JOURNAL OF BIOMEDICAL INVESTIGATION (JBI) VOLUME 13 NUMBER 3: NOVEMBER-DECEMBER 2025

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ASSESSMENT OF DIAGNOSTIC ACCURACY OF CLINICAL EXAMINATION AND ULTRASONOGRAPHY IN DETECTING BREAST CANCER IN KIGALI REFERRAL HOSPITALS

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ABSTRACT

Background: Breast cancer remains a major public health issue in Rwanda, with high mortality rates largely due to late-stage diagnosis.

Aim: This study aimed to assess the accuracy of clinical breast examination (CBE) and ultrasonography in diagnosing breast cancer among patients at Kigali referral hospitals.

Methods: A retrospective cross-sectional study was conducted using a sample of 286 patients, determined via Clinical Breast Examination and selected through simple random sampling. Data on findings, breast ultrasound results, and histopathology outcomes were collected using a checklist. Histopathology served as the gold standard. T-test statistics were used to test three hypotheses at the 0.05 level of significance.

Results: Clinical breast examination showed 70.8% sensitivity, 86.7% specificity, and 77.6% overall accuracy. Sensitivity and specificity of clinical breast examination did not differ significantly in their influence on breast cancer detection in the sampled patients ($t = 1.462$; $p = 0.145$). Breast ultrasound had a sensitivity of 93.4%, specificity of 84.1%, and an overall accuracy of 88.1%. Both sensitivity and specificity contributed similarly to the diagnostic capability of breast ultrasonography in detecting breast cancer within the studied referral hospitals ($t = 0.794$; $p = 0.331$). Clinical breast examination and breast ultrasound demonstrate comparable predictive performance in detecting breast cancer within the studied referral hospitals ($t = 1.248$; $p = 0.213$).

Conclusion: Clinical breast examination demonstrated a high sensitivity, specificity of 86.7% and overall accuracy indicating effective identification of most breast cancer cases. Additionally, breast ultrasound also exhibited a high sensitivity, specificity and overall accuracy confirming its effectiveness in accurately detecting breast cancer and correctly identifying benign cases, highlighting the potential of breast ultrasound as a reliable diagnostic tool.

Recommendations: Access to ultrasound services should be expanded particularly in underserved areas; integrating into primary healthcare for early detection and management of breast cancer.

Keywords: ultrasound, breast, Kigali, cancer, data

INTRODUCTION

Clinical breast examination (CBE) refers to a systematic evaluation conducted by a healthcare professional to examine and evaluate the breasts for any abnormalities or signs of potential breast health concerns. It involves a combination of techniques, such as visual inspection, palpation, and gathering medical history, to assess the overall breast health and detect any possible signs of breast disease or conditions[1]. Breast ultrasound is a medical imaging technique that uses ultrasound waves (sound wave with frequency higher than 20 KHz) to produce detailed images of the internal structures of the breast. It is a non-invasive procedure that provides valuable information about breast tissue and is commonly used as a diagnostic tool in breast healthcare. The images produced by a breast ultrasound can help assess various aspects of the breast, such as the size, shape, and composition of lumps or masses[2].

Breast Imaging Reporting and Data System (BI-RADS) is a standardized reporting system developed by the American College of Radiology (ACR) to provide a consistent and uniform way of categorizing and reporting findings from breast imaging (mammograms, breast ultrasounds, and breast MRI scans)[3]. The purpose of BI-RADS is to improve communication between healthcare providers and to ensure a common language for describing breast imaging findings.

Sensitivity is a measure of how well a test can correctly identify individuals who have the condition or disease of interest and is used to explain the ability of clinical breast examination and breast ultrasound to correctly identify individuals with breast cancer. Sensitivity is mathematically calculated as (True Positives / (True Positives + False Negatives)) × 100. A high sensitivity value indicates that the test has a low rate of false negatives, meaning it can accurately identify a large proportion of individuals with the condition[4].

Specificity is a measure of how well a test can correctly identify individuals who do not have the condition or disease of interest. It represents the proportion of true negative results (correctly identified individuals without the condition) relative to the total

number of individuals who do not have the condition and explains the ability of either clinical breast examination or breast ultrasound to correctly identify individuals without breast cancer. Mathematically, specificity is calculated as (True Negatives / (True Negatives + False Positives)) × 100. A high specificity value indicates that the test has a low rate of false positives, meaning it can accurately identify individuals who do not have the condition[4].

The main objective of this study was to assess the accuracy of clinical breast examination and breast ultrasound in detecting breast cancer in Kigali referral hospitals. The specific objectives were to identify the sensitivity of clinical breast examination and breast ultrasound in detecting breast cancer using histopathology as gold standard for confirming breast cancer; evaluate the specificity of clinical breast examination and breast ultrasound in detecting breast cancer by referring to histopathological findings; and calculate the predictive value(s) of clinical breast examination and breast ultrasound in detecting breast cancer.

Without known accuracy of diagnostic method, healthcare providers may encounter uncertainty when interpreting examination results and making clinical decisions. This uncertainty can lead to potential misdiagnosis or delayed diagnosis of breast cancer, ultimately impacting patient outcomes and treatment efficacy[5,6]. Thus this study attempted to bridge the gap in knowledge on the accuracy of clinical breast examination and breast ultrasound in detecting breast cancer in our settings.

MATERIAL AND METHODS

This research was conducted in Kigali referral hospitals, including CHUK, RMH, and KFH. These hospitals were selected because they had established efficient methods for storing patient information in their Radiology Departments such as PACS. Clinical information was accessible through OpenClinic and NAPIER, facilitating the retrieval of patient data. Moreover, these hospitals received a substantial number of patients from various parts of the country, making the sample from these hospitals' representative of the study population.

Study Design and Area

This retrospective cross-sectional study with a quantitative approach was carried out on only female patients, irrespective of their age, who attended the Radiology Department of Kigali Referral Hospitals (CHUK, RMH, KFH) from March 2018 to March 2023. These women had undergone clinical breast examination and breast ultrasound and had histopathological results in hospital database.

Study Population and Time Frame

The study population consisted of women who had visited the Radiology Department at Kigali referral hospitals between March 2018 and March 2023. These women were identified as having benign breast condition or suspected to have breast cancer based on clinical examination and breast ultrasound, with BI-RADS scores of 2 up to 5 where BI-RADS format was utilized. These individuals underwent breast biopsy, and

their histopathological results were found in the hospital database.

We excluded cases of women who attended the Radiology Department at Kigali referral hospitals (CHUK, RMH, KFH) between March 2018 and March 2023 with breast condition but lacked histopathological findings in the electronic medical record system. Furthermore, patients who had already been diagnosed with breast cancer before visiting the Radiology Department were also excluded from the study.

Procedure

Patient data were retrieved from hospital electronic medical records after getting permission from Ethical Committees and Hospital Administrations. All relevant information from representative samples of women who met all the inclusion criteria, including demographic information, breast ultrasound findings, results from histopathology and clinical breast examination was recorded according to the checklist. Data collection adhered to ethical guidelines and patient confidentiality.

Total study population (N) was estimated to 1000. Sample size was calculated for this study using the Yamane formula with estimated total population of 1000 and a 5% margin of error is approximately 286.

Data Analysis

A descriptive statistical analysis was performed using cross-tabulation to obtain a number of true positive, true negative, false positive and false negative. The information obtained was used to calculate sensitivity, specificity, negative predictive value, and positive predictive value for CBE findings and breast ultrasound in accurately detecting breast cancer. In addition, inferential statistics of t-test was used to test the three hypotheses formulated at 0.05 level of significance.

RESULTS

The youngest age groups (under 19 and 20-30) had the lowest occurrences of breast cancer, while the older age groups showed a higher prevalence. The highest number of breast cancer cases was found in the age group of 41-50 years. Table 1 illustrates the relationship between age group and breast cancer prevalence. The data reveal a clear pattern of increased breast cancer prevalence with age.

The mean age of menarche was calculated as 14.71 years, with a median age of 15 years. The range of ages at menarche ranged from 11 to 18 years, illustrating the variability in the time for first menstrual period within the study select cases.

Breast lump or mass has a sensitivity of 95.24% indicating high ability to correctly identify individual with breast cancer, it has a specificity of 14.79% a. where Nipple discharge shows the highest specificity at 92.16% indicating that nipple discharge mostly found in breast cancer. Negative predictive value (NPV) measures the probability that individuals with a negative test result truly do not have breast cancer, with nipple discharge demonstrating the highest NPV at 71.43%. Positive predictive value (PPV) indicates the probability that individuals with a positive test result actually have breast cancer, where nipple discharge and retraction exhibit the highest PPV at 85.45% and 81.82%, respectively.

These metrics collectively highlight the varying diagnostic accuracies of different clinical features, emphasizing the importance of considering multiple factors in breast cancer diagnosis and screening protocols.

The analysis of Breast Clinical Examination (BCE) findings in relation to pathology conclusions reveals notable diagnostic performance of BCE. The sensitivity of BCE in detecting malignant neoplasms is approximately 70.8%, indicating its effectiveness in correctly identifying a majority of malignant cases. It also indicates the confidence in the ability of BCE, when it shows a positive result, to accurately identify patient who have a condition without incorrectly classifying those who actually have the condition as not having it.

On the other hand, the specificity is approximately 86.7%. Based on true negative rates, BCE can accurately identify patient who don't have breast cancer. The positive predictive value (PPV) of BCE is approximately 87.6 %, indicating confidence in a BCE capacity to accurately distinguish between those who have a breast cancer and those who do not, when it yields a positive result. The negative predictive value (NPV) is approximately 69.1%, demonstrating the reliability of a BCE to correctly identify individuals without a breast cancer when it shows a negative result.

Overall, the accuracy of BCE in classifying neoplasms is approximately 77.6%, highlighting its utility as a diagnostic tool in clinical practice.

Table 1: **Relationship between age group and breast cancer prevalence**

		Pathology results		
		Benign	**Malignant**	**Total**
Age	20-30	30	3	33
	31-40	49	16	65
	41-50	35	61	96
	51-60	14	14	28
	61-70	5	18	23
	above 70	9	19	28
	below 19	9	2	11
Total		151	133	286

Table 2: **Age at menarche distribution in study participants**

		Frequency	Percentage
Menarche at:	11.00	1	1.1
	12.00	10	10.6
	13.00	17	18.1
	14.00	10	10.6
	15.00	17	18.1
	16.00	29	30.9
	17.00	8	8.5
	18.00	2	2.1
	Total	94	100.0

Table 3: **Accuracy of breast clinical examination findings in detecting breast cancer**

BCE Feature	***Sensitivity%***	***Specificity%***	***PPV%***	***NPV%***
Breast Lump/mass	95.24	14.79	49.79	77.78
Breast Pain	47.47	71.67	58.02	62.32
Breast Tenderness	42.66	54.04	42.66	54.04
Nipple Discharge	92.16	55.56	85.45	71.43
Nipple Retraction	75.00	71.43	81.82	62.50
Axillary Lymph Nodes	64.44	65.63	66.46	63.64
Skin Change (ulceration)	42.52	51.39	42.52	51.39

Table 4: Comparison between breast clinical examination impression and histopathology conclusion

			Pathology		
			negative	positive	Total
BCE	Negative	Count	85	13	98
		%	86.7%	13.3%	100.0%
	Positive	Count	38	92	130
		%	29.2%	70.8%	100.0%
Total		Count	123	105	228

This study analyzed the echo-patterns of lesions identified by ultrasound. The majority (76, 53%) were hypoechoic, followed by heterogeneous (18, 12.4%) and anechoic (12, 8.4%). Notably, echo-pattern was not documented for 95 lesions. Malignancy was most frequent among hypoechoic lesions (53/129, 41.1%), followed by heterogeneous lesions (8/18, 44.4%). Conversely, all anechoic lesions were benign (12/12, 100%) Tables 5 and 6 show varied performance characteristics in distinguishing between benign and malignant pathologies. Notably, findings such as spiculated margins and internal vascularity exhibit high sensitivity (0.929 and 0.951, respectively), indicating their effectiveness in detecting true positives among malignant cases.

The analysis reveals that the sensitivity of breast ultrasound in detecting breast cancer is approximately 93.4%, indicating its high effectiveness in identifying most cases of breast cancer. Furthermore, the specificity is approximately 84.1%, suggesting that most benign cases are accurately identified. The positive predictive value (PPV) of breast ultrasound in detecting breast cancer is approximately 81.3%, indicating that when an ultrasound result is malignant, it is likely to be accurate. Conversely, the negative predictive value (NPV) is approximately 94.6%, demonstrating a high likelihood of correctness when an ultrasound result is benign. Overall, the accuracy of breast ultrasound in detecting breast cancer is approximately 88.1%, underscoring its reliability as a diagnostic tool in clinical practice.

Table 5: **Summary of Sonographic Features and Pathology Outcomes in Breast Imaging**

Sonographic Feature	***Present in***	***Benign***	***Malignant***
Micro-calcification	23	8	15
Calcification	21	12	9
Well-defined//circumscribed Margins	106	104	2
Spiculated Margins	63	11	52
Indistinct Margins	35	19	16
Vascularity	107	10	97
Architectural Distortion	43	5	38
Enlarged axillary Lymph nodes	71	28	43

Table 6: **Diagnostic accuracy of ultrasound findings in detecting breast cancer**

Ultrasound Finding	***Sensitivity***	***Specificity***	***PPV***	***NPV***
Micro-calcification	0.833	0.385	0.652	0.625
Calcification	0.429	0.368	0.429	0.368
Well-defined/circumscribed Margins	0.071	0.224	0.019	0.536
Spiculated Margins	0.929	0.857	0.825	0.943
Indistinct Margins	0.762	0.844	0.457	0.954
Internal Vascularity	0.951	0.655	0.907	0.791
Architectural Distortion	0.791	0.583	0.883	0.412
Ductal Dilatation	0.636	0.714	0.304	0.909
Enlarged axillary Lymph nodes	0.565	0.750	0.605	0.717

Table 7: **Relationship between echo pattern of neoplasm and histopathology conclusion**

		PATHOLOGY		
		Benign	**Malignant**	**Total**
ECHOPATTERN	Anechoic	12	0	12
	Heterogenous	10	8	18
	Hyperechoic	1	2	3
	Hypoechoic	76	53	129
	Isoechoic	6	0	6
	mixed (cystic and solid)	15	8	23
	Not documented	33	62	95
Total		153	133	286

Table 8: Correlation between breast ultrasound findings and histopathology results

			Pathology		
			Benign	**Malignant**	**Total**
Ultrasound	Benign	Count	122	7	129
		%	94.6%	5.4%	100.0%
	Malignancy	Count	23	100	123
		%	18.7%	81.3%	100.0%
Total		Count	145	107	252

Table 9 shows the independent t-test comparing the mean sensitivity and specificity values obtained from clinical breast examination among the 286 patients. The findings showed that the mean sensitivity value was slightly higher than the mean specificity value, but this difference was not statistically significant. The calculated t-value of 1.462 with a corresponding p-value of 0.145 indicated that the observed difference could have occurred by chance. Since the p-value was greater than the 0.05 significance level, the null hypothesis was retained. This indicates that sensitivity and specificity of clinical breast examination did not differ significantly in their influence on breast cancer detection in the sampled patients. The result implies that both measures contribute comparably to the diagnostic performance of the clinical examination in the studied referral hospitals.

Table 10 shows the independent t-test examining whether diagnostic sensitivity and specificity of breast ultrasonography differed in their influence on breast cancer detection among the 286 patients. The mean sensitivity score was slightly higher than the mean specificity score, indicating marginally better performance of sensitivity. However, the difference was not statistically significant, as shown by the t-value of 0.974 and a p-value of 0.331. Since the p-value exceeded the 0.05 threshold, the null hypothesis was retained. This finding suggests that both sensitivity and specificity contribute similarly to the diagnostic capability of breast ultrasonography in detecting breast cancer within the studied referral hospitals.

The independent t-test assessed whether the predictive values of clinical breast examination and breast ultrasound differed in their contribution to breast cancer detection among the 286 patients. Although the predictive value of breast ultrasound showed a slightly higher mean than that of clinical breast examination, the difference was not statistically significant. The obtained t-value of 1.248 and p-value of 0.213 indicate that the variation between the two diagnostic approaches could be due to chance. Since the p-value exceeded the 0.05 significance level, the null hypothesis was upheld. This suggests that both clinical breast examination and breast ultrasound demonstrate comparable predictive performance in detecting breast cancer within the studied referral hospitals.

Table 9: Independent sample t-test means for sensitivity and specificity of clinical breast examination

Variable	N	Mean	SD	Df	t-value	p-value	Decision
Sensitivity	286	0.78	0.12				
Specificity	286	0.75	0.14	284	1.462	0.145	NS

NS = Not Significant

Table 10: Independent sample t-test equality means for sensitivity and specificity of breast ultrasound

Variable	N	Mean	SD	Df	t-value	p-value	Decision
Ultrasound sensitivity	286	0.91	0.08				
Ultrasound Specificity	286	0.89	0.10	284	0.974	0.331	NS

NS = Not Significant

Table 11: Independent sample t-test equality means for predictive values of clinical breast examination and breast ultrasound

Variable	N	Mean	SD	Df	t-value	p-value	Decision
Predictive values of clinical breast examination	286	0.72	0.15	284	1.248	0.213	NS
Predictive values of clinical breast ultrasound	286	0.75	0.13				

NS = Not Significant

DISCUSSION

Out of the 286 participants, 133 had breast cancer while 153 had benign condition. By studying the relationship between age groups and prevalence of breast cancer, this revealed a pattern of increasing prevalence of breast cancer with age. This is consistent with another research conducted by Meredith et al.[7] In this study, breast cancer is more presented in age group of 41-50 years, this is consistent with study highlighted that women between the ages of 40 and 44 and 45 and 49 accounted for the majority of breast cancer cases (77.3%).[7] In addition, as reflected in Table 9 with t-value = 1.462, and p-value = 0.145, this implies that the observed difference could have occurred by chance. However, since the p-value was greater than the 0.05 significance level, sensitivity and specificity of clinical breast examination did not differ significantly in their influence on breast cancer detection in the sampled patients. Sensitivity and specificity contribute similarly to the diagnostic capability of breast ultrasonography in detecting breast cancer within the studied referral hospitals. Also, clinical breast examination and breast ultrasound demonstrate comparable predictive performance in detecting breast cancer within the studied referral hospitals. This study demonstrated a reliable accuracy of Breast Clinical Examination (BCE) in detecting breast cancer. The sensitivity of BCE in detecting malignant neoplasms is approximately 70.8%, indicating its effectiveness in correctly identifying a majority of malignant cases. On the other hand, the specificity is approximately 86.7%, suggesting that BCE can accurately identifying patient with non-malignant breast condition. The positive predictive value (PPV) of BCE for detecting malignant neoplasms is approximately 87.6%, emphasizing the high likelihood of accurate malignancy detection when BCE indicates a positive result. The negative predictive value (NPV) is approximately 69.1%, demonstrating the reliability of BCE in correctly identifying non-malignant cases. Overall, the accuracy of BCE in classifying neoplasms is approximately 77.6%, highlighting its utility as a diagnostic tool in clinical practice. These findings emphasize

the significance of BCE in guiding the diagnosis and management of breast neoplasms. Chen et al. reported a higher sensitivity of 100% and a higher specificity of 94.6% for CBE, implying that their examination process effectively detected all cases of carcinoma breast while maintaining a high level of specificity in correctly identifying non-malignant cases.[8]

While this study demonstrated respectable sensitivity and specificity values for CBE, there is still room for enhancement, particularly in reducing false positive and false negative rates. However, our results align closely with those reported in the other studies, further reinforcing the consistency and validity of clinical examination across different research contexts.

The positive predictive values (PPV) of various sonographic features in detecting breast cancer provide valuable insights in interpreting sonographic findings. Features with higher PPVs, such as spiculated margins (82.5%), internal vascularity (90.7%), and architectural distortion (88.3%), indicate a strong likelihood of malignancy when these features are present on ultrasound. These findings suggest that cases showing spiculated margins or significant internal vascularity are highly likely to be malignant and should prompt further investigation or biopsy. Conversely, features like well-defined/ circumscribed margins (1.9%) and ductal dilatation (30.4%) exhibit lower PPVs, indicating that presence of ductal dilatation and well circumscribed margins does not indicate presence of malignancy and highlighting the need for caution in interpreting these findings alone. These findings emphasize the relevance of specific sonographic features in predicting breast pathology outcomes, emphasizing the importance of detailed and standardized breast imaging protocol in clinical practice.

In this study, breast ultrasound exhibited a sensitivity of approximately 93.4% in detecting breast cancer, demonstrating its high effectiveness at identifying most cases of breast cancer. The specificity was approximately 84.1%, indicating that it is effective at correctly identifying a majority of benign cases based on a benign ultrasound result. The positive predictive value was approximately 81.3%, suggesting high accuracy when an ultrasound result is malignant. The negative predictive value was approximately 94.6%, indicating a high likelihood of correctness when an ultrasound result is benign. Overall, the accuracy of breast ultrasound in detecting breast cancer was approximately 88.1%, confirming its reliability as a diagnostic tool. A previous study[9] reported a higher sensitivity of 100% but a lower specificity of 80.4%. While their sensitivity suggests a perfect ability to detect malignant lesions, the lower specificity implies a higher rate of false positives, potentially leading to unnecessary interventions or anxiety for patients. Their PPV and NPV were 83.9% and 100%, respectively, indicating a slightly higher rate of false positives but perfect identification of benign cases.[9]

Another study[10], reported significantly lower sensitivity and specificity values compared to both our study and the aforementioned research. With a sensitivity of only 26% and a specificity of 58.2%, their ultrasound findings had limited accuracy in

distinguishing between malignant and non-malignant lesions. However, their NPV of 53.3% indicates a relatively high rate of correctly identifying benign cases, despite the low sensitivity.[10] Comparatively, we see that our study did well in all areas, like sensitivity, specificity, PPV, and NPV. This means that our research gives strong evidence for justifying the use breast ultrasound in detecting breast cancer in our settings. It is good at finding cancerous lumps accurately and also gives confidence in saying when lumps are not cancerous.

CONCLUSION

While mammography was not studied in this study due to its scarcity in our setting, our findings shed light on the reliability of CBE and breast ultrasound in this regard, demonstrating a sensitivity of 70.8% and specificity of 86.7% with overall accuracy of 77.6%, indicating effective identification of most breast cancer cases while reliably distinguishing benign cases. Additionally, breast ultrasound exhibited a high sensitivity, and specificity and overall accuracy, confirming its effectiveness in accurately detecting breast cancer and correctly identifying benign cases. These results highlight the potential of breast ultrasound as a reliable diagnostic tool, providing strong evidence for its utilization in hospitals, especially where mammography is not readily available.

RECOMMENDATIONS

Based on the findings presented, it is recommended to further integrate and refine breast clinical examination and ultrasound in routine diagnostic practices in hospitals and at primary health care level. The strengths of each modality, as highlighted in the study, can be leveraged to create a complementary diagnostic approach that maximizes sensitivity and specificity. It is recommended that:

i. There is a need to increase access to ultrasound services, especially in areas with limited availability.

ii. Integrating breast ultrasound services into primary healthcare settings to improve early detection and management of breast abnormalities, especially breast cancer.

iii. Collaboration of stakeholders with researchers in developing national ultrasound protocols for standardized and effective use of ultrasound in diagnosing breast cancer and other breast conditions.

iv. Invest in capacity building initiatives, such as training programs for healthcare workers in Clinical Breast Examination (CBE) and increasing the number of trained sonographers.

v. Encourage universities, like the University of Rwanda, to enhance hands-on training opportunities for breast ultrasound to ensure proficiency among healthcare professionals.

Implementing these recommendations can collectively advance the accuracy and efficiency of breast examinations, fostering early detection and improving overall patient care.

Ethical Approval and Consent to Participate

Patient confidentiality was strictly maintained throughout the study. Patient information was entirely reserved for the researchers and will be not be made public after publication. Ethical clearances were obtained from the ethical committees of study sites and University of Rwanda CMHS/IRB/404/2023 (College of Medicine and Health Sciences) prior to data collection: approval from KFH with reference number of KFH/2023/ 122/IRB, approval from CHUK ethical committee with reference number of EC/CHUK/185/2023

Conflict-of-Interest: Non-Declared

Source-of-Funding: Private

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ASSOCIATION BETWEEN FASTING BLOOD GLUCOSE AND LIPID PROFILE STATUS AMONG NEWLY-DIAGNOSED TYPE 2 DIABETICS OF THE THREE MAJOR ETHNIC GROUPS IN NIGERIA

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ABSTRACT

Background: Atherogenic dyslipidaemia and the risk of CVD in type 2 diabetics has been widely investigated. Few studies in Nigeria have been carried out among different ethnic groups to understand the pattern of dyslipidaemia and its link with hyperglycaemia in type 2 diabetics.

Aim: The present study aimed to determine the influence of hyperglycaemia on the lipid profile parameters in type 2 diabetes of the three major ethnic groups in Nigeria.

Materials and Methods: This was a cross-sectional study in which Blood glucose and lipid profile parameters were determined using standard laboratory tests. Ranges for TC < 200 mg/dl, TG < 150 mg/dl, LDL-C < 100 mg/dl, 51-60 mg/dl for females, and HDL-C 41- 60 mg/dl for males were considered dyslipidaemia. One-way analysis of variance (ANOVA) followed by Bonferroni multiple comparison test to evaluate the mean difference among the groups. Independent samples t-test was used to evaluate the mean difference in FBG and BMI with lipid profiles between groups. Analysis was with statistical significance set at <0.05.

Results: The FBS was associated with increased TC, TG and LDL-c (r = 0.483269, 0.623952 and 0.390193 respectively). Similarly, BMI was found to be associated with increased TC, TG and LDL-c (r=0.432284, 0.569792 and 0.339701 respectively) with p=0.000001) However, both FBS and BMI were associated with decreased serum HDL-c levels (r= -0.278993 and -0.247900 respectively) with p=0.000001. Correlation between FBG and serum

lipid profile parameters was observed. FBS levels were positively linked with TG, TC, and LDL-c but negatively associated with HDL-c.

Conclusion: A correlation between FBG and serum lipid profile parameters has been observed. FBS levels were positively linked with TG and TC, but negatively associated with HDL-C, the good cholesterol. It can be suggested that failure to control type 2 diabetes leads in dyslipidaemia. Thus, effective control of hyperglycaemia in type 2 diabetics and periodic check and treatment of dyslipidaemia can prevent associated adverse health outcomes.

INTRODUCTION

Type 2 diabetes (T2DM) is a severe global health issue that has reached alarming levels. Recently, more than half a billion (536.6million people) have been reported to have DM worldwide and about 14.2 million of them lived in Africa (IDF, 2021).[1] According to WHO (2015), Nigeria has the greatest number of people living with diabetes in Africa, with more than 1.5 million cases.[2] The occurrence of T2DM may be due to abnormalities in carbohydrate, protein and lipid metabolism mainly due to insulin resistance (in overweight and obese individuals) or insulin deficiency.[3] T2DM is often characterized by defects in lipids metabolism and dyslipidemia that trigger cardiovascular disease (CVD), coronary artery disease (CAD) and macrovascular-related complications.[4] Alteration of lipoproteins and lipid profile parameters in T2DM contributes to oxidative stress and formation of free radicals which damage the endothelial tissue and accelerate the progression of atherosclerosis in blood vessels and the subsequent CVD. It is estimated that, over 30 percent of patients admitted for CVD had T2DM in sub-Saharan Africa.[3, 4] The important risk factor, in the development of CVD in type 2 diabetics, is abnormalities of the lipid profile parameters and comorbidity with high blood pressure in old age.[5] The risk increases with increased blood levels of TC, TG, LDL-C, and decreased HDL-C.[6] Diabetes-related CVD are decreased by the normal lipid profiles especially HDL-c that circulate in the blood because of its role of removing bad cholesterol (LDL-C) from the blood. The decreased uptake of free fatty acids that circulate in the blood may be increased by TG hydrolysis owing to the lipase action. This may increase free fatty acids that play in favor of insulin resistance and atherogenic dyslipidemia.[7,8] Elevated remnant lipoprotein is correlated with high TG because it is composed of high cholesterol and TG.[9, 10] Extensive research on atherogenic dyslipidemia and the risk of CVD in type 2 diabetics has been carried out in developed countries. However, in Nigeria, few studies have been carried out to understand the pattern of dyslipidemia and its link with hyperglycemia in type 2 diabetics.[9,10] The aim of the present study was to determine the influence of hyperglycemia on the lipid profile parameters in type 2 diabetes. Thus, the regulation of dyslipidemia to reduce CVD-related mortality in diabetes could be further elucidated.

MATERIALS AND METHODS

Site of Study

The study participants were recruited from males and females with type 2 diabetes attending diabetic clinics at Specialist

Hospital Sokoto, Women and Children Hospital Sokoto, Maryam Abacha Hospital Sokoto, Sir Yahayya Memorial Hospital Birnin Kebbi and Kebbi Medical Centre. Apparently healthy individuals used as controls (age-, sex, ethnic- matched) were recruited within the metropolis.

Study Population

Nigeria as a multinational state is inhabited by more than 250 ethnic groups speaking over 500 distinct languages, all identifying with a wide variety of cultures. The study was conducted among the three major ethnic groups: the Hausa/Fulani, Yoruba and Igbo situated in the north, west, and east respectively, together comprising over 70% of the total population.

Study Design

The total of 300 participants recruited comprised of 100 participants each for Hausa, Igbo and Yoruba ethnic groups, aged 18-54 years. The total of 174 were Males and 126 were Females. The participants were divided into six groups (3 groups were type 2 diabetics and 3 groups were healthy control). Of the 174 male participants Hausa/Fulani has 56 males, Igbo has 64 males and Yoruba has 54 males. The remaining 44 participants, 36 participants and 46 participants were female for Hausa/Fulani, Igbo and Yoruba respectively. Ethnic backgrounds were identified through clinical records, informed consent form and questionnaire administered.

Ethical consideration and clearance

Ethical approvals were duly sought obtained from Kebbi State Ministry of Health and Sokoto State Ministry of Health with reference numbers: MOH/KSREC/VOL,1/56 and SMH/1580/V.IV respectively.

Informed consent

Informed consent for inclusion into the study was duly obtained from each participant using standard protocol prior to recruitment.

Instrument for data collection

Standard self-administered questionnaire was prepared and administered to all the study participants to obtain their socio-demographic characteristics including, gender, age, tribe, occupation, life style, family history of DM, history of DM, etc,.

Sample Size Determination

Calculation of sample size of the study, using Cochran formula (1977).[11]

$n=Z^2P(1-P)/d^2$

Where, n= desired sample size

P= Prevalence rate of diabetes in Nigeria = 7.0% = 0.07 (Michael *et al.*, 2024).[12]

Z= 95% confidence interval=1.96

d= degree of accuracy= 0.05

Therefore,

n=118, approximately 150

Inclusion Criteria

Newly diagnosed type 2 diabetics (with first diagnosis< 2 years), of both sexes (aged between 18 to 54 years old) with apparently non-existing complication and without any disease condition were included for this study. Age-, sex-, tribe- and BMI-matched healthy individuals who have given their informed consent served as controls.

Exclusion Criteria

Patients with other disease conditions such as hypertension, HIV/AIDS, tuberculosis, thyroid disorder and other apparently

diabetes-related complications were excluded from the study. Pregnant women were excluded. Other disease conditions such as hypertensive retinopathy, hypertensive nephropathy, and alcoholic neuropathy were also excluded from the study.

Sample Collection and Analytical Techniques

Blood pressure was measured using the Belsk digital blood pressure monitor (Northfield, IL 60093 USA). The body height and weight were also measured using appropriate instrument (Mechanical Brecknell HS-200M scale, UK). A systolic blood pressure of ≥ 140 mmHg and diastolic blood pressure of ≥ 90 mmHg were considered hypertensive.[13] Body mass index (BMI) was calculated using the equation: Weight in (Kg)/Height (M^2). Values of 20-25, <30 but > 25, >30 and <20 were considered Normal, overweight, obese and underweight respectively. Blood glucose was tested in capillary blood samples by glucose oxidase and peroxidase methods using Accu-Check Aviva (68305 Mannheim, Germany).[14] Four milliliters of venous blood were collected in a red top tube with a clot activator collected from each participant by venipuncture using a 21G needle. It was then subjected to centrifugation at 3000 rpm for five min and the obtained serum was used to test TG, TC, and HDL.[15] Humastar 80 Auto Analyzer (Human, Wiesbaden. Germany) was utilized to measure the concentration of TC, TG, and HDL in the sera. The Friedewald formula (modified) was used to determine LDL-C (mg/dl) = (Non-HDL-C × 90%) – (TG × 10%). The concentration of TC, TG, and HDL was measured at 546 nm.[16] The normal reference values were defined as: < 200 mg/dl for TC, < 150 mg/dl for TG, < 100 mg/dl for LDL-C. For, HDL-C, values considered were 41- 60 mg/dl for males, and 51-60 mg/dl for females.15 All reagents, controls, and calibrators were stored in a fridge at 2-8 °C.

Statistical Analysis

All statistical analyses were performed with IBM SPSS V1 (IBM corporation, Armonk, New York, US) and Microsoft 2010 based Excel (Microsoft, US). All data were presented as mean and standard deviation. One-way analysis of variance (ANOVA) followed by Bonferroni multiple comparison test to evaluate the mean difference of the data between the groups (control and type 2 diabetics). The correlations were investigated using Pearson's coefficient of correlation (r) between study variables. Analysis was done at the 95% confidence level and the statistical significance was considered when p-value <0.05.

RESULTS

Table 1 shows the Socio-demographic and clinical characteristics of the study participants. The total of 300 participants recruited for this study comprised of 174 (58%) Males and 126 (42%) Females. Of the 174 male participants, Hausa/Fulani has 56 (56%), Igbo has 64 (64%) and Yoruba has 54(54%); while females were 44(44%), 36(36%) and 46(46%) for Hausa/Fulani, Igbo and Yoruba respectively. Table 1 further presents the body mass index (BMI) of the study participants. The mean average BMI of the diabetics was higher compared to control subjects (F=2.741; p <0.05). No

statistically significant difference observed in mean systolic and diastolic blood pressure for diabetics of the ethnic groups compared to their corresponding control (F=0.127; p>0.05).

Table 2 shows the mean comparisons of FBG (mg/dl) across the groups. The mean values for FBG and lipid profile parameters of the study ethnic groups were nearly the same. The level in the diabetics group was significantly higher than those in the control group (p<0.0001). The mean comparisons of TC, TG and LDL-c across the groups were significantly higher in diabetics compared to their corresponding control groups (p<0.0001). However, the mean comparisons of HDL-c (mg/dl) across the groups were lower in diabetics of different ethnic groups compared to their corresponding control groups (F=6.015; p<0.05).

The FBS was associated with increased TC, TG and LDL-c (r = 0.483269, 0.623952 and 0.390193 respectively). Similarly, BMI was found to be associated with increased TC, TG and LDL-c (r=0.432284, 0.569792 and 0.339701 respectively) p=0.000001) Table 3. However, both FBS and BMI were associated with decrease in serum HDL-c (r= -0.278993 and -0.247900 respectively) p=0.000001(Table 3).

Table 1: Socio-demographic and clinical characteristics of the study participants

Groups		**Mean age (Years)**	**Gender Male N (%)/Female N (%)**	**BMI (Kg/m^2)**	**SBP (mmHg)**	**DPB (mm/Hg)**	**Hypertensive: no N(%)/yes N(%)**
All Participants (n=300)	T2DM (n=150)	55.04±6.64*†	87(58)/63(42)$^{\&}$	30.71±5.15*†	128.95±9.47	80.80±5.01	150(100)/0(0)
	Control(n=150)	45.97±7.58	87(58)/63(42)	24.15±4.18	114.06±7.08	79.24±4.57	150(100)/0(0)
Hausa/Fulani (n=100)	T2DM(n=50)	55.57±7.39*†	28(56)/22(44)	27.59±4,90	123.74±9.30	78.17±5.00	50(100)/0(0)
	Control(n=50)	42.27±8.11	28(56)/22(44)	23.21±4.62	115.18±7.78	69.78±3.80	50(100)/0(0)
Igbo (n=100)	T2DM(n=50)	55.04±6.34	32(64)/18(36)	32.6±4.60*†	129.37±8.36	72.64±4.19	50(100)/0(0)
	Control(n=50)	46.98±7.48	32(64)/18(36)	25.6±3.71	116.71±6.41	74.48±4.89	50(100)/0(0)
Yoruba (n=100)	T2DM(n=50)	54.51±6.18	27(54)/23(46)	29.81±5.50	131.74±8.42	78.57±4.60	50(100)/0(0)
	Control(n=50)	48.65±7.14	27(54)/23(46)	23.6±2.72	110.30±5.94	71.48±4.18	50(100)/0(0)
	F value	6.015	3.474	2.751	0.122	0.127	4.384
	P value	<0.05	>0.05	<0.05	>0,05	>0.05	>0.05

Values are mean ± Standard Deviation of the mean of age, SBP, DBP and BMI ,n= number of participants, T2DM= type 2 diabetics, %= percentage, Kg/m^2, kilogram per meter square; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; BP. Blood pressure, n, sample size; %, percentage,;†= comparison between patients and control, &= comparison within the group, and *=p≤0.05 was considered significant in the statistical analysis t-test.

Table 2: FBG and lipid profiles of the study population

Groups		FBG (mg/dL)	TC (mg/dL)	TG (mg/dL)	HDL-c (mg/dL)	LDL-c (mg/dL)
All Participants (n=300)	T2DM(n=150)	122.20±17.60**†	207.99±89.82**†	183.16±60.76**†	37.26±12.90	101.27±52.19*†
	Control(n=150)	93.26±8.68	161.68±27.64	137.14±24.18	46.64±11.15*†	90.16±28.14
Hausa/Fulani (n=100)	T2DM(n=50)	127.70±23.48**†	203.74±89.49**†	181.21±59.85**†	34.92±13.72	100.05±51.46*†
	Control(n=50)	92.80±8.95	159.71±27.70	132.91±18.89	46.61±11.23*†	93.51±30.30
Igbo (n=100)	T2DM(n=50)	118.12±10.62**†	216.51±90.35**†	189.41±63.40**†	39.72±12.37	104.82±53.98*†
	Control(n=50)	91.68±8.94	162.60±27.15	139.38±26.00	46.55±11.19*†	89.10±26.95
Yoruba (n=100)	T2DM(n=50)	120.80±14.98**†	203.71±90.29**†	178.87±0.67**†	37.15±12.68	98.94±51.67*†
	Control(n=50)	95.31±7.82	162.74±28.35	139.14±26.66	46.75±11.18*†	87.88±27.13
	F- value	17.384	17.474	14.083	6.015	9.815
	P-value	<0.01	<0.01	<0.01	<0.05	<0.05

Values are mean ± Standard Deviation of the mean of FBS, LP, lipid profile n, number of participants; %, percentage,; †= comparison between patients and control, *=p≤0.05 and **=p≤0.01 were considered significant in the statistical analysis t-test.

Table 3: Correlation between FBG and BMI with serum lipid profiles of the study population

Parameters		TC (mg/dL)	TG (mg/dL)	HDL-c (mg/dL)	LDL-c (mg/dL)
FBG (mmol/L)	r value	0.483269**	0.623952**	-0.278993**	0.390193**
	P value	0.000001	0.000001	0.000001	0.000001
Body Mass Index (kg/m^2)	r value	0.432284**	0.569792**	-0.247900**	0.339701**
	P value	0.000001	0.000001	0.000001	0.000001

**** Correlation is significant at the < 0.01 level (2-tailed).**

DISCUSSION

The current study involved three hundred (300) participants, hundred (100) from each of the three ethnic groups (Hausa/Fulani, Igbo and Yoruba). Each group has fifty (50) diabetics and fifty (50) control subjects, non-hypertensive with significant increased BMI in diabetic groups compared to their corresponding control groups ($p<0.05$). The mean values for FBG and lipid profile parameters of the study ethnic groups were nearly the same. The current study determined the mean FBG concentration in the diabetics of the study groups to be significantly higher compared to their corresponding control groups ($p< 0.01$). Significantly increased serum levels of TC and TG were observed for diabetic groups compared to their corresponding control groups (both $p<0.01$). Similarly, increased serum level of LDL-c and decreased serum level of HDL-c among diabetics of the study groups were observed compared to their corresponding control groups (both $p<0.05$). The glucose levels in this study groups are consistent with the glycaemic condition of type 2 diabetes as defined by ADA, (2022).[17] In type 2 diabetes, hyperglycaemia is the result, initially, of the inability of the body cells to fully respond to insulin (insulin resistance). With the onset of insulin resistance, the hormone is less effective which, in due course, prompts an increase in insulin production. Over time, inadequate production of insulin can develop as a result of failure of the pancreatic beta cells to keep up with demand leading to overt hyperglycaemia.[1] Hyperglycaemia may have many effects on the vascular endothelium which leads to the development of dyslipidemia.[18]

In the current study, a significant positive correlation between FBG and TC, TG and LDL-c (r=0.483269, 0.623952 and 0.390193 respectively) and a significant negative correlation (r= -0.278993) between FBG and HDL-c were observed. The hardening of the blood artery wall may be caused by the LDL-c because it accumulates LDL in the artery wall which narrows the blood vessel. This abnormality of LDL which is known as bad cholesterol may result from the

impairment of blood glucose.[18] These findings agree with those reported in several other studies providing further evidence that in poor glycemic control, the likelihood of a high concentration of LDL-C would be increased.[19] Therefore, blood glucose could be utilized to predict the level of LDL-C in diabetic patients because blood glucose can cause the defect of LDL which leads to the formation of plaque inside the artery.

A significant positive correlation was observed between the BMI and TC, TG, and LDL-c, but negative association with HDL-c. The same observation was reported by another study.[10] The association between BMI and lipid profile parameters may therefore be due to lifestyle changes. Several studies reported that increased in BMI accounted for lipid abnormality.[10] Similarly, this study showed a significant link between BMI and lipid profile status. It is evident that dyslipidemia is among the consequences of high BMI.[20] The levels of serum HDL-C were significantly lower in diabetics compared to controls. Contrasting results were reported in other studies.[21, 22] However, similar to this study, the levels of serum HDL-C were significantly lower in patients than in control.[22]

Diabetics may possess various lipid abnormalities predisposing to the threat of cardiovascular ailments. This condition arises owing to especially glucose catabolism, resulting in hyperglycemia and dyslipidemia.[23] Other studies have reported a non-significant weak positive correlation between FBG and TC in type 2 diabetics.[24, 25] Ultimately, the results imply that uncontrolled diabetes leads to defects in cholesterol metabolism. Thus, periodic check and treatment of dyslipidemia can prevent associated adverse health outcomes. Triglycerides transport adipose fat and blood glucose to and from the liver where they are produced.[26] Hypertriglyceridemia contributes to the plaque formation inside of the walls of blood vessels leading to their narrowing and preventing normal blood flow with increased risk of cardiovascular complications.[27] Several other studies reported a moderate positive and significant correlation between triglyceride and blood glucose.[28, 29] Therefore, the concentrations of blood glucose may be utilized in prognosticating the serum TGs and cardiovascular risk due to alteration of lipoprotein caused by hyperglycemia. Hardening of the blood artery wall may be caused by the LDL-c which accumulates LDL in the artery wall that narrows the blood vessel. The abnormality of LDL, a bad cholesterol, may results from the impairment of blood glucose.[28] Several other studies provided further evidence that in poor glycemic control, the LDL-c increases.[9, 10, 30] Therefore, blood glucose could be utilized to predict the level of LDL-c in diabetics because blood glucose may lead to defect in LDL which results in the formation of plaque inside the artery.

CONCLUSION

A correlation between FBG and serum lipid profile parameters has been observed. FBS levels were positively linked with TG and TC, but negatively associated with HDL-C, the good cholesterol. It can be suggested that failure to control type 2 diabetes leads in dyslipidemia. Thus, effective control of

hyperglycemia in type 2 diabetics and periodic check and treatment of dyslipidemia can prevent associated adverse health outcomes.

Limitation the study

Standard biochemistry procedures were employed in this study to quantify TC, HDL-c, TG, LDL-c, and FBG. The present results, therefore, are correct and valid. Study patients in the present research were from the three major ethnic groups in Nigeria. The present results therefore may serve as Nigerian estimates even though, there are more 250 ethnic groups in Nigeria.

ACKNOWLEDGEMENTS

The authors thank Sir Yahaya Hospital, Birnin Kebbi and Specialist Hospital Sokoto for giving us an opportunity to conduct this study. Special thanks to the Clinicians/Nurses for the help in recording patient clinical information.

Financial support and sponsorship: Nil.

Conflicts of interest: There are no conflicts of interest.

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COMPARISON OF LIQUID BACTEC MGIT 960 AND SOLID LOWENSTEIN-JENSEN CULTURE FOR DETECTION OF *Mycobacterium tuberculosis* IN CLINICAL SAMPLES AT ANAMBRA STATE, NIGERIA.

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ABSTRACT

Background: Pulmonary tuberculosis is a major public health problem. Rapid and accurate detection of *Mycobacterium tuberculosis* (*M.tb*) and its drug resistance is fundamental for effective control.

Aim: This study evaluated the diagnostic performance, agreement and drug susceptibility testing (DST) using MGIT 960 compared with LJ culture of 530 clinical samples for detection of *Mycobacterium tuberculosis*.

Materials and Methods: A cross-sectional study was conducted at NAUTH, GHO and COOUTH in Anambra state. Sputum samples of suspected TB participants were collected and cultured using liquid MGIT-960 and solid LJ media. Drug sensitivity testing of positive *Mycobacterium tuberculosis* isolates against Streptomycin, Isoniazid, Rifampicin and Ethambutol, was performed. Data was analyzed using Epi-info.

Results: MGIT-960 detected 428/530 (80.7%) positive *M.tb*, 62 (11.7%) negatives, 36 (6.8%) contaminated, and 4 (0.8%) NTM with detection time 11 (6) days compared to LJ that detected

411/530 (77.5%) positive *M.tb*, 92 (17.4%) negatives, 22 (4.2%) contaminated and 5 (0.9%) NTM with detection time 30 (11) days, 456(86.0%)were concordant positive *M.tb* isolates. Assuming LJ as the gold standard, MGIT 960 showed a sensitivity of 94.6%, specificity of 63.2%, positive predictive value of 90.9%, negative predictive value of 75.3%, and accuracy of 86.0%. DST was performed on 389 culture-positive samples. Concordance of resistance between MGIT and LJ varied by drug, Streptomycin 73.8%, Isoniazid 81.8%, Rifampicin 71.4%, and Ethambutol 71.1%. Total prevalence of MDR-TB 20.8%, Concordance MDR-TB for both method 35.8% and discordant MDR-TB 64.2%, MDR-TB 6.4% by MGIT-960 and 6.9% by LJ while Susceptibility was high for all drugs.

Conclusion: MGIT 960 demonstrated high diagnostic yield for *M.tb*, and identified additional resistant isolates not detected by solid LJ medium. Combined use of both methods improves accurate diagnosis of TB, drug resistance, reduce contamination and guide effective treatment strategies.

Keywords*: Mycobacterium tuberculosis*, Liquid BACTEC MGIT 960 system, Solid Lowenstein-Jensen medium, Accuracy, Drug susceptibility testing.

INTRODUCTION

Tuberculosis (TB), caused by *Mycobacterium tuberculosis* (*M.tb*) remains a significant global health burden, particularly in low- and middle-income countries causing morbidity and mortality, it is estimated to have caused disease in 10.6 million new cases and 1.3 million deaths were reported in 2022[1]. Despite global efforts toward TB control, case finding, HIV, multidrug-resistant TB (MDR-TB) remains a significant challenge in diagnosis, treatment and public health surveillance[2]. The main problem is that, clinical specimen often contains very few mycobacterium bacilli, in which the slow growth rate limits detection using conventional method. Multidrug-resistant TB is the most challenging forms of resistance because treatment with the second-line drugs is more toxic, more expensive and must be administered for a longer period of time than standard first-line drug therapy[1,3] . World Health Organization**,** reported Nigeria among one of the 30 high TB, multidrug-resistant TB (MDR-TB) and TB/HIV co-infection burden countries[1]. The estimated prevalence rate of multidrug-resistant TB in Nigeria is 4.3% of new tuberculosis cases and 25% of previously treated TB cases, though the rate varies according to countries or regions, based on various susceptibility testing methods available especially for first-line anti-TB drugs[2,3]. A study within Southeast Nigeria, reported 8% MDR-TB in cultured sputum of 180 patients[4]. In developed countries, TB diagnosis depends on culture of sputum in liquid and solid

media while in developing countries diagnosis depends only on microscopic examination of sputum stained for acid-fast bacilli (AFB), though used as international standard for TB diagnosis but the emergence of the HIV and multidrug-resistant TB epidemics uncovered its limitations since HIV-infected TB patients frequently have negative sputum smears[5].

