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Original Article

Demographic, Clinical, and Lifestyle-Related Factors Associated with Body Mass Index in Ghana: WHO STEPS Household Survey, 2014-2025

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Abstract

Introduction: Globally, several populations suffer from obesity, which is defined as a body mass index (BMI) of at least 30 kg/m², and it is up to 25% in some regions of Ghana. Factors such as lifestyle behaviours, dietary intake, and some chronic illnesses.

Objective: This study analysed data from a household survey to determine the predictors of high body mass index in Ghana, 2014-2025.

Methods: This is a secondary data analysis of a study conducted across Ghana, involving both rural and urban communities to ensure national representativeness. The population included 4,464 adults aged 18 years and older, with 2,643 from rural areas and 1,821 from urban areas. Socio-demographic characteristics, clinical diagnoses, and selected lifestyle behaviours were extracted to inform the design of an effective, age-appropriate intervention. Bivariate and multivariable regression analyses were estimated. Statistical significance was determined at $p < 0.05$, and CI was set at 95%. Results from the analysis were presented as text, tables, and figures.

Results: The median age is 58 years (IQR: 18.0). The median BMI is 22.94 kg/m² (IQR: 6.23). The median SBP is 124.33 mmHg (IQR: 27.33). The median SBP is 76.67 mmHg (IQR: 16.33). The prevalence of obesity in the study was 12.05% (95% CI: 11.13, 13.04). Most of the population were of normal weight, 2,468 (55.29%). High BMI (obesity) was higher among adults (14%) and middle-aged adults (16%), while underweight (low BMI) was higher among older adults (19%) and the oldest adults (28%). Increased age (β: -0.04, 95% CI: -0.04, -0.019) predicted lower BMI. Female sex (β: 1.91, 95% CI: 1.52, 2.25), urban dwelling (β: 1.98, 95% CI: 1.54, 2.23), and education (β: 0.96, CI: 0.59, 1.34) were all found to significantly increase BMI. However, alcohol, tobacco, and blood pressure were significant on bivariate tests ($P < 0.05$).

Conclusion: Obesity in the population varies with age. Education on lifestyle modification, screening of at-risk groups, and control of the identified factors are necessary interventions. Ongoing surveillance and longitudinal research are essential to clarify causal pathways and to inform the development of effective, culturally tailored prevention and management strategies for obesity and related chronic diseases.

Keywords: "body mass index", "factors", "Ghana", "obesity".

Introduction

The increase in non-communicable and chronic diseases in the developing world is an issue of major concern and can lead to reduced quality of life and premature deaths (Obirikorang et al., 2015a). Obesity is a classified risk factor for several of the world's leading causes of death. It represents the 5th and 6th major level two public health problem among women and men, respectively, leading to the toll of death and disability worldwide (L Murray et al., 2020). Obesity is an independent risk factor for cardiac diseases, some types of cancer, and type-2 diabetes and leads to a high mortality rate. Moreover, it aggravates chronic diseases such as cholelithiasis, hypertension, hypercholesterolemia, and arthritis (So & Seo, 2013a).

The prevalence of obesity has increased in pandemic dimensions over the past 50 years with 650 million adults, 340 million adolescents and 39 million children classified as obese. As the obesity pandemic continues, estimates indicate that approximately 167 million adults and children will become less healthy due to being overweight or obese by 2025 (Blüher, 2019). Especially in developing countries, the possible implications of obesity on current and future population health and healthcare spending are likely to be enormous (Obirikorang et al., 2024a). The prevalence of overweight and obesity has risen steadily in Ghana over the years and is reportedly more common in the capital city, Accra where urbanization is highest (Lartey et al., 2019).

The Body Mass Index (BMI) is the crude method of measure of overweight and obesity but there are other popular but simple measures of obesity employed in recent times, which include the waist-to hip ratio (WHR), waist-to-height ratio (WHtR) and the percentage body fat (BF%), whose validity has been supported. Estimates from the World Health Organization (WHO) in 2008 showed that more than 1.4 billion adults, 20 years and older, were overweight with an overall 10% of the world's population being obese (Obirikorang et al., 2018a). There are reports on the prevalence of obesity across countries in and outside Africa and in Ghana, suggesting that obesity is increasing in prevalence (Doku & Neupane, 2015a; Lartey et al., 2019). Obesity is mainly caused by a mismatch between caloric intake and energy expenditure. The factors related to the nature of energy imbalance are numerous and complex; they include the socioeconomic environment, smoking, drinking, mental stress, sleep duration, sedentary lifestyle, eating habits, and the interactions between these factors (So & Seo, 2013a). For this reason, many obese people attempt to balance energy intake and expenditure or promote energy expenditure through diverse efforts in order to reduce and control their obesity.

Furthermore, previous studies have provided evidence that lifestyle-related factors that promote health are associated with decreased obesity (Al-Hazzaa, Abahussain, et al., 2012a), Also, obesity is a multifactorial condition influenced by many variables, including genetic, demographic and lifestyle factors. Genetic and demographic variables such as family history of obesity, age, ethnicity and sex cannot be modified while obesity-associated lifestyle factors such as sedentary behaviours, physical inactivity and unhealthy dietary choices are often modifiable (Doku & Neupane, 2015a).

