.453$, $p = 0.001$), ($r = 0.408$, $p = 0.001$ and $r = 0.420$, $p = 0.001$).
Conclusion: Dominant body somatotypes as well as gender differences had a very significant

influence on handgrip strength.

Keywords: *Somatotype, Anthropometry, Kinesiology, Body Mass Index. Biomechanics*

Introduction

Somatotype is a quantitative term used to characterize the human body's current structure and makeup; it is based on a variety of features[1,2,3]. Also, it is a person's current morphological configuration[2]. According to Slankamenac *et al*., The Heath and Carter method is the approach to somatotype assessment that is most frequently utilized and this approach is most effective for sports science and is typically utilized in its anthropometric version which can reveal information about the body's proportions, composition, and shape [2,3]. Slankamenac *et al*. further proceeded to suggest that with respect to body height, the somatotype is made up of three basic elements: Endomorphy, Mesomorphy and Ectomorphy[2].

Ectomorphy is a body type in which people have small frames, quick metabolisms, minimal body fat, and a bony structure, narrow shoulders, are naturally thin, and genetically find it more difficult to put on weight or develop muscular mass than other body types[3]. Walden also defined Mesomorphy as a body type with a medium frame and bone structure, slender, muscular body mass, and naturally athletic body types. Mesomorphs typically have a growth hormone-dominant phenotype[3]. They are physically predisposed to gaining muscle easily and naturally maintaining reduced body fat levels as a result. Endomorphy was defined according to Walden, as a body type with a slower metabolism than other body types, a higher body fat ratio, and a naturally "softer" body mass[3]. Endomorphs are also insulin dominant, meaning their bodies readily store energy as body fat and yet, endomorphs can also easily put on muscle[3]. Endomorphs can maximize their performance by properly utilizing their natural strength with the right training and nutrition[3].

Nonetheless, body somatotype changes occur from birth through adulthood, although they can be influenced by nutrition and/or training[4]. Body somatotype varies greatly between individuals and may be influenced by calorie consumption, physical activity, sex, age, genetic variability, and the sociocultural environment, among other factors [5]. Somatotype assessment quantifies the body's shape and makeup, including the relative fatness, the relative robustness of the musculoskeletal system, and the relative linearity of the body[6]. For example, a 3-5-2 gives the magnitude of each of the component in fixed order, endomorphy is 3 mesomorphy is 5 while ectomorphy is 2. These figures give the magnitude of each of the three components. Ratings for each component that were less than or equal to ½-2½ were judged low, 3-5 are moderate or usual, while 5½ -7 are high[1].

The differences in physical features between males and females are referred to as "sex or gender difference"[7]. The limitations of human performance are assumed to vary depending on the physical and physiological distinctions between males and females [8]. According to Bhargava *et al*., understanding sex differences in disease etiology, therapies and outcomes begins with an awareness of differences in baseline physiology and associated mechanisms [9]. In this regard, both the general and athletic populations have repeatedly shown that men and women have different physical somatotypes, with men being more mesomorphic and women having a higher endomorphy rating [7,10].

Hand Grip strength (HGS) refers to the ability of muscle or group of muscles to exert or generate maximal voluntary force in relation to motor fitness and total body strength [11,12]. The Hand Grip Strength is a

dependable, easy-to-use, and non-invasive test that evaluates the power of the hand muscles utilized for grasping or gripping [13]. Infact, literature supports the claim that HGS is an impartial and valid indicator of physical ability, frailty and risk of disability among adults as is associated with cardiovascular, respiratory, and cancer outcomes and also with mortality[14]. In this sense, handgrip strength refers to the amount of power needed to grab an object, which is essential for daily activities [15]. It is an essential source of energy for actions related to work. Stronger HGS indicates a firmer grasp or grip[15]. Moreover, according to Liao, HGS forecasts changes in muscle strength, physical movement, and capacity for daily activities as well as upper extremity function[15].

According to De *et al*., many variables, including age, gender, limited range of motion, nutritional health, muscle strength, pain, and fatigue, have an impact on the strength of the grip[16]. Additionally, later studies established that gender, age, height, weight, and handedness affect the strength of the hand grip, hence making gender and age common and consistent variables in HGS performance[15,17]. Studies have even shown that there is a high preponderance for poor HGS amongst females as compared to males [18,19]. In spite of all these, there have been very few studies done on the influence of dominant body somatotypes and gender difference on the hand grip strength.

The quantification of grip strength is too great an importance. Given that it helps in identification and determination of the effectiveness of different treatment strategies in rehabilitation of the hand[20]. According to Depp, having good wrist and hand strength is a marker for overall muscle strength[21]. In athletes, it's important to have a strong grip to improve athletic performance and to help prevent injuries, but it's just as important in healthy adults[21]. Low grip strength can predict an increased risk of functional limitations and disability as we get older [21].

Also, in a study on healthy adults, it was observed that grip strength was lower in individuals with diagnosed and undiagnosed diabetes and hypertension[22]. Faris Almashaqbeh confirms the effect of gender difference on maximal hand grip strength, with a higher grip strength reported in males than that of females[23]. Despite all these, most studies compare HGS of males and females across different ages but fail to consider the dominant body somatotypes of these participants. Therefore, knowledge of dominant body somatotypes and different sexes on the hand grip strength of young adults will help in identification and determination of effectiveness of different treatment strategies in hand rehabilitation and also provide normative values for young adults, providing a basis for effective assessment of physicality as is affected by body somatotypes and gender difference. With the earlier highlighted gaps in mind, the researchers sought to bridge this gap by further determining the influence of dominant body somatotypes and gender on HGS amongst undergraduates of a South-Eastern Nigerian University.

Consequently, clinicians may better monitor the effectiveness of surgical and non-surgical hand problems across body somatotypes and different sexes[24]. This study may as well help clinicians have a reference data on the function of the upper extremity and changes in muscle strength, physical movement and ability to undertake activities of daily living, aiding the overall rehabilitation process of individuals with hand injuries[15]. This study may also provide a basis for comparison of handgrip strength in cases of hand injuries of right and left hands in hand rehabilitation, the data gotten from this study will give a clear identification for distinguishing normality

and abnormality across different body somatotypes of different sexes on handgrip strength.

Materials and methods

The research design was an ex-post facto research design in which the attribute of the participants was measured once and for all. The research was conducted on both male and female undergraduates of a Nigerian University who were 200-500 level students of all departments under the Faculty of Health Science and Technology with a gross total of 2,901 students. These departments in no specific order include Medical Rehabilitation (562 students), Radiography (785 students), Nursing Science (402 students), Medical Laboratory Sciences (756 students), and Environmental Health Sciences (396 students).

Inclusion Criteria

All apparently healthy male and female undergraduates of the Faculty/Departments as listed above.

Exclusion Criteria

Members of the Faculty excluded from this research included pregnant students, and students apparent hand deformities.

Sampling Technique

The sampling technique was a proportionate stratified random sampling technique where participants were selected at random according to each stratum.

Sample Size

The sample size[25] of 162 participants was arrived at using G-power software version 3.1.9 with a 90% power to detect a large effect size at an alpha level of 0.05.

In no particular order, 31 students were recruited from the Department of Medical Rehabilitation, 43 students were recruited from Radiography, 22 students were recruited from Nursing Science, 44 students were recruited from Medical Laboratory Sciences and 22 students were recruited from Environmental Health Science. Using the formula (***No. of students/No. of students in faculty) * Sample size*** with all sub strata represented significantly.

Research Instruments

i. Height meter (locally made, Nigeria): This was used to measure the height of the participants to the nearest 0.1cm.

ii. Bathroom weighing Scale (HANA model, China): This was used to measure the weight in kilograms (kg) of the participants.

iii. Skinfold calipers (Slim guide model, China): This was used to measure the skinfold of the triceps, subscapular, supraspinale and medial calf skinfold in millimetres.

iv. Sliding/Venier calipers (Vogel, Germany): This was used to the biepicondyle breadth of the humerus and femur of the participant in millimetres.

v. Flexible Tape (butterfly brand, Nigeria): This was used to measure the girth circumference of the participants in centimetres.

vi. Tip felt marker (Nigeria): This was used to make marks on the area identified for measurement.

vii. Gripx Electronic Hand Dynamometer (EH101BL, made in China): This was used to measure hand grip strength by measuring the amount of tension produced in kilograms (kg) of the participants.

Procedure for Data Collection

Prior to commencement of this study, Ethical approval was sought and obtained from the Ethical Review Committee of the Faculty of Health Sciences and Technology, the Protocol number of the ethical approval is FHSTREC/023/00113. Before the commencement of the study, the researchers ensured all research instruments were well calibrated before use. The participants were fully informed about the purpose of the study and consent was sought and obtained before taking the measurements.

Measurement of handgrip strength

This was measured using a Gripx electronic handheld dynamometer (EH101BL). Each participant was instructed to sit on a chair with elbow flexed at 90 degrees and the forearm in semi-pronated position resting on the armrest of the chair. The participant was then asked to hold and squeeze the dynamometer for at least 3 seconds in order to get the maximal voluntary contraction. This was then read and recorded for both hands. It was measured three times with the mean average recorded as the handgrip strength in kilograms (kg).

Measurement of body somatotype

The body somatotype of each participant was assessed using the ***Heath-Carter anthropometric somatotype instruction manual.***

The Ten anthropometric dimension used to calculate the anthropometric somatotype were:

i. **Height:** This was taken against a height scale. Take height with the participant standing straight against an upright wall, touching the wall with the heels, buttocks and back.

ii. **Weight:** This was taken with a weighing scale with the participants wearing a minimal clothing and standing with shoes off.

iii. **Triceps skinfold:** A fold was raised at the back of the arm halfway along a line connecting the acromion and the olecranon process. This was taken with the participant's arm hanging loosely in the anatomical posture.

iv. **Subscapular skinfold:** The fold was raised on a line from the inferior angle of the scapula in a direction that is obliquely downwards and laterally at 45 degrees.

v. **Supraspinale skinfold:** The fold was raised 5-7cm (depending on the size of the participant) above the anterior superior iliac spine on a line to the anterior axillary border and on a diagonal line going downwards and medially at 45degrees.

vi. **Medial calf skinfold:** A vertical skinfold was raised on the medial side of the leg, at the level of the maximum girth of the calf.

vii. **Biepicondylar breadth of the humerus (right):** With the shoulder and elbow were bent to 90 degrees, this is the distance between the medial and lateral epicondyles of the humerus. The calipers was used at an angle that approximately bisects the elbow's angle.

viii. **Biepicondylar breadth of the femur (right):** The participant sat with the knee bent at right angle. The greatest distance between the lateral and medial epicondyle of the femur was measured with firm pressure on

the crossbar in order to compress the subcutaneous tissues.

ix. **Upper arm girth (right):** With the elbow flexed to 45 degrees and tensed, shoulder flexed to 90 degrees and hand clenched, elbow flexors and extensors maximally contracted, measurement of the greatest girth was taken with a tape.

x. **Calf girth (right):** The participant stood with feet slightly apart. The tape was placed around the calf and the maximum circumference was measured.

Method of calculating body somatotype from the Heath-Carter anthropometric somatotype instruction manual.

The equation to calculate Endomorphy is:

Endomorphy = - 0.7182 + 0.1451 (X) - 0.00068 (X 2) + 0.0000014 (X 3)

Where X = (sum of triceps, subscapular and supraspinale skinfolds) multiplied by (170.18/height in cm). This is called height-corrected endomorphy and is the preferred method for calculating endomorphy.

The equation to calculate mesomorphy is:

Mesomorphy = 0.858 x humerus breadth + 0.601 x femur breadth + 0.188 x corrected arm girth + 0.161 x corrected calf girth – height 0.131 + 4.5.

The equation to calculate Ectomorphy:

There are three different equations used to calculate ectomorphy according to the height-weight ratio (HWR):

If HWR is greater than or equal to 40.75 then;

Ectomorphy = 0.732 HWR - 28.58

If HWR is less than 40.75 but greater than 38.25 then

Ectomorphy = 0.463 HWR - 17.63

If HWR is equal to or less than 38.25 then

Ectomorphy = 0.1

Data Analysis

The data collected from this study was summarized using descriptive statistics of frequency distribution and percentage count, mean and standard deviation.

The inferential statistics of:

1. Pearson's Product Moment Correlation was used to analyze the correlation between hand grip strength of left and right hands and the height and weight of the participants
2. One-Way ANOVA was used to analyze the statistical effect of dominant body somatotype on handgrip strength.
3. Two-Way ANOVA was then used to analyze the statistical effect of dominant body somatotype and sex difference on handgrip strength to an alpha level of 0.05.

Results

The purpose of this study was to determine the Hand grip strength (HGS) using an Electronic Hand Dynamometer across various dominant body somatotypes of male and female apparently healthy undergraduates of the Faculty of Health Sciences and Technology, Nnamdi Azikiwe University. One Hundred and Sixty-Two (162) undergraduate students participated in the study. They comprised of 80 males (49.4%) and 82 females (50.6%). Their mean Heights (X = 169.08, SD = 8.58) and Weight (X = 69.01, SD = 13.26) were taken, their Height-Weight Ratio (HWR) were also calculated. The magnitude of each participant's components was expressed in 3 categories Endomorphy (48.1%), Mesomorphy (25.3%) and Ectomorphy (26.5%). After this, the hand grip strength of both hands was measured. Exactly 65.4% of the participants had right hand grip strength

dominance, 32.7% of the participants had left hand grip strength dominance, while 1.9% were ambidextrous.

Table 1 summarizes the socio-demographic and physical characteristics of the participants.

Table 2 reveals a statistical significant influence of dominant body somatotype on the left and right hand grip strength ($F = 11.959$, $p = 0.001$ and $F = 9.817$, $p = 0.001$) respectively.

A post-hoc analysis revealing the interacting effect between dominant body somatotypes and left and right-hand grip strength using the Turkey HSD test can be seen in Table 3. The interaction between Endomorphy and Mesomorphy was the only one of which had a significant difference with hand grip strength of the left and right hands.

Table 4 reveals that gender differences and dominant body somatotypes on the Left HGS did not significantly influence HGS ($F_2 = 0.821$, $p = 0.442$) but there was a statistically significant influence of dominant body somatotype ($F_1 = 149.188$, $p = 0.001$). However, the effect size was small (Partial Eta Squared = 0.010).

Table 5 also reveals that the interaction effect between gender difference and dominant body somatotype on the Right HGS was not statistically significant ($F_2 = 0.553$, $p = 0.576$); although a statistically significant main effect for between males and females was seen ($F_1 = 135.552$, $p = 0.001$). However, there was a small effect size (Partial Eta Squared = 0.007).

The post-hoc comparison using Turkey HSD test in Table 6 indicates an all-round statistical significance in the interaction effect of different body somatotypes and hand grip strength of the left and right hands, with dominant body somatotype significantly of main effect.

Table 7 reveals the correlation between the anthropometric variables of height and weight and HGS. There were significant positive correlations between height and right and left HGS ($r = 0.45$, $p = 0.001$ and $r = 0.48$, $p = 0.001$). Also, significant correlations between weight and right and left HGS ($r = 0.41$, $p = 0.001$ and $r = 0.42$, $p = 0.001$) were respectively revealed.

Table 1: Sociodemographic and Physical Characteristics of Participants

Variables	N	X±SD	Minimum	Maximum
Height (cm)	162	169.08 ± 8.58	146.00	196.00
Weight (kg)	162	69.01 ± 13.26	40.00	116,00
HWR	162	41.47 ± 2.18	34.33	46.55

KEYS: N – Number of Participants, X – Mean, SD – Standard Deviation, HGS – Hand Grip Strength, Endo – Endomorphy, Meso – Mesomorphy, Ecto – Ectomorphy

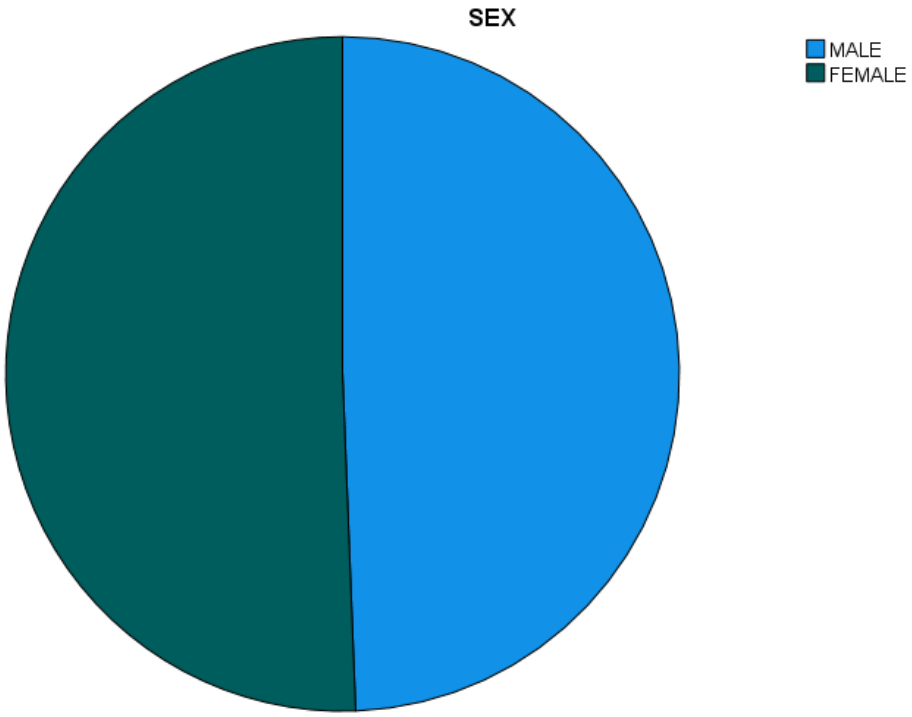

Figure 1: Gender distribution of participants

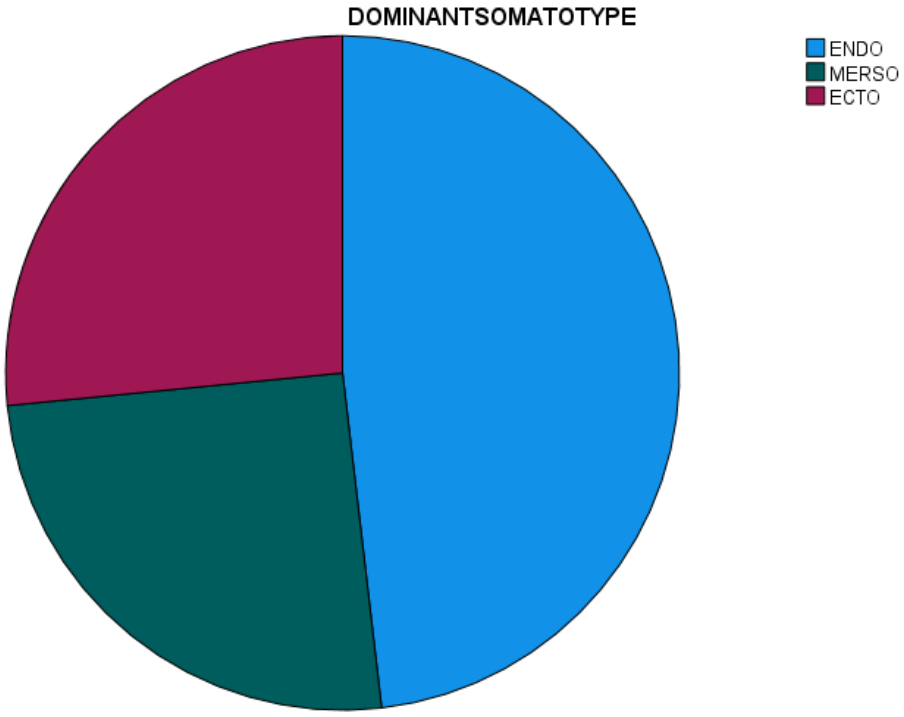

Fig 2 Distribution of Dominant body somatotype

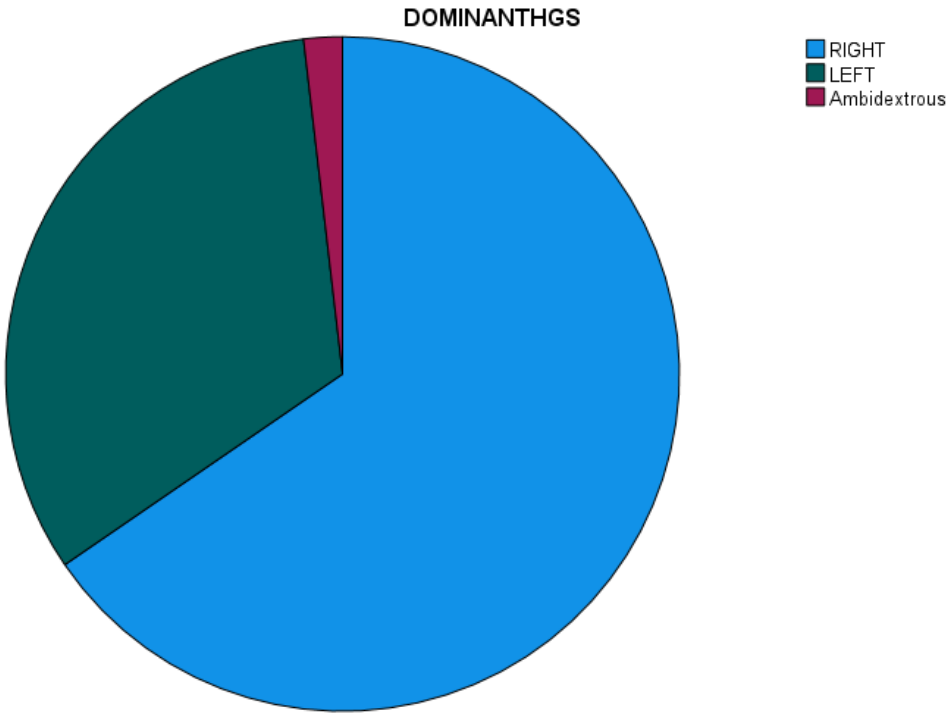

Figure 3: Distribution of Hand dominance of participants.

Table 2: Analysis of Variance Showing the Effect of Dominant Body Somatotype on Hand Grip Strength (Left and Right)

Variables	Dominant Body Somatotype	N	X ± SD	F-value	p-value
Left HGS (Kg)	Endo	78	31.80 ± 9.56		
	Meso	44	41.49± 11.98	11.959	0.001*
	Ecto	43	36.11 ± 9.99		
Right HGS (Kg)	Endo	78	33.86 ± 9.98		
	Meso	41	43.20± 12.34	9.817	0.001*
	Ecto	43	38.48± 11.68		

KEY

* - Significant at α = 0.05, N – Number of Participants, X – Mean, SD – Standard Deviation, HGS – Hand Grip Strength, Endo – Endomorphy, Meso – Mesomorphy, Ecto – Ectomorphy

Table 3: Post Hoc Analysis Using Turkey HSD Test Showing Interactive Effect of Dominant Body Somatotypes on Hand Grip Strength (Left and Right)

Variable		Dominant Body Somatotype (I)	Dominant Body Somatotype (J)	MD	p-value
HGS	**Left**	Endo	Meso	-9.6892	0.001*
			Ecto	-4.3108	0.075
		Meso	Endo	9.6892	0.001*
			Ecto	5.3784	0.048*
		Ecto	Endo	4.3108	0.075
			Meso	-5.3784	0.048*
	Right	Endo	Meso	-9.3408	0.001*
			Ecto	-4.6136	0.075
		Meso	Endo	9.3408	0.001*
			Ecto	4.7272	0.127
		Ecto	Endo	4.6136	0.075
			Meso	-4.7272	0.127

KEY

MD – Mean Difference, *- Significant at α = 0.05, N – Number of Participants, X – Mean, SD – Standard Deviation, HGS – Hand Grip Strength, Endo – Endomorphy, Meso – Mesomorphy, Ecto – Ectomorphy

Table 4: Two-Way ANOVA Showing Effect of Sex and Dominant Body Somatotype on Left Hand Grip Strength (HGS)

HGS	Sex	Dominant Body Somatotype	N	X ± SD	F_2-value	p-value	Comment
Left	Male	Endo	23	42.19 ± 7.79			
		Meso	28	47.30 ± 9.05			
		Ecto	29	40.84 ± 7.63			
					0.821	0.442	ns
	Female	Endo	55	27.45 ± 6.34			
		Meso	13	28.96 ± 6.72			
		Ecto	14	26.30 ± 6.61			

KEY:
MD – Mean Difference, *- Significant at α = 0.05, N – Number of Participants, X – Mean, SD – Standard Deviation, HGS – Hand Grip Strength, Endo – Endomorphy, Meso – Mesomorphy, Ecto – Ectomorphy, NS – Not Significant, S - Significant

Table 5: Two-Way Anova Showing Effect of Sex and Dominant Body Somatotype on Right Hand Grip Strength (HGS)

HGS	Sex	Dominant Body Somatotype	N	X ± SD	F_2 - value	p-value	Comment
Right	Male	Endo	23	44.46 ± 7.84			
		Meso	28	48.39 ± 10.40			
		Ecto	29	44.50 ± 8.41			
					0.553	0.576	NS
	Female	Endo	55	29.43 ± 7.01			
		Meso	13	32.05 ± 8.16			
		Ecto	14	25.99 ± 6.29			

KEY:
MD – Mean Difference, *- Significant at α = 0.05, N – Number of Participants, X – Mean, SD – Standard Deviation, HGS – Hand Grip Strength, Endo – Endomorphy, Meso – Mesomorphy, Ecto – Ectomorphy, NS – Not Significant, S – Significant

Table 6: Post Hoc Analysis Using Turkey HSD Test Showing Multiple Comparison Of Sex Difference And Dominant Body Somatotype On Hand Grip Strength (HGS) of Left And Right Hands

HGS	Dominant Body Somatotype (I)	Dominant Body Somatotype (J)	MD	p-value
Left	Endo	Meso	-9.6892	0.001*
		Ecto	-4.3108	0.007*
	Meso	Endo	9.6892	0.001*
		Ecto	5.3784	0.003*
	Ecto	Endo	4.3108	0.007*
		Meso	-5.3784	0.003*
Right	Endo	Meso	-9.3408	0.001*
		Ecto	-4.6136	0.009*
	Meso	Endo	9.3408	0.001*
		Ecto	4.7272	0.022*
	Ecto	Endo	4.6136	0.009*
		Meso	-4.7272	0.022*

KEY:
MD – Mean Difference, *- Significant at α = 0.05, N – Number of Participants, X – Mean, SD – Standard Deviation, HGS – Hand Grip Strength, Endo – Endomorphy, Meso – Mesomorphy, Ecto – Ectomorphy, NS – Not Significant, S - Significant

Table 7: Pearson Correlation between Anthropometric Variables (Height and Weight) and Hang Grip Strength (Left and Right)

Variables	HGS	p-value	r-values
Height	Right	0.001*	0.45
	Left	0.001*	0.48
Weight	Right	0.001*	0.41
	Left	0.001*	0.42

KEY:
*- Significant at α = 0.05, HGS – Hand Grip Strength

Discussion

The aim of this study was to determine the influence of dominant body somatotypes and gender difference on handgrip strength among young adults in a University in Southeast Nigeria.

Their grip strength was measured using an electronic hand dynamometer. The outcome of this research revealed that dominant body somatotype and gender difference had no significant influence on Handgrip strength. The dominant body somatotype among the male participants was ectomorphs and mesomorphs while those of the female participants was endomorphy. This was in line with a study done by Gaur *et al*., whose work enlisted 218 boys and 220 girls for participation[26]. The study concluded that there were notable sex differences in the dominant body somatotypes of adolescents with girls being significantly more endomorphic and boys being more mesomorphic, it was revealed in the study that this was because females had overall more fat deposits than males[26], possibly because of the somewhat less physically strenuous life female students lived in the school. The result of this study was also similar to the work of Awotidebe et al., whose interpretations were also that males were majorly ectomorphs and mesomorphs while females were dominantly endomorphic[27]. Findings from this research revealed positive significant correlation between anthropometric variables of height and weight and hand grip strength (HGS) of right and left hand of the participants. This is in line with the findings of Amaral *et al*., who found a positive correlation between hand grip strength, weight and height among adult and elderly populations in Rio Branco, Brazil[28]; and in line with the results of Awotidebe et al. which revealed that some selected anthropometric characteristics like body weight, height and body mass index had significantly positive correlation with HGS. This result was consistent with the findings of previous studies indicating that body compositions is related to muscle mass and distribution of fat deposit in human[15,17,27,29,30] .

The results also revealed endomorphy as a more predominant somatotype among the research population with females having a higher endomorphy rating than males. From the findings of this study, dominant body somatotype had a significant statistical effect on hand grip strength. Mesomorphs who were characterized with muscular body mass and naturally athletic body had higher handgrip strength on both left and right hands than ectomorphs who had small frames and bony structures, and endomorphs with their higher body fat ratio respectively in that order . This finding is in line with that of Awotidebe *et al*., which found that body somatotype influenced the degree of handgrip strength[27]. This was revealed in a cross-sectional survey involving 385 young adults which showed that mesomorphs and then ectomorphs had higher hand grip strength in that order than endomorphs. This may be due to the fact that mesomorphs were characterized by muscular dominance than the other body somatotypes[27].This was consistent with the works of previous authors who noted that there is little doubt that variations in human anatomical structures could influence certain differences[31, 32]. This indicated that endomorphs expresses degree of adiposity development greater in females than in males whereas mesomorphs reflects muscle development known to be positively associated with strength and motor performance greater in males than females [18,19,27,31, 32].These findings are consistent with the findings of previous studies that mesomorphy is associated with males while endomorphy is related to female body shapes [27, 33]. A likely elucidation for these results may be due to the increased fat

content in females for endomorph and increased muscular development in males for mesomorph[33].

Males had significantly higher handgrip strength on both right and left hands as compared to females as seen from the result of this study. This was in line with the study done by Ibikunle *et al.*, whose research revealed that boys showed more strength in their peak grip and grip strength on both dominant and non-dominant hands more than females[34].This is also in line with other authors who revealed from their works that Males had higher HGS than females[15, 17,18,19]. This might be attributed to the obvious physical disparity between males and females, the males presented more with a dominant mesomorphic body somatotype as compared to the females whose dominant somatotype was endomorphy[31, 32].

The result of this study also revealed a non-significant effect of sex-difference on the HGS across various dominant body somatotypes. However, though not significant the mesomorphs still had higher HGS than Endomorphs and ectomorphs in that order.

Conclusion

The following conclusions were drawn from the findings of this study: there were significant correlations between hand grip strength of the right and left hands with height and weight among the participants. Also there were significant differences in the hand grip strength of the right and left hands across different dominant body somatotypes of participants. However, across different dominant body somatotypes, there was no significant influence of gender on the handgrip strength of left and right hands of the participants.

Recommendation

Based on the findings of this study, it is recommended that:

1. There is a need for continuous research on the influence of dominant body somatotype across different genders on hand grip strength among a larger and more diverse population.
2. More sensitization should be carried out on clinicians, enlightening them on the effect of dominant body somatotype on measures associated with hand rehabilitation.

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Declaration of conflicting interests

The authors declare no conflict of interest.

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KNOWLEDGE, ATTITUDE AND PRACTICE OF HEALTH PROMOTION AMONG PHYSIOTHERAPISTS IN SELECTED TERTIARY HOSPITALS IN SOUTH EAST, NIGERIA

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Abstract

Background: Health promotion involves multiple strategies that can be employed to improve the individual's life condition generally, and the physiotherapy profession has been shown to be highly relevant in health promotion activities.
Aim: The aim of this study was to determine the level of knowledge, attitude and practice of health promotion among physiotherapists in South-East Nigeria.
Method: One hundred and five (105) participants involving 56 males and 49 females in selected tertiary hospitals in the South-East Nigeria were recruited through a consecutive sampling technique. The study utilized a 19- item self-reported questionnaire adapted from a previously validated instrument utilized in Nigeria. Obtained data were summarized using descriptive Statistics presented in frequency distribution tables.
Results: A total of 105 physiotherapists responded with majority demonstrating a good knowledge of health promotion (97%). Most of the respondents (72.4%) agreed that their training offered lots of opportunity to explore health promotion. About 73.3% disagree that health promotion is less important than other aspects of the physiotherapist's role. Also 96.2% believe that health promotion is a fundamental part of physiotherapy. About 50.5% usually actively incorporate aspects of health promotion into delivery of care to clients, while 43.8% of them have good attitude towards health promotion.
Conclusion: The outcome of this study showed that the participants have good knowledge, a fair attitude and possess good practice of health promotion.

Keywords: *Health Promotion, knowledge, attitude, practice, physiotherapists*

Introduction

Health as defined by Proulx,[1] is the intersection of one's physical, mental, emotional economic and spiritual state of being at any particular point in time. The most established modern day definition of health is that which has been termed by the World Health Organization[2] and it defines health as a state of complete physical, mental and social well-being, not merely the absence of disease and infirmity.

Health promotion is described as those activities that are undertaken to enhance well-being that are directed towards actualizing an individual's potential[3]. WHO has termed health promotion as "the process of enabling people to increase control over, and to improve their health and it also goes beyond a focus on one's attitude towards a wide range of social and environmental interventions that are made to benefit and protect individual people's health and quality of life by addressing and preventing the main causes of ill health, and not just focusing on its treatment and cure[4]. One of the major control measures to the rising prevalence of non-communicable diseases (NCDs) in the world according to the World Health Organization [5] is by the primary prevention aspect of health promotion, which is specifically aimed at doing the following; eradicating, eliminating or minimizing the impact of diseases through comprehensive population based programs.