Hence, timely diagnosis and reliable drug susceptibility testing (DST) is crucial for guiding appropriate treatment and limit the spread of resistant strains particularly in region like Southeast Nigeria where diagnostic delays and treatment failures contribute to ongoing transmission[4,6]. Rapid molecular assays like Gene Xpert MTB/RIF have improved early detection but the recommended method for DST remain the gold standard for definitive diagnosis of *M. tuberculosis* performed on solid Lowenstein-Jensen (LJ) culture, an egg-based medium that supports *M. tuberculosis* growth, allows for visual observation of colony morphology, isolation of viable organisms, phenotypic drug testing and ease of preparation[7]. However, limitation due to slow growth of colonies for result interpretation makes the agar proportion LJ method take long incubation time 4–8 weeks, thus causes delay DST results that can hinder timely initiation of appropriate therapy, its lower sensitivity especially in paucibacillary or early-stage cases and contamination risks if media are improperly prepared or stored, influence their utility in resource-limited settings[7,8]. While solid culture methods are more common due to cost constraints, there is growing interest in transitioning to liquid culture for faster and more accurate diagnosis. WHO recommended the use of liquid culture systems like Mycobacteria Growth Indicator Tube 960 (MGIT 960 system) media for TB diagnosis and DST where feasible, especially in reference or tertiary laboratories, though most secondary and tertiary hospitals rely on ZN microscopy [9,10]. The BACTEC MGIT 960 system has been evaluated for the detection of resistance to first-line anti-TB drugs against the standard proportion solid LJ method and has shown good concordance, a sensitivity of 100% for Rifampicin (RIF) and Isoniazid (INH), specificity ranging from 89% to 100% [11,12].

Nevertheless, comparing liquid MGIT 960 and solid LJ culture methods for DST of *Mycobacterium tuberculosis* is crucial to guide diagnostic optimization, inform national TB control strategies on whether to replace one method for the other or to run the two in parallel to improve patient outcomes especially in resource-limited settings like Anambra state, Nigeria. This study compared diagnostic yield, time to detection, contamination rate, and DST accuracy using liquid MGIT 960 and solid LJ culture media for detection of *Mycobacterium tuberculosis* from sputum specimen at secondary and tertiary health care facilities in Anambra state.

MATERIAL AND METHODS

Study design: This was a cross-sectional study, conducted at Nnamdi Azikiwe University Teaching Hospital (NAUTH) Nnewi, General Hospital Onitsha (GHO) and Chukwuemeka Odimegwu Ojukwu University Teaching Hospital (COOUTH)

Awka in Anambra state, Nigeria. The aim was to compare the isolation rate of *Mycobacterium tuberculosis* from sputum and the level of agreement of DST to first-line anti-TB drugs using BACTEC-MGIT-960 and solid LJ culture methods

Ethical approval: The study was ethically cleared by Institutional Ethics Committee of Nnamdi Azikiwe University Teaching Hospital Nnewi (reference number: NAUTH/CS/66/VOL.3/35). Information on the purpose of the study was provided to all the participants. The confidentiality of data and freedom to withdraw from the study at any time were assured to participants. All individual participants provided written consent to participate, following the procedures approved by the ethics committees. Specimen were collected after getting written informed consent from all the study participants.

Specimen collection: A total of 530 sputum specimen of 318 males and 212 females within the age of 2 to 80 years old were collected from both new suspected TB cases and those who had failed first-line anti-TB treatment were enrolled. Patients unable to produce sputum or did not provide informed consent to participate, smear-negative cases and specimen with poor quality sputum or saliva or insufficient quantity for both cultures were excluded.

Based on the assumption that solid Lowenstein-Jensen culture is the gold standard allows calculation of diagnostic accuracy, the sample sizes were calculated to assess the prevalence of drug resistance in new and retreatment patients using Lawson method, assuming that 10% of newly diagnosed patients and 40% of the patients failing first-line treatment would be resistant to one or more of the first-line drugs tested[13]. Concordance, discordance, and diagnostic accuracy of MGIT 960 were evaluated, accounting for contaminated and non-tuberculosis mycobacteria (NTM) results.

Sample processing: Three sputum specimen were collected from each patient with their bio-data and submitted for direct Ziehl Neelsen (ZN) smear microscopy[4] . All muco-purulent sputum specimen were sent for culture and DST at Zankli Medical Research Laboratory (ZMRL) at Abuja within seven days of collection. Samples were refrigerated prior to shipment and maintained in cold-chain during shipment with ice packs. At ZMRL, each specimen was decontaminated using Petroff's method (4% NAOH) and inoculated onto Lowenstein-Jensen medium and on an automated MGIT-960 system[13]. Two hundred microliters of decontaminated sputum were inoculated on each of two glycerol enriched solid LJ slopes and 500µl in MGIT-960 7H9 bottles. The MGIT 960 was enriched with supplement (OADC) and antibiotics (PANTA) prior to inoculation. Cultures were incubated at 37°C for up to 8 weeks on LJ and 42 days in MGIT 960 media. Solid LJ cultures were checked weekly and MGIT 960 cultures were monitored continuously through the automated system as direction from manufacturers[12]. Isolates were confirmed as *M.tb* by ZN smear, also isolates were observed by serpentine cords typical of *M.tb*, with the nitrate reduction test and temperature liability of the catalase test[4,13]. These were the only available tests in the

laboratory at the time of the study. The time for *M.tb* detection on solid LJ and MGIT 960 media were recorded. MGIT time to detection was defined as the interval between inoculation and the bottle being flagged as positive by the machine while LJ time to detection was defined as the time between inoculation and the culture being considered positive by naked eye reading[8].

Antibiotic susceptibility testing: Drug sensitivity testing for Streptomycin (STR), Isoniazid (INH), Rifampicin (RIF) and Ethambutol (EMB) was performed on positive *M.tb* isolates using the proportion method on solid LJ medium according to the Clinical and Laboratory Standard Institute (CLSI) procedures and recommended critical concentrations[14] and in MGIT 960 bottles according to the manufacturers recommendations[4]. Drug concentrations in LJ were 8.0 µg/ml for STR, 0.2 µg/ml for INH, 40 µg/ml for RIF and 2.0 µg/ml for EMB. While MGIT 960 were 1.0 µg/ml for STR, 0.1 µg/ml for INH, 1.0 µg/ml for RIF and 1.0 µg/ml for EMB[4,13]. Concisely, 1.0 McFarland standard isolate suspension was serially diluted 10-fold, from 10^{-1} to 10^{-4}, in sterile distilled water, inoculated onto L-J slants with and without drugs, and incubated at 37°C[15]. Results were read at 28 days and up to 42 days, depending on control growth. An isolate was considered resistant to a given drug when growth of 1% or more above the control was observed in drug-containing medium[8]. A susceptible *M. tuberculosis* H37Rv reference strain and a known ZMRL determined MDR -*M. tuberculosis* isolates (resistant to STR, INH, RIF, and EMB), were used for quality control[13]. Zankli Medical Research Laboratory undergoes external quality assurance of DST for first-line anti-TB drugs through the WHO Supranational reference laboratory network (SRLN) for MGIT 960 and solid LJ media. The external quality assurance (EQA) of the laboratory results was 100% for STR, INH, RIF and EMB when using MGIT 960; 100% for STR, RIF and EMB and 80% for INH when using solid LJ media[4,13].

Data analysis: All data were analyzed using Epi-info, proportions were compared using Chi squared tests. The degree agreement of the DST results obtained using both methods were compared using Cohen's Kappa (K) statistics [8,16]

RESULTS

Socio-demographic characteristics of the study participants:

A total of 530 sputum samples were collected from suspected TB participants, aged 2 to 80 years with a mean age of 34 ± 15 years or a median of 32 years. They were examined using two cultured methods, Liquid BACTEC MGIT 960 and solid Lowenstein-Jensen media. The majority of TB cases were within the age group 25–44years (52.8%), indicating that tuberculosis predominantly affected the economically active population. The male-to-female ratio was 1·5:1, with 318 (60%) males and 212 (40%) females, suggesting that males were more affected than females, possibly due to greater exposure risks and health-seeking behavior differences (Table 1).

Culture results on liquid MGIT-960 and Solid LJ Media

Of the 530 sputum samples cultured MGIT-960 system detected 428 (80.7%) positive *M.tb*, 62 (11.7%) negatives, 36 (6.8%) contaminated, and 4 (0.8%) NTM with detection time of 11 (6) days. While the solid LJ medium detected 411 (77.5%) positive *M.tb*, 92 (17.4%) negatives, 22 (4.2%) contaminated, and 5 (0.9%) NTM, with detection time of 30 (11) days. MGIT 960 detected 17 (3.2%) more positive *M.tb* cases and 14(2.6%) more contaminants compared to solid LJ alone. Solid LJ detected 30 (5.7%) more negative *M.tb* cases and less contaminated compared to MGIT 960 alone. The culture diagnostic yield of both methods showed that, of 530 sputum samples, 456 (86.0%) were positive for *M. tuberculosis*, 57 (10.8%) negatives, 14 (2.6%) contaminated and 3 (0.6%) NTM. Using both methods together increased detection of 28 (5.3%) *M.tb* by MGIT-960 alone and detection of 45(8.5%) *M.tb* by solid LJ alone. Also combined culture detected 57(10.8%) negative *M.tb,* minimizing false negative results, 14(2.6%) Contaminants restraining Contamination rate (Table 2a).

.**Table 1: AGE AND SEX DISTRIBUTION OF STUDIED PARTICIPANTS**

AGE GROUP	**GENDER**		
Years	**Male (%)**	**Female (%)**	**Total (%)**
0- 24	22 (4.1%)	18(3.4%)	40(7.5%)
25- 44	190 (35.8%)	90(17.0%)	280(52.8%)
45-64	60 (11.3%)	60(11.3%)	120(22.6%)
65- 80	46(8.8%)	44 (8.3%)	90(17.0%)
Total	318 (60%)	212 (40%)	530(100%)

Table 2a: **FREQENCY OF ISOLATION OF *Mycobacterium tuberculosis*** ON **LIQUID MGIT-960 AND SOLID LJ**

Culture result	MGIT 960 %	LJ %	MGIT960/LJ%
Positive *M.tb*	428 (80.7%)	411(77.5%)	456 (86.0%)
Negative *M.tb*	62(11.7%)	92(17.4%)	57(10.8%)
Contaminant	36(6.8%)	22(4.2%)	14(2.6%)
NTM	4(0.8%)	5 (0.9%)	3(0.6%)
Total	530	530	530 (100%)

Comparison of BACTEC-MGIT-960 and LJ, ** p = 0.057. ***($\chi^2 = 3.63, p = 0.057$)***

M.tb-Mycobacterium tuberculosis. NTM-Non tuberculosis mycobacteria. MGIT-960-Mycobacteria Growth Indicator Tube-960. LJ- Lowenstein–Jensen. %-Percentage.

Culture results of BACTEC MGIT 960 and solid LJ methods by individual hospitals:

Out of 240 (45.3%) sputum samples from NAUTH, MGIT 960 detected 183 (34.5%) positive *M.tb* ,37 (7.0%) negatives, 2(0.4%)NTM and 18(3.4%) contaminants while solid LJ detected 176 (33.2%) positive *M.tb* , 50(9.4%) negative, 3(0.6%) NTM and 11(2.1%) contaminants. GHO identified 200 (37.7%) sputum samples cultured, MGIT 960 detected 160(30.2%) positive *M.tb*, 23(4.3%) negatives, 2(0.4%) NTM and 15(2.8%) contaminants while solid LJ detected 156(29.4%) positive *M.tb*, 35 (6.6%) negatives, 2(0.4%) NTM and 7 (1.3%) contaminants. COOUTH identified 90(17.0%) sputum samples, MGIT 960 detected 85(16.0%) positive *M.tb*, 2(0.4%) negatives, 0(0%) NTM and 3(0.6%) contaminants, whereas solid LJ detected 79(14.9*%)* positive *M.tb* , 7(1.3%) negatives, 0(0%) NTM and 4 (0.8%) contaminants, (Table 2b).

Concordant and discordant positive *Mycobacterium tuberculosis* isolates by MGIT960 and LJ method

In comparison of culture results, only patients with positive results for both culture methods were included. The BACTEC-MGIT-960 and solid LJ culture results were concordant in 456 (86.0%) samples, with substantial agreement between the methods (Kappa = 0.70, Standard error = 0.046)[16], confirming that both methods perform consistently under standardized laboratory conditions. The distribution of agreement included both cultures being positive for *M. tuberculosis* in 389 samples, 50 negatives, 13 contaminated and 4 NTM samples. The high proportion of concordant positive cultures reflects the overall reliability and comparable efficiency of both MGIT-960 systems and LJ media in detecting *M. tuberculosis*. In comparison, discordant or discrepant results showed 74 (14.0%) samples for both two cultures method.

Among these, 12 samples were positive on LJ but negative on MGIT-960, while 30samples were negative on LJ but positive on MGIT-960. 10 samples were positive on LJ but contaminated on MGIT 960 and 12 samples were negative on LJ but contaminated on MGIT 960. Also 9 were contaminated on LJ but positive on MGIT 960 and one NTM sample on LJ but contaminated on MGIT 960. The largest proportion of additional 30 cases were LJ culture negative and BACTEC-MGIT-960 culture positive. This high difference in LJ-negative/ MGIT- 960 positive cases demonstrated the greater sensitivity of the MGIT-960 system and both methods resulted in an additional yield of cultures, likely due to the enhanced nutrient composition and automated growth detection in the liquid medium. Conversely, the few 12 LJ-positive/MGIT-negative samples may reflect, slow-growing or low bacterial load specimens not detected within the MGIT incubation period. Similarly, a total of 58 cultures were contaminated, 22 samples by LJ method and 36 samples by MGIT-960 method while only 13 samples were contaminated by both methods. (Table 3).

Table 2b. CULTURE RESULT OF BACTEC MGIT 960 AND LJ MEDIA BY STUDIED SITES

	Hospitals							
	NAUTH		GHO		COOUTH		Total %	
Result	MGIT960 %	LJ %	MGIT960%	LJ %	MGIT960%	LJ%	TMGIT960%	TLJ%
Positive	183(34.5%),	176(33.2%)	160(30.2%),	156(29.4%)	85(16.0%),	79(14.9%)	428(80.7%)	411(77.5%).
Negative	37 (7.0%),	50(9.4%)	23(4.3%)	35(6.6%)	2 (0.4%)	7(1.3%)	62(11.7%)	92(17.4%).
NTM	2 (0.4%)	3(0.6%)	2(0.4%)	2 (0.4%)	0 (0%)	0(0%)	4(0.8%)	5 (0.9%).
Cont	18(3.4%)	11(2.1%)	15(2.8%)	7(1.3%)	3 (0.6%)	4(0.8%)	36 (6.8%)	22(4.2%).
Total	240(45.3%)	240 (45.3%)	200(37.7%)	200(37.7%)	90(17.0%)	90(17.0%)	530 (100%)	530(100%)

NTM=Non-tuberculosis mycobacteria. Cont=contaminant. T=Total *

Table 3a: CONCORDANT AND DISCORDANT POSITIVE *Mycobacterium tuberculosis* ISOLATES BY MGIT 960 AND SOLID LJ CULTURE METHODS

	LJ				
MGIT 960	**Positive**	**Negative**	**Contaminated**	**NTM**	**Total**
Positive.	389	30	9	0	428
Negative.	12	50	0	0	62
Contaminated.	10	12	13	1	36
NTM.	0	0	0	4	4
Total	411	92	22	5	530

Concordant results (agreement)**:** 456/530 (86.0%). K = 0.70. Discordant results (disagreement)**:** 74/530 (14.0%)

Table 3b: COMPARISON OF CONCORDANT AND DISCORDAND RESULTS

Pattern of Concordant	**Results**	**%**	**Pattern of discordant**	**Result**	**%**	**Total sample**
Both positive *M.tb*	389	73.4	MGIT positive/LJ negative	30	5.7	
Both negative *M.tb*	50	9.4	MGIT negative/LJ positive	12	2.3	
Both contaminated	13	2.9	MGIT positive/LJ contaminated	9	1.7	
Both NTM	4	0.7	MGIT contaminated/LJ positive	10	1.9	
--	--	--	MGIT contaminated/LJ negative	12	2.3	
--	--	--	MGIT contaminated/LJ NTM	1	0.1	
Total concordant	456	86.0%	Total discordant	74	14.0%	530

Diagnostic performance of liquid MGIT 960 and solid LJ culture:

The diagnostic accuracy of liquid BACTEC MGIT 960 culture was compared to solid Löwenstein–Jensen (LJ) culture as the gold standard, BACTEC MGIT 960 demonstrated a sensitivity of 94.6% *(*95% CI: 91.7–96.3*),* but the two methods were complementary rather than interchangeable ***(χ^2 = 3.63, p = 0.057)***. The specificity was 63.2% *(*95% CI: 58.3–75.1*)* and overall diagnostic accuracy of 86.0% *(*95% CI: 85.4–91.0*)*. The positive predictive value was 90.9% (95%CL:87.8-93.3) and negative predictive value 75.3% (95%CL:68.6-84.7) (Table 4).

Table 4: **DIAGNOSTIC PERFORMANCE OF LIQUID MGIT 960 AND SOLID LJ CULTURE WITH 95% CONFIDENCE INTERVAL (CL)**

Measure	Formula	Calculation	Estimate	%	95%CL
Sensitivity	TP/TP + FN	389/411	0.946	94.6%	91.7-96.3
Specificity	TN/TN + FP	67/106	0.632	63.2%	58.3-75.1
PPV	TP/TP + FP	389/428	0.909	90.9%	87.8-93.3
NPV	TN/TN + FN	67/89	0.753	75.3%	68.6-84.7
Accuracy	TP + TN/TP + FP + FN + TN	456 /530	0.860	86.0%	85.4-91.0

($\chi^2 = 3.63$,, $p = 0.057$) .PPV- positive predictive value; NPV- negative predictive value; CI, confidence interval., TP- True positive, TN- True negative, FP- False positive. FN- False negative

Drug Susceptibilty Test

DST was successfully performed on 389 concordant culture-positive *M. tuberculosis* isolates recovered from both MGIT-960 and LJ medium. The DST results demonstrated variable levels of resistance to first-line anti-tuberculosis drugs, including Streptomycin, Isoniazid, Rifampicin, and Ethambutol. Patterns of drug resistance showed that, resistance was most frequently observed in Ethambutol (32.9%), followed by Isoniazid (28.3%), Streptomycin (27.5%), and Rifampicin (14.4%). The highest proportion of resistance detected in both methods was in Ethambutol (23.4%). Mono-resistance detected only by one method varied slightly, and MGIT-960 detected more unique resistant isolates for Streptomycin 20(5.1%) and Rifampicin 11(2.8%) than LJ culture while LJ detected more unique resistant isolates for Isoniazid 15(3.9%) and Ethambutol 20 (5.1%) than MGIT 960. Among the 389 concordant *M. tuberculosis* isolates, 81 isolates were classified multidrug-resistant tuberculosis (MDR-TB), defined as resistance to at least Isoniazid and Rifampicin, highlighted a total prevalence rate of 20.8%. Of these, 29 (7.5%) were MDR-TB by both MGIT-960 and LJ methods, 27 (6.9%) MDR-TB by LJ culture and 25 (6.4%) by MGIT-960 only. Specific concordance for individual drugs showed, 81.8% Isoniazid, 73.8% Streptomycin, 71.4% Rifampicin and 71.1% Ethambutol resistant isolates detected by both MGIT960 and LJ cultures. Ethambutol showed slightly lower concordance compared with Isoniazid and concordance varies by drug. Concordance of MDR-TB detected 29/81 (35.8%), while discordant of MDR-TB detected the remaining 52/81(64.2%) by only one method (27 LJ, or 25 MGIT 960 only). The remaining 308 (79.2%) isolates were fully susceptible concordance to all four first-line drugs and Cohen's Kappa indicated good agreement (K = 0.70) as summarized on (Table 5).

Table 5. **DST AGREEMENT OF CONCORDANT POSITIVE SAMPLES ON LIQUID MGIT 960 AND SOLID LJ METHODS.**

	RESISTANT PATTERN						
Drugs	RLJ (%)	RMGIT960 (%)	RLJ/MGIT960 (%)	TRLJ/MGIT960 (%)	CRLJMGIT (%)	TSLJMGIT (%)	TTESTED
STR	8(2.1%)	20(5.1%)	79 (20.3%)	107(27.5%)	73.8	282 (72.5%)	389
INH	15(3.9%)	5(1.3%)	90 (23.1%)	110(28.3%)	81.8	279(71.7%)	389
RIF	5 (1.3%)	11(2.8%)	40 (10.3%)	56(14.4%)	71.4	333(85.6%)	389
EMB	20 (5.1%)	17(4.4%)	91(23.4%)	128(32.9%)	71.1	261(67.1%)	389
MDR-TB	27(6.9%)	25(6.4%)	29(7.5%)	81(20.8%)	35.8	308(79.2%)	389

NB; Total MDR-TB prevalence = 81/389 = 20.8%, Concordance of MDR-TB resistance = 29/81 = 35.8%, Discordant MDR-TB = 27 (LJ-only) + 25 (MGIT-only) = 52/81 = 64.2%.
RLJ =Resistant LJ, RMGIT=Resistant MGIT, RLJ/MGIT=Resistant LJ/MGIT, TRLJMGIT=Total resistant LJ/MGIT, CRLJ/MGIT- Concordance resistance LJ/MGIT, TSLG/MGIT, Total susceptible LJ/MGIT, TTested =Total tested, STR=Streptomycin, INH=Isoniazid, RIF=Rifampicin, EMB= Ethambutol.

DISCUSSION

Proper diagnosis and early treatment of tuberculosis provide easy cure for patients, thus reduce the spread of TB. This study compared automated Liquid BACTEC MGIT 960 system to solid LJ culture for isolation of *Mycobacterium tuberculosis* in clinically suspected pulmonary tuberculosis cases and the results demonstrated a substantial correlation between the two culture systems in detecting *Mycobacterium tuberculosis*. Out of 530 sputum samples cultured, 86.0% samples showed concordant results, indicated substantial agreement using both MGIT-960 and solid LJ culture methods (Kappa = 0.70, SE = 0.046), suggested that both methods are dependable for routine mycobacterial culture. The majority of TB cases 52,8% were within 25–44 years age group, showed that tuberculosis predominantly affects the economically active population. The male-to-female ratio was 1.5:1, suggested that males were more affected than females, possibly due to greater exposure risks and health-seeking behavior differences. The MGIT-960 method demonstrated a slightly higher positive detection rate reflecting its enhanced sensitivity 80.7% and faster detection time 11 (6) days, compared to 77.5% detection rate by solid LJ culture with longer detecting time 30 (11) days. The mean time to detection was shorter with liquid culture and its monitoring can be automated to facilitate large number of specimens, but implementation challenges in

resource-constrained settings require a stable and sustainable diagnostic approach. This is consistent with findings from similar studies that highlighted the superior recovery, 76%, 81.8%, 94% of *Mycobacterium tuberculosis* and shorter detection time of liquid MGIT 960 culture systems, demonstrating its advantage in rapid diagnosis [10,11,24]. The slightly higher contamination rate 6.8% was observed in MGIT-960 compared to LJ culture with lower contamination rate of 4.2%. A finding reported in previous study, emphasizes the need for improved specimen processing, strict adherence to decontamination protocols when using liquid media, though the contamination rate in MGIT 960 system was within acceptable limits and may reflect its increased nutrient-rich environment which can support growth of contaminants but regular quality assurance measures are recommended to minimize contamination in liquid culture systems. Thus, MGIT 960 detected 3.2% more positive *M.tb* cases and 2.6% more contaminants cases while solid LJ detected 5.7% more negative *M.tb* cases compared to MGIT 960 alone. However, false positives or negative due to poor sample quality and technical errors can affect the sensitivity, thus the need for dual testing in high-priority cases. However, LJ culture remains valuable due to its lower cost, reduced maintenance demands, and is more accessible for peripheral laboratories particularly in hospital or research settings lacking automated equipment[19]. The culture diagnostic yield of both methods showed that, 86.0% were positive for *M. tuberculosis*, and using both methods together increased detection of 5.3% *M.tb* by MGIT-960 alone and detection of 8.5% *M.tb* by solid LJ alone. Comparing individual TB detection rate by hospitals, the rate was observed slightly higher in NAUTH 33.2%, followed by GHO 29.4% compared to COOTH 14.9% TB cases, these were similar with reported lower rates, 10.6%, 24% and 41% *M. tuberculosis* isolated in MGIT 960 cultures system[20,22.23.]. These variations could be due to differences in population and mycobacterial characteristics, sampling techniques and microbiological methods applied. The inclusion of samples from three different hospitals increases the generalized findings and highlights variability in sample handling, contamination, and processing infrastructure. The diagnostic accuracy of liquid BACTEC MGIT 960 culture was compared to solid Löwenstein–Jensen (LJ) culture as the gold standard, BACTEC MGIT 960 demonstrated a sensitivity of 94.6%, but there was no statistically significant difference ***($\chi^2 = 3.63$, $p = 0.057$)***, between MGIT and LJ results after accounting for concordant contaminated and NTM samples, emphasizing that the two methods are complementary rather than interchangeable. For MGIT960 detecting significantly more TB cases than LJ culture, highlighted its superior sensitivity, although at the expense of a higher contamination rate and NTM samples. These findings support the use of MGIT 960 as a highly sensitive diagnostic tool, ideally in combination with solid culture to optimize accuracy[8]. The specificity was moderate 63.2% reflecting additional TB detection by MGIT 960 not identified by solid LJ culture and overall diagnostic accuracy of 86.0%, for detection of *Mycobacterium tuberculosis*, reflecting

good agreement with LJ culture results. The positive predictive value was 90.9% and negative predictive value 75.3%, indicated that MGIT-negative results were reliable.

The DST results further revealed considerable resistance to first-line anti-TB drugs, particularly Ethambutol (EMB) and Isoniazid, this showed emerging resistance patterns in the population and both methods identified similar proportions of ethambutol mono-resistance with 5.1% LJ and 4.4% MGIT 960 respectively. MGIT 960 has previously been reported to detect ethambutol resistance more accurately due to liquid medium penetration and consistent drug concentration[24]. However, the variation seen with STR and EMB suggested method-specific differences in drug concentrations or interpretation protocols[24]. This is consistent with findings that, MGIT 960 is more sensitive for EMB detection, while LJ may overestimate STR resistance due to inactivation of the drug during media preparation[23,24]. MGIT 960 offer better sensitivity in mono-resistant streptomycin 5.1% cases, confirming its highly resistance prevalent. The total MDR-TB prevalence of 20.8% is concerning and highlights the need for continuous surveillance and effective infection control measures. The close similarity in MDR -TB 7.5% for Rifampicin and Isoniazid detection between the two methods indicated good agreement and reliability of both systems for DST performance but lower for streptomycin and ethambutol. In MDR-TB detection, concordance was lower, 35.8% of MDR-TB resistant isolates were detected by both MGIT and LJ, this means there is substantial discordance and the remaining 64.2% were resistant in only one of the two methods, 27 resistant showed 6.9% MDR-TB cases was found in LJ only, and 25 resistant indicated 6.4% MDR-TB in MGIT 960 only. The slightly higher number of MDR- TB detections by LJ suggests that some resistant strains may grow more slowly or show delayed detection in liquid culture. This highlighted important discrepancies which may have arisen from borderline minimum inhibitory concentrations (MICs), inoculum size variations, susceptibility testing method used, as well as the geographic setting, the prevalence of these strains or differences in critical concentration interpretations between solid and liquid systems. Ethambutol shows slightly lower concordance compared with Isoniazid; this is consistent with known variability in phenotypic DST for this drug[24,25]**.** This shows that using only one method would miss a majority of MDR-TB cases, highlighting the importance of dual-method testing, emphasizing the complementary role of MGIT and LJ in programmatic surveillance. It has been reported that the susceptibility of *M. tuberculosis* to ethambutol and streptomycin is less reliable and reproducible using solid medium[25,26,27]. Advance training in DST interpretation and harmonization of critical concentrations can reduce discrepancies in clinical decision making[27]. The discordance in MDR-TB detection emphasizes the need for dual-method confirmation, particularly in surveillance and treatment of resistant TB management. For all drugs, the majority of samples were susceptible in both methods especially rifampicin 85.6%. This strong

concordance supports the validity of DST results and builds confidence in using either method for ruling out resistance. The problem is that, Nigeria has a growing burden of drug-resistant TB and faces several TB diagnostic challenges especially in Southeast region, particularly Anambra state, this includes limited laboratory capacity and access to rapid diagnostics, inconsistent power supply, high reagent costs, lack of trained personnel and poor access to second-line DST. The National TB and Public health programs should help to implement these lacking resources in particular, tertiary hospital and peripheral health centers in Anambra state. Adoption of MGIT 960 system as the primary culture system due to its faster turnaround and higher sensitivity especially for smear-negative and paucibacillary cases and use of LJ culture simultaneously is very fundamental to maximize TB detection, improve diagnostic accuracy, and reduce false-negative results. Also, LJ culture should be maintained as a complementary method where MGIT960 is unavailable and ensuring access to culture-based diagnosis particularly where contamination is problematic or verification of results is vital, since WHO has endorsed the use of both culture system based on its advantages though with some limitations[8]. Public health programs should also respond to the high MDR-TB prevalence with appropriate treatment regimens and conduct periodic performance evaluation to monitor diagnostic efficiency, especially in MDR-TB surveillance, strengthen decontamination protocols to reduce MGIT960 contamination rates and **e**nhance laboratory capacity, including biosafety, quality assurance, and technician training.

CONCLUSION

This study established that, Clinical laboratories should employ routine use of combined MGIT 960 and LJ culture methods for reliable isolation and drug susceptibility testing of *Mycobacterium tuberculosis,* due to its significant improved TB detection, reduced false-negative results, improved turnaround times, early detection of drug-resistant TB to enhance treatment outcomes and ensure a more complete diagnosis. The substantial agreement between the two methods indicated comparable diagnostic accuracy. Liquid BACTEC MGIT 960 demonstrated a higher diagnostic yield when compared to solid Löwenstein–Jensen culture alone, though with moderate specificity but detected additional positive *Mycobacterium tuberculosis* and mono-resistant cases, with a higher contamination rate 6.8%. The MDR-TB rate of 20.8% highlights the ongoing challenge of drug-resistant TB in Anambra state. In Nigeria, solid culture remains relevant, especially in peripheral laboratories or resource-limited settings for optimizing laboratory performance and strengthen contamination control but is very essential to implement standardized DST protocols and cross-validation to minimize discrepancies in resistance profiles.

Acknowledgement
We appreciate Zankli Medical Centre Utako, Abuja, Nigeria for being part of sponsors of this research.

Competing Interests.
The authors declare that; there exists no conflict of interests.

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KNOWLEDGE AND COPING STRATEGIES FOR OCCUPATIONAL STRESS AMONG HEALTHCARE WORKERS IN UMUAHIA, NIGERIA

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ABSTRACT

Background: Occupational stress and its coping strategies remain a significant challenge among healthcare workers globally, particularly in Nigeria, where healthcare professionals face increasing workload, poor staffing and managerial constraints that may predispose them to work-related stress.

Aim: To assess the knowledge and coping strategies for occupational stress among healthcare workers in Umuahia, Abia State, Nigeria.

Material and Methods: This descriptive cross-sectional design involved a stratified random sampling technique used to select 420 healthcare workers from selected hospitals who met the inclusion criteria. The data collected were summarized using descriptive and analysed using inferential statistics of Chi-square, one-way ANOVA and paired t-test at significance level of $p< 0.05$.

Results: Participants showed a high level of knowledge of occupational stress (3.03 ± 0.85). The major coping strategy employed by healthcare workers was positive attitude to work (3.12 ± 0.86) followed by time management and prioritizing tasks (3.09 ± 0.87) and identifying sources of stress in the workplace (3.06 ± 0.88) The Chi-square analysis revealed a statistically significant association between knowledge of occupational stress and coping strategies ($x^2 = 24.615$, P = 0.001), while t-test analysis showed a statistically significant association between occupational stress and demographic variables (gender: t = 3.314, p = 0.001 and marital status: t = -2.641, p = 0.009). The result of one-way ANOVA revealed a statistically significant difference in the use of coping strategies across the three hospitals (F=2, 417; = 12.98, P = 0.0001).

Conclusions: Level of knowledge of occupational stress was high and significantly influenced the coping strategies adopted by healthcare workers in Umuahia. Occupational stress was also significantly influenced by gender and marital status.

Keywords: Occupational stress; Healthcare workers; Coping strategies; Umuahia; Nigeria.

INTRODUCTION

Occupational stress is defined as harmful physical and emotional responses that occur when job demands exceed available resources [1]. According to the World Health Organization, occupational stress has become one of the leading occupational health risk globally, particularly, in high-demand sectors such as health care [2,3]. The International Labour Organization (I.L.O) further reports that prolonged occupational stress contributes significantly to burnout, reduced productivity, absenteeism, and compromised service delivery [4,5]. Healthcare workers are especially vulnerable to occupational stress due to emotionally demanding nature of their work, exposure to life-threatening conditions, shift duties, and high responsibility for patient outcomes [6]. Globally, studies[7,8] conducted across Europe, Asia, and Africa have consistently shown high stress prevalence among healthcare workers, ranging from 45% to 75%. Burnout syndrome, characterized by emotional exhaustion and depersonalization, has been strongly linked to chronic occupational stress among healthcare professionals [8]. Occupational stress is a significant concern among healthcare workers globally, impacting on mental health, job performance, and patient care.

In Nigeria, healthcare workers face unique challenges such as inadequate staffing, limited resources, heavy workloads, poor remuneration, poor working condition, and weak healthcare infrastructure. [9]. Healthcare workers are frequently required to manage a high patient load with minimal support which significantly increases their psychological strain and vulnerability to work-related stress [10]. It is reported that over 60% of healthcare professionals experienced moderate to severe occupational stress due to heavy patient workload and poor management support [11]. Studies have shown that prolonged exposure to stress in healthcare settings can lead to burnout, anxiety, depression, and decreased quality of patient care. [11,12]. Similarly, role conflict, long working hours, and limited decision-making autonomy have been identified as major stress predictors [12].

When individuals are stressed, they develop certain mechanisms to help them manage the stress and its effects as it is difficult to remain in a state of constant tension. These mechanisms known as coping strategies, is the application of psychological and physical resources to reduce the negative effects of stress, which is very crucial in the study of occupational stress. A critical determinant of coping mechanisms that has been identified sparsely in research, is the knowledge of occupational stress [13]. In the context of occupational health, the relationship between stress knowledge and coping behavior has gained increasing scholarly attention. Knowledge of occupational stress encompasses an individual's understanding of workplace stressors, early warning signs, and potential health implications [14]. Although coping mechanism may contribute to experiential understanding of stress over time, current evidence suggests that knowledge of occupational stress plays a more foundational role in shaping coping behaviors, making it a critical determinant of how individuals cope with workplace stress [15].

Gender differences in occupational stress have also been widely documented as significantly influenced by knowledge of occupational stress and coping strategies. Female healthcare workers often report higher stress levels due to dual work-family responsibilities and workplace discrimination [16]. In addition, marital status

has been found to influence stress perception, with married healthcare workers sometimes experiencing additional family-role pressures that interact with occupational demands [17].

Recent global workforce report according to Razai[18] highlighted the urgent need for health organization at all levels to aim at protecting healthcare workers' mental health and other impacts of occupational stress. Evidence suggests that institutional coping strategies, improved staffing ratios, and participatory leadership demand approaches that significantly reduce occupational stress levels [19,20]. The healthcare facilities in this region experience increasing patient loads without proportional expansion of workforce capacity, potentially predisposing staff to elevated stress levels [21]. Despite these challenges, there is limited data on healthcare workers' knowledge of occupational stress and coping strategies in Umuahia.

This study is anchored on the Transactional Model of stress and coping [23] which explains stress as a dynamic process arising from the interaction between individuals and their work environment. According to this model, stress is not merely the presence of demanding conditions, but rather the individual's cognitive appraisal of those conditions and their perceived ability to cope with them. Within this framework, occupational stressors such as workload, role conflict, time pressure, and organizational environment represent external demands placed on workers. These stressors trigger a process of cognitive appraisal, where individuals evaluate the significance of the stressor (primary appraisal) and their available resources to manage it (secondary appraisal).

Knowledge of occupational stress is conceptualized in this study as a critical cognitive resource that influences this appraisal process. Individuals with adequate knowledge are more likely to correctly identify sources and symptoms of stress, understand its potential consequences, and be aware of appropriate coping mechanisms. This knowledge enhances their ability to evaluate stress situations accurately and select effective responses. The framework assumes that knowledge of occupational stress plays a mediating role between exposure to stressors and the coping strategies adopted. Workers with higher level of knowledge are more likely to engage in adaptive coping strategies, such as problem-solving, time management, seeking social support, and relaxation techniques. In contrast, those with limited knowledge may rely on maladaptive coping strategies, including avoidance, denial, or withdrawal, which may exacerbate stress and negatively affect well-being and job performance. Thus, the relationship between occupational stress and coping strategies is not direct but is significantly influenced by the individual's level of knowledge. Understanding occupational stress and coping strategies can inform interventions to improve healthcare worker's well-being, job satisfaction, performance and patient outcomes. Identifying gaps in knowledge and effective coping strategies can also guide policy development, training programs, and support services, ultimately enhancing healthcare delivery in Umuahia and similar settings. Additionally, the study's findings can contribute to the broader understanding of occupational stress in low-resource healthcare environments, informing strategies to mitigate its impact. This study therefore examines how knowledge of occupational stress shaped the selection and effectiveness of coping strategies among workers

MATERIALS AND METHODS

Study Design

The study employed a descriptive cross-sectional research design to assess knowledge and coping strategies among healthcare workers. The design was appropriate because it allowed for the collection of data at a single point in time and enabled the determination of relationships between variables using inferential statistics.

Study Area

The study was conducted in selected healthcare facilities in Umuahia, the capital city of Abia State, Nigeria. Umuahia serves as a major administrative and healthcare hub in the state, with both public and private health institutions providing primary, secondary and tertiary healthcare services.

Study Population

The study population comprised healthcare workers including doctors, nurses, pharmacists, laboratory scientists, and physiotherapists working in the selected healthcare facilities in Umuahia.

Inclusion Criteria

Registered and licensed healthcare workers, those who had worked for at least one year in the facility and those who consented to participate while non-registered and licensed healthcare workers, non-consenting healthcare workers and those who had not worked up to one year were excluded.

Sample Size Determination

The sample size for the study was determined from the target population of 1,333 using Taro Yamene's [22] formula shown as $n = N/ (1 +N(e)^2)$. The minimum sample size was calculated as 400 and with 10% for attrition/non-response, the sample size became 440. This was distributed proportionately among the healthcare workers in the selected hospitals.

Sampling Technique

A multistage sampling technique was adopted. Healthcare facilities were first stratified by level of care and hospital type (Federal, State and Private). A stratified random sampling technique was used to select participants. Within each facility, healthcare workers were randomly selected proportionally from each professional category to ensure representativeness. In the three hospitals, the proportion of healthcare workers recruited in each of the hospital was made to be proportional to the number they contribute to the total population of healthcare workers in each hospital.

Instrument for Data Collection

The instrument for data collection was a questionnaire organized in accordance with the research objectives and hypothesis. The questionnaire was divided into three sections: A, B, and C. Section A was structured to obtain information on socio-demographic characteristics (Age, gender, professional category, and years of experience. Section B also structured to obtain information on Knowledge of occupational stress by the respondents. Section C was used to obtain information on respondents' coping strategies for occupational stress. Questions were rated on a four-point rating scale of 4 - Strongly Agree, 3 – Agree, 2 – Disagree and 1 - Strongly Disagree.

Validity of the Instrument

Face and content validity was ensured by giving the instrument to supervisor and two senior lecturers in the Department of Nursing Science. In line with the objectives of the study, the Validators' criticism, advice and suggestion guided structuring of the instrument.

Reliability of the Instrument

Reliability of the instrument was ensured by pilot study of the instrument using forty-two healthcare workers in Abia State Teaching Hospital Aba which is a tertiary hospital. Data collected were subjected to split-half reliability coefficient statistics. The reliability of the instrument was established using Cronbach's Alpha. The reliability index arising from this method achieved a high degree of internal consistency of the instrument. Section A and B yielded reliability coefficient of 0.87 and 0.84 respectively, signifying a considerable reliability.

Ethical Consideration

Ethical approval was obtained from the health Research Ethics Committee of the selected hospitals (FMC/QEH/G.596/VOL.10/674, ABHDC/ADM/177/26). A written informed consent was obtained from the respondents before administering the questionnaire, ensuring the principle of confidentiality.

Data Collection Procedure

Research assistants distributed questionnaires to participants during working hours. Participants were briefed on the purpose of the study and written informed consent obtained as a confirmation of their participation. Completed questionnaires were collected on the same day or within 48 hours. The response rate was 95%.

Data Analysis

The data collected were summarized using frequency counts, percentages, mean score and standard deviation; and analyzed using Chi-square test, paired t-test, one-way ANOVA and Tukey HSD post-hoc test. The mean of the options of the four-point Likert scale (1+2+3+4)/4 = 2.5, was used as an index to determine the cut-off point between low level and high-level perception of respondents to the question items. The interpretation of Likert scale means: the following benchmark was adopted: 1.00 - 1.49 = very low, 1.50 – 2.49 = low, 2.50 – 3.49 = moderate, 3.50 - 4.00 = high. In this study. All statistical analysis were performed at $p < 0.05$ level of significance

RESULTS

The socio-demographic characteristics of the respondents showed that nearly half of the participants (47.62%) were within the age bracket of 26-35 and the majority were females (57.1%) and about three quarters were married (61.9%). Nurses constituted the largest professional group (61.2%). while majority of them (40.95%) were still within 10 years of working experience.

The result of healthcare workers' knowledge of occupational stress revealed, a grand mean of 3.03±0.91. Majority of the respondents agreed they understood the importance of self-care (3.09 ±0.84) while others were more aware of prioritizing tasks to manage stress (3.06 ±0.79) and aware of the signs and symptoms of stress (3.05± 0.88).

The result of coping strategy used by healthcare workers showed a grand mean score of 3.03±0.89. The major coping strategy employed by healthcare workers was Positive attitude 3.12±0.85 followed by time management and prioritizing tasks (3.09±0.86) and identification of sources of stress (3.06 ±0.87), while use of relaxation technique scored lowest (3.00±0.90).

A Chi-square was used to determine if there were significant relationship between knowledge of occupational stress and coping

strategies. As shown in table 4, the result revealed a statistically significant association between knowledge of occupational stress and coping strategies (x^2 = 24.615, P = 0.001).

Independent t-test analysis conducted to determine if there were significant difference between occupational stress and demographic variables (gender and marital status) was shown in Table 5. The result revealed a statistically significant association between gender and occupational stress (t = 3.314, p = 0.001) and between marital status and occupational stress (t = -2.641, p = 0.009).

As shown is Table 6a, Hospital A recorded the highest mean of total coping score of112.28±17.36 followed by Hospital B with 106.96±19.4, while Hospital C recorded the lowest mean of 96.60± 19.88

The result of one-way ANOVA presented in Table 6b revealed a statistically significant difference in the total use of coping strategies across the three hospitals (F= 12.98, P <0.0001.

Table 1: Demographic characteristics of respondents

Variable	Options	Frequency	Percentage
Age (years)	18 - 25	136	32.38
	26 – 35	200	47.62
	36 – 45	62	14.76
	46 and above	22	5.24
Gender	Male	180	42.8
	Female	240	57.1
Marital Status	Single	160	38.1
	Married	260	61.9
Professional	Doctors	84	20.0
Category	Nurses	257	61.19
	Pharmacists	34	8.10
	Laboratory Scientists	25	5.95
	Physiotherapist	20	4.76
Years of Experience	1 – 10	172	40.95
	11 – 20	84	20.00
	21 – 30	84	20.00
	31 and above	80	19.05

Table 2: Mean Score and Standard Deviation of respondents on the Knowledge of Occupational Stress (n = 420

Item	Mean± SD	Decision
I understand the importance of self-care	3.09 ±0.84	High
I Prioritize tasks to manage workload	3.06 ±0.79	High
I am aware of signs and symptoms of stress	3.05± 0.88	High
I understand what occupational stress is.	3.04± 0.92	High
I know the common sources of stress	3.04± 0.93	High
I am aware of the resources available to support healthcare professionals	3.04± 0.91	High
Grand mean	3.03±0.91	High

Decision rule: Mean > 2.50 = High knowledge and Mean < 2.49 =low knowledge

Table 3: Mean score and standard deviation on coping strategies for occupational stress

Item	Mean+_ SD	Decision
Positive attitude to work	3.12±0.85	High
Time management and prioritization of tasks	3.09±0.86	High
Identification of source of stress	3.06 ±0.87	High
Delegation of duty where possible	3.03±0.88	High
Adequate sleep and rest	3.02±0.90	High
Use of relaxation techniques	3.00±0.90	High
Grand mean	3.03±0.89	High

Decision Rule: Mean > 2.50 = High use and Mean < 2.49 low use

Table 4: Relationship between knowledge of occupational stress and coping strategies

Variable	x^2	Df	p-value	Decision
Occupational stress and coping strategies	24.615	4	0.001	Sig

Significance level: $p < 0.05$

Table 5: Association between occupational stress and demographic variable (gender and marital status)

Variable	t-value	Df	p-value
Gender and occupational stress	3.314	418	0.001
Marital status and occupational stress	-2.641	418	0.009

Significance level: $p < 0.05$

Table 6a: Descriptive statistics of Brief COPE score across hospitals

Hospital	N	Mean	Std. Deviation	Std. Error	95% CI for Mean	Min	Max
FMC	268	112.28	17.36	1.06	110.19-114.37	65	112
GHA	126	106.96	19.04	1.70	103.60-110.32	59	112
MCH	26	96.60	19.88	3.00	88.58-104.62	58	112
Total	420	109.20	18.48	0.90	107.43-110.97	58	112

Higher scores indicate greater overall frequency of coping strategy use

Table 6b: One-Way ANOVA Summary for Coping Strategy Use Across Hospitals

Source Variation	**Sum of Square (SS)**	**df**	**Mean Square (MS)**	**F**	**p-value**	**n^2**
Between groups	8487.512	2	4243.756	12.98	.000*	.059
Within Groups	136314.888	417	326.895			
Total	144802.400	419				

p < .05, significant at 5% level of significance

The Tukey HSD post-hoc test in Table 6c further showed that all pairwise mean differences were statistically significant. Healthcare workers in FMC reported significantly higher coping use than healthcare workers in GHA, mean difference = 5.32, p = 0.018 and MCH mean difference = 15.68, p = 0.000. Similarly, GHA healthcare workers scored significantly high than MCH healthcare workers, mean difference = 10.36, p = 0.021. The calculated effect size $n^2 = 0.059$ indicated a medium effect according to Cohen's guidelines. This implied that 5.9% of the variability in coping strategy use among healthcare workers is attributable to hospital type.

Table 6c: Tukey HSD Post-Hoc Test for Total Brief COPE Score

(I) Hospital	(J) Hospital	Mean Difference (I-J)	Std. Error	Sig.	95% CI
FMC	GHA	5.32*	1.934	.018	0.76 – 9.88
FMC	MCH	15.68*	3,696	.000	6.99 – 24.37
GHA	MCH	10.36*	3.836	.021	1.33 – 19.39

- The mean difference is significant at 0.05 level

DISCUSSION

The study conducted among healthcare workers in Umuahia revealed high knowledge of occupational stress, this could largely be attributed to substantial awareness of its importance of self-care, sources, and symptoms. This finding is consistent with the finding of Okwuosa and Okonkwo who reported that healthcare workers in southeast Nigeria were knowledgeable about both physical and psychological symptoms of occupational stress, reinforcing the importance of such awareness in promoting timely intervention [23]. Similarly, this finding aligned with report from the World Health Organization, which identified healthcare professionals as one of the occupational groups most vulnerable to work-related stress due to high job demands and emotional strain [3], and the contemporary Nigerian and global research indicating that health system constraints significantly contribute to workplace stress [4].