According to the Ghana Demographic and Health Survey of 2014, the percentage of Ghanaian overweight/obese women rose from 30% in 2008 to 40% in 2014 (Amugsi et al., 2017). These direct and indirect effects of obesity impose an economic burden on the healthcare budget of countries and individual citizens (Tremmel et al., 2017a). Lifestyle changes related to westernization and socio-economic improvement are responsible for this increasing phenomenon of obesity and overweight. Reduction in physical activity has been identified as a risk factor, which on the global level causes 3.2 million related deaths. Another major cause of obesity is a relatively higher amount of calories consumed compared to that which is expended. In Sub-Saharan Africa, increased body fat composition is regarded as beautiful and a sign of wealth, which is a cultural driver for the increasing prevalence of overweight and obesity in young adults (Muthuri et al., 2014; Agyei et al., 2022).

We have mainly relied on the routine use of the body mass index (BMI) as an obesity measure. However, BMI has a limitation in differentiating between body composition and body fat distribution (Obirikorang et al., 2018). Alternative measures, including the bioelectrical impedance analysis (BIA) and BIA-derived body fat indices, like the body adiposity index and relative fat mass (RFM), have been proposed (Freedman et al., 2012b). These measures aim to address the limitations of BMI and serve as cost-effective indices to accurately identify individuals, with accuracy close to that of underwater weighing and dual-energy X-ray absorptiometry. In particular, RFM and total percentage body fat (TPBF) have been validated as being a more accurate measure compared to BMI to estimate whole-body fat percentage, in addition to improving body fat-defined obesity misclassification among different population groups(Obirikorang et al., 2024b). Hence the need for this study and the knowledge will go a long way in informing strategies to combat the obesity epidemic and hopefully, related medical conditions among the general Ghanaian population.

This study assessed the association between demographic (age, sex, education, marriage), clinical (blood pressure, history of stroke, depression, angina, arthritis, asthma and chronic lung disease), and lifestyle-related factors (salt diet, physical activity, alcohol, tobacco) factors and body mass index (BMI) in a Nationally representative survey of Ghanaian adults, using age-standardized anthropometric methods.

Methodology

Study Setting

This study was conducted across Ghana, involving both rural and urban communities to ensure national representativeness. The study population included 4,464 adults aged 18 years and older, with 2,643 from rural areas and 1,821 from urban areas.

Study Design and Sampling

This cross-sectional analytical study was conducted over four months. The sample size was calculated using the formula for comparative quantitative outcomes, adjusted for a 10% non-response rate, resulting in a total of 4,464 participants, with 2,643 from rural areas and 1,821 from urban areas. Simple random sampling was employed, selecting participants from nationally representative community lists to ensure unbiased representation.

Study Variables

The independent variables in this survey include sociodemographic variables (age, sex, location, marital status, level of education and school), clinical variables **(**presence of arthritis, angina, stroke, asthma, depression, systolic blood pressure, diastolic blood pressure, chronic lung disease, and health status); and lifestyle-related variables (salt intake, physical activity, alcohol consumption, and tobacco use). The outcome variable is the body mass index (BMI), which was used to assess obesity.

Data Collection Instruments and Procedures

Data were extracted from the WHO STEPSwise household survey to collect information on the variables. The questionnaire was pre-tested for clarity and reliability before data collection. Anthropometric measurements were taken following age-standardised protocols to ensure consistency. Height was measured to the nearest 0.1 cm using a stadiometer, with participants wearing minimal clothing and no shoes. Weight was recorded to the nearest 0.1 kg using a calibrated scale. Systolic and Diastolic blood pressures were measured three times in a seated position using a mercury sphygmomanometer and stethoscope, following American Heart Association guidelines, with the average of the last two readings recorded. All measurements were performed by trained personnel to ensure accuracy and reliability.

Data Management

BMI was calculated as weight (kg) divided by height squared (m^2). BMI was categorised using the WHO standard defined as: underweight (<18.5 kg/m^2), normal weight (18.5-24.9 kg/m^2), overweight (25.0–29.9 kg/m^2), and obese (≥30.0 kg/m^2). Ages were categorized based on the WHO

ageing distribution as: 18-24 years (young adults), 25-44 years (adults), 45-59 years (middle-aged adults), 60-74 years (young older adults), 75-84 years (middle older adults), and 85 years and above (oldest older adults) for age-sensitive interpretations and interventions.

Systolic Blood pressure was categorized according to the International Society on Hypertension (ISH) as: less than 90 mmHg (low SBP), 90-119 mmHg (normal SBP), 120-139 mmHg (pre-hypertension), 140 mmHg and above (Systolic Hypertension). Diastolic Blood pressure was graded as: less than 60 mmHg (low DBP), 60-79 mmHg (normal DBP), 80-89 mmHg (pre-hypertension), 90 mmHg and above (Diastolic Hypertension). For the purpose of analysis, both were further coded into binary as 1 (for hypertension) and 0 (for non-hypertensive). Marital status was further dichotomized into currently married (coded as 1) and not currently married (coded 0).