All health professionals, irrespective of their site or area of practice as noted by Zenzano,[6] have a key role to play in health promotion, either as individual practitioners or as a member of an inter-professional health care team. The Physiotherapy profession has been shown to be highly relevant in health promotional activities and there's a strong need for the Physiotherapists to make health promotion practice a priority[7]. The Ontario Physiotherapy Leadership Consortium (OPLC) described the model of health promotion in physiotherapy practice as one in which the Physiotherapists have high input in health promotion at the level of the determinants of health, and this includes lifestyle and disease, injury and illness[8]. Dean *et al*,[9] also noted that in order to educate patients and clients on the basic principles of health promotion, Physiotherapists need knowledge in the epidemiology of injury and disease, risk factors, and the major factors that are influencing the safety and injury prevention. This study thus served to identify and bridge the gaps between the required knowledge needed for proper health promotion and the accepted practice of health promotion among physiotherapists.

Materials And Methods

Study Design and Setting

This study was a cross- sectional survey. The study population comprised of physiotherapists in federal tertiary hospitals in South-East Nigeria. A purposive sampling technique was used and the physiotherapists were consecutively selected. The total number of physiotherapists in the selected Federal tertiary hospitals in the South-East Nigeria was 124.

Inclusion Criteria

Physiotherapists who have at least two years of working experience in the selected federal tertiary hospital in South-East Nigeria and who were willing to participate in the study.

Study Location

The study was conducted in selected federal tertiary hospitals in the South-East region of Nigeria. The South-East region is one of the six geo-political zones in Nigeria and consists of five states; Abia, Anambra, Ebonyi, Enugu and Imo. There are seven Federal tertiary hospitals in South-East Nigeria, which are: Alex Ekwueme Federal Teaching Hospital, Abakaliki (FETHA); Federal Medical Center (FMC), Owerri;

Federal Medical Center (FMC), Umuahia; Federal Neuropsychiatric Hospital Enugu; National Orthopaedic Hospital, Enugu (NOHE); Nnamdi Azikiwe University Teaching Hospital (NAUTH), Nnewi; and University of Nigeria Teaching Hospital (UNTH), Enugu. All except the Federal Neuropsychiatric Hospital offer Physiotherapy services; therefore, these six hospitals; FETHA; FMC, Owerri; FMC, Umuahia; NOHE; NAUTH and UNTH were selected for the study.

Research Instruments

The Knowledge, Attitude and Practice (KAP) on Health Promotion Questionnaire was used for this study. The Questionnaire was adopted from a previous study KAP of Physiotherapists towards Health promotion in Nigeria by Abaraogu et al.,[10]. It is a 19 item self-reported Questionnaire comprising of four sections.

Section A: This section includes seven questions relating to the socio-demographic characteristics. It gathers information on the physiotherapist's age, gender, qualified years of practice as a Chartered Physiotherapist, city of the Physiotherapist, post qualification, work population group and total years of practice of the Physiotherapist.

Section B

This includes three sub questions on Knowledge of Health promotion by the Physiotherapists answered using a Likert scale for 'Agree, neutral and disagree'

Section C

This includes three sub questions on Attitude of Physiotherapists towards Health promotion answered using a Likert scale for 'often, sometimes, rarely and Never'

Section D

This includes seven questions on the Practice of Physiotherapists towards Health promotion answered using a Likert scale for 'often, sometimes, rarely and Never'

Psychometric Properties

Validity: This instrument has been content Validated in a previous study carried out in the Nigerian environment, which was piloted, modified and used by McMahon and Connolly to assess the Health promotion knowledge, Attitude and Practice of Chartered Physiotherapists in Ireland, and the authors granted Abaraogu[10] the permission to modify and use the version.

Reliability: This instrument was also reported to have a good test-retest reliability with a coefficient range between 0.6 and 1.0 for sub-domains and 0.71 for the whole instrumental at 2- weeks interval among 18 Nigerian Physiotherapists that were chosen to represent different genders, practice orientations, and years of practice indicated a range of moderate to excellent scores[10]

Questions contained all through the survey instrument were adapted to suit the peculiarities of the Nigerian environment[10]

Procedure for Data Collection

Ethical approval was obtained from the Ethics Review committee of Faculty of Health science and Technology, Nnamdi Azikiwe University Nnewi before the commencement of the study. The informed consent of the participants was obtained prior to the study. Confidentiality of the participants was fully ensured using their initials instead of their full names. Afterwards, the researchers met with the participants in their clinics to explain the reason for the research to them and explain the questionnaire to them for clarity sake. The questionnaire was distributed to the various facilities by the principal researcher through recruited research assistants and each respondent was given three days to respond and return questionnaire via already mapped out courier services which were finally weigh-billed down to the location of the principal researcher.

Analysis of Data

Descriptive statistics of frequency, mean and standard deviation were employed to summarize the data.

Results

Participant's profile

Out of the one hundred and twenty-four (124) total population, a total of one hundred and five (comprising 56 males and 49 females) participated in this study with a response rate of 76.44%. Fifty participants (47.6%) had Bachelor degree, 35persons (33.3%) had an MSc degree, 20 persons (19.0%) had PhD degree. In the population group, musculoskeletal specialty was l2 (39%), Neurology 31(37.1%), Peripheral arterial disease/Geriatrics 16 (10.5%), In-patient 5(4.8%), women health 8 (7.5%), others 1(1.0%). The result also showed that majority of the participants fall in the age range 30-39 (57.1%), had 16 years of practice (15.2%) and had been licensed physiotherapists within the range of 10-19 years (52.4%) as shown in table 1.

Majority of the respondents (95.2% 100 of 105) agreed that health promotion enables people to cope with health problems and attain the best possible quality of life; 92.4% believe that health promotion is about preventing disease; 72.4% believe that health promotion seeks to create a more equitable society; 76.2% believe that the role of health promotion is to alleviate the economic strain on the health service; 70.5 % believed that health promotion is predominately concerned with changing people's behavior; 60% believe that health promotion is about changing public policy; 61.9% believe that health promotion is about empowering individuals; 91.4% believe that health promotion aims to reduce health inequalities; 97.1% believe that all Health professionals should promote a healthy lifestyle as shown in table 2 below.

About 95.2 %(100 of 105) believe that health promotion requires the physiotherapist to have an understanding of the clients' lives; 96.2% believe that health promotion is a fundamental part of physiotherapy; details of the respondents' attitudes toward health promotion are presented in Table 3. Furthermore, slightly fewer than half (43.8%, 46 of 105) had sometimes worked with physiotherapists who had a health promotion remit; 49.5% sometimes work with health professionals who were responsible for health promotion; 38.1 % usually worked with physiotherapy managers who saw health promotion as an important aspect of physiotherapy, and 50.5% usually actively incorporated aspects of health promotion into delivery of care to clients. Table 4 presents details of respondents' practice of health promotion.

Majority of the respondents (72.4%; 76 of 140) agreed that their training offered lots of opportunity to explore health promotion. Only 52.4% agreed that health promotion was well embedded in their course structure. A total of 79% agreed that health promotion should be incorporated into students' physiotherapy courses as a part of their undergraduate program in third or fourth year, and the majority (87.6%; 92 of 132) agreed that health promotion should be offered as a clearly de-fined theme. A total 84.8% agreed that careful consideration of policy implementation and guidelines for health promotion should be included in physiotherapy training programs as seen in table 5 below.

Table 1: Respondent's Sociodemographic Data

Variables	Category	Frequency	Percentage (%)
Sex	Male	56	53.3
	Female	49	46.7
Age	20-29	17	16.2
	30-39	60	57.1
	40-49	27	25.7
	50-59	1	1.0
PG	Musculoskeletal	12	39.0
	Neurology	31	37.1
	PAD/Geriatrics	16	10.5
	Women's health	8	7.6
	In-patients	5	4.8
	Others	1	1.0
Licensed Physiotherapist	<5yrs	25	23.8
	5-9	23	21.9
	10-19	55	52.4
	>20	2	1.9
Qualification	Bachelor degree	50	47.6
	Master's degree	35	33.3
	Doctor of Philosophy	20	19.0

Table 2: Respondent's Knowledge of Health Promotion

What is Health Promotion?	**Agree**	**Neutral**	**Disagree**	
Health promotion enables people to cope with health problems and attain the best possible quality of life.	100(95.2)	3(2.8)	2(1.9)	
Health promotion is about preventing disease	97(92.4)	2(1.9)	6(5.7)	
Health promotion seeks to create a more equitable society	76(72.4)	25(23.8)	4(3.8)	
The role of health promotion is to alleviate the economic strain on the health service.	60(76.2)	16(15.2)	9(8.6)	
Health promotion is predominately concerned with changing people's behavior	74(70.5)	13(12.4)	18(17.1)	
Health promotion is about changing public policy	63(60.0)	22(21.0)	20(19.0)	
Health promotion is about empowering individuals	65(61.9)	31(29.5)	9(8.5)	
Health promotion aims to reduce health inequalities.	96(91.4)	4(3.8)	5(4.8)	
All Health professionals should promote a healthy lifestyle.	102(97.1)	2(1.9)	1(1.0)	
How often do you encounter the following publications in your practice?	**Often**	**Sometimes**	**Rarely**	**Never**
The National Health Promotion Strategy documents	11(10.5)	24(22.9)	37(35.2)	33(31.4)
Changing Cardiovascular Health: National Cardiovascular Health Policies	14(13.3)	26(24.8)	31(29.5)	34(32.4)
The Ottawa Charter for Health Promotion (WHO, 1986)	13(12.4)	23(21.9)	34(32.4)	35(33.3)
Survey of Lifestyles, Attitudes and Nutrition	12(11.4)	36(34.3)	29(27.6)	28(26.7)
Do you believe you have sufficient knowledge of the following to engage in HP with clients?	**Yes**	**No**		
Concepts and Principles of Health Promotion	77(73.3)	26(24.8)		
Biological Determinants of Health	80(76.2)	25(23.2)		
Psychological Determinants of Health	76(74.3)	26(24.8)		
Socioecological Determinants of Health	70(66.7)	35(33.3)		
Behavior Change Theory	63(60.0)	42(40.0)		
Key Action Areas of Health Promotion	72(65.3)	38(31.0)		

Table 3. Respondent's attitude towards health promotion and Health Promotion Action

Questions	Agree	Neutral	Disagree
Health promotion requires the physiotherapist to have an understanding of the clients' lives	100(95.2)	3(2.9)	2(1.9)
Health promotion is a fundamental part of physiotherapy	101(96.2)	3(2.9)	1(1.0)
Health promotion is less important than other aspects of the physiotherapist's role	15(14.3)	13(12.4)	77(73.3)
Physiotherapists are well placed to respond to the client's health promotion needs	78(74.3)	16(15.2)	10(9.5)
Physiotherapists usually have too much else to do to be able to offer health promotion	26(24.8)	32(30.5)	47(44.8)
Physiotherapists empower clients to change unhealthy aspects of their lives	97(92.4)	5(4.8)	3(2.9)
Physiotherapists' health promotion practice is not supported by a strong evidence base	26(24.8)	26(24.8)	53(50.5)
Physiotherapists should 'model' good health behavior in order to give health promotion advice	98(93.3)	2(1.9)	5(4.8)
Physiotherapists should be required to engage in health promotion as part of government policy	101(96.2)	1(1.0)	3(2.9)
All physiotherapists should promote a healthy lifestyle	102(97.1)	1(1.0)	2(1.9)
A physiotherapist who is a smoker is just as good a health promoter as one who is not a smoker	35(33.3)	23(21.9)	47(44.8)
Physiotherapy students have a role in health promotion	99(94.3)	6(5.7)	2(1.9)
Physiotherapists should enable individuals, groups, communities and organizations to build capacity for health promotion action to improve health and reduce health inequities.	103(98.1)	2(1.9)	0(0.0)
Physiotherapists should advocate with, and on behalf, of individuals, communities and organizations to improve health and well-being and build capacity for health promotion action.	100(95.2)	5(4.8)	1(1.0)
Physiotherapists should work collaboratively across disciplines, sectors and partners to enhance the impact and sustainability of health promotion action.	103(98.1)	1(1.9)	2(1.0)
Physiotherapists should communicate health promotion action effectively, using appropriate techniques and technologies for diverse audiences.	102(97.2)	3(2.9)	0(0.0)
Physiotherapists should contribute to the development of a shared vision and strategic direction for health promotion action.	103(98.1)	2(1.9)	0(0.0)

Table 4. Respondent's practice of Health Promotion

Questions	Often	Sometimes	Rarely	Never
How often have you worked with physiotherapists who have a specific health promotion remit?	21(20.0)	46(43.3)	34(32.4)	4(3.8)
How often have you worked with health professionals who are responsible for health promotion?	30(28.6)	52(49.5)	18(17.1)	5(4.9)
How often have you worked with physiotherapy managers who see health promotion as an important aspect of physiotherapy?	40(38.1)	36(34.3)	27(25.7)	2(1.9)
Do you actively incorporate aspects of health promotion in the delivery of care to clients?	49(45.7)	53(50.5)	2(1.9)	1(1.0)

Table 5. Respondent's view on Physiotherapy Training

	Agree	Neutral	Disagree
My training offered lots of opportunities to explore health promotion	76(72.4)	12(11.4)	16(15.2)
Health promotion was well embedded in the course structure	55(52.4)	27(25.7)	23(21.9)
Health promotion was predominantly taught in one module	48(45.7)	28(24.8)	30(28.6)
Specific health promotion module in first year	61(58.1)	22(21.0)	22(21.0)
Specific health promotion module in third or fourth year	83(79.0)	12(11.4)	10(9.5)
Health promotion offered as a clearly defined theme within key modules on the course	92(87.6)	6(5.7)	7(6.7)
Specific health promotion placements	89(84.8)	11(10.5)	5(4.8)
Careful consideration of policy implementation/guidelines	89(84.8)	12(11.4)	3(2.9)
Exploration of how policy relates to practice	88(83.8)	13(12.4)	4(3.8)

Discussion

This is a descriptive study that determined the knowledge, attitudes, and practice of health promotion among physiotherapists in the South-East of Nigeria. From the result, majority of the population possess sound knowledge and implement health promotion in their practice. First, we considered how the sample represents the population of physiotherapists in South-East Nigeria, then we related the findings with the result from other studies.

The male population was slightly more the females in the work force by 6.6%. This is consistent with the reports from other studies conducted in Nigeria [10,11,12,13]. This is because the pioneers of physiotherapy training in Nigeria were men. Although, the population sample is a good representation of physiotherapists in South-East Nigeria.

Almost all the participants gave a positive response on having the knowledge of health promotion in aiding people to manage health problems and achieve good quality of life. There was also positive affirmation from the physiotherapists about being aware that health promotion prevents the occurrence of disease and modifies the lifestyles of individuals to improve their health status. This is consistent with the results from other studies that indicated the role of health promotion in improving peoples' lifestyles and this should be the basis in tackling critical health issues in third world countries particularly Nigeria[10,14,15,16]

Moreover, the study participants are majorly aware that health promotion reduces health inequalities and can lead to individual empowerment. Hence, it is capable of reducing the economic stress on the health service and creating an equitable society. An

interesting part of this analysis is that most of the respondents were aware that policy changes should be considered in order to encourage health promotion practices and activities. This is in contrast with the result from the study conducted by Abaraogu *et al*,[10] which stated that few respondents are of the opinion that health promotion activities involved public policy changes to aid the improvement of practice. Explaining further on this, the study indicated that the reason behind this response is due to the fact that these respondents lack absolute knowledge of the factors that are known as the determinants of health.

Hence, it would be appropriate to state that the physiotherapists in South-East Nigeria have good knowledge about the determinants of health. Most of the respondents have positive attitude towards the implementation of health promotion activities in their clinical practice. This was evident in the response from 97% of the study population that physiotherapists empower clients to change some unhealthy lifestyles. In addition, it was indicated that physiotherapists should give health promotion advice by modelling good health behavior. This was supported by McMahon *et al*,[14] that revealed that Physiotherapists appear to possess positive attitudes towards health promotion by adopting it as one of their basic role in clinical practice. It further identified some barriers that are against its implementation such as time constraints, absence of health promotion training and attitudes of patients towards the adoption of health promotion activities. However, the responses from the physiotherapists regarding whether they were adequately equipped with health promotion knowledge during their undergraduate training, have been inconclusive. Although 72.4% of the respondents agreed that their undergraduate training had offered them many opportunities to explore health promotion, only 45.7% of them stated that health promotion was incorporated in the course structure, while 58.1% indicated that health promotion was taught in one of the modules. Following this, it's hereby suggested that health promotion training be incorporated into the education of health professionals at all levels, both undergraduate and postgraduate. This was supported by Mooney *et al*,[17] they emphasized the need to include health promotion in the training of nursing students. It is important that Physiotherapy training should be well tailored to ensure that there is proper adoption of health promotion into their clinical practice, by exposing them to opportunities for the acquisition of knowledge about health promotion[18,19]. This training enhances students' confidence in applying the acquired health promotion knowledge and skills to their clinical practice[20]. There is also need for physiotherapists to go for continuing education programs that would improve their knowledge of health promotion practice[21]. In addition, physiotherapists' knowledge, expertise, and confidence in health promotion activities can be improved via entry-level curriculum reviews that bring in modules that are specific to health promotion into the system[22]

Furthermore, majority of the respondents are of the opinion that health promotion is the basic aspect of physiotherapy. Hence, physiotherapists should adopt health promotion roles that would aid in improving health and minimize inequalities. On the other hand, most of them believed that they are in good position to respond to their clients' needs relating to health promotion and that they should participate in other health promotion activities, such as smoking cessation, physical activity and reduced alcohol consumption. The findings are consistent with the results from other studies that stated that physiotherapists play an

important role in several health promotion activities[23,14]

The responses from the Physiotherapists in this study have various implications for the professional practice and role of physiotherapists in South-East Nigeria. Their positive attitudes towards health promotion was obvious in their clinical practice such that a greater part of the population indicated that they are actively implementing the practice of health promotion activities in client management. Although, this study did not determine the various inputs, processes or outcome measures for the health promotion activities that were delivered by the physiotherapists because it's not within the scope of this study.

According to The Ontario Physiotherapy Leadership Consortium model[8], physiotherapists should have health promotion activities at the level of determinants of health; lifestyle, disease prevention, injury and the general health and wellness of the body. The inputs at the level of the determinants of health; lifestyle; and disease, injury, and illness through promotion, prevention, and rehabilitation to see outcomes at both the individual and the population level of health and wellness.

Conclusions

From the study, it can be concluded that Physiotherapists in South-East Nigeria have good knowledge of health promotion. Their attitude towards health promotion was fair and they possessed a good implementation of health promotion in their practice. Furthermore, the respondents also indicated that their undergraduate training had offered them many opportunities to explore health promotion.

Recommendations

More emphasis should be made in health promotion in the undergraduate curriculum in lieu of enhancing multidisciplinary team empowerment with regard to health promotion in both public and private sectors. While this research considered the knowledge of practicing physiotherapist, more extensive research should be emphasized in conducting and planning evidence based approach in the line of health promotion among physiotherapists.

Furthermore, the use of health promotion models should be implemented for research purposes in physiotherapy profession when practicing health promotion or primary healthcare in order to discover indicators as a measuring tool and to provide constant follow-ups in developing the program. More media involvement to educate masses in both rural areas as part of capacity building with regard to health promotion, education and prevention.

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EFFECT OF ORAL ADMINISTRATION OF BITTER EXTRA ON THE HISTOMORPHOLOGY OF THE CEREBELLUM

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Abstract

Background: Bitter Extra is a brand of herbal product adjudged to be efficacious in the treatment of various ailments. This study is aimed at investigating the effect of Bitter Extra on the histomorphology of the cerebellum.
Method: Sixteen adult male Wistar Rats with weight between 158-230g were used for this study and were divided into four groups (1,2,3 and 4) with four (4) rats in each group. Group 1 served as the control while groups 2, 3 and 4 were taken as the test groups. Group 2 was administered 1.35ml/kg (low dose), group 3 was administered 2.7ml/kg (Medium dose), Group 4 was administered 5.4ml/kg (High dose) of Bitter Extra through an orogastric tube for a period of four weeks while group 1 was given water and feed only. The rats were fed and administered these amounts of drugs daily with their weights recorded after a week interval for four weeks.
Results: There were no significant changes ($P>0.05$) in the mean body weights of the rats in the test groups administered with Bitter Extra. However, in the histology of the cerebellum, rats in Group 4 showed increased proliferation of the Purkinje cell bodies into the molecular layer. Group 3 showed focal increase in Purkinje cell proliferation and hypertrophy of the Purkinje cell layer with pushing of the molecular layer, while Group 2 showed increased hypertrophy of Purkinje cell bodies and hyperplasia of cells in the granular layer.
Conclusion: These finding suggest that consumption of Bitter Extra does not in any way affect the body weight. High dosage consumption of Bitter Extra may cause hyperplasia, hypertrophy of the cells of the cerebellum which may lead to observable cerebellar dysfunction features.

Keywords: *Bitter Extra, Body Weight, Cerebellum, Rats, Cerebellar Dysfunction*

Introduction

According to World Health Organization (WHO) herbal medicine is defined as any part of the plant that can be used for therapeutic purposes or as precursors for the synthesis of important drugs[1]. Based on the information from WHO, the use of herbal medicine worldwide has surpassed the use of conventional therapies by two to three times[2]. Plants, (herbs or ethno botanicals) have been used from the beginning of human race and are still used throughout the world for promotion of health and treatment of diseases[3]. Plants and herbs form the basis of today's modern medicine and have contributed enormously to the commercial drug preparations manufactured today[4]. It has been discovered that about 25% of the drugs prescribed worldwide are synthesized from plants[2]. In most developing countries, herbs rather than conventional drugs are often used in health care services. For some individuals, herbal medicine is the preferred method of treatment, while for others; herbs are used as adjunct to therapy with conventional pharmaceuticals. However, in most developing countries, traditional medicine of which herbal medicine is a core part is the only system of health care that can be assessed and is affordable[5].

Herbal medicine has been reportedly used by about 80% of the world population both in the developing and developed countries where modern medicines are predominant[6,7]. A study by Ibrahim[8] showed that more than 60% of the surveyed population claimed to have used some herbal mixture either alone or in combination with other medicines. The rising popularity of phytomedicines could be attributed to the alleged advantages of being efficacious and also a more affordable source of medical care. In contrast, there is growing disillusion with modern medicines coupled with the misconception that herbal supplements might be devoid of adverse and toxic effects, which are associated with conventional and allopathic medicines. But recent reports have raised concerns that indiscriminate use of packaged herbal bitters may have a toxic effect on the spleen, pancreas, heart and other structures of the body[9].

In addition, herbal supplements are administered in most clinical conditions over a long period of time, without taking cognizance of their toxic effects which might result from a prolonged usage[10]. In most cases, these herbal products are not often prescribed by a physician and neither were they dispensed by a pharmacist. The individual reports of any potential adverse effect are mostly absent or inaccurate[11]. Therefore, the danger associated with the potential toxicity of many of these herbal products of which Bitter Extra is a brand and other herbal therapies, which are being used over long period of time demands that the practitioners and even the general public be kept abreast of the reported incidence of any tissue toxicities.

Bitter Extra as a brand of bitters contains complex carbohydrates and alkaloids. Alkaloids are groups of naturally occurring chemical compounds that mostly contained basic nitrogen atoms. It also contains weak acidic properties[12]. The alkaloids have a wide range of pharmacological activities including antimalaria (e.g. quinine), antiasthma (e.g. ephedrine), anticancer (e.g. homoharringtonine)[13]. It also has other activities like cholinomimetic (e.g. galantamine)[14], vasodilatory (e.g. vincamine), antiarrhythimic (e.g. quinidine), analgesic (e.g. morphine)[12], antibacterial (e.g. chelerythrine)[15], and antihyperglycemic activities (e.g. piperine)[16]. It is mostly used as a cleanser drug for purifying the entire body system.

The cerebellum is a vital part of the brain that plays a cognitive role and in balanced

movements. Any distortion in the histo-architecture or morphology of the cerebellum may lead to cerebellar dysfunction resulting in uncoordinated movements and other neurological consequences. In a related study on the effect of Yoyo Cleanser Bitters on the cerebellum of adult male Wistar Rats by Shugaba[11]), marked changes with the width of the granular layer was observed.

There are growing incidences of many chronic diseases which affect some vital organs of the body coupled with high consumption rate of bitter products in our locality, it however, becomes imperative to carry out a study on the possible effect that may occur in the cerebellum following Bitter Extra administration.

Materials And Methods

Drugs

The herbal product, Bitter Extra was purchased from a registered pharmaceutical shop, in Abakaliki, Ebonyi State, South-East Nigeria. The Bitter Extra was ascertained to have been registered with the National Agency for Food, Drug Administration and Control (NAFDAC). The manufacture and expiry date of the product were inspected and all were confirmed not to have been expired. The manufacturer's seal was also inspected to ascertain that its originality was intact. Each bottle contains 200 ml of the content.

Experimental animals

A total number of sixteen (16) adult male Wistar rats with their initial weight ranging from 155g-230g were procured from the Animal House of the Department of Veterinary Medicine, College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria. The animals were handled in accordance with the guide for use of experimental animals by the Faculty of Medicine Pre-Clinical Research and Ethics Committee of Ebonyi State University, Abakaliki, Ebonyi State, Nigeria with ethical code number: MPC/1704/02/001. They were kept in well ventilated poly-ethylene cages in the animal house. The animal house was kept properly ventilated, cleaned and disinfected at an interval of 3 days to ensure a healthy environment. Also, they were kept in a suitable experimental condition within room temperature and a normal light cycle (12 hour light and 12 hour dark) during the period of the experiment. The animals were allowed free feeding with a standard diet and drinking water *ad libitum*.

Experimental design

Grouping of animals

The animals were divided into four (4) experimental groups, each consisting of four rats and treated for a period of four (4) weeks as follows: Group 1 was given distilled water (Control), Group 2 was administered with 1.35ml/kg of Bitter Extra (Low dose), Group 3 was administered with 2.7ml/kg of Bitter Extra (Medium dose) whereas Group 4 took 5.4ml/kg of Bitter Extra (High dose). The graded daily doses gave the opportunity of studying the effect of the low, medium and higher doses of Bitter Extra. They were all weighed daily with BRECKNELL EPB500 Pocket Balance Digital Scale with its calibrations in grams and their weights recorded.

Drug administration

The Bitter Extra was administered to the animals through an orogastric tube for a period of four weeks (28 days). The dosage of bitter extra was as per the human recommended dose of 0.45 ml/kg body weight.

Collection of experimental specimen and histological analysis

The animals were sacrificed by anesthetizing them in a jar containing cotton wool soaked in diethyl ether 24 hours after the last dose administration of their respective treatment and their various cerebellum harvested. The tissues were blotted dry using blotting paper. They were further subjected to normal routine histological procedures, stained with Hematoxylin-Eosin and examined using the light microscope. The main significant histological changes were noted and recorded.

The method employed to process the cerebellar tissue of all the groups was the paraffin wax method. The following steps are involved:

1) Decalcification of the rat's head using decalcifier. The reason for decalcification is to;

   a. Remove calcium component of bone, hence makes it softer.

   b. For easy removal of the brain from the skull.

2) Fixation using 10% formaldehyde. The reason for fixation are as follows; a. To coagulate blood protein. b. It facilitates staining. c. It hardens the tissue. d. Helps to prevent autolysis. e. Helps to prevent putrefaction.

3) Rinsing, that is, excess fixative removal with water.

4) Dehydration (in increasing alcohol e.g. ethanol from 70% to absolute alcohol) for 2 h each. This helps to remove water from the tissue.

5) Clearing (to replace ethanol with a solvent miscible with both ethanol and paraffin wax) with xylene used for 2 h.

6) Embedding (impregnation of tissue in molten paraffin wax and subsequently hardening by cooling). The tissue may have microscopic holes which are filled by the paraffin wax. This helps to harden tissues for easy sectioning.

7) Sectioning (slicing the wax-impregnated tissue on a microtome).It aids easy fixing of tissue on glass slide.

8) A fixing section of glass slide (usually with egg albumin). The albumin helps to hold tissue on glass slide.

9) The prepared slide is dewaxed using xylene for 5mins.

10) The tissue is then hydrated with decreasing grades of alcohol (e.g. from absolute alcohol to 70%) for 5mins each.

11) The tissue is transferred to Distilled H_2O for 1 min.

12) Staining of tissue is done using haematoxylin stain for 7mins. The staining is most widely used and important in general-purpose stain combination. Haematoxylin is a basic nuclear stain.

13) After staining with Haematoxylin, the tissue is washed in Distilled H_2O for 2 mins.

14) Differentiate in 1% acid alcohol for 20 s to remove excess stain that water cannot remove. 15) Wash in water for 1 min.

16) Blue with Scott's Tap Water until it turns blue within 5mins. This helps to increase the intensity of the stain (Haematoxylin) and make the tissue blue.

17) Counter stain with Eosin for 12 mins. Eosin is the acidic cytoplasmic counter stain which helps to stain the cytoplasm,

as the Haematoxylin helps to stain the nucleus.

18) Wash in tap water for 2 mins to remove excess counter stain.

19) Dehydrate in increasing grade of alcohol (e.g. 70%, 80% to absolute alcohol) for 5 mins each. This enable the removal of excess stain and water, because stain contains water and water is an agent of microorganism.

20) Clear in xylene for 5mins to remove or replace alcohol.

21) Mount with dipropanyl xylene (DPX) with cover slip which helps tissue to stick to slide, and allowed to dry overnight.

22) Finally it is observed under the light microscope.

Statistical analysis

Students' t-test was employed and the mean was presented in Mean ±SD. P values less than 0.05 were considered to be statistically significant.

Result

Mean body weight

The mean body weight of the animals in the test groups were measured and compared with that of the control group to ascertain the changes associated with their body weights as shown in Table 1. The mean body weights were not significant (P>0.05) among and within the test groups and the control. The histological results obtained are discussed and are based on the comparison between the histological features of the control group and those of the Bitter Extra treated groups.

Table 1: Changes in Mean Body Weight of the Rats

		N	Minimum	Maximum	Mean± SD	*P*-value
Week 1	G1	4	149.20	160.70	154.70±4.74	0.12
	G2	4	163.90	233.30	189.18±30.35	
	G3	4	149.60	198.00	169.98±20.44	
	G4	4	157.20	230.00	196.55±32.68	
	Total	16	149.20	233.30	177.60±27.80	
Week 2	G1	4	157.60	163.60	161.30±2.61	0.33
	G2	4	154.90	235.30	189.33±33.51	
	G3	4	138.90	202.90	172.63±26.30	
	G4	4	157.30	237.80	195.05±34.96	
	Total	16	138.90	237.80	179.58±28.28	
Week 3	G1	4	155.50	172.80	162.38±7.83	0.11
	G2	4	178.60	233.50	201.57±28.53	
	G3	4	182.60	203.10	190.67±10.93	
	G4	4	160.20	230.10	198.53±29.46	
	Total	16	155.50	233.50	187.16±25.25	
Week 4	G1	4	155.90	175.20	163.83±8.14	0.30
	G2	4	176.30	238.20	198.20±34.69	
	G3	4	173.20	199.60	184.10±13.79	
	G4	4	149.90	217.40	189.20±28.87	
	Total	16	149.90	238.20	182.79±24.51	

Histological features

The normal cerebellum has a smooth molecular layer and a rough granular layer separated by the Purkinje layer. The control, Group 1 has a smooth molecular layer while other groups have some degree of hypertrophy and hyperplasia. In Group 4 (Plate 4), increased stimulation of proliferation of the Purkinje cell bodies into the molecular layer was observed. Focal increase in Purkinje cells proliferation into the molecular layer and hypertrophy coupled with spreading of the molecular layer was observed in Plate 3 of Group 3 administered with normal dose. It was observed in Plate 2 of Group 2 an increased hypertrophy of Purkinje cell bodies within the molecular layer and hyperplasia of cells in the granular layer.