The findings of this study showed that healthcare workers utilized several strategies to cope with occupational stress. The grand mean score revealed a moderate to high level of adoption of coping strategies under high level of stress. Among the strategies adopted, suggested that healthcare workers are aware of the importance of adopting coping mechanisms to maintain their psychological well-being and job effectiveness despite the demanding nature of healthcare services. This finding is in agreement with the result obtained by Dall'Ora, Ball, Reinius and Griffiths[12] who observed resource availability and institutional culture to have strongly influenced the adoption of coping strategies among healthcare workers [7].

The Chi-square revealed a statistically significant association between healthcare workers' level of knowledge of occupational stress and coping strategies. This suggests that knowledge of occupational stress plays a role in shaping coping behaviors, making it a critical determinant of how individuals cope with workplace stress [15]. The finding further suggested that the type of coping strategy adopted by healthcare workers varies significantly with their level of knowledge of occupational stress. Healthcare workers with higher knowledge of occupational stress are more likely to adopt effective coping strategies. This is in line with the finding of Adeolu et al.[2] who found a strong relationship between occupational stress awareness and consistent use of preventive coping strategies among nurses in Enugu State. Similarly, Nwankwo et al.[8] observed that knowledge-based interventions enhanced the adoption of healthy coping behavior among healthcare providers in Nigeria.

The present study found a statistically significant association between occupational

stress and demographic variable (gender). This gender-based vulnerability of occupational stress as documented in healthcare settings suggests that women often experience greater emotional exhaustion compared to their male counterparts [20]. This finding supports the finding by Abiodun [16] who reported higher levels of knowledge of stress among female healthcare workers due to dual role responsibilities and workplace pressure. Marital status showed a significant association with occupational stress. This implied that marital status has been found to influence stress perception, with married healthcare workers sometimes experiencing additional family-role pressures that interact with occupational demands [17]. This finding is consistent with Olatunji et al.[17] who observed that married healthcare workers reported higher stress levels due to competing family and professional responsibilities. Psychosocial role theory suggests that additional domestic obligation may amplify perceived job strain, especially in demanding healthcare environment [4].

The result of one-way ANOVA revealed a statistically significant difference in the use of coping strategies across the three hospitals. The null hypothesis was rejected. Possible reasons could be that Federal Medical Centre faces higher patient influx, and more complex medical cases, which may compel healthcare workers to adopt structured or individual coping strategies. This suggests that the variability in coping strategy use among healthcare workers is attributable to hospital type and administrative demands. This result is consistent with the finding of Abiodun et al.[16] who posited that healthcare workers in Federal, State and Private hospitals hardly face similar stressors with fewer coping resources than tertiary hospitals.

CONCLUSION

The study has shown that healthcare workers possessed a high level of knowledge of occupational stress in Umuahia, Abia State. The knowledge of occupational stress significantly predicted the coping behaviors adopted by healthcare workers. Furthermore, occupational stress was significantly influenced by demographic factors and coping strategies employed.

Acknowledgements

The author acknowledges the management of the participating facilities and all healthcare workers who participated in the study.

Conflict of interest: The author declares no conflict of interest

Funding: This research received no specific grant from any funding agency in the public, commercial, or not-for profit sectors.

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CORRELATION OF SERUM MYOGENIN WITH DISEASE SEVERITY IN SUBJECTS WITH SICKLE CELL DISEASE IN STEADY STATE

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ABSTRACT

Background: Sickle cell disease (SCD) is a hereditary haemoglobinopathy with a high prevalence in sub-Saharan Africa. It is characterised by chronic haemolysis, inflammation, and recurrent vaso-occlusive episodes that contribute to progressive organ dysfunction. Skeletal muscle involvement in SCD is increasingly recognised, yet the role of muscle regulatory factors such as myogenin, a key transcription factor involved in muscle differentiation and regeneration remains poorly understood.

Aim: This study aimed to evaluate the relationship between serum myogenin levels and disease severity in individuals with SCD in steady state, and to assess its association with anthropometric indices across different haemoglobin phenotypes.

Materials and Methods: This cross-sectional comparative study included 90 participants recruited using simple random sampling method: 30 HbSS patients in steady state, 30 HbAS individuals, and 30 HbAA controls, aged 10-50 years. Serum myogenin levels were measured using enzyme-linked immunosorbent assay (ELISA). Anthropometric parameters were assessed

and body mass index (BMI) calculated. Disease severity in HbSS participants was evaluated using the Adegoke and Kuti sickle cell disease severity scoring system. Individuals with cardiovascular disease, inflammatory disorders, renal disease, obesity, pregnancy, or metabolic conditions were excluded. Statistical analyses included ANOVA and correlation testing to evaluate group differences and relationships between variables.

Results: HbSS participants had significantly lower BMI compared to HbAA and HbAS groups ($p = 0.001$), despite no significant difference in age across groups. However, serum myogenin levels did not differ significantly among HbAA, HbAS, and HbSS participants ($p = 0.967$). Correlation analysis showed no significant association between myogenin and BMI ($r = 0.017$, $p = 0.931$) or age ($r = 0.003$, $p = 0.986$), although BMI was strongly positively correlated with age ($r = 0.801$, $p = 0.001$). Notably, there was a statistically significant moderate inverse correlation between myogenin levels and disease severity in HbSS patients ($r = –0.387$, $p = 0.035$).

Conclusion: This study demonstrated that while individuals with SCD exhibit significant nutritional deficits, as reflected by reduced BMI, these do not directly influence circulating myogenin levels. The observed inverse relationship between myogenin and disease severity may suggest that myogenin may serve as a marker of disease-related muscle dysfunction rather than genotype or nutritional status.

Recommendations: Further longitudinal and mechanistic studies are needed to validate myogenin as a biomarker and explore its potential role in therapeutic strategies targeting muscle health in SCD.

Key words: Sickle cell anaemia, Sickle cell disease, myogenin, biomarkers, body mass index.

INTRODUCTION

Sickle cell disease (SCD) represents one of the most prevalent monogenic disorders globally, affecting approximately 50 million people worldwide, with Nigeria bearing the highest burden of 4-6 million cases.[1] The disease results from a point mutation in the β-globin gene, leading to the production of abnormal hemoglobin S (HbS), which polymerizes under deoxygenated conditions, causing red blood cells to assume a characteristic sickle shape.[2]

The clinical manifestations of SCD extend beyond hematological complications, significantly impacting multiple organ systems, including the musculoskeletal system.[3] While vaso-occlusive crises and chronic hemolysis are well-documented pathophysiological mechanisms, the role of muscle-specific factors in disease progression and severity remains poorly understood.[4] This knowledge gap is particularly significant given that muscle wasting, and reduced muscle mass are common complications in SCD patients, contributing to reduced quality of life and increased morbidity.

Myogenin, a muscle-specific transcription factor belonging to the MyoD family, plays a crucial role in skeletal muscle development, repair, and regeneration. Previous studies have demonstrated elevated

myogenin expression in various muscle-wasting conditions and skeletal myopathies, suggesting its potential role as a compensatory mechanism in muscle repair.[5] However, despite the prevalence of muscle-related complications in SCD, no previous studies have investigated the relationship between myogenin level and disease severity in SCD patients.

This study aimed to bridge this critical knowledge gap by examining the relationship between serum myogenin level and disease severity in patients with homozygous sickle cell disease (HbSS). Understanding this relationship could provide new insights into disease progression mechanisms and potentially identify novel therapeutic targets for managing SCD-related muscle complications.

MATERIALS AND METHODS

Study Site This research was carried out in Nnamdi Azikiwe University Teaching Hospital (NAUTH), Nnewi, Anambra State, Nigeria.

Study Design: A cross-sectional comparative study was carried out to evaluate the serum level of myogenin and its correlation with disease severity in subjects with sickle cell disease in their steady state. Selection of sickle cell subjects as well as the control group (HbAS and HbAA) was done by simple random sampling method. A total of 90 subjects were recruited for the study which includes 30 homozygous sickle cell (HbSS) subjects, 30 heterozygous sickle cell (HbAS) and 30 normal subjects (HbAA). The selection of the steady state group was dependent on subjects not experiencing crisis for at least 2 weeks; not receiving blood transfusion for at least 3 months, and not having fever for at least 2 weeks prior to the study. Full blood count results of the subjects were used alongside other criteria, to estimate severity scoring in homozygous sickle cell disorder in steady state and crisis.

Sample Size Determination

Sample size was calculated using the Cochran's Formula as given below:

$$n = \frac{Z^2 p(1-p)}{d^2}$$

Where n= sample size; Z = 1.96 (95% confidence level); p = 0.04 (SCD prevalence in Nigeria = 4.0% [6] and d = 0.05 (absolute error or precision level).

$$n = \frac{1.96^2 * 0.04(1 - 0.04)}{0.05^2}$$

n= 59.00

However, in order to increase the statistical power of the study, a total of 90 participants were recruited into the study using simple random sampling method. Participants were comprised of 30 homozygous sickle cell (HbSS) subjects, 30 heterozygous sickle cell (HbAS) and 30 normal subjects (HbAA).

Inclusion Criteria: Homozygous sickle cell disease (HbSS) subjects in steady state, heterozygous sickle cell subjects (HbAS) and normal healthy subjects (HbAA) all within the age range 10-50 years.

Exclusion Criteria: Subjects outside the age range of 10-50 years, individuals with known cardiovascular and inflammatory disease (autoimmune disease), obesity, renal disease, pregnancy, metabolic disorders such

as diabetes, subjects on medication such as non-steroidal anti-inflammatory drugs (NSAIDs).

Ethical Approval: The ethical approval for this research was obtained from Nnamdi Azikiwe University Teaching Hospital Ethics Committee in agreement with the Helsinki declaration by the World Medical Association (WMA) on the ethical principles for medical research involving human subjects.[7]

Informed consent: Prior to the study, written informed consent from the subjects and or their guardians (for minors) was obtained.

Sample Collection Blood samples were collected aseptically, and serum was separated and stored at -20°C until analysis. Hemoglobin genotype was determined using cellulose acetate electrophoresis.[8]

Parameters Assayed: Serum myogenin levels were measured using an enzyme-linked immunosorbent assay (ELISA). Disease severity in HbSS participants was evaluated using the Adegoke and Kuti sickle cell disease severity scoring system based on anemia, complications, white blood cell counts, transfusion rates, and crisis frequency in line with prior studies.[9]

Body mass index (BMI) measurement: BMI was calculated using the following formula[10,11] as given below:

$$BMI = \frac{\text{Weight}(kg)}{Height^2\,(m^2)}$$

A measuring tape was fastened to a piece of wood to determine height, and an electronic weighing scale was used to estimate the body weight.

Statistical Analysis The data were presented as mean±SD and the mean values of the control and test group were compared by Analysis of Variance (ANOVA), posthoc test and Pearson's correlation coefficient using Statistical package for social sciences (SPSS) (Version 26.0) software. Statistical significance was set at $p < 0.05$.

RESULTS

Table 1 show the mean anthropometric values in different blood genotype groups (HbAA, HbAS and HbSS). The One-way ANOVA revealed no statistically significant difference in the mean age between groups ($F = 0.525$; $p = 0.593$). However, a significant difference was observed in the BMI ($F = 16.927$; $p=0.001$) when compared across the groups. The BMI did not differ significantly in the HbAA participants when compared to the HbAS group ($p = 0.906$), but the BMI was significantly decreased in sickle cell patients when compared with HbAA and HbAS subjects ($p = 0.001$), respectively.

Table 2 shows the mean level of Myogenin in subjects with homozygous sickle cell disease (HbSS) and the control groups (HbAA and HbAS). There was no significant difference in the mean serum level of myogenin in the different hemoglobin phenotype groups ($F = 0.034$; $p = 0.967$). No significant difference was observed in the mean level of myogenin between HbAA and HbAS subjects ($p = 0.963$), between HbAA and HbSS ($p = 0.989$) and between HbAS and HbSS ($p = 0.992$).

Table 3 shows the correlation of BMI, age and Myogenin with disease severity in subjects with sickle cell anemia in steady state. No correlation was observed between BMI and Myogenin in sickle cell anemic patients (r = 0.017, p = 0.931). A positive correlation was observed between BMI and age in sickle cell anemic patients (r = 0.801, p = 0.001). No correlation was observed also between age and Myogenin in sickle cell anemic patients (r = 0.003, p = 0.986).

Table 4 shows the correlation of BMI, age and Myogenin with disease severity in subjects with sickle cell anemia in steady state**.** No correlation was observed between disease severity and Age (r = -0.068, p = 0.719) and between disease severity and BMI (r = -0.174, p = 0.356) in sickle cell anemic patients. A negative correlation was observed between disease severity and Myogenin level (r = -0.387, p = 0.035).

Table 1: Anthropometric values in different blood genotype (HbSS, HbAA and HbAS)

Groups	N	Age(years)	BMI (kg/m^2)
AA	30	25.03 ± 5.72	23.81 ± 2.77
AS	30	23.50 ± 4.54	23.49 ± 2.19
SS	30	24.93 ± 8.52	19.90 ± 3.52
F-value		0.525	16.927
P-value		0.593	0.001*
AA vs AS (p-value)		0.632	0.906
AA vs SS (p-value)		0.998	0.001*
AS vs SS (p-value)		0.669	0.001*

**P*-value is statistically significant at <0.05.

Table 2: Mean level of Myogenin in different haemoglobin phenotype groups (HbSS, HbAA and HbAS)

Groups	Myogenin
AA (n=30)	31.70 ± 9.13
AS (n=30)	31.97 ± 6.00
SS (n=30)	31.47 ±6.98
F-value	0.034
P-value	0.967
AA vs AS (p-value)	0.963
AA vs SS (p-value)	0.989
AS vs SS (p-value)	0.992

**P*-value is statistically significant at <0.05.

Table 3: Correlation of anthropometric values (BMI and Age) with Myogenin in subjects with sickle cell anaemia in steady state

Variable	N	r- value	p-value
BMI VS MYOGENIN	30	0.017	0.931
BMI VS AGE	30	0.801**	0.001*
AGE VS MYOGENIN	30	0.003	0.986

**P*-value is statistically significant at <0.05.

Table 4: Correlation of Myogenin, BMI and Age with disease severity in subjects with sickle cell anaemia in steady state

Variable	N	r- Value	p-value
Disease Severity Vs Myogenin	30	-0.387*	0.035*
Disease Severity Vs Age	30	-0.068	0.719
Disease Severity VS BMI	30	-0.174	0.356

**P*-value is statistically significant at <0.05.

DISCUSSION

Sickle cell disease (HbSS) is an autosomal recessive genetic disorder of the red blood cell.[12] affecting approximately 1-3% of populations within the states in Nigeria.[13] Increasing evidence points towards oxidative stress, vasooclusion and the resulting inflammatory response as responsible for the increased organ and system involvement in sickle cell disease.[14] The activity and expression of myogenin has been shown to be controlled by the balance between the pro-inflammatory and anti-inflammatory cytokines and these factors are

shown to be induced in sickle cell anemia. Skeletal muscle hypertrophy and hypotrophy have been documented to occur due to imbalance between these group of cytokines.[15]

This study examined anthropometric characteristics, myogenin levels, and their association with disease severity in individuals with sickle cell disease (SCD) in steady state. While our findings provide useful insights, a more critical evaluation highlights both agreements and inconsistencies with existing literature, particularly regarding the complex regulation of muscle regeneration in chronic disease.

The anthropometric findings (Table 1) showed that individuals with HbSS had significantly lower BMI compared to HbAA and HbAS groups ($p = 0.001$), despite similar age distribution. This is consistent with established evidence that SCD is associated with chronic undernutrition, increased resting energy expenditure or metabolic demand, and recurrent illness, all of which contribute to reduced body mass and impaired growth. However, a critical observation is that despite this significant reduction in BMI, there was no corresponding alteration in myogenin levels (Table 2) or correlation with BMI (Table 3). This contrasts with evidence from muscle-wasting conditions where nutritional deficits and catabolic states are often associated with dysregulation of myogenic regulatory factors, including myogenin.[16, 17] The lack of association observed in this study suggests that BMI may be an insufficient proxy for muscle mass or muscle metabolic activity, and therefore may not adequately reflect the biological processes regulating myogenin expression. It also raises the possibility that in steady-state SCD, nutritional deficits alone are not sufficient to disrupt muscle differentiation pathways.

The lack of significant difference in myogenin levels across haemoglobin phenotypes (Table 2) also warrants critical consideration. While this suggests that genotype alone does not influence myogenin expression, it contrasts with findings in chronic inflammatory and degenerative muscle conditions, where myogenin is often altered as part of a regenerative response.[18,19] One possible explanation is that the study population was in a steady state, where acute inflammatory or hypoxic triggers that typically activate myogenic pathways are minimal. Another possibility is methodological, suggesting that circulating myogenin may not accurately reflect tissue-level activity, where most myogenic processes occur. This limitation has been highlighted in studies showing that myogenin expression is tightly regulated within muscle tissue and may not be reliably detected in systemic circulation.[20]

The correlation analysis (Table 3) revealed no relationship between myogenin and BMI or age, despite a strong correlation between BMI and age. This finding reinforces the concept that myogenin reflects localized cellular processes such as satellite cell differentiation rather than systemic or demographic variables.[20] However, this also limits its clinical applicability, as it suggests that myogenin cannot be easily inferred from routine clinical parameters. Furthermore, ageing is known to impair muscle regenerative capacity and reduce

expression of myogenic markers under stress conditions.[21,22] The absence of such an effect in this study may reflect the relatively young population studied of our participants, or maybe due to the insufficient age variability in our sampled population.

The most significant finding of this study is the inverse relationship between myogenin and disease severity (Table 4). This suggests that as disease severity increases, myogenin levels decrease, indicating impaired muscle regenerative capacity. This is supported by mechanistic studies showing that chronic inflammation and inflammatory mediators such as TNF-α can suppress myogenin expression and inhibit myogenic differentiation.[23] Similarly, reduced myogenin expression has been associated with impaired muscle repair and muscle wasting in chronic disease states.[16,24]

However, this finding is not entirely consistent across the literature. Some studies report increased myogenin expression in response to muscle injury, reflecting an adaptive or compensatory mechanism response.[18,25] This apparent contradiction may be explained by disease stage. For example, in early or moderate disease, myogenin may be upregulated to promote repair, whereas in chronic or severe disease, persistent inflammation and repeated injury may exhaust regenerative capacity, leading to reduced expression. This concept is supported by studies showing that in prolonged disease states, muscle regeneration becomes ineffective despite ongoing damage.[26,27] Therefore, the negative correlation observed in this study likely reflects advanced impairment of muscle regenerative signalling in more severe SCD.

Another critical limitation is that myogenin is primarily a tissue-specific marker, and its measurement in serum may not accurately represent its functional activity within muscle. This methodological issue may partly explain the weak or absent associations with BMI, age, and genotype. This is because some studies have shown that direct tissue-level assessment provides more reliable insight into myogenic activity[20], suggesting that future research should incorporate muscle biopsies or even more sensitive molecular techniques.

Generally, our findings indicate that while HbSS individuals exhibit clear nutritional deficits (Table 1), these do not directly influence myogenin levels, and genotype alone is not a determinant of myogenin expression (Table 2). Instead, myogenin appears to be more closely linked to disease burden and chronic pathophysiological stress, as reflected in its inverse association with disease severity (Table 4). This suggests that myogenin may serve as a marker of functional muscle impairment rather than structural or nutritional status.

Conclusion

This study provides important insight into the relationship between myogenin and disease severity in sickle cell disease. While significant reductions in BMI were observed in HbSS individuals, these did not correspond to changes in myogenin levels, highlighting the limitation of anthropometric measures in reflecting muscle biology. Critically, the study demonstrates a significant inverse relationship between myogenin and disease severity, suggesting that worsening SCD is associated with

impaired muscle regenerative capacity. When interpreted alongside existing evidence, our finding suggests progressive exhaustion of muscle repair mechanisms under chronic inflammatory and hypoxic stress. Hence, our study challenges the assumption that myogenin is influenced by genotype or nutritional status alone and instead positions it as a potential marker of disease-related muscle dysfunction. However, its clinical utility is limited by methodological constraints and variability in its expression across disease states. Therefore, we recommend that future studies should adopt longitudinal designs and integrate molecular, functional, and inflammatory markers to better define the role of myogenin in SCD progression and its potential as a therapeutic target.

Acknowledgment: The authors are sincerely grateful to the participants in this study.

Author contributions: MPO, OCE, OMO conceptualised and designed the study. ESC, OEC, IAC, OSC, MCC-M, OSE, NEE and OVC contributed to implementation of the project and revision of the manuscript. All authors were involved in the writing and revision of the manuscript. The authors read, approved the final manuscript and agree to be accountable for all aspects of the work.

Data availability: The data used to support the findings of this study are available from the corresponding author upon reasonable request.

Funding: Self-funded.

Conflict of Interest: The authors declare no conflict of interest in the conduct and publication of this work.

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MOLECULAR CHARACTERIZATION AND MULTI-DRUG-RESISTANT PROFILES OF UROPATHOGENIC *ESCHERICHIA COLI* FROM ADULTS ATTENDING NATIONAL ORTHOPEDIC HOSPITAL ENUGU, NIGERIA

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ABSTRACT

Background: Antimicrobial resistance (AMR) in *Escherichia coli* poses a significant challenge in both community and hospital settings.

Aim: The study aimed to ascertain the prevalence, molecular types and susceptibility pattern of multidrug resistance patterns of *E.coli* isolated from urine of adults with urinary tract infections attending National Orthopaedic Hospital, Enugu Nigeria.

Materials and Methods: Two hundred and thirty (230) urine samples were collected from adults aged 18-65 years attending the hospital using sterile screw capped containers. Structured questionnaire was used to obtain the demographic data of the participants. Urine samples were inoculated directly onto MacConkey agar, CLED and Chrome agar using streak plate method and incubated at 37^0C for 24 hrs. Colonies were identified based on their cultural, morphological and biochemical characteristics. Antimicrobial susceptibility and resistance of the isolates were determined by Kirby Bauer standard disc diffusion method on Mueller Hinton agar. Molecular analysis was done using multiplex and Linear PCR on multidrug resistant *E. coli* isolates.

Results: Out of the 230 samples analysed, 60(26.08%) were positive for *E. coli,* of which 42(24.9%) were obtained from the female participants, suggesting a gender-based predisposition to UTIs($p>0.05$). Age group 35-45 years had the highest prevalence (31.14%) of infection. Out of the 60 samples positive for *E.coli*, 25(41.66%) were multidrug resistant *E. coli*. The result shows that there was no statistically significant ($p>0.05$) association between gender, age in multidrug resistant *E. coli.* The *E.coli* isolates were highly resistant (86.6%) to Streptomycin and Penicillin, and had least resistance to Nitrofurantoin (1.66%). Molecular analysis identified the presence of blaCTX-M and blaTEM resistance genes, while blaSHV was not detected. All the isolates resistant to Streptomycin and Penicillin were positive for blaTEM genes, while the isolates resistant to Nalidixic acid, Amoxicillin-clavulanic acid and Trimethoprim-Sulfamethoxazole were positive for blaCTX-M genes.

Conclusion: The high level of resistance to commonly used antibiotics and the detection of resistance genes such as blaCTX-M and blaTEM highlights the growing threats of antimicrobial resistance in the area.

Keywords: *Multidrug resistant, molecular characterization, E.coli, UTIs*

INTRODUCTION

Escherichia coli (E. coli) is an important pathogen causing infections ranging from mild cases to life threatening conditions, it is linked to increased rate of morbidity and mortality due to multidrug resistance *E. coli*, it is also known as one of the major threats to global health in the recent times.[1,2] *Escherichia coli* is a versatile commensal and pathogenic member of the human microflora. As the primary causative pathogen in urosepsis, *E. coli* places an immense burden on healthcare systems worldwide.[3] Also, the major resistance mechanism of the Enterobacteriaceae resistance is primarily due to the production of beta-lactamase hydrolyzing enzymes like extended spectrum beta-lactamases (ESBL), AmpC beta-lactamases, and carbapenemases (Carbapenemase-Producing Enterobacteriaceae (CPE). These resistance genes are found on plasmids that can spread between Enterobacteriaceae, causing infections. These plasmids often contain additional resistance genes, making them difficult to treat. Asymptomatic carriage in healthy children and community-acquired infections, especially with ESBL, are increasingly reported.[4]

Escherichia coli is a commensal bacterium of the family Enterobacteriaceae. It is a common cause of urinary tract, blood stream, food borne infections, cholecystitis, respiratory illnesses and meningitis, and is linked with community associated as well as nosocromial infections.[5,6] It can also serve as a genetic reservoir for plasmid-mediated antibiotic resistance that can be transferred to other pathogens and thus, represent on important index pathogen for understanding the epidemiology of antibiotic resistance.[7]

Urinary tract infections(UTI) are the major common bacterial infection in humans after respiratory tract infection because of a wide used of broad-spectrum antibiotics and indiscriminate use of antibiotics.[8] Thus, it accounts for approximately 200 million cases across the globe yearly, with females having higher (40%) prevalence rates than males (12%) that experiencing one symptomatic episode of the urinary tract infection in their lifetime.[9] Infections with *E. coli* is associated with increased length of hospital stay, higher cost of care, drain on limited resources, and high rates of morbidity and mortality.[2] UTIs are mostly caused by Gram-negative bacteria; thus, the foremost pathogen responsible for uncomplicated cystitis and pyelonephritis is *Escherichia coli* followed by other species of *Enterobacteriaceae*, like *Proteus mirabilis* and mostly *Klebsiella pneumoniae*, and Gram-positive pathogens includes *Enterococcus faecalis* and *Staphylococcus saprophyticus*.[10] Further, high recurrence rates and increasing antimicrobial resistance among uropathogens threaten to greatly increase the economic burden of these infections.[10] Also, UTIs are the most frequent infectious diseases affecting humans and represent an immense public health threat with a substantial economic onus. However, UTIs are responsible for approximately 7 million physician visits annually with 15% of all community-prescribed antibiotics.[11]

Multidrug-resistant organisms (MDROs) pose a global public health threat, increasing in community-acquired and hospital-acquired infections, with prevalence varying by region.[12] Multidrug-resistant bacteria are the principal cause of failure in the treatment of infectious diseases, resulting in increases in the term and magnitude of mobility, higher rates of mortality, and a greater health cost burde.[13]

Multidrug resistance (MDR) is the ability of a microorganism (like bacteria, viruses, fungi, or parasites) or a cell (like a cancer cell) to resist the effects of multiple different drugs.

MATERIALS AND METHODS

This study was conducted at the National Orthopaedic Hospital, Enugu State, Nigeria. The National Orthopaedic Hospital, Enugu (NOHE), established on January 17, 1975, is a federal government specialty hospital located in Enugu State, Nigeria. A descriptive and cross-sectional study design was used in this study which involved adults with urinary tract infections. A consecutive convenience sampling technique was used to recruit participants for urine samples from adults with urinary infections.

Method of Sample Collection

Every adult with urinary infections who came to the hospital was recruited. The clean-catch midstream technique was used to obtain a sterile, uncontaminated sample in a sterile universal bottle was employed. A structured questionnaire was used in the study to collect demographic data and medical history of the participants involved in the study. For individuals with an indwelling urinary catheter, the tube is clamped before the sample is extracted using a sterile procedure.

Microbial Analysis

Isolation and Identification of *E. coli* was done using MacConkey agar, CLED and Chrome agar culture media. The selective media show unique peculiarities for *E. coli*, while MacConkey agar media is a lactose fermenter, which has the potency of fermenting lactose and gives a pink to red coloration colonies.

Isolation of *Escherichia coli*

Urine samples were collected from the participants and inoculated directly onto MacConkey, CLED, and Chrome agar using the streak plate method. The plates were thereafter incubated at 37^0C for 24 hours in an inverted position. Also, identification of the *E. coli* was done using biochemical test procedures

Purification of the isolates

The culture plates that showed discrete colonies were selected after 24 hours, and aseptically, the colonies were streaked on a sterile culture plates that were prepared according to the manufacturer's description. The streaked plates were placed in a bacteriological incubator in inverted positions at 37^0C for 24 hours as described in.[14]

Characterization of the pure isolates.

The pure isolates were characterized using morphological and biochemical characteristics:

Gram staining, motility test, and biochemical reactions.

The following biochemical tests were carried out: indole test, catalase test, urease test, oxidase test, citrate test, coagulase test, and the following sugar fermentations were done: H_2S production, glucose, maltose, sorbitol, methyl red, voges-proskauer etc

Antimicrobial Susceptibility Testing

Antimicrobial Susceptibility Testing (AST) is a laboratory procedure that determines the effectiveness of antimicrobial drugs, such as antibiotics, against specific bacteria causing an infection. The antibiotics used include: ofloxacin (OFX), pefloxacin (PEF), ceftriaxone (CEF), nalidixic acid (NA) and trimethoprim-sulfamethoxazole (SXT), ciprofloxacin (CPX), penicillin (PN), cefuroxime (CEP), amoxicillin-clavulanate (AU), gentamicin (CN), streptomycin (S), nitrofurantoin (N).

Modified Kirby-Bauer disc diffusion method was used to determine the antibiotic susceptibility of isolates already identified and confirmed by biochemical tests.

Discrete colonies of isolates on solid agar media was emulsified in 3ml of sterile physiological saline and the turbidity adjusted to 0.5 McFarland standards. The standardized suspensions were inoculated on Muller Hillton agar using a sterile swab to ensure even distribution and confluent growth. The antibiotic disc was aseptically placed using antibiotic dispenser. A pre-diffusion time of 30 minutes was allowed and the plate incubated at 37^0 C for 24 hours. After incubation, the inhibition zone diameter produced by each antibiotic disc was measured to the nearest millimeter and recorded.

The results were interpreted according to the Clinical and Laboratory Standards and Institute

guideline (CLSI 2024). This diameter was then compared to pre-defined clinical breakpoints.

Ethical Approval

An ethical approval was obtained from the research ethics committee of Orthopedic hospital Enugu, Enugu State, Nigeria.

Sample Size Calculation

A prevalence rate of 18.8% was adopted based on the findings of Okafor and Nweze[16], who investigated the occurrence and antibiotic susceptibility of *Escherichia coli* isolated from patients diagnosed with urinary tract infections (UTIs) in Nsukka, southeastern Nigeria. This figure reflects the burden of UTI within the studied population and provides a reliable estimate to be used in calculating the sample size for similar epidemiological research. Utilizing this prevalence in the sample size formula ensures that the study is adequately powered to detect meaningful associations or differences while minimizing the risk of Type II error. Thus, the sample size was calculated using the[41] formula using a true prevalence of the said population.

N= $(Z^2 \times P \times (1 - P)) / d^2$

Z: confidence level, P: expected prevalence, d: margin of error, and N= sample size

Sample size determination for patients with urinary tract infection using the prevalence as shown above:

N= $(1.96)^2$ X 0.188 X (1-0.188) / 0.05^2

N= 0.586 / 0.0025

N= 234.4

So the Sample size calculated was **234**.

Genotypic identification of resistance genes and sequencing of the isolates

The multidrug isolates that were positive for ESBLs were selected and screened for blaTEM, blaSHV, and blaCTX-M for amplification of resistance genes using the polymerase chain reaction technique. The protocol for the molecular analysis was done according to the method described by.[42] The polymerase chain reaction (PCR) was carried out using the One Taq Quick Load 2X Master Mix with Standard Buffer (New England Biolabs, MA, U.S.A.), which is composed of 20 mM Tris-HCI, 1.8 mM MgCl2, 22 mM NH4Cl, 22 mM KCI, 0.2 mM dNTPs, 5%glycerol, 0.06% IGEPAL CA-630,0.05% Tween 20,Xylene Cyanol FF,Tartrazine, and 25 units/ml of Taq DNA polymerase. This was vortexed at low speed and placed in a thermal cycler machine, with cycling parameters and primers used.

Multiplex PCR was used to detect the genes for SHV and CTX-M, while conventional linear PCR was used for the BlaTEM type ESBL gene.

The PCR products were analyzed on a 1.5% agarose gel stained with ethidium bromide (1µg/mL), with a 100 bp DNA ladder (New England Biolabs, USA) used as the DNA molecular weight marker. Electrophoresis was carried out at 90 volts until the dye front reached the other end of the gel (about 1 hour 20 mins). The gel was visualized under an ultraviolet trans-illuminator.

Data Analysis

Data obtained was summarised using descriptive statistics of mean and standard error of the mean. Risk factors, prevalence and susceptibility, and their associations

with multi-drug resistance were analysed using Chi-square, Pearson's correlation, and multiple logistic regression analysis. Values were considered significant at $p \leq 0.05$.

RESULTS

The cultural and morphological characteristics of the isolates (A's, B's, C's, D's, and E's) were determined, and it was revealed that the isolates appeared indifferent. Moreover, they all varied in colony sizes and elevations. All isolates were gram-negative rods arranged in single pairs, except for isolate C's, which was cocci in clusters (Table 1).

Table 2 show the biochemical characteristics of the isolates, presenting the results of the catalase test, oxidase test, coagulase test, indole test, citrate test, and sugar fermentation reactions.

The confirmed bacteria were *Escherichia coli* (60), *Klebsiella pneumoniae* (23), *Staphylococcus aureus*(90), *Proteus spp.* (17), and *Pseudomonas aeruginosa* (17).

Figure 1 shows the distribution of other bacterial organisms co-isolated with *Escherichia coli* from the urine culture plates. A total of 230 urine samples were obtained from adults diagnosed with UTI. Out of the 230 samples, 26.08% (60/230) were *E. coli*. The most frequently co-isolated organism was *Staphylococcus aureus* 39.13% (90/230), followed by *Pseudomonas aeruginosa* and *Proteus species*, which had 7.39% (17/230) each; while *Klebsiella pneumoniae* and others accounted for 10% (23/230). Therefore, the *E. coli* (60) isolates were used for further study.

Figure 2 illustrates the proportion of multidrug-resistant (MDR) and non-MDR *Escherichia coli* isolated from urine samples of adult patients with urinary tract infections. Out of a total of 60 *E. coli* isolates, 35 (58.33%) were non-MDR, while 25 (41.67%) were classified as multidrug-resistant. The drug susceptibility pattern of MDR *E. coli* (n=60) in the urine of adults with urinary tract infections is represented in Table 3.

Isolates showed high resistance to Penicillin and Streptomycin (86. 6% each), Nalidixic acid (85.0%), and Trimethoprim-Sulfamethoxazole and Amoxicillin-Clavulanate (83.3% each). Moderate resistance was observed for Cefuroxime (45.0%), Ofloxacin (23.3%), Ceftazidime (25.0%), and Ceftriaxone (20.0%), with low resistance to Pefloxacin (10.0%), Ciprofloxacin and Gentamycin (3.3%) respectively and Nitrofurantoin (1.66%).

Table 4 shows the prevalence and percentage of multidrug resistant *E. coli* isolates with demographic characteristics. Out of the 230 participants, 169 (73.47%) were females and 61 (26.52%) were males. Out of the sixty (60) isolated *E. coli*, forty-two (42) were from females, with eighteen 18 (42.85%) positive for multidrug resistance, while eighteen (18) were from males, with seven 7 (38.88%) positive for multidrug resistance. From the results, females had more positive urinary tract infections with a higher number of multidrug-resistant *E. coli*.

The participants ages ranged from 18 to 65 years, with the highest representation in the 36 – 45 years age group (31.14%), followed by 46 – 55 years (28.0%), 18 – 25 years

(24.0%), 26 – 35 years (21.27%), and 56 – 65 years (25.0%). Regarding the duration of illness, 23.07% had UTIs for 1 – 6 months, 36.36% for 7 to 12 months, and 50% for more than 12 months. In term of the level of education of the participants, primary education accounted for 30.76%, secondary for 23.39%, while tertiary education accounted for 34.7%. The majority of the sample types were midstream urine.

Table 5 shows the association of demographic clinical factors with MDR *E. coli* in adult urine. The results show that there was no statistically significant ($p>0.05$) association between gender and age in non- MDR *E. coli.* Moreover, the duration of illness shows a higher percentage (50.0%) in those with illness lasting over 12 months. There was no statistically significant ($p>0.05$) association between the duration of illness and MDR *E. coli*.

Figure 3 illustrates the genetic analysis of the MDR *E. coli* isolates. The gel bands show that all samples tested positive for the blaTEM gene except for samples 1 and 6, which did not exhibit visible bands at the 459 bp region. The presence of the bla_{TEM} gene in the majority of isolates suggests a high prevalence of ESBL-producing *E. coli*, which contributes significantly to antibiotic resistance.

Figure 4 presents the gel electrophoresis image showing the amplification results for the blaCTX-M (560 base pairs) and blaSHV (398 base pairs) genes among the samples. The image reveals that only samples 3, 4, and 5 showed positive bands for the blaCTX-M gene, indicating the presence of this ESBL gene in those isolates. In contrast, none of the samples exhibited bands at the 398 bp region, suggesting that all tested isolates were negative for the blaSHV gene.

Table 1: Cultural and Morphological Characteristics of the Isolates

PARAMETERS	**A's**	**B's**	**C's**	**D's**	**E's**
Appearance	Pink/Red	Cream	Golden yellow	Colourless	Greenish
Motility	Motile	Non-mobile	Non-motile	Highly motile	Motile
Shape	Rods	Rods	Cocci	Rods	Curved rods
Arrangement	Single/Pairs	Single/Pairs	Cluster	Single/Pairs	Single/Pairs
Edge	Smooth	Mucoid	Smooth	Swarming	Metallic
Size	1-3µm long by 0.4 – 0.7 µm wide	1 - 2 µm long by 0.5 – 0.8 µm wide	0.8 - 1 µm	0.4 – 0.6 µm wide by 1-3 µm long	1.5 - 3 µm long by 0.5-0.8 µm wide
CLED	Yellow	Yellow/muoid	Yellow	Blue/green	Green
MacConkey	Pink/Red (hf)	Pink/Mucoid	No growth	Colourless/Pale(hf)	Colourless
Chrom Agar	Pink/Magenta	Blue-green, Mucoid	Golden-Yellow	Brownish/light amber	Greenish/Grey
Bacterium	***E. coli***	***Klebsiella Pneumonia***	***Staphlococcus aureus***	***Proteus spp***	***Pseudomonas Aeruginosa***

Table 2: Biochemical Characteristics

Parameter	**A's**	**B's**	**C's**	**D's**	**E's**
Gram reaction	Gram negative rod	Gram negative rod	Gram positive cocci (cluster)	Gram negative rod	Gram negative rod
Catalase	+	+	+	+	+
Oxidase	-	-	-	-	+
Urease	weakly+	+	-	Strong+	-
Indole	+	-	-	+	-
Methyl red	+	-	-	+	-
Voges-Proskauer	-	+	-	+	-
Citrate	-	+	-	+	+
H_2S production	-	-	-	+	-
Coagulase	-	-	+	-	-
Glucose	+	+	-	+	-
Maltose	+	+	+	+	-
Xylose	+	+	-	+	-
Dulcitol	-	+	-	Variable	-
Inositol	-	+	-	Variable	-
Sorbitol	+	+	Variable	Variable	-
Bacterium	***E. coli***	***Klebsiella Pneumonia***	***Staphyloccus aureus***	***Pseudomonas aeruginosa***	***Proteus spp***

Key: Variable= reaction depend on strain/specie. Positive (+) =reaction, Acid(medium twin yellow) and/or gas (bubble in durham tube). Negative(-)= No reaction, No change or alkaline(medium remain red/pink)

Fig 1: The prevalence of other bacteria Isolated with *E. coli*

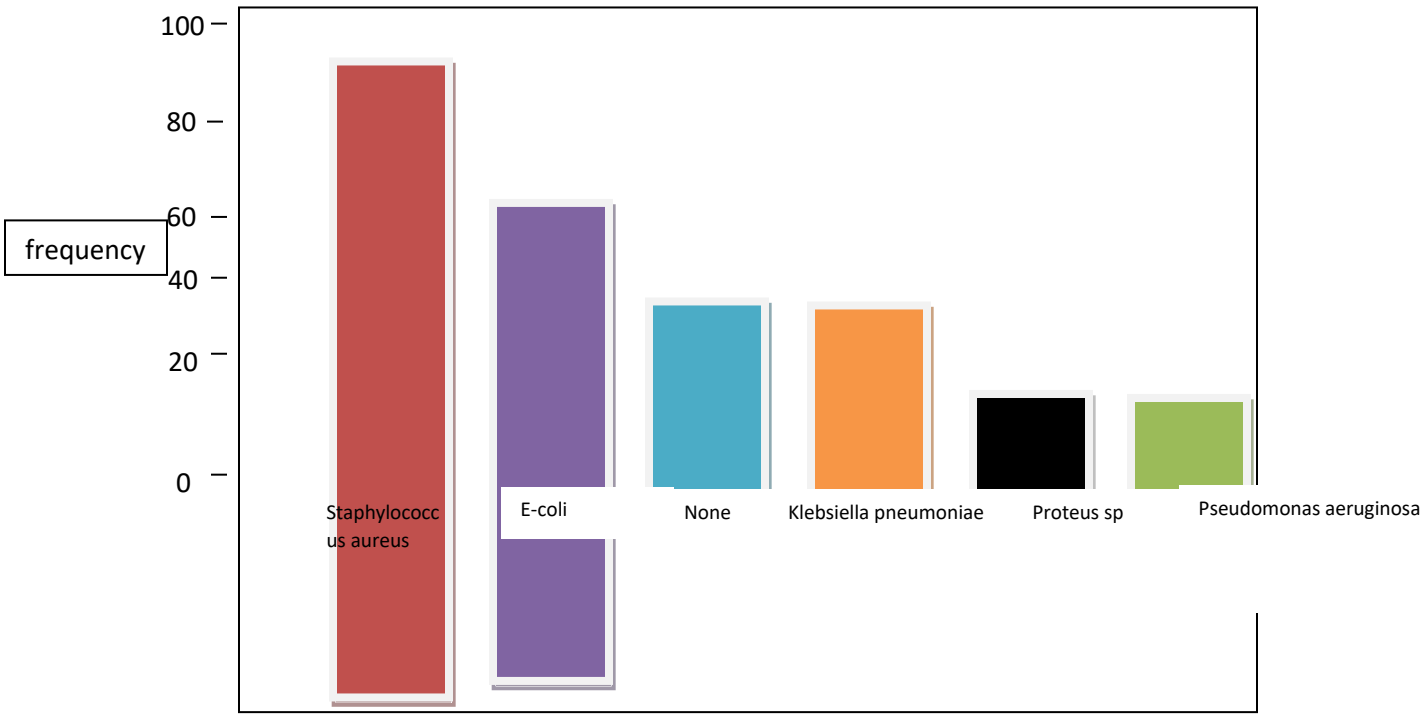


Fig 2: The prevalence of MDR *E. coli* in urine of Adults with Urinary Tract Infections

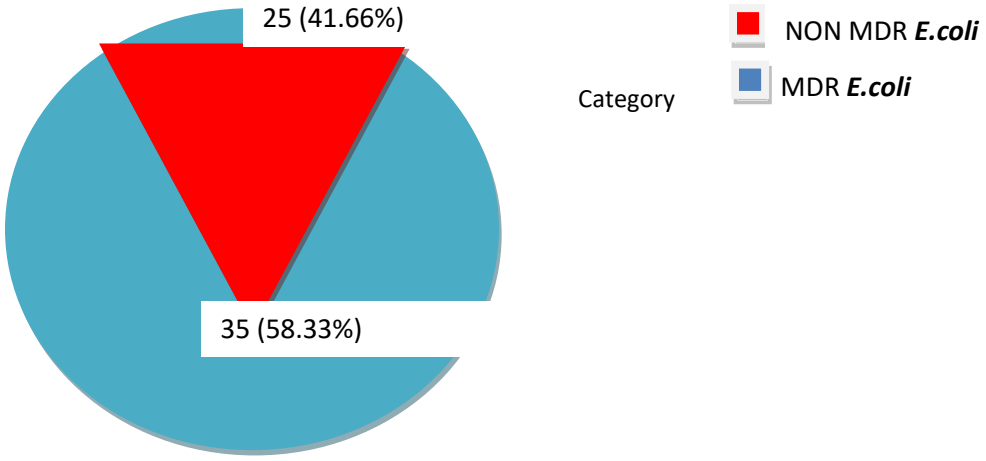


Table 3: **Drug Susceptibility Pattern Of MDR *E. coli* In Urine Of Adults With Urinary Tract Infections.**

Antibiotics	Number Of *E. coli* Isolated (n=60)			
	Sensitive	Intermediate	Resistant	% Resistance
CPX	57	1	2	3.3
N	59	-	1	1.66
NA	1	2	57	85
CN	57	1	2	3.3
S	6	2	52	86.6
SXT	2	8	50	83.3
PN	5	3	52	86.6
AU	6	4	50	83.3
PEF	53	1	6	10.0
OFX	46	-	14	23.3
CEP	33	-	27	45.0
CEF	38	10	12	20.0
CFZ	35	11	15	25.0

Key: CPX= Ciprofloxacin, N=Nitrofurantoin, NA= Nalidixic acid, CN=Gentamicin, S=Streptomycin, SXT=Trimethoprim-Sulfamethoxazole, PN=Penicillin, AU=Augmentin-Clavulanate, PEF= Pefloxacin, OFX=Ofloxacin, CEP=Cefuroxime, CEF=Ceftriaxone, CFZ=Ceftazidine.

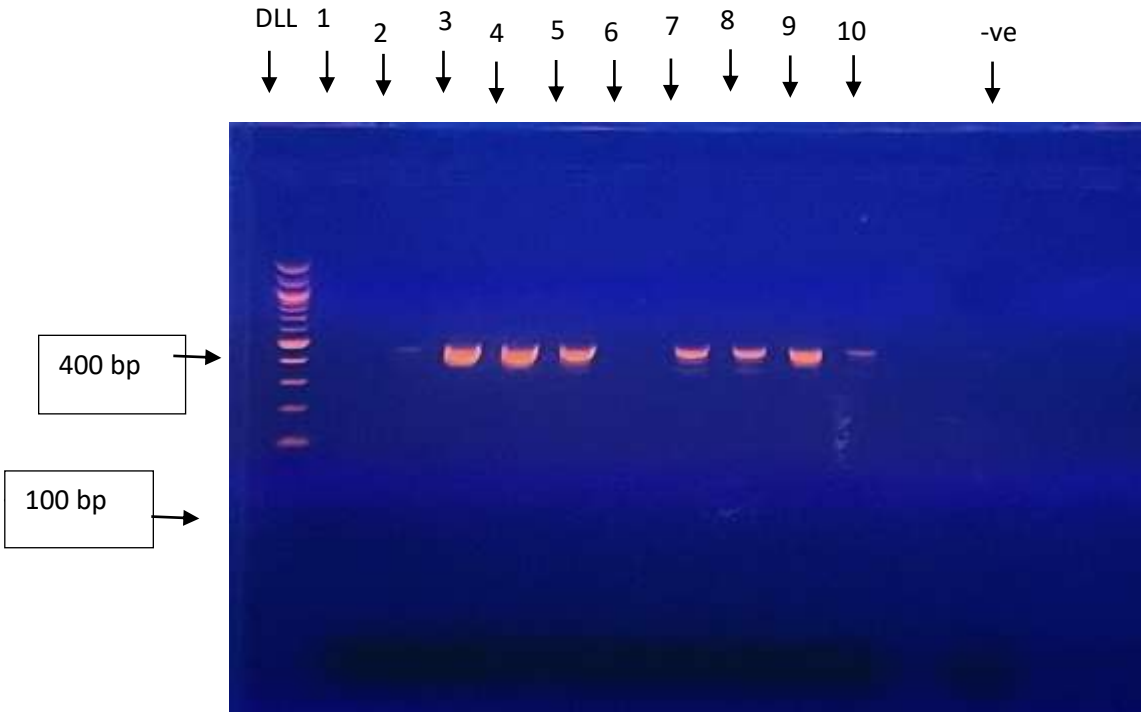
Table 4: PREVALENCE OF MULTIDRUG RESISTANCE *E. COLI* ISOLATES WITH DEMOGRAPHIC CHARACTERISTICS.