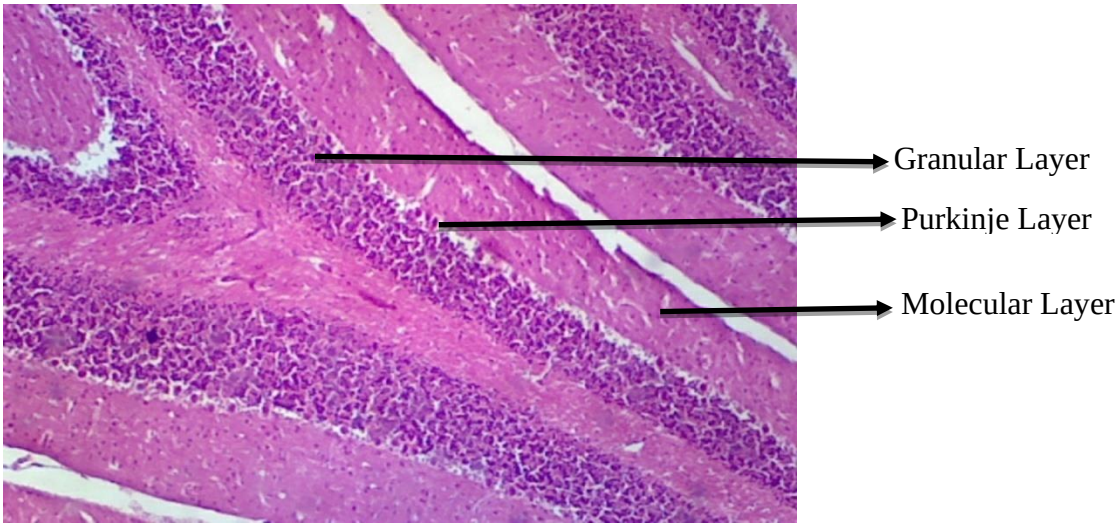

Figure 1: A section of cerebellum of rat (control) showing smooth Molecular Layer, Purkinje Layer and rough Granular Layer. Stain: **H & E, X100**

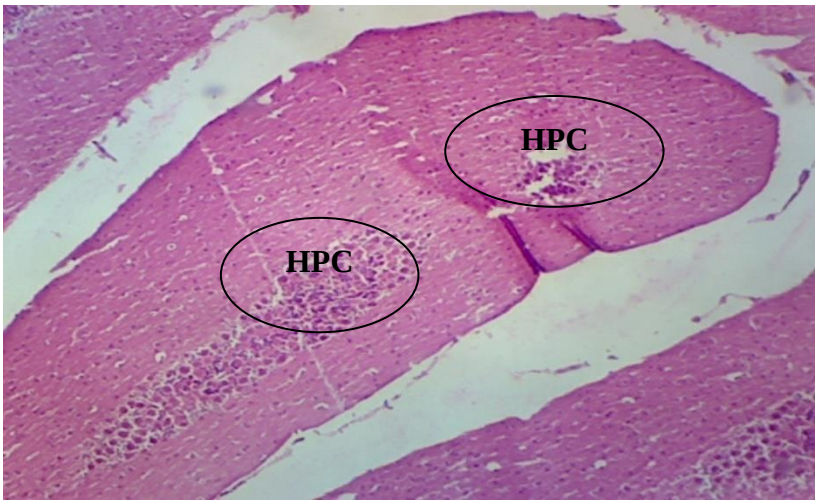

Figure 2: A section of cerebellum of rat treated with 5.4ml/kg (High dose) of Bitter Extra. Showing increased Hypertrophy of Purkinje Cell (HPC) bodies and hyperplasia of cells in the Granular Layer. Stain: **H & E, X100.**

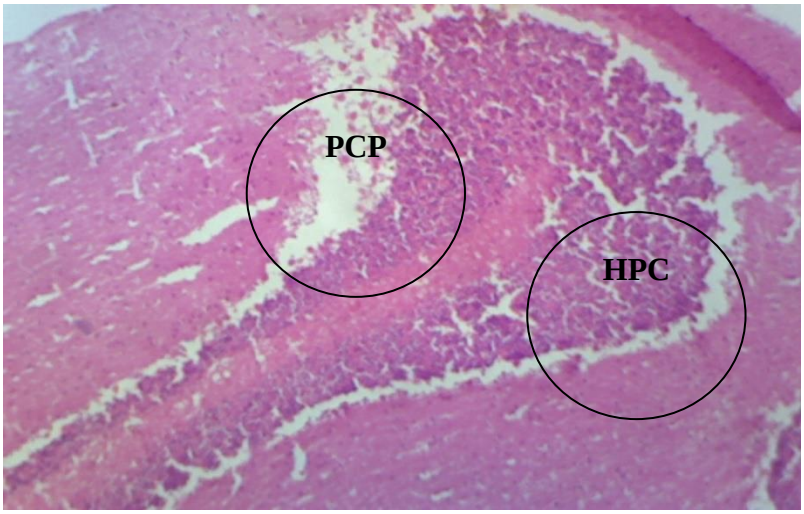

Figure 3: A section of cerebellum of rat treated with 2.7ml/kg (Normal dose) of Bitter Extra. Showing focal increase in Purkinje Cells Proliferation (PCP) and Hypertrophy of the Purkinje Cell (HPC) layer with pushing of the Molecular Layer. Stain: **H &E, X100**

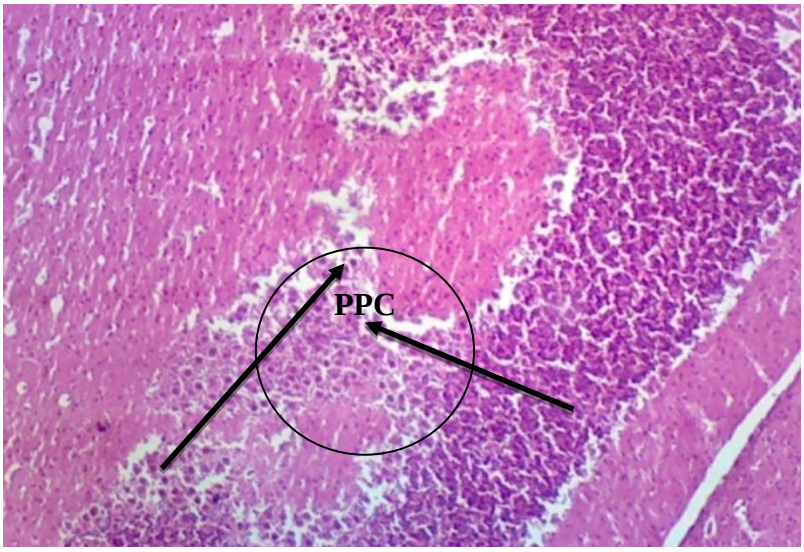

Figure 4: A section of cerebellum of rat treated with 1.35ml/kg (Low dose) of Bitter Extra. Showing increased Proliferation of Purkinje Cell (PPC) bodies into the Molecular Layer.

Stain: **H & E, X100.**

Discussion

Globally and indeed in Nigeria, local medicinal herbs are employed in the management of various diseases. The effect of Bitter Extra on the cerebellum and body weight of Wistar rats has been investigated in this study. The body weights of rats administered with Bitter Extra was not statistically significant when compared with the control, an indication that Bitter Extra has no effect on the body weight. This is in agreement with Shugaba[11] on the effect of Yoyo Cleanser bitters on the cerebellum of adult Wistar rats which revealed that overdose group showed hypertrophy of the granular layer of the cerebella cortex with a corresponding increase in granular cells. In this study, the result of the experimental groups when compared with the control group revealed a proliferation of purkinje cell bodies within the molecular layer which became more pronounced as the administered dose increases, a hypertrophy of the purkinje cell layer coupled with hyperplasia of the cells within the granular layer. The hypertrophy observed suggests increase in the size of the purkinje cells which may affect their proper functioning, hence, delays in sending inhibitory projections to the deep cerebellar nuclei and also affect the output of all motor coordination in the cerebellar cortex. The hyperplasia as seen in the granular cells is the increase in the number of cells of the granular layer which is an indication of a compromised function in the area of processing visual and motor information to learning and memory. In addition, when Bitter Extra is consumed excessively as observed in the overdose group, and possibly for a long period of time, it can lead to an observable cerebellar damage and cerebellar dysfunction features. This is in line with the findings that bitters can cause cerebellar damages and dysfunctions[17].

In a similar study using Nature Cure Bitters (NCB), the preliminary results associated with the toxicity studies did not produce severe toxicological effects on organ weights, biochemical and haematological indices as given at normal therapeutic doses. The graded doses of NCB were administered on a daily basis (100, 200 and 400 mg/kg) to rats for 28 days and the effects on body weight, organ weight, clinical signs, gross pathology, haematology, histology and serum biochemical parameters were evaluated. The relative weights of the heart, liver and testes of treated rats were unaffected in contrast to a significant increase in the relative weights of the lungs, kidneys and spleen[18]. Physiologically, the packed cell volume and haemoglobin concentrations were significantly reduced, whereas total leucocyte counts and glucose levels were remarkably increased. The calculated therapeutic index was >37.5 and histological findings did not reveal any treatment related effects[18].

Bitter Extra facilitates digestion, and therefore too much consumption can lead to poor absorption of vitamin B12 which is the main cause of pernicious anemia[19]. The deficiency of vitamin B12 has neurological consequences as it is an essential vitamin for the proper functioning and development of the brain and nerve cells[20]. Vitamin B12 plays an important role in the maintenance of the myelin sheaths that cover and protect the nerves of the central nervous system.

Also the effects of feeding of four vegetables commonly consumed in Thailand, namely, flowers of the neem tree (Azadirachta indica var. siamensis), fruits of Thai and the Chinese bitter gourd (Momordica charantia Linn.) and leaves of sweet basil (Ocimum basilicum Linn) on the levels of phase I enzymes, which include Cytochrome P_{450} (P_{450}), aniline hydroxylase (ANH) and aminopyrine-N-demethylase (AMD) as well as the capacity to activate

the mutagenicities of aflatoxin B1 (AFB1) and benzo[a]pyrene (BaP), and to induce the phase II enzymes [i.e. glutathione S-transferase (GST)] in rat liver. It was found that feeding of the diets containing 12.5% neem flowers and Thai bitter gourd fruits for 2 weeks strongly enhanced GST activity, 2.7- and 1.6- fold of the pair-fed control values, respectively, while resulting in a marked reduction of the levels of most phase I reactions[21]. The results in the study clearly demonstrated that Neem flowers and Thai bitter gourd fruits contain monofunctional phase II enzyme inducers and compounds capable of repressing some monooxygenases, especially those involved in the metabolic activation of chemical carcinogens. Sweet basil leaves contain compounds, probably bifunctional inducers, capable of inducing both phase I and phase II enzymes. Chinese bitter gourd fruits contain only compounds capable of repressing some monooxygenases[21].

Furthermore, Gentianae Radix, the dried root and rhizome of Gentiana lutea L. (Gentianaceae), has long been used as a remedy for liver and stomach inflammation, eye troubles, etc. Here, the gastro protective effects of the methanol extract of Gentian root (GM) were studied using different gastric lesion models. In pylorus-ligated rats, administration of GM in the duodenum suppressed gastric juice secretion and total acid output in a dose-dependent manner[22]. Oral or duodenum administration of GM showed significant protection against acute gastric ulcer induced by aspirin plus pylorus ligation, water-immersion restraint stress-induced ulcers, and gastric mucosal injury induced by ethanol[22]. Antimicrobial evaluation of acute and subchronic toxicity studies in rodents, of a Nigerian polyherbal formulation called Leon Bitters. Leon Bitters is prepared with Gongronema latifolia (climbing stem), Cocos nucifera (coconut) roots and Parinari curatellifolia seeds. Toxicity of the polyherbal preparation was evaluated in Swiss albino mice by administering to the animals oral graded doses of the lyophilized drug in the ranges of 1.0 to 20.0 g/kg body weight and observed continuously for the first 4 h and hourly for the next 12 h, then 6 hourly for 56 h (72 h, acute toxicity).

Wistar rats were also fed with different doses of the lyophilized drug for 30 days and the effects of the drug on some tissues - heart, liver, kidney and testes - were microscopically examined. Also the effects on the biochemical and haematological parameters were evaluated (sub-chronic toxicity model). The median acute toxicity value (LD_{50}) of the polyherbal medicine was determined to be 7.2 g/kg body weight. No significant increase in the body weight was observed in the groups treated with the drug compared to the control. The drug significantly reduced ($p \leq 0.05$) triglyceride (TG) level while low density lipoprotein (LDL)-cholesterol level was not altered, but led to increase in high density lipoprotein (HDL)–cholesterol in the treated groups compared to the control. There was no significant change in the mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration compared to the control. The study showed that the drug exhibited hypolipidemic activity and good reducing effects on cardiovascular factors. Since, most of the work carried out is on bitters associated to the digestive system. Here the work is extended beyond the digestive system to include the Nervous system, a part called Cerebellum.

Finally, the results revealed that the administration of Bitter Extra to the experimental groups has no significant effect on the weights of the animals as compared to the control. Histologically, the results of the experimental groups compared with control for the plates revealed that the cells

in the granular layer in the over dose group is increased, while the cells in the granule layer is reduced in the under dose group which confirms that Bitter Extra drug has a great effect on the cerebellum. And when Bitter Extra is consumed excessively it can lead to observable cerebellar damage with time like; ataxia, dysmetria, dysdiadochokinesia, scanning speech, intention tremor and nystagmus, and possible Neocerebellar Syndrome and cerebellar dysfunctional features[23,24].

Conclusion

The result of this study reveals that Bitter Extra has no significant effect on the weight of Wistar rats, while histologically it is observed that consumption of Bitter Extra in high doses may have a significant effect on the morphology of the cerebellum. Therefore, it is advised that consumption of Bitter Extra in high doses should be discouraged as it can affect the nervous system considering the fact that it is a cleanser drug for purifying the entire body system.

Conflict Of Interest

Authors declared they have no competing interest.

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Abstract

Background: Millions of people suffer from hypertension; and considering that it poses a significant risk for a number of serious health-related problems (such as stroke, kidney, and heart diseases), lifestyle modifications activities like exercise, healthier diet, and stress reduction has been shown to effectively control hypertension and lower the risk of problems.

Aim: This study examined the knowledge and awareness of the predisposing factors to hypertension as a measure of lifestyle modification influencing the management of hypertension among hypertensive patients.

Materials and Methods: one hundred and fifty (150) hypertensive patients attending Delta State University Teaching Hospital Oghara, Delta State, Nigeria were recruited using a descriptive cross-sectional research design. Data was collected using a questionnaire designed by the researchers. A face-to-face administration of the questionnaire was used. Data was summarized using frequencies and percentages and analysed using the spearman's rank order correlation at p-value significant at <0.05 level.

Results: This study revealed that most participants were knowledgeable about the causes of hypertension and considered stroke as a complication of hypertension. Most participants were aware that a person with hypertension should practice a healthy diet, reduction of chronic stress level and should participate in aerobic exercise, while adopting cessation of smoking as measure for the control hypertension complications. Similarly, most of the respondents accepted the following factors are the influence of lifestyle modifications in the management of hypertension: having enough rest, mild to moderate exercise, low consumption of low salt diet, and high consumption of fruits and vegetables.

Conclusion: Most of the participants were knowledgeable about hypertension and the associated causative factors; and aware of various positive lifestyle modification strategies in the management of hypertension. However, the level of awareness of the respondents still needs to be increased, as it would improve the lifestyle modifications.

Keywords: *Awareness, Hypertension, Lifestyle modifications, Hypertensive patients*

Introduction

Hypertension is a chronic medical condition characterized by elevated blood pressure in the arteries. Blood pressure is the force of blood against the walls of the arteries as the heart pumps it around the body. It is recorded as two values: systolic pressure (the pressure when the heart beats while pumping blood) and diastolic pressure (the pressure when the heart is at rest between beats). Hypertension results if the systolic blood pressure is greater than 140mmHg and diastolic pressure greater than 90mmHg based on the average of two or more accurate blood pressure measurements taken during two or more contacts with a health care provider[1]. A blood pressure of less than or equal to 120/80mmHg is normal, with values of 120 - 129mmHg systolic and 80 - 89mmHg diastolic are prehypertension. Individuals with consistent blood pressure readings of 140 mmHg systolic and 90mmHg diastolic or higher are classified as hypertension. It is important to state that hypertension is a non-communicable disease, which may develop as a result of certain behavioural or lifestyle practices[2]. Hypertension is a leading risk factor for mortality in both developed and developing countries and directly affects 972 million people worldwide; and is a contributing factor for another 370 million with cardiovascular-related diseases[3].

The prevalence of hypertension varies widely, ranging from a minimum of 3.2% to a maximum of 10.1% for children and adolescents, and from a minimum of 21.5% to a maximum of 78.5% for adults[4]. According to a previous study, the predictions of increased prevalence in the future persist and by 2025, up to 1.58 billion adults worldwide are likely to suffer complications of hypertension[5]. The causes of hypertension can be primary or secondary[6]. Hypertension could be primary (high blood pressure from unidentified causes) or secondary (hypertension related to identified causes such as narrowing of the renal arteries, hyperaldosteronism, etc). Invariably, the kind of high blood pressure that typically develops gradually over many years is called primary (essential) hypertension. Many people have high blood pressure for unknown reasons. Conversely, secondary, or non-essential, hypertension is a form of hypertension caused by the consequences of other illnesses. They include; congenital anomalies, pyelonephritis, obstruction in renal artery, acute or chronic glomerulonephritis, aortic stenosis, tumours in the adrenal gland (Pheochromocytoma), Cushing syndrome, hyperthyroidisms, sleep apnea and drugs such as NSAIDs, oestrogen, sympathomimetics, steroids, antidepressants, etc. Developing countries are highly affected by hypertension, of which Nigeria is not exempted[7].

The predictions of a high and ongoing incidence of hypertension in Africa, especially in rural areas, suggests that it is a major risk factor for cardiovascular disease, including stroke, heart attack, and heart failure[8]. Age-related increases in hypertension are steady. For example, in Africa, the frequency among older people is almost two to four times higher than that of younger adults[9]. Hypertension may not give any symptom, therefore, it is referred to as a “silent killer” Otherwise symptoms such as severe headache, shortness of breath, dizziness, and chest pain, only occurs when blood pressure is already high. If left uncontrolled, hypertension can lead to stroke, dementia, coronary heart disease, kidney failure, and ultimately, sudden cardiac death[3]. Adequate knowledge of lifestyle modifications as preventive and management measures of hypertension is essential in reducing its prevalence, damaging effects, and associated complications[10]. Blood pressure levels can

be reduced with antihypertensive drugs, which are effective in reducing the risk or severity of cardiovascular disease. However, drug therapy is not the only means of preventing and treating high blood pressure. Non-pharmacological approaches have been effective in the prevention and treatment of hypertension. Awareness of the risk factors associated with hypertension, such as obesity, high sodium intake, sedentary lifestyle, and chronic stress, can motivate people to adopt lifestyle modifications.

A good knowledge of the preventive measures of hypertension is vital. Adequate preventive measures for high blood pressure can effectively reduce the risks of stroke and other complications such as myocardial infarction, chronic kidney disease and heart failure[11]. Lifestyle modifications recommended for both the treatment and prevention of hypertension include dietary changes or a healthy diet, increased physical activity, or participation in aerobic exercises such as brisk walking, swimming, or cycling to facilitate weight reduction, moderation of alcohol consumption, and stress management considering that chronic stress contributes to elevated blood pressure. According to Sefah et al.[12], some lifestyles and attitudes which include excessive alcohol consumption, sedentary lifestyles, smoking cigarettes, may contribute to the rising levels of hypertension predisposition. In addition, Adekoya & Sodeinde[3], pointed out that unhealthy lifestyle choices include smoking, drinking alcohol, leading a sedentary lifestyle, having a high level of job strain, poor diet (food containing too much salt and fat), obesity and having a family history of hypertension also makes one prone to the condition[13].

The role of knowledge and awareness is pivotal lifestyle modifications for the management of hypertension. Such a role include; educating patients about the importance of lifestyle modifications, awareness of risk factors associated with hypertension, and access to resources such as educational materials, support groups, and nutritional counselling[14]. Despite the importance of knowledge and awareness, there several challenges and barriers to implementing lifestyle modifications for hypertension management. These may include socioeconomic factors such as limited financial resources, limited social support or encouragement from family members, friends, or community networks, and cultural or traditional dietary preferences[15]. By addressing these factors, people with hypertension can be better equipped to make sustainable lifestyle changes that contribute to improved overall health and well-being[16]. Hence, the conceived need to examine the knowledge of hypertensive patients at the Delta State University Teaching Hospital Oghara on lifestyle modifications as a measure for the management of hypertension.

Materials and Methods

Research Design

A descriptive cross-sectional survey was used. Data was collected about different variables from the sample at one point of time in order to reveal the relationship between these variables, therefore it was considered for this study.

Study Setting

The study was carried out at the Delta State University Teaching Hospital (DELSUTH). Reputably established and accredited, DELSUTH serves as a teaching hospital for Delta State University (DELSU), Abraka. It is situated in Oghara, Ethiope West Local Government Area of Delta State in the South-South region of Nigeria. Initially, the hospital was intended to be an ultramodern, 180 bed specialist facility. Oghara, a town in the Ethiope West Local Government Area, shares borders with the Mosogar Kingdom

to the east via the Ethiope River, Sapele Local Government Area to the south via an adjacent tributary of Ethiope, Delta State to the North via the Osiomo/Ologbo River, and Koko in the Warri North Local Government Area of Delta State to the South-West, Nigeria.[17-18]

Population of the study

The target population covered by this study included hypertensive patients attending DELSUTH, Oghara, Delta State. The average population of patients at the time of the study was 240 hypertensive patients registered at the facility. Information about the participants were obtained from the patients in male and female medical units, out-patient department of the hospital.

Sample and sampling technique

The sample size for this study was calculated using the Taro Yamane method described as follows;

$$n = \frac{N}{1 + N(e)^2}$$

Where; n is the sample size, N is the population size, and e is the level of precision that is usually 0.10, 0.05, or 0.01 (i.e. 10%, 5% or 1%). Using a level of precision of 5% and the population size of 240

$$n = \frac{240}{1+240\,(0.05)^2} = 150$$

Hence, the sample size for this study is 150 patients. A purposive sampling technique was used to recruit the participants.

Instrument for data collection

Data as obtained using a structured questionnaire designed by the researchers. The items in the questionnaire were generated according to the objectives set for the study. For the purpose of this study, the questionnaire was constructed in four (4) sections. Section A contains sociodemographic data, Section B contains data on knowledge of hypertension, Section C contains data on level of awareness of lifestyle modifications and section D contains data on the various lifestyle modifications used by hypertensive patients.

Validity of the instrument

To ensure valid results, the instrument was validated for face and content validity by experts who were staff of the Department of Measurement and Evaluation, Delta State University, Abraka. Face validity, which is a form of self- evident measure, was used to make sure the questionnaire was appealing to sight and it was presentable to the respondents irrespective of the format. The validity of the content was determined to ensure that the questionnaire measures what it should measure. The final draft of the questionnaire was administered to the participants.

Method of data collection

A face-to-face method of data administration of the questionnaire was used. The researchers went to the hospital, met with the respondents, and distributed the questionnaires to them after informing them about the study and obtaining consent to participate. The researcher administered questionnaires allowing a period of 30 minutes to 1 hour to fill the questionnaires before the same was retrieved from them. The data collection process lasted for a period of one week to allow the researcher to cover all participating subjects.

Ethical considerations

Ethical approval was obtained from the Ethical Review Committee of the DELSUTH, Oghara to carry out this study with permit number; HREC/PAN/2022/041/0537. The distribution of the questionnaire to the

respondents was made after the objective of the study was explained to gain informed consent. Anonymity, privacy, and confidentiality were maintained throughout the study.

Data analysis

Findings are presented using percentages, frequency distribution, and tables. The formulated hypothesis was tested using Spearman's rank order correlation at the $p< 0.05$ level of significance.

Results

From table 1 below, most of the respondents (40 or 26.6%) were 60-69 years old; 80 (53.3%) of the respondents were Christians, 60 (40%) were females, and 80 (53.3%) were Urhobo, 5 (3.3%) were Igbo, 15 (10%) were Hausa, and 10 (6.6%) were Yoruba, while 40(26.7%) were from other tribes. With respect to marital status, 90 (60%) were married, 25 (16.7%) were singles, 20 (13.3%) were widowed, and 15 (10%) were divorced. Based on educational background, most (46.7%) of the respondents had at least a secondary school education while 30 (20%) had no formal education; 45 (30%) of the respondents were self-employed, with 55 (36.6%) being farmers.

As shown in table 2, The majority of the respondents (69.3%) had adequate knowledge that hypertension is sustained elevated blood pressure; 88 (58.6%) of the respondents had agreed that consumption of high salt diet causes hypertension. Similarly, most respondents (69.3%) agreed that consumption of high fat causes hypertension; while 80 (53.3%) of the respondents agreed that lack of exercise causes hypertension. Furthermore, 50 (33.3%) recognized stroke as a complication of hypertension compared to majority that did not recognize stroke as a complication 100 (66.7%). In the same vein, 42 (28%) of the respondents accepted that hypertension cannot be cured compared to 108 (72%) that felt it can be cured.

From Table 3 it can be observed that most of the respondents (85.3%) were aware that a person with hypertension should practice a healthy diet; while 100 (66.7%) were aware that hypertensive patients should participate in aerobic exercise. Also, most of the respondents (61.3%) were aware that cessation of smoking help control hypertension complications; 78 (52%) were aware that chronic stress contributes to elevated blood pressure; while 102 (68%) were aware hypertensive patients should eat low salt diet. Additionally, 90 (60%) were aware that hypertensive patients should moderate their alcohol consumption.

Table 4 showed the result of lifestyle modifications used by respondents including the influence of some lifestyle modifications. While 60 (40%) of the respondents rejected the use herbs and routinely consume alcohol to control their blood pressure, 102 (68%) accepted that having enough rest helps to control their blood pressure. Also, 100 (66.6%) of the respondents sometimes eat low or no salt diet to reduce complications. Most of the respondents accepted that the following factors may influence lifestyle modifications in the management of hypertension: having enough rest (128, 85.3%), mild to moderate exercise (102, 68%), low consumption of low salt diet (102, 68%), and high consumption of fruits and vegetables (90, 60%) while 40(26.62%) accepted that cessation of smoking was not influenced.

Table 5 presents the correlation between level of awareness of lifestyle modifications and knowledge of lifestyle modifications in the management of hypertension. The results revealed that the two variables were positively and significantly correlated ($p<0.0001$).

Table 1: Sociodemographic characteristics of the respondents

Variables	Frequency	Percentage (%)
Age (years)		
20-29	10	6.6
30-39	25	16.7
40-49	15	10
50-59	35	23.3
60-69	40	26.7
70-79	25	16.7
Total	**150**	**100**
Religion		
Christianity	80	53.3
Muslim	5	3.3
Traditionalist	35	23.3
Others	30	20
Total	**150**	**100**
Gender		
Male	90	60
Female	60	40
Total	**150**	**100**
Tribe		
Urhobo	80	**53.3**
Igbo	5	3.3
Hausa	15	10
Yoruba	10	6.6
Others	40	26.7
Total	**150**	**100**
Marital status		
Married	90	60
Single	25	16.7
Divorced	15	10
Widowed	20	13.3
Total	**150**	**100**
Level of Education		
Primary	35	23.3
Secondary	70	46.7
Tertiary	15	10
No formal education	30	20
Total	**150**	**100**
Profession		
Employed	50	33.3
Self employed	45	30
Students	20	13.3
Unemployed	35	23.3
Total	**150**	**100**
Occupation		
Trader	45	30
Farmer	55	36.6
Business	50	33.3
Total	**150**	**100**

Table 2: Knowledge of hypertension among respondents

Variables	YES		NO	
	Frequency	Percentage	Frequency	Percentage
Hypertension is sustained elevated blood pressure	104	69.3%	46	30.7%
Hypertension is caused by consumption of high salt diet	88	58.6%	62	41.4%
Hypertension is caused by smoking	88	58.6%	62	41.3%
High fat consumption can cause hypertension	104	69.3%	46	30.7%
Lack of exercise is a cause of hypertension	80	53.3%	70	46.7%
Stroke is a complication of Hypertension	50	33.3%	100	66.7%
Hypertension cannot be cured	42	28%	108	72%

Table 3: Level of awareness of lifestyle modifications among respondents.

Variables	YES		NO	
	Frequency	Percentage	Frequency	Percentage
A person with Hypertension should practice a healthy diet	128	85.3%	22	14.7%
Hypertensive patients should participate in aerobic exercise	100	66.7%	50	33.3%
Cessation of smoking help control hypertension complications	92	61.3%	58	38.7%
Chronic stress contributes to elevated blood pressure	78	52%	72	48%
Hypertensive patients should eat low salt diet	102	68%	48	32%
Hypertensive patients should moderate their alcohol consumption	90	60%	60	40%

Table 4: Lifestyle modifications used by Respondents

Variables	YES		NO	
	Frequency	Percentage	Frequency	Percentage
I take herbs to control my blood pressure	60	40%	90	60%
I have enough rest to control my blood pressure	102	68%	48	32%
I routinely consume alcohol to control my blood pressure	60	40%	90	60%
I sometimes eat low or no salt diet to reduce complications	100	66.6%	50	33.3%
Influence of lifestyle modifications in Hypertension management				
Does enough rest lower your blood pressure?	128	85.3%	22	14.2%
Does mild to moderate exercise help control your blood pressure?	102	68%	48	32%
Did routine consumption of low salt diet lower your blood pressure?	102	68%	48	32%
Did Cessation of smoking help control your blood pressure?	40	26.6%	110	73.4%
Did high consumption of fruits and vegetables help lower your blood pressure?	90	60%	60	40%

Table 5: Correlation between the level of awareness of Lifestyle modifications and knowledge of lifestyle modifications in the management of hypertension.

Variables	N	Mean	Std	Std Error	r-cal	DF	p-value
Level of awareness of lifestyle modification	150	0.3413	0.23235	0.01897	0.0810	148	0.0001*
Knowledge of lifestyle modification	150	0.3840	0.20921	0.01708			

Discussion

The sociodemographic characteristics explored revealed that a higher proportion of the respondents were in the age range of 60-69 years, while the least represented age group was 20-29 years. Majority of the respondents were Christians. This could be attributed to the fact that the study location used is predominated by Christians. The majority of the respondents were from Urhobo ethnicity. Delta State University Teaching Hospital is in the Oghara community and majority of the residents and the nearby communities are Urhobo speaking communities and most participants were married, which could be attributed to the age range of the majority of participants in this study. Most of the respondents only attained secondary education, followed by those who attained primary education, and the least attained their tertiary education. Most of the respondents were farmers, followed by into business and trading. 60% of the respondents were male, 40% were female. Yang et al.[11], identified male sex as one of the main determinants of the value high blood pressure. From this study, 33.3% of the respondents were employed, 30% were self-employed, 23.3% were unemployed, and 13.3% were students. A previous study has equally reported that lifestyle practices such as high job pressure, environmental and mental stress, equally make one prone to the condition[3,19].

In the present study, most of the respondents have adequate knowledge about the concept and causes of hypertension. For example, most respondents accepted that consumption of high fat causes hypertension (69.3%), lack of exercise causes hypertension and stroke as a complication of hypertension. This is consistent with a previous study that reported that 54.2% of participants in their study did not know the reasons or consequences of hypertension. Likewise, 52.5% of patients whose blood pressure was not under control did not know the causes or complications of hypertension[20]. In the present study, the level of awareness of lifestyle modifications in the management of hypertension among respondents was high. Majority of the respondents, 65.85% were aware of lifestyle modifications in hypertension management while 34.14% were not aware. This agrees with an existing study that reported that healthy lifestyle behaviours play significant role in the control of hypertension among adult hypertensive patients[21]. The results of this study also showed that those who were aware, but not using any antihypertensive medications are able to control their level of blood pressure better than those using medications. This study attests that the level of awareness about lifestyle modifications is important in the management of hypertension. The study conducted by Modey et al.[20], concluded that increased physical activity, abstaining from alcohol and cessation of smoking, increased intake of fruits and vegetables, and reduced intake of carbohydrates, meat, and fat have a positive influence on blood pressure control. As such, modifying factors play a key role in complementing pharmacotherapy in hypertension control[21].

The results of the present study on the influence of lifestyles changes in the management of hypertension which include; 128(85.3%) respondents answered yes to the question ‘did having enough rest help lower your blood pressure?’, 102(68%) answered yes to the question ‘does mild to moderate exercise help control your blood pressure?’, 90(60%) also answered yes to the question ‘does consumption of fruits and vegetables help lower your blood pressure?’. The results of the present study showed some positive influence of lifestyles in the management of hypertension. Alhalaiqa et al.[22] in a study of adherence to lifestyle modification factors among hypertensive

patients stated that addressing behavioural risk factors (physical inactivity, high blood sugar increased cholesterol levels, obesity, exposure to stress, and poor stress management) has a positive influence on preventing and controlling hypertension. Also, Buda *et al*.[23], stated that lifestyle modifications through changes in eating patterns, abstinence from alcohol, weight management, smoking cessation and regular physical activity are part of important and effective treatment strategies for hypertension.

Conclusion

This study has shown that most of the respondents have good knowledge of hypertension, and are aware of lifestyle changes and its influence. Most of the respondents also practiced various lifestyle modifications. Also, a large percentage of the participants are aware of various positive lifestyle strategies in the management hypertension. However, the level of awareness of the respondents still needs to be increased as it would improve the lifestyle modifications that they use.

Conflict of interest

No conflict of interest.

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COMPARATIVE ASSESSMENT OF URINE LIPOARABINOMANNAN ASSAY AND ZIEHL NEELSEN SPUTUM SMEAR MICROSCOPY AS TUBERCULOSIS DIAGNOSTIC TOOLS IN NNEWI, NIGERIA

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Abstract

Introduction. Tuberculosis (TB) remains a major public health issue worldwide, with about 8.0 million new cases and 2.0 million deaths yearly, the burden being more in the developing countries partly due to missed or improper diagnosis. Most available methods for TB diagnosis have one shortcoming or the other. The current detection rate of TB is far below set target, making it imperative for diagnostic tests that are rapid and accurate.
Aim: To assess the accuracy of urinary lipoarabinomannan (LAM) antigen and ZN microscopy assays in detecting MTB using Gene Xpert MTB/RIF as the gold standard.
Methodology: A total of 200 participants, 109 males and 91 females, accessing care at the TB (DOTS) Center of Nnamdi Azikiwe University Teaching Hospital, Nnewi were tested for TB using urinary LAM ELISA, ZN sputum microscopy and Xpert MTB/RIF. All the participants were recruited sequentially as they presented at the DOTS clinic. This is an experimental cross sectional study and all the data obtained were analysed using SPSS (statistical package for social sciences), version 20.
Results: ZN microscopy with a sensitivity of 80.0%, specificity of 91.1%, positive predictive value of 90.0%, negative predictive value of 82.2%, and diagnostic accuracy of 85.6% was found to be comparatively superior to LAM ELISA which had sensitivity of 13.3 %, specificity of 71.1% , positive predictive value of 31.6%, negative predictive value of 45.1 and diagnostic accuracy of 42.2%. Positivity rate with LAM ELISA was higher in HIV-positive patients (30.8 %) than in the HIV negative patients (20.0%).

Conclusion: LAM antigen assay does not appear to be very useful as a stand-alone test for TB due to its variable sensitivity and specificity compared to the gold standard in TB diagnosis which is smear microscopy. Smear microscopy should be optimized for enhanced performance in view of its superiority over LAM ELISA as noted in this study.

Key words: *Tuberculosis, Gene Xpert MTB RIF Assay, ZN Staining, LAM ELISA Assay*

Introduction

Tuberculosis (TB) is an air-borne infectious disease caused by various strains of Mycobacteria that usually attack the lungs. TB remains a major public health issue worldwide, with an estimated 8.0 million new cases and 2.0 million deaths annually. The burden is particularly pervasive in the developing regions of Sub- Sahara Africa and South East Asia, both of which have the countries with the world's highest TB burden, accounting for 80% of global TB cases. This situation is partly attributed to delayed, missed or improper diagnosis in these resource-limited countries[1]. Undiagnosed and misdiagnosed TB drives the TB epidemic by creating and maintaining a continuous pool of transmission of the TB organism. There is postponement of initiation of treatment, which potentially leads to increased morbidity and mortality[2].

Rapid and accurate laboratory diagnosis is critical to effective TB treatment[3,4]. The Mantoux tuberculin skin test is only a screening test that measures delayed-type hypersensitivity response to purified protein derivative. Its results may be interfered by Bacilli Calmette- Guerin (BCG) vaccination. It is not confirmatory and a negative result does not rule out TB disease, as may be seen in those with HIV, sarcoidosis and malnutrition. Also, the administration and reading of the TST require a certain amount of expertise that, when lacking, may result in erroneous interpretations[5]. Gamma interferon release assays provide an alternative for the diagnosis of latent TB infection. They show higher sensitivity and specificity, better correlation with exposure to the TB organism, lower cross-reactivity with BCG, hence fewer false-positives compared to Mantoux test. However, there is not yet adequate evidence for the accuracy of these tests for specific populations, including HIV-infected or other immunocompromised patients and children[3,6].

The old Ziehl-Neelsen sputum smear microscopy method remains the method of choice in most resource poor countries because of its relative cost effectiveness in terms of equipment and reagents. However, the method has technical and operational characteristics that limit the quality and scope of its applications. It is tedious and time consuming, thus limiting the number of slides that can be examined per day. It also has the problem of comparative low sensitivity. It detects fewer than 60% of all new TB infections and as few as 20-35% of HIV/TB infections[7]. In Nigeria there is little or no facility at the primary health care centers for reliable diagnosis of TB. Skilled and trained manpower, infrastructure and electricity supply are in short supply or completely non-existent making it difficult to diagnose TB. The method also requires repeated visits to the hospital for sample submission and result collection. Patients drop out in the diagnostic process is common with the result that many TB cases remain undetected and untreated[8]. Use of culture is the gold standard. It allows for

proper identification of the isolated organism and drug sensitivity testing. However, this is not feasible in resource limited settings due to its cost, technicality and prolonged turnaround time[9]. There have been recent impressive advances in the field of TB diagnosis. Several automated molecular techniques (including the Gene Xpert MTB/Rif assay and Line Probe assay) with reduced turn over time have been developed but their high costs or sophisticated infrastructure requirement makes them unaffordable for large scale routine use[10,11].

Perhaps, as part of the solution to this challenge, rapid diagnostic tests that detect TB antigens or antibodies have been developed. These tests are quite attractive because they are easy to use, rapid and relatively inexpensive and without the need for sophisticated laboratory infrastructure. Some formats can be performed in the fields or at the point of care by non-technical staff. So many of these[rapid test kits] are commercially available, but most are not endorsed by any international body[12]. Yet, they are packaged, offered for sale and are widely used in many TB high burden countries[13]. There are contradictory reports in support and against the use of some of these kits, based on their highly variable sensitivity (0- 100%) and specificity (31-100%)[14].

There has been an addition to the list of these assay kits, the urinary TB lipoarabinomannan (LAM) antigen test, which detects the presence of LAM antigen in urine of TB patients. The test is easy to perform and very convenient as urine is easier to collect, and may be less variable in quality and safer to handle. Urine is a particularly more attractive specimen in children who do not have forceful expulsion of sputum. The collection procedure is not invasive. Again, there is no repeated visits to the hospital for sample submission and result collection. The amount of LAM in the urine is said to reflect the bacterial load, hence the assay permits a semi-quantitative assessment of the infectious status[15]. The assay is said to be useful in people living with HIV and children who are disproportionately affected by smear-negative and extra pulmonary TB[16].The increasing sensitivity of urine LAM testing with progressive immune suppression (as reflected by falling CD4 cell counts) is a major distinguishing feature from other TB diagnostics such as sputum smear microscopy which loses sensitivity with worsening immune suppression[17]. As total Mycobacterial burden increases with progressive immune suppression, there is a concomitant increase in the urinary LAM excretion[18].

The Directly Observed Treatment Short course (DOTS) strategy has recorded significant improvement in TB detection in Nigeria. However, the set target for the TB detection rate (at least70%) has not been achieved. The current detection rate of 16% [19] is far below the target and constitutes an impediment in the fight to stop TB. There is the need for early case detection with diagnostic tests that are rapid and accurate if the set TB control targets must be achieved. Most conventional methods for TB diagnosis have one shortcoming or the other as aforementioned. Rapid TB diagnostics such as the urinary TB LAM antigen test has the potentials to close the gap between this current detection rate and target detection rate. The test has many appealing properties for use in resource-limited settings, including cost effectiveness and speed. The lateral flow urine LAM assay (the routine diagnostic) version takes about 25minutes[16,20,21].

However, even as promising as the assay may appear, it is still very important that the

kit be evaluated before it is endorsed for use in Nigeria. This is because evaluation of several rapid TB serological tests has shown that these tests have variable performances in different epidemiological settings[22]. This study was aimed to compare the sensitivity, specificity and diagnostic accuracy of ZN microscopy and urinary LAM Ag in detecting *MTB* using Gene Xpert MTB/RIF as the gold standard.

Methodology

A total of 200 pulmonary TB suspects comprising 109 males and 91 females, of mean age of 29.7 years, 65 of whom were HIV positive and 135 HIV negative and with cough ≥ 2 weeks accessing care at Nnamdi Azikiwe University Teaching Hospital (NAUTH), Nnewi were used for this study. Patients already enrolled in TB treatment prior to commencement of this study were excluded. Written informed consent was obtained from each of the subjects. Ethical clearance was obtained from the Ethics Committee of NAUTH. Each patient submitted two samples of sputum, one on the spot on first day and the other as early morning sample the second day, according to the national algorithm[5]. Each sample was collected in sterile, wide- mouthed, transparent, leak-proof sputum cups. Each patient also collected early morning urine sample into sterile universal container for detection of urinary TB LAM antigen. Urine samples were frozen- stored as soon as received in the laboratory.

Smear measuring 1cm × 2cm was made from each sputum sample by spreading the sputum in an oval shape, allowed to air dry, heat-fixed and stained by the hot Ziehl Neelsen staining procedure as adopted by National Tuberculosis Programme[5]. Positive and negative control slides were included in each batch of staining. Each stained smear was examined under ×100 oil objective lens and scored according to the WHO/IUATLD system (1980)[23].

Each of the early morning sputum sample for Xpert assay was diluted 2:1(v/v) with the sample reagent, mixed by shaking vigorously 10-20 times and incubated at room temperature for 15 minutes to achieve sample liquefaction and inactivation of *Mycobacteria tuberculosis.* Two (2.0) ml of the diluted sputum was transferred into the Xpert MTB/RIF cartridge, scanned with bar-code scanner to determine its content integrity/expiry date. The cartridge with diluted sample was placed into the module for real time polymerase chain reaction. Results were recorded as: MTB not detected (Negative), MTB detected/RIF resistance not detected (Positive), MTB detected/RIF resistance detected (MDRTB), accordingly at the end of the processing[24].

The urinary LAM ELISA assay procedures were conducted according to the kit producer's instructions. The optical density was measured at 450 nm using a microtiter plate reader within 15 minutes to obtain the LAM concentration. The average concentration for each sample was compared to the cut-off value of 0.98mg/100ml to determine positivity or negativity[25].

The statistical analysis of results was done using SPSS (statistical package for social sciences), version 20, to carry out chi square tests and MedCal software to compare sensitivity and specificity as well as the positive predictive values (PPV), negative predictive values (NPV), likelihood ratios and diagnostic accuracy of ZN AFB microscopy and LAM ELISA methods with Gene Xpert as the gold standard. P value was calculated at 95% confidence level and P values less than 0.05were considered statistically significant.

Results

Ninety (45%) of the 200 patients were diagnosed TB positive using the Gene Xpert MTB/Rif assay, while ZN microscopy and Urinary LAM ELISA detected TB in 82 and 47 of 200 respectively. ZN microscopy showed a sensitivity of 80.0%, specificity of 91.1%, positive predictive value of 90.0%, negative predictive value of 82.2%, and diagnostic accuracy of 85.6% over urinary LAM ELISA with a sensitivity of 13.3 %, specificity of 71.1% , positive predictive value of 31.6%, negative predictive value of 45.1 and diagnostic accuracy of 42.2% as shown in figure 1.

The association between HIV status of the patients and results of the Gene Xpert MTB/RIF assay is shown in Table 1. Eighteen (18) (27.7%) of the 65 HIV positive TB suspects had HIV-TB co-infection. The association between HIV status of the patients and results of the Ziehl Neelsen AFB microscopy technique is shown in Table 2. The Ziehl Neelsen technique was seen to be more sensitive in HIV negatives (48.9%) than in HIV positives (24.6%) (p - value: 0.001). The association between HIV status of the patients and results of urinary LAM ELISA assay which is shown in Table 3 indicates that the positivity rate with LAM ELISA was higher in the HIV positives (30.8%) than in the HIV negatives (20.0%) (p - value:0.092).

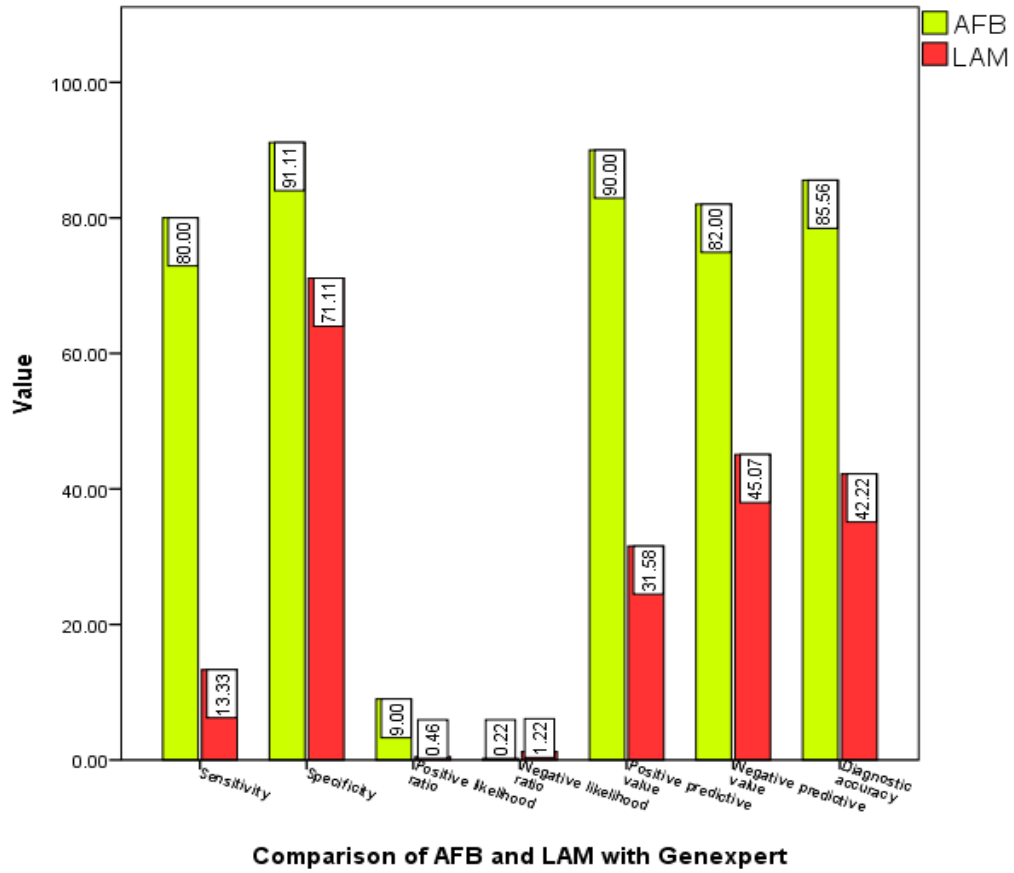

Figure 1 : Comparison between ZN AFB microscopy and LAM ELISA.

Table 1: Association between TB status by Gene Xpert MTB/ RIF and HIV status of patients.

			HIV Status		Total	P-value
			Positive	Negative		
Gene Xpert	Positive	Count (% within HIV Status)	18 (27.7%)	72 (53.3%)	90 (45.0%)	0.001
	Negative	Count (% within HIV Status)	47 (72.3%)	63 (46.7%)	110 (55.0%)	
Total		Count (% within HIV Status)	65 (100.0%)	135 (100.0%)	200 (100.0%)	

Table 2: Association between TB status by AFB and HIV Status of patients.

			HIV Status			P-value
			Positive	Negative	Total	
AFB	Positive	Count(% within HIV status)	16 (24.6%)	66(48.9%)	82 (41.0%)	0.001
	Negative	Count (% within HIV status)	49 (75.4%)	69 (51.1%)	118 (59%)	
Total		Count (% within HIV status)	65 (100%)	135(100%)	200(100%)	

Table 3: Association between TB status by LAM ELISAA and HIV status of patients.

			HIV Status			P-value
			Positive	Negative	Total	
LAM	Positive	Count (% within HIV Status)	20 (30.8%)	27 (20.0%)	47 (23.5%)	0.092
	Negative	Count (% within HIV Status)	45 (69.2%)	108 (80.0%)	153 (76.5%)	
Total		Count (% within HIV Status)	65(100 %)	135 (100%)	200 (100%)	

Discussion

In this study ZN AFB smear microscopy was found to be more sensitive and specific than the LAM antigen test. Smear microscopy has a sensitivity of 80.0% and specificity of 91.1% higher than sensitivity of 13.3% and specificity of 71.1% of LAM ELISA assay. This finding tallies with those of another studies[26-28]. On the other hand some previous studies have evaluated urine LAM antigen test and found out that its sensitivity and specificity were superior to that of sputum smear microscopy[16, 29-35] in Nigeria documented that urine LAM testing was more sensitive (77.8%) than the AFB smear microscopy (33.3%). These variations may be related to operational and technical issues surrounding LAM assay and which impacts its sensitivity and specificity. These include the involved different patient population, technical issues such as the collection of more than one sputum samples or use of sputum concentration or fluorescence microscopy method which are said to increase diagnostic sensitivity of sputum smear microscopy.

Urinary LAM assay has been evaluated globally with widely varying sensitivity and specificity. A meta-analysis of studies using urine LAM assays in patients with microbiologically confirmed TB, reported variable sensitivity ranging from 13% to 93% and specificity ranging from 87% to 99% [36]. The underlying causes of the variable sensitivity and specificity of urine LAM testing are not adequately understood and this has been an obstacle to its wider application because it makes it difficult to compare performance characteristic of LAM across various settings. According to earlier studies, the variability in sensitivity may be attributed to the study design, study populations, hospitalized versus out-patients, HIV status, degree of immunosuppression, sample patient selection, humoral immune response and the proportion of urine LAM derived from either renal or extra-renal TB sources [37]. For instance, sensitivity of LAM assay generally has been found to be higher among hospital inpatients than among outpatients (58-67% versus 17-32%) [16,29,36,38,39]. The patients in this study were not hospitalized or stratified by the severity of their illness. This limitation could have impacted the LAM ELISA sensitivity in this study.

Other factors that can influence the sensitivity of LAM antigen assays include the characteristics and standardization of the

test-capture antibody, variable concentration of the urine sample, unprocessed or centrifuged urine, use of fresh urine versus frozen urine, cut-off grade for positive result and performance of the test by state certified medical laboratory personnel versus trained but not certified hands [37]. LAM ELISA tests that incorporate poly-clonal antibodies for LAM antigen capture are more likely to recognize the multiple antigenic epitopes of LAM compared with those using monoclonal antibodies that are targeted at a single epitope[40] and that was what was used in this present study.

Sample containers, collection, storage and processing could also affect the results of LAM ELISA. Cross reaction of perineal or fecal bacteria can occur in urine contaminated from unsterile container. Microbiological contamination of urine sample in sufficient quantity may affect the specificity of the assay[41,42.] To prevent the possibility of this ugly occurrence in this study urine sample were collected in sterile containers. Concentrating the urine before carrying out LAM ELISA assay is said to increase the sensitivity of the LAM ELISA, but decreases specificity[43].

In this study, LAM ELISA was not performed on fresh but on frozen urine samples and without centrifugation and it is unclear if this could impact the obtained results. However, careful instructions were given to subjects such as recapping the sterile container soon after collection and submitting the sample to the laboratory as soon as possible and the samples were stored frozen until they were processed. Other possible reasons for the variable specificity in LAM ELISA studies include the sensitivity of the gold or reference standard used in the different studies[41]. Some used composite reference standards[44,39] while others used microbiological reference standards which could be culture[21,31] or Gene Xpert MTB/RIF assay[35].

In this study LAM ELISA detected TB in 20 (30.8%) of the 65 HIV patients while Gene Xpert MTB/RIF and ZN microscopy techniques detected TB in 18(27.7%) of the 65 and 16 (24.6%) of the 65 HIV positive patients respectively. Positivity rate with LAM ELISA was higher in HIV-positive patients (30.8 %) than in the HIV negative patients (20.0%), a finding that tallies with those of some previous studies [16,45,46]. It has been observed that urine LAM detection appears to have greater diagnostic accuracy in patients with HIV co-infection with lowered CD4 count than in the HIV negative patients. Studies stratifying urine LAM by CD4 cell count have shown sensitivities of 56- 85% among patients with CD4 <50 cells/⌠l as against a sensitivity less than 25% in HIV-uninfected populations [36]. LAM concentration in urine of HIV negative TB patients is said to be extremely low, in many cases in the range of pico-gram per ml, hence the urine LAM assay which detects LAM in the range of nano gram per ml, may not be sensitive enough for diagnostic use in such unselected TB suspects[15] used in this present study. The stage of HIV infection or severity of immunosuppression of the HIV positive patients in this study was not determined nor was the CD 4 count done [16-18.]

Conclusion

Irrespective of the simplicity and rapidity, the current commercially available generation of LAM antigen assay does not appear to be very useful as an independent stand-alone diagnostic test for TB in view of its variable sensitivity and specificity. And until the Gene Xpert machine becomes adequately available; perhaps, the use of the conventional sputum smear microscopy should be optimized to further enhance its performance considering its superiority over urinary LAM ELISA as observed in this study. Smear microscopy is quite attractive

for public health programs. It is relatively cheap, specific enough and can provide visual evidence of the bacterial burden. Attention should be focused on techniques for optimization of smear microscopy to improve its sensitivity and to reduce diagnostic defaulting by assessing the feasibility of diagnosing PTB with two sputum samples on a single day known as 1-day protocol or front-loading, particularly for patients who are more likely to default. Training/retraining for laboratory scientists in smear microscopy and external quality assessment will be needful.

Recommendation

There is the need for further researches to.

1. Increase the evidence base for the diagnostic accuracy in the appropriate clinical populations by characterization of patients using parameters such as symptom profile, CD4 cell count, blood hemoglobin, body mass index as well as morbidity tools such as Modified Early Warning Score (MEWS). These may help in the interpretation and comparison of study data in future studies.
2. Define the optimum methods of urine collection to minimize the likelihood of urine contamination and optimum storage if analyses are to be done.
3. Incorporate the assay into the national diagnostic algorithm.
4. Standardize the cut-off mark.
5. To optimize the conventional sputum smear microscopy to further enhance its performance
6. Ensure wider distribution of the Gene Xpert Machine to more facilities.
7. To enhance the sensitivity of smear microscopy.

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COMPARATIVE EFFECTS OF MAGNESIUM SULPHATE IONTOPHORESIS ON POST-STROKE ELBOW FLEXORS SPASTICITY

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Abstract

Background: Controversy continues to trail the best physiotherapeutic approach to adopt in effectively managing hypertonicity in stroke patients. Magnesium sulphate is a muscle relaxant whose efficacy is relatively unexplored, and is unknown if it will be more effective than conventional cryotherapy.

Aim: The aim of this study was to compare the effects of cryotherapy, magnesium sulphate iontophoresis and the combination of both on the spastic elbow flexor muscles of stroke survivors.

Material and methods: Fifty-two stroke survivors were purposively recruited for the pre and post experimental study. They were randomly assigned to three groups, and the interventions were cryotherapy alone, magnesium sulphate iontophoresis (40 mA- mins) only and combination of both. The interventions were administered twice a week for six weeks for each group. The Modified Ashworth scale was used to grade the spasticity at baseline, 3rd and 6th week. Descriptive and Inferential statistics of Kruskal Wallis and Friedman tests were used to analyze the data obtained at $p<0.05$.

Results: Cyotherapy and Magnesium sulphate iontophoresis significantly ($p = 0.003$ and $p = 0.02$ respectively) reduced elbow flexors spasticity at 6th week. However, there were no significant differences ($p > 0.05$) when the three interventions were compared.

Conclusion: The three interventions were effective in alleviating spasticity of the elbow flexors of stroke survivors but none was superior over the other.

Keywords: *Stroke, Spasticity, Elbow Flexors, Cryotherapy, Magnesium sulphate Iontophoresis*

Introduction

Spasticity is best described as an increased, involuntary and velocity-dependent muscle tone that causes resistance to movement after damage to the central nervous system and this is as a result of an imbalance in the inhibitory and excitatory signals to the muscles[1]. Spasticity is one of a multitude of factors, leading to hypertonia, subsequently; there is hyper-reflexia, an exaggerated deep tendon reflex[2]. There are also features of involuntary movements, pain, decreased functional abilities and delayed motor development. Furthermore, spasticity had been reported to have significant impact on motor functions; hence, there is likelihood that it may affect Activities of Daily Living[3].

Spasticity can be highly debilitating as a result of flexor pattern of spasticity in the upper limbs and the extensor pattern in the lower limbs and it has been considered to be a major determinant of activity limitations[4]. It has been reported that spasticity restricts functional abilities and reduces quality of life[5,6]. These have made the management of muscle hypertonicity to be considered as an important goal in post-stroke patient care and rehabilitation[4,7]. The aims of interventions are to improve functions by managing spasticity so as to prevent contractures and correct deformities[8].

There are both pharmacological and non-pharmacological means of managing spasticity. Most mild and moderate spasticity cases are effectively managed with the conservative measures using available oral therapies such as Dantrolene, Diazepam tizanidine, Baclofen, Magnesium sulphate and Clonazepam. However, in cases of severe spasticity, there is less responsiveness to drug therapies[9]. Amongst drugs being considered for therapy is Magnesium Sulphate ($MgSO_4$), which is a naturally occurring mineral and it is often seen in the heptahydrate sulphate mineral epsomite. Magnesium sulphate has been known to be a muscle relaxant and vasodilator for over a century and it is considered physiologically to be an antagonist of calcium. In a systematic review reported by Nepal et al, it was found that magnesium sulphate was able to relieve muscle spasms in cases of tetanus[10.] Claudia et al, also observed that it was relevant in anesthesiology as an adjuvant for sedation, analgesia, neuromuscular relaxation, motor relaxation [11]. Ante-natal magnesium sulfate was also associated with a significant reduction in the risk of cerebral palsy by preventing preterm birth[12]. There are also reports of effectiveness in treating myalgia's, neuritis, deltoid bursitis, low back pain spasm and anti-arrhythmia in cases of cardiac arrest[13].

The quest to find best and effective techniques to adopt to reduce muscle spasticity is a continuing process[5]. Considering side effects of most drugs, the use of alternate means to administer medications is gaining wider acceptance. This includes the use of electromotive forces to administer medications through the skin without passing through the gastro-intestinal system[14,15,16]. Electromotive Drug Administration (EMDA) is a technique in which an electric current is used to deliver a medicine or other chemical through the skin. It is basically referred to as an 'injection without needle'[17]. Iontophoresis is a non-

invasive technology that uses controlled low level electrical energy to transport ionized drugs through the skin in a safe and effective manner[17].

Cryotherapy is a conventional approach usually administered to manage spasticity. There are documentations with the potentiality of significantly reducing spasticity, and improving hand functions in children with spastic cerebral palsy[8] Also, a not too old literature, reported that long-term cryotherapy induced prolonged inhibitory effects on the clonus, thereby resulting in muscle relaxation[18]. Physiotherapists are still facing challenges on best approach to adopt to alleviate muscle spasticity arising from upper motor neuron lesions. Cryotherapy is a non-pharmacological therapy but iontophoretic administration of topical drug relaxant is an emerging trend in physiotherapy. Spruyt et al, and Hassan et al reported that magnesium sulphate has therapeutic benefits in controlling muscle spasms, alleviating pain and reducing autonomic instability[19,20]. However, there are very few clinical trials in the use of magnesium sulphate to alleviate muscle spasticity among stroke survivors[21].Onigbinde et al. observed that magnesium sulphate iontophoresis alone was not significantly better than the application of cryotherapy alone in the reduction of biceps brachii spasticity among stroke survivors[21]. The generalization of the finding Onigbinde et al is limited as a result of the small sample size of stroke survivors that participated in the study. There is still dearth of empirical data to ascertain the combined effects of therapies, that is, combining magnesium sulphate iontophoresis and cryotherapy. Aside from these, contradiction of findings is still trailing the effects of cryotherapy in alleviating spasticity[22]. Hence, the need to investigate the most effective between cryotherapy and magnesium sulphate iontophoresis in alleviating posts stroke elbow flexor spasticity.

Methodology

Participants

The participants in this study were stroke survivors receiving treatment at the Physiotherapy Department of Obafemi Awolowo University Teaching Hospital Complex, Ile- Ife. Sixty-one stroke survivors were recruited for the study; however, 52 stroke survivors completed the study.

Inclusion and Exclusion Criteria

The major inclusion criteria were that the participants must have developed spasticity post-stroke and should not be on any anti-spasticity medication. Also, the duration of onset must not be more than 3 months and the blood pressure must be relatively stable at 140/90mm/Hg. Excluded criteria were having co-morbidities like diabetes mellitus, impaired skin sensation, metallic implants, cardiac pacemakers and those with history of allergy to magnesium sulphate.

Sampling Technique

The sample size for this study was determined using a sample size formula (Cohen, 1988). A total of 61 stroke survivors were purposively recruited for the study and were randomly assigned to three groups using fish bowl technique. Seven were excluded for not meeting the inclusion

criteria/irregular clinic attendance. Only 52 stroke survivors completed the study. Seventeen participants completed the study in cryotherapy group while there were 15 in the magnesium sulphate iontophoresis group while the combined therapy group had 20 (Figure).

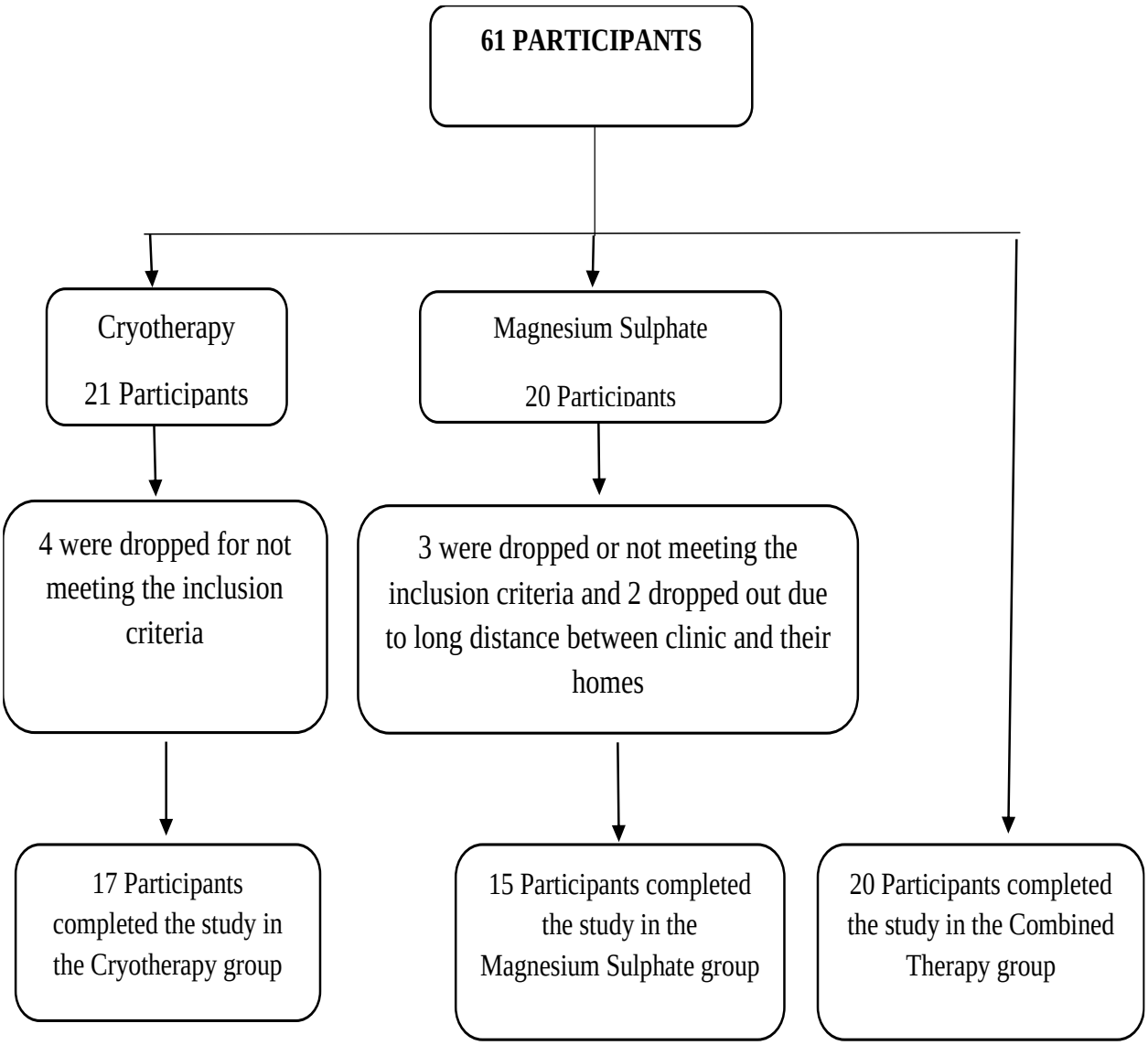

Fig: Flow chart for participants' recruitment

Instruments

The major test instrument was an Electrical stimulator with surface circular electrodes (Model: Endomed 582, India). The Magnesium Sulphate gel was compounded using the method of Onigbinde et al[20] while ice flakes were used for the application of cryotherapy, wrapped in a clean towel. Other instruments were mercury sphygmomanometer, stop watch, cotton wool, methylated spirit (Lifesign Healthcare Limited) and gauze bandage.

Research Design

The study was a pre and post experimental design.

Procedure

Ethical clearance was obtained from the Ethics and Research Committee, Obafemi Awolowo University Teaching Hospitals Complex. Participants were informed about the purpose of the study and they consented to participate. The surface area of the skin where electrodes were placed was cleaned with cotton wool soaked in 3ml of 95% ethanol[20].

The blood pressure of each participant was measured with a mercury sphygmomanometer and pulse rate was taken using the radial pulse; and recorded. Skin sensation was assessed from response to pin prick test. Thermal and cold sensations were assessed using test tubes filled with hot and cold water to ascertain normal skin sensation. A pre- treatment assessment of spasticity was carried out by passively flexing and extending the elbow while the patient was in a seated position. The level of spasticity was graded using the Modified Ashworth Scale[23]. The level of spasticity was graded as: 0- No increase in muscle tone (scored as Grade 0), 1- The presence of slight increase in muscle tone characterized by a catch and release at the end of range of motion when affected part is moved in flexion or extension (scored as Grade 1), 1^{+}- The presence of slight increase in muscle tone characterized by a catch followed by minimal resistance throughout the range of motion (scored as Grade 2), 2- The presence of marked resistance in muscle tone throughout the ROM but affected part can be easily moved (scored as Grade 3), 3- The presence of considerable increase in muscle tone and passive movement is difficult (scored as Grade 4), 5- Affected part is rigid in flexion and extension (scored as Grade 5).