Parameter (Total Number)	*E. coli* Isolated(%)	MDR E. coli (%)
Gender		
Female	42 (24.9)	18 (42.85)
Male	18 (29.51)	7 (38.88)
Total	60 (26.08)	25 (41.66)
Age		
18 – 25 years	12 (24.0)	6 (42.85)
26 – 35 years	10 (21.27)	5(41.66)
36 – 45 years	18 (31.14)	5 (30.0)
46 – 55 years	14 (28.0)	7 (57.14)
56 – 65 years	3 (25.0)	2 (40.0)
Duration of illness		
1 – 6 months (182)	42 (23.07)	18 (42.85)
7 – 12 months (44)	16 (36.36)	6 (37.5)
Above 12 months (4)	2 (50.0)	1 (50.0)
Sample type		
Midstream (228)	59 (25.43)	24 (41.37)
Catheterization (2)	2 (100)	1 (50.0)
Level of Education		
Primary (13)	4 (30.76)	3 (75.0)
Secondary (171)	40 (23.39)	16 (40.0)
Tertiary (46)	16 (34.7)	6 (37.5)
Mean duration +/- SD		

Table 5: Association of Demographic and Clinical factors with MDR *E. coli* in Adult Urine Samples

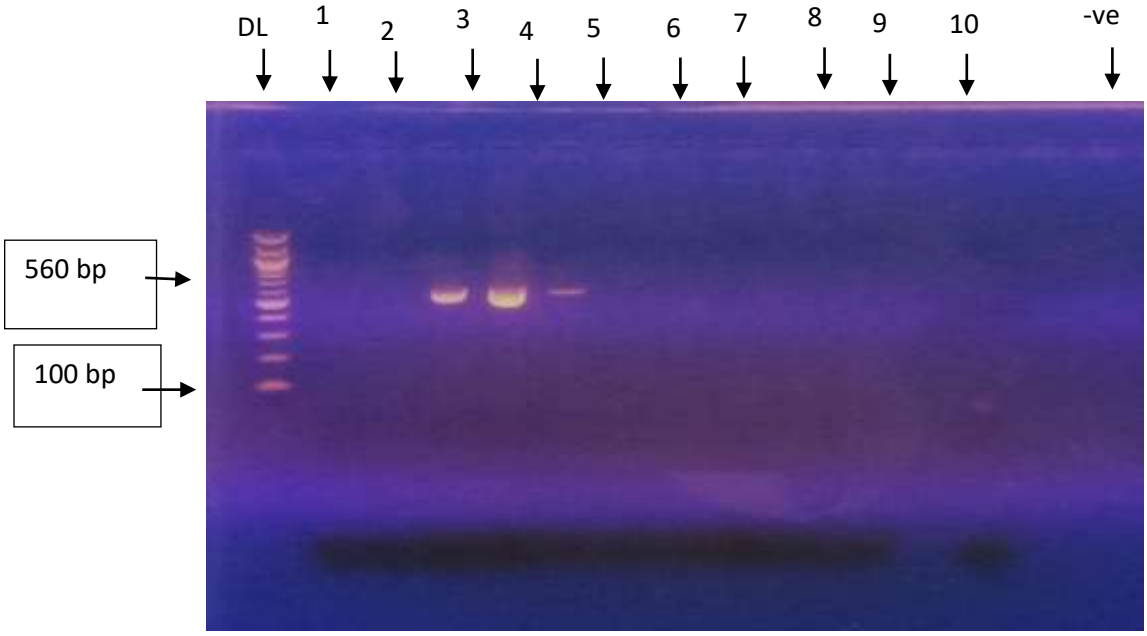
Par ammeters	Non-MDR *E. coli*	MDR *E. coli*	Total	χ^2	p-value
Gender					
– Female	24 (40.0%)	18 (30.0%)	42	0.03	0.866
– Male	11 (18.33%)	7 (11.66%)	18		
Age Range					
– 18–25 Years	6 (17.1%)	2 (8.0%)	8	7.75	0.051
– 26–35 Years	5 (14.3%)	4 (16.0%)	9		
– 36–45 Years	13 (37.1%)	12 (48.0%)	25		
– 46–55 Years	8 (22.9%)	4 (16.0%)	12		
– 56– 65 Years	3 (8.6%)	3 (12%)	6		
Sample Type					
– Catherterization	2(3.1%)	2 (5.0%)	4		
– Midstream	33 (96.9%)	23(95.0%)	56		
Duration Of Illness					
– 1–6 Months	6 (23.07%)	6 (25.0%)	12	29.35	0.000*
– 7–12 Months	12 (36.36%)	6 (25.0%)	18		
– Above 12 Months	18 (50.0%)	13 (50.0%)	30		

Fig 3: blaTEM Gene (459bp) Gel Image



Key:
DL= DNA Ladder
-ve= Negative Control.

Fig 4: blaCTX-M Gene(560bp) and blaSHV gene (398bp) gel image



Key:
DL= DNA Ladder
-ve= Negative Control

DISCUSSION

Escherichia coli are very important pathogens of public health importance affecting both children and adults worldwide.[17] A total of 230 adult urine samples were processed to determine the prevalence, molecular types, and susceptibility rate of multidrug resistance patterns of *Escherichia coli* in the study area. One hundred and sixty-nine (73.47%) were female and sixty-one (26.52%) were male, aligning with prior studies that documented that urinary infections (UTIs) are more common in females due to anatomical predisposition, such as a shorter urethra and proximity to the anal region.[18,19] The work also agrees with,[20] which also reported a high prevalence rate of *E. coli* in females.

The age distribution was broad with most participants falling within the age brackets of 36 – 45 years (31.14%) and 46 - 55 years (28.0%). This middle-aged group is often exposed to factors such as hormonal changes, chronic illness, and hospital visitations that increase UTI risk.[21]

The study highlighted that 42 (23.07%) participants with *E. coli* isolates had UTI cases that persisted between 1 and 6 months, and 16 (36.36%) persisted between 7 and 12 months, while 2 (50.0%) persisted for more than 12 months. This chronicity may reflect delayed healthcare-seeking behavior, empirical antibiotic use, or recurrent infections linked to resistant

organisms. According to,[22] recurrent UTIs, particularly in women, are often exacerbated by self-medication and inappropriate antibiotic usage, factors prevalent in low-resource settings like Nigeria. The isolation of *E. coli* from 26.08% of adult urine samples reinforces its role as one of the common uropathogens, as substantiated by previous findings that *E. coli* accounts for the majority of UTIs globally.[23,24]

This study also revealed that participants whose level of education was secondary school had a higher number of *E. coli* isolates, followed by those with tertiary education, with sixteen (16) isolated *E. coli.* Out of the sixty (60) *E. coli* isolates, 25 (41.66%) were multidrug-resistant.

In this study, the highest drug resistance (86.6%) in *E. coli* was recorded for penicillin and streptomycin, respectively. Higher resistance (81% - 100%) of *E. coli* to penicillin has been reported by many researchers.[20,25,26,27,28 and 29] It has been suggested that high resistance to penicillin may have been caused by the presence of the beta-lactamase enzyme in the bacteria or as a result of the reduction of affinity of existing penicillin binding proteins.[30] Another possible reason for the high resistance to penicillin is the frequent use or misuse due to its easy availability and affordability.[31] Also implicated in antibiotic resistance is non-adherence to standard treatment procedures, especially in sub-Saharan Africa, the use of fake or substandard drugs, and the unauthorized sale of antibiotics without appropriate prescriptions.[32,33]

A relatively low resistance level was observed with nitrofurantoin (1.66%), and ciprofloxacin (3.3%). This result is in line with the studies of[34 and 35] who also recorded a low resistance rates against these antibiotics.

Out of the twenty-five (41.66%) MDR *E. coli* isolates, ten (10) were selected for further analyses to identify the genes responsible for the resistance using multiplex and linear PCR.

The molecular findings from the gel electrophoresis images showed that among the Extended Spectrum Beta-Lactamases (ESBL) genes screened, blaCTX-M (560 bp) was present in three isolates, while no bands were observed for blaSHV (398 bp).

This corroborates findings by[36] who documented a surge in blaCTX-M-15 among Nigerian isolates, especially in multidrug-resistant (MDR) strains.

Interestingly, the blaTEM gene was also detected in the isolates, with a band at 459 bp, confirming its role in beta-lactam resistance, as observed by.[37] These ESBL genes are often plasmid-mediated and associated with horizontal gene transfer, contributing to the rapid spread of resistance among Enterobacteriaceae.[38,7] The predominance of these genes in a specialized tertiary hospital population—such as an orthopedic hospital—highlights the risk posed by nosocomial infections and prolonged catheterization.[39]

Furthermore, the lack of detection of blaSHV aligns with observations in other Nigerian studies, where CTX-M has increasingly displaced older ESBL genotypes such as SHV and TEM in terms of frequency and distribution.[8, 23] This evolutionary shift may be attributed to selective antibiotic pressure in the hospital

environment and the clonal expansion of successful lineages such as ST131, which are commonly associated with CTX-M-15 production.[40]

CONCLUSION

This study concludes that *Escherichia coli* remains the most prevalent causative agent of urinary tract infections among adults attending the National Orthopedic Hospital, Enugu, with a concerning level of multidrug resistance. The bacterial isolates demonstrated high carriage rates of resistance genes such as blaCTX-M and blaTEM, confirming the spread of extended-spectrum beta-lactamase (ESBL)-producing strains within the hospital environment. The absence of the blaSHV gene suggests a possible regional decline in its prevalence, in contrast to the growing dominance of CTX-M genes. The predominance of these ESBLs, especially blaCTX-M, reflects the global trend of the rapid emergence of resistant clones like ST131, known for their clinical severity, persistence, and limited therapeutic options. The findings of this study underscore the threat that multidrug-resistant *E. coli* poses to the effective management of UTIs in both hospital and community settings.

The study also reinforces the need for the integration of molecular diagnostic tools into clinical microbiology laboratories for early detection of resistance genes and targeted therapy. In conclusion, this research emphasizes the urgent necessity for institutional and national strategies that include antimicrobial stewardship, public health education, improved diagnostic infrastructure, and infection control protocols to contain the threat of antimicrobial resistance, particularly in specialized hospital settings like orthopedic units.

Competing Interest: Authors have no conflict of interest.

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PHYSICAL ACTIVITY LEVEL, PERCEIVED BARRIERS AND FACILITATORS AMONG WOMEN IN NNEWI, ANAMBRA STATE

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Abstract

Background: Physical inactivity is a major public health concern and a significant risk factor for non-communicable diseases, particularly among women in low- and middle-income countries where sociocultural and environmental demands may limit physical activity participation.

Aim: This study assessed the levels of physical activity, perceived benefits, and perceived barriers to physical activity among adult women residing in Nnewi, Anambra State, Nigeria.

Materials and methods: A cross-sectional study design involving 400 adult women selected from the four quarters of Nnewi was adopted. The International Physical Activity Questionnaire-Short Form (IPAQ-SF) assessed physical activity level, while the Exercise Benefits and Barriers Scale (EBBS) assessed perceived benefits and barriers. Data was summarized using frequency counts, charts, mean and standard deviation while the Kruskal-Wallis test was used for analysis at the <0.05 level of significance.

Results: The mean age of participants was 30.77±11.15 years. Participants demonstrated high perceived exercise benefits (mean score 89.12±20.05) and moderate perceived barriers (36.10±9.15). Modal reported walking and moderate physical activity duration was less than 30 minutes to 1 hour. Respondents who reported the highest sitting period also recorded the highest benefits (p=0.003) yet more barriers to walking (p<0.001).

Conclusions: Women in Nnewi exhibit favourable perceptions of exercise benefits but relatively low engagement in vigorous physical activity, with notable barriers linked to sedentary behaviour and daily responsibilities. Targeted interventions addressing contextual barriers and promoting active lifestyles are recommended to improve physical activity participation among women in this population.

Keywords: Physical activity, Exercise benefits, Exercise barriers, Women, Sedentary behaviour, Nnewi, Nigeria

INTRODUCTION

Physical activity (PA) refers to any bodily movement produced by skeletal muscles that requires energy expenditure(1). World Health Organization (WHO) guidelines recommend that adults aged 18–64 do at least 150–300 minutes of moderate-intensity or 75–150 minutes of vigorous-intensity aerobic activity per week (or an equivalent combination) plus muscle-strengthening activities on two or more days each week [2]. Regular physical activity provides significant health benefits by preventing and managing non-communicable diseases such as heart disease, stroke, diabetes, and some cancers, while also improving mental health and overall well-being [1].

However, about one in three adults globally does not meet the recommended PA levels, with women generally being less active than men, as surveys indicate that approximately 5% more women than men fail to reach these levels[1]. In Nigeria, physical inactivity appears to be rising, with a 2022 meta-analysis estimating that about 52% of adults are insufficiently active, with higher rates in women (56%) than in men (approximately 49%), and particularly in urban areas (nearly 57%) compared to rural areas (about 19%) [3].

In sub-Saharan Africa (SSA), increasing urbanization, changes in transportation patterns, occupational shifts, and limited supportive environments for walking and recreation have been linked with reduced movement and increasing sedentary lifestyles. Evidence suggests that transport-related physical activity (e.g., walking/cycling) is a meaningful contributor to overall wellness in SSA, but it depends strongly on safety, infrastructure and built-environment support [4]. Among women of reproductive age in urban SSA, determinants of PA commonly include time constraints, competing responsibilities, socioeconomic

conditions, perceptions of safety, social norms, and access to facilities [5].

Gender norms and expectations in some communities may discourage women from engaging in adequate PA or allocating personal time for PA. Sociocultural factors such as heavy household and caregiving responsibilities also affect Nigerian women's PA, potentially limiting their time for leisure exercise [6]. Unfortunately, inadequate PA level is an important modifiable risk factor for cardiovascular diseases and several other chronic non-communicable diseases.

Nnewi, a major urban-industrial city in Anambra State with a population of over one million, is known for its vibrant trade and industry but has limited public recreational infrastructure. Although community exercise gatherings reflect a strong sports culture, the lack of accessible public sports facilities, combined with women's responsibilities at work, household duties, and childcare, as well as social norms that emphasize domestic roles over formal exercise, highlights the absence of published data on how these factors affect women's physical activity [7].

Therefore, this study aimed to assess the levels of physical activity, perceived benefits, and perceived barriers to physical activity among adult women residing in Nnewi, Anambra State, Nigeria.

MATERIALS AND METHODS

Study Design

This study employed a cross-sectional survey design.

Study Population

The respondents of this study were adult women aged 18–59 years residing in Nnewi town, Anambra State of Nigeria who gave their consent to be used for the study.

Inclusion Criteria

Women aged 18–59 years who were residents of Nnewi for at least one year and provided informed consent were included.

Exclusion Criteria

Women with physical or cognitive impairments that prevented them from engaging in physical activity or completing the questionnaire were excluded.

Sampling Technique

A quota sampling technique was used to select an equal number of women from selected communities from each of the four quarters of Nnewi namely: Otolo, Uruagu, Nnewi-ichi, and Umudim. The fishbowl method was also used to randomly select two communities from each quarter. Female residents of the selected communities who met the inclusion criteria were consecutively recruited into the study.

Sample Size Determination

The sample size was determined using Taro Yamane's formula:

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

Where:

- n = required sample size
- N = total population of women aged 18–59 in Nnewi North (55,493)[15]
- e = margin of error (0.05 for 95% confidence level)

$n = 397.22$

Thus, the minimum required sample size was 400 women.

Research Instruments

International Physical Activity Questionnaire-Short Form (IPAQ-SF): The IPAQ-SF is a standardized 7-item self-report instrument for adults that assesses physical activity over the past 7 days by asking respondents to report the number of days and time spent in walking, moderate-intensity, and vigorous-intensity activities (each lasting at least 10 minutes), as well as daily sitting time [8]. The IPAQ-SF has demonstrated acceptable test-retest reliability with intra-class correlations of 0.642–0.789 for total physical activity [9].

Exercise Benefits and Barriers Scale (EBBS): The EBBS is a 43-item instrument designed to assess individuals' perceptions of the benefits of and barriers to engaging in physical activity. It comprises two subscales: 29 items measuring perceived benefits and 14 items measuring perceived barriers. Each item is rated on a 4-point Likert scale, ranging from "strongly disagree" (1) to "strongly agree" (4) [10]. Psychometric evaluations of the EBBS indicate strong reliability with Cronbach's $\alpha \approx 0.82$ for the overall EBBS [11].

Data Collection Procedure

Ethical approval was obtained from the ethical review committee of the Faculty of Health Sciences and Technology, Nnamdi Azikiwe University, Nnewi Campus before the commencement of this study (Protocol Number: FHST/REC/024/1063). Research assistants were recruited and trained by the researcher. The details of the study were explained to the participants. Participants' confidentiality was assured, and informed consent was obtained. Voluntary participation and freedom to withdraw from the study were duly emphasised. The questionnaires were distributed to the participants by the researcher and retrieved after completion.

Data Analysis

The obtained data were summarized using descriptive statistics of mean, frequency table, percentage, range, standard deviation, and bar chart. The Kruskal-Wallis test was used to test the hypotheses. The level of significance was set at 0.05.

RESULTS

Sociodemographic Characteristics of Participants

A total of 400 female adult residents of Nnewi participated in this study. The mean age of the participants was 30.77 ± 11.15 years, with ages ranging from 17 to 65 years. Regarding marital status, 49.8% were single, 45.3% were married, 3.5% were widowed, 1.0% were divorced, and 0.5% were classified under other categories. In terms of educational attainment, 63.5% had secondary education, 27.8% had tertiary education, 5.0% had primary education, 3.0% had postgraduate education, and 0.3% had no formal education. Occupational distribution showed that participants were engaged in diverse economic activities: business (25.5%), trading (17.5%), students (14.8%), fashion industry (11.3%), beauticians (10.8%), caterers (7.2%), healthcare providers (4.5%), civil servants (3.8%), and unemployed (2.8%). Participants were almost evenly distributed across the four quarters of Nnewi: Nnewichi (24.8%), Uruagu (24.5%), Umudim (25.5%), and Otolo (25.3%) (Table 1).

Table 1 Participants' Sociodemographic Profile

Variable	Class	Frequency	Percentage
Marital Status	Single	199	49.8
	Married	181	45.3
	Widowed	14	3.5
	Divorced	4	1.0
	Other	2	0.5
Educational Level	No formal education	1	0.3
	Primary education	20	5.0
	Secondary education	254	63.5
	Tertiary education	111	27.8
	Postgraduate education	12	3.0
Occupation	None (unemployed)	11	2.8
	Trader	70	17.5
	Business	102	25.5
	Student	59	14.8
	Fashion industry	45	11.3
	Caterer	29	7.2
	Healthcare provider	18	4.5
	Beautician	43	10.8
	Civil servant	15	3.8
	Others	6	1.5
Quarter of Residence	Nnewichi	99	24.8
	Uruagu	98	24.5
	Umudim	102	25.5
	Otolo	101	25.3

Physical Activity Levels, Exercise Benefits and Barrier Scores

The mean benefit score recorded by participants was 89.12 ± 20.05, while the mean barrier score was 36.10 ± 9.15 (Table 2). The modal number of days participants engaged in vigorous physical activity was 1 day per week, while the modal number of days for moderate physical activity or walking was 7 days per week.

Regarding duration of physical activity, about one-third of the participants who engaged in vigorous physical activity did so for less than 30 minutes, while a quarter of them engaged in vigorous physical activity for a time range between 30 minutes to 1 hour (Fig. 1). For moderate physical activity, the modal time frame was 30 minutes to 1 hour (39.3%) (Fig. 2). The majority of participants reported walking duration of less than 30 minutes to 1 hour (Fig. 3). The modal sitting time on weekdays was 3 to 6 hours (20.8%) (Fig. 4).

Perceived Exercise Benefits, Barriers, and Associations with Physical Activity Levels

The majority of participants agreed or strongly agreed with most benefit statements, indicating high awareness of the positive effects of exercise. Notable findings include: 89.0% agreed or strongly agreed that "Exercise increases my muscle strength"; 92.5% agreed or strongly agreed that "Exercise increases my level of physical fitness"; 90.8% agreed or strongly agreed that "Exercise improves the functioning of my cardiovascular system"; 87.5% agreed or strongly agreed that "Exercise helps me sleep better at night"; 86.8% agreed or strongly agreed that "I will live longer if I exercise"; 91.7% agreed or strongly agreed that "Exercise improves overall body functioning for me"; and 89.0% agreed or strongly agreed that "Exercise improves the way my body looks."

Regarding perceived barriers, the most commonly endorsed barriers included: "Exercise tires me" (48.3% agreed or strongly agreed); "Places for me to exercise are too far away" (50.6%); "Exercise facilities do not have convenient schedules for me" (38.5%); "I am fatigued by exercise" (39.0%); "Exercise is hard work for me" (30.3%); and "There are too few places for me to exercise" (47.8%). Encouragingly, social barriers such as lack of spousal or family encouragement were less commonly endorsed (18.1% and 17.5%, respectively).

The present study found no significant associations between exercise benefit scores and vigorous physical activity ($p = 0.783$), moderate physical activity ($p = 0.057$), or walking ($p = 0.059$). However, there was a significant association between weekday sitting duration and perceived exercise benefits ($p = 0.003$), with the highest mean rank recorded among persons who reported the highest sitting periods (>12 hours). Conversely, moderate physical activity ($p = 0.039$), walking ($p < 0.001$), and weekday sitting duration ($p = 0.028$) each showed significant associations with perceived barrier scores.

A summary of the key findings on perceived benefits, barriers, and their associations with physical activity levels is presented in Table 3.

Table 2 Mean Age, Benefit and Barrier Scores

Variable	Minimum	Maximum	Mean	Std. Deviation
Age (years)	17	65	30.77	11.145
Days of vigorous PA (per week)	1	7	1.87	0.983
Days of moderate PA (per week)	0	7	4.40	2.221
Days of walking (per week)	1	7	5.41	1.919
Exercise Benefits score	2	116	89.12	20.049
Exercise Barriers score	0	56	36.10	9.150

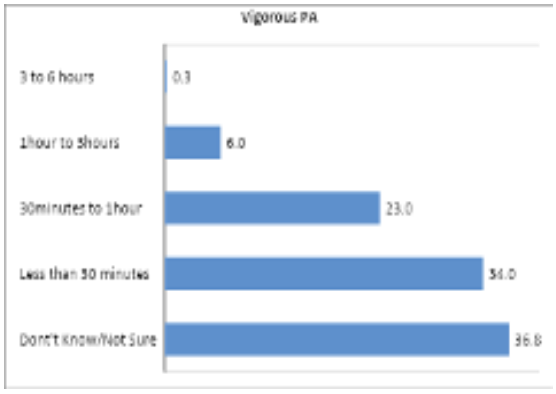

Fig 1 Level of Vigorous PA

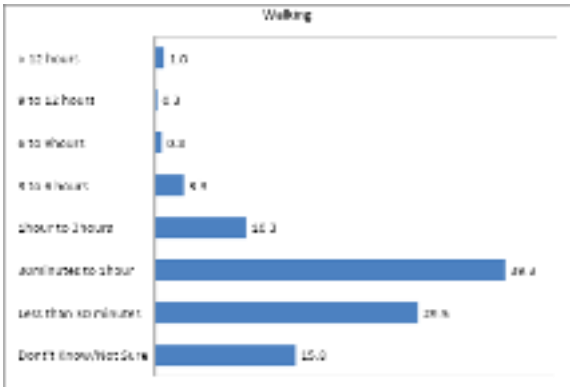

Fig 3 Walking

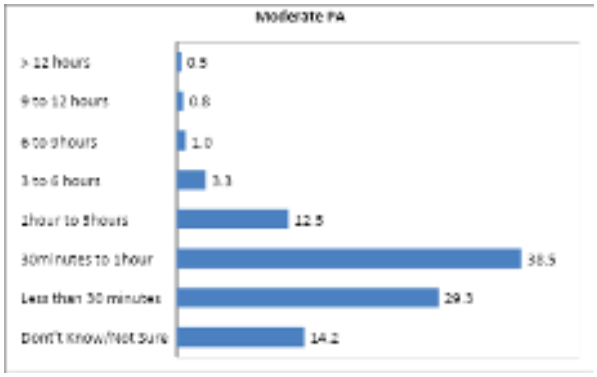

Fig 2 Moderate PA

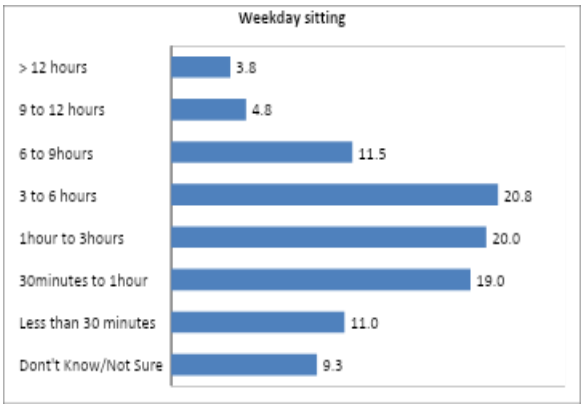

Fig 4 Weekday Sitting

Table 3 Summary of Perceived Exercise Benefits, Barriers, and Associations with Physical Activity Levels

Category	Variable / Item	Finding	tatistical Value
Perceived Benefits	Mean benefit score (max 116)	89.12 ± 20.05	–
	Exercise increases muscle strength	89.0% agreed/strongly agreed	–
	Exercise increases level of physical fitness	92.5% agreed/strongly agreed	–
	Exercise improves cardiovascular functioning	90.8% agreed/strongly agreed	–
	Exercise helps sleep better at night	87.5% agreed/strongly agreed	–
	I will live longer if I exercise	86.8% agreed/strongly agreed	–
	Exercise improves overall body functioning	91.7% agreed/strongly agreed	–
	Exercise improves the way my body looks	89.0% agreed/strongly agreed	–
Perceived Barriers	Mean barrier score (max 56)	36.10 ± 9.15	–
	Exercise tires me	48.3% agreed/strongly agreed	–
	Places to exercise are too far away	50.6% agreed/strongly agreed	–
	Exercise facilities lack convenient schedules	38.5% agreed/strongly agreed	–
	I am fatigued by exercise	39.0% agreed/strongly agreed	–
	Exercise is hard work for me	30.3% agreed/strongly agreed	–
	There are too few places to exercise	47.8% agreed/strongly agreed	–
	Lack of spousal encouragement	18.1% agreed/strongly agreed	–
	Lack of family encouragement	17.5% agreed/strongly agreed	–
Associations with Benefits	Vigorous PA vs. Benefits	Not significant	$p = 0.783$
	Moderate PA vs. Benefits	Not significant	$p = 0.057$
	Walking vs. Benefits	Not significant	$p = 0.059$
	Weekday sitting duration vs. Benefits	Significant	$p = 0.003$
Associations with Barriers	Vigorous PA vs. Barriers	Not significant	$p = 0.575$
	Moderate PA vs. Barriers	Significant	$p = 0.039$
	Walking vs. Barriers	Significant	$p < 0.001$
	Weekday sitting duration vs. Barriers	Significant	$p = 0.028$

DISCUSSION

This study aimed to determine the levels of physical activity as well as perceived exercise benefits and barriers among adult women residing in Nnewi, Anambra State. The study found that most participants had at least secondary school education. Participants reported high perceived benefits of exercise alongside moderate perceived barriers. Most women engaged frequently in moderate-intensity activities and walking, whereas vigorous exercise was much less common.

The predominance of moderate physical activity and walking among women in Nnewi suggests that their activity could be largely incidental and derived from daily functional tasks such as transportation, trading, household chores, and occupational movement rather than structured exercise. This pattern is consistent with reports among Nigerian women where intentional exercise participation remains limited [3]. This finding supports existing evidence that women may meet some physical activity requirements through daily routines but still fall short of recommended levels of vigorous or structured exercise [2].

Perceived benefits of exercise were generally high among participants, reflecting good awareness of its positive effects on physical fitness, cardiovascular health, mental well-being, and overall quality of life. This is consistent with previous findings that women often recognise these benefits even when their participation is suboptimal [10]. The high mean exercise benefit score indicates that participants generally held positive perceptions of physical activity, a finding consistent with studies from low- and middle-income countries showing high awareness of exercise benefits despite low participation levels [3,12].

The lack of significant associations between perceived benefits and most physical activity measures suggests that awareness alone does not increase participation, aligning with behavioural models that highlight environmental and psychosocial constraints on health behaviour change [13]. Women may value exercise but still be unable to prioritise it due to competing demands such as work, childcare, and household responsibilities.

Interestingly, women who reported longer sitting periods also demonstrated higher perceived exercise benefits, which may reflect greater health awareness among those engaged in sedentary occupations such as office work or trading. Similar observations have been reported in urban African populations, where sedentary workers often possess greater health knowledge but face structural barriers to physical activity engagement [3].

Perceived barriers to physical activity were moderately high and significantly associated with moderate activity, walking, and sitting duration, highlighting their influence on actual physical activity behaviour. Barriers such as lack of time, fatigue, distance to exercise facilities, and cost have been consistently identified as major determinants of inactivity among women [14]. In Nigeria, time constraints related to economic activities and

domestic roles remain prominent barriers, particularly for women of reproductive and working age [12].

CONCLUSION

Physical activity among women in Nnewi was marked by low participation in vigorous activities, with higher engagement in moderate activities and walking. Women in Nnewi generally have a high perception of the benefits of physical activity, especially in relation to physical health, mental well-being, and functional capacity. Perceived barriers to physical activity exist at a moderate level and commonly include time constraints, fatigue, limited access to exercise facilities, and cost-related factors. There was no significant relationship between physical activity levels and perceived benefits, indicating that awareness of benefits alone may not be enough to increase participation. Perceived barriers show a significant association with moderate physical activity, walking, and sedentary behaviour, suggesting that barriers influence physical activity participation more strongly than perceived benefits.

RECOMMENDATIONS

Based on the findings of this study, the following recommendations are made:

1. Community-based exercise programmes that respect the culture and traditions of women in Nnewi should be created to help them stay active.

2. Healthcare professionals such as physiotherapists should design practical programmes on how to fit exercise into a busy schedule, even when feeling tired.

3. Further research should be carried out using intervention-based designs and repeated measures to identify effective strategies that can reduce perceived barriers and increase physical activity participation among women.

Ethical Approval and Consent to Participate

Ethical approval for this study was obtained from the ethical review committee of the Faculty of Health Sciences and Technology, Nnamdi Azikiwe University, Nnewi Campus (Protocol Number: FHST/REC/024/1063). The study was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants prior to enrolment. Participants were assured of anonymity and the right to withdraw from the study at any time without consequence.

Availability of Data and Materials

The datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing Interests

The authors declare that they have no competing interests.

Authors' Contributions

NOC and AIA conceptualised the study, NOC and OCC performed data collection, AIA supervised the study, provided methodological guidance,

performed data analysis. APO and AIA drafted the manuscript. OCC and NPO revised the manuscript critically for important intellectual content. NPO supervised the study and provided administrative support. All authors read and approved the final manuscript.

Acknowledgements

The authors thank all the women who voluntarily participated in this study.

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EFFECT OF A SIX-WEEK SUPERVISED AXILLARY CRUTCH WALKING PROGRAM ON SELECTED PHYSIOLOGICAL PARAMETERS IN PATIENTS WITH LOWER LIMB FRACTURES IN ENUGU, NIGERIA

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ABSTRACT

Background: Axillary crutches are commonly prescribed for patients with lower limb fractures to facilitate protected ambulation. Although crutch-assisted gait increases acute metabolic and cardiovascular demand, its longitudinal physiological effects remain unclear. This study investigated whether a six-week supervised axillary crutch walking program would produce measurable changes in selected physiological parameters compared with usual care.

Methods: A randomized pretest–posttest-controlled trial was conducted at the National Orthopaedic Hospital, Enugu, Nigeria. Forty-five patients with lower limb fractures (22 males, 23 females) prescribed axillary crutches were randomized to an experimental group (n = 29), which received supervised crutch walking training three times weekly for six weeks, or a control group (n = 16), which received usual physiotherapy care. Outcome measures included resting systolic and diastolic blood pressure, resting heart rate, estimated VO_2max (derived from resting heart rate), and percentage body fat measured via bioelectrical impedance. Analysis of

covariance (ANCOVA) was used to compare post-intervention outcomes while adjusting for baseline values ($p < 0.05$).

Results: No statistically significant between-group differences were observed after six weeks. Resting systolic blood pressure ($F = 0.363$, $p = 0.64$), diastolic blood pressure ($F = 2.153$, $p = 0.15$), resting heart rate ($F = 0.96$, $p = 0.758$), estimated VO_2max ($F = 0.2$, $p = 0.65$), and percentage body fat ($F = 0.4$, $p = 0.5$) showed no significant group effects.

Conclusion: The six-week supervised axillary crutch walking program did not produce significant cardiovascular or body composition adaptations beyond usual physiotherapy care in patients with lower limb fractures.

INTRODUCTION

Lower limb fractures are among the most common musculoskeletal injuries worldwide and represent a significant cause of functional impairment, reduced mobility, and increased healthcare utilization. Globally, lower limb fractures involving the patella, tibia, fibula or ankle are the most common and burdensome, with incidence rates highest among older adults[1]. Omoke and Ekumankama[2] reported an incidence of 22.6/1000/year for extremity fractures in a Nigerian Teaching Hospital over 12 months from 2016 to 2017, with road traffic accidents and falls from height being the most prevalent causative factors. Following definitive fracture management, such as internal fixation or plaster cast, patients are usually prescribed assistive devices to enable mobility while protecting the healing limb[3, 4].

Axillary crutches are among the most prescribed ambulatory aids in clinical practice, especially for patients with lower extremity injuries requiring non-weight bearing or partial weight bearing on the injured limb. They serve to redistribute body weight from the injured lower limb to the upper extremities, widen the base of support, enhance balance, and reduce ground reaction forces on the affected side[5] Proper crutch fitting is critical to avoid secondary complications such as axillary nerve compression and brachial plexus injury; this is typically 2 inches below the axillary fold, with approximately 30° of elbow flexion, and the tip of the crutch 6 inches away from the fifth toe[6, 5].

The transition from lower-limb to upper-extremity weight-bearing during crutch-assisted gait fundamentally disrupts natural biomechanical efficiency, thereby imposing a significantly higher metabolic and cardiovascular burden[7, 8]. Canter *et al.*[8], reported that, oxygen consumption during axillary crutch ambulation was 7.35 kcal/min compared to 3.06 kcal/min for unassisted walking at a similar pace, representing a 2.4-fold increase in metabolic cost. Heart rate increases are also notable, with post-activity heart rates around 122 bpm for axillary crutches compared to 107 bpm for hands-free crutches and even lower for unassisted walking[7].

Despite these findings, limited evidence exists regarding whether repeated exposure

to crutch-assisted ambulation over several weeks produces measurable chronic physiological adaptations in patients recovering from lower limb fractures. Most studies have focused on acute metabolic responses or gait mechanics in healthy volunteers[9, 8, 10] few have systematically evaluated how sustained crutch ambulation over weeks affects cardiovascular function, body composition indices, or oxygen utilization in patients recovering from lower limb fractures. In clinical practice, crutch use may occur in either structured, supervised formats or as part of routine, unstructured daily mobility. Whether a supervised crutch walking program confers additional cardiovascular or body composition benefits beyond usual care remains unclear, particularly in low-resource settings. Therefore, this study aimed to investigate the effect of six weeks of axillary crutch walking on selected physiological parameters among patients with lower limb fractures in Enugu, Nigeria.

MATERIALS AND METHODS

Study Design

This study employed a randomized pretest–posttest controlled trial design to evaluate the effect of a six-week supervised axillary crutch walking program on selected physiological parameters (resting systolic blood pressure, resting diastolic blood pressure, resting heart rate, VO_2 max and percentage body fat) in patients with lower limb fractures. Participants were age-matched and randomly allocated to either an experimental group or a control group.

Study Setting

The study was conducted at the Physiotherapy Department of National Orthopaedic Hospital Enugu, located in Enugu State, Nigeria. The hospital is a tertiary referral centre for orthopaedic and trauma cases in southeastern Nigeria.

Participants

The target population comprised patients with lower limb fractures prescribed axillary crutches for ambulation at National Orthopaedic Hospital Enugu during the study period (N = 264; 104 males, 160 females).

A total of 45 participants (22 males, 23 females) were recruited. Participants were randomized via simple balloting into the experimental group (n = 29) and control group (n = 16).

Inclusion Criteria

Participants who were;

i. Male and female patients aged ≥12 years
ii. Diagnosed lower limb fracture
iii. Recently mobilized from immobilization
iv. Prescribed axillary crutches for ambulation

Exclusion Criteria

Participants who had:

i. Pathological fractures
ii. Known cardiovascular, pulmonary, neurological, renal, or metabolic disease (such as diabetes, parkinson's disease, chronic obstructive pulmonary disease)

iii. Pre-existing hypertension or coronary artery disease
iv. Active malignancy
v. Degenerative or inflammatory musculoskeletal disorders
vi. Undergone joint replacement surgery

Outcome Measures

i. **Blood pressure:** Resting blood pressure was measured using a calibrated aneroid sphygmomanometer and Littmann stethoscope using the standard auscultatory technique. Measurements were taken in the seated position after 5 minutes of rest.
ii. **Heart rate:** Resting heart rate was measured in beats per minute using a Polar RS800CX heart rate monitor.
iii. **VO_2max:** VO_2max ($mL \cdot kg^{-1} \cdot min^{-1}$) was estimated from resting heart rate using established prediction equations (Nieman, 2011):
Males: $VO_2max = 111.33 - (0.42 \times HR)$
Females: $VO_2max = 65.81 - (0.1847 \times HR)$
iv. **Body composition:** Percentage body fat was measured using a bioelectrical impedance analyzer (Omron HBF-306). Height was measured using a stadiometer, and demographic variables (age, sex, weight) were entered into the device prior to measurement.

Intervention Protocol

The experimental group participated in supervised axillary crutch walking training three times per week (Monday, Wednesday, Friday) for six weeks. Each session lasted approximately 45–60 minutes and included:

1. Warm-up Phase
   i. Active mobilization exercises for upper and lower limbs
   ii. Joint range-of-motion activities
2. Skill Acquisition Phase
   i. Wall bar-assisted non-weight-bearing training
   ii. Parallel bar ambulation practice
   iii. Instruction in appropriate crutch gait technique
3. Main Exercise Phase
   i. Ambulation with axillary crutches over 50 meters
   ii. Four repetitions per session
   iii. Controlled breathing exercises
4. Cool-down Phase
   i. Supine relaxation
   ii. Passive and active lower limb mobilization
   iii. Controlled breathing exercises (10 minutes)

The control group received usual physiotherapy care without structured crutch walking training.

Data Analysis

Descriptive statistics (mean ± standard deviation) were calculated for all variables. Analysis of covariance (ANCOVA) was used to compare post-intervention outcomes between groups, adjusting for baseline differences. Statistical analyses were performed using SPSS Version 20.0 (IBM Corp., USA). Significance level was set at $p < 0.05$.

RESULTS

Following 6 weeks of crutch walking, resting systolic blood pressure showed a minimal increase in the experimental group and a larger increase in the control group; however, the between-group effect was not statistically significant ($F = 0.363$, $p = 0.64$). Resting diastolic blood pressure decreased slightly in the experimental group but increased in the control group, with no significant group effect ($F = 2.153$, $p = 0.15$). Resting heart rate declined in both groups, with a greater reduction observed in the experimental group; this difference was not statistically significant ($F = 0.96$, $p = 0.758$). VO_2max improved modestly and similarly in both groups, with no significant between-group difference ($F = 0.2$, $p = 0.65$). Percentage body fat increased slightly in both groups, and the group effect was not statistically significant ($F = 0.4$, $p = 0.5$). This is presented in Table 1.

Table 1: Effect of crutch walking on physiological parameters after 6 weeks of crutch walking

	Pre-test	Post-test	Mean Diff.	F	p-value
Resting Systolic BP (mmHg)					
Experimental	124.9 ± 18.5	125.3 ± 13.9	0.4	0.363	0.64
Control	121.5 ± 21.3	128.7 ± 16.1	7.2		
Resting Diastolic BP (mmHg)					
Experimental	81.7 ± 9.7	80.9 ± 10.6	0.8	2.153	0.15
Control	78.0 ± 10.9	83.8 ± 11.9	5.8		
Resting HR (bpm)					
Experimental	73.9 ± 7.8	66.9 ± 4	7	0.96	0.758
Control	70.6 ± 8.2	66.9 ± 5.3	3.7		
Estimated VO_2max (mL·kg ^{1}·min ^{1})					
Experimental	69.6 ± 14	71.4 ± 14.7	1.8		
Control	65.4 ± 14.7	67.1 ± 15	1.8	0.2	0.65
Percentage Body Fat (%)					
Experimental	25.8 ± 10	26.6 ± 11.8	1.7	0.4	0.5
Control	30.5 ± 14.5	31.7 ± 14.6	1.2		

DISCUSSION

This study evaluated whether a six-week supervised axillary crutch walking program produced measurable changes in resting cardiovascular parameters, estimated VO_2max, and percentage body fat among patients with lower limb fractures. The findings demonstrated no statistically significant between-group differences in resting systolic blood pressure, resting diastolic blood pressure, resting heart rate, estimated VO_2max, or percentage body fat following the intervention period. These results suggest that supervised crutch ambulation, as implemented in this study, did not confer additional cardiovascular or body composition benefits beyond usual physiotherapy care. Studies on the effect of crutch-assisted walking on physiological parameters have largely focused on its acute effects[11, 8, 10], thereby limiting direct comparison of the findings of our study with existing literature.

In the present study, although modest reductions in resting heart rate and diastolic blood pressure were observed in the experimental group, these changes were not statistically significant. The absence of meaningful between-group differences may be explained by several factors. Firstly, both groups were prescribed axillary crutches as part of routine care. The control group therefore continued ambulating during daily activities, potentially reducing the contrast between both groups. The training stimulus may also be insufficient to elicit long-term cardiovascular adaptation. While crutch walking increases acute metabolic demand, the total workload performed during each session was relatively modest. Cardiovascular adaptation typically requires sustained moderate-to-vigorous aerobic stimulus, which may not have been achieved under the present protocol[12].

Both groups demonstrated small, similar increases in estimated VO_2max, with no statistically significant group effect. Given the modest training load, the absence of significant improvement in aerobic capacity is not unexpected. Hands-free crutch gait has been linked with acute elevation in peak oxygen uptake compared to normal gait following a 6-minute walk test, indicating a higher aerobic demand[13]. For chronic effects, training primarily increases VO_2max through adaptations such as increased stroke volume, cardiac output, and enhance muscle capillarization which are influence by factors such as training volume, intensity and duration[14, 15].

Percentage body fat increased slightly in both groups without significant between-group differences. Changes in body composition typically require either sustained caloric deficit or prolonged higher-intensity aerobic training. The six-week duration and the relatively low exercise volume likely limited the potential for measurable changes in fat mass. While crutch use increases cardiovascular demand acutely, its impact on long-term changes in body fat has not been directly studied. Walking as a form of physical activity has been shown to reduce body fat percentage[16]. However, such programs typically involve sustained moderate-intensity activity performed for longer durations than the crutch ambulation protocol implemented in the present study. The minimal ambulatory

volume and the clinical context of fracture recovery, which may involve prolonged periods of reduced mobility outside supervised sessions, likely limited the potential for favourable changes in body composition. Additionally, dietary intake and overall physical activity levels were not controlled, which may have influenced body fat outcomes.

CONCLUSION

This study found that six weeks of supervised axillary crutch walking did not result in statistically significant changes in resting systolic blood pressure, resting diastolic blood pressure, resting heart rate, estimated VO_2max, or percentage body fat compared with usual care.

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PREVALENCE OF EYE DISEASES AMONG WELDERS IN ONITSHA, NIGERIA

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ABSTRACT

Background: Welders are prone to radiation, mechanical and chemical eye injuries which could cause visual loss. Information on the prevalence of ocular disorders among welders is essential for planning eye care services.

Aim: To determine the prevalence of eye diseases among welders in Onitsha, Nigeria.

Materials and Methods: Eligible welders at Bridge Head Market Onitsha, selected by simple random sampling, had ophthalmic assessment using a pre-tested questionnaire, visual acuity assessment, external eye examination, refraction, and ophthalmoscopy.

Results: Two hundred and five adult male welders, aged 18 – 70 years, median - 42 years, were studied. Job experience was 6 months - 35 years, median - 15 years; 70 (34.1%) participants were married.

All the participants complained of having had at least one eye disease symptom; light sensitivity, 80 (39.0%), and ocular pains, 62 (30.2%) were the commonest symptoms. Ninety four (45.9%) were diagnosed with ocular disorders; allergic conjunctivitis, 40 (42.5%) and pterygium, 24 (25.5%), ametropia, 12 (12.8%), were the commonest disorders. No participant was bilaterally blind but 13 (6.3%) had monocular blindness caused by corneal opacity, 6 (2.9%), cataract, 4 (2.0%), glaucoma, 2 (1.0), and pterygium, 1 (0.5%). Twenty-nine (14.1%) had moderate low vision and 30 (14.6%), severe low vision in the better eyes.

Conclusion: The prevalence of eye diseases, low vision and monocular blindness among the welders are high but the causes are avoidable. Eye health education for welders is recommended.
Key words: welders, eye diseases, prevalence

INTRODUCTION

The Nigeria National Blindness and Visual Impairment Study[1] had reported the prevalence and causes of blindness nationwide. Earlier on, both hospital-based[2] and population-based[3] studies had documented the burden of visual loss in Anambra State. However, none of these studies focused on welders. It is known that certain occupations, of which welding is one, are at increased risk of ocular disorders as part of occupational hazard[4,5].

Welding is an occupation in which workers are predisposed to eye diseases via exposure to thermal radiation as well as mechanical and chemical injuries. The ensuing ocular disorders include anterior segment inflammation such as conjunctivitis and photokeratitis[4] as well as degenerative changes including pingueculum and pterygium[5]; the welder's arc light could damage the retina leading to irreversible visual loss[6].

However, the prevalence of eye diseases among welders in Anambra State is unknown. The need to fill this gap in knowledge is not in doubt. Therefore, to solve this unmet need, we embarked on a survey of eye diseases and their causes among welders in Onitsha Nigeria. This article presents the prevalence of eye diseases among welders at the Bridge Head Market, Onitsha, Nigeria.

MATERIALS AND METHODS

This cross-sectional study of welders at the Bridge Head Market, Onitsha, was conducted between August and October 2024. Approval for this study was obtained from the Anambra State Ministry of Health Ethics Committee. Permission was also obtained from the leadership of the welders union; the consent of each participant was equally obtained.

Using the Leslie Kish formula with an adjustment for population <10000[8], a minimum sample size of 186 was calculated at 95% confidence interval. However, allowance was made for 10% attrition thus bringing the sample size to 205. Welders aged ≥18 years who willingly consented to participate in the study were enrolled. Welders who did not give consent or who were absent during the study period or who were too ill to participate were excluded.

Simple random sampling technique was used to select participants as follows: from the register of the welder's union, the names of the welders were extracted and written on a 2cm by 2cm piece of paper and each folded. The folded papers were put in a bag and mixed several times. An assistant not involved with writing names and folding papers picked out the folded papers until the calculated sample size was attained. The selected papers were then unfolded and the workers whose names appeared on the papers constituted the study subjects.

The tools used for the study comprised a pre-tested questionnaire on the participants' socio-demographic characteristics, eye disease symptoms and eye health-seeking behaviour. The questionnaire and the ophthalmic insturments used for the study were pre-tested among welders in Oba, more than 10 kilometres away from the study site. Ocular examination was included visual acuity measurement with the Snellen chart, pen-torch examination of the ocular adnexa and the anterior segment, refraction and direct ophthalmoscopy. The study team comprised ophthalmologists and ophthalmic nurses.

In this study, ocular diagnosis was recorded in terms of persons and not in terms of eyes; where more than one lesion co-existed, the one likely to have caused visual loss was taken as the diagnosis. For instance in a participant having glaucoma and refractive error, glaucoma is taken as the main diagnosis. Blindness was defined as visual acuity <3/60 and low vision as acuity of <6/18 – 3/60. Data obtained were analysed using descriptive statistics.

RESULTS

A total of 205 welders participated in the study. All the participants were males with age range of 18 – 70 years and a median of 42 years; the 31 -50 age bracket constituted 99 (48.2%). Table 1 shows the age distribution of the participants. While 70 (34.1%) participants were married, no participant was divorced or separated. The job experience was 6 months to 35 years with a median of 15 years. Although all the participants knew that use of protective eye wear during welding would minimize eye disorders, not many participants consistently used the protective eye wear. Reasons for non-use included on-availability and visual blurring during use. The relationship between use of protective eye wear and the incidence of eye diseases among this cohort of welders is the subject of another article.

In Table 2 is shown the self-reported eye disease symptoms. While all the participants complained of having had at least one eye disease symptom, some complained of multiple symptoms. Light sensitivity, 80 (39.0%), and ocular pains, 62 (30.2%) were the commonest symptoms reported, constituting more than two-thirds of the complaints.