Procedure for the Administration of Magnesium Sulphate Iontophoresis`

Patients were instructed to lie on the treatment couch, facing upward (supine position) and the surface area of the biceps brachii muscle was cleaned with cotton wool and methylated spirit. The electrodes were wrapped in wet gauze. 0.5g of magnesium sulphate gel (Finger Tip Unit) was applied to the positive electrode (anode). The electrodes (both positive and negative) were placed on the bicep brachii muscle using the labile electrode placement method. The electrical stimulator was preset to positive polarity and the cables of the electrodes were plugged into it and the current intensity was gradually increased using a dose of

40mA-min for 15 minutes[14,15, 21] in each participant. A clean towel was used to clean the area after treatment. After a brief period of 5 minutes, participants received 10 repetitions of passive stretching of the biceps brachii muscle and then the level of spasticity was assessed using the Modified Ashworth Scale pre and post intervention at onset, and post intervention at 3rd and 6th week respectively.

Application of Cryotherapy

This procedure was also carried out with the patient lying supine, relaxed and well positioned. A clean towel was used to wrap the ice flakes and it was placed on the bicep brachii muscle for 15 minutes[22]. The participants in this group also received 10 repetition of passive stretching of the arm flexors after application of the ice which was baseline for all the groups. The spasticity was also assessed using the Modified Ashworth scale and recorded pre- and post-intervention at onset, and post intervention at 3rd and 6th week.

Administration of Both Magnesium Sulphate Iontophoresis and Cryotherapy

The participants in this group received both interventions of groups 1 and 2. First to be administered was magnesium sulphate iontophoresis followed by the application of cryotherapy with 5 minutes' interval in between. This was to allow the permeation of magnesium sulphate gel through the skin and avoid washing away effect that may arise from the application of cryotherapy. All documentations were recorded.

Data analysis

The data were analyzed using descriptive statistics of frequency, means, standard deviation and percentage. Analysis of Variance (ANOVA) was used to compare age, height and weight of the groups. The Wilcoxon signed rank test was used to determine the immediate effects of intervention, Friedmann test was utilized for comparing pre and post intervention scores within the groups while Kruska-Wallis was used to compare the spasticity grades across the 3 groups. SPSS version 23 was used and Alpha level was set at $p < 0.05$.

Results

There were 10 (58.8%) males and 7 (41.2%) females among participants in the cryotherapy group. The sex distribution of participants in magnesium sulphate iontophoresis group and the group that had a combination of both magnesium sulphate iontophoresis and cryotherapy are presented in table 1. Twelve stroke survivors (70.6%) in the cryotherapy group had ischemic stroke; while five (29.4%) presented with hemorrhagic stroke. In the magnesium sulphate iontophoresis group, 12 participants (80%) and three (20%) presented with ischemic and hemorrhagic stroke respectively. In the combined therapy group, there were 14 (77.8%) ischemic and four (22.2%) hemorrhagic stroke patients. The type of stroke and side of affectation are presented in Table 2.

The mean ages of participants in cryotherapy, magnesium sulphate iontophoresis and the combined therapy groups are 55.35±4.60, 56.53±5.42 and 59.11±4.39 years respectively. The vital signs of the stroke survivors are presented in Table 3. The result of the Analysis of Variance showed that there were no significant differences in the cardiovascular parameters of the participants in the three groups, at the onset of interventions (Table

3). The mean height and weight of the participants in the cryotherapy group were 1.69±0.13m and 79.11±8.15kg respectively. Other means of height and weight of participants in the magnesium sulphate iontophoresis and combined therapy groups are also presented in Table 3. The results of the Analysis of Variance (ANOVA) showed that there were no significant differences in the height and weight of the participants.

Table 1: Gender distribution of participants in the groups

Groups	Male		Female	
	F	%	F	%
Cryotherapy	10	58.8	7	41.2
Magnesium Sulphate	10	66.7	5	33.3
Combined Therapy	11	61.1	7	38.9

Table 2: **Pattern of distribution of Types of Stroke**

Types		Cryotherapy		Magnesium Sulphate		Combined Therapy	
		F	%	F	%	F	%
Ischemic		12	70.6	12	80	14	77.8
Hemorrhagic		5	29.4	3	20	4	22.2
Affectation:	Right	3	17.6	1	6.7	3	16.7
	Left	14	82.4	14	93.3	15	83.3

Table 3: Age, cardiovascular parameters, height and weight of the participants

Variables	CRYO	MSI	CMT	F	p
Systolic BP (mmHg)	125±21.83	122.80±10.68	126.94±12.14	0.28	0.76
Diastolic BP (mmHg)	76.06±7.58	73.80±6.05	75.89±6.20	0.56	0.57
Pulse rate (bpm)	80.71±8.30	76.67±4.87	76.00±6.17	2.49	0.09
Height (in m)	1.69±0.13	1.70±0.13	1.74±0.12	0.86	0.43
Weight (in Kg)	79.12±8.15	79.73±7.72	79.72±5.74	0.40	0.96

*Key: **CRYO:** Cryotherapy **MSI**: Magnesium Sulphate Iontophoresis **CmT**: Combined Therapy group*

The mean ranks of biceps brachii muscle at onset and immediately after first treatment session are presented in table 4. The results showed that there was significant difference in the spasticity grades immediately after cryotherapy intervention (Z =3.32, p= 0.001). Similarly, significant differences were observed after interventions using magnesium sulphate iontophoresis and combination of both therapies (Z =-3.05, p= 0.002 and Z =-3.61, p=0,001) respectively (Table 4).

The mean rank at onset, 3rd week and 6th week post intervention was 35.03, 21.94 and 21.03 respectively. For the magnesium sulphate iontophoresis group the mean rank at baseline, 3rd and 6th week was 30.43, 18.33 and 20.23 respectively (Table 5). There are significant differences in the reduction of spasticity within cryotherapy, magnesium sulphate iontophoresis and combined therapy groups (T = 11.33, p = 0.003; T = 8.42; T= 22.81, p = 0.001 respectively). There were no significant differences in the level of spasticity across the groups at baseline, 3rd and 6th week of intervention (Table 6).

Table 4: Comparison of immediate effects of Interventions for cryotherapy group

Period		N	Mean rank	Sum of ranks	*Z*	p
Cryotherapy						
At onset	Negative ranks	11[a]	6.00	66.00	-3.32	0.001
Post Intervention	Positive ranks	0[b]	0.00	0.00		
Magnesium Sulphate						
At onset	Negative ranks	10[a]	5.50	55.00	-3.05	0.002
Post Intervention	Positive ranks	0[b]	0.00	0.00		
Combined Therapy						
At onset	Negative ranks	13[a]	7.00	91.00	-3.61	0.001
Post Intervention	Positive ranks	0[b]	0.00	0.00		

a. *Spasticity grade immediately after Intervention < spasticity at onset*

b. *Spasticity grade immediately after Intervention > spasticity at onset*

Table 5: Results of Friedman test comparing the spasticity grades at baseline, 3rd and 6th week post intervention

Groups	Mean spasticity grades			T*	P
	Baseline	3rd week	6th week		
Cryotherapy	35.03	21.94	21.03	11.33	0.003*
Magnesium Sulphate	30.43	18.33	20.23	8.42	0.02*
Combined Therapy	40.50	20.72	.28	22.81	0.001*

***Friedman test*

Table 6: Comparison of spasticity grades across the groups

	Cryotherapy	Magnesium Sulphate	Combined Therapy		
	Mean rank	Mean rank	Mean rank	H	p
Baseline	23.44	26.63	26.50	0.839	0.66*
3rd week	26.62	25.50	24.44	0.234	0.89*
6th week	24.74	27.43	24.61	0.470	0.79*

Discussion

The singular application of cryotherapy, Magnesium Sulphate iontophoresis or the combination of both therapies, resulted to reduction in the arm flexor spasticity of stroke survivors in each group. The current outcome of this study provided empirical support to numerous previous documentations on the effects of cryotherapy in decreasing spasticity[8,18,22,25,26,27,28,29,30]. The application of cold packs in combination with other physiotherapeutic modalities enhances muscle relaxation, including cases of spasticity. The administration of magnesium sulphate alone through iontophoresis also reduced the grade of arm flexors spasticity after six weeks of intervention. The outcome of this study is similar to the reports of Onigbinde et al and Geneviva et al, although, magnesium sulphate was administered through infusion in that of the later report[21,31]. Similarly, the finding is not different from that of when magnesium sulphate was administered orally[32.]

There are reports that magnesium was not toxic when administered through skin and it does not also vaporize easily[33]. The neurophysiological effect of magnesium sulphate is that it blocks peripheral neuromuscular transmission by reducing acetylcholine release at myoneural junction resulting in inhibition of skeletal muscle contraction[34]. The pharmacological form of magnesium, is a physiological voltage-dependent blocker of *N*-methyl-D-aspartate (NMDA)-coupled channels and there documentation that it has anti-nociceptive role, blocks calcium influx, which inhibits central sensitization and decreases preexisting pain hypersensitivity[16]. Magnesium is a cofactor for enzymatic reactions which play important role in neurochemical transmission and muscular excitability and the ions elevate the firing threshold in both myelinated and unmyelinated axons[35]. Magnesium results into the reduction of acetylcholine release and dimishes motor endplate sensitivity to acetylcholine coupled with reduction in the amplitude of the motor endplate potential[36,37]. However, it is noteworthy that magnesium levels should be kept at a serum range between 0.75 and 0.95mmol/L, and this should be applicable even when administering through iontophoresis, especially in the elderly because several changes of magnesium (mg) metabolism have been reported with aging, including diminished Mg intake, impaired intestinal absorption and renal wasting[38]. The combination of cryotherapy and magnesium sulphate iontophoresis to alleviate spasticity was comparable to the singular application of either cryotherapy or magnesium sulphate iontophoresis. This study is in a verdant area of physiotherapy, hence, little comparison could be made with previous studies.

The clinical implication of this study is that there is no added advantage to combine cryotherapy with magnesium sulphate in the management of spasticity of the arm flexors among stroke survivors, despite that the two techniques have different neurophysiological effects on spasticity.

Conclusion

It was concluded that combined therapies showed no superiority over individual use of cryotherapy and magnesium sulphate iontophoresis in alleviating spasticity among stroke survivors. The outcome of this study, however, provides preference of choice for patients who may have hypersensitive allergy to either cold or magnesium.

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Curcuma longa AND *Moringa oleifera* ARE SYNERGISTICALLY ANTIPROLIFERATIVE BY DOWNREGULATING p63 GENE IN TESTOSTERONE-INDUCED BENIGN PROSTATE HYPERPLASIA IN RATS

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Abstract

Background: Although many drugs exist for Benign Prostate Hyperplasia (BPH) today, it remains the most common prostatic disease affecting older men. Herbal medicine has become more common as an affordable alternative therapy for curing and preventing tissue-related pathologies in hopes of overcoming the side effects of existing synthetic pharmaceutical products and surgical procedures. This study evaluated the effects of single and combined ethanolic extract of *Curcuma longa* and *Moringa oleifera* on testosterone-induced BPH, urothelial, and testicular toxicity in albino rats.

Method: In this experimental study BHP was induced in albino rats and treated with *C. longa*, *M. oleifera*, *C. longa*/*M. oleifera*, and Tamsulosin hydrochloride. Serum prostate-specific antigen (PSA) and tissue p63 expression were estimated by the ELISA and Avidin-Biotin Complex (immunohistochemistry) methods. Differences in PSA levels among the Groups were assessed using ANOVA and significance set at $p< 0.05$.

Result: Biochemical findings showed significantly increased serum PSA levels in BPH induced group (0.59 ± 0.07 nmol/L) without treatment compared with BPH-induced group treated with a single extract of *C. longa*, *M. oleifera*, combined extracts of *C. longa* and *M. oleifera* and standard drug (0.12 ± 0.06 nmol/L, 0.16 ± 0.04 nmol/L, 0.11 ± 0.02 nmol/L, and 0.20 ± 0.04 nmol/L, respectively) at $p< 0.05$. Histology revealed giant cell formation in the BPH-induced group without treatment. Combined administration of a mixture of *C. longa* and *M. oleifera*

showed an observable reduction of the p63 protein expression in urothelial cells when compared with the single use of either of the plants.
Conclusion: This study revealed that combining both *C. longa* root and *M. oleifera* seed extracts has curative potential on experimentally induced BPH. It suggests that standardized herbal medicine could be used as an alternative to orthodox medicine, especially in low-resource settings.

Keywords*:* *Curcuma longa, Moringa oleifera*, Benign Prostate Hyperplasia, p53 protein, testes

Introduction

Benign Prostate Hyperplasia (BPH) is the proliferation of stromal and glandular cells, characterized by an excessive increase in the number of smooth muscles within the prostate region.[1] Protein 63 (p63) is found in the nuclei of basal cells of normal urogenital cells. It is a key regulator in cell proliferation and cell differentiation in stratified squamous epithelium.[2] It is overexpressed in proliferating basal cells of epithelial layers in the epidermis, cervix, urothelium, nasal epithelium and prostate.[3] More than 300 active components have been identified in Turmeric (*Curcuma longa*) but the component of interest is curcumin (diferuloylmethane).[4] It is obtained from its roots, and it is commonly used to treat wounds, inflammation, and some tumours due to its antioxidant content.[5] *Curcuma longa* reduces testicular damage caused by an increase in glutathione (GSH), testosterone levels, and glucose-6-phosphate dehydrogenase activity and a decrease in malondialdehyde (MDA) levels.[6] The main limitation of using Curcumin as an antiproliferative agent is its low oral bioavailability due to its extensive first-pass metabolism and its poor aqueous solubility.[7] An adjuvant known as piperine (the most active component of *Piper nigrum*, black pepper), which is a known inhibitor of hepatic and intestinal glucuronidation has been advocated. Simultaneous administration of piperine (20 mg/kg) increased the serum concentration of curcumin with a bioavailability of 154% in rats and 2000% in humans with concomitant decreases in its clearance.[8,9] *Moringa oleifera* is cultivated worldwide for its nutritional properties.[10] Its leaves and seeds are traditionally used to treat various ailments, including abdominal tumours, scurvy, paralysis, prostate and bladder problems, sores, and skin infections due to its antiproliferative, antioxidant and anti-inflammatory.[11,12] Studies have shown that *M. Oleifera* and *C. longa* complement each other in terms of the amount of inherent phytochemical compounds and minerals: Anthocyanin, Carotenoids, Cardiac glycoside, Saponins, Alkaloids, Steroids, Flavonoid, Terpenoids, Tannins and Anthraquinone, and Zinc, Iron, Phosphorus, Magnesium, Potassium, Calcium and Nitrogen.[13-15] In recent times, combination therapy has been practised to improve disease conditions which were mildly affected by single therapy. One such therapy included a combination of genistein and curcumin which prevented cellular proliferation caused by a single or mixture of pesticides. This study mainly evaluated the combined effect of *C. longa*, and *M. oleifera* on artificially induced BPH and Testicular toxicity.

Materials and Methods

Animal handling

A total of 54 male albino rats weighing 150 to 180 g were used for this study. The animals were purchased and bred in the

Animal Faculty of Babcock University. Rats were housed in wooden cages, under standardized housing conditions 12 hours light and 12 hours darkness. The rats were fed twice daily with clean tap water and chow *ad libitum*. They were allowed to acclimatize for 14 days before the experiment. The actual experiment lasted for 2 weeks. The experimental procedures adopted were following the Babcock University Health Research Ethics Committee (BUHREC530/18), Babcock University, Ilishan-Remo, Ogun State and the US National Institute of Health Guidelines for Care and Use of Laboratory Animals in Biomedical Research.

Plant collection, preparation, and extraction

Tumeric *Curcuma longa* roots, *M. Oleifera* seeds and black pepper *Piper nigrum* fruit were obtained from the Ilishan community market and identified at the Department of Agriculture, Babcock University. The plants were dried at 40^{o}C, and pulverized into powder, mixed with 70% ethanol (in a ratio of 1:8) and with intermittent agitation for 72 hours. The solids were then filtered out using a filter and a vacuum pump for accelerated filtration. The solution was concentrated with a rotary evaporator at 60^{0}c. After most of the ethanol had been removed, the solution was poured into a glass petri dish and placed in an oven overnight at 40^{0}c to remove moisture further.

Study design

Following acclimatization, 54 male Albino rats were randomly divided into 9 groups and treated as follows: Group 1 (control): 1 ml/kg of distilled water for 14 days, Group 2: 5 mg/kg of Testosterone Propionate (TESTOST^{TM}; Laborate Pharmaceuticals Ltd., India) for 7 days and sacrifice after another 7 days (Li et al., 2018), Group 3: 25 mg/kg of *C. longa*+ 25 mg/kg of piperine (Pp)for 7 days, Group 4: 5 mg/kg of Testosterone Propionate for 7 days and 25mg/kg of *C. longa* + 25 mg/kg of piperine for another 7 days, Group 5: 50 mg/kg of *M. oleifera* for 7 days, Group 6: 5 mg/kg of Testosterone Propionate for 7 days and 50 mg/kg of *M. oleifera* for 7 days, Group 7: 25 mg/kg of *C. longa* + 25 mg/kg of piperine+25 mg/kg of *M. oleifera* for 7 days, Group 8: 5 mg/kg of Testosterone Propionate for 7 days and 25 mg/kg of *C. longa* + 25 mg/kg of piperine+25 mg/kg of *M. oleifera* for 7 days. Group 9: 5 mg/kg Testosterone Propionate for 7 days and 50 mg/kg of Tamsulosin hydrochloride (standard BPH drug from Contiflo XL^{TM}; Ranxaby Ltd., United Kingdom). Administration of Testosterone Propionate was done subcutaneously. Distilled water, *C. longa* root extract, *M. oleifera* seed extract, piperine, and Tamsulosin hydrochloride were administered orally with the aid of an oral cannula. The study lasted for 28 days. Of note, piperine was used to improve the bioavailability of *C. longa*.

Sample Collection and Investigation

After two weeks, the animals were subjected to an overnight fast and anaesthetized by drop method. Blood samples were collected through the jugular vein and discharged in plain tubes. Blood samples were allowed to clot, and the sera were separated. Animals were then sacrificed by cervical dislocation and organs (prostate and testes) were harvested, weighed, fixed in neutral buffered formalin, and processed. Photomicrographs of the tissues were taken for documentation. The serum level of Prostate Specific Antigen (PSA) was determined by ELISA method using a commercial kit (Accu-Bind^{TM}; Monobind Inc., USA). The Avidin-Biotin Immuno-peroxidase method, as described by Okoye et al.,[16] was used to demonstrate the presence of p63 gene expression in basal

cells of the prostate by using kits: 1) Anti-p63 [BSH-3006-S, clone BSR6, Nordic Biosite AB, Sweden/Ref.: TL-925-QPB160718], 2) DAB Quanto substrate [Ref.: TA-125-QHSx160718; Thermo-Fisher scientific], 3) Citrate buffer [Ref.: AP-9003-125; Thermo-Fisher scientific], 4) Phosphate buffered saline buffer with Tweenzo [Ref.: ab64028], DAB chromogen [ab80436, GR171088-3; Abcam Nigeria], and 5. Hydrogen peroxide Solution B [Batch № 2356; Samstella Ind. Nig Ltd, Nigeria].

Statistical Analysis

The differences between the experimental groups were assessed using ANOVA (SPSS, version 20). The prostate and testis indices were calculated by dividing the organ weight by the body weight multiplied by 100.

Results

The administration of testosterone propionate induced benign prostate hyperplasia with a concomitant increase in the prostate index ($F= 0.92$, $p= 0.51$) and testes index ($F= 0.99$, $p= 0.46$), though the increases were not statistically significant ($p>0.05$) when compared to other groups. Results showed significantly elevated levels of PSA among animals in group 2 (BPH untreated group) compared with other treatment groups and control ($F= 2.19$, $p= 0.05$) and an insignificant decrease in body weight of animals in group 2 ($F= 1.86$, $p= 0.10$) (table 1). The single administration of *M. oleifera* or *C. longa* extract resulted in reduced levels of PSA after the experiment while administration of a combination of both extracts resulted in a minimal increase in PSA level when compared with the control group. Results also showed an observable correlation between biochemical, histopathological, and immunological findings (Figure 1).

Table 1: Comparison of PSA levels and Body weights before and after the experiment (mean ± SD)

Group (G)		Parameters					
	Description of Groups	**PSA before experiment (PB; ng/ml)**	**PSA after experiment (PA; ng/ml)**	**Mean Diff. in PSA levels (PA-PB; ng/ml)**	**Body weight (WB; g)**	**PI (%)**	**TI (%)**
G1	Control (D/w)	0.01 ± 0.01	0.06 ± 0.03[b]	0.05 ± 0.02	194.2 ± 15.99	0.24	1.61
G2	TP 7 days, sacrifice after 7 days	0.10 ± 0.03	0.59 ± 0.07	0.49 ± 0.71	173.20 ± 14.45	0.37	2.06
G3	*C. longa* (CL) for 7 days	0.06 ± 0.02	0.02 ± 0.02[a]	-0.04 ± 0.00	203.60 ± 11.89	0.25	1.74
G4	TP 7 days, Cl for 7 days	0.05 ± 0.03	0.12 ± 0.06[a]	0.07 ± 0.03	201.60 ± 16.79	0.27	1.68
G5	*M. oleifera* (Mo) for 7 days.	0.15 ± 0.05	0.02 ± 0.01[b]	-0.13 ± -0.04	192.80 ± 15.93	0.29	1.56
G6	TP 7 days, Mo for 7 days	0.04 ± 0.02	0.16 ± 0.04[a]	0.12 ± 0.03	208.33 ± 27.70	0.22	1.46
G7	Cl/Mo for 7 days	0.06 ± 0.04	0.08 ± 0.03[b]	0.02 ± 0.00	203.75 ± 11.93	0.23	1.67
G8	TP 7 days, Cl/Mo for 7 days	0.06 ± 0.02	0.11 ± 0.02[b]	0.05 ± 0.00	208.20 ± 20.87	0.24	1.58
G9	TP for 7 days, STD for 7 days	0.04 ± 0.02	0.20 ± 0.04[a]	0.16 ± 0.02	201.60 ± 17.05	0.28	2.02

Statistically significant at P< 0.05= a, P<0.01= b, ANOVA, N= 54, n= 6

KEY: G; Group**, Diff.; Difference, CL;** ***C. longa*****, Mo**; *M. oleifera,* **TP**; Testosterone Propionate, SD; Standard deviation, **STD**; Standard Drug (Tamsulosin hydrochloride), **N;** total number of animals, **n**; number of animals per group, **D/w**; Distilled water, TI; Testis index, PI; Prostate Index

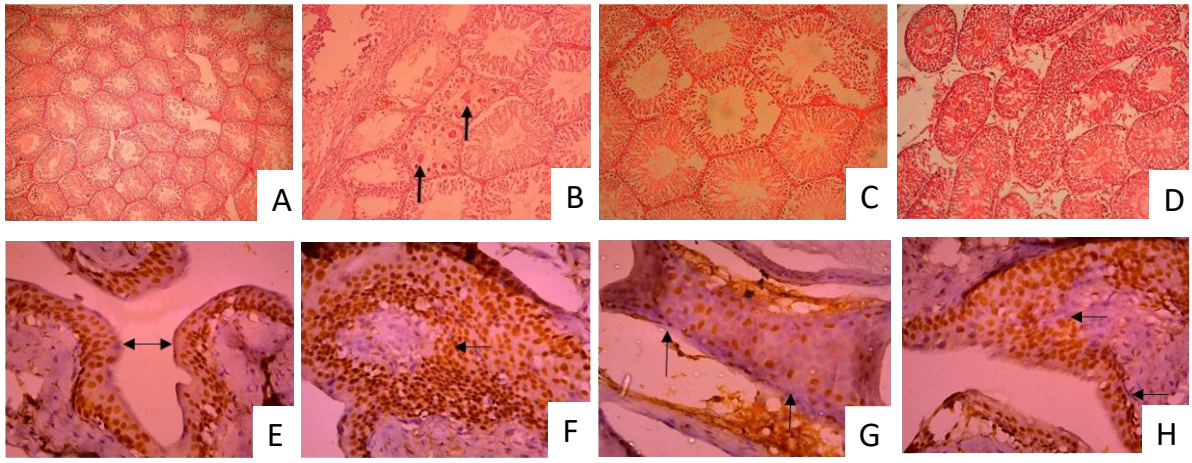

Figure 1: Histology of Testis and urethra

Figure 1A: Photomicrograph of a testis section from the control group (G1) showing normal maturation and well-differentiated seminiferous tubules (Stained by H&E technique, X100 magnification). Figure 1B (G2; BPH induced group without treatment) shows moderate depletion but severely distorted seminiferous tubules, mild haemorrhage, and giant cell formation (black arrows). Figure 1C (BPH-induced group treated with *C. longa/M. oleifera* treated group; G8): shows moderately depleted and distorted seminiferous tubules. Figure 1D (G9; BPH induced group, treated with standard drug): Photomicrograph shows mildly distorted seminiferous tubules (Stained by H&E technique, X200 magnification). Figure 1E: Photomicrograph of a urethra section negative for stromal expression of p63 protein. Figure 1F: Photomicrograph of urothelial cells (G2) with evidence of high p63 protein expression. Figure 1G shows mild p63 protein in urothelial cells following treatment with *C. longa/M. oleifera.* Figure 1H: moderate expression of p63 protein in urothelial cells after treatment with the standard drug (Stained by immunohistochemical technique, X200 magnification).

Discussion

The prevalence of BPH rises markedly with age with a histological prevalence of 8%, 50% and 80% in the 4th, 6th and 9th decades of life, respectively.[17] A survey carried out among men above 40 years in Nigeria (South-West) showed a BPH prevalence of 23.7%.[18] Although many drugs exist to cure BPH today, it remain the most common prostatic disease affecting older men.[19] Natural drugs have become more common as an alternative method for curing and preventing BPH in hopes of overcoming the side effects of existing synthetic pharmaceutical products and surgery procedures. Since the administration of *C. longa* root and *M. oleifera* seed extracts resulted in a considerable reduction in PSA levels in the experimental animals, it was hypothesized that a combination of both may further reduce serum PSA levels. This present study assessed the effect of the combined administration of *C. longa* and *M. oleifera* on testosterone-induced benign prostate hyperplasia and testicular injury in male albino rats. It estimated serum PSA level, prostate index and testes index as parameters for confirming BPH induction.[20-22] The observed high PSA level, prostate index, testes index, and epithelial hyperplasia were obvious indications that BPH was successfully achieved following the administration of testosterone propionate for seven days. The increased testes index in the untreated group when compared with the control group is an indication of BPH because of the relationship existing between the testes and prostate; testicular androgens contribute to the growth of the prostate gland.[23,24]

The serum PSA level of all treatment groups decreased differently after 7 days of administering the plant extracts and standard drug. The extracts, therefore, seem to have inhibited cell proliferation and induced apoptosis in the animals following treatment. Biochemical findings in this study show that turmeric and *M. oleifera* when administered singly reduced serum PSA levels. Histological results were also in concordance with the biochemical findings since there was decreased hyperplasia and papillary formation.[25] The potential of *M. oleifera* seed extract to reduce PSA levels in the experimental animals is in concordance with an earlier study which showed that ethanolic leaf extract of *M. oleifera* forestalled artificially induced BPH by improving the antioxidant effect and inhibiting inflammatory mediators.[26] Despite the reduced serum PSA levels observed among animals that received single therapy of *C. longa* or *M. oleifera*, the combination of both extracts did not show any significant decrease in the assayed PSA levels. However, histological findings in the prostate and testes suggest otherwise in that there was a marked reduction in observable papilloma and testicular injury. The immunohistochemical result suggests that combined therapy of *C. longa* root and *M. oleifera* seed extracts reduced the expression of the p63 gene more than the single therapy of either of the two compared with the standard drug.[27-30]

Conclusion

This study revealed that combining both *C. longa* root and *M. oleifera* seed ethanolic extracts are less toxic, reduces PSA levels and downregulates the p63 gene. Thus, *C. longa* and *M. oleifera* should be incorporated into the meals of BPH patients and high-risk individuals to reduce the progression of prostate disease and patients' mortality.

Competing interest

No competing interests exist in this study.

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UTILIZATION OF DIAGNOSTIC EFFICACY OF A-METHYLACYL COA RACEMASE (AMACR) AND P63 IN DIFFERENTIAL DIAGNOSIS OF PROSTATE CANCER AND BENIGN PROSTATIC HYPERPLASIA

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Abstract

Background: Diagnosis of prostatic tumours, especially the suspicious cases of precancerous lesions are usually subjective, using conventional morphology in Haematoxylin and Eosin stained tissue section. This method is also prone to diagnostic errors or misdiagnosis both in benign and malignant cases. Morphological investigation via light microscopy remains the gold standard for the diagnosis of prostatic carcinoma. Intra-observer variability in diagnosis and difficult cases may benefit from immunohistochemical staining using panel of markers.
Aim: The potentials of p63 and α--methylacyl CoA racemase (AMACR) in differentiating cases of benign prostatic hyperplasia (BPH) from prostate cancer was studied.
Method: Eighty-five previously diagnosed archived prostate tumour tissues comprising of 41 malignant and 44 benign lesions were retrieved for the Histopathology Laboratory of a tertiary healthcare facility. The samples were reviewed and processed for immunostaining (IHC) using p63 and AMACR monoclonal antibodies.
Results: There was 85.9% (73) agreement between morphological diagnosis using conventional Haematoxylin and Eosin technique and IHC, and 14.10% (12) discordance. Of this discordance, 4 (33.30%) were found in cancer of the prostate and 8 (66.70%) were found in benign prostatic

hyperplasia; 37 (90.2%) of previously H&E diagnosed CAP showed strong (3+) immunoreactivity for AMACR while 4(9.8%) were positive for p63. Similarly, previously H&E diagnosed BPH showed 36(81.8%) strong immunoreactivity for p63 and 8(18.2%) for AMACR. Grade V cancers occurred highest with 41% while grade II was the lowest with 5% occurrence. The Gleason's scores ranges for 4+4 to 5+5, while the age of patients ranges from 48 to 86 years with mean age of 68.3 years.

Conclusion: Whereas morphological method remains the gold standard for diagnosis of prostatic lesions, it is not devoid of diagnostic errors. Therefore, p63 and AMACR biomarkers may be of great value in definitive diagnosis and confirming small foci of adenocarcinoma, resolving suspicious lesions and excluding benign mimickers.

Key Words: Prostate, Cancer, Benign, p63, AMACR

Introduction

Prostate cancer is the most frequently diagnosed malignancy in men worldwide after lung cancer, and the 5th leading cause of cancer related deaths in men[1]. Worldwide, the incidence and mortality of prostate cancer increases with increasing age [2]. Black men of African descent have the highest prostate cancer incidence and mortality rates and are more likely to develop disease earlier in life when compared to other racial and ethnic groups[3].

Tissue examination of a prostate needle biopsy or transurethral resection specimen from prostate is mandatory for the diagnosis of prostate cancer and allows patients to receive appropriate therapy. The diagnosis of prostate tumours using conventional Haematoxylin and Eosin staining method may be subjective and prone to misdiagnosis both in benign and malignant cases[4]. Misdiagnosis could be traced to difficult and inaccurate tissue diagnosis due to the increased presence of very small cancer focus leading to either limited amount of suspicious glands and minimal atypism[5], the presence of many benign mimickers of malignancy or as a result of sampling variations[6]. This is more so because a single morphologic feature dose not reliably establish prostatic adenocarcinoma diagnosis. The establishment of a pathologic diagnosis requires the presence of a combination of multiple histologic features of tumor cells which include; pattern of growth, nuclear atypia, absence of basal cells, and the presence of characteristic extracellular material in malignant glands[7]. The consequences associated with incorrect diagnosis, such as unnecessary prostatectomy or radiation associated with adverse complications owing to a false-positive diagnosis or delay of effective treatment owing to a false-negative diagnosis, are undesirable. Although the light microscopic morphological findings remain adjudged as the gold standard for the diagnosis of prostatic carcinoma, intra-observer variability in diagnosis and difficult cases may benefit from immunohistochemical studies[5]. The accuracy of pathologic diagnosis of prostate cancer may be improved by the application of a more objective and reliable tumour-specific markers[6].

PSA is not a cancer-specific marker, as it is present in benign and malignant prostatic epithelial cells[8]. Serum PSA levels frequently are elevated in benign conditions such as benign prostatic hyperplasia (BPH)

and prostatitis[8]. Consequently, patients with an elevated serum PSA level must undergo a biopsy to confirm or exclude the presence of prostate cancer. Other biomarkers, including prostate acid phosphatase (PAP), prostate-specific membrane antigen, prostate inhibin peptide, PCA-1, PR92, prostate-associated glycoprotein complex, PD41, 12-lipoxygenase, p53, p27, hepsin, PIM-1 kinase, and EZH2 are expressed in prostate carcinoma[9]. However, up to now, these markers are not usually used by pathologists to distinguish benign from malignant glands because of lack sensitivity or specificity for prostate carcinoma in formalin-fixed tissue samples[10].

Benign prostate glands contain secretory epithelial cells that express PSA and PAP and basal cells that lie beneath the secretory cells[11]. Basal cells are oriented parallel to the basement membrane and might be inconspicuous in benign glands[12]. Because basal cells are absent in prostate adenocarcinoma, high-molecular-weight cytokeratin (34ßE12) and p63 immunostains specific for basal cells have been used as ancillary tools for the diagnosis of prostate cancer[13]. The identification of the basal cells of prostate glands indicates the presence of benign glands[13]. However, a limitation of using this negative marker for the diagnosis of carcinoma is that basal cells can have a patchy or discontinuous distribution in some benign lesions (adenosis). Consequently, negative staining for basal cells in a few glands suggestive of cancer is not proof of their malignancy[14]. P63 has advantages over 34betaE12, because 34betaE12 is highly susceptible to effect of formalin fixation and IHC procedures such as antigen retrieval pre-treatment, resulting in variable staining arising from loss of staining in the benign glands and can cause misdiagnosis of prostatic adenocarcinoma[15].

P504S was a 382-amino-acid protein, which had been identified as human α-methylacyl coenzyme A racemase (AMACR)[16]. AMACR is an essential enzyme in the beta-oxidation of branched-chain fatty acids. High expression of AMACR protein is found in prostate adenocarcinoma but not in benign prostate tissue by immunohistochemical staining in paraffin-embedded tissue[16]. The expression of AMACR is also detected in prostate premalignant lesions, such as prostate intraepithelial neoplasia (PIN)[16]. The p63 protein, a homologue of the tumor-suppressor p53, is highly expressed in the basal or progenitor layer of many epithelial tissues[17]. P63 is detected in prostate basal cells in normal prostate glands and PIN. However, it is negative in prostate adenocarcinoma[18]. Thus p63 is useful as a differential marker for benign prostate glands and adenocarcinoma (negative marker). The combination of AMACR and p63 may be extremely useful for diagnosing PIN and small focus adenocarcinoma, especially in difficult or suspicious cases for malignancy and cases with limited tissues. This antibody cocktail may eliminate the need for high-molecular-weight cytokeratin (34ßE12).

This study therefore, sought to evaluate the specificity and sensitivity of p63 (negative marker) which is specific for basal cells in benign glands and a more objective and reliable molecular marker for prostate adenocarcinoma such as AMACR (positive marker). The study was also aimed to substantiate the existence of diagnostic inaccuracies or misdiagnosis using only the conventional H&E staining of needle biopsy and transurethral resection of prostate (TURP) samples.

Materials And Methods

Study design

A 5-year retrospective study explored the immunohistochemical evaluation of AMACR and P63 in previously diagnosed

samples of benign and malignant formalin processed, paraffin wax embedded prostate tissue blocks from 2013 to 2018 retrieved from the Histopathology Department of Nnamdi Azikiwe University Teaching Hospital Nnewi was carried out. Also retrieved from the available records were patients' biodata. Ethical approval for the study was obtained from the ethics committee of the hospital before commencement of the study.

Sample collection

Excluding damaged tissue blocks and tissue blocks that were not core needle biopsies, a total of 85 tissue blocks were selected from the hospital archives. All the cases were reviewed during which the tissue blocks were categorized into two, namely 41 malignant and 44 benign cases. The paraffin blocks were trimmed, 3 microns thick sectioned using rotary microtome and three serial sections were mounted on three different slides.

Haematoxylin and Eosin (H&E) Staining[19]

The sections were stained using H&E staining method and photomicrographs of sections taken using Amscope digital camera eyepiece attached to an Olympus optical microscope. Two independent blind reviewers reviewed the slides to confirm morphological diagnosis.

Immunohistochemical (IHC) staining[20]

Tissue sections were subjected to IHC evaluation of AMACR and P63 using anti-AMACR and anti-P63 monoclonal antibodies along with positive and negative controls. Immunoractivity were detected using rabbit horseradish antiperoxidase/diamino benzidene (HRP/DAB) detection IHC kit. Immunoractivity was semi-quantitatively scored[21]. Antibodies and detection kits were products of ABCAM Plc Uk, sourced through Biotec Nigeria.

Data Analysis

Data obtained were analyzed and results presented in tables, pie charts, bar charts and plates.

Results

There was 73(85.9%) agreement between H&E and IHC staining methods and 12(14.10%) discordance (Figure 1). Of this discordance, 4(33.30%) were found amongst cancer of the prostate while 8(66.70%) were found in benign prostatic hyperplasia (Figure 2). Thirty-seven (90.2%) of previously H&E diagnosed CAP showed strong (3+) immunoreactivity for AMACR while 4(9.8%) were strongly positive for p63. Similarly, previously H&E diagnosed BPH showed 36(81.8%) strong immunoreactivity for p63 and 8(18.2%) for AMACR (Table 1). Grade V cancers occurred highest with 41% while grade II was the lowest with 5% occurrence (figure 3). The Gleason's scores ranges for 4+4 to 5+5, while the age of patients ranges from 48 to 86 years with mean age of 68.3 years. The morphology and the ICH expression patterns are shown in plates 1-3.

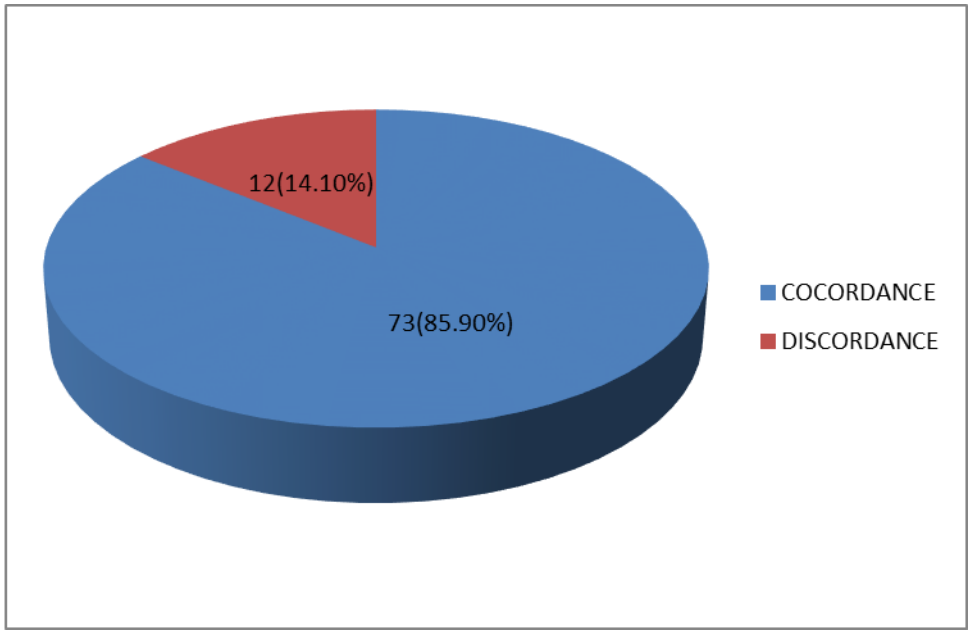


Figure 1: Degree of agreement (concordance) and disagreement (discordance) Between H and E and IHC.

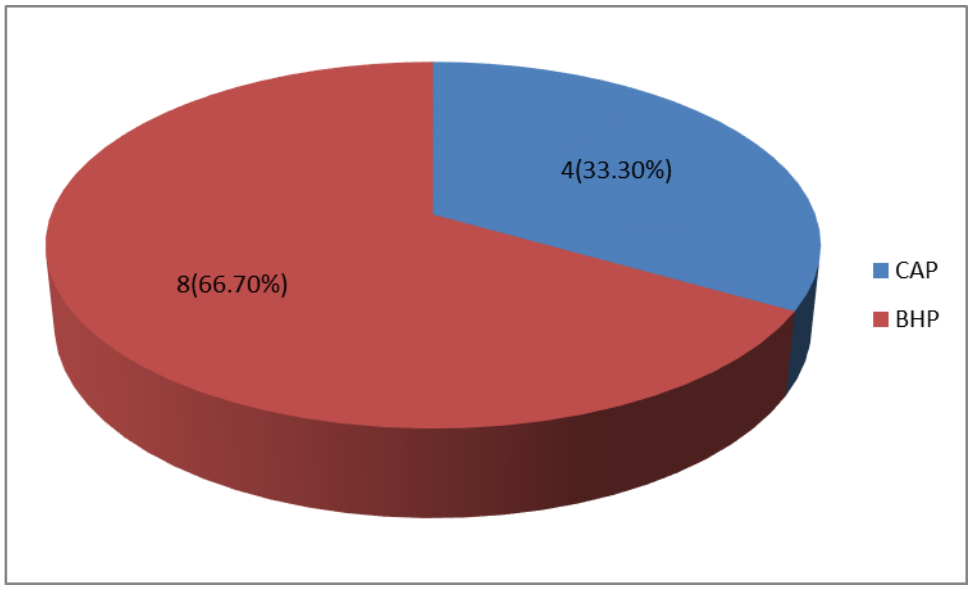


Figure 2: Percentage of discordance between H and E diagnosed CAP and BPH after application of AMACR and P63 markers.

Table 1: Differential Diagnosis of IHC on Benign Prostatic Hyperplasia (BPH) and Cancer of the prostate (CAP) using AMACR and p63

Lesion type/staining techniques	H&E		AMACR		P63	
	Positive n(%)	Negative n(%)	Positive n(%)	Negative n(%)	Positive n(%)	Negative n(%)
BPH	44(100)	0 (0.0)	8(18.2)	36(81.8)	36(81.8)	8(18.2)
CAP	41(100)	0 (0.0)	37(90.2)	4(9.8)	4(9.8)	37(90.2)

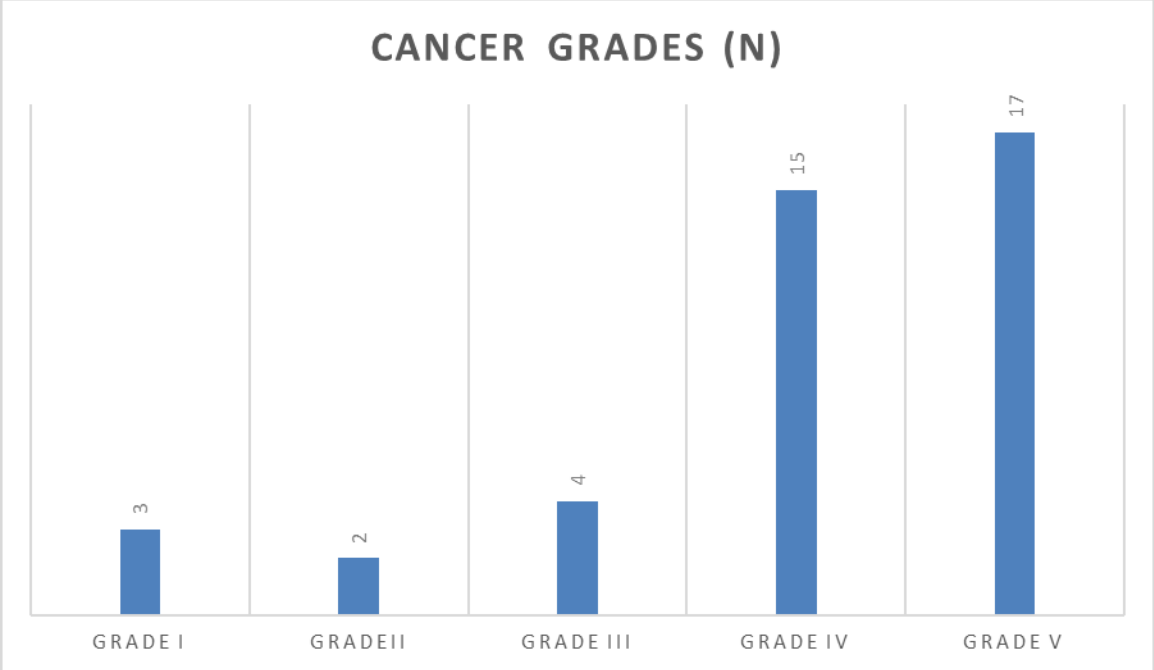
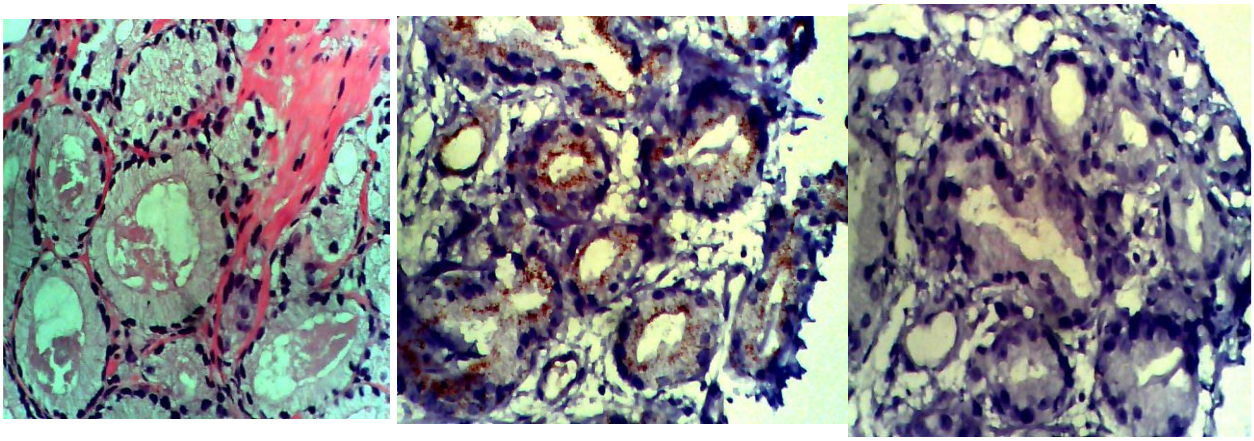
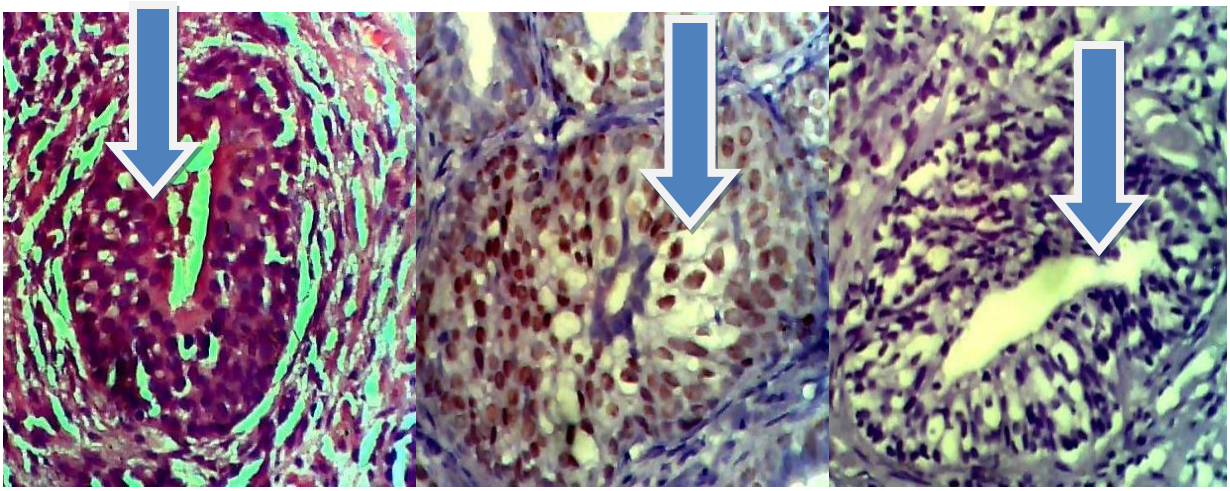


Figure 3: Grades of CAP samples



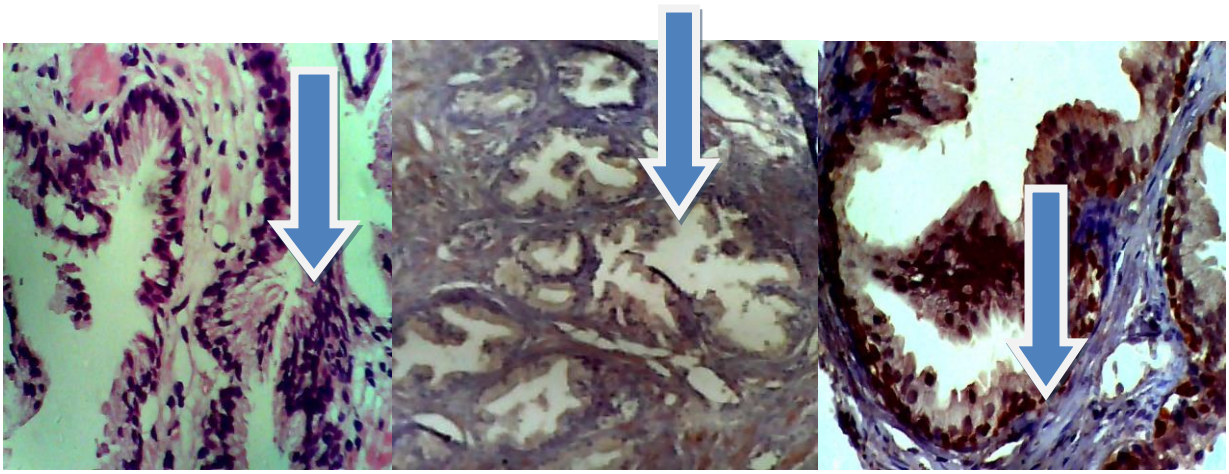
BPH H and E Diagnosis AMACR= POSITIVE P63= NEGATIVE

PLATE I Photomicrograph of a case with a BPH at H and E diagnosis mimicking an Adenosis was finally diagnosed as LOW GRADE ADENOCARCINOMA (Discordance) using AMACR and P63 Immunohistochemistry.x40. There was immunostaining in the luminal cells of the malignant lesions by anti AMACR monoclonal antibodies.No staining by anti p63 monoclonal antibodies .Note AMACR POSITIVE and P63 NEGATIVE.



CAP H and E P63=POSITIVE AMACR= NEGATIVE

PLATE II Photomicrograph of a case with a Cancer of the prostate (intraductal type) at H and E diagnosis was finally diagnosed as BASAL CELL HYPERPLASIA (Discordance) using AMACR and P63 Immunohistochemistry.x40. The basal cell nucei of the benign lesions were immunostained positive by anti-p63 monoclonal antibodies.No immunostaining was seen in the luminal cells of the malignant lesions.Note: AMACR NEGATIVE and P63 POSITIVE.



BPH H and E AMACR =NEGATIVE P63= POSITIVE

PLATE III Photomicrograph showing a case with BPH diagnosis at H and E was also supported (concordance) using AMACR and P63 Immunohistochemistry.x40. The basal cell nucei of the benign lesions were immunostained positive by anti-p63 monoclonal antibodies.No immunostaining was seen in the luminal cells of the malignant lesions by anti AMACR monoclonal antibodies: Note AMACR NEGATIVE and P63 POSITIVE.

Discussion

In agreement with the findings of this study there was an increased significant differential diagnosis in benign prostatic samples and malignant prostatic samples when AMACR and p63 were used as adjunct diagnostic tools, corroborating the report of Rashed *et al*[22] who observed increased statistically significant difference in AMACR index between benign and malignant prostatic lesions. This finding is also in congruence with the finding of Okonkwo *et al*[23] who also carried out the similar study on routinely diagnosed prostatic carcinoma and equivocal diagnoses. The above researchers, though, not undermining the value of morphological diagnosis, revealed a major shortcoming while advocating for inclusion of adjunct IHC markers.

The present study revealed high percentage of diagnosis agreement of 73/85(85.9%) and lower disagreement of 12/85(14.10%) between H&E and IHC methods. This agreed with a previous by Singh *et al*[24] who reported 27/40(67.5%) agreement and 13/40(32.5%) discordance between H&E and IHC. The high level of agreements reported from both studies further proved that H&E method still remains very sensitive diagnostic method. Nonetheless, the record of 12% discordant diagnosis may be an indication of the vulnerability of the method to minimal misdiagnosis, due largely to its subjectivity nature. The higher percentage of discordance in the benign lesions compared to the malignant ones, reported in the current study also corroborates the finding of Singh *et al*[24], who reported 11/13 benign to malignant and 1/13 malignant to benign. One could adduce that the likelihood of having false negative benign results in prostatic diagnosis using only the routine H&E technique is more than false negative malignant results. This portends grave consequences and underscores the importance of using IHC ancillary technique alongside H&E.

The sensitivity and specificity results on diagnostic utility of p63 and α-methyl acyl Co A racemase (AMACR) in resolving suspicious foci in prostatic needle biopsy and transurethral resection of prostate specimens obtained from the current study was generally high and this finding is in agreement with several other studies[25,26,27,28,29]. These authors in separate studies reported high immunoreactivity of AMACR in prostate cancer as compared with benign lesions of prostate.

The current study reported that immonoreactivity of AMACR favours only malignant lesions while only benign lesion showed p63 immunostaining with no case of cross reactivity. This is in congruence with the report of Okonkwo *et al*[23] and Herawi and Epstein[18]. There is abundant literature evidence that luminal cells of high grade prostate cancer do not express p63 but express AMACR whereas basal cells of benign prostatic lesions express p63, they do not express AMACR. This further underscores the value of these panel of tumour markers in definitive diagnosis of prostate lesions. While not jettisoning the efficacy of morphological diagnosis using H&E, including AMACR and p63 in the routine diagnosis regimen may sensitivity of prostatic lesion diagnosis to 100%. This not improves patients' outcome through appropriate management but save time and cost.

The reported patients' mean age of 68.3 years agrees with the generally agreed and reported vulnerable age for prostatic lesions. The pattern grades and the Gleason's scores observed in the current study corroborates the report of earlier studies[23,30].

Conclusion

Morphological diagnosis of prostatic lesions showed high level of sensitive and specific

but with an unacceptable percentage of discordant diagnosis. P63 and AMACR tumour markers showed high percentage of sensitive and specificity for detection of BPH and CAP respectively, when compared to routine H&E method. Therefore, to achieve all times definitive diagnosis of prostate lesions, effective resolution of suspicious cases of prostate cancer and BPH, promote early diagnosis, make uncertain diagnoses less frequent and obviate the need for a number of repeated biopsies, inclusion of p63 and AMACR IHC panel in routine diagnosis may be the answer.

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IMPACT OF *FUSARIUM SOLANI* ON BRAIN, LIVER AND SPLEEN OF MOUSE INFECTION MODELS

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Abstract

Background: *Fusarium* species infection is on the increase leading to morbidity and mortality in immunocompromised hosts. There have been several reports of disseminated infection of *Fusarium* species in immunosuppressed patients over the last 25- 30 years.
Aim: The study aimed to determine the histopathological impact of the isolated *Fusarium solani* on the histomorphology of brain, liver and spleen tissue of albino mice.
Material and Methods: Isolates of *Fusarium solani* from humans and plants were obtained from clinical samples of patients and different plant products respectively. All the samples collected, were cultured immediately on Sabouraud Dextrose Agar slant containing 50 mg/l of chloramphenicol and 5 mg/l of gentamicin. Identification of isolates was carried out macroscopically and microscopically using standard mycological methods. Test mice were challenged with isolated conidia of *Fusarium solani* while the control mice were unexposed. After 30 days, randomly chosen surviving test mice and control were sacrificed. The brain, liver and spleen of the animals were aseptically excised for tissue burden estimation and further histological processing for light microscopical examination.
Result: *In vivo* virulence studies of *Fusarium solani* on mice brain, liver and spleen organs revealed disseminated infection of multiple organs and mortality. The presence of fungal propagules was detected in all organs, with the highest concentration found in the spleen. There were intense inflammations of the internal organs especially excretory organs compared to the control where there was no inflammation seen. Histopathological findings on the brain showed extensive oedemas with blurring of the white and grey matter junction and extensive destruction of the Purkinje cells. Splenic tissues also revealed complete destruction of the splenic bulb and presence of extensive destructive granulomas with many Langhans giant cells formed. There was

also evidence of infections in the liver tissue ranging from intense hepatocyte swelling with hyperchromatic nuclei to intense periportal mononuclear cell infiltrates arising from diffuse toxic hepatic injury.
Conclusion: This study showed strong evidence of *Fusarium solani* pathogenicity in mice from various pathological manifestations and physical signs observed

Keywords: *Fusarium*, mycosis, virulence, resistance, immunosuppressed

Introduction

Currently there is increase in wave of invasive infections caused by *Fusarium* spp in patients with underlying immunosuppression. *Fusarium* species is a common fungal mold that causes wide spectrum of opportunistic infections in man and is normally found in soil, plants, and air (ubiquitous fungus). It is also a significant plant pathogen and has been steadily documented as emerging human pathogen due to its ability to cause systemic toxicity and mortality in heavily immunocompromised individuals.[1,2] *Fusarium* spp is known to be the second common cause of invasive mold infection in immunosuppressed patients.[1,3,4] *Fusarium solani* has been recorded as the highest frequent cause of invasive disease, among *Fusarium* species, especially in patients with hematological malignancies, stem cell transplant recipients, and prolonged neutropenia. [5, 6] *Fusarium* spp is capable of producing a number of different mycotoxins, which include trichothecenes (T-2 toxin, HF-2 toxin), deoxynivalenol (Don) and nivalenol, zearalenone and fumonisms. The key function of these virulence factors is suppression of humoral and cellular immunity. Fumonisin B1, the most common, has been linked with cerebral invasion; resulting in neural axon degeneration as well as abnormal mitochondrial function.[7] Fumonisins are acutely toxic to the liver and kidney of a wide range of experimental animals

Fungal meningitis is rare globally but is a serious complication in transplant recipients. Fungal brain abscesses can develop after a fungus disseminate from any part of the body to the brain or spinal cord. *Histoplasma*, *Blastomyces*, *Cryptococcus*, *Candida* and *Coccidioides* are well known causes of fungal meningitis. Disseminated infection had been associated with cases of cerebral fusariosis and affected individual may develop single or multiple brain abscesses, endophthalmitis, meningitis, cutaneous nodules, chorioretinitis and fungemia.[8,9] The most implicated species are *F. solani*, *F. moliniforme* and *F. oxysporum* complexes [1]

Early and rapid diagnosis of *Fusarium* infection is very crucial for successful antifungal chemotherapy and survival of the patient. The organism is noted for high level of multi-drug resistance to several antifungal agent, hence combination therapy is advocated.[10,6] Decisive diagnosis is made by direct histopathological examination and identification of the organism in tissue (brain biopsy, CSF, vitreous fluid). Mycological diagnosis of fungemia can be on the basis of positive blood cultures.[6]

Fusarium infections in the patients with liver cirrhosis remain rare.[11] However, a case of disseminated *F. solani* infection with hepatic localization was reported by in a liver transplant patient[12]. The frequency of *F. solani* invasion of spleen is on the increase

among patients with haematological malignancy due to more intensive chemotherapeutic treatment of the malignancy. These patients most often present with persistent pyrexia and associated with unresponsive to broad spectrum antibiotics. The diagnosis of *Fusarium* infection is principally based on mycology and histopathology. Invasive fusariosis treatment is very challenging due to the infection severity, multi-drug resistant and profound immunosuppression of the host. The present study aimed to determine the histopathological impact of isolated *Fusarium solani* on histomorphology of brain, liver and spleen tissue of albino mice

Materials and Methods

Specimens used for this work were obtained from both humans and plants. Clinical isolates of *Fusarium solani* from humans were obtained from mycological clinical samples of patients seen at different hospitals within Enugu, Nigeria. The *F. solani* from plants were isolated from different plant products. Ethical approval for the study was obtained from the Ethical Committee of the Department of Pharmaceutics, University of Nigeria, Nsukka.

The clinical samples (nail clippings, skin scrapings and corneal scrapings) and plants samples (pawpaw, avocado pear, carrot, plantain, water-melon, pepper, tomato seedlings, Irish potato, sweet potato, banana and palm fruit), obtained were cultured immediately in triplicate on Sabouraud Dextrose Agar (SDA) slant containing 50 mg/l of chloramphenicol and 5 mg/l of gentamicin. The cultures were incubated at 28°C for 1 week in the dark. Identification of isolates was carried out macroscopically and microscopically according to modified method of [13] with little modifications. Further characteristics for identification of the isolates was by slide cultures using SDA from the pure cultures

Animal infection

Animal experimentation was done using International Standard Procedure Guide for the Care and Use of Laboratory Animals.[14] One hundred and forty test mice and 7 controls (73 males and 74 females,(including 4 pregnant mice)); with a weight range of 32-34g obtained from the laboratory animal centre of the College of Medicine, University of Nigeria, Enugu Campus, Enugu State. After certification of the health conditions of the mice and approval by the committee for animal experiments of our institute, the mice were housed (seven per cage) in aluminum cages, with corncob bedding and free access to food and water, under standard conditions.

All isolates were first cultured on SDA for 7-10 days at 28°C. Test mice were infected by intravenous injection of a 0.2 ml of 10^6 conidia/ml suspension of *F. solani* into the lateral tail vein while the control mice were unexposed using modified method of [15]. Mice were observed for 4 weeks (30 days) post-challenge and mortality was recorded daily. The estimation of Mean survival time (MST) was done using Kaplan-Meier method in the SPSS software (version 15) and log-rank test was used for comparison among the groups.

Tissue Burden and Histopathology

After 30 days, 10 randomly chosen surviving test mice and control were sacrificed. The brains, livers and spleens of the animals were aseptically excised for further histological processing and light microscopic examination. One half of each organ was weighed and homogenized in 1 ml of sterile saline. Ten-fold serial dilutions of this homogenate were spread onto SDA plates and incubated at 28°C for 72hrs. After incubation, colonies were counted and

expressed as number of colony forming units CFU) per gram for each organ. The Log_{10} values of counted colonies were calculated and compared using the analysis of variance test. Data were analyzed with the software SPSS for windows version 15.

The remaining half of the organs were fixed in 10 % neutral buffered formaldehyde for period of 10 days, they were further treated by embedding in molten paraffin wax and automatically processed. Sections of the tissues were stained with hematoxylin-eosin for light microscopy observations. In addition, samples of spleen, brain, and liver from infected mice were homogenized, treated with KOH and stained with lactophenol cotton blue to check for fungal elements (hyphae and spores).[15]

Results

Isolation and identification of *Fusarium solani*

Fusarium solani colonies culturally grew easily and rapidly within three to five days on Sabouraud dextrose agar beginning as a white patch and quickly developed into pinkish shade. There was no pigment production on the reverse side of the tube (Figure 1). Identification was by observation of cultural and morphological characteristics, and by comparison with standard descriptions given by [16]

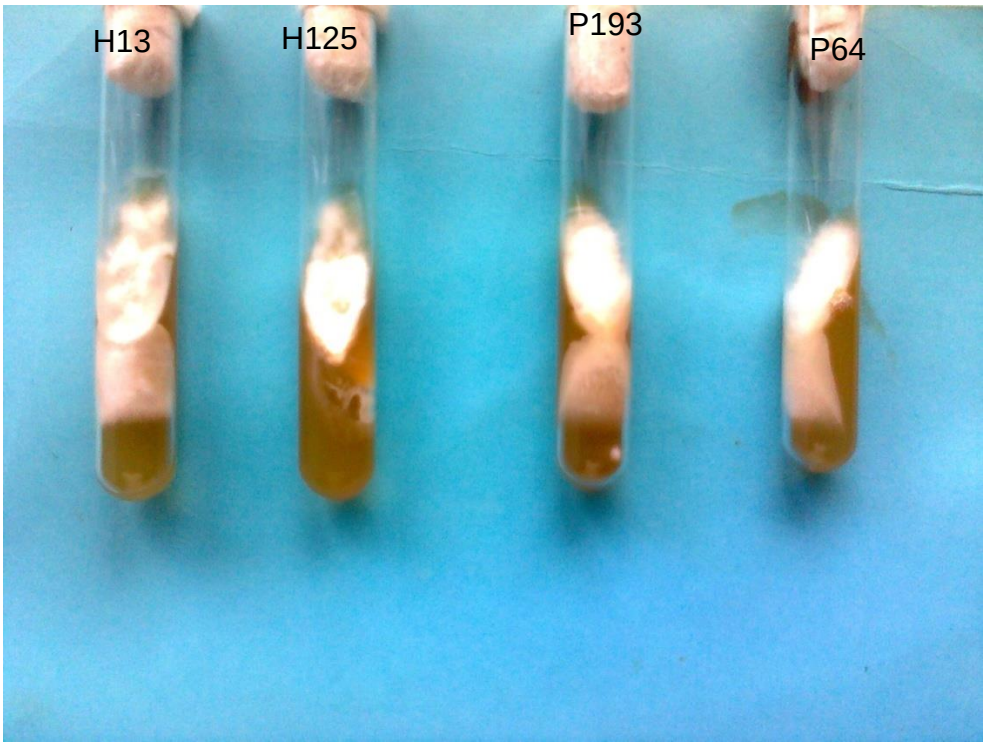

Fig. 1: Colonial morphology of *Fusarium solani* isolated from humans and plants
H- *Fusarium solani* isolates from humans
P – *Fusarium solani* isolates from plant products

Macroscopic Examination (Colonial Morphology)

Fusarium solani colonies culturally grew easily and rapidly within three to five days on Sabouraud dextrose agar beginning as a white patch and quickly developed into pinkish shade. There was no pigment production on the reverse side of the tube (Figure 1). Identification was by observation of cultural and morphological characteristics, and by comparison with standard descriptions given by [16]

Pathology

We observed that mice that received 0.2 ml of 10^6 conidia / ml conidia suspension of *F. solani* through intravenous injection into the lateral tail vein developed rapid systemic infection, with all mice becoming severely ill, very dull, malnourished, and not feeding well for three days, after which, they picked up again. They developed pinkish ring -like lesions on the upper, undersurfaces of their bodies, towards the waist in some of the mice and on the tails in some others. Generally, there were slight weight loss and the signs of the infections became intense after each inoculation. Some mice developed solid granulomas which were very large (as large as the stomach), from the neck up to the thorax and upper abdomen on the right (Fig.2). The granulomas were about 7.5cm in their longest diameter. Generally, all the infected mice become severely ill (very dull and unable to feed well for three days), after which some picked up again.