Table 3 shows the ocular diagnosis. Of the 205 participants, 94 had ocular disorders thus giving a prevalence of 45.9% among this cohort of welders. Allergic conjunctivitis, 40 (42.5%) and pterygium, 24 (25.5%), and ametropia (refractive errors), 12 (12.8%) were the commonest disorders. None of the participants with refractive errors had optical aids; of these ametropic participants, 5 also had uncorrected presbyopia. The degree of ametropia was ±0.50 - ±3.00 dioptres.

Table shows the presenting visual acuity in both the better and worse eyes of the participants. No participant was blind in the better eye but 13 (6.3%) were blind in one eye only i.e. 6.3% prevalence of uniocular blindness. The causes of uniocular blindness were corneal opacity, 6 (2.9%), cataract, 4 (2.0%), glaucoma, 2 (1.0), and pterygium, 1 (0.5%). All the cases of corneal opacity were due to ocular trauma. With regard to low vision, 29 (14.1%) had moderate low vision (acuity 6/24 – 6/60) and 30 (14.6%) severe

low vision (acuity <6/60 – 3/60) in the better eyes; similarly moderate, 47 (22.9%) and severe low vision, 35 (17.1%) were also recorded in the worse eyes of the participants.

Table 1: Age distribution

Age (years)	No.	%
≤20	21	10.2
21 – 30	38	18.5
31 – 40	41	20.0
41 – 50	58	28.4
51 – 60	26	12.7
61 – 70	21	10.2
Total	205	100.0

Table 2: Self-reported eye disease symptoms*

Symptom	No.	%
Light sensitivity	80	39.0
Ocular pain	62	30.2
Tearing	40	19.5
Redness	30	14.6
Sandy sensation	18	8.8
Blurred vision	16	7.8

*% based on 205

Table 3: Ocular diagnosis

Disorder	No.	%
Allergic conjunctivitis	40	42.5
Pterygium	24	25.5
Refractive error	12	12.8
Corneal opacity	8	8.5
Cataract	6	6.4
Glaucoma	4	4.3
Total	94	100.0

Table 4: Presenting visual acuity

Visual acuity (Snellen)	Better eye (%)	Worse eye (%)
6/4 – 6/9	110 (53.7)	60 (29.3)
6/12 – 6/18	36 (17.6)	50 (24.4)
6/24 – 6/60	29 (14.1)	47 (22.9)
<6/60 - 3/60	30 (14.6)	35 (17.1)
<3/60 – LP*	0 (0.0)	13 (6.3)
Total	205 (100.0)	205 (100.0)

*LP = light perception

DISCUSSION

Although a nationwide blindness and visual impairment survey had been conducted in Nigeria[1], problems peculiar to some occupational group are yet to be specifically addressed. Thus, an ophthalmic survey of welders is essential as their peculiar needs relative to their occupation were not particularly captured in the national blindness and visual impairment study.

The 45.9% prevalence of eye diseases recorded in the present study points at the huge burden of ocular ailments among the welders. A similarly high prevalence was recorded by Ajayi and Omotoye[5] in Ile-Ife, southwest Nigeria and Atakunda et al[8] in Uganda. Although none of the participants was bilaterally blind, the 6.3% prevalence of uniocular blindness is more than 6-fold the blindness rate within the general population[1]. Of great importance is that welding requires good stereopsis. Thus a monocular welder is prone to poor judgement of depth with attendant loss of precision when joining metals; loss of stereo-acuity also increases the tendency to work-related injuries.

Eye injuries constitute a common cause of ocular morbidity among welders[9]. While the present study did not primarily focus on ocular trauma, the finding that nearly half of the uniocular blindness were due to trauma-related corneal opacity supports the preponderance of eye injuries as cause of visual loss among welders. But work-related eye injuries are preventable if the welders use protective eye wear (goggles) during welding.

While cataract and glaucoma are common causes of visual impairment and blindness in Nigeria, they are treatable especially if the afflicted seeks help early. But late presentation to hospital had been reported as being common among ophthalmic patients in Onitsha Nigeria[10]. The finding that pterygium counted among the causes of blindness among the participants is an important indicator of the very poor state of eye health among welders. Although pterygium as a cause of blindness had been previously documented in some Nigerian

communities, these were often among the underserved rural communities[11,12]. But the present study was conducted among urban dwellers. Onitsha is the foremost urban city in Anambra State with a tertiary public eye hospital that offers optical, medical, surgical and preventive ophthalmic services. However, none of the participants had accessed eye care in this hospital. Thus, urban habitation alone may not be a panacea to prevention of avoidable blindness.

Lack of awareness, cost of treatment and location of healthcare facilities are among the factors that may cause visual loss even among urban dwellers. Therefore, while the known barriers should be addressed, an aggressive eye health education programme should be instituted for the welders and all artisans in order to reduce the incidence and prevalence of avoidable blindness among these occupational groups.

Allergic conjunctivitis was the commonest disorder among the participants. While not a cause of visual loss, allergic conjunctivitis causes distressing symptoms including persistent itching, increased lacrimation (tearing) and redness of the eyes. Some of these symptoms may keep the afflicted off duty and this will, in turn, negatively impact productivity.

Similarly, uncorrected refractive errors including presbyopia were common. This is not surprising for a population that sparingly accessed eye care. Akin to what had been documented in previously[13], the degree of ametropia among the participants were of low to moderate degrees. However, it is conceivable that correction of ametropia among welders would enhance their efficiency.

Given that welders are usually exposed to radiant energy from the arc light, it was expected that many participants would have had dry eyes. But no case of diagnosis of dry eye disease was made in this study. This was because the tools for definitive diagnosis of dry eye disease were not employed in the present study. Therefore, the apparent absence of dry eye disease among the participants should be interpreted with caution.

In conclusion, our study has documented a high prevalence of eye diseases and low vision among the welders studied. While no participant was bilaterally blind, there was a high rate of monocular blindness. The causes of the eye diseases and visual impairment are avoidable in the sense that they either preventable or treatable. Therefore, frequent eye health education is recommended to enable the welders and indeed all artisans achieve and maintain optimal eye health.

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JOURNAL OF BIOMEDICAL INVESTIGATION (JBI) VOLUME 13 NUMBER 3: NOVEMBER-DECEMBER 2025

PREVALENCE OF LOW BACK PAIN AND AWARENESS OF PHYSIOTHERAPY INTERVENTIONS AMONG WELDERS IN NNEWI, ANAMBRA STATE, NIGERIA: A CROSS-SECTIONAL STUDY

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ABSTRACT

Background: Low back pain (LBP) is a major occupational health problem among workers in physically demanding jobs. Welders are particularly susceptible due to prolonged bending, awkward postures, and heavy lifting. Despite the established effectiveness of physiotherapy in managing musculoskeletal disorders, awareness and utilization of physiotherapy services remain critically low among informal sector workers in Nigeria.

Aim: This study investigated the prevalence of LBP and assessed whether welders in Nnewi North Local Government Area (L.G.A.), Anambra State, were aware of physiotherapy interventions.

Materials and Methods: A descriptive cross-sectional survey was conducted among 67 male welders (mean age 34.25 ± 7.79 years) selected via purposive sampling. Data were collected using the Modified Nordic Musculoskeletal Questionnaire (NMQ; Cronbach's α = 0.79) and a

structured Awareness of Physiotherapy Questionnaire (adapted from Maruf et al., 2012; Cronbach's α = 0.81). Descriptive statistics (frequencies, percentages) and inferential statistics (Chi-square and Fisher's Exact tests) were used for analysis (α ≤ 0.05).

Results: The 12-month prevalence of LBP was 53.7%, with 40.3% reporting activity limitation due to pain. Only 13.4% of respondents were aware of physiotherapy, with family/friends as the exclusive source of information (100%). Among the nine respondents who were aware, none demonstrated any knowledge of what physiotherapy involves or the conditions it manages. A statistically significant association was found between experiencing LBP and awareness of physiotherapy ($\chi^2 = 7.10$, $p = 0.008$).

Conclusion: LBP is highly prevalent among welders in Nnewi North L.G.A., yet awareness of physiotherapy interventions is critically low. Awareness is predominantly reactive, arising from personal pain experience rather than structured occupational health education. Targeted outreach and integration of physiotherapy into primary and occupational healthcare services are urgently needed.

Keywords: Low Back Pain; Metal Workers; Cross-Sectional Studies; Occupational Health; Prevalence; Nigeria; Health Education; Surveys and Questionnaires; Posture; Informal Sector

INTRODUCTION

Low back pain (LBP) is defined as pain or discomfort localized between the lower margin of the 12th rib and the gluteal folds, often accompanied by stiffness or functional limitation.[1] It is a global public health concern and the leading cause of years lived with disability (YLDs) worldwide.[2] The global point prevalence of LBP is estimated at 7.5%, with lifetime prevalence ranging from 70% to 85%.[3] In Nigeria, 12-month prevalence rates vary between 32.5% and 73.5%, reflecting substantial burden across occupational groups.[4]

Welders represent a high-risk occupational group due to prolonged static postures, repetitive trunk flexion, and heavy lifting.[5] These biomechanical demands place excessive mechanical stress on the lumbar spine, predisposing welders to musculoskeletal disorders including LBP. Physiotherapy plays a crucial role in LBP management through exercise therapy, manual therapy, and ergonomic education.[6] However, awareness and utilization of physiotherapy services remain low in many developing countries, particularly among informal sector workers.[7]

Despite the high physical demands of welding, no known study has focused specifically on welders in Nnewi North L.G.A., Anambra State, creating a significant gap in the occupational health literature. This study therefore sought to answer two sequential research questions: (i) Are welders in Nnewi North L.G.A. aware of physiotherapy interventions? and (ii) Among those who are aware, what is the

extent of their knowledge of physiotherapy? This approach recognizes the conceptual distinction between awareness (knowing that a construct exists) and knowledge (understanding what it entails), as well as the methodological principle that the level of knowledge should be assessed only among those already confirmed to be aware of the subject matter. Accordingly, this study aimed to determine the prevalence of LBP and to assess awareness — and, among aware respondents, the extent of knowledge — of physiotherapy interventions among welders in this region.

MATERIALS AND METHODS

Study Design and Setting

A descriptive cross-sectional survey was conducted among welders in Nnewi North L.G.A., Anambra State, Nigeria, from January to June 2024. Nnewi North is a major commercial and automotive artisan hub in Anambra State, with a dense concentration of welding workshops in its informal industrial clusters.

Participants and Sampling

A purposive sampling technique was used to recruit 67 male welders. Sample size was calculated using Taro Yamane's formula (1967). **Inclusion criteria:** age ≥18 years, at least one year of welding experience, and welding as the primary occupation. **Exclusion criteria:** history of back or spine surgery, and presence of hip region structural abnormalities diagnosed by a clinician. Note: 'less than one year of experience' was not retained as an exclusion criterion, as it is logically redundant with the inclusion criterion requiring at least one year of experience — including it as both an inclusion and exclusion criterion would introduce a logical contradiction.

Instruments

Two validated instruments were used: **(1) The Modified Nordic Musculoskeletal Questionnaire (NMQ)** — a widely used tool for assessing regional musculoskeletal pain prevalence (Cronbach's α = 0.79; test-retest reliability r = 0.82 in comparable populations), used here to assess LBP prevalence, activity limitation, work absenteeism, and healthcare consultation. **(2) Awareness of Physiotherapy Questionnaire** — a structured questionnaire adapted from Maruf et al. (2012) to assess: (a) whether respondents were aware of physiotherapy (binary yes/no), (b) source of awareness, and (c) among those who were aware, their knowledge of what physiotherapy entails and the conditions it addresses. The adapted tool demonstrated acceptable internal consistency (Cronbach's α = 0.81) and test-retest reliability (ICC = 0.78) in a pilot sample of 10 welders prior to the main study.

Data Collection and Analysis

Ethical approval was obtained from the Research and Ethics Committee of Nnamdi Azikiwe University, Faculty of Health Sciences and Technology, Nnewi Campus (Approval No.: NAUTH/CS/66/VOL.16/VER.3/032/2024). Questionnaires were researcher-administered in person to each eligible welder to ensure comprehension, given that the majority had secondary-level formal education. For respondents who could not read English fluently, the questionnaire was

read aloud and responses recorded by the researcher. All respondents provided written informed consent before participation.

Data were analyzed using IBM SPSS Statistics version 26 (IBM Corp., Armonk, NY). Descriptive statistics — frequencies, percentages, means, and standard deviations — were computed for all variables. For inferential analysis, a 2×2 contingency table was constructed to examine the association between LBP status (yes/no) and awareness of physiotherapy (yes/no). The Pearson Chi-square test with Yates' continuity correction was applied, and Fisher's Exact Test was used given the small expected cell frequencies. Statistical significance was set at $p \leq 0.05$.

RESULTS

Socio-Demographic Characteristics

A total of 67 male welders participated (mean age 34.25 ± 7.79 years). The majority were aged 30–39 years (52.2%), were single (53.7%), and had completed secondary school education (76.1%). No respondent had primary school as their highest level of education; 23.9% had vocational/technical training as their highest qualification. Mean years of welding practice was 11.37 ± 7.87 years. Full socio-demographic data are presented in Table 1.

Prevalence and Impact of Low Back Pain

The 12-month prevalence of LBP was 53.7% (n = 36/67). Among all respondents, 40.3% reported that LBP had prevented normal daily activities or work within the same period, and the 7-day point prevalence was also 40.3%. Regarding work absenteeism attributable to LBP, 59.7% reported no days lost, 16.4% lost 1–7 workdays, and 23.9% lost 8–30 workdays. Only 29.9% had ever consulted a healthcare professional for their LBP (Table 2).

Awareness of Physiotherapy

Only 13.4% (n = 9/67) of respondents indicated awareness of physiotherapy. The sole source of this awareness was family members or friends (100%), with no exposure through healthcare professionals, workplace programmes, or mass media. Among the nine respondents who were aware, none could accurately describe what physiotherapy involves or identify any conditions that physiotherapy addresses. Awareness and knowledge data are presented in Table 3.

Association between LBP Experience and Awareness of Physiotherapy

All nine respondents who were aware of physiotherapy had experienced LBP (100%). Among those with LBP (n = 36), 25.0% were aware of physiotherapy, compared to 0% among those without LBP (n = 31). Chi-square analysis with Yates' continuity correction ($\chi^2 = 7.10$, df = 1) and Fisher's Exact Test revealed a statistically significant association between experiencing LBP and awareness of physiotherapy ($p = 0.008$). The test statistic, degrees of freedom, expected values, and p-value are presented in Table 4.

Table 1: Socio-Demographic Profile of Respondents (N = 67)

Variable	Category	Frequency (n)	Percentage (%)
Age (Years)	20–29	12	17.9
	30–39	35	52.2
	40–49	20	29.9
	Mean ± SD = 34.25 ± 7.79 years		
Marital Status	Single	36	53.7
	Married	31	46.3
Educational Level	Primary	0	0.0
	Secondary	51	76.1
	Vocational/Technical	16	23.9
Years of Practice	1–10	37	55.2
	11–20	12	17.9
	21–30	18	26.9
	Mean ± SD = 11.37 ± 7.87 years		

SD = Standard Deviation

Table 2: Prevalence and Functional Impact of Low Back Pain (N = 67)

Questionnaire Item	Response	Frequency (n)	Percentage (%)
1. Had LBP in last 12 months?	Yes	36	53.7
	No	31	46.3
2. Did LBP prevent ADL/Work?	Yes	27	40.3
	No	40	59.7
3. Had LBP in the last 7 days?	Yes	27	40.3
	No	40	59.7
4. Total days unable to work	None	40	59.7
	1–7 days	11	16.4
	8–30 days	16	23.9
5. Consulted a healthcare professional for LBP?	Yes	20	29.9
	No	47	70.1

ADL = Activities of Daily Living; LBP = Low Back Pain

Table 3: Awareness of Physiotherapy and Source of Information (N = 67)

Item	Response	n (%)
Are you aware of physiotherapy?	Yes	9 (13.4)
	No	58 (86.6)
Source of awareness (n = 9)	Family/Friends	9 (100.0)
	Healthcare professional	0 (0.0)
	Workplace/Media	0 (0.0)
Do you know what physiotherapy involves? (n = 9)	Yes	0 (0.0)
	No/Not Sure	9 (100.0)
Do you know conditions physiotherapy treats? (n = 9)	Yes	0 (0.0)
	No knowledge	9 (100.0)

Note: Knowledge items (rows 3–4) were assessed only among the nine respondents who indicated awareness of physiotherapy.

Table 4: Cross-tabulation and Chi-Square Analysis: LBP Status vs. Awareness of Physiotherapy

LBP Status	Aware of Physiotherapy: No	Aware of Physiotherapy: Yes	Total
LBP: No	31 (100%)	0 (0%)	31
LBP: Yes	27 (75.0%)	9 (25.0%)	36
Total	58 (86.6%)	9 (13.4%)	67

χ^2 = 7.10 (Yates' continuity corrected), df = 1; Fisher's Exact Test p = 0.008.

Expected cell frequencies: LBP Yes & Aware Yes = 4.84; LBP Yes & Aware No = 31.16; LBP No & Aware Yes = 4.16; LBP No & Aware No = 26.84.

DISCUSSION

This study investigated the prevalence of LBP and awareness of physiotherapy among welders in Nnewi North L.G.A. The 12-month LBP prevalence of 53.7% aligns with previous findings among Nigerian industrial workers and artisans, where rates ranging from 44.7% to 65.5% have been documented.[8] This high prevalence is attributable to the occupational demands of welding — specifically, prolonged standing, sustained trunk flexion, and repetitive lifting — which collectively increase mechanical loading on the lumbar spine and predispose workers to musculoskeletal injury.[9]

The finding that only 13.4% of welders were aware of physiotherapy is strikingly low and is consistent with reported awareness deficits in other comparable groups of Nigerian informal sector workers. For instance, Odebiyi et al.[10] reported that physiotherapy awareness was under 20% among artisans and market traders in Lagos, while Akinpelu et al.[7] documented similarly poor awareness among members of the general Nigerian public without formal health education. The present study extends these findings specifically to welders in the Southeast, a group previously unstudied in this context.

The exclusive sourcing of physiotherapy awareness through family and friends, with no contributions from healthcare professionals or workplace training, reflects a systemic failure in occupational health outreach for informal sector workers. The current study did not specifically survey whether welders' associations in the area were engaged with any health promotion initiatives; this represents an important area for future investigation and targeted intervention. While it is plausible that welders without physiotherapy awareness may rely on self-medication or traditional remedies — a pattern widely documented in the Nigerian informal sector[11] — this study

did not directly measure health-seeking behaviour for LBP, and caution is warranted in making that causal attribution from awareness data alone.

A significant association ($\chi^2 = 7.10$, $p = 0.008$) was found between experiencing LBP and awareness of physiotherapy, suggesting that awareness is reactive — driven by personal pain experience and the search for relief — rather than arising from proactive occupational health education. This finding is consistent with Ojukwu et al.,[12] who observed that artisans in Enugu typically seek rehabilitation services only after conventional and informal treatments fail. It is noteworthy that the study design (cross-sectional) does not allow causal inference from this association; the directionality — whether pain precedes or follows awareness — cannot be established from these data.

The clinical and public health implications are substantial. Low awareness contributes to delayed presentation, the progression of acute LBP to chronic disabling conditions, reduced work capacity, and lost economic productivity.[13] Given that 40.3% of respondents reported that LBP limited their activities of daily living and 23.9% lost 8–30 workdays, the functional and economic burden on individual welders and their households is considerable. Early physiotherapy intervention — including exercise prescription, ergonomic advice, and manual therapy — has been demonstrated to reduce disability and prevent chronicity in work-related LBP.[6]

Study Limitations

This study has several limitations. The sample was restricted to one L.G.A. and comprised only 67 participants, which constrains generalisability to welders in other Nigerian states or regions. The use of purposive sampling, while appropriate for this exploratory study, introduces selection bias. The cross-sectional design precludes any causal inference between LBP experience and physiotherapy awareness. Additionally, the study did not assess health-seeking behaviour directly, nor did it engage welders' associations prior to data collection to assess existing health promotion activities. Future studies should employ larger, multi-regional, probability-based samples and incorporate qualitative components to explore health-seeking behaviours, barriers to physiotherapy utilization, and the role of informal occupational networks in health information dissemination.

CONCLUSION

Low back pain is highly prevalent among welders in Nnewi North L.G.A., Anambra State, yet awareness of physiotherapy interventions is critically low. Among the small proportion who were aware of physiotherapy, no meaningful knowledge of the profession's scope or the conditions it manages was demonstrated. Awareness is predominantly reactive, occurring in the context of personal pain experience rather than structured occupational health education. There is an urgent need for targeted physiotherapy outreach, integration

of physiotherapy into accessible primary healthcare services, and structured health education programmes directed at informal sector workers.

Recommendations

1. Physiotherapy professional bodies and councils should design and deploy community-based outreach and ergonomic training programmes within welding clusters and informal trade hubs.
2. The Federal and State Ministries of Health should expand physiotherapy services within primary healthcare facilities to ensure accessibility for informal workers.
3. Future researchers and implementers should establish formal partnerships with welders' associations and occupational health bodies prior to intervention design, to understand existing health knowledge, practices, and association structures.
4. Mass media, social media, and peer-health education strategies should be leveraged to raise public awareness of physiotherapy's role in managing work-related musculoskeletal disorders, targeting artisan communities specifically.

Conflict of Interest: None declared.

Funding: This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Acknowledgments: The author thanks the welders of Nnewi North L.G.A. for their participation

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ESTIMATION OF CHOLESTEROL AND URIC ACID LEVEL IN MENOPAUSE

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ABSTRACT

Background: Menopause is a natural physiological process characterized by the permanent cessation of menstruation resulting from the loss of ovarian follicular activity. It commonly occurs between the ages of 45 and 55 years and is associated with progressive hormonal and metabolic changes, particularly a decline in oestrogen production. These hormonal alterations may significantly affect lipid and purine metabolism, thereby increasing the risk of metabolic and cardiovascular disorders.

Aim: This study was designed to evaluate serum cholesterol and uric acid levels in postmenopausal women and compare them with those of premenopausal women in order to determine whether the observed changes are associated with menopause or aging.

Materials and Methods: A comparative cross-sectional study was carried out involving 100 postmenopausal women aged 45–78 years and 50 apparently healthy premenopausal women aged 20–45 years who served as controls. Serum cholesterol levels were determined using the enzymatic cholesterol oxidase-peroxidase method, while uric acid was estimated using the

uricase-peroxidase method. Statistical analysis was performed using Student's *t*-test with level of significance set at $p < 0.05$.

Results: The results showed that serum cholesterol and uric acid levels were significantly higher in postmenopausal women compared with premenopausal controls ($p < 0.05$). However, no statistically significant differences were observed when menopausal women were stratified according to age groups ($p > 0.05$). Similarly, no significant age-related differences were observed among the control group.

Conclusion: menopause significantly influences serum cholesterol and uric acid levels due to hormonal changes associated with oestrogen deficiency. Regular monitoring of these biochemical parameters, together with lifestyle modifications such as healthy diet, physical activity, and weight management, is recommended for postmenopausal women.

Keywords: Menopause, Cholesterol, Uric Acid, Oestrogen, Hyperuricaemia, Postmenopausal Women

INTRODUCTION

Menopause, which is a normal part of aging, marks the end of reproductive years and the termination of cyclic ovarian functions, which are indicated by cyclic menstruation [1]. Menopausal transition, a time when the endocrine, biochemical, and clinical signs of impending menopause start, is what heralds it [2]. Every woman experience this "change of life" in a distinctive way, and it often happens about age 51. Menopause is seen as "natural" and a typical aspect of aging when it happens between the ages of 45 and 55 [2]. However, some women may go through menopause early due to ovarian damage from chemotherapy or surgical procedures like hysterectomy. Premature menopause is defined as menopause that happens before the age of 45, regardless of the reason. Genetics, autoimmune diseases, and medical procedures can all cause premature menopause. Some women will just have the end of their menstrual cycle as a symptom, while others will face more serious mental and physical difficulties, such as memory loss, hot flashes, night sweats, mood swings, hurting joints, and thinning hair. Menopause can also lead to the development of a number of chronic illnesses, including an increased risk of cardiovascular disease, osteoporosis, and gout [3].

However, some women may go through menopause early due to ovarian damage from chemotherapy or surgical procedures like hysterectomy. Premature menopause is defined as menopause that happens before the age of 45, regardless of the reason [3]. Genetics, autoimmune diseases, and medical procedures can all cause premature menopause. Some women will just have the end of their menstrual cycle as a symptom, while others will face more serious mental and physical difficulties, such as memory loss, hot flashes, night sweats, mood swings, hurting joints, and thinning hair. Menopause can also lead to the development of a number of chronic illnesses, including an increased risk of cardiovascular disease, osteoporosis, and gout. When the ovaries are completely empty of eggs and cannot be

stimulated by the regulating hormones, menopause takes place. Because they lack the hormone oestrogen, menopausal women typically have higher levels of uric acid and cholesterol in their blood [3]

A crucial part of the cell membrane's lipid bilayer is cholesterol. The phospholipid molecules, which are the other main component of the lipid layer of the cell membrane, are positioned between the cholesterol molecules. According to Sembulingam and Sembulingam (2010), cholesterol is responsible for the structural integrity of the cell membrane because it aids in packing the phospholipids, which are soft and oily structures, in the membrane. It is actively required for the manufacture of estrogen because it acts as a precursor to steroids. Menopause causes the synthesis of estrogen to stop, which raises the plasma level of cholesterol because it is no longer needed for synthesis [4].

In primates, including humans, purines and nucleic acids are metabolised primarily to produce uric acid (Yu *et al*., 2023). The enzyme xanthine oxidase converts xanthine into uric acid via a common route. It is further converted into the more water-soluble version of allantoin in the majority of other mammals, but humans are unable to undergo this transformation. Humans are more susceptible to the clinical consequences of hyperuricaemia, such as gout, due to its poor solubility. Man is vulnerable to the clinical consequences of hyperuricaemia, such as gout and kidney impairment, due to its low solubility. Oedema and joint discomfort are signs of gout [5,6]. This study's primary objective is to investigate menopausal cholesterol and uric acid levels.

Recent years have been attempts to look at how menopause affects serum levels of uric acid and cholesterol. Compared to younger women, older women are more likely to have conditions linked to high levels of uric acid and cholesterol. Is this related to menopause or aging?

The aim of this study was to determine the serum levels of uric acid and cholesterol in menopausal women and compare them with those of a control group. Specifically, the study sought to evaluate the effect of menopause on serum uric acid and cholesterol levels and to determine the relationship between menopause duration, serum cholesterol, and uric acid levels.

MATERIALS AND METHODS

Study Population

The study population consisted of women attending Iyi-Enu Mission Hospital. A total of 150 female participants were recruited and categorized into two groups. The test group comprised 100 postmenopausal women aged 45–78 years with a mean age of 62 years, while the control group consisted of 50 apparently healthy premenopausal women aged 20–44 years with a mean age of 33 years. Participants with known histories of dyslipidaemia, gout, liver disease, renal disease, tuberculosis, heart failure, pregnancy, lactation, or harmful alcohol/substance use were excluded from the study to minimize confounding variables.

Sample Size Determination

The sample size for the study was determined using a convenience sample

approach based on the availability of eligible participants who met the inclusion criteria during the study period. A total of 150 participants were considered adequate to provide comparative data between postmenopausal and premenopausal women for the biochemical parameters under investigation.

Sampling Method and Participant Recruitment

A purposive sampling technique was employed for participant recruitment. Eligible postmenopausal women attending the hospital during the study period were consecutively recruited after screening for eligibility criteria. The control group of premenopausal women was selected from hospital staff, students, and other apparently healthy female volunteers within the hospital community. Information about the study objectives and procedures was explained to all participants, and written informed consent was obtained prior to sample collection and data acquisition.

Inclusion Criteria

Only post-menopausal women were selected.

Exclusion Criteria

The exclusion criteria were determined through a combination of medical history assessment, clinical evaluation, and review of participants' medical records. Pregnant women and lactating mothers were identified through self-reporting and clinical history. Participants with liver failure, kidney failure, tuberculosis, or heart failure history were excluded based on documented medical diagnoses, hospital records, current medications, and physician reports where available. History of harmful alcohol intake or substance use was assessed through structured interview questions and self-reported social history during participant recruitment. These measures were taken to minimize confounding factors that could influence the biochemical and clinical parameters under investigation.

Specimen Collection and Processing

Blood samples (4mls) were collected from each subject by venipuncture using sterile needles and syringes after disinfecting the site with methylated spirit. The sample was carefully dispensed into sterile plain containers and labelled appropriately. These were allowed to clot after which they were carefully dislodged with a dropper pipette (avoiding lysis) and were centrifuged at 3000rpm for 10minutes. The sera were frozen until the time of analysis.

Chemicals and Reagents

The following chemicals and reagent were used for this study; 4-aminoantipyrine, phenol, peroxidase, cholesterol esterase, cholesterol oxidase, pipes buffer, hepes buffer, 3,5-Dichloro-2-hydroxyl-bezenesulfonic acid, 4-aminophenazone, peroxidase and uricase. All regents were of analytical grade.

Method of analysis

Cholesterol estimation

The serum levels of cholesterol were determined using the method described by Trinder (1967), with kit assay system (Randox, United Kingdom).

Principle: The principle of this method was based on the enzymatic hydrolysis of cholesterol ester by cholesterol esterase to form cholesterol and fatty acids. The cholesterol is being oxidized in the second stage of the reaction by cholesterol oxidase

to form cholestene -3- one and hydrogen peroxide. The hydrogen peroxide formed reacts in the third stage of the reaction with phenol and 4-Aminoantipyrine in the presence of peroxidase to form a pink quinoneimine whose intensity measured colorimetrically at 500nm is directly proportional to cholesterol concentration.

Procedure: 0.01ml (10µL) of the sample was added to 1.0ml (1000µL) of the reagent. The solution was well mixed and then incubated for 5minutes at 37ºC. The absorbance of the test sample was read colorimetrically at 500nm against water blank. The cholesterol concentration was calculated using the standard formula.

Reference range: < 5.17mmol/L (200mg/dl).

Uric Acid Estimation

The serum level of uric acid was determined using the method described by Fossat *et al* (1980), with kit assay system (Randox United Kingdom).

Principle: The principle of this reaction was based on the conversion of uric acid by uricase to allantoin and hydrogen peroxide which under the catalytic influence of peroxidase, oxidizes 3,5-Dichloro-2-hydroxylbenzenesulfonic acid and 4-aminophenazone to form a red-violet quinoneimine compound whose intensity measured colorimetrically at 520nm is directly proportional to the uric acid concentration in the sample

Procedure: 0.02ml (20µL) of the sample was added to 1.0ml (1000µL) of the reagent. The solution was well mixed and then incubated for 5minutes at 37ºC. The absorbance of the test sample was read colorimetrically at 520nm against water blank. The uric acid concentration was calculated using the standard formula.

Reference range: 2.4 – 5.7mg/dl

Data Analysis

The data obtained from this study were analyzed using appropriate statistical methods. Mean values and standard deviations were calculated for all variables. Comparisons between menopausal women and the control group were performed using the Student's t-test with Statistical Package for Social Sciences (SPSS) Windows version 16.0. Statistical significance was set at a p-value of ≤0.05.

RESULTS

The results obtained from this study are presented in Tables 1–3.

Table 1: Shows the mean cholesterol values were significantly higher in menopausal women than the control subjects. Also, the mean uric acid values were significantly higher in menopausal women than the control subjects.

Table 2: also discloses the mean serum cholesterol and uric acid levels of the age groups 45-60 years and 61-78 years in menopausal women, showed no statistically significant difference ($p>0.05$) in the values obtained of age group 45-60 years when compared with age group 61-78 years

Table 3: also discloses the mean serum cholesterol and uric acid levels of the age groups 20-35 years and 36-44 years in control subjects, showed no statistically significant difference ($p>0.05$) in the values obtained of age group 20-35 years when compared with age group 36-44 years.

TABLE 1: Presents the serum concentrations of cholesterol and uric acid in menopausal women compared with the control subjects in the study

Parameters	Menopausal women (n=100)	Control (n=50)	t-value	p-value
CHOL (mg/dl)	216.20 ± 59.71	182.4 ± 39.4	**4.20**	P<0.01
U.A (mg/dl)	10.38 ± 3.49	7.20 ± 3.16	**6.10**	P<0.01

KEY: n = number of subjects, P<0.05 = statistically significant, P>0.05 = not statistically significant, CHOL = Cholesterol and U.A = Uric acid

TABLE 2: shows the age-related distribution of serum total cholesterol and uric acid levels among menopausal women within the age groups of 18–60 years and 61–78 years in the study area

Parameters	Menopausal women 45-60 years (n=45)	Menopausal women 61-78 years (n=55)	t-value	p-value
CHOL (mg/dl)	215.20 ± 63.36	213.70 ± 51.00	**0.131**	0.311
U.A (mg/dl)	9.20 ± 2.34	9.70 ± 2.60	**0.568**	0.582

TABLE 3: Illustrates the age-related distribution of serum total cholesterol and uric acid levels among the control subjects within the age groups of 20–35 years and 36–44 years in the study area.

Parameters	Menopausal women 20-35 years (n=27)	Menopausal women 36-44 years (n=23)	t-value	p-value
CHOL (mg/dl)	196.70 ± 63.36	206.31 ± 48.20	**0.127**	0.220
U.A (mg/dl)	6.91 ± 2.05	7.02 ± 2.10	**0.510**	0.515

DISCUSSION

Menopause is the permanent cessation of menstruation resulting from the loss of ovarian follicular activity and decline in reproductive hormone production. It marks the end of a woman's reproductive phase and is clinically diagnosed after 12 consecutive months of amenorrhea in the absence of other physiological or pathological causes. Menopause commonly occurs between the ages of 45 and 55 years. [12].

The study's findings demonstrate that menopausal women's levels of uric acid and total serum cholesterol were altered. Regardless of age, menopausal women's total cholesterol and uric acid levels were not significantly different from those of premenopausal women (menstruating women) [5;3;6;9]. Menopausal women's elevated levels of uric acid and total cholesterol have been linked to hormonal changes and follicular development failure, where the plasma oestrogen levels that lower LDL while raising HDL and are also in charge of uric acid excretion in the urine are lower than those of premenopausal women [7;11]. Also due to Steatotic liver disease in premenopausal women refers to the accumulation of excess fat in the liver of women who have not yet undergone menopause. It is now broadly classified under the term Metabolic Dysfunction-Associated Steatotic Liver Disease (MASLD), previously known as non-alcoholic fatty liver disease [4].

CONCLUSION

The findings of this study indicate that menopause is associated with alterations in serum uric acid and total cholesterol levels. However, the results showed no statistically significant difference in these parameters between menopausal and premenopausal women across the studied age groups. The observed variations in uric acid and cholesterol levels among menopausal women have been attributed to hormonal changes, particularly the decline in oestrogen production due to follicular depletion. Since oestrogen plays a role in regulating lipid metabolism and enhancing uric acid excretion, its reduction during menopause may contribute to metabolic changes, even though these changes were not statistically significant in this study.

RECOMMENDATIONS

Menopausal women can lower their cholesterol and uric acid levels by making certain lifestyle changes, such as maintaining a healthy weight by engaging in at least 30 minutes of moderate-to-intense exercise each day; dietary changes, such as eating more fish, whole grains and fibre, less refined sugar, less red meat and high-fat dairy products, more mono- and polyunsaturated fatty acids, and more fruits, vegetables, and legumes; Tran's fats should be avoided or reduced, and saturated fat intake should be less than 7% of daily calories.

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PREVALENCE AND PATTERNS OF MUSCLE STRAIN INJURIES AMONG AMATEUR MALE FOOTBALLERS IN NNEWI, ANAMBRA STATE, NIGERIA: A CROSS-SECTIONAL STUDY

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ABSTRACT

Background: Lower limb muscle strain injuries represent a significant burden in amateur football, yet epidemiological data from Nigerian community settings remain limited.

Aim: This study determined the prevalence, anatomical patterns, and factors associated with lower limb muscle strain injuries among amateur male footballers in Okofia, Nnewi, Nigeria.

Materials and Methods: A descriptive cross-sectional study was conducted among 173 amateur male football players at the College of Health Sciences, Okofia, Nnewi. Participants were recruited using simple consecutive sampling. Data on sociodemographic characteristics, football participation history, injury characteristics, circumstances, management, and prevention practices were collected via a validated structured questionnaire. Descriptive statistics summarized the data; chi-square tests examined associations between injury severity and selected factors at the $P<0.05$ level of significance.

Results: The mean age of participants was 22.94 ± 3.33 years. The prevalence of lower limb muscle strain injuries was 58.4% (101/173). The most commonly affected sites were the thigh (12.1%) and hamstring (12.1%), followed by the calf (11.6%), knee (9.8%), ankle (7.5%), and groin (5.2%). Injuries occurred more frequently during competitions (32.9%) than during

training (25.4%). Perceived causes included fatigue (13.9%), overtraining (12.1%), poor warm-up (9.8%), and sudden movements or sprinting (8.1%). Most players (86.1%) believed injuries were preventable, and 74.6% reported regular post-activity stretching. Significant associations ($p < 0.001$) were found between injury severity and history of injury, injury site, number of previous injuries, time since last injury, circumstance of injury, perceived cause, and performance impact. No significant associations were observed with training frequency ($p = 0.874$), training duration ($p = 0.074$), or warm-up practice ($p = 0.574$).

Conclusion: Lower limb muscle strain injuries, particularly affecting the thigh and hamstring, are highly prevalent among amateur male footballers in this Nigerian community setting. Injury history, anatomical site, and recurrence are strongly associated with injury severity.

Keywords: *lower limb muscle strain injuries; amateur football; prevalence; injury patterns; Nigeria; sports injury prevention*

INTRODUCTION

Football is the most widely played sport globally, with an estimated 250 million active participants [1]. In Nigeria, football serves not only as a major recreational pursuit but also as a talent development platform, particularly among young males [2]. Despite its numerous physical and social benefits, football is associated with a high incidence of musculoskeletal injuries, making it a significant public health and sports medicine concern [3].

Lower limb muscle strain injuries—commonly referred to as "pulled muscles"—involve overstretching or tearing of muscle fibres or their musculotendinous junctions [4]. These injuries range from mild micro-tears (Grade I) to complete ruptures (Grade III) and are frequently precipitated by sudden acceleration, high-speed sprinting, forceful kicking, or abrupt directional changes—movements that are integral to football performance [5]. Lower limb muscle strains account for approximately 18–23% of all time-loss injuries in football, with the hamstrings, quadriceps, adductors, and calf muscles representing the most vulnerable muscle groups [6].

Globally, 30–50% of football-related injuries involve lower limb muscle strains [7,8]. Amateur footballers have been shown to sustain higher injury rates than their professional counterparts, primarily attributable to irregular training, inconsistent conditioning, inadequate recovery, and limited access to sports medicine services [9,10]. In Nigeria, previous studies have reported that lower limb muscle strains account for over 40% of all football-related injuries among amateur male players in the southwest [11], and that approximately 78% of injuries occur in the lower extremities among amateur players in the north [12]. Contextual factors—including poor pitch surfaces, inadequate warm-up routines, and absent medical supervision—further compound the injury burden in Nigerian community football settings [13].

Despite growing evidence of football-related injuries in Nigeria, epidemiological data on

lower limb muscle strain injuries in specific community settings, including Okofia, Nnewi, remain limited. This study, therefore, aimed to determine the prevalence, anatomical patterns, and factors associated with lower limb muscle strain injuries among amateur male footballers at the College of Health Sciences, Okofia, Nnewi, with a view to informing context-specific prevention strategies and sports health planning.

MATERIALS AND METHODS

Study Design and Setting

A descriptive cross-sectional study was conducted at the College of Health Sciences, Okofia, Nnewi, Anambra State, Nigeria, between July and September 2025. The College comprises eight departments offering health sciences programmes, each with active amateur football teams.

Study Population and Sampling

The target population comprised 306 registered male amateur football players distributed across eight departments: Medical Rehabilitation (n = 60), Medical Laboratory Science (n = 60), Radiography (n = 66), Environmental Health Science (n = 25), Nursing Science (n = 22), Anatomy (n = 24), Physiology (n = 24), and Medicine (n = 23). Using the Taro Yamane formula [$n = N / (1 + N(e^2))$] with a 5% margin of error, the minimum required sample size was calculated as 173. Participants were recruited using simple consecutive sampling until the target was achieved.

Inclusion and Exclusion Criteria

Players were included if they had been actively participating in organised football for a minimum of one year at the College of Health Sciences. Players were excluded if their injuries were sustained during activities unrelated to football competitions or training.

Data Collection Instrument

A structured 23-item Muscle Strain Injury Questionnaire for Amateur Male Footballers was developed and validated for this study. The instrument collected data in the following domains:

i. Sociodemographic characteristics (age, department, study level)
ii. Football participation history (playing duration, training frequency, session duration, warm-up practices)
iii. History of lower limb muscle strain injury (occurrence, anatomical site, frequency, timing)
iv. Circumstances and perceived causes of injury (training vs. competition)
v. Management and recovery (treatment-seeking behaviour, rest period, performance impact)
vi. Injury prevention beliefs and practices

Content, construct, and face validity were established through expert review. Test-retest reliability and internal consistency (Cronbach's alpha) were confirmed prior to administration.

Data Collection Procedure

Ethical approval was obtained from the Nnamdi Azikiwe University Teaching Hospital Ethical Committee (NAUTHEC). Written informed consent was obtained from all participants prior to data collection. Questionnaires were administered either directly by the researcher or self-administered according to participant preference.

Data Analysis

Descriptive statistics—including frequencies, percentages, means, and standard deviations—were used to summarise participant characteristics and injury patterns. Pearson chi-square tests were applied to examine associations between injury severity (operationalized as the rest period required before return to football) and the following independent variables: training frequency, training duration, warm-up practice, injury history, injury site, number of prior injuries, time since last injury, circumstance of injury, perceived cause, and performance impact. The level of statistical significance was set at $p < 0.05$.

RESULTS

Participant Characteristics

A total of 173 male amateur football players participated in the study, representing a response rate of 100%. The distribution by study level was: 100 level (15.6%), 200 level (20.2%), 300 level (20.2%), 400 level (23.7%), and 500 level (20.2%). The mean age was 22.94 ± 3.33 years (range: 18–28 years). Departmental representation and football participation characteristics are presented in **Table 1**.

With respect to football participation history, 8.7% of participants had been playing for less than one year, 32.9% for 1–3 years, 33.5% for 4–6 years, and 24.9% for more than six years. Training frequency was reported as 1–2 sessions per week by 27.2%, 3–4 sessions per week by 48.2%, and five or more sessions per week by 24.9% of participants. Session duration was less than one hour for 26.6%, one to two hours for 47.4%, and more than two hours for 26.0%. Pre-activity warm-up was practised by 76.9% of participants.

Table 1: Sociodemographic and Football Participation Characteristics (n = 173)

Variable	Category	n	%
Study Level	100 level	27	15.6%
	200 level	35	20.2%
	300 level	35	20.2%
	400 level	41	23.7%
	500 level	35	20.2%
Age (years)	Mean ± SD	22.94 ± 3.33 years (range: 18–28)	
Department	Medical Rehabilitation	34	19.7%
	Medical Lab Science	34	19.7%
	Radiography	37	21.4%
	Environmental Health	14	8.1%
	Nursing Science	12	6.9%
	Anatomy	14	8.1%
	Physiology	14	8.1%
	Medicine	14	8.1%
Playing Duration	<1 year	15	8.7%
	1–3 years	57	32.9%
	4–6 years	58	33.5%
	>6 years	43	24.9%
Training Frequency	1–2 times/week	47	27.2%
	3–4 times/week	83	48.2%
	≥5 times/week	43	24.9%
Session Duration	<1 hour	46	26.6%
	1–2 hours	82	47.4%
	>2 hours	45	26.0%
Warm-up Practice	Yes	133	76.9%
	No	40	23.1%

Prevalence and Anatomical Patterns of Lower Limb Muscle Strain Injuries

A history of lower limb muscle strain injury was reported by 101 participants, yielding a prevalence of **58.4%**. Among those with an injury history, the most frequently affected anatomical sites were the thigh (12.1%) and hamstring (12.1%), followed by the calf (11.6%), knee (9.8%), ankle (7.5%), and groin (5.2%). The frequency of injury episodes was: once (27.2%), twice (17.9%), and three or more times (13.3%). The most recent injury occurred within the previous three months for 18.5% of participants, between three and six months ago for 22.0%, and more than six months ago for 17.9%. Full injury prevalence and pattern data are presented in **Table 2**.

Circumstances and Perceived Causes of Injury

Lower limb muscle strain injuries occurred during training in 25.4% of cases and during competition or matches in 32.9% of cases. The most commonly identified perceived causes were fatigue (13.9%), overtraining (12.1%), poor warm-up (9.8%), sudden movement or sprinting (8.1%), previous injury (6.9%), and uncertainty about the cause (7.5%).

Management, Recovery, and Prevention

Among injured participants, 37.0% sought formal treatment, distributed as follows: physiotherapy clinic (11.6%), sports therapist (8.7%), hospital (7.5%), and self-treatment (9.2%). Rest periods before return to football were less than one week (17.3%), one to two weeks (22.0%), and more than two weeks (19.1%). Lower limb muscle strain injuries affected sporting performance in 46.8% of injured players. Regarding prevention, 86.1% of all participants believed lower limb muscle strain injuries are preventable, and 74.6% reported regular post-activity stretching.

Factors Associated with Injury Severity

Pearson chi-square analyses revealed statistically significant associations ($p < 0.001$) between injury severity—defined as the rest period required before return to football—and the following factors: history of injury ($\chi^2 = 173.000$), injury site ($\chi^2 = 193.863$), number of previous injuries ($\chi^2 = 180.801$), time since last injury ($\chi^2 = 178.343$), circumstance of injury—training versus match ($\chi^2 = 189.127$), perceived cause of injury ($\chi^2 = 185.784$), and performance impact ($\chi^2 = 175.278$). No statistically significant associations were found with training frequency ($p = 0.874$), training duration ($p = 0.074$), or warm-up practice ($p = 0.574$). Full results are presented in **Table 3**.

Table 2: Prevalence, Patterns, Circumstances, and Management of Lower Limb Muscle Strain Injuries (n = 173)

Variable	Category	N	%
History of lower limb muscle strain injury	Yes	101	58.4%
	No	72	41.6%
Injury site (among injured, n=101)	Thigh	21	12.1%
	Hamstring	21	12.1%
	Calf	20	11.6%
	Knee	17	9.8%
	Ankle	13	7.5%
	Groin	9	5.2%
Number of injury episodes	Once	47	27.2%
	2 times	31	17.9%
	≥3 times	23	13.3%
Time since last injury	<3 months	32	18.5%
	3–6 months	38	22.0%
	>6 months	31	17.9%
Injury occurrence	During training	44	25.4%
	During competition/match	57	32.9%
Perceived cause	Fatigue	24	13.9%
	Overtraining	21	12.1%
	Poor warm-up	17	9.8%
	Sudden movement/sprint	14	8.1%
	Previous injury	12	6.9%
	Unsure	13	7.5%
Treatment sought	Yes (any)	64	37.0%
	Physiotherapy clinic	20	11.6%
	Sport therapist	15	8.7%
	Hospital	13	7.5%

Variable	Category	N	%
	Self-treatment	16	9.2%
Rest period (return to football)	<1 week	30	17.3%
	1–2 weeks	38	22.0%
	>2 weeks	33	19.1%
Injury prevention belief	Injuries are preventable	149	86.1%
Post-activity stretching	Regular	129	74.6%

Table 3: Factors Associated with Lower Limb Muscle Strain Injury Severity (Rest Period Before Return to Football)

Factor	χ^2	p-value	Significance
History of injury	173.000	**<0.001**	**Significant**
Injury site	193.863	**<0.001**	**Significant**
Number of previous injuries	180.801	**<0.001**	**Significant**
Time since last injury	178.343	**<0.001**	**Significant**
Circumstance of injury (training vs. match)	189.127	**<0.001**	**Significant**
Perceived cause of injury	185.784	**<0.001**	**Significant**
Performance impact	175.278	**<0.001**	**Significant**
Training frequency	2.450	0.874	Not significant
Training duration	11.499	0.074	Not significant
Warm-up practice	1.994	0.574	Not significant

Note: Significance level set at $p < 0.05$.

DISCUSSION

This study determined the prevalence and anatomical patterns of lower limb muscle strain injuries among amateur male footballers at a Nigerian health sciences college, and examined factors associated with injury severity. The prevalence of 58.4% is substantial and consistent with previous Nigerian studies reporting high injury rates among amateur football populations [11,12]. This figure markedly exceeds the 31% prevalence reported in professional football [14], corroborating the observation that amateur players sustain disproportionately higher injury rates due to irregular training schedules, inconsistent conditioning, inadequate recovery periods, and limited access to sports medicine services [10].

The predominance of thigh (12.1%) and hamstring (12.1%) involvement is consistent with global literature identifying these structures as the most common sites of lower limb muscle strain in football [7,15]. The biomechanical demands of sprinting, forceful kicking, and sudden directional changes impose high eccentric loads on these muscle groups, rendering them particularly susceptible [4]. Tsametis et al. [16] reported analogous findings among Greek amateur soccer players, with posterior thigh injuries being most frequently affected. The comparatively lower prevalence of groin injuries (5.2%) in the present study, relative to some published reports [17], may reflect differences in competitive intensity or overall match exposure within this collegiate amateur cohort.

Injuries occurred more frequently during competition (32.9%) than during training (25.4%), consistent with findings by Kekelekis et al. [18], who reported substantially higher injury incidence during matches compared to training sessions in amateur soccer. This disparity likely reflects the higher physical demands, greater contact forces, and elevated psychological pressure characteristic of competitive match play. Fatigue (13.9%) and overtraining (12.1%) were identified as the leading perceived causes of lower limb muscle strain injuries, underscoring the importance of appropriate load management and structured recovery in amateur settings where players frequently participate in consecutive matches without adequate recuperation [19].

The finding that 86.1% of participants believed lower limb muscle strain injuries are preventable, and 74.6% reported regular post-activity stretching, indicates a positive awareness of injury prevention. Nevertheless, a gap between knowledge and the implementation of structured, evidence-based prevention programmes remains evident. Comparable observations have been reported in Nigerian amateur football, where awareness of injury prevention principles exists without consistent adoption of structured protocols such as the FIFA 11+ warm-up programme [20].

Significant associations between injury severity and prior injury history, injury site, and the number of previous episodes ($p < 0.001$) are consistent with findings by Ekstrand et al. (14), who identified previous injury as the strongest independent predictor of future injury in football. Incomplete rehabilitation, premature return to play, and

residual biomechanical deficits are well-recognized mechanisms underpinning injury recurrence [21]. In the present study, 31.2% of injured players reported two or more episodes, emphasizing the critical need for structured, phase-based rehabilitation protocols and objective return-to-play criteria in amateur football environments.

The absence of a significant association between injury severity and training frequency, duration, or warm-up practice (all $p > 0.05$) warrants careful interpretation. These findings may reflect the relative homogeneity of training practices within this collegiate population, or alternatively, they may be attributable to the inherent limitations of a cross-sectional design in establishing causal relationships. It is possible that the quality, rather than the quantity, of training and warm-up practice is the more critical determinant of lower limb muscle strain injury severity [22].

The finding that only 37.0% of injured players sought formal treatment—with a notable proportion relying on self-treatment (9.2%)—highlights a significant gap in medical access for amateur footballers in this setting. Similar challenges have been documented across Nigerian amateur football communities, where limited availability of physiotherapy and sports medicine services contributes to incomplete recovery and elevated reinjury risk [12,13].

This study has presented some original epidemiological data on lower limb muscle strain injuries in an understudied amateur football population within a Nigerian community setting, employing a validated structured questionnaire and examining a comprehensive range of injury-related variables. However, several limitations should be acknowledged. The cross-sectional design precludes causal inference. Self-reported data are susceptible to recall bias. The single-site focus limits the generalizability of findings to broader Nigerian amateur football populations. Furthermore, the absence of clinical verification of injury diagnoses may affect diagnostic accuracy.

CONCLUSION

This study demonstrates a high prevalence (58.4%) of lower limb muscle strain injuries among amateur male footballers at the College of Health Sciences, Okofia, Nnewi, Nigeria. The thigh and hamstring are the most commonly affected anatomical sites. Injuries occur more frequently during competition than during training, with fatigue and overtraining identified as the principal perceived causes. Prior injury history, anatomical injury site, recurrence, and injury circumstance are strongly associated with injury severity.

RECOMMENDATIONS

Based on the findings of this study, the following recommendations are proposed:

i. Structured warm-up programmes: Coaches and team officials should enforce evidence-based warm-up routines (e.g., FIFA 11+) prior to all training sessions and competitive matches, incorporating dynamic stretching, neuromuscular activation, and progressive eccentric

strengthening of the hamstrings and quadriceps.

ii. Load management: Training schedules should incorporate adequate rest days, periodization principles, and systematic monitoring of training load to mitigate the risk of overtraining and fatigue-related lower limb muscle strain injuries.

iii. Education and awareness programmes: Regular educational sessions should be delivered to players, coaches, and support staff on injury risk factors, early symptom recognition, the importance of complete rehabilitation, and appropriate return-to-play criteria.

Conflicts of Interest:
None declared

Funding: None received

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JOURNAL OF BIOMEDICAL INVESTIGATION (JBI) VOLUME 13 NUMBER 3: NOVEMBER-DECEMBER 2025

EYE HEALTH-SEEKING BEHAVIOUR OF SAWMILL WORKERS IN ONITSHA, NIGERIA

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ABSTRACT

Background: A hazard of sawmilling is ocular affliction which may eventuate in visual impairment. The visual loss could be ameliorated if eye care is sought early. But timeous treatment is a function of eye health-seeking behaviour of the afflicted.

Aim: To determine the eye health-seeking behaviour of sawmill workers in Onitsha, Nigeria.

Materials and Methods: Consenting adult male sawmill workers at Onitsha, selected by simple random sampling were studied using pre-tested a questionnaire on eye disease symptoms, and eye health-seeking behaviour. Examination included visual acuity assessment, external eye examination, refraction, and ophthalmoscopy.

Results: Eighty-five sawmill workers with an age range 18 – 61 years; median – 42 years participated; 50 (58.8%) were self-employed. Eighty (94.1%) had symptoms of eye disease. Tearing, 75 (88.2%), and foreign body sensation, 62 (72.9%), were the common symptoms. Sixty-five (76.5%) participants sought eye care only when they consider the symptoms serious; 20 (23.5%) never did. Self-medication, [58 (68.2%)] and consulting traditional healers, [9 (10.6%)] were popular practice. Cost of treatment, [58 (68.2%)] and a feeling that the symptom was not serious, [44 (51.8%)] were the commonest barriers.

Conclusions: The eye health-seeking behaviour of this cohort of sawmill workers is poor. Treatment cost and not taking eye disease symptoms were the two most important barriers to

seeking eye care. To improve the eye health-seeking behaviour of these workers, subscribing to health insurance scheme and eye health education are recommended.

Key words: sawmill workers, eye health, barriers

INTRODUCTION

Eye diseases and blindness are of public health concern in Nigeria. The nationwide eye disease survey has documented the burden of blindness and eye diseases among Nigerians[1]. No population or geographic segment is spared. Some of the studies have also documented late presentation, and seeking eye care from unqualified persons as factors that contribute to poor outcome of treatment of eye diseases[2,3]. But visual loss can be minimized if the afflicted is takes proactive measures to address the problem by seeking eye care early.

Sawmill workers constitute a distinct occupational group with eye diseases especially those associated with ocular and adnexal trauma as occupational hazard[4]. But the attitude of sawmill workers in Anambra State to eye care is unknown. In an effort to bridge this knowledge gap, a study of eye health-seeking behaviour, among sawmill workers in Onitsha was conducted.

MATERIALS AND METHODS

This was a cross-sectional study of sawmill workers at the Bridge Head Onitsha Anambra State Nigeria. The study was conducted between August and September 2024. Ethical clearance was obtained from the Anambra State Ministry of Health Ethics Committee. Permission was obtained from the chairman of the sawmill workers union. Each participant gave informed consent. A minimum sample size of 77 participants, based on 95% confidence interval was calculated using the Leslie Kish formula, adjusted for population <10,000[5]. Allowing for 10% attrition, the final sample size was 85 participants. Included in the study were sawmill workers aged ≥18 years who willingly consented to participate in the study. Excluded were sawmill workers too ill to participate, those who were absent during the study duration, and those who refused to give consent.

The selection of the participants was by simple random sampling technique viz: using the register of the sawmill workers union, the names of the workers were extracted and written on a 2cm by 2 cm piece; the paper was folded and put in a bag. The bag was churned several times and an assistant not involved with writing the names picked the folded papers from the bag until the calculated sample size was met. The papers were unfolded and the workers whose names were on the papers were interviewed and examined.

The study tool included a questionnaire on socio-demographic profile, eye disease symptoms, and eye health-seeking behaviour. This questionnaire was pre-tested among saw mill workers at Nkwo Nnewi, more than 35 kilometres from our Onitsha study site. Examination included recording

visual acuity separately for each eye, penlight examination of the anterior segment and ocular adnexa, refraction, and ophthalmoscopy.

RESULTS

A total of 85 sawmill workers participated in the study. All participants were of the male gender. The age range 18 – 61 years; median – 42 years; 70 (82.4%) were married. While 7 (8.3%) did not attain formal education, 3 (3.5%) had tertiary education and 50 (58.8%) were self-employed. Table 1 shows the socio-demographic profile of the participants.

Table 2 shows the participants' self-reported symptoms of eye diseases. Eighty (94.1%) had symptoms of eye disease. Some reported more than one symptom. Tearing (watery eyes) was the commonest symptom reported by 75 (88.2%) participants; the least was visual blur reported by 10 (11.2%).

While 65 (76.5%) participants went for eye care consultation only when they consider the symptoms serious, 20 (23.5%) including 15 (17.6%) that had eye disease symptoms never sought any eye care. In Table 3 is shown the where the participants went for eye care. Some consulted multiple healthcare facilities. While self-medication, reported by 58 (68.2%) was the commonest practice, 9 (10.6%) consulted traditional healers.

Table 4 shows barriers to eye care consultation. Some participants mentioned multiple barriers. Cost of treatment, 58 (68.2%), and a feeling that the symptom was not serious, 44 (51.8%), were the commonest barriers.

Table 1: Socio-demographic profile

Variable	No.	%
Age (years)		
≤20	2	2.4
21 – 30	11	12.9
31 – 40	27	31.8
41 – 50	23	27.0
51 – 60	17	20.0
≥61	5	5.9
Total	85	100.0
Marital status		
Married	70	82.4
Single	15	17.6
Total	85	100.0
Educational level		
Non-formal	7	8.3
Primary	45	52.9
Secondary	30	35.3
Tertiary	3	3.5
Total	85	100.0
Employment status		
Self-employed	50	58.8
Apprentice	35	41.2
Total	85	100.0

Table 2: Self-reported eye disease symptoms*

Symptom	No.	%
Tearing	75	88.2
Foreign body sensation	62	72.9
Pain	25	29.4
Glare	22	21.9
Redness	18	21.2
Blurred vision	10	11.8

*% based on 85

Table 3: Health facilities patronized for eye care*

Facility	No.	%
Self-medication	58	68.2
Public hospital	37	43.5
Chemist	26	30.5
Private hospital	13	15.2
Traditional healer	9	10.6

*% based on 85

Table 4: Self-reported barriers to eye care*

Barrier	No.	%
Cost of treatment	58	68.2
Symptom not serious	44	51.8
Lack of time	32	37.6
Lack of awareness	28	32.9
Fear of diagnosis	17	20.0
Distance to health facility	8	9.4

*% based on 85

DISCUSSION

Sawmill workers are an important segment of the population that contribute to the economic development of any nation. However, part of the occupational hazards of sawmilling is proneness to eye disorders[6]. The commonest eye disease symptoms reported by the participants were tearing (watery eyes) and foreign body sensations. The findings were not surprising as these symptoms may result from sawdust particles generated during work. Some of the disorders underlying the symptoms of eye diseases among sawmill workers may lead to visual impairment. On the other hand, the ensuing visual loss could be mitigated by early treatment. Therefore, assessing the eye health-seeking behaviour of sawmill workers is important.

That 94.1% of the participants in the present study had symptoms of eye disease symptoms points at a high burden of eye diseases among sawmill workers. On the other hand, with 76.5% of the participants seeking eye care only when the symptoms were considered serious and the rest of the participants not going for eye care at all, the eye health-seeking behaviour of this cohort

of sawmill workers is poor. Although some participants consulted orthodox healthcare facilities, the finding that up to 68.2% indulged in self-medication and another 10.6% relied on traditional healers for eye care, also constitute cause for worry. These findings portend danger for the eye health of this vital occupational group.

Cost of treatment, reported by 68.2% of the participants, was the commonest barrier to accessing eye reported by the study participants. Treatment cost and other hidden or indirect cost had been reported as constituting significant hindrance to gaining access to healthcare services, including eye care, especially in countries without health insurance scheme[6]. The Nigeria National Health Insurance Scheme[7] was signed into law in 1999 and superseded by the National Insurance Authority (NHIA) Act of 2022[7,8]. The scheme was designed to cater for the health needs of Nigerians with regard to offsetting treatment bills. But scheme commenced with registration of workers in the formal sector (mainly civil servants). Its effect on the informal sector where most of the sawmill workers belong is for now very limited. However, the Anambra State Health Insurance Agency (ASHIA)[9] has recently embarked on enrolling citizens in the informal sector of the economy. It is therefore recommended that sawmill workers in Anambra State should register and access healthcare services underwritten by the Anambra State Insurance scheme in order to minimize out-of-pocket expenditure when obtaining eye care services.

Another important barrier to eye care reported by 72.9% of the participants was the feeling that 'the eye symptom was not serious. This misconception is common among the populace especially when the symptom, especially at the initial stages, does not cause pain or redness of the eyes. But it is known that some blinding diseases like glaucoma and diabetic retinopathy cause neither redness nor pains at the initial stages. Glaucoma has the highest prevalence among the Igbo ethnicity in Nigeria and this study was conducted among the Igbos[1]; similarly, diabetic retinopathy previously considered rare in Nigeria now has a hospital incidence of up to 33% and up to 7% within the general population. Therefore, targeted health education is required in order to persuade sawmill workers to minimize self-medication and seek eye care early. The available eye health care facilities should also be made known and accessible to them.

CONCLUSION

This study has revealed a poor eye health-seeking behaviour among sawmill workers in Onitsha, Nigeria. Treatment cost and not taking eye disease symptoms were the two most important factors found to encourage the poor attitude. While subscribing to health insurance scheme would provide financial relief to the sawmill workers, targeted eye health education is needed in order to effect attitudinal change and encourage the workers to seek eye care early even when symptoms may appear trivial.

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AFRICAN'S GROWING CONTRIBUTION TO ANTIRETROVIRAL DRUG RESISTANCE RESEARCH: BIBLIOMETRIC ANALYSIS OF 2004 TO 2024

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ABSTRACT

Background: The threat of antiretroviral drug resistance developing and spreading is of greater public health importance as antiretroviral therapy is scaled up. Therefore, this study was designed to assess the growing contribution to Africa's antiretroviral drug resistance Research.

Materials and Methods: This investigation presents a bibliometric analysis of all research articles on antiretroviral drug resistance published from May, 2004 to May 2024, indexed in the PubMed database.

Result: The overall number of articles published was 1,601 papers, which has an average annual productivity of 123.15. South Africa accounted for the highest productive outputs with the total number of publications of 216, followed by the Switzerland (n = 56), Uganda (n = 52), Kenya (n = 18), Zimbabwe (n = 18), Netherlands (n = 15), Cameroon (n = 12), Botswana (n = 10), Malawi (n = 10), Zambia (n = 6), Ethiopia 6 (n = 6), and Nigeria (n = 5). Since 2015, publications on ARV Drug Resistance Research have fluctuated, with the highest number in 2020. Following that trend, the number of productive outputs dramatically reduced from 2021 to

2024. The University of Cape Town, South Africa, emerged as the leading institution in ARV Drug Resistance Research. In Nigeria, ARV Drug Resistance Research is the least prolific.

Conclusion: The overall trend in this field is characterized as decreasing yet evolving. This suggests that while fewer studies are being published overall, the focus of the research is shifting, likely toward these previously understudied areas rather than repeating established research.

Keywords: Antiretroviral drug, AIDS, Africa, Drug resistance

INTRODUCTION

The genetic diversity of Human Immunodeficiency virus (HIV) poses a significant challenge to the global management of HIV infection [1-7]. Even with the increasing availability of antiretroviral (ARV) medications, treatment failure is still a frequent issue among patients [8-10]. Besides, other factors such as; absence of plasma viral load and CD4 T lymphocyte count monitoring, drugs interactions, treatment disruptions due to stock shortages, and the use of substandard antiretroviral treatment regimens play role in the development of drug resistance in Africa [11-15].

Though, the emergence of drug-resistant variants of HIV-1 has been linked to mutations in the HIV-1 pol genes, which encode the key targets for major antiretroviral (ARV) medications [16, 17]. Evidences indicate that the effectiveness of ARV therapy is also affected by both the viral subtype and pre-existing mutations [18, 19]. Additionally, it has been suggested that the pathways leading to drug resistance might be influenced by pre-existing polymorphisms across different HIV-1 subtypes [20-22].

It had been reported that older age, ART regimen, lower CD4 count, higher viral load (VL) and inadequate adherence were predictors of virologic failure (VF), which is defined as 2 consecutive VL > 1000 copies/mL after at least 6 months of ART and intensive adherence counselling could lead to the accumulation of HIV drug-resistance-associated mutations [23-26]. Previous studies showed that ARVs status, CD4 <100 cells/mm^3, Viral subtype G is more likely to have Drug Resistance Mutation (DRM) than subtype Circulating Recombinant Form 2 (CRF02)_AG, frequent use of Non-nucleoside Reverse Transcriptase inhibitors (NNRTI), and ART duration are predisposing factors to Human Immunodeficiency Virus Drug Resistance (HIVDR) [27, 28].

The threat of HIV drug resistance developing and spreading is a greater public health concern as ART is scaled up [29-32]. World Health Organization (WHO) supports monitoring HIVDR, whether acquired or transmitted, to increase the efficacy of treatment programs for individuals living with HIV [33, 34]. Numerous studies suggest implementing specific measures in areas where the prevalence of NNRTI pre-treatment resistance is greater than or

equivalent to 10% [35]. These measures include initiating antiretroviral therapy (ART) with dolutegravir or selecting antiretroviral drugs based on a genotypic resistance test [12, 24, 36-42]. It is speculated that one in ten individuals starting antiretroviral therapy (ART) had a virus with one or more resistance mutations, with females two times more likely to be affected than males [1, 43, 44]. However, there is relatively limited information available from developing nations where non-B subtypes are more prevalent. In Africa, where the epidemic is primarily caused by non-B subtypes, studies on HIV drug resistance and polymorphisms have mostly investigated in resistance to non-nucleoside reverse transcriptase inhibitors (NNRTIs) and nucleoside reverse transcriptase inhibitors (NRTIs), while the resistance to protease inhibitors (PIs) has not been thoroughly examined [10, 45-48].

Bibliometric studies are valuable tools for assessing a specific discipline over a designated period, both socially and scientifically. It acts as an indicator of research activities in a particular field [49]. These studies analyze advancements and highlight research deficiencies. The performance evaluation of a chosen area of study is frequently conducted through bibliometrics and social network analysis (SNA). While bibliometric data provides fundamental metrics, social network analysis sheds light on the impact of social connections and interactions. This research aimed to examine the trends and contributions of Africa in the field of ARV Drug resistance mutation research. The results of this research are intended to assess progress, pinpoint gaps in Antiretroviral Drugs Resistance Mutation within Africa, and guide future research efforts and funding opportunities.

METHOD

We examined bibliometric data on Antiretroviral (ARV) drugs resistance mutations in Africa research published in PUBMED from May 13, 2004, to May 14, 2024.

The search was conducted on January 18, 2025. Utilizing advanced search features in PUBMED, we employed “MESH” terms for “HIV Antiretroviral drugs Resistance mutations” and “ AIDS Antiretroviral drugs Resistance mutations” and incorporated the following keywords: “HIV Antiretroviral drugs Resistance mutations” [Title/Abstract] OR “AIDS Antiretroviral drugs Resistance mutations” [Title/Abstract] OR “Acquired Immunodeficiency Syndrome Antiretroviral drugs Resistance mutations” [Title/Abstract] OR “Human Immunodeficiency Virus Antiretroviral drugs Resistance mutations” [Title/Abstract] OR “polymorphism of non-subtype B HIV strain” [Title/Abstract]AND “Africa” [Title/Abstract] OR “Sub-Sharan Africa” [Title/Abstract] We retrieved all relevant data according to the pre-established search criteria without restrictions on the type of articles. The collected data were used to calculate bibliometric measures. Since PUBMED does not maintain citation records, we obtained citation details for authors and articles through Google Scholar. We also re-conducted searches in PUBMED using the previously mentioned search terms along with “Retraction” and “Expression of

Concern." Furthermore, we searched the Retraction database with the location specified as Africa.

Screening protocol and criteria

Only articles specifically addressing Antiretroviral drugs Resistance mutations and polymorphism of non-sub type B HIV strains in Africa were considered for inclusion. Articles that mentioned Antiretroviral drugs in that manner but didn't focus on it were excluded, as were those that did not pertain to neither Africa nor Sub-Sharan Africa. There were no limitations regarding the type of article accepted. Duplicate articles were also eliminated. Two separate review teams among the authors independently conducted the article selection. Any disagreements were resolved through consensus between both groups.

Visualization of social network analysis

We employed VOS viewer (Center for Science and Technology Studies, Leiden University, The Netherlands) version 1.6.18 to create a visual representation of "ARV Drug resistance mutation", "Polymorphism non-subtype B HIV strains", "Africa" terminology and collaboration based on the data obtained from PUBMED.

Impact factor

The impact factor (IF) serves as an indicator of a journal's influence and was first established by the Institute for Scientific Information in Philadelphia, PA, USA, as a bibliometric measure. It is refreshed yearly in the Journal Citation Report (JCR) published by Clarivate Analytics, and the figure is frequently seen as a sign of prestige. We referenced the JCR data from 2024.

Authors/Institution participations index

We assessed the total scientific publications from 2004 to 2024 related to "ARV Drug resistance mutation", "Polymorphism non-subtype B HIV strains", "Africa". This reflects the count of documents authored by an individual or institution on the subject of Antiretroviral drugs Resistance mutations and polymorphism of non-sub type B HIV strains in Africa compared to the overall publications in that field.

Keyword analysis

We utilized keyword evaluation to confirm the trends of discussion and research regarding the "ARV Drug resistance mutation", "Polymorphism non-subtype B HIV strains", "Africa"

Co-authorship analysis

Co-authorship signifies the collaboration among authors who contribute to a specific area of research. The collaboration between authors can be observed through the co-authorship of publications (26). The co-authorship network diagram created by VOS viewer illustrates the collaborative social network within research domains.

Bibliometric mapping

Bibliometric mapping consists of two components: co-authorship mapping and co-occurrence mapping. Co-authorship highlights the collaborative efforts of authors within institutions contributing to the field of research, while co-occurrence

emphasizes the relationships among keywords.

The visualization of co-authorship network analysis employs the following interpretative keys: The size of the nodes or bubbles (circles) in the network signifies the frequency or quantity of documents associated with an author or institution. Furthermore, the lines or arcs connecting nodes represent the existence and strength of the co-authorship relationship. Lastly, the color of the node is determined by the VOS viewer clustering algorithm, which assigns colors based on the calculated similarity measure among them. As a result, it can be concluded that nodes sharing the same color are interconnected. Moreover, the proximity of two (2) nodes indicates the closeness of their relationship.

RESULTS

Publications output

We gathered a total of 1,601 papers publications, after excluding 65 publications that were either irrelevant to Africa (only mentioning Antiretroviral drugs briefly) or not directly focused on neither Antiretroviral drug resistance in Africa nor mutation in Africa. Among these, 54.3 % (N = 576) consisted of original articles, 22.4% (N = 238) were Clinical Trials, 22.1 % (N= 235) were narrative reviews, 14.7 % (N= 164) were Randomized Controlled Trial, 10.2% (N = 163) were Book and Document, 7.9 % (N = 84) were Comparative study, 5.6 % (N = 59) were Observation study, 4.3% (N= 46) were systematic reviews, and 2.5 % (N = 27) were Medical. Analysis Additional types of publications identified included Newspaper (0.4%), Expression of interest (0.3%), conference/Retracted paper (0.1%) (table 1).

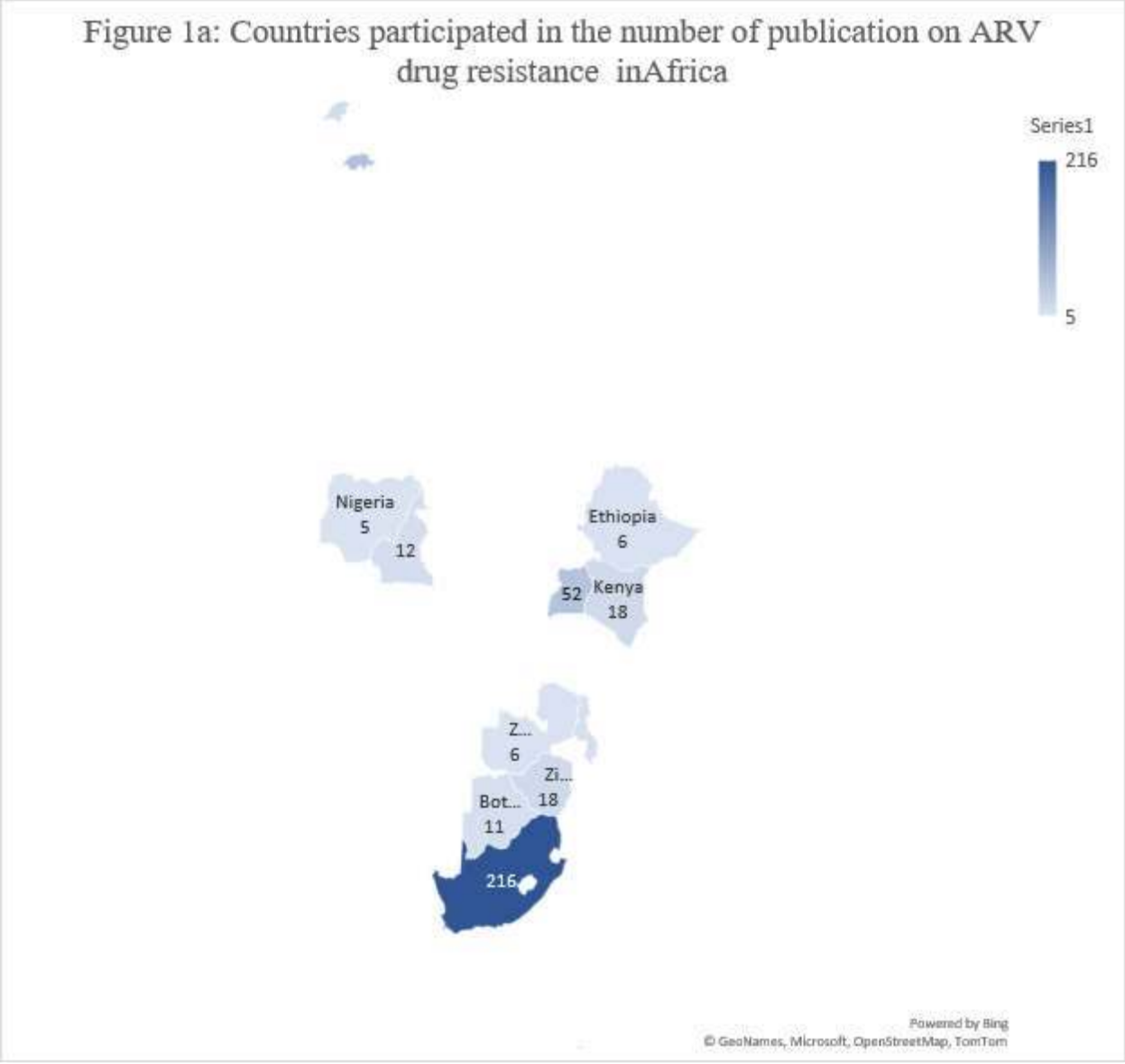
Table 1: Demonstration of Research out within study period with respect to article types

Type of Articles	Frequency (%)
Original articles	576 (54.3)
Clinical Trial	238 (22.4)
Narrow Review	235(22.1)
Randomized Controlled Trial	164(14.7)
Book and Document	163(10.2)
Comparative study	84(7.9)
Observation study	59(5.6)
Systematic Review	46(4.3)
Medical Analysis	27(2.5)
Newspaper	4(0.4)
Expression of Interest	3(0.3)
Clinical Conference	1(0.1)
Retracted paper	1(0.1)

Analysis of Africa with the highest publications

The current prevalence rate of publications on ARV Drug resistance in Africa and stratification based on countries showed the highest productive outputs in South Africa 216 (48.6%), Switzerland 56 (12.6%), Uganda 52 (11.7%), Kenya 18 (4.0 %), Zimbabwe 18 (4.0 %), Netherlands 15 (3.4%), Cameroon 12 (2.7%), Botswana 10 (2.5%), Malawi 10 (2.3%), Zambia 6 (1.4 %), Ethiopia 6 (1.4%), Nigeria 5 (1.1%) (Figure 1a and Figure 1b)

Figure 1a: Countries participated in the number of publication on ARV drug resistance inAfrica



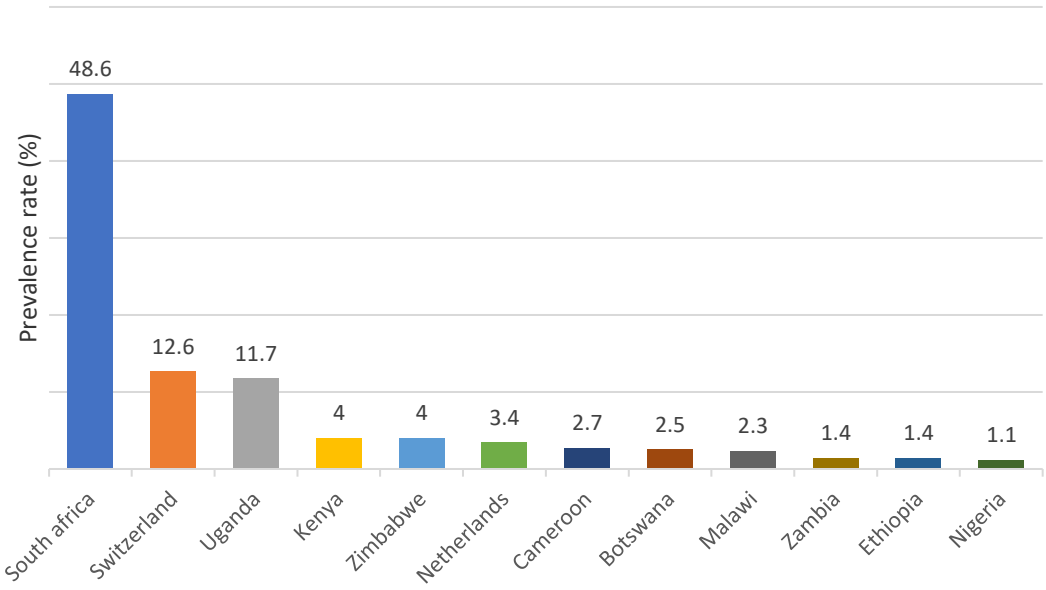
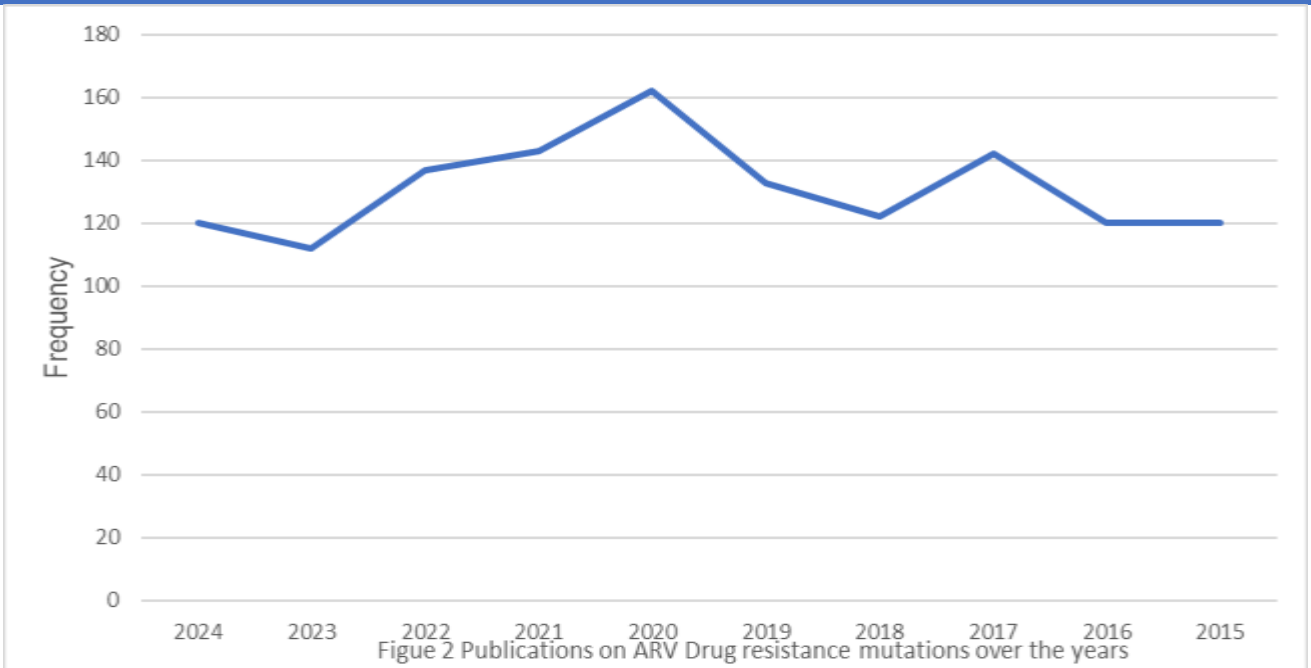


Figure 1b:Prevalence rate of countries participated in publications on ARV Drug resistance

It is speculated that there was a scarcity of publications on ARV Drug resistance in Africa from 2004 to 2014 until when 120 publications were released in 2015. Following that trend, the output of ARV Drug resistance in Africa -related literature experienced a slow increase until 2020, when there was a dramatic rise of over 40 times. Since then, the pace of research has been maintained to the initial publication, with a steady output of at least 120 publications annually after 2023 (figure 2)



Figue 2 Publications on ARV Drug resistance mutations over the years

Evaluation of the distribution of articles based on author count

Our findings indicated a wide range in the number of authors per publication, from single-author to those with more than 4 authors. Additionally, the results revealed that over 90% (n = 1726) of the published papers were produced through collaboration more than 4 authors (Table 2).

Table 2: Distribution of articles based on Author number

Number of Authors	Number of Publications
1 author	26(1.3%)
2 authors	72(3.7%)
3 authors	105(5.4%)
> 4 authors	1726(89.6%)

Evaluation of the institutions with the highest productivity in ARV Drug resistance mutation in Africa research

The institutions in Africa with the highest productivity in ARV Drug resistance mutation in Africa. The University of cape town, cape town emerged as the leading institution (n = 42), followed closely by the University of the Witwatersrand, Johannesburg (n =36), Joint clinical research Centre, Kampala, (n = 22), University of Zurich, Zurich (n =17), among others. The top six institutions alone accounted for over 5% of the total published literature. It is significant that both the University of cape town, cape town and its associated university (University of the Witwatersrand, Johannesburg) are among the most productive institutions. Likewise, the Joint clinical research Centre, Kampala also feature on the list of top institutions.

Among the leading 20 institutions, there are 9 institutions found in South Africa, 5 institutions found in Switzerland, 3 institutions found in Uganda, 1 institution located in Cameroon, and other countries (Table 3)

Table 3: Top 20 most productive institutions in ARV Drug resistance mutation in Africa research

Participating institutions	**Countries**	**No.of documents** (%)
University of cape town, cape town	South Africa	42 (15.6)
University of the witwatersrand, johannesburg	South Africa	36 (13.3)
Joint clinical research centre, kampala,	Uganda	22 (8.2)
University of zurich, zurich	Switzerland	17 (6.3)
University of basel, basel	Switzerland	15 (5.6)
University of yaoundé i, yaoundé	Cameroon	14 (5.2)
University of kwazulu-natal, durban	South Africa	13 (4.8)
Africa health research institute, durban	South Africa	12 (4.4)
Mrc/uvri uganda research unit on aids, entebbe	Uganda	11 (4.1)
Right to care, johannesburg	South Africa	10 (3.7)
Hiv department, world health organization, geneva	Switzerland	9 (3.3)
stichting hiv monitoring, amsterdam	Netherlands	9 (3.3)
University of kwazulu-natal, durban	South Africa	8 (2.9)
Ndlovu research consortium, elandsdoorn	South Africa	8 (2.9)
University of bern, bern	Switzerland	8 (2.9)
Uganda virus research institute,entebbe	Uganda	8 (2.9)
Josha research, bloemfontein	South Africa	8 (2.9)
National health laboratory service, johannesburg	South Africa	7 (2.6)
Swiss tropical and public health institute, basel	Switzerland	7 (2.6)
Africa health research institute, kwazulu-natal	South Africa	6 (2.2)

Analysis of publications from sources with the most output

Table 4 illustrates the journals that have published the highest volume of research related to ARV Drug resistance mutation research in Africa. The top sources include PLoS ONE, AIDS Research Human and Retroviruses, Aids, Journal of Acquired Immune Deficiency Syndromes, Journal of Antimicrobial Chemotherapy, Lancet Infectious Disease, Clinical Infectious Disease, Antiviral Therapy, Journal of International AIDS Society, AIDS Reviews, HIV Medicine, Viruses, Pediatric Infectious Disease Journal, Current Opinion Virology, PLoS Med, Antimicrobial Resistance Infectious Control, Medicine (Baltimore), South African Medical Journal Pan African Medical Journal, Of these sources, five journals have an impact factor (JCR 2024). Three of them, Lancet Infectious Disease, Clinical Infectious Disease, Current Opinion Virology have an impact factor exceeding 5. Ten of the journals are based in United State of America while Five and four of the journals were published in United Kingdom and Africa respectively.

Table 4: Analysis of sources with the highest number of publications in Africa

Article Source	No of Document	Impact factor	Country of Origin	Abbreviation
PLoS One	160	2.9(2023)	USA	PLoS One,
AIDS Research Human and Retroviruses	136	N/A	USA	AIDS Res Hum Retroviruses
Aids	110	4.6 (2021)	USA	Aids
Journal of Acquired Immune Deficiency Syndromes	96	3.863(2018)	USA	Acq.Imm.Def.Syn
Journal of Antimicrobial Chemotherapy	91	5.2 (2023)	UK	J Antimicrob Chemother
Lancet Infectious Disease	80	36.4 (2024)	UK	Lancet Infect Dis
Clinical Infectious Disease	71	8.2(2024)	USA	Clin Infect Dis
Antiviral Therapy	50	1.3(2025)	USA	Antivir Ther
BMC Infectious Disease	50	N/A	UK	BMC Infect Dis
Journal of International AIDS Society	48	5.3(2020)	USA	J Int AIDS Soc
AIDS Reviews	46	0.5(2024)	Spain	AIDS Rev
HIV Medicine	32	3.0(2022)	UK	HIV Med
Viruses	30	4.0(2023)	Switzerland	Viruses
Pediatric Infectious Disease Journal	30	3.4(2023)	USA	Pediatr Infect Dis J
Current Opinion Virology	22	5.7(2024)	Netherlands	Curr Opin Virol
PLoS Med	20	10.5(2023)	USA	PLoS Med
Antimicrobial Resistance Infectious Control	18	4.6 (2023)	UK	AntimicrobResist Infect Control

Medicine (Baltimore)	17	4.2(2024)	USA	Medicine (Baltimore)
South African Medical Journal	16	3.0(2023)	South Africa	S Afr Med J
Pan African Medical Journal	15	0.9(2023)	Nigeria	Pan Afr Med J

Analysis of co-authorship among involved institutions

The collaborative network among institutions conducting ARV Drug resistance mutation research in Africa is depicted in Fig 3a and Fig 3b. The mapping criterion was established at a minimum of 5 collaborative efforts. Out of the 6139 institutions that met the criteria, only 66 (%) were interconnected through collaborations. The institutions with the highest collaboration totals include the Joint Clinical Research Centre, Kampala, (18 link strength), HIV Department, World Health Organization, Geneva (8 link strength), Africa health research institute, Durban (8 link strength), Institute of Medical Virology, University of Zurich, Zurich, Switzerland (15 link strength), Division of Clinical Pharmacology, University of Cape Town, South Africa (11 link strength), Center for the aids programme of Research in South Africa (13 link strength), Department of Molecular Medicine And Haematology, Johannesburg (14 link strength), University of Witwaterand Right to care, Johannesburg, South Africa (10 link strength), Desmond tutu, HIV centre, University of cape town, South Africa (15 link strength), and University of Basel, Basel, Switzerland (10 link strength) Among the notable collaborations of the Joint Clinical Research Centre, Kampala are: Division of Clinical Pharmacology, University of Cape Town, South Africa, Division of infection and immunity, University College, London, United Kingdom, Department of Molecular Medicine And Haematology, Johannesburg, National Health Laboratory Service, Johannesburg, South Africa, Stitching HIV monitoring, Amsterdam, the Netherland, Josha Research, Bloemfontein. Nonetheless, the strongest overall collaboration, with a link strength of 9, occurred between the Division of Infection and Immunity, University College, London, United Kingdom, and the Joint Clinical Research Centre, Kampala (Fig 3a and Fig 3b)

The leading first authors in ARV Drug resistance mutation in Africa research include Fokam J, Kityo C, Wallis CL, Kaleebu P, Moyo S, Ndembi N, Sigaloff KCE, Van zyl G, Manasa J, Hunt G, Aghokeng AF, and Steegen K. Out of the 20 authors listed, 11 are based in Nigeria while the remaining 9 are affiliated with institutions in the USA, Indonesia, United Kingdom, and France respectively (Table 5 and Table 6).

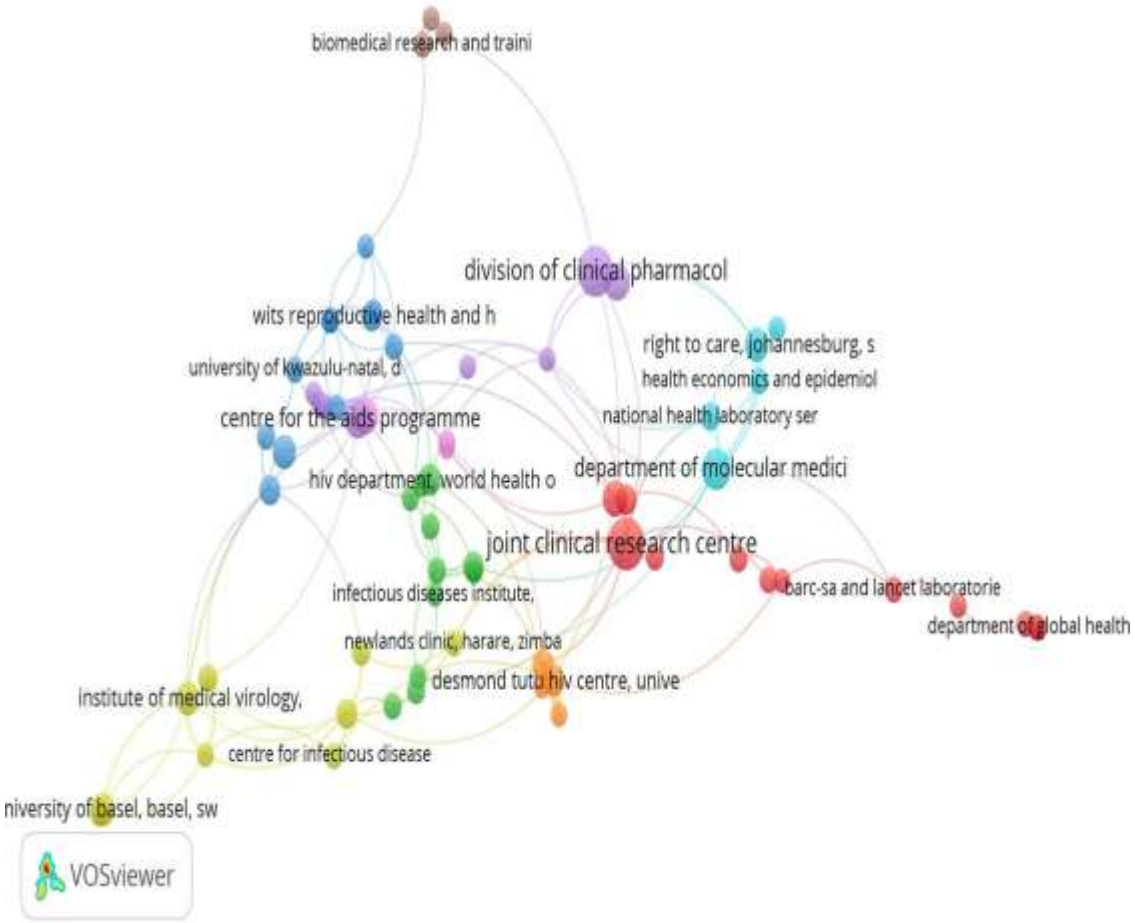


Fig.3a: Collaborative network among institutions publishing Drug resistance mutation related research in Africa

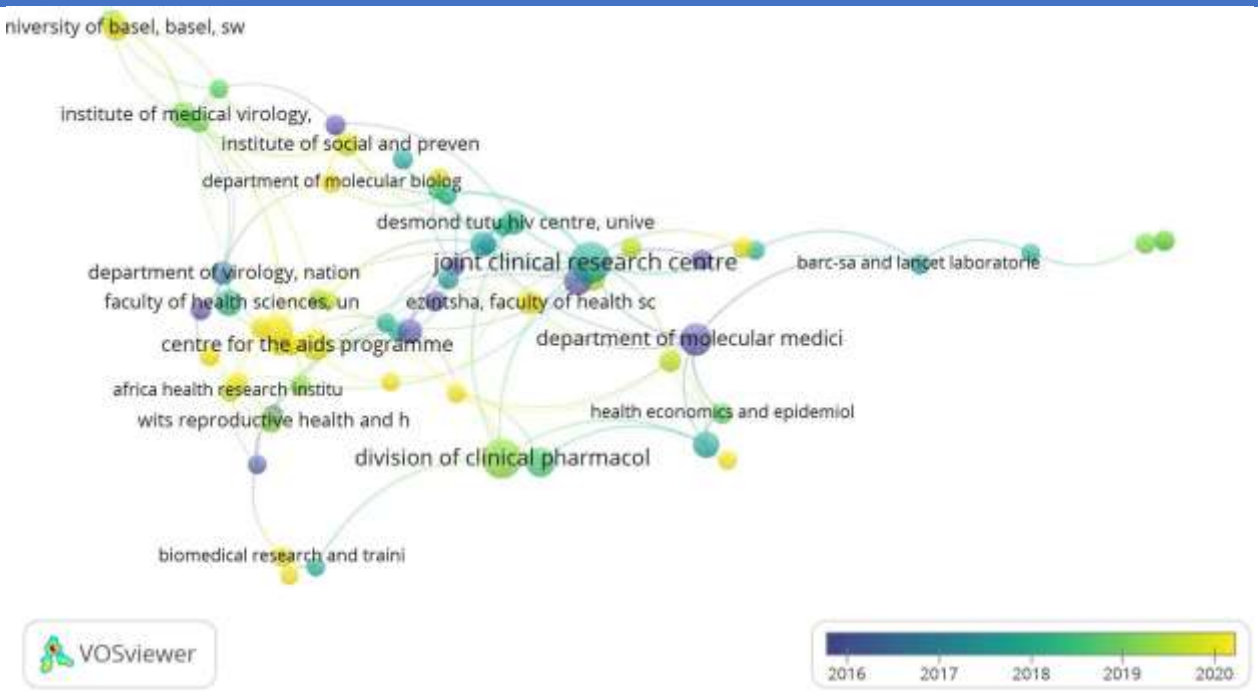


Fig. 3b: Overlay visualization of collaborative network among institutions publishing Drug resistance mutation related research in Africa

Table 5: Top 20 most productive Principal author (First author) in ARV Drug resistance mutation in Africa research

Author	h-index	Country	Affiliation	Documents (%)
Fokam, J	N/A	Cameroon	University of Buea,Buea, Cameroon	45(8.0)
kityo, C	N/A	Uganda	Joint Clinical Research Center	40 (7.1)
Hamers, RL	44	Indonesia	Oxford University Clinical Research	39 (6.9)
Wallis, CL	28	South African	Lancet Laboratories, Johannesburg	39 (6.9)
Jordan, MR (6.2)	N/A	USA	Tufts University and Tufts Medical Center	35
Kaleebu, P	47	Uganda	Uganda Virus Research Institute	32 (5.7)
Eshleman,SH	52	USA	Johns Hopkin University School of Medicine	32 (5.7)
Moyo,S	41	South African	Botswana-Harvard AIDS Institute partnership	30 (5.3)
Gupta, RK	63	United Kingdom	University of Cambrigde	27 (4.8)
Ndembi,N	37	Nigeria	Institute of Human Virology,Abuja	26 (4.6)
Sigaloff,KC	15	Netherlands	Amsterdam UMC	25 (4.4)
Van zyl, G	25	South Africa	University of Cape town and National Health Laboratory	24 (4.2)
Lockman,S	182	USA	Harvard University, Cambridge	23 (4.0)
Charpentier, C	47	France	University of Paris	23 (4.0)
Manasa,J	19	Zimbabwe	University of Zimbabwe	21(3.7)
Hunt,G	30	Switzerland	National Institute for Communicable Diseases	20 (3.5)
Aghokeng,AF	NA	Cameroon	Virology Laboratory CREMER-IMPM	20 (3.5)
Abrams, EJ	NA	USA	Columbia University Medical Center	20 (3.5)
Steegen,K	18	South Africa	National Health Laboratory Service	20 (3.5)
Meintjes,G	90	South Africa	University of Cape town	20 (3.5)

Table 6: Mapping the most influential Authors through co-Authorship collaboration Networks

Author	Number of documents	Total Link strength	Affiliation
Kityo, C	40	151	Joint Clinical Research Center
Fokam, J	45	133	University of Buea,Buea, Cameroon
Wallis, CL	39	117	Lancet Laboratories, Johannesburg
Moyo, S	30	108	Botswana-Harvard AIDS Institute
Chimukangara,B	18	66	National Institutes of Health (NIH)
Hunt, G	35	85	National Institute for Communicable Diseases
Charpentier,C	23	35	University of Paris
Meintjes, G	20	27	University of Cape town

Overall analysis of co-authorship among authors

Figure 4a and 4b illustrates the network and overlay visualization of co-authors comprised of individuals who have published at least 10 (ten) research articles related to ARV Drug resistance mutation and polymorphism non-subtype B strains in Africa research. The network consists of 145 nodes, 988 co-authorship links, a total link strength of 2902, and is divided into 8 clusters.