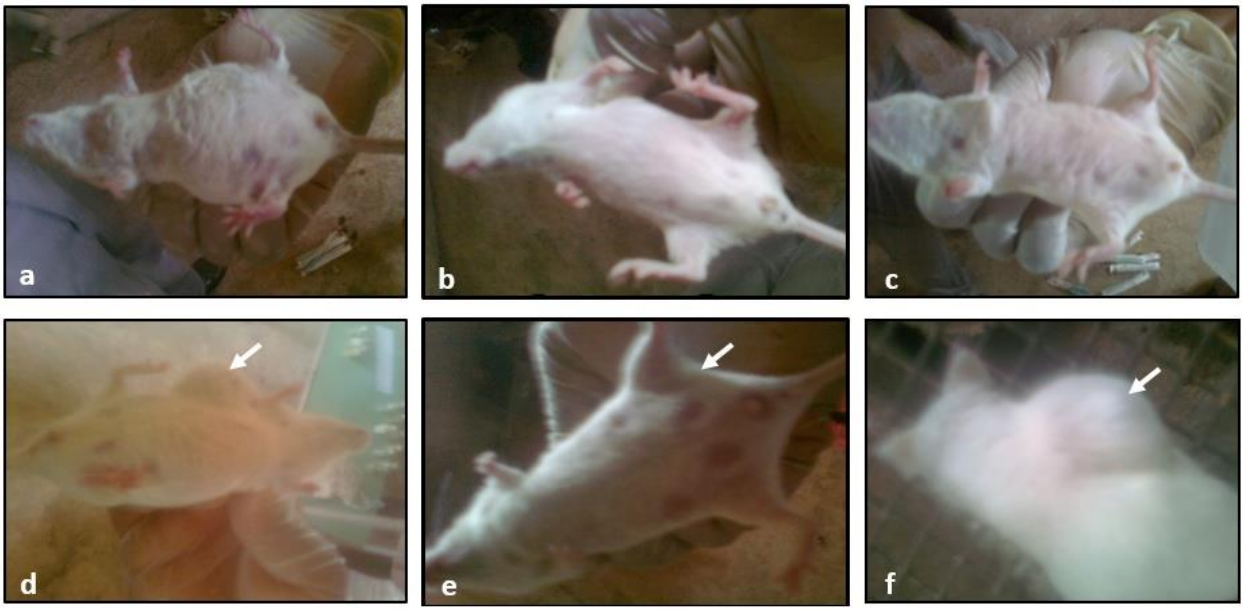

Fig.2: *Mouse infection models.* Signs of infection are noted in all mice (a – f). Mice a, b, c and d were infected with *Fusarium* conidia (P189, P196, H7, P193 and H133 strains respectively). Hair loss is observed in (a). Large granuloma is noted in (d); and (f) is a male mouse with very large solid granuloma after infection with *Fusarium solani* conidia from human.

Mortality

Mortality recorded daily for 30 days showed that a total of 42 mice died out of 140 test mice used. The mortality rate per week is as shown in Figures 2a and 2b. The ability of the *Fusarium solani* isolated from humans and plants to survive and reproduce in mice, colonize multiple organs and finally lead to the death of the host suggests that *Fusarium solani*possess pathogenicity determinants needed to cause disease in mammalian hosts.

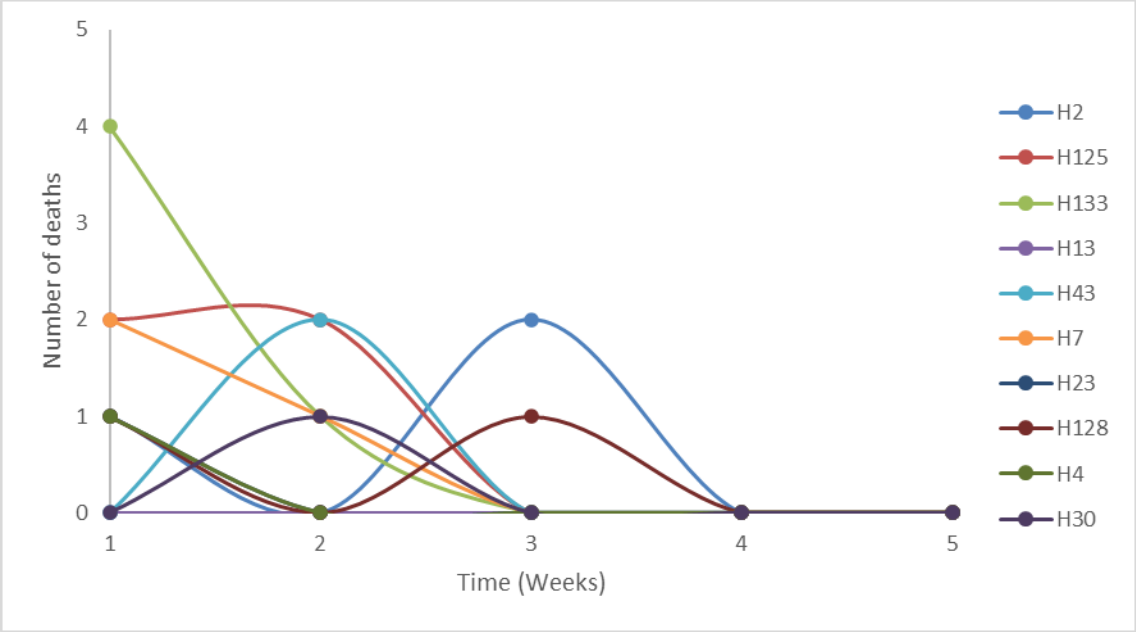

Fig 3a: Mortality Rate of Mice Inoculated with *Fusarium* Conidia from Humans, within a 30-day Period

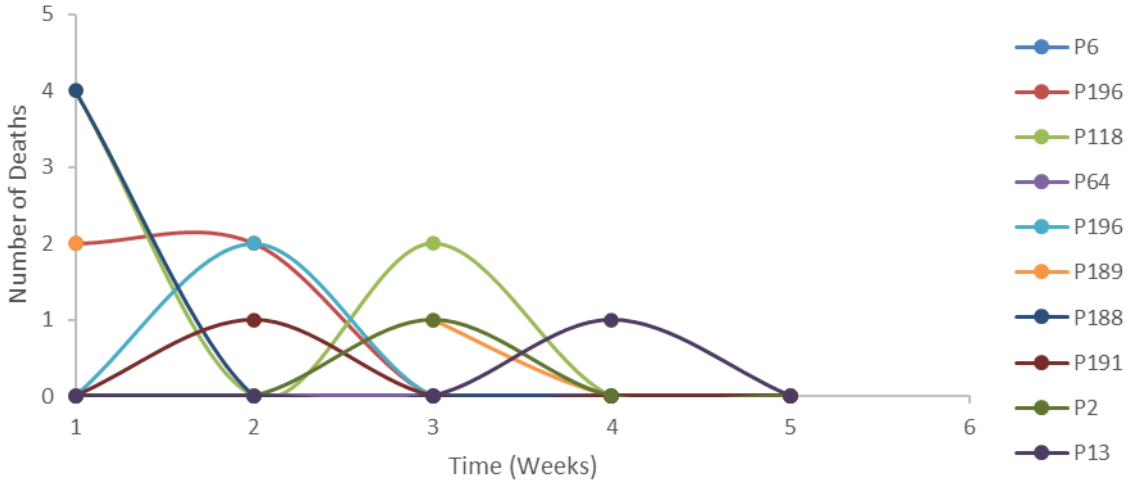

Fig 3b: Mortality Rate of Mice Inoculated with *Fusarium* Conidia from Plants, within a 30-day Period

Host organs invasion

The fungal propagules were detected in multiple organs, with the highest concentration found in the spleen followed by liver, while the brain had the least (Table 1). The internal organs of most of the sacrificed mice showed intense inflammations. There were no statistically significant (P = 1.000) differences in weight among organs and between cages

The number of colony forming units (CFUs) of the conidia per gram, calculated from the spleen was significantly higher (P = 0.013) than that from the brain and liver but there was no significant difference between the colony count in the liver and that in the brain (P = 0.753).

However, the brain had the lowest colony count. The *F. solani* inoculated into the different mice were recovered again from the various organs of the sacrificed mice

Table 1: Colony counts of *Fusarium solani* recovered from different mice organs

TREATMENT MICE GROUPS	BRAIN			LIVER			SPLEEN		
	Colony count after 3 days	Numbers of CFU per gram of organ	Colony count (log_{10})	Colony count after 3 days	Numbers of CFU per gram of organ	Colony count (log_{10})	Colony count after 3 days	Numbers of CFU per gram of organ	Colony count (log_{10})
1	1	3	0.4771	708	545	2.7364	600	1200	3.0792
2	300	1000	3.0000	200	133	2.1239	500	1000	3.0000
3	10	33	1.5185	400	267	2.4265	400	1333	3.1248
4	16	53	1.7243	1000	1000	3.0000	12	40	1.6021
5	11	37	1.5682	600	600	2.7782	490	2450	3.3892
6	1	3	0.4771	300	375	2.5778	58	193	2.2856
7	8	40	1.60206	400	400	2.6021	48	160	2.2041
8	18	45	1.6532	290	290	2.4624	390	780	2.8921
9	2	7	0.8451	201	201	2.3032	38	190	2.2788
10	5	17	1.2304	560	373	2.5717	659	2197	3.3418

Histopathology of the Mice Organs

In vivo virulence studies of *Fusarium solani* on mice brain, liver and spleen organs resulted in disseminated infection on the organs and mortality.

Brain: Brain of mice infected with microconidia of *F*. solani were equally damaged, with some mice showing diffuse encephalitis with total destruction of the Purkinje cells and fibrosis extending to the white matter and no demarcation between the grey and white matter. In others, there were extensive brain oedemas or widening of both white matter and Purkinje cells, with blurring of the white and grey matter junction and extensive destruction of the Purkinje cells. Severe encephalitis with balloon degeneration of the Purkinje cells was also noticed in others. Some strains were more toxic to the brain than others, showing gliosis (fibroid tissues) with heavy loss of brain tissue (Loss of Purkinje cells) and extensive destruction of the neurons. Fig.4a showed normal brain of control mice while Fig4b -4j revealed specific damage to the brain by *F. solani* isolated from both humans and plants.

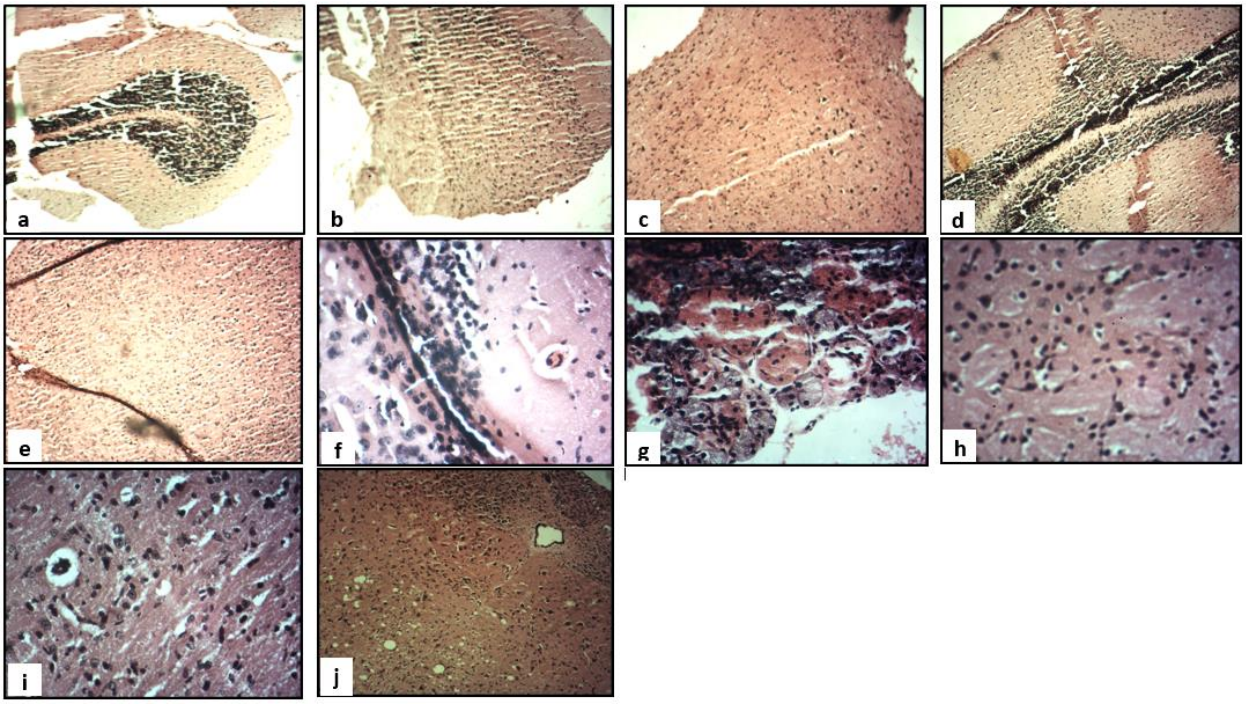

Fig.4: *Photomicrographs of Brain sections of control and Fusarium species infection models;* (a) **Normal control:** Section shows outer white matter and inside grey matter (Purkinje cells) devoid of inflammation with distinct and sharp borders. (b**) H2 strain:** Extensive brain oedema or widening of both white matter and Purkinje cells, with blurring of the white and grey matter junction. Extensive destruction of the Purkinje cells is also observed. (c) **H125 strain:** Complete destruction of the Purkinje cells and fibrosis extending to the white matter with no demarcation between the grey and white matter. Strain is more toxic than strain H1. (d) **P193 strain:** Almost normal brain with slight reduction in the number of the Purkinje cells and extensive oedema of the white matter. (e) **H133 strain:** Diffuse encephalitis with complete destruction of the Purkinje cells. (f) **H13 strain:** Moderate loss of Purkinje cells, Oedema of the white matter and presence of giant cell. (g) **H132 strain:** Severe encephalitis with ballooning degeneration of the Purkinje cells. (h) **H43 strain**: Gliosis (pink fibroid tissues) with heavy loss of brain tissue (Purkinje cells) and extensive destruction of the neurons. (i) **P64 strain:** Near complete loss of Purkinje cells with encephalitis (with cyst). (j) **P6 strain:** Moderate loss of Purkinje cells with severe (gliosis) (Fibrosis with cyst).

Liver: Mice livers infected with conidia of *F*. solani isolated from humans and plants also showed evidence of infections, ranging from intense hepatocyte swelling with hyperchromatic nuclei to intense periportal mononuclear cell infiltrates from diffuse toxic hepatic injury. In some mice, complete loss of hepatocytes was noticed while others showed ballooning degeneration of the hepatocytes with intra cytoplasmic eosinophilic accumulations (microconidia) with severe oedema and hepatitis (inflammation of the liver), as well as fused and hazy cells. The presence of microconidia in the cytoplasm was also noticed. Fig.5a shows the control liver while Fig.5b- 5k revealed various damages caused by the *F. solani* to the liver of infected mice.

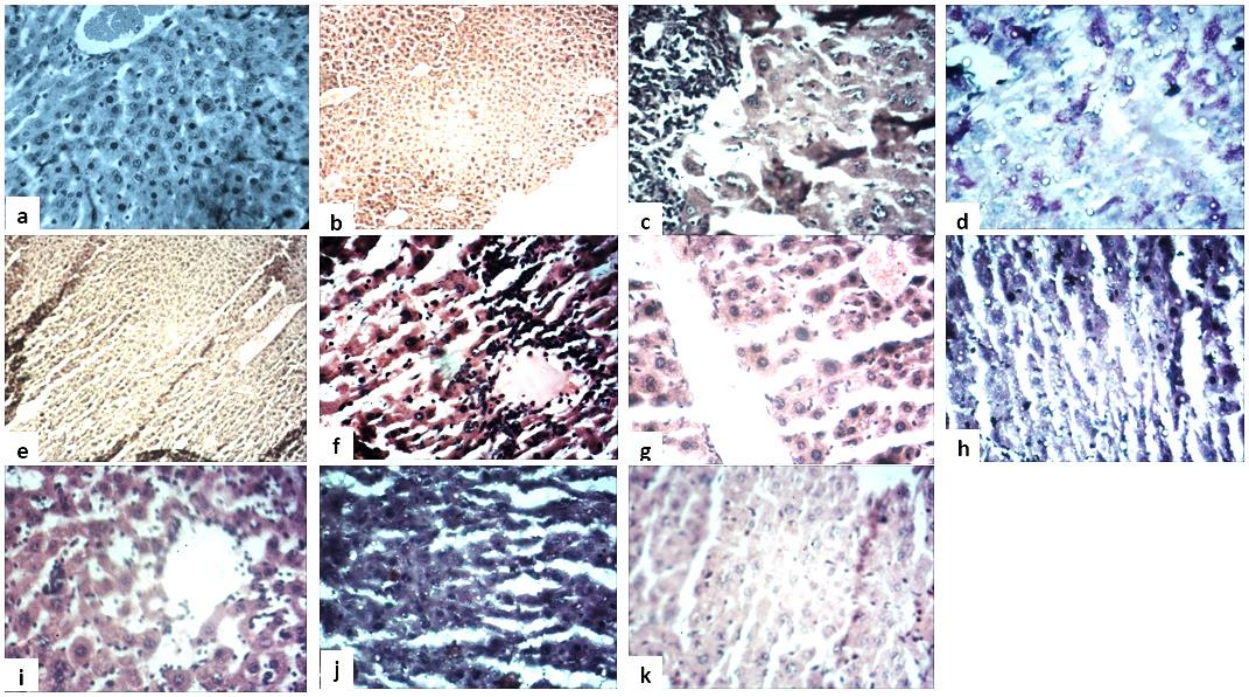

Fig. 5: *Photomicrographs of Liver sections of control and Fusarium species infection models;* (a) **Normal control:** Section shows normal central veins, portal tracts, sinusoids and hepatocytes. (b**) H2 strain:** Portal tracts are not visualized and there is loss of periportal hepatocytes; however, centrilobular hepatocytes are better preserved. (c) **H125 strain:** Intense hepatocyte swelling with hyperchromatic nuclei and intense periportal mononuclear cell infiltrates from diffuse toxic hepatic injury. Note the presence of microconidia within the cytoplasm. (d) **P189 strain:** Ballooning degeneration of the hepatocytes with intra cytoplasmic eosinophilic accumulations (microconidia), severe odema, and fused hazy cells. (e) **H193 strain:** Extensive hepatocyte toxic injury with swelling. (f) **H133 strain:** Mild swelling of hepatocytes with centrilobular inflammation. (g) **H13 strain:** Hepatocytes with granular or ground glass cytoplasm and reactive changes including binucleation. (h) **H132 strain**: Mild reactive changes in the liver section. (i) **H43 strain:** Evidence of generative liver changes and hepatitis (inflammation of the liver). (j) **P64 strain:** Liver ground glass hepatocytes with ballooning degeneration. (k) **P6 strain:** Ballooning degeneration of hepatocytes.

Spleen: The inoculated *F*. solani also disseminated to the spleen of the infected mice with evidence of damage such as extensive destructive granulomas with many Langhans giant cells formed from type IV hypersensitivity reaction. Some strains showed complete destruction of the splenic pulp with giant cell granulomas seen diffusely (Quite toxic), while others showed balloon degeneration of the splenic cells and foamy macrophages (splenitis). Fig6a showed normal spleen while plates 6b- 6j revealed damages from different *Fusarium* strains.

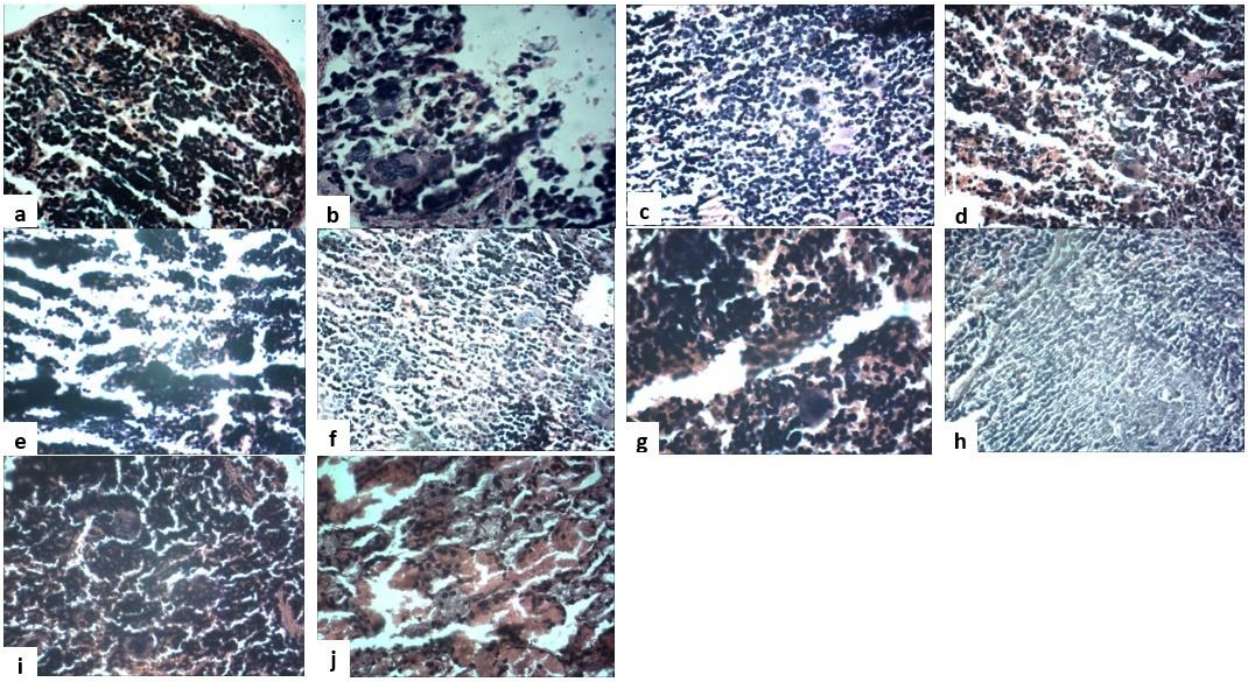

Fig. 6: *Photomicrographs of Spleen sections of control and Fusarium species infection models;* (a) **Normal control:** showing normal red pulp and sinusoids. (b**) H2 strain:** showing extensive destructive granulomas with many Langhans giant cells. Complete destruction of the splenic pulp is also observed. (c) **H125 strain:** Diffuse granulomatous inflammation in keeping with a type IV hypersensitivity reaction. Note presence of numerous Langhans giant cells. (d) **P189 strain:**showing granulomatous inflammation and presence of numerous giant cells. (e) **P193 strain:** shows no obvious pathological change. The histoarchitecture of the tissue appears normal. (f) **H133 strain:** shows granulomatous splenitis and the presence of giant cells. (g) **H13 strain:** showing mild splenic granuloma with few giant cells. (h) **H132 strain**: showing essentially normal spleen with mild splenitis but absence of giant cells. (i) **H43 strain:** Diffuse giant cell granulomas (Quite toxic). (j) **P64 strain:** Ballooning degeneration of the splenic cells and foamy macrophages (splenitis).

Discussion

Fusarium are fungi genus that commonly inhabit soil, and had been documented that they cause both plant disease [17,18] as well animal/human infections.[19,20] Tissue invasion of this organism in human with immunocompromise condition had been well reported by researchers.[21] Thus early diagnosis can help in rapid antifungal therapies which are essential for patient survival. In resource limited environment, cultural identification of this fungi still remained an essential tool. In this study, cultural characterization of one of the *Fusarium* species (*F. solani)*, were done. The identification technique involved observation of cultural and morphological characteristics, through comparing the isolates with standard fungal atlas using as stated by [16] One of the striking observations in this study was that infected mice showed poor eating and drinking habit as well as inactive behavior and weight loss. It was reported previously that animals infection do results in inactivity, weight loss and poor feed habits.[22] This had been documented as markers of severe infection.[22] Though majority of the mice became active after few

days, the cause of the death of those that could not survive could be attributed to systemic infection. Similar report was also documented by [23] that injection of *F. solani* into lateral vein of the mice tail results in death of some of the mice. In addition, the mice demonstrated some degrees of infection which includes pinkish ring-like lesions on the upper, undersurfaces of their bodies towards their inguinal region and tails. This might depict inflammation/necrosis at the site were the organism had disseminated into. [24] had stated that the *Fusarium* multiple necrotizing lesion are usually notice in the trunk and the extremities especially in human and that this can be an important and an early hint in the diagnosis.

Survival of the fungi in animal model had been linked with its ability to cause infection in immunosuppressed mice. 15 Despite the fact that immunocompetent mice were used in this study unlike other study, animal mortalities were still recorded. A previous work has stated that immunosuppression of animals prior to infection with *F. solani* affects the severity of disease in experimental animals [25]; this might explain lower death rate recorded in this study against high death rate in other study. [20]

Dissemination of *Fusarium* infection in human had been documented [26-28], and Murine model had been demonstrated. [29] Dissemination of the organism into spleen, liver and brain was observed in affected animals. This was noticed through the detection of the fungal propagules in the organs mentioned; this was in concordance with the reports of [20], who also observed that the organism germinated in the kidney of the mice while ungerminated microconidia of *F. solani* were found in liver and brain.

The internal organs of most of the sacrificed mice showed intense inflammations.

This result confirmed the work of [15], where fungal popagules were also recovered in multiple organs although their work was on *F. oxysporium*. In that study, spleen also recorded the highest concentration, followed by the liver. There were no statistically significant (P = 1.000) differences in weight among organs and between cages

The number of colony forming units (CFUs) of the conidia per gram, calculated from the spleen was significantly higher (P = 0.013) than that from the brain and liver but there was no significant difference between the colony count in the liver and that in the brain (P = 0.753). However, the brain had the lowest colony count. The *F. solani* inoculated into the different mice were recovered again from the various organs of the sacrificed mice. In human however, skin is the most frequent affected organ in fusarium dissemination infection in immunocompromised individuals. [27]

Conclusion

This result of this study expresses high occurrence of *Fusarium solani* in both humans and plants and also the ability to disseminate into various organs causing morbidity and mortality. This confirmed that it possesses very potent virulence factors which might include mycotoxin production.

Competing Interest

There exists no conflict of interests.

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EFFECTS OF MALARIA INFECTION ON THE HAEMATOLOGICAL AND BIOCHEMICAL PARAMETERS OF PREGNANT WOMEN IN ONITSHA, ANAMBRA STATE, NIGERIA

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Abstract

Background: Malaria remains a major public health problem in sub-Saharan Africa especially among pregnant women and unborn infants.
Aim: The effects of malaria infection on the haematological and biochemical parameters of pregnant women were investigated in Onitsha, Anambra State, Nigeria.
Materials and Methods: Venous blood samples were collected from 1500 pregnant women who attended antenatal clinic, in Onitsha, Anambra State, Nigeria. The haematological and biochemical parameters of the malaria positive samples were analysed using automated analysers and Giemsa stained blood films. Data was analysed using Chi-square analysis at level of significance of $p<0.05$.
Results: A total of 423 were positive for malaria. Haemoglobin and White blood cell (total and differential) counts were significantly associated with malaria infection ($P<0.05$). A total of 28 pregnant women (6.6%) who had malaria were found to be anaemic with highest prevalence of anaemia observed: among those less than 20years old (43.3%; $p<0.05$), in first trimester (20.0%; $p<0.05$), and among Secundigravidae (7.8%; $p<0.05$). Case wise, there were 68.3% leucocytosis and 1.4% leucopenia. Decreased platelet count was observed among malaria infected women, with 16(3.8%) having thrombocytosis while 24(5.7%) had thrombocytopenia. The biochemical parameters showed a significant difference between pregnant women with normal values and those with abnormal values ($p<0.05$). A total of 24(5.7%) of them had elevated ALT, 19(4.5%) had elevated AST, 15(3.6%) had elevated ALP and 32(7.6%) had elevated total bilirubin. Pregnant women less than twenty years old had the highest prevalence 9(30.0%) of elevated

ALT, 7(23.3%) of elevated AST, 6(20%) of elevated ALP and 11(36.7%) of elevated total bilirubin.
Conclusion: Malaria in pregnancy is a major public health problem affecting women. Pregnant women below 20 years of age are particularly vulnerable to the disease and its associated morbidities including decreased haemoglobin (Hb), platelets counts, full blood counts, resulting in increased risk for anaemia.

Keywords: *Placental malaria, liver function test, low birth weight, Plasmodium falciparum*

Introduction

Malaria remains a major public health problem in sub-Saharan Africa especially among pregnant women[1]. The World Health Organization (WHO) reported 241 million cases and 627 thousand deaths from malaria in 2020[2]. Malaria in pregnancy has severe consequences for the woman and her unborn infant. An infant born to a mother with malaria is more likely to have low birth weight (LBW), which is the single greatest risk factor for death during the first few months of life. The risk of maternal death also increases considerably as a pregnant woman suffering from malaria is likely to develop severe maternal anaemia[3,4]. Parasites in peripheral blood without symptoms are commonly seen in hyper endemic areas and usually associated with Placental sequestration and chronic anaemia[5]. Approximately one in four pregnant women present with malaria infection during delivery and this has been evidenced by findings from clinical studies, suggesting that placental malaria is common[6]. There is increased susceptibility to malaria and other infections during pregnancy due to the suppression of the immune system[7].

Haematological changes are common complications of malaria in pregnancy. These changes involve the major cell lines such as red blood cells, leucocytes and thrombocytes Haematological abnormalities such as anaemia, thrombocytopenia and leukocytosis or leucopenia have been observed in patients with malaria[8]. The biochemical parameters of pregnant women are also impacted especially if the woman is having malaria. In pregnancy, liver synthesis and metabolic functions are affected by increased serum estrogen and progesterone levels. Increased metabolic and physiological stress of pregnancy, could cause previously been subclinical liver disorders to become symptomatic[9]. Women who are infected with malaria parasite during pregnancy are at risk of miscarriage, still birth, low birth weight of the foetus, maternal anaemia and intra-uterine growth retardation[4]. It therefore became imperative that regular malaria parasite test be included in the routine tests for pregnant women, and liver function and haematological parameters are assayed for pregnant women who test positive for malaria, to enable better management of the condition and prevent adverse consequences. This study was hence carried out to ascertain the impact of malaria infection on haematological and liver function parameters of pregnant women in Onitsha metropolis.

Materials And Methods

Study Area

Onitsha is an urban city, a commercial, educational and religious centre as well as a river port on the Eastern bank of the River Niger in Anambra State, Southeastern

Nigeria. It has an estimated population of 1,003,000 people[10]. Onitsha lies between the latitudes 6°7'N and 6°47'E and longitudes 6°17'N and 6°78'E in the rainforest belt of Nigeria. Onitsha town is situated about 5 miles (8km) Northeast of Asaba and 25 miles (40km) Southeast of Awka, the capital city of Anambra State. Onitsha experiences two distinct seasons - a wet season which begins in March/April and ends in October or early November with annual rainfall range of 2000mm to 3000mm and about four/five months of dry season which lasts from November to February/March. The daily temperature ranges from 22°C to 36°C. The indigenous people of Onitsha are Igbos and they speak Igbo language. The occupational groups in Onitsha include traders, civil servants and artisans.

Study Population and Sample size

A total of 1500 pregnant women attending clinic at Holy Rosary Specialist Hospital and Maternity, Onitsha, and General Hospital, Onitsha both in Anambra State volunteered and participated in the study. Sample size formula $n = N/1+N(e^2)$ was used to determine sample size for the study[11].

Study Design

The study design was a cross sectional study of pregnant women that attended clinic at Holy Rosary Specialist Hospital and Maternity Onitsha and General Hospital Onitsha within a period of 12 months.

Ethical Consideration

Ethical approval for the study was obtained from the ethical committee of Anambra State Hospitals Management Board (SHMB/AD196/VOL.V/124) and the ethical committee of NnamdiAzikiweUniversityTeachingHospital Nnewi NAUTH/CS/66/VOL.14/VER.3/294/2021/067). The informed consent of the pregnant mothers was obtained through careful explanation of the project intentions and advantages.

Sample Collection

A total of 1500 blood samples were collected for the study. From each participant, 5ml of blood was withdrawn. 2ml of blood was dispensed into an Ethylenediaminetetra-aceticacid (EDTA) container; the remaining 3ml was transferred to a clean dry test tube. The sample in EDTA container was used for thick and thin blood film preparation, and full blood count test. The samples in the clean dry test tube were used for Liver function test (LFT).

Preparation of Thick Blood Films, Staining and Examination

Thick blood films were prepared, stained and examined following standard haematological techniques[12].

Haematological assay

Automated haematology analyzer by Mindray BC-5300 was used to analyse haematological parameters of haemoglobin concentration, platelet count, total white blood cell and differential count.

Biochemical Assays (Liver Function Test)

The liver function tests were determined using automated mindray chemistry analyzer (BS-230).

Analysis of Data

Chi-square analysis was used to compare the association among the different groups for significant difference. 95% level of confidence was used. Questionnaire was also used for other relevant information.