Each node symbolizes an author, with the size of the node indicating the author's activity or number of publications, while the connections between authors represent their collaborative relationships. Notably, 160 (19.80%) of the 10140 authors who satisfied the minimum requirements (at least 10 publications) were found to have no collaborative connections. Regarding total link strength, Kityo, Cissy (151; blue cluster), Fokam Joseph (133; Brown cluster), Wallis, Carole l (117; purple cluister), Moyo, Sikhulile (108; orange cluster), Chimukangara, Benjamin (66; yellow cluster), Hunt, Gillian (85; red cluster), Charpentier, Charlotte (35; green cluster), and Meintjes, Graeme (27; turquoise), were the leading authors in the network of ARV Drug resistance mutation in Africa research.

Analysis of the authors with the greatest impact based on collaborative co-authorship relationships (including both principal and co-author roles), the most productive authors were Fokam, Joseph (n= 45), Kityo, Cissy (n= 40), Wallis, Carole l (n= 39), Hunt, Gillian (n= 35), Moyo, Sikhulile (n= 30), Charpentier, Charlotte (n = 23), Meintjes, Graeme (n= 20), Chimukangara, Benjamin (n= 18), listed in that order. Kityo, Cissy and Fokam, Joseph appeared to be the most collaborative authors and achieved the highest total link strength, with affiliations to the Tufts University and Tufts Medical Center, USA; Uganda Virus Research Institute; Institute of Human Virology, Nigeria and Virology Laboratory CREMER-IMPM, Cameroon respectively (Table 7).

Interestingly, Kityo, Cissy (from Joint Clinical Research Center, Uganda) held the

2nd position for the most productive author based on principal author analysis (n = 40) and ranked as the 1st most collaborative author (n = 151), while Fokam, joseph secured the 2nd highest total link strength. Likewise, Wallis, Carole l and Moyo, Sikhulile maintained both lists as the 3rd and 4th most published principal author as well as the most collaborative author, total link strength (Tables 5 and 6).

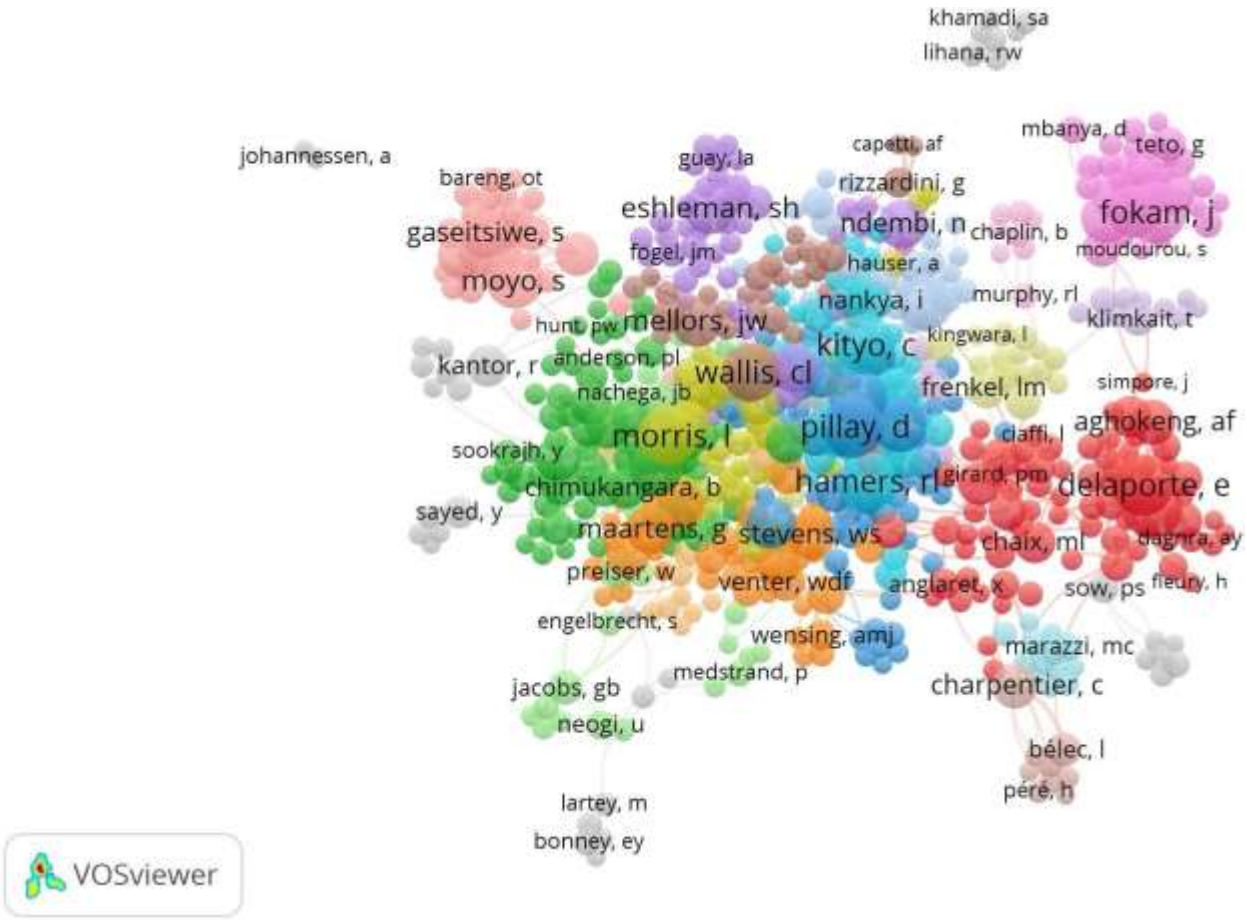


Fig. 4a: Co-authorship network among authors publishing Drug resistance mutation related articles in Africa

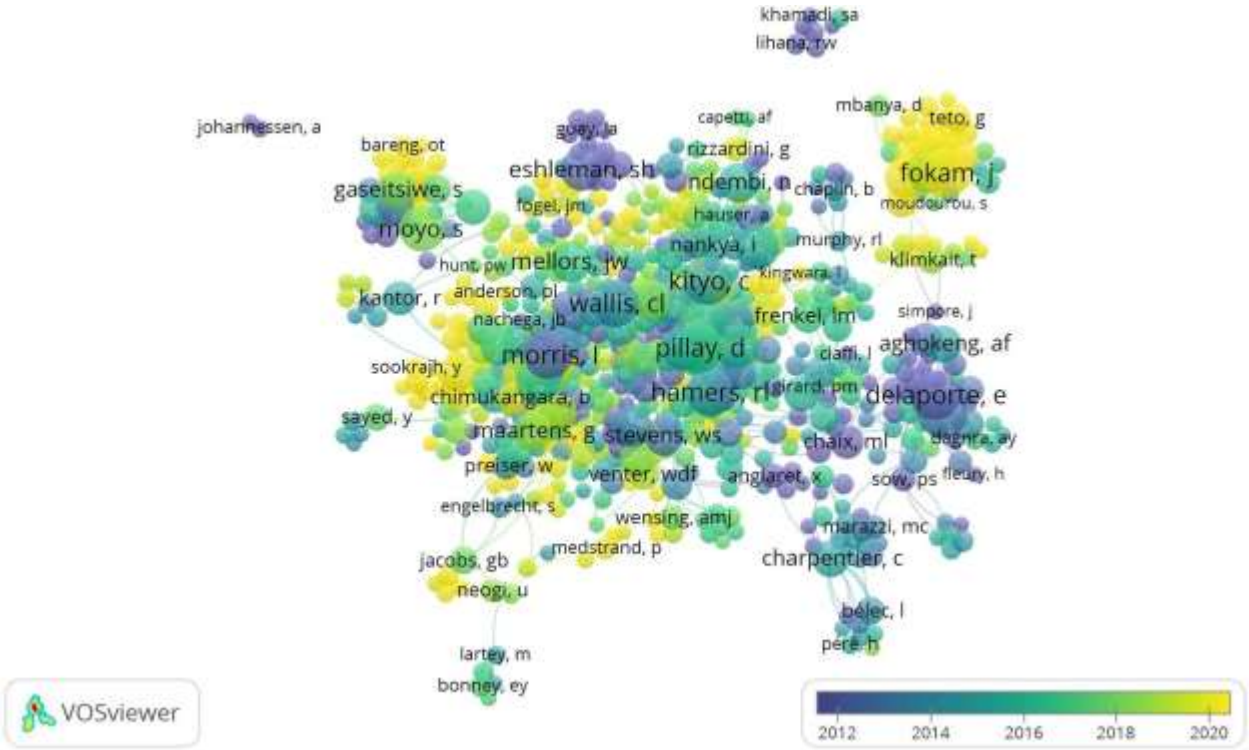


Fig. 4b: Overlay visualization of Co-authorship among authors publishing article per year related to drug resistance mutation research in Africa.

Analysis of keywords and hotspots

The hotspot analysis of author keywords used in ARV Drug resistance mutation related research conducted in Africa is displayed in Figure 5a and Figure 5b. The map contains keywords that appeared more than 5 times. For this, precisely 501 keywords were eligible. The keywords were categorized into 6 clusters by the network visualization. female (n=1224), human (n= 1849), Antiretroviral agent (n=383), CD4 Lymphocyte count (n= 267), risk factors (n=93), Medical Adherences (n=104), acquired immunodeficiency syndrome (n= 58), HIV (n=349) and PMTCT (n=6) were all represented by Cluster 1 (red). The two most prevalent keywords in cluster 1 were Female (n=1224), and human (n= 1849) respectively. Nevertheless, while Antiretroviral agent, CD4 lymphocyte counts, Risk factors and medical adherence were less common although there was a higher total link strength of Antiretroviral agent (4126), CD4 Lymphocyte count (3534), risk factors (1059), Medical Adherences (1276), acquired immunodeficiency syndrome (534), and HIV (3636) respectively.

Antiviral Drug resistance (n=1030), HIV-1 (1017), Mutation (n=446), Genotype (n=

327), Phylogeny (n=145), Missense mutation (n=101), DNA sequency analysis (n=125), genetic polymorphism (n= 53), Amino acid sequence (n=28), Molecular sequence data (n=128), HIV reverse transcriptase (n= 121), HIV protease inhibitor (107) were the main topics of Cluster 2 (green). Cluster 3 (blue) represented keywords related to molecular detection of antiretroviral drug resistance. The most common keywords in cluster 3 were Adolescent (n= 435), child (n=301), genotyping technique (61), HIV integrase (n= 48), Dolutegravir (n=28), Acquired drug resistance (n=5).

Cluster 4 (yellow) stood for mother-to-child transmission on antiretroviral drugs and drug resistance mutations -related terms. In cluster 4, the most common keywords were newborn infant (n= 84), nevirapine (n= 142), pregnancy (n= 184), pregnancy complications (n= 92), vertical infectious disease transmission (n=153), HIV 1-drug resistance (n=14), pretreatment drug resistance (n=9), High through put nucleotide sequence (n= 21), Drug Resistance mutations (n=11). In contrast, conduct in category 4 exhibited the strongest correlation (when combined with other terms).

Cluster 5 (purple) depicted keywords related to viral load and prevalence of young adult. In cluster 5, the most often occurring keywords were viral load (n=625) and young adult (n=368). Overall, the most used keywords were those that dealt with human, female related to hiv-1 and mutation (Fig. 5a).

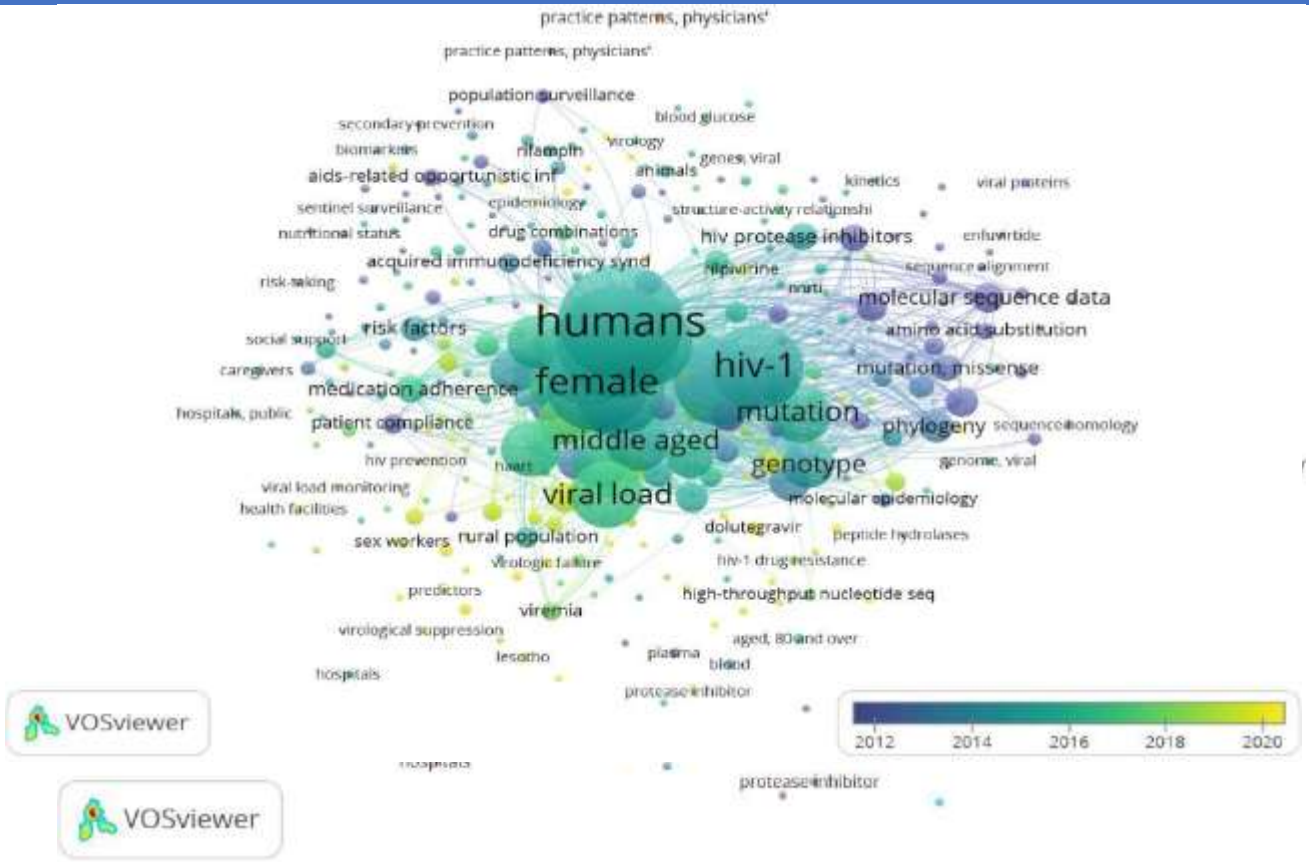


Fig. 5a: Network visualization of Keyword hotspots

Under overlay visualization, VOSviewer highlighted keywords included in the map with multiple colors based on the average year of keywords' appearance (Fig. 5b). Green keywords were more prominent than purple and yellow ones. In more recent years, keywords from yellow cluster emerged, indicating that Adherence, HIV integrase inhibitors, pyridines, antiretroviral therapy, HIV drug resistance, and 3-ring heterocyclic compound are now being discussed in ARV Drug resistance mutation related research in Africa (2012 to 2020) (Fig. 5b).

Research areas are still available for additional studies because keywords like virological failure, adolescent, HIV drug resistance, HIV integrase, high through put nucleotide sequencing, acquired drug resistance, drug resistance mutations, pretreatment drug resistance and dolutegravir did not provide any links.

Fig. 5b: Overlay visualization of Keyword hotspots

DISCUSSION

The trend of research output on ARV Drug resistance in Africa showed a very low output on ARV Drug resistance mutation in Africa from 2004 to 2014 until when a few publications were released in 2015. Following that trend, the output of ARV Drug resistance mutation in Africa -related literature experienced a slow increase until 2020, when there was a dramatic rise of over 40 times. Since then, there was a gradually decline in research output which continued to decrease until it maintained lag phase in 2024.

The prevalence rate of publications on ARV Drug resistance mutations in Africa based on countries indicated that South Africa recorded the highest productive outputs followed by Switzerland and Uganda while, Nigeria contributed to the lowest publications. Only the top five countries from the African regions—South Africa, Uganda, Kenya, and Zimbabwe—rank favorably on a global scale in terms of the number of publications concerning ARV Drug resistance mutation research, occupying the 1st, 2nd, 3rd, and 4th positions, respectively.

This study indicated that Nigeria nation is falling short compared to other countries and many African countries regarding the volume of publications on ARV Drug resistance mutation despite the high incidence rates of the disease in this country. For instance, a cross-sectional survey conducted in 2018 found that 19% of Nigerian adults with unsuppressed viral loads had evidence of drug-resistant mutations (DRMs) [50, 51]. The majority of DRMs observed conferred resistance to non-nucleoside reverse transcriptase inhibitors (NNRTIs) (17.6%) and nucleoside reverse transcriptase inhibitors (NRTIs) (11.2%) [50]. HIV drug resistance was associated with antiretroviral therapy (ART) experienced, longer duration on ART, and lower CD4 count [50]. However, a 2020 study published in the Journal of Acquired Immune Deficiency Syndromes reported a low prevalence of transmitted drug resistance among HIV-1-infected individuals in Switzerland [52, 53]. The study found that only 6.3% of participants had at least one drug-resistant mutation, with the majority being resistant to NRTIs (4.2%) [52, 53].

In addition, South Africa remains the most productive country in this research, together

with the highest prevalence rate of ARV Drug resistance mutation. Currently, a high prevalence of HIV-1 drug resistance mutations among individuals failing first-line ART in South Africa is reported [54]. The study found that 76.2% of participants had at least one drug resistance mutation, with the majority being resistant to NNRTIs (63.2%) and NRTIs (44.7%) [54].

Subsequently, Uganda ranked 3rd position in publication outputs and became 2nd position in the prevalence rate of ARV drug resistance mutation based on a study published in 2019 that reported a high prevalence of HIV drug resistance mutations among individuals failing first-line ART in Uganda [55]. The study found that 71.4% of participants had at least one drug-resistant mutation, with the majority being resistant to NNRTIs (57.1%) and NRTIs (40.0%) (55).

Kenya secured both 4th productive output country and the prevalence rate of ARV Drug resistance mutation according to a 2019 study published in the Journal of Acquired Immune Deficiency Syndromes reported a moderate prevalence of HIV-1 drug resistance mutations among individuals failing first-line ART in Kenya [56]. The study found that 44.1% of participants had at least one drug resistance mutation, with the majority being resistant to NNRTIs (34.6%) and NRTIs (23.1%) [57].

Unfortunately, the majority of the African countries that provided low publication outputs experienced a high prevalence rate of ARV Drug resistance mutation. Notably, a recent study found a high prevalence of HIV-1 drug resistance mutations among non-citizen adults living with HIV in Botswana [58, 59]. The study reported that 78.4% of participants had major HIV drug resistance mutations, with 83.3% of treatment-experienced individuals showing acquired drug resistance to any antiretroviral drug [60]. The most frequent drug resistance mutations were M184V (62.1%), V106M (41.4%), and K103N (34.4%) [60]. Furthermore, previous study reported a high prevalence of HIV-1 drug resistance mutations among individuals failing first-line antiretroviral therapy in Zambia [61]. The study found that 67.9% of participants had at least one drug-resistant mutation [62].

Limited institutions in the Africa have been previously integrated into ARV Drug resistance mutation research Centers such as University of Buea, Buea, Cameroon[35]; Joint Clinical Research Center, Uganda [18]; Lancet Laboratories, Johannesburg, South Africa [47]; Botswana-Harvard AIDS Institute partnership, South Africa[58]; Amsterdam UMC, Netherlands; University of Cape town and National Health Laboratory, South Africa; Institute of Human Virology, Abuja, Nigeria[50]; University of Zimbabwe, Zimbabwe; National Institute for Communicable Diseases, Switzerland[63]; Virology Laboratory CREMER-IMPM, Cameroon[64]; National Health Laboratory Service, South Africa ; University of Cape town, South Africa; discussed in various ARV Drug resistance mutation-related fields such as First line drugs [35-37], HIV drug resistance, integrase inhibitors [35], HIV Drug Resistance Mutations in Non-B Subtypes, Transmitted antiretroviral drug resistance[65], Protease inhibitor Resistance [37], HIV-1 drug resistance mutations in drug-naive patients[64].The research systems related to ARV drug resistance mutation in the

Africans are generally regarded as lacking productivity. South Africa stands out as the leading country in the number of publications related to ARV Drug resistance mutation research in Africa. One possible reason for this is the high prevalence of HIV/AIDS in the country, which has experienced numerous risk factors for HIV transmissions.

When compared to the global research output, the Nigeria has contributed less to the study of Drug resistance mutation. This discrepancy may stem from the generally struggling economies present in the low-income Economies countries, as indicated by the World Bank's online database [64]. Moreover, a high poverty-growth elasticity in most Africa, related to population size and gross domestic product (GDP) per capita, can result in insufficient funding for ARV Drug resistance mutation in Africa. Therefore, it is essential for governments in the African nations to prioritize ARV Drug resistance mutation research by providing increased manpower and resources to support it. Additionally, the developed world should be encouraged to establish more collaborative initiatives with Africa nations and to secure additional funding for research areas like virological failure, adolescent, HIV drug resistance, HIV integrase, high through put nucleotide sequencing, acquired drug resistance, drug resistance mutations, pretreatment drug resistance and dolutegravir are still available for further studies which did not provide any links.

Female, human, Antiretroviral agent, CD4 Lymphocyte count, risk factors, Medical Adherences, acquired immunodeficiency syndrome, HIV, Assessment of medical adherence, and PMTCT were all are the primary trending topics which reflected in the research emphasized in the most frequently cited publications for research in this field on a global scale and within the Africa [10, 35, 38-40].

Additionally, the main subjects of interest in the current study are Antiviral Drug resistance, Mutation, Genotype, Phylogeny, Missense mutation, DNA sequency analysis, genetic polymorphism, Amino acid sequence, Molecular sequence data, HIV reverse transcriptase, HIV protease inhibitor, genotyping technique, HIV integrase, virological failure, Tenofovir, Dolutegravir, Acquired drug resistance which provide significant and insightful perspectives on the publications and themes driving research advancements in this area over time[7, 9, 13, 31, 66-70].

The current study has some limitations that are typical of bibliometric analyses. Firstly, the criteria set by the PUBMED database dictate the outcome of the materials examined. Additionally, local journals that were not included in PUBMED during the study period could have been overlooked. It’s possible that we missed ARV Drug resistance mutation research articles in Africa. if the authors did not use our specific search terms. Finally, since we were restricted to using PUBMED, a publicly accessible database, we may have overlooked articles that are indexed only in other databases. Nonetheless, we believe the findings accurately reflect the research trends in the area of study.

CONCLUSION

This research offers an original review of the existing studies related to drug resistance mutation in the Africa region and their connections to global findings. In conclusion, this study analyzed twenty years of drug resistance mutation publication outputs within the Africa. The results revealed that South Africa led in total publication numbers in the drug resistance mutation field as documented by PubMed during the analyzed timeframe, followed by the Uganda, Kenya, and Zimbabwe. Furthermore, the University of cape town, cape town emerged as the leading institution, followed closely by the University of the Witwatersrand, Johannesburg, Joint clinical research Centre, Kampala and University of Zurich, Zurich. Additionally, the developed world should be encouraged to establish more collaborative initiatives with Africa nations and to secure additional funding for research areas like virological failure, HIV drug resistance, HIV integrase, high through put nucleotide sequencing, acquired drug resistance, drug resistance mutations, pretreatment drug resistance and dolutegravir are still available for further studies which did not provide any links. Finally, the findings presented in this study through a novel approach depict a clear overview of the progress made in drug resistance mutation research and may assist relevant researchers and clinicians in guiding global drug resistance mutation in HIV/AIDS patients, particularly in African nations where the disease is prevalent.

Funding:

There was no specific funding for this Research

Availability of data and materials

The datasets produced and analyzed in this study are included within the article. The main data source, PUBMED, is publicly accessible.

Declarations

Ethics approval and consent to participate

This study utilized analyses based on secondary data, thereby not necessitating ethical clearance.

Consent for publication

Not applicable.

Competing interests

The authors state that there are no competing interests.

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EVALUATION OF TRANSAMINASE ACTIVITIES IN PATIENTS WITH DIABETIC NEPHROPATHY

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ABSTRACT

Background: Nephrotic syndrome and widespread glomerulosclerosis are the hallmarks of diabetic nephropathy, a progressive kidney disease brought on by angiopathy of capillaries in the kidney glomeruli.

Aim: Thhis study aimed to assess the activity of transaminases, specifically alanine aminotransferase (ALT) and aspartate aminotransferase (AST), in patients with diabetic nephropathy.

Materials and Methods: Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activity were measured in patients with diabetic nephropathy, diabetes mellitus (without

nephropathy), and control participants who appeared to be in good health. A total of 150 participants were involved in this study. Three groups of 50 patients each who had diabetes mellitus, diabetic nephropathy, and control subjects who appeared to be in good health. The Reitman and Frankel technique of analysis was used to measure ALT and AST activity.

Results: Diabetic nephropathy was associated with a substantial increase in enzyme activity above the control value ($P<0.05$). While ALT activity was not significant [ALT ($P>0.05$)], diabetes mellitus was also associated with a little increase in AST activity above the control value. Patients with diabetic nephropathy had mean ALT and AST activity that was statistically significant ($P<0.05$) when compared to patients with diabetes mellitus who did not have nephropathy.

Conclusion: The study demonstrated that both ALT and AST levels were significantly elevated in patients with diabetic nephropathy and diabetes mellitus compared with control subjects

Key words: diabetic nephropathy, Alanine aminotransferase, diabetes mellitus

INTRODUCTION

Diabetic nephropathy is one of the most serious microvascular complications of diabetes mellitus and remains a leading cause of chronic kidney disease and end-stage renal failure worldwide. It is characterized by progressive deterioration of renal function due to persistent hyperglycaemia, leading to structural and functional changes in the kidneys. In addition to renal impairment, diabetic nephropathy has also been linked to alterations in liver function, suggesting a broader metabolic disturbance affecting multiple organ systems [1,2]. Published clinical evidence has reported that biochemical markers of liver integrity, particularly the transaminases alanine aminotransferase (ALT) and aspartate aminotransferase (AST), may be affected in diabetic conditions. However, there is limited or no documented study specifically investigating these biochemical alterations among patients with diabetic nephropathy and diabetes mellitus attending Iyi-Enu Mission Hospital, Anambra State, Nigeria.

ALT and AST are intracellular enzymes that play essential roles in amino acid metabolism and are widely distributed in the liver, heart, kidneys, skeletal muscles, and other tissues. ALT is primarily localized in the liver and is considered a more specific indicator of hepatocellular injury, while AST is found in both hepatic and extrahepatic tissues, making it a less specific but still valuable marker of tissue damage [3,4]. Under normal physiological conditions, these enzymes are present in low concentrations in the blood. However, when there is cellular injury or increased membrane permeability, such as in diabetes mellitus and its complications, elevated levels may be detected in the serum. This makes ALT and AST useful biochemical

markers in assessing metabolic and organ dysfunction.

The relationship between diabetes mellitus, diabetic nephropathy, and altered transaminase activity is an important area of clinical investigation[5,6]. Chronic hyperglycaemia can induce oxidative stress, inflammation, and metabolic derangements that may affect both hepatic and renal tissues. These pathological processes can lead to leakage of intracellular enzymes into the bloodstream, resulting in abnormal serum levels of ALT and AST[7]. Understanding these alterations is important for early detection, monitoring, and management of diabetic complications.

Given the increasing burden of diabetes mellitus and its complications in developing countries such as Nigeria, there is a need for localized studies that provide region-specific data. Investigating the activity of transaminases among patients with diabetic nephropathy and diabetes mellitus attending Iyi-Enu Mission Hospital will help bridge this knowledge gap. Such data will not only contribute to existing scientific literature but will also provide valuable insight for clinicians managing diabetic patients in the region.

The aim of this study is to assess the activity of transaminases (ALT and AST) in patients with diabetic nephropathy. The specific objectives are to investigate the levels of ALT and AST in diabetic nephropathy, determine transaminase activity in diabetes mellitus, and compare the differences in transaminase activities between diabetic nephropathy and diabetes mellitus. The findings from this study are expected to demonstrate the prevalence of abnormal enzyme levels and provide empirical evidence that may assist in improving the clinical management and monitoring of diabetes mellitus and diabetic nephropathy.

The study was designed to investigate the levels of these transaminases in individuals diagnosed with diabetic nephropathy in order to understand possible biochemical alterations associated with the condition. In addition, it seeks to determine the activity of ALT and AST in patients with diabetes mellitus, thereby providing a comparative baseline for enzyme levels in uncomplicated diabetic cases.

Furthermore, the study compared the differences in transaminase activities between patients with diabetic nephropathy and those with diabetes mellitus. This comparison is intended to highlight any significant variations that may be associated with the progression of diabetic complications, particularly renal involvement. By achieving these objectives, the study will contribute to a better understanding of the relationship between liver enzyme activity and diabetic disease states, which may be useful in clinical monitoring and management.

MATERIALS AND METHODS

Subjects

A total number of one hundred and fifty (150) adult subjects aged 18-70 years, were randomly selected. fifty (50) were already diagnosed diabetes mellitus patients and the rest fifty (50) were diabetic kidney disease patients attending Iyi-Enu Mission Hospital

(IEH), Anambra State with their 50 control individuals.

Ethical Approval

Ethical approval for this study was obtained from the Ethics Committee of Iyi-Enu Mission Hospital Ethics Committee before the commencement of the research. The study was conducted in accordance with established ethical principles guiding research involving human participants. Informed consent was obtained from all participants prior to sample collection and data acquisition. Participants were adequately informed about the purpose of the study, the procedures involved, and their right to withdraw from the study at any stage without any consequences. Confidentiality and privacy of participants' information were strictly maintained throughout the study, and all data obtained were used solely for research purposes.

Inclusion Criteria

Already diagnosed diabetes mellitus patients, diabetic nephropathy patients and apparently healthy volunteered male and female adults who are not diabetic were recruited for the study.

Exclusion Criteria

Non-adult diabetes mellitus patients and non-adult control subjects were excluded from the study.

Sample Collection

venous blood sample (4mls) was collected from each subject by vein puncture from antecubital vein using a 5mls sterile disposable syringe. The area was first swabbed with cotton wool dipped in 70% alcohol. The blood was delivered into properly labelled lithium heparin bottle with the subject's name, age and sex. It was capped and mixed well with the anticoagulant by gentle inversion. The blood was spun for 5 minutes at 3000 rpm. The serum was separated from the red cells using a dry clean pipette into a dry clean plain specimen container. The serum was then stored at -70°C. Enzyme activity for AST and ALT were estimated.

Analytical Methods

The tests were performed in batches of samples daily alongside standards. Each test sample, the standard, and the blank had their respective tubes.

Estimation of Plasma ALT AND AST (Reitman and Frankel, 1957)

Analytical Procedure

For the determination of alanine aminotransferase (ALT) activity, two test tubes were prepared for each specimen and appropriately labelled as T (test) and B (blank). Into each tube, 0.5 ml of buffer solution containing the substrate mixture composed of L-alanine and α-oxoglutarate was carefully pipetted. Subsequently, 0.1 ml of the sample was added only into tube T.

The contents of both tubes were mixed thoroughly and incubated at 37°C for exactly 30 minutes to allow the enzymatic reaction to proceed. At the end of the incubation period, 0.5 ml of 2,4-dinitrophenylhydrazine (DNPH) reagent was

added into both tubes to terminate the reaction and facilitate colour development. Thereafter, 0.1 ml of the sample was also introduced into tube B, after which both tubes were mixed again and allowed to stand for exactly 20 minutes at room temperature (20–25°C).

Following incubation, 5 ml of 0.4N sodium hydroxide (NaOH) solution was added to each tube and mixed thoroughly. The absorbance of the resulting solution was then measured spectrophotometrically after 5 minutes at a wavelength of 546 nm, using the blank as reference. The activity of ALT in the sample was subsequently determined by referring to the standard table provided in the manufacturer's manual.

Analytical Procedure

The determination of aspartate aminotransferase (AST) activity was carried out using a colorimetric method under carefully controlled laboratory conditions. For each specimen analysed, two clean test tubes were prepared and appropriately labelled as T (test) and B (blank). Into each tube, 0.5 ml of buffer solution containing the substrate mixture composed of L-aspartate and α-oxoglutarate was carefully pipetted. Thereafter, 0.1 ml of the patient's sample was added only into tube T, while tube B was initially left without the sample to serve as a control for background correction.

Both tubes were thoroughly mixed to ensure proper interaction of the reagents and then incubated at 37°C for exactly 30 minutes to allow the enzymatic reaction to occur. At the end of the incubation period, 0.5 ml of 2,4-dinitrophenylhydrazine (DNPH) reagent was added to each tube to terminate the reaction and facilitate the formation of a coloured complex. Subsequently, 0.1 ml of the sample was also added into tube B, and both tubes were mixed again and allowed to stand at room temperature (20–25°C) for exactly 20 minutes.

After the standing period, 5 ml of 0.4N sodium hydroxide (NaOH) solution was added to each tube to develop the final colour. The contents were mixed thoroughly, and the absorbance of the test solution was measured spectrophotometrically after 5 minutes at a wavelength of 546 nm, using the blank as reference. The AST activity in each sample was finally determined by comparing the absorbance values with standard reference tables provided in the manufacturer's manual.

Data Analysis

The results obtained in this study were analyzed statistically. The mean and standard errors of mean values were calculated in each case. Students' t-test statistical method was employed for comparisons. A p-value equal less than 0.05 ($p < 0.05$) was considered statistically significant.

RESULTS

Table 1: show the mean ALT activity of patients with diabetes Nephropathy was found to be 11.80 ± 1.29 U/L and AST activity was 20.40 ± 3.90 U/L. That of the control subjects was found to be 9.80 ± 0.80 for ALT and 7.60 ±0.46 L for AST. This table also reveals that a comparison of the

mean transaminase activities of patients with diabetic nephropathy and the control subjects was statistically significant (P< 0.05).

Table 2: reveals the mean Transaminase activities of patients with diabetes mellitus and the control subjects. From this table, the mean ALT activity of patients with diabetes mellitus was found to be 11.80 ± 1.40 U/L and the mean AST activity was 20.40 ± 3.10 U/L . While that of the control subjects was found to be 9.20 ± 0.92 U/L for ALT and 7.60 ± 0.46 U/L for AST.

Table 3: indicates the mean Transaminase activities of patients with diabetes mellitus and diabetic nephropathy. This table reveals that a comparison of the mean Transaminase activities of patients with diabetes mellitus and diabetic nephropathy was statistically significant (P< 0.05).

Table 1: Shows the mean Transaminase Activities in Patients with Diabetic Nephropathy and Control Subjects

	Diabetic Nephropathy (n=50)	Control (n=50)	t-Value	P-Value
ALT(U/L)	32.90 ±7.10	9.80 ± 0.84	4.0	P< 0.00
AST(U/L)	45.0 ±6.20	7.60 ± 0.46	7.0	P< 0.00

Table 2: Mean ALT and AST Activities in Diabetes Mellitus patients and the Control Subject

Parameter	Diabetes Mellitus n=50	Control Subjects n=50	t-value	P-value
ALT (U/L)	11.80 ± 1.40 U/L	9.20 ± 0.92 U/L	1.32	P > 0.00
AST (U/L)	20.40 ± 3.10 U/L	7.60 ± 0.46 U/L	4.4	P < 0.01

Table 3: Mean ALT and AST Activities of Diabetes Mellitus patients and Diabetic nephropathy patients

Parameter	Diabètes Mellites patients (n=50)	Diabetic nephropathy patients (n= 50)	t-value	P-value
ALT (U/L)	11.80 ± 1.40 U/L	32.00 ± 7.20 U/L	3.34	P < 0.01
AST (U/L)	20.40 ± 3.10 U/L	45.04 ± 6.11 U/L	3.65	P < 0.00

DISCUSSION

Persistent albuminuria, a steadily declining glomerular filtration rate (GFR), and high arterial blood pressure are the hallmarks of diabetic nephropathy, a clinical illness. This study's main goal was to evaluate transaminase activity in diabetic nephropathy. Among the transaminases are aspartate amino transferase (AST) and alanine aminotransferase (ALT) [6,15].

The test group (diabetic nephropathy) showed elevated ALT and AST activity. This indicates that diabetic nephropathy has an excitatory influence on the transaminases' plasma level by raising their activities over the reference range's upper limit and control values [11,14,16]. It can be the result of kidney injury. Damage to cells increases permeability, which allows cytosolic and mitochondrial enzymes to leak into the interstitum and then into the peripheral circulation. When a kidney with high amounts of ALT and AST is injured, these enzymes can seep into the bloodstream [6].

In diabetic mellitus (without nephropathy), there was a little increase in transaminases above the control value. While AST was substantial ($P<0.05$), the increase in ALT was not significant ($P>0.05$) [9,10,11,12]. Increased transaminase activity in diabetes mellitus is thought to be an indication of impaired insulin signalling rather than only hepatocyte dysfunction. or possibly as a result of increasing hepatocyte injury. Hepatocytes are known to be directly harmed by the excess free fatty acids present in the insulin-resistant state, and proinflammatory cytokines such tumour necrosis factor α (TNF ALPH) may also be involved in hepatocellular damage [1,14,15,17,18,19].

Because ALT and AST activities were higher in diabetic nephropathy than in diabetes mellitus, there was a substantial difference in transaminase activities between the two conditions. One explanation could be that the enzymes leak from both renal damage and hepatocellular injury, leading to a marked rise in the enzymes' plasma activity.

The results suggest progressive hepatic and metabolic involvement in diabetic conditions. These findings align with reports that diabetes mellitus is associated with altered transaminase activity due to metabolic stress and tissue damage. Monitoring these enzymes may therefore be useful in assessing disease progression and complications in diabetic patients.

CONCLUSION

In conclusion, the study demonstrated that both ALT and AST levels were significantly elevated in patients with diabetic nephropathy and diabetes mellitus compared with control subjects, with AST showing a more pronounced increase.

RECOMMENDATIONS

Transaminase testing is crucial for patients with diabetic nephropathy in addition to urine albumin testing. This is advised for the early detection of nephropathy in patients. Additionally, to evaluate the disease's course and response to treatment.

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JOURNAL OF BIOMEDICAL INVESTIGATION (JBI) VOLUME 13 NUMBER 3: NOVEMBER-DECEMBER 2025

REVIEW OF LABORATORY BIOMARKERS OF INFLAMMATORY BOWEL DISEASE

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ABSTRACT

Inflammatory bowel disease (IBD), a cluster of chronic, immune–related and long–term disorders that cause fever, abdominal pain/cramping, fatigue, severe and recurrent diarrhoea, rectal bleeding and weight loss, and characterised by inflammation of gastrointestinal tract (GIT), is categorised as crohn's disease (CD), ulcerative colitis (UC) or IBD unclassified (IBDU). Assessing the diagnosis, severity and monitoring of IBD is largely based on the combined effects of clinical presentations, endoscopy, radiology, histology and laboratory biomarkers. Some laboratory biomarkers of IBD such as C–reactive protein (CRP), erythrocyte sedimentation rate (ESR), platelets etc are associated with systemic and gastrointestinal inflammation or disease activity, i.e., active or quiescent IBD. Others are linked to genetic predisposition, e.g., autophagy genes, nucleotide–binding oligomerization domain–containing protein 2 (NOD2), interleukin–23 receptor (IL23R) etc, correlated with the neoplastic transformation, e.g., M2–pyruvate kinase, miRNAs, mucosal chitinase–3–like protein 1 (CH13L1) etc, and coupled to drug metabolism in relation to therapeutic intervention e.g., Thiopurine Methyltransferase (TPMT) and 6–Thioguanine Nucleotide (6TGN). There are also those such as anti–neutrophil cytoplasmic antibodies (ANCAs), and anti–saccharomyces cerevisiae antibodies (ASCAs) that are related to the type of IBD, i.e., either CD, UC or IBDU. This review summarises the characterisation of laboratory biomarkers of IBD including a narrative on their future perspectives from the standpoint of diagnosis, prognosis, monitoring the disease course and a practical algorithm for the practical application of these biomarkers in the assessment of IBD.

Keywords: Biomarker, Inflammatory Bowel Disease, Crohn's Disease, Ulcerative Colitis, S100 Proteins, Calprotectin, S100A12, C–reactive Protein.

INTRODUCTION

Inflammatory bowel disease (IBD), a cluster of chronic, immune–related and long–term disorders that cause fever, abdominal pain/cramping, fatigue, severe and recurrent diarrhoea, rectal bleeding and weight loss, and characterised by inflammation of the gastrointestinal tract (GIT), is categorised as crohn's disease (CD), ulcerative colitis (UC) or IBD unclassified (IBDU).[1–3]

Epidemiologically, IBD has witnessed considerable changes with consequent annual variations in the incidence by geographical region and increase in the disease burden globally. With worldwide prevalence at ≈5 million, the incidence per 100,000 population in Europe stood at ≈10.5–46.1%, Middle East at ≈23.7–39.8%, North America at ≈7.3–0.2%, Oceania at ≈0.21–3.67% and Asia at ≈1.37–1.5% , there appears to be an increasing trend with age in the disease burden in adults (exceeding 1% in those >70 years) relative to children and adolescents, and in female (≈3.9 million) compared to male (≈3.0 million) population.[1–3]

The advent of more convenient, available and affordable laboratory biomarkers for the diagnosis, prognosis and treatment of IBD is expected to totally change the way that patients presenting with the disease are treated[1]. Although the recent scientific and technological advances in the development, optimisation and analytical validation of immunoassay kits have somewhat eased the practical issues associated with the application of laboratory biomarkers in the assessment of IBD, there still exist some uncertainties over the gains recorded with the advances in genomic, proteomic and metabolics arrays in relation to clinical laboratory testing[1].

Recent advances in immunoassay technologies have necessitated the categorisation of these laboratory biomarkers of IBD into those biomarkers that are associated with systemic and gastrointestinal inflammation including disease activity, i.e., active or quiescent IBD; those biomarkers that are linked to genetic predisposition of IBD; those biomarkers that are correlated with the neoplastic transformation of IBD; those biomarkers that are coupled to drug metabolism in relation to therapeutic intervention of IBD; and those biomarkers that are related to type of IBD, i.e., either CD, UC or IBDU.[1–3]

More information may be needed on the many aspects of laboratory biomarkers to diagnose IBD, differentiate between disease states (CD vs. UC), evaluate disease activity (active vs. quiescent), confirm disease sites or locations as in small intestine: ileal and upper (A1) vs. colonic and ileo–colonic (A2); large intestine: proctitis (E1) vs. distal colitis (E2) vs. pancolitis (E3), predict the disease course and relapse, and monitor follow–up and response to treatment.[1]

The diagnosis, severity and monitoring of IBD are usually based on a combination of clinical presentations, endoscopy, radiology, laboratory biomarkers and, if appropriate histology. Laboratory biomarkers should be non–invasive, cheap, simple, objective, rapid, easy to perform and reproducible *(table 1)*. An ideal biomarker for IBD that would combine all these characteristics is not available.[1,2] Serum biomarkers of systemic inflammation are shown in *table 2* and faecal biomarkers of gastrointestinal inflammation are shown in *table 3*.

Faecal biomarkers may be regarded as the 'gold standards' in intestinal inflammation. They are however, limited by certain factors including

lack of specificity for IBD; lack of validated, optimal and varying assay cut–offs make it difficult to characterize active inflammatory disease, distinguish IBD from irritable bowel syndrome (IBS), predict clinical remission and mucosal healing, and assess response to treatment; levels of calprotectin in stool samples are dependent on diverse physiological considerations such as age and clinical comorbidities; significant overlap in faecal calprotectin levels (50–150 µg/g) in both IBD and IBS patients presents an ambiguous situation regarding the decision to refer a patient to endoscopy or not, and intra–individual and inter–individual variability, spot variability in the same sample and reluctance of some patients to provide stool samples.[1–3] These issues may be overcome by using serum samples for the measurement of calprotectin and S100A12[1]. This offers the prospect that serum calprotectin and serum S100A12 could replace or supplement faecal calprotectin and faecal S100A12 in the identification and assessment of IBD.

Whilst it is important for laboratory biomarkers of IBD to be non–invasive, cheap, simple, objective, rapid, easy to perform and reproducible, the 'real–world' experience with these biomarkers is increasing together with the increasing number of patients with IBD for which the usefulness of these laboratory biomarkers to assess the disease have become necessary.[1–3] The 'ideal biomarker' that would integrate all the preceding characteristics for evaluating the analytical performance and diagnostic accuracy of these laboratory biomarkers in IBD is not available.[1] Therefore, the search for the availability of a single laboratory biomarker that would demonstrate the attributes of an 'ideal biomarker' for use in assessing IBD continues.

This objectives of this review were to present a summary of the characterisation of laboratory biomarkers of IBD including a narrative on their future perspectives from the standpoint of diagnosis, prognosis and monitoring the disease course. A practical algorithm for the practical application of these biomarkers, both current and potential future biomarkers, in the assessment of IBD will also merit discussion. A conclusion will be added.