Results

A total of 28(6.6%) pregnant women who had malaria were found to be anaemic. The highest prevalence 13(43.3%) of anaemia was observed among those <20 years. No case 0(0.0%) of anaemia was observed among those who are 41+ years as shown in Table 1. Prevalence of anaemia in relation to age was statistically different among the age groups (P<0.05).The highest prevalence 15(20.0%) of anaemia was observed in pregnant women in their first trimester while the least 7(2.8%) was observed in pregnant women in their third trimester. There was a statistical difference in the prevalence of anaemia in relation to trimester (P<0.05).The highest prevalence 8(7.8%) of anaemia was observed in secundigravidae while the least 15(6.1%) was observed in the primigravidae. Prevalence in relation to gravidity was not statistically different (P>0.05).

Malaria infection in pregnant subjects affected their Total WBC counts (TWBC) (Table 2). Of the 423 infected subjects, 128(30.3%) had TWBC within the normal range, while the remaining 295 subjects recorded 289(68.3%) leukocytosis and 6(1.4%) leucopenia. The difference between normal and abnormal TWBC were statistically significant (P<0.05) across the age groups. Age group <20 recorded highest percentage 24(80.0%) of abnormal count while age group 41+ recorded the least 5(62.5%).

A total of 383(90.5%) had normal count of thrombocyte while 40(9.5%) had abnormal thrombocyte count. Malaria-infected pregnant women that were 41+ years and above had the highest cases of abnormal thrombocytes 5(62.5%) while the age group 21 - 30 had the least cases of abnormal thrombocytes 12(5.2%) as shown in (Table 3). Statistical analysis showed a significant difference in the platelet count of malaria-infected pregnant women studied across the age groups. (P<0.05).

Table 4 showed a total of 399(94.3%), 404(95.5%) and 408(96.4%) had normal ALT, AST and ALP values respectively, while 24(5.7%), 19(4.5%) and 15(3.6%) had abnormal ALT, AST and ALP values respectively. A total of 391(92.4%) and 395(93.4%) had normal value of total bilirubin and direct bilirubin respectively, while 32(7.6%) and 28(6.6%) had abnormal total bilirubin and direct bilirubin values respectively. Age group (<20years) recorded the highest percentage abnormality for all the liver enzymes assayed; ALT - 9(30.0%), AST – 7(23.3%),

ALP – 6(20.0%), Total Bilirubin – 11(36.7%), Direct Bilirubin – 10(33.3%), while the least percentage abnormality, 0 (0.00%) for ALT, AST and ALP were recorded in age group (41+years), but for Total and Direct bilirubin age group (21-30years) recorded least abnormality 11(4.7%) and 9(3.8%) respectively. The differences in the liver function parameters across the age groups in malaria infected pregnant women were statistically significant at (p<0.05).

Table 1: Prevalence of anaemia in relation to age, trimester and gravidity of the malaria infected pregnant women studied.

Variables	*Number examined*	*Number positive (%)*	*Number anaemic (%)*	*Number not anaemic (%)*
Age (years)				
<20	48	30 (62.5)	13(43.3)	17(56.7)
21-30	774	234(30.2)	10(4.3)	224(95.7)
31-40	600	151(25.2)	5(3.3)	146(96.7)
41+	78	8(10.3)	0(0.0)	8(100.0)
(P<0.05, x^2 =172.99, df = 3, P-value=0.000)				
Trimester				
First	300	75(25.0)	15(20.0)	60(80.0)
Second	429	99(23.1)	6(6.1)	93(93.9)
Third	771	249(32.3)	7(2.8)	242(97.2)
(P<0.05, x^2 =20.46, df =2, P-value=0.000)				
Gravidity				
Primigravidae	630	246(39.0)	15(6.1)	231(93.9)
Secundigravidae	330	102(30.9)	8(7.8)	94(92.2)
Multigravidae	540	75(13.9)	5(6.7)	70(93.3)
(P>0.05, x^2=4.08, df=2, P-value=0.130)				
Total	**1500**	**423(28.2)**	**28(6.6)**	**395(93.4)**

Table 2: Total WBC count (TWBC) of malaria infected pregnant women in relation to age

Age (years)	No. Inf. (%)	No. with Normal range of TWBC (Leucocyte) (%)	No with abnormal TWBC count (%)	No. above normal range TWBC (Leucocytosis) (%)	No. below normal range TWBC (Leucocytopenia) (%)
<20	30(62.5)	6(20.0)	24(80.0)	21(70.0)	3(10.0)
21-30	234(30.2)	65(27.8)	169(72.2)	168(71.8)	1(0.4)
31-40	151(25.2)	54(35.8)	97(64.30	96(63.6)	1(0.7)
41+	8(10.3)	3(37.5)	5(62.5)	4(50.0)	1(12.5)
Total	**423(28.2)**	**128(30.3)**	**295(69.7)**	**289(68.3)**	**6(1.4)**

(p<0.05, x^2=29.21,df=3, p-value=0.000).

Table 3: Platelet count of malaria infected pregnant women in relation to age

Age (years)	No. Inf. (%)	No. with Normal range of Platelets (%)	No with abnormal platelet count (%)	No. above normal range of platelets (Thrombocytosis) (%)	No. below the normal range of platelets (Thrombocytopenia) (%)
<20	30(62.5)	16(53.3)	14(46.6)	4(13.3)	10(33.3)
21-30	234(30.2)	222(94.9)	12(5.2)	6(2.6)	6(2.6)
31-40	151(25.2)	142(94.0)	9(6.0)	3(2.0)	6(4.0)
41+	8(10.3)	3(37.5)	5(62.5)	3(37.5)	2(25.0)
Total	**423(28.2)**	**383(90.5)**	**40(9.5)**	**16(3.8)**	**24(5.7)**

($p<0.05$, $x^2=91.72$, df=6, p-value=0.000).

Table 4: Liver function parameters of malaria infected pregnant women in relation to age

Age (years)	No examined	No. inf. (%)	No. with normal range of ALT (5-35 IU/L) (%)	No with abnormal range of ALT (%)	No. with normal range of AST (0-35 IU/L) (%)	No with abnormal range of AST (%)	No. with normal range of ALP (41-133 IU/L)	No with abnormal range of ALP (%)	No. with normal range of Total Bilirubin (2-12µmol/L)	No with abnormal range of total bilirubin	No. with normal range of Direct Bilirubin (<12µmol/L)	No with abnormal range of direct bilirubin
<20	48	30(62.5)	**21(70.0)**	**9(30.0)**	23(76.7)	7(23.3)	24(80.0)	6(20.0)	19 (63.3)	11(36.7)	20(66.7)	10(33.3)
21-30	774	234(30.2)	224(95.7)	10(4.3)	227(97.0)	7(3.0)	229(97.9)	5(2.1)	223(97.9)	11(4.7)	225(96.2)	9(3.8)
31-40	600	151(25.2)	146(96.7)	5(3.3)	146(96.7)	5(3.3)	147(97.4)	4(2.6)	142(94.0)	9(6.0)	144(95.4)	7(4.6)
41+	78	8(10.3)	8(100.0)	0(0.0)	8(100.0)	0(0.0)	8(100.0)	0(0.0)	7(87.5)	1(12.5)	6(75.0)	2(25.0)
Total	**1500**	**423(28.2)**	**399(94.3)**	**24(5.7)**	**404(95.5)**	**19(4.5)**	**408(96.4)**	**15(3.6)**	**391(92.4)**	**32(7.6)**	**395(93.4**	**28(6.6)**

Discussion

Various hematological abnormalities have been associated with the prevalence of malaria. They include anemia, leucopenia, leucocytosis, thrombocytosis and thrombocytopenia[13,14]. The results of this study showed that the presence of malaria infection in the subjects studied brought about some increases/ decreases on some hematological parameters evaluated.

This study showed that haemoglobin (Hb) was significantly lower among younger age groups in the malaria-infected women. This could be attributed to destruction of both parasitized and unparasitized erythrocytes that take place in the spleen during malaria infection[15]. Haemoglobin is the major biomolecule that is metabolized by the malaria parasite. This results in destruction of red blood cells, a condition known as anaemia. This finding is in line with the reports of which indicated that infected patients tended to have significantly lower haemoglobin and red blood cell count[13,16]. The World Health Organization has stated that anaemia is present in pregnancies when Haemoglobin concentration is less than 11 g/dL[17].

The overall prevalence of anaemia among pregnant women in this study is lower than that reported in other studies[18,19]. The factors strongly associated with anaemia in this study such as age, trimester and gravidity; are similar to those reported in many other studies in different geographical locations[19]. Pregnant women less than 20 years of age were observed from this study to have the highest prevalence of anaemia than women in the other age bracket although pregnant women that were 41+ years and above had no cases of anaemia. This may be because teenage pregnancy is usually unintended, unplanned, sometimes outside wedlock in our setting with associated poor nutrition, poor health-seeking behaviour and poor antenatal care. Compared with similar studies, a decrease in haemoglobin of pregnant women in relation to trimester was shown in this study[16,20]. Pregnant women in their first trimester were found to be more anaemic than others in their second trimester and third trimesters.

This is in agreement with findings from similar studies which reported that pregnant women in their first trimester were more anaemic than those in their second and third trimesters.[21] Nevertheless, Wogu et al.,[22] reported progressive decline in haemoglobin (Hb) concentration from the first to third trimester, has been reported and attributed it to an increase demand for iron as pregnancy progresses. More iron is required to meet the expansion of maternal Hb mass and the needs of fetal growth. In relation to gravidity, it was observed in this study that secundigravids have a higher prevalence of anaemia than the primigravids and multigravids. This finding is in line with Akinbami et al.,[23] who reported that women with at least one previous birth or pregnancy were more likely to have anaemia than women who have not given birth. On the contrary it has also been observed that prevalence of anaemia was highest among primigravidae and decreased with subsequent pregnancies and hence complication rates were higher in primigravidae as compared to multigravidae patients[24].

Other haematological abnormalities / changes were linked to malaria prevalence in pregnancy and include leucocytosis and leucopenia. In this study leukocytosis was found higher than leucocytopenia among malaria infected pregnant women. However, Obebe et al.,[25] reported an elevation of leucocytes in malaria parasitized pregnant women in agreement with the findings in this study. Nlinwe et al.,[26] affirmed that leucocytosis occurring during pregnancy may be due to the physiologic stress induced by the pregnant state. However, contrary to this, some researchers observed leukopenia in their various studies[27]. This study showed low percentage of thrombocytopenia as one of the haematological changes in malaria parasitaemia. This is in line with observations of a significant reduction in the platelet counts of more than half of their study population[26]. Similar findings of increased thrombocytopenia among malaria subjects were reported in Kenya[8]. The plausible reason could partly be due to haemodilution and partly due to increased platelet activation and accelerated clearance.

This study showed an elevated value of the liver enzymes (biochemical parameter) in some of the malaria infected pregnant women especially the <20 years old. Malaria infection may cause liver disease of varying severity evident in the levels of serum aminotransferases at some stages of the infection[27].

This increased activities of liver enzymes observed among malaria infected ones in this study could be as a result of leakage of transaminases and alkaline phosphatase from parenchymal and hepatocytes

membrane respectively into the circulatory system or exchanged cord blood between the mother and the child as reported by Bawah et al.[28].

Conclusion

Malaria in pregnancy is a major public health problem affecting women in Onitsha, Anambra State, Nigeria. Pregnant women below 20 years of age are particularly vulnerable to the malaria infection and its associated morbidities including decreased haemoglobin (Hb), resulting in increased risk for anaemia. Therefore, there is need to conduct malaria parasite test, full blood count test and liver function test on pregnant women attending antenatal to ensure prompt management and prevention of adverse consequences.

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Competing Interest: Authors declare that there exists no conflict of interests.

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AQUEOUS EXTRACT OF *SOLANUM NIGRUM* LEAF REVERSED REPRODUCTIVE DYSFUNCTIONS ASSOCIATED WITH ANASTROZOLE-INDUCED POLYCYSTIC OVARIAN SYNDROME IN RATS

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Abstract

Background: Polycystic Ovarian Syndrome (PCOS), also referred to as Stein-Leventhal syndrome, is a common endocrine disorder that impacts 12-21% of women in their reproductive years. The aqueous extract of *Solanum nigrum* leaves (AEoSNL) was investigated for its therapeutic effects on reproductive dysfunctions associated with anastrozole-induced PCOS in Wistar rats.

Method: Sixteen female Wistar rats (160.46 ± 4.11 g) were divided into four groups (A-D): group A were not induced into PCOS, while those in groups B, C and D were induced into PCOS by oral administration of 0.5 mg/kg body weight of anastrozole dissolved in 1% CMC (2 mL/kg) daily for 21 days. Animals in groups A and B both received 0.5 mL of distilled water, C and D received 0.5 ml co-administration of metformin (7.14mg/kg/day) and clomiphene citrate (2mg/kg/day) metformin, and 200 mg/kg body weight (bw) of AEoSNL once daily for fourteen days post-induction. Vaginal cytology, ovarian histology and levels of some reproductive hormones in the serum were determined.

Result: Anastrozole administration resulted in disrupted oestrous cyclicity, ovarian cyst formation and altered hormonal levels thereby replicating PCOS-like symptoms. The administration of 200mg/kg (bw) AEoSNL to PCOS rats significantly decreased ($P \leq 0.05$) serum testosterone, follicle stimulating hormone, progesterone and luteinizing hormone (LH)

concentration but there was no significance difference (P≥0.05) in the prolactin level when compared with the control. The AEoSNL reversed the hyperandrogenemia, LH hypersecretion and irregular estrous cycle in PCOS-induced rats.
Conclusion: This study suggests that, 200 mg/kg(bwt) of AEoSNL exhibit therapeutic functions in anastrozole-induced PCOS in rats and can be explored in an anti-PCOS drug design subject to further experimentation.
Key words: Anastrozole, Clomiphene citrate, Metformin, Oestrous, Polycystic Ovarian Syndrome, *Solanum nigrum*

Introduction

Polycystic Ovary Syndrome (PCOS) is a hormonal disorder prevalent in females primarily in those of reproductive age[1]. PCOS is characterized by an imbalance in sex hormones, particularly an excess of androgens (male hormones like testosterone) in the body and this hormonal imbalance can disrupt the normal functioning of the ovaries[1]. One of the hallmark features of PCOS is anovulation, which connotes that the ovaries do not consistently release eggs during the menstrual cycle. This can lead to irregular or absent menstrual periods[2]. PCOS can manifest with various symptoms, including irregular menstrual periods, heavy or prolonged bleeding, acne, excess facial and body hair (hirsutism), hair thinning on the scalp, weight gain as well as metabolic disorders such as insulin resistance, type 2 diabetes, and cardiovascular problems[3]. The available treatment options involve lifestyle changes (dietary modifications), drugs (such as metformin), exercise to help with weight management, medications to regulate the menstrual cycle (clomiphene citrate), and medications to manage the hormonal and metabolic aspects of the condition[1]. However, some of the medications used in the treatment of PCOS have been reported to have various side effects[4]. This has necessitated the need scientifically explore alternative therapies for the treatment of PCOS. Folklore medicine has been in existence for years and its usage by women has been on the increased in the recent times[5].

Solanum nigrum (Black nightshade) is a multipurpose plant species belonging to Solanaceae family[6]. The leaves of *Solanum nigrum* are notable for their anti-inflammatory, antioxidant, antibacterial, anti-diabetic, and maybe anti-cancer properties[6,7,8]. Some studies have reported *Solanum nigrum* having the potential to improve insulin resistance and enhance lipid metabolism[9]. However, in the management of polycystic ovary syndrome and fertility disorders, there is limited scientific reports on the efficacy of *Solanum nigrum* leaves on reproductive dysfunctions. Thus, this study was aimed at evaluating the therapeutic effect of *Solanum nigrum* leaves on certain reproductive dysfunctions associated with anastrozole-induced PCOS in female Wister rats.

Materials And Methods

Plant Material

Fresh leaves of *Solanum nigrum* plant were collected from Mountain Top University permanent site, Ogun State, Nigeria. The plant leaf was authenticated at the

Department of Plant Biology, University of Lagos, Nigeria. A voucher specimen number LUH 10037 was prepared and deposited at the herbarium of the department.

Animals

Sixteen healthy female Wistar rats (190.56±5.35g) were obtained from the animal holding Centre of the Mountain Top University, Ogun State, Nigeria. The animals were kept in a well-ventilated house condition (temperature: 22±3°C; photoperiod: 12h/12h light/dark cycle; humidity: 45-50 %) and fed with rat pellets (New Hope, Grand Cereals, Lagos, Nigeria) and water *ad libitum*.

Drugs, Assay Kits, and Chemicals

Progesterone, Insulin, Prolactin, Follicle Stimulating Hormone, Testosterone and Luteinizing Hormone assay kits were manufactured by Perkin Elmer Laboratories, Freiburg, Germany. Anastrozole was a product of Ani Pharmaceuticals, Minnesota, USA. All other reagents used were from Sigma Chemicals, St. Louis, USA.

Preparation of Extract

A known weight (2 kg) of *Solanum nigrum* leaves was washed, air-dried and pulverized in a warring electric blender. The powdered leaves (715 g) was extracted in distilled water using the ratio (1:4)[3] for 48 hours and filtered with Whatman No. 1 filter paper. The filtrate was lyophilized to give a yield of 17.4 g which corresponded to 2.43%. The resulting powder was reconstituted in distilled water to obtain the dose, 200 mg/kg body weight used in this study.

Animal grouping and extract administration for pharmacological study

A total of 16 female rats were acclimatized for 2 weeks and divided into 4 groups of four (4) animals each (A-D). PCOS was induced in twelve female Wistar rats in groups designated B - D with 1 mg of anastrozole described by Yakubu and Ibiyo (2013)[10] for a period of 21 days. The extract administration was done as follows:

Group A (non PCOS induced control) received 0.5ml of distilled water

Group B (untreated PCOS) received 0.5ml of distilled water

Group C (PCOS induced) received 7.14mg/kg body weight of metformin and 2mg/kg

body weight clomiphene citrate (Reference drug)

Group D (PCOS induced) received 200mg/kg body weight of *Solanium nigrum* leaves extract.

The extract, distilled water and reference drugs were once daily administered for fourteen days. Twenty-four hours after the last administration (end of the experimental period), the rats were anesthetized using diethyl ether and sacrificed by jugular puncture. The blood samples and ovaries were collected using the procedures described by Femi-Olabisi *et al.* (2023)[11]. Thereafter, the serum was used to carry out hormonal assays.

Vaginal Cytology

Vaginal Cytology Using a light microscope, the vaginal cytology of the stages of estrous cycle in female rats was monitored to observe the predominant cell type in the vaginal smears which were obtained daily for 21 days during the induction period[12].

Determination of reproductive hormones

The concentrations of Progesterone, Insulin, Prolactin, Follicle Stimulating Hormone, Testosterone and Luteinizing Hormone, were determined by adopting standard procedures. Also, Histopathology of the ovaries were determined

Histopathological examination

The histological examination was carried out as described by Yakubu and Ibiyo (2013)[10]. The animals were quickly dissected and the ovaries were excised from each rat, cleaned of fatty layers and fixed in 10% formalin for at least 24 hr before the slides were prepared according to the procedure described by Drury and Wallington (1980)[13].

Statistical Analysis

Data were expressed as the mean ± standard error mean of four determinations and data were analysed for statistical significant at P≤ 0.05 using One Way Analysis of Variance and Duncan Multiple Range Test performed with Statistical Package for Social Sciences, version 21.0 (IBM Inc., Chicago, USA).

Results

The administration of anastrozole significantly increased (p≤ 0.05) the serum testosterone and LH concentrations while the serum progesterone, FSH and prolactin concentrations were significantly decreased (p≤ 0.05) when compared with that of normal control. The administration of 200mg/kg B.W of AEoSNL significantly decreased (p≤0.05) the elevated levels of testosterone and LH in anastrozole-induced PCOS animals in a manner similar to the reference drug-treated group. compared favourably the glucose level to the anastrozole -treated rats (Table 1). The reduction in the prolactin content of the animals at 200 mg/kg body weight of the AEoSNL as well as PCOS animals treated with MET and CC compared favourably with the distilled water treated non-PCOS animals (Table 1). However, the extract significantly decreased the FSH levels of the PCOS rats while the reference drug increased the serum levels of FSH compared to the normal control animals (Table 1).

The ovarian histology of the normal control rats revealed developing follicles with numerous corpora lutea in the ovarian stroma whereas the distilled water-treated anastrazole-induced PCOS animals had very few developing follicles and corpora lutea (Plates 1a and 1b). The ovaries of the anastrozole-induced PCOS rats treated with metformin and clomiphene citrate and AEoSNL had numerous developing follicles, many corpora lutea and few atretic follicles (Plates 1c and 1d)

From the obtained as plotted in the line graphs, the control rats showed regularity of

the estrous cycle and were in the diestrus phase three times over the period of the experiment. The control rats had 4-6 days of oestrus cycle (Figure 1). In contrast to the normal control animals, group B, the distilled water-treated PCOS rats, had irregular cycles characterized with persistent estrus and metestrus phases, and cyclical disruptions ranging from 5-9 days (Figure 2). Compared to the control group, the PCOS-induced rats were consistently at the metestrus phase, with irregular intervals for extended periods within the experiment. In the proestrus phase, all the rats exhibited similar cyclical patterns. The administration of extract at 200mg/kg body weight reversed the trend of persistent presence of cornified epithelial cells in the oestrous cycle of the anastrozole induced rats in a manner that was similar to that of the control group and the reference drug-treated PCOS animals (Figure 3 and 4).

Table 1: Effect of aqueous extract of *Solanum nigrum* leaves on reproductive hormone

Parameters / Groups	Testosterone (nMol/L)	Progesterone (nMol/L)	Follicle Stimulating Hormone (mIU/ml)	Luteinizing Hormone (mIU/ml)	Prolactin (mg/mL)
Control	6.13 ± 0.125^{a}	44.00 ± 0.41^{a}	62.50 ± 1.44^{a}	25.00 ± 0.04^{a}	9.50 ± 0.29^{a}
PCOS +Distilled water	27.75 ± 1.30^{d}	33.50 ± 0.29^{c}	51.25 ± 1.25^{d}	30.03 ± 0.20^{b}	9.00 ± 0.01^{b}
PCOS+MET+CC	14.38 ± 0.13^{b}	31.50 ± 0.87^{b}	151.75 ± 1.18^{b}	27.01 ± 0.13^{c}	9.25 ± 0.14^{c}
PCOS + 200 mg/kg b.wt. of AEoSNL	23.99 ± 0.01^{c}	34.50 ± 0.29	33.75 ± 1.25^{c}	$27.05\pm0.03c$	9.50 ± 0.29^{a}

Data are means of four determinations ± SEM. Values with different superscripts in each column are significantly different ($P \leq 0.05$). **Metformin- MET, clomiphene citrate- CC**

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 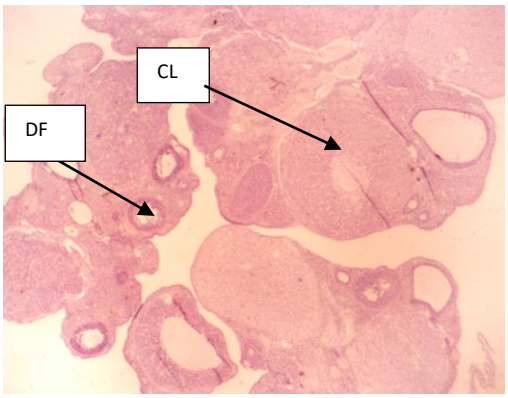

Plate 1a: Photomicrograph of cross section of ovary of normal rat administered carboxymethyl cellulose (×400; H & E) CL-corpus luteum, DF-developing follicles</td>
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 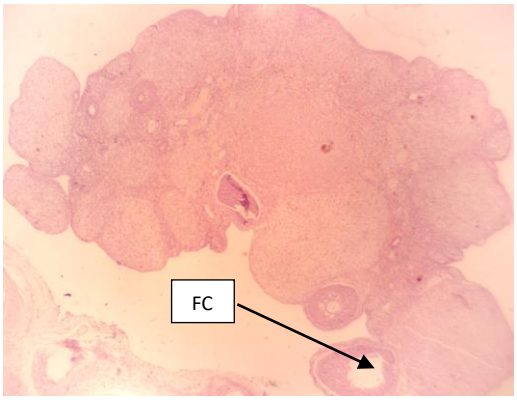

Plate 1b: Photomicrograph of cross section of ovary of the untreated anastrozole-induced PCOS rat administered distilled water (×400; H & E) FC- follicular cyst</td>
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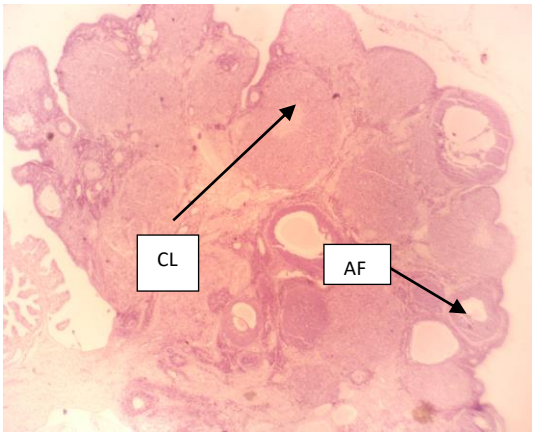
 

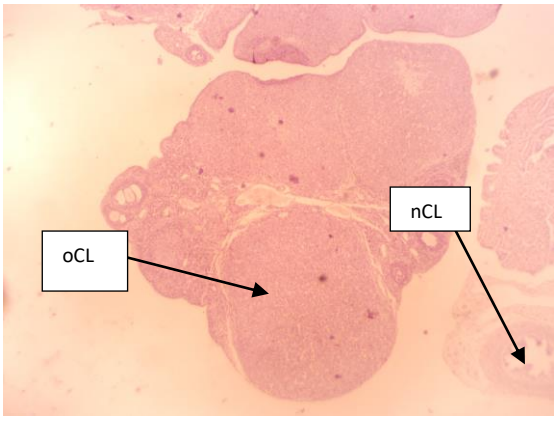
Plate 1c: Photomicrograph of cross section of ovary of anastrozole-induced PCOS rat co-administered metformin and clomiphene citrated (×400; H & E) CL-corpus luteum, AF-Atretic follicles</td>
<td>4
 

Plate 1d: Photomicrograph of cross section of ovary anastrozole-induced PCOS rat administered 200 mg/kg bwt. Of AEoSNL (×400; H & E) oCL- old corpus luteum, nCL-new Corpus Luteum</td>
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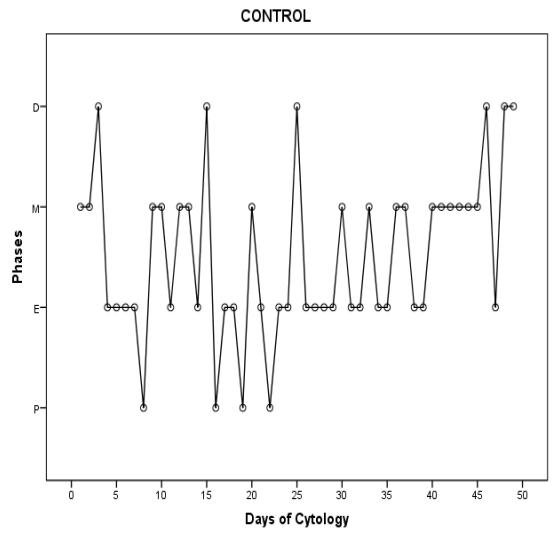

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Figure 1: Oestrous cyclicity of rats of normal control rats

Proestrous, E: Estrous, M: Metaestrous, D: Diestrous

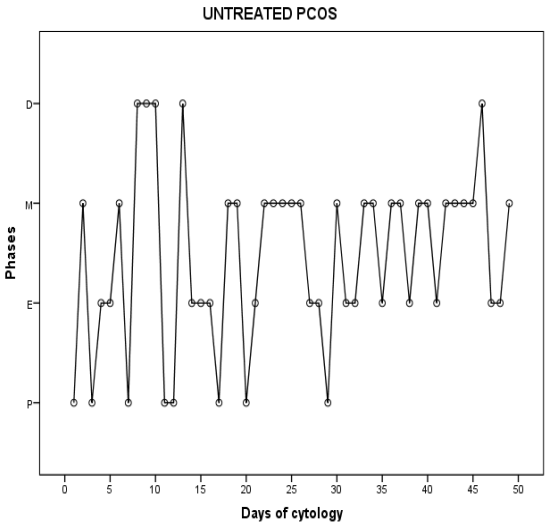

Figure 2: Oestrous cyclicity of anastrozole -induced female rats untreated group

Proestrous, E: Estrous, M: Metaestrous, D: Diestrous

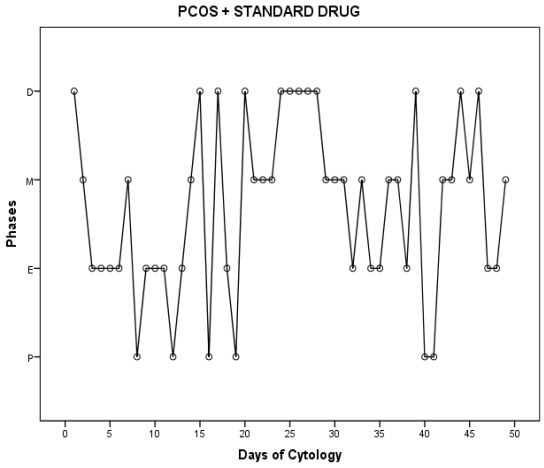

Figure 3: Oestrous cyclicity of anastrozole - induced female rats treated with reference drugs (metformin and clomiphene citrate).

: Proestrous, E: Estrous, M: Metaestrous, D: Diestrous

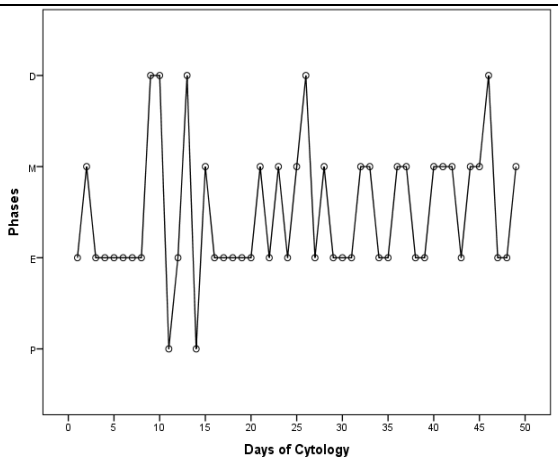

Figure 4: Oestrous cyclicity of anastrozole - induced female rats treated with 200mg/kg body weight of AESN LEAVES

P: Proestrous, E: Estrous, M: Metaestrous, D: Diestrous

Discussion

There non-availability of cure for PCOS necessitated the use of drugs to manage this condition symptomatically but without a variety of adverse effects. Drugs made from natural plant products may help treat PCOS because several plant extracts have been widely recognized to attenuate the symptoms of PCOS[14]. Measurement of sex hormone levels reveal the presence of PCOS and this is a consistent parameter needed to diagnosis a woman with PCOS exhibiting elevated serum testosterone, LH concentrations, and low progesterone and FSH levels[15]. High testosterone levels indicated a buildup of androgens, presumably as a result of the inhibition of androgen substrate conversion to estrogens[16]. Increased testosterone secretion, the elevated serum LH concentrations could be related to a reduction in estrogen synthesis in the brain and pituitary and this validates the usage of anastrozole for the induction of PCOS[17]. Elevated blood levels of androgens are also the one of the major etiologies behind PCOS. Therefore, therapeutics with anti-androgen activity are used in the treatment of PCOS[18]. The decrease in the testosterone and LH concentration in PCOS rats administered 200 mg/kg body weight of AEoSNL suggests that the extract possesses anti-androgenic activity (Table 1). Changes in prolactin levels and hormonal imbalances will have a significant impact on ovulatory cycles. Decreasing prolactin levels or improving the hormonal balance have a positive impact on ovulatory cycles and the treatment of PCOS. The prolactin levels of PCOS rats treated with the extract compared favourably with the control and also reversed the acyclicity in their oestrus cycle characterized by persistent estrus and metestrus phases and this implies that the AEoSNL possesses anti-PCOS activity in ameliorating hormonal imbalances as well as reversing acyclicity in the ovulatory cycles. Metformin's insulin-sensitizing qualities have been found in studies to enhance menstrual periods and ovulation rates in women with PCOS especially when co-administered with Clomiphene citrate. Metformin have been reported affect hyperandrogenism, metabolic changes, and, most critically, fertility[19]. The extract may have similar mechanism of action with metformin due to its effects on hyperandrogenism and improvement of the oestrus cycle (Figure 3 and 4).

Acyclicity and the presence of polycyctic ovary are crucial parameters used in measuring reproductive abnormalities in PCOS[20]. The control animals exhibited regular 3–5 days oestrus cycle, and their histologically examined ovaries contained new corpora lutea which indicates recent ovulations (Plate 1a). The untreated PCOS rats ovaries had follicular cyst which are atretic and this establishes the induction of PCOS using anastrozole. The administration of AEoSNL to PCOS rats reflected in the ovarian histology with the presence of few old atretic follicles and new corpus lutea which indicates that ovulation has recently occurred (Plate 1d).

Conclusion

The administration of 200 mg/kg BW of aqueous extract of *Solanum nigrum* leaf to anastrole-induced PCOS rats reversed reproductive dysfunctions such as elevated testosterone, LH hypersecretion, oestrous acyclicity and the presence of cystic ovaries which are features common with PCOS. Therefore, the use of aqueous extract of *Solanum nigrum* leaf can be explored in novel drug design for the treatment of PCOS subject to further experimentation.

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