A. Laboratory biomarkers with widespread application

1. Serum C–reactive protein and cytokines

C–Reactive Protein (CRP), a pentameric protein synthesized predominantly in the liver but also in vascular walls and adipose tissue, is a well–established biomarker of acute inflammation. During acute phase response, hepatocytes rapidly increase production of CRP under the influence of interleukin–6 (IL–6), tumour necrosis factor–alpha (TNF–α), and interleukin–1β (IL–1β). Serum CRP, therefore, rises rapidly up to 100 to 1000–fold, peaking in two days and then decreases rapidly with the resolution of the acute insult as it has a half–life of 19 hours. Functionally, CRP is recruited into the complement activation process when it binds to organisms or particles that contain phosphocholine which enables it to opsonise infectious agents and damaged cells.[4–6] In healthy individuals, CRP circulates in low concentration (about 1 mg/L). CRP concentrations around 10 to 40 mg/L may be seen in chronic inflammation.[7,8]

CRP is generally increased in IBD, but appears to respond differently to inflammation in UC and CD. A rise in serum CRP level correlates well with the disease activity in CD. CRP, however, may not be raised or mildly raised in

the presence of increased disease activity in UC even though increased levels of IL–6, IL–1β or TNF–α are observed.[9] Other inflammatory markers such as β_2–microglobulin correlate better with histology scores in UC.[10,11] The main reason given for this differential CRP response in IBD is that inflammation is limited to the mucosa in UC and, less likely to provoke a systemic response to inflammation when compared to transmural inflammation in CD. Other possible reasons include increased IL–6 levels in CD compared to UC[12] and CRP gene polymorphism in UC and CD.[13–15] CRP levels in IBD patients, however, are not associated with CRP gene polymorphism.[16]

Table 1 – Characteristics of an ideal biomarker for use in the assessment of IBD[1]

Performance	Criteria Characteristics
Simple	Disease specific: Identify individuals with IBD; Able to differentiate IBD from non–IBD cases; Able to predict the remission or relapse; Monitor the effect of treatment; Prognostic value in assessing morbidity/mortality
Ease of performance	Ability to objectively measure disease activity without ambiguity
Invasiveness	Not invasive or minimally invasive
Cost	Affordable
Rapid	Quick turnaround time
Reproducibility	Assay results not showing discrepancies between individuals and clinical laboratories.

Table 2 – Serum acute phase proteins and their responses to IBD and Other inflammatory processes[1]. *α_2–macroglobulin shows a different response in animals in comparison to humans, both in positive and negative acute phase protein respectively.

Acute Phase Protein	Increased (↑)	Decreased (↓)
Proteinase Inhibitors	α_1–Antitrypsin, α_1–Antichymotrypsin, α_2–Macroglobulin*	
Coagulation and Fibrinolytic Proteins	Fibrinogen, Prothrombin, Factor VIII, Plasminogen, Tissue Plasminogen Activator Antithrombin	Factor XII
Complement System	C1s, C2, C3, C4, C5, C1 Inhibitor, C9	Albumin, Transferrin
Transport Proteins	Haptoglobin, Haemopexin, Caeruloplasmin	Insulin–like Growth Factor, α– Fetoprotein, Cholinesterase
Other Acute Phase Proteins	C–reactive protein, Serum Amyloid A, Ferritin, Fibronectin, Orosomucoid (α_1–Acid Glycoprotein).	

Table 3 – Faecal biomarkers in clinical use for the diagnosis and differential diagnosis of IBD including its discrimination from IBS[3].

Faecal Biomarker	Major Source or Origin
Calprotectin (S100A8/S100A9)	Neutrophils, Monocytes and Epithelial Cells.
Calgranulin C or EN–RAGE (S100A12)	Neutrophils
Lactoferrin	Mucosal Epithelial Cells and Neutrophils
M2–Pyruvate Kinase (M2PK)	Expressed by rapidly dividing cells
Neopterin	Activated Macrophages
Metalloproteinases (MMPs)	Different Cell types including Activated Neutrophils
Myeloperoxidases (MPOs)	Activated Neutrophils
Polymorphonuclear Elastase (PMN)	Activated Neutrophils

CRP is generally increased in IBD, but appears to respond differently to inflammation in UC and CD. A rise in serum CRP level correlates well with the disease activity in CD. CRP, however, may not be raised or mildly raised in the presence of increased disease activity in UC even though increased levels of IL–6, IL–1β or TNF–α are observed.[9] Other inflammatory markers such as β_2–microglobulin correlate better with histology scores in UC.[10,11] The main reason given for this differential CRP response in IBD is that inflammation is limited to the mucosa in UC and, less likely to provoke a systemic response to inflammation when compared to transmural inflammation in CD. Other possible reasons include increased IL–6 levels in CD compared to UC[12] and CRP gene polymorphism in UC and CD.[13–15] CRP levels in IBD patients, however, are not associated with CRP gene polymorphism.[16]

The utility of CRP as a serum biomarker in the assessment of IBD is still unclear. CRP is useful in assessing IBD patients with the active disease. However, there is the disadvantage that the inclusion of CRP in IBD assessment carries the increased risk of being restricted to IBD patients with high concentration of the protein.[1] Although the application of CRP may offer the promise of an increased prospect of response to treatment with a reduced rate for placebo considerations, it is however, important to emphasise the fact that the United States Food and Drug Administration (FDA) placed restrictions on using CRP in IBD assessment.[1] CRP presents a fair evaluation of the follow–up effect of therapeutic intervention in IBD patients. The ambiguity of CRP cut–off values in assessing IBD makes the utility of CRP very problematic.[1]

2. Circulating haemopoietic biomarkers: erythrocyte sedimentation rate (ESR), platelet count, mean platelet volume (MCV) and red cell distribution width (RDW)

ESR is an indirect measure of systemic inflammation. ESR measures the rate of migration of erythrocytes through the plasma, and is increased in the presence of increased proteins including acute phase proteins. ESR, compared to CRP, is slow to peak in response to inflammation and slow to decline after resolution of inflammation and is therefore of limited value in IBD assessment.[1,17]

Inflammation processes, including IBD, cause an increase in platelets and changes in their morphology. Platelet count increases in patients with IBD, particularly in UC when reticulated platelets are taken into account.[18,19] It, however, is not a useful biomarker of IBD, given the wide range of a normal platelet count.[1,20]

MPV has been reported as decreased in active IBD and has a negative correlation with CRP, while other studies have found no correlation between a fall in MPV and the disease activity.[21,22] Likewise, leucocytes lack specificity as a biomarker for IBD,[23] and are influenced by treatments in IBD with drugs such as glucocorticoids or azathioprine (increased) and 6–mercaptopurine (decreased).[1]

RDW is a measure of size variability and heterogeneity of erythrocytes in peripheral blood. RDW increases in active IBD, and particularly in CD compared to UC. RDW at a diagnostic cut–off value of 13.8% in non–anaemic UC patients has a sensitivity and specificity of 76% and 86% respectively, and in non–anaemic CD patients at a diagnostic cut–off value of 14.1% has a sensitivity and specificity of 82% and 83% respectively.[23,24]

3. Other serum acute phase proteins

Other acute phase reactants (Table 1.3) such as fibrinogen, sialic acid, α_1–acid glycoprotein (orosomucoid), α_1–antitrypsin, α_2–globulin, β_2–microglobulin, serum amyloid A (SAA) and albumin as biomarkers of IBD have not been widely studied because of their apparent inferiority to CRP. α_1–acid glycoprotein, shows good correlation with IBD disease activity but its half–life of 5 days makes it unsuitable as IBD biomarker.[1,17]

4. Faecal lactoferrin

Lactoferrin is an iron–binding protein found in neutrophil granulocytes. In acute intestinal inflammation, increased mucosal infiltration of neutrophils and subsequent secretion of lactoferrin into the intestinal tract results in increased faecal lactoferrin.[25,26] Lactoferrin has anti–bacterial activity by limiting availability of iron, and causes direct damage to bacterial cell membrane. Faecal lactoferrin is resistant to degradation and proteolysis, although less so than calprotectin, making it a useful biomarker of intestinal inflammation. The significant proportion of faecal lactoferrin in stool is, therefore, a reflection of intestinal inflammation.[27–29]

Lactoferrin is stable in stool for up to 5 days, does not deteriorate with repeated freezing and thawing, and can be quantitatively measured by the enzyme–linked immunosorbent assay (ELISA) technique. It is a non–specific intestinal biomarker being raised not only in active IBD but in other inflammatory intestinal disorders including infective diarrhoea, colon cancer and non–steroidal anti–inflammatory drugs (NSAIDs) enteropathy. Faecal lactoferrin correlates well with the clinical, endoscopic and histological grading of IBD disease activity and therefore, may distinguish between active and inactive IBD, and between IBD patients and healthy controls.[26,30–36] For predicting relapse, faecal lactoferrin has 46% sensitivity and 61% specificity for UC, and 77% sensitivity and 68% specificity for CD.[37] Comparable diagnostic accuracy of lactoferrin and calprotectin exist in patients with IBD and in patients with irritable bowel syndrome (IBS) and healthy controls[38]. Calprotectin and lactoferrin had similar sensitivity (78% vs. 80%), specificity (83% vs. 85%), overall diagnostic accuracy of 80% vs. 81% and are both significantly elevated in children with IBD.[38,39]

5. Faecal neopterin

Neopterin is a metabolite of biopterin released by activated macrophages and monocytes. Faecal neopterin correlates well with disease activity in IBD particularly with the severity of mucosal lesions. Faecal neopterin shows better correlation with endoscopic scores in UC (r = 0.72; $p < 0.0001$) than in CD (r = 0.47; $p < 0.0001$). Faecal neopterin is significantly higher in clinically and endoscopically active IBD compared to inactive IBD. The diagnostic accuracy of faecal neopterin to predict endoscopic activity in IBD compares favourably with faecal calprotectin in CD (74%) and in UC (87%) and, like calprotectin and lactoferrin, release of neopterin is non–specific as it could be triggered in response to disease conditions of viral infection and cell–mediated immune response during the early phase of inflammation.[40,41]

6. Faecal metalloproteinases (MMPs)

MMPs are zinc–dependent endopeptidases secreted by various cell types. MMP–9 is the MMP released in highest concentrations by activated neutrophils during intestinal inflammation such as in IBD. While MMP–1, MMP–2 and MMP–3 are detected in significantly higher amount in UC, only MMP–9 levels are significantly higher in active UC than in IBS or healthy controls thereby making it a reliable biomarker in distinguishing between UC and IBS based on 85% sensitivity and 100% specificity.[42] Faecal MMP–9 correlates well with other faecal biomarkers including calprotectin, lactoferrin and neopterin in endoscopically assessed disease activity in IBD and also demonstrates comparable diagnostic accuracy with faecal calprotectin.[43]

7. Faecal myeloperoxidases (MPOs)

MPOs are lysosomal proteins released by activated neutrophils in response to inflammation. The diagnostic accuracy of faecal MPOs to assess endoscopic disease activity in IBD is inferior to calprotectin and polymorphonuclear (PMN) elastase.[44]

8. Faecal polymorphonuclear (PMN) elastase

PMN elastase is released by activated neutrophils during inflammation, and is stable for up to 4 days in stool at ambient temperature. Faecal PMN elastase may be a useful biomarker in IBD as it is significantly higher in patients with active IBD compared to inactive IBD. This is reflected in its diagnostic accuracy of 84% sensitivity and 87% specificity for IBD that increases to 96% sensitivity and 100% specificity when combined with calprotectin and lactoferrin.[35,45]

9. Faecal M2–pyruvate kinase (M2PK)

Faecal levels of M2PK, a multi–functional protein found in undifferentiated and proliferating cells, are associated with active IBD, and correlate well with calprotectin in distinguishing IBD from IBS. M2PK is a superior biomarker to calprotectin, lactoferrin and S100A12 in predicting steroid refractoriness in severe paediatric UC. It also holds promise as a potential screening biomarker for colorectal cancer (CRC) in UC.[46–49]

10. The S100 Proteins: Faecal and serum S100A12

The family of low molecular weight S100 proteins or calgranulins *(figure 1)* have several functions including roles in cellular inflammation, proliferation, differentiation, apoptosis, signal transduction, calcium homeostasis and energy metabolism.[50–53] In chronic inflammation, the S100 protein family

are actively expressed in activated granulocytes.[54–56]

The cytokine–like extracellular functions of S100 proteins such as chemotactic activities related to inflammation and the acute phase response are exhibited mainly by S100A8 (calgranulin A), S100A9 (calgranulin B) and S100A12 (calgranulin C), and these are commonly referred to as the calgranulins or myeloid–related proteins (MRP).[57–59] The name 'S100 proteins' was derived from their ability to be 100% soluble in a saturated solution of ammonium sulphate at neutral pH. These proteins bind calcium, and are characterized by two calcium–binding motifs called the elongation factor (EF) hand. There are over 600 members of EF–hand super family.[60–63] The S100 proteins are expressed in myeloid cells including neutrophils, monocytes, and dendritic cells.[64]

S100A12 *(figure 2)* is a 10.4 *kilodalton (kDa)* molecular weight, 91–amino acids protein. It is a calcium–binding, proinflammatory protein predominantly expressed and secreted by neutrophil granulocytes, and represents 5% of total cytosolic protein.[65,66] S100A12 is a ligand for the receptor for advanced glycation end products (RAGE) and therefore, also named as extracellular newly identified receptor for advanced glycation end products binding protein (EN–RAGE). Other alternative names for S100A12 are calgranulin C, migration inhibition factor–related protein 6 (MRP–6) and calcium–binding protein in amniotic fluid (CAAF–1).[57,67,68] The binding of S100A12 to RAGE modulates these extracellular functions in disease. In inflammatory disease, serum S100 correlates with disease activity parameters and in these conditions S100 proteins may be superior to conventional laboratory biomarkers of inflammation including CRP and ESR.[69–71]

Serum S100A12 have been reported in increased concentrations in various biological materials including synovial fluid, synovial tissue and serum of patients with inflammatory arthritis.[72–75] Serum S100A12 is also raised in neurodegenerative diseases, diabetes mellitus, rheumatoid arthritis, osteoarthritis, cancerogenesis, familial mediterranean fever, idiopathic pulmonary fibrosis, cardiovascular diseases and atherosclerosis.[76–82]. S100A12 levels were also elevated in patients with peripheral radiographic features ($p = 0.036$), but did not correlate with clinical variables of disease activity in psoriatic arthritis.[83] In haemodialysis patients, levels of S100A12 are linked to cardiovascular disease.[84,85] Elevated concentrations of S100A12 and its receptors are found in pulmonary tissue and broncho–alveolar lavage fluid in acute lung injury.[86,87] Both S100A8 and S100A9 hetero–complexes are actively expressed preceding prostate tumour genesis and subsequent development, progression and enlargement of prostate carcinomas. Alone, serum S100A9 is increased significantly in prostate cancer patients compared to healthy controls or patients with benign prostatic hyperplasia (BPH); thereby underlining its role as a useful biomarker in differentiating prostate carcinoma and BPH.[88]

Faecal S100A12 have been shown to be a novel non–invasive biomarker of IBD in paediatric populations.[89,90] S100A12 levels in stool can be used as an indicator of disease activity in chronic IBD and to gauge the degree of gastrointestinal tract inflammation. As a biomarker of neutrophil activation, faecal S100A12 could play a significant role as a non–invasive biomarker of intestinal inflammation.

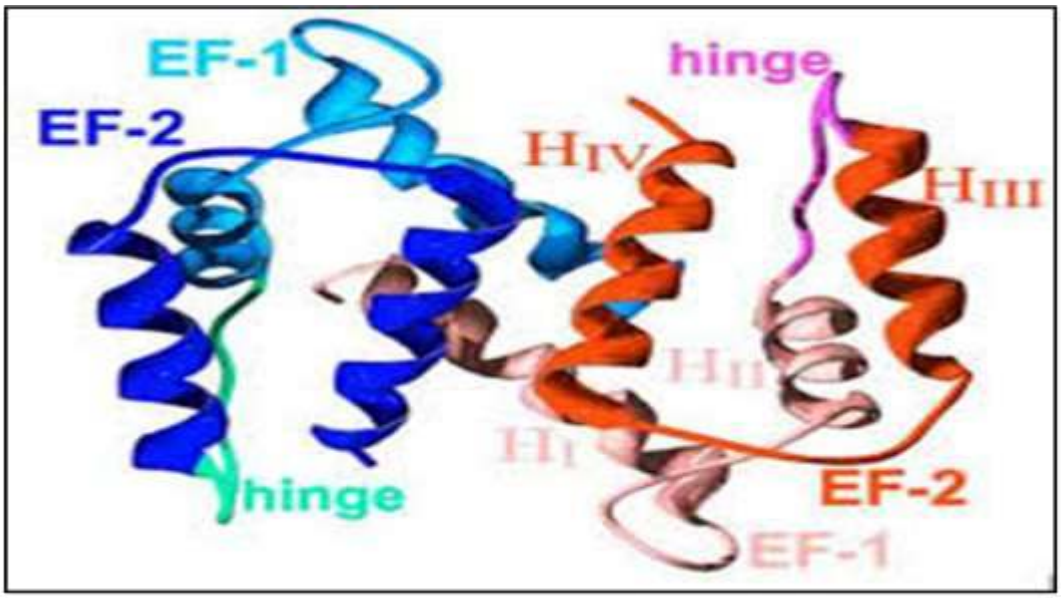

Figure 1 – Dimer structure of S100 protein. S100 proteins are small proteins with a molecular weight of 10 – 12 kDa. Each S100 protein consists of two EF–hand helix–loop–helix structural motifs, which are arranged in a back–to–back manner and linked with a flexible hinge.[95]

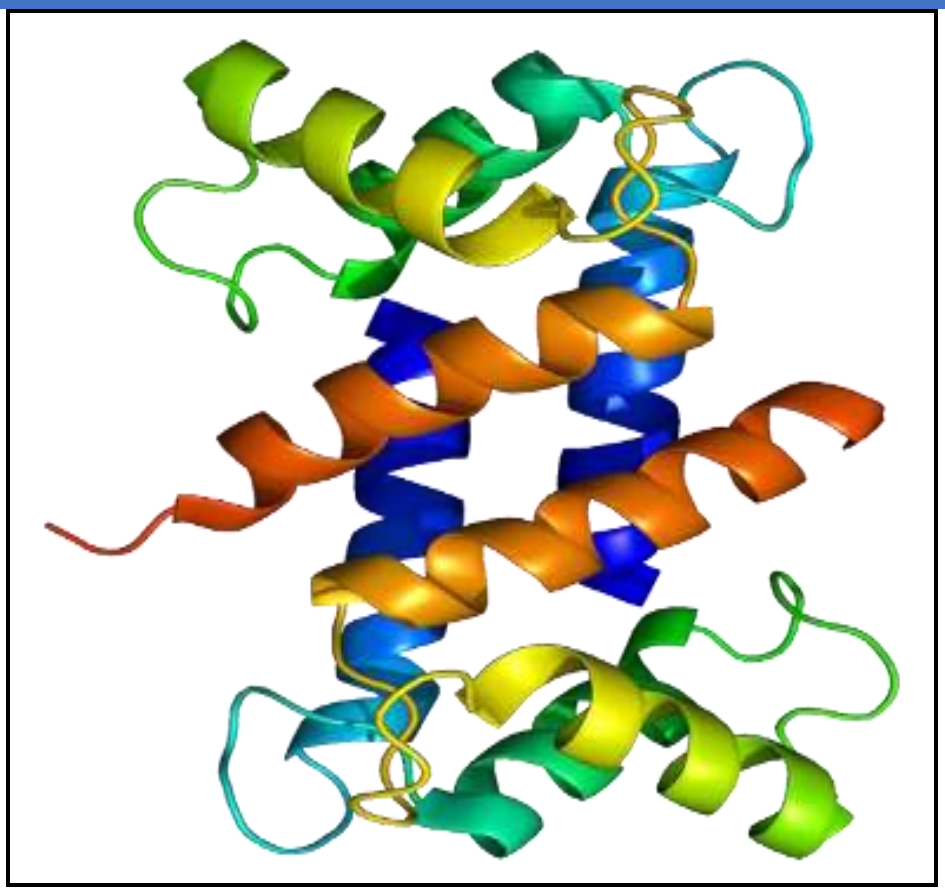

Figure 2 – Structure of S100A12. A three–dimensional model of the crystal structure of the S100A12 protein in a hexameric form and its proposed role in receptor signalling. The stereo view of the Ca^{2+} and Cu^{2+}–S100A12 dimer in ribbon representation, with individual subunits shown in red and blue. The chain topology, subunit arrangement, and juxtaposition of metal–binding sites are typical of metal–bound S100 proteins.[91–94]

11. Faecal and serum calprotectin

Calprotectin *(figure 3),* is a 36 kilodalton (kDa) calcium– and zinc–binding protein composed of two heavy 14 kDa S100A9 (MRP14) and one light 8 kDa S100A8 (MRP8) subunits which are members of the EF–hand motif containing S100 family of proteins. Like all S100 proteins, the genes that code for calprotectin are located within the gene cluster on chromosome 1q21 region. Calprotectin is expressed predominantly in neutrophils and monocytes – in which it constitutes up to 60% of the cytosolic protein. It is also expressed in macrophages, keratinocytes, epithelial cells and endothelial cells.[50,96-100]

Calprotectin was first characterised in 1980 as the Leucocyte protein candidate 1 (L1), to reflect *in–vivo* granulocyte turnover.[101-104] L1 was renamed calprotectin in recognition of its antimicrobial activity[105], calcium–binding roles and subsequent involvement in intracellular signal transduction and regulatory functions in acute phase response and inflammatory processes[96,106,107]. Neither S100A8 nor S100A9 subunits in isolation have anti–microbial

characteristics. It is possible that the high affinity of calprotectin subunits, S100A8 and S100A9, for zinc–binding site could explain the reduction of zinc concentration sufficiently to allow calprotectin to inhibit microbial growth.[106,108-111]

Alternative names for calprotectin include: MRP–8 and MRP–14 (MRP8/MRP14), p8/14, p34 and S100A8/S100A9. Calprotectin is composed of two heterocomplexes: S100A8 (also known as MRP–8, Calgranulin A and CP–10 in mouse) and S100A9 (also known as MRP–14 and Calgranulin B).

Calprotectin is found in plasma, saliva, cerebrospinal fluid, urine and faeces. Circulating calprotectin is commonly raised in inflammatory diseases including acute coronary syndromes, cystic fibrosis, multiple sclerosis, human immunodeficiency virus, rheumatoid arthritis, reactive arthritis, juvenile chronic arthritis, juvenile idiopathic arthritis, psoriatic arthritis, polymyalgia rheumatica, systemic lupus erythematosus and acute rejection in kidney allograft transplantation[103,112–130]

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Calprotectin expression correlates with microglial activation in cerebral malaria; and serum levels are prognostic biomarkers in recurrent infection and survival in alcoholic liver disease.[131-133] Increased calprotectin concentrations in faecal samples of patients with IBD that reflect granulocyte migration through the inflamed intestinal wall has been reported.[134-136] Measurement of faecal calprotectin now enjoys wide application in the diagnosis and monitoring of IBD, and an increased concentration indicates organic intestinal disorders. Calprotectin is an extremely stable protein, and remains unaltered in stool samples left unprepared for longer than seven days[137-139]. Studies have focused on the accuracy of faecal calprotectin in the diagnosis and monitoring of IBD. A meta–analysis reported 95% sensitivity and 91% specificity of faecal calprotectin in the identification of IBD. This study also reported that faecal calprotectin was superior to CRP and ESR[140].

The United Kingdom's National Institute for Health and Care Excellence (NICE) recommends faecal calprotectin as a diagnostic tool to help in the differential diagnosis of IBD and IBS[141]. A normal faecal calprotectin excludes IBD, whereas an elevated faecal calprotectin is an indication for colonoscopy, thereby reducing referrals for unnecessary endoscopic evaluation. A meta–analysis of 13 studies concluded that faecal calprotectin testing would result in a 67% reduction in the number of adults requiring endoscopy, but with a delayed diagnosis in 8% of adults because of false negative results[142].

One area of controversy surrounding faecal calprotectin testing is the determination of an appropriate cut–off value, above which the result is deemed as positive. In most centres, a relatively low level of 50 µg/g is used. A cohort of adult patients undergoing faecal calprotectin testing in primary care was studied[143]. At a cut–off value of 50 µg/g, faecal calprotectin had a negative predictive value (NPV) of 98% and positive predictive value (PPV) of 28%. Increasing the cut–off value to 150 µg/g gave a very comparable NPV of 97%, but a much

higher PPV of 71%. Given these values, it was calculated that increasing the cut–off value to 150 µg/g, would reduce colonoscopy and flexible sigmoidoscopy bookings by 10% at the cost of 4 missed cases of IBD[143].

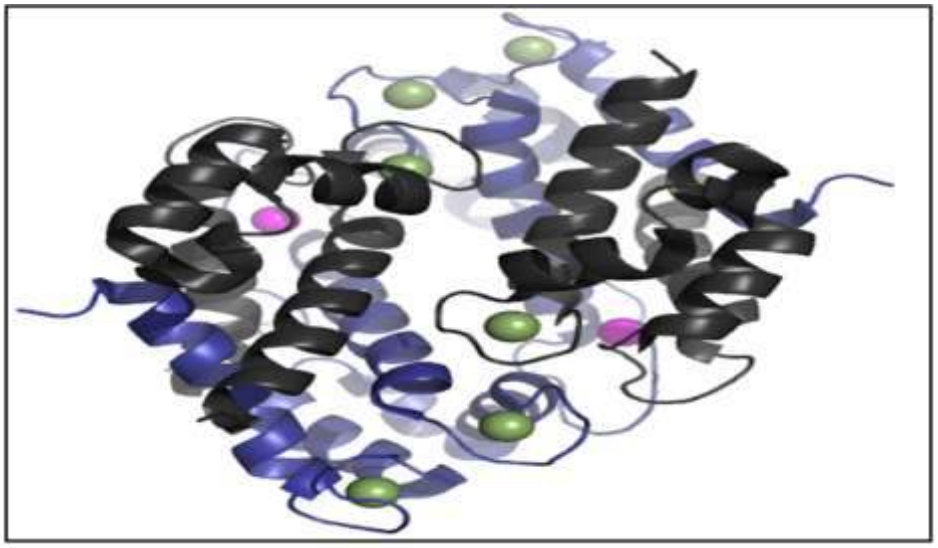

Figure 3 – Structure of calprotectin. A three–dimensional model of the crystal structure of Mn^{2+} and Ca^{2+} loaded calprotectin protein, showing two heterodimers: S100A8 and S100A9 as determined by X–ray diffraction. The grey and blue chains represent S100A8 and S100A9, respectively. The purple spheres represent Mn^{2+} and green spheres represent Ca^{2+}. Only one manganese ion can bind per calprotectin heterodimer. The modified figure was taken from the protein data bank (PDB) at: http://www.rcsb.org/pdb

12. Blood autophagy genes and nucleotide–binding oligomerization domain–containing protein 2 (NOD2)

Family history of CD is a risk factor for CD[144]. Genome–wide association studies have reported that NOD2 and Autophagy genes are associated with CD risk while IL–23/IL–17 is associated with increased risk of both CD and UC[145].

13. Serum anti–neutrophil cytoplasmic antibodies (ANCAs) and anti–saccharomyces cerevisiae antibodies (ASCAs)

It may be difficult to differentiate CD from UC due to their overlapping pathological, endoscopic and clinical features. Biomarkers like ANCAs, which are antibodies against granules of neutrophil cytoplasm and ASCAs, which are antibodies against mannan found in the cell walls of saccharomyces cerevisiae (S. cerevisiae) have been used to help differentiate CD from UC[146].

The sensitivity and specificity of perinuclear ANCA (pANCA) for the diagnosis of ulcerative colitis is 63% and 86% respectively. ASCAs for

diagnosing CD had a sensitivity of 72% and a specificity of 82%[147]. The utility of these serological tests in differentiating UC from CD is therefore limited but may be of value in studying disease heterogeneity and disease epidemiology.

Other serologic tests investigated to improve the diagnosis and differential diagnosis of IBD include: antibodies to Escherichia coli outer membrane porin C (anti–OmpC), antibodies against laminaribioside (ALCAs), antibodies against chitobioside (ACCAs), Pseudomonas fluorescens–associated sequence 12, antibodies to mannobioside (AMCAs), pancreatic autoantibodies (PAB), and anti–flagellin CBir1 but all are of limited diagnostic utility.[148-153]

B. Laboratory biomarkers with limited application

The foregoing biomarkers enjoy widespread application in IBD assessment. However, there are some that are not extensively used, or those whose utility for efficacy in IBD assessment can be enhanced only when in combination with one or more biomarkers of similar or different biochemical composition.[49]

These include: adenosine deaminase (ADA), mopterin, nitric oxide, substance P, micro ribonucleic acids (MicroRNAs), lipopolysaccharide–binding protein (LPBP) and cluster of differentiation (CD14), abnormal lectin–based immunoglobulin G (IgG) glycosylation, soluble triggering receptor expressed on myeloid cells–1 (sTREM–1), Soluble suppression of tumorigenicity 2 (ST2), Quantitative fecal immunochemical test (QFIT), activated thrombin activatable fibrinolysis inhibitor (TAFIa), chitinase 3–like–1, mucosal cytokine, mucosal indoleamine 2,3 dioxygenase–1 (IDO1) and angiogenin[49].

Others are: genes, antibodies toward infliximab, mucosal CHI3L1, urine salicylate level, and thiopurine methyltransferase and 6–thioguanine nucleotide (TPMT)[49].

14. Inflammatory and serological biomarkers in differential diagnosis of inflammatory bowel disease

The differential diagnosis of IBD includes colon cancer, ischaemic colitis, diverticulitis, bacterial and viral infection. These alternatives or co–existing disorders must be ruled out prior to confirmation of intestinal inflammation as a first step in the differential diagnosis of IBD *(Figure 4)*.

The likelihood of CD, UC and Moderate/High Clinical Suspicion are subjected to further radiographic, endoscopic and histological investigations to complete the differential diagnosis. While the above illustration *(figure 4)* provides the necessary information to aid in test selection, interpretation, diagnosis and overall patient management decisions, it is not a substitute for the clinician's underpinning knowledge of IBD in patient assessment based on clinical expertise.[154-157]

Accurate diagnosis of CD and UC is therefore dependent on not only laboratory test results but also on the patient's clinical history and examination, histology, imaging results (X–ray, CT and/or MRI scans) and endoscopy[154,158].

Inflammation in UC is relatively superficial because it affects the mucosa. It is confined to the colon starting at the rectum, with rare extension to the terminal ileum. There is, however, a 10 to 15% chance of misdiagnosis due to difficulty in distinguishing UC from CD. Accuracy in differential diagnosis is critical because while inflammation in CD extends deeper into the tissues and could affect any portion of the gastrointestinal tract, associated

symptoms like abdominal pain, fever, malnutrition and severe bloody diarrhoea are common to both[159].

15. Inflammatory biomarkers in differential diagnosis of inflammatory bowel disease

The initial step in the identification of IBD is exclusion of other organic disorders. Serum CRP, ESR, faecal calprotectin and faecal lactoferrin form the available inflammatory biomarkers.

Many laboratory biomarkers have been evaluated as the ideal replacement for, or supplement to faecal calprotectin in IBD studies but CRP and ESR remain the most widely used and assessed tests[1]. Other biomarkers used include leucocyte counts, platelet counts, albumin levels and orosomucoid concentrations[160], but there is no evidence that they are of greater benefit compared to CRP in the identification of IBD and monitoring its disease activity[161].

CRP is routinely available as it is rapid, inexpensive and a simple testing technique. CRP's reliability as a biomarker of choice in routine clinical practice is well established[162]. Serum CRP may help in differentiating IBD from other functional bowel disorders[163]. However, normal CRP levels (≤ 5 mg/L) at diagnosis occur in majority of UC patients and in 25% of CD patients.[164] There is, however, a correlation between CRP and endoscopic activity in IBD and this may have a useful role in monitoring responses to therapy.[165,166]

In summary, increased CRP is more common in CD than in UC, thereby supporting CRP to be a useful biomarker of disease activity in CD than in UC.[1,164,165] CRP, however, like most serologic biomarkers, is a non–specific biomarker of systemic inflammation.[1]

16. Serological biomarkers in differential diagnosis of inflammatory bowel disease

In the presence of inflammation, ANCAs and ASCAs may be useful in the identification of IBD.[154,155] A positive result will necessitate radiology, endoscopy and histological investigation to confirm the diagnosis of IBD and differentiation of CD and UC. If serological markers are negative in the presence of a high clinical suspicion of IBD, then radiologic, endoscopic and histological findings could be used to diagnose or exclude IBD and, if present, to distinguish CD and UC.

ASCAs and atypical pANCA are the two serologic markers most commonly used to distinguish CD and UC.[167] A positive ANCAs result is reflexed to determine the relevant pattern(s): cytoplasmic (cANCA), perinuclear (pANCA) or atypical pANCA patterns and their titres. Whereas cANCA and pANCA are found in vasculitis, atypical pANCA is present in IBD. While atypical pANCA is detected in only 5% to 25% of patients with CD, it occurs in about 55% to 80% of those with UC.[157,168] Conversely, ASCAs is detected in about 60% to 70% of CD patients and only in about 6% to 15% of UC patients.[168,169]

A combination of pANCA and ASCAs has a sensitivity of 53% and specificity of 93% for both CD and UC; and may be of value in assessing IBD in patients that cannot be distinguished as CD or UC on the basis of indeterminate colitis or established criteria.[156,157]

Atypical pANCA and ASCAs may help stratify CD. Positive atypical pANCA in CD indicate colonic involvement and an association with a clinical phenotype analogous to UC or UC–like CD, while a positive ASCAs result is linked with the non–UC–like CD.[169,170]Serologic biomarkers may be of value in children. In children at high risk of IBD, pANCA and

ASCAs may help to identify those children with IBD and to avoid invasive assessment.[157,171-174]

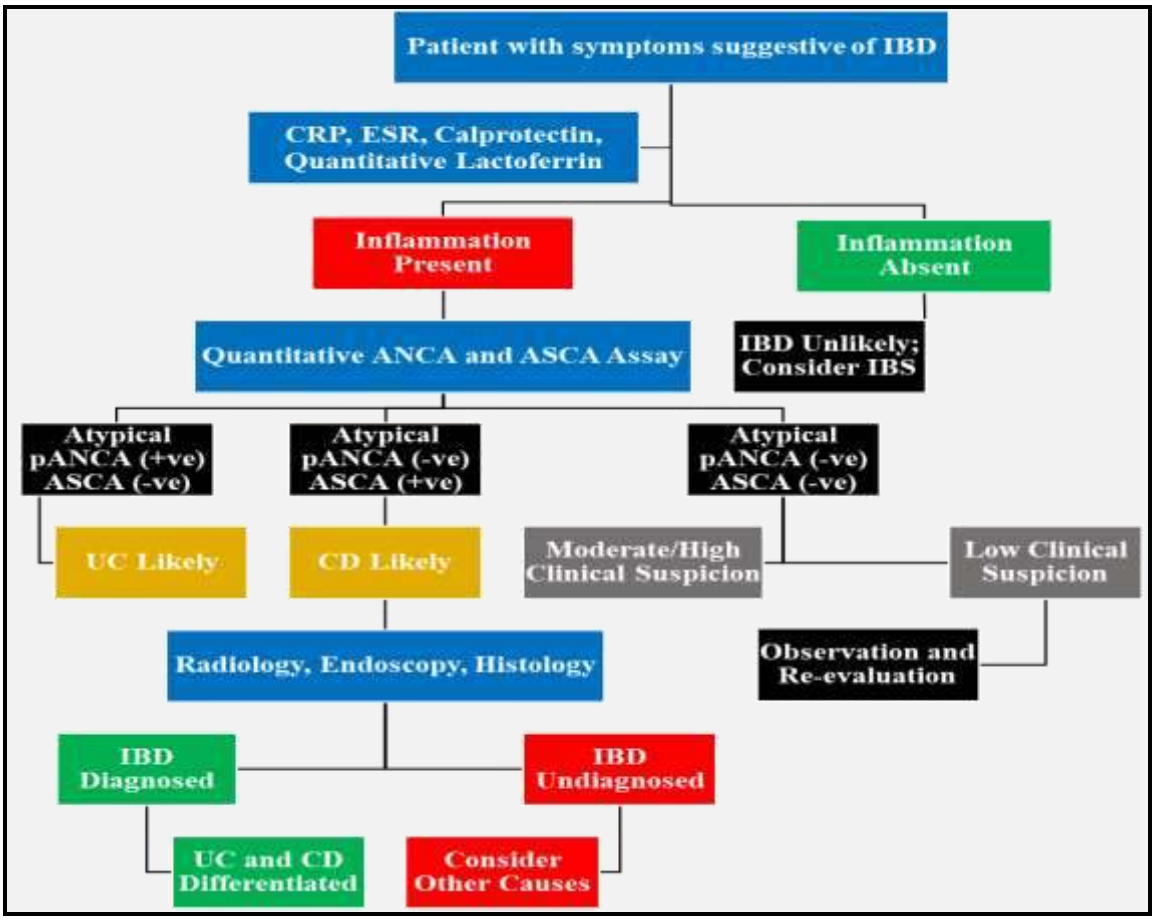

Figure 4 – The interplay of inflammatory (CRP, ESR, Calprotectin and Lactoferrin) and serological (pANCA and ASCA) biomarkers in the differential diagnosis of inflammatory bowel disease.

Management of disease activity in inflammatory bowel disease

Achieving mucosal healing with subsequent improvement in natural course of the disease state in patients with IBD remains the overall goal of treatment, including the use of immunomodulators and biological agents. Monitoring for efficacy of treatment and relapse of disease activity, therefore, becomes an important consideration.[175,176]

No single test procedure or examination has been able to satisfy all the necessary requirements for the clinical management of IBD patients. Assessing disease activity for the foreseeable future involves the use of laboratory tests, radiology, endoscopy, clinical examination and symptoms.[177] This should not, however,

detract from the search for a reliable, non–invasive, highly sensitive and reproducible biomarker of disease activity.[1,178]

The faecal excretion of ^{111}Indium–labelled (^{111}In) granulocytes is considered to be the 'gold standard' method for measuring the degree of neutrophilic infiltration into the intestinal mucosa in IBD and hence the disease activity particularly in patients with small bowel CD.[179,180] The unwieldy nature of radio–labelling techniques in addition to being expensive and involving exposure to radiation restricts their routine clinical use.

Endoscopic assessment of disease activity in IBD, particularly via ileo–colonoscopy is regarded as the 'gold standard' because it has the added advantage of enabling biopsy sampling for histopathological examination apart from offering the opportunity for direct mucosal visualization, endoscopic management of complications, assessing the success of various treatment regimens and predicting the course of disease.[181-187] Ileo–colonoscopy, however, is invasive, time–consuming and is limited by expense, risks of complications, patient discomfort and variation in interpretation between endoscopists.[188,189] This underscores the desirability for an easier method to monitor disease activity.

I. Active versus inactive disease state in inflammatory bowel disease

Increased serum CRP is an indication of active disease in CD patients and it compares favourably with endoscopic disease activity.[30] Endoscopic assessment should be considered in patients with UC if elevated serum CRP concentrations fail to normalise with or without underlying symptomology.[190]

In the patients with endoscopically confirmed active IBD, faecal calprotectin and lactoferrin levels correlate with endoscopic disease activity in both CD and UC patients.[30] When compared to serum CRP (49%), both faecal calprotectin (88%) and lactoferrin (82%) are more sensitive because of their ability to correlate better with colonic (proctitis: E1 vs. distal colitis: E2 vs. pancolitis: E3) than ileal (ileal and upper: A1 vs. colonic and ileo–colonic: A2) disease activity.[154,155]

The relatively high sensitivity of both faecal biomarkers (i.e., calprotectin and lactoferrin) in patients with active disease underscores their usefulness in managing patients with IBD[191] and thus avoiding endoscopy in patients with a high clinical suspicion of active disease with raised faecal biomarkers. As a corollary, a negative faecal biomarker result may not rule out active disease and therefore endoscopy may be required if clinically indicated.[191] Similarly, with CRP, endoscopic assessment should be considered in patients with UC if elevated faecal biomarkers fail to normalise irrespective of underlying symptoms.[190]

II. Relapse, remission, and disease course in inflammatory bowel disease

An increase in serum CRP levels predict relapse in disease activity in patients with CD following medically induced remission.[155,190]

Faecal biomarkers, particularly calprotectin, play a crucial role in predicting relapse in IBD particularly in UC than in CD.[192] Elevated faecal calprotectin level is a common feature in patients with CD who relapse compared to those who remain in remission over a 12–month follow–up.[193] Low faecal calprotectin levels help identify IBD patients who remain in stable remission during follow–up.[194]

Based on a cut–off value of 167 µg/g, faecal calprotectin with a sensitivity of 69% and specificity of 75% appears to be the best

biomarker for predicting IBD relapse following remission.[154,193] Faecal calprotectin levels may also be particularly valuable in predicting relapse in patients with CD who have undergone surgical resection.[154,195]

Faecal lactoferrin also appears to have a role in predicting IBD relapse. Elevated faecal lactoferrin levels have a sensitivity of 62% and specificity of 65% in predicting early disease relapse in paediatric patients.[37,193] In some paediatric patients with CD, pANCA and ASCAs levels also predict complicated disease courses.[154,155]

III. Response to therapy in inflammatory bowel disease

A normal CRP level in CD patients undergoing treatment is linked to a favourable response to therapy.[190] Whereas normalization of CRP levels may serve as a reliable biomarker to measure the response to therapy, there are currently a paucity of studies investigating the roles of faecal calprotectin, lactoferrin, pANCA and ASCAs in predicting response to therapy.

In summary, more information may be needed on the many aspects *(i.e., analytical and technical factors: sensitivity and specificity, accuracy and precision, reproducibility, calibration, limits of the blank, detection and quantitation; pre–analytical and biological factors: sample handling, patient characteristics, timing, analyte/assay stability; clinical factors and research design variables: purpose, validation, surrogate endpoint, reference ranges)* of laboratory biomarkers to diagnose IBD, differentiate between disease states (CD vs. UC), evaluate disease activity (active vs. quiescent), confirm disease sites or locations as in the small intestine: ileal and upper (A1) vs. colonic and ileo–colonic (A2); large intestine: proctitis (E1) vs. distal colitis (E2) vs. pancolitis (E3), predict the disease course and relapse, and monitor follow–up and response to treatment.

Summary and future perspectives

It is noteworthy that not all the biomarkers of IBD discovered through clinical research that could be available for routine application in clinical practice. Many of them fail the rigorous protocols of the clinical testing criteria and assay processing through assay development, optimisation and analytical validation, and therefore, are deemed not to be feasible and acceptable for use as laboratory biomarker for assessing IBD. However, for those biomarkers that meet the acceptable target criteria and 'fitness for purpose' via the analytical methods, there is also the added laborious intervening timeline of more than 10 years approximation that span from discovery to routine clinical use to consider.[196,197]

It is a common occurrence for newly discovered biomarkers to fail short of expectation in terms of good analytical performance and diagnostic accuracy during assay validation studies. Such are the demonstrable challenges that are intrinsic in the development and clinical application of biomarkers as articulated in a recent multicentre, open–label randomised controlled trial (RCT) for PRedicting Outcomes For Crohn's disease using a moLecular biomarker (PROFILE).[197]

The practical application of laboratory biomarkers in the assessment of inflammatory bowel disease was the subject of a recent study that reviewed the utility of current and potential future biomarkers *(figure 5)*.[198] The study reaffirmed the contemporary knowledge of the utility of currently available biomarkers such as CRP and faecal calprotectin to differentiate IBD from functional gastrointestinal pathology and to monitor the disease course. However, it presented such novel biomarkers as Oncostatin M, anti–integrin antibody, faecal MPOs, faecal

microRNAs and glycome profiling, as future biomarkers that could potentially address the risk stratification of individuals not presenting with the disease (i.e., pre–IBD) including screening those with asymptomatic IBD.[198]

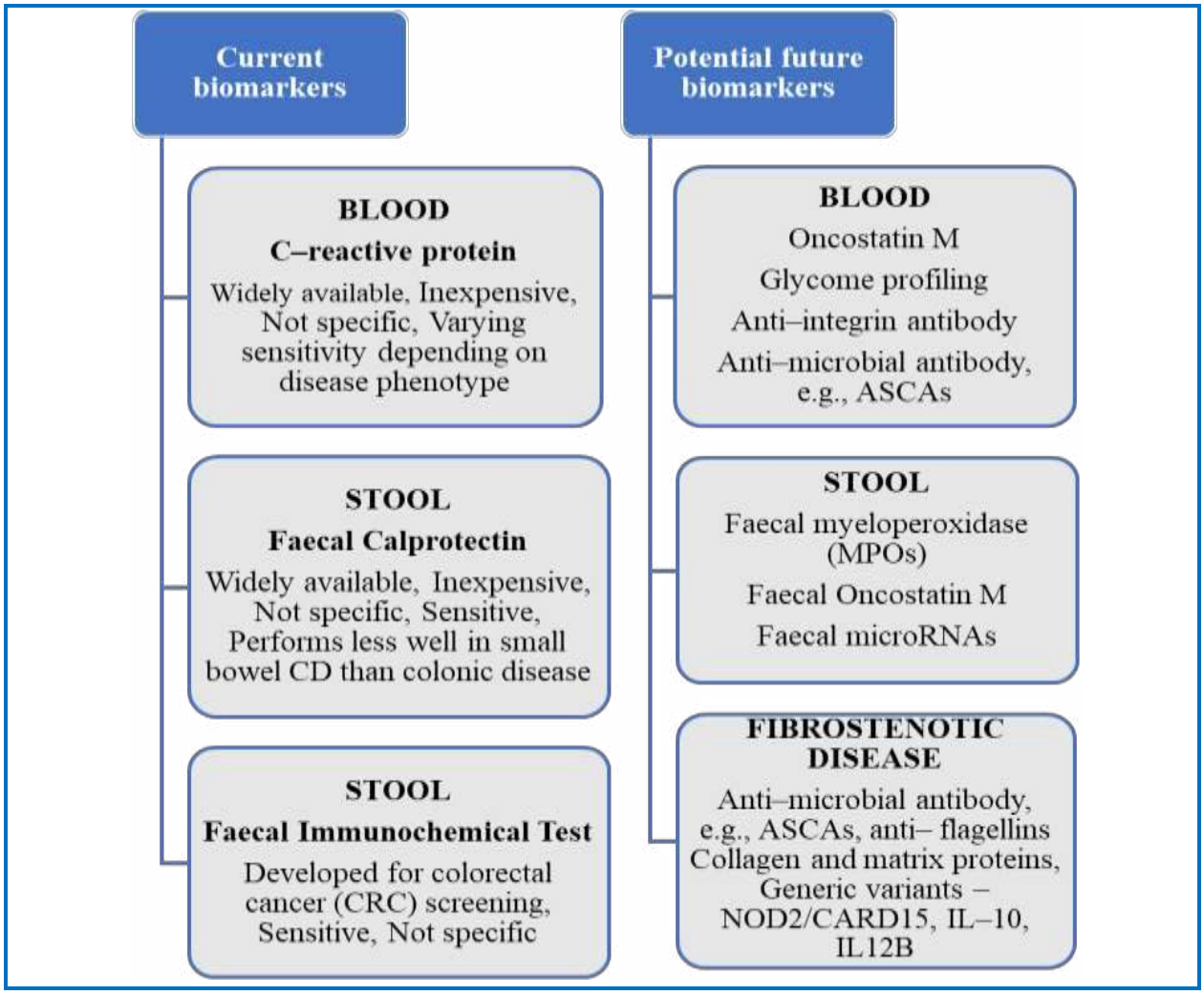

Figure 5 – An algorithm for the practical application of laboratory biomarkers in the assessment of inflammatory bowel disease. A narrative of current and potential future biomarkers for diagnosis, prognosis and disease monitoring.[198]

The future perspective on the utility of laboratory biomarkers in assessing IBD seems to tilt towards deploying the probable influence of artificial intelligence (AI) to integrate metabolomic, proteomic, genetic and transcriptomic outputs of biomarker analysis[199-201]. With the continuous growth in complexity of available data, and rapid advances in the availability and affordability of technology, the resort to AI 'to expand the horizon for biomarker

discovery, enabling the integration of multi–modal data from existing datasets to discover new biomarkers'[201] will be realised.

CONCLUSION

While it is important for laboratory biomarkers of IBD to be non–invasive, cheap, simple, objective, rapid, easy to perform and reproducible, the 'real–world' experience with these biomarkers is increasing together with the increasing number of patients with IBD for which the usefulness of these laboratory biomarkers to assess the disease have become necessary. The 'ideal biomarker' that would integrate all the preceding characteristics for evaluating the analytical performance and diagnostic accuracy of these laboratory biomarkers in IBD is not available. Therefore, the search for the availability of a single laboratory biomarker that would demonstrate the attributes of an 'ideal biomarker' for use in assessing IBD continues.

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