Statistical Analysis

Data were analysed using STATA version 18. Continuous variables (age and blood pressures) were assessed for normality using Jarque-Bera Skewness and Kurtosis (sktest). Being not normally distributed, they were summarised with median and interquartile range (IQR). The categorical variables were summarised with frequencies and proportions.

Bivariate analysis was conducted to determine any relationship between each of the independent variables and the dependent variable (BMI). BMI was tested for normality using the sktest. It was not normal, thus, non-parametric tests were employed. Hence, the relationship between the categorical variables (such as age, sex, salt intake, physical activity, and stroke) and BMI was assessed each using the Wilcoxon-Mann-Whitney test. However, the Kruskal-Wallis test was used for health status, which had more than two levels.

Spearman's rank test of correlation was used for the relationship between the continuous variables (age and Blood pressure) and Body Mass Index. Statistical significance was set at $p < 0.05$.

Multivariate ordinal least squares (linear) regression was used to test the extent of prediction of BMI by the significant factors above. The residual from the BMI was generated and tested for normality using the sktest and the Shapiro–Wilk normality test (swilk). It was not normally distributed. Hence, the quantile regression model was used. It was also tested for equality of variance using the Cook-Weisberg test for heteroscedasticity. The variances were not equal; thus the regression model was fitted with heteroskedasticity-robust standard errors (RSE) using a variance-covariance estimate (VCE). The endogeneity assumption was tested using the omitted variable test (OVT), which showed no omission. Multicollinearity was checked using the variable inflation factor (VIF) test, and school was found to be collinear (with education), thus not included in the model. Each factor was reported using the coefficient and confidence interval, adjusting for the others.

The dataset was extracted by the Department of Biostatistics after permission and provided for a term paper while other ethical considerations were followed as appropriate for a secondary data analysis.

Results

Sociodemographic Characteristics

The median age is 58 years (IQR: 18.0), median BMI is 22.94 kg/m ^{2} (IQR: 6.23), median SBP is 124.33mmHg (IQR: 27.33), and median DBP is 76.67mmHg (IQR: 16.33).

Table 1 presents the socio-demographic characteristics of the respondents. The largest age group was middle-aged adults (1,468; 32.89%) while older olds constituted the smallest (130; 2.91%) of the study population. About 2,521 (56.47%) were married, most living rurally (2,643; 59.21%), with a female: male ratio of about 3:2. Among the respondents who had received formal education, primary school education was the most common level attained (668; 25.69%), while tertiary education had the lowest representation (141; 5.42%).

Table 1: Sociodemographic Characteristics of the participants

Variable	N	Frequency	Percentage
Age group (yrs)	4464		
Young adults		218	4.88
Adults		676	15.14
Middle-aged		1.468	32.89
Young olds		1,440	32.26
Middle olds		532	11.92
Older olds		130	2.91
Marital status	4464		
Not married		1,943	43.53
Married		2,521	56.47
Location	4464		
Rural		2,643	59.21
Urban		1,821	40.79
Sex	4464		
Female		2,610	58.47
Male		1,854	41.53
Schooling	4,464		
Yes		2,600	58.24
No		1,864	41.76
Education	2,600		
Below primary		626	24.08
Primary		668	25.69
Junior Sec.		667	25.65
Senior Sec		498	19.15
Tertiary		141	5.42

N: Number of participants

Table 2 presents the clinical factors. A total of 3,011 respondents were assessed for arthritis, 236 (7.84%) reported having arthritis. Angina was reported by 61 (2.03%) participants. Similarly, stroke prevalence was low, with only 50 (1.66%) participants reporting a previous stroke. Asthma was reported by 97 (2.18%). Depression was the least reported condition, affecting only 27 (0.90%).

For systolic blood pressure (SBP), 31 (0.70%) participants had low SBP, 1,756 (39.47%) had normal SBP, 1,533 (34.46%) were classified as pre-hypertensive, and 1,129 (25.38%) had systolic hypertension. For diastolic blood pressure (DBP), only 234 (5.29%) had low DBP, while over a half (2,415, 54.64%) had normal DBP.

More than half of the participants, 2,389 (53.92%), rated their health as good, while 631 (14.24%) considered their health very good. Moderate health status was reported by 1,041 (23.49%) participants. Only a small proportion rated their health as bad (8.35%).

Body mass index (BMI) assessment showed that 2,468 (55.29%) participants had normal weight. However, 960 (21.51%) were overweight and 538 (12.05%) were obese, indicating that approximately one-third of the participants had excess body weight. In contrast, 498 (11.16%) participants were underweight.

Table 3 presents the lifestyle-related aspects of the participants. Tobacco use was relatively low, with only 273 (6.14%) participants reporting tobacco use, while the vast majority, 4,176 (93.86%), did not use tobacco. Alcohol consumption was more common, with 1,306 (29.35%) participants reporting alcohol use compared to 3,143 (70.65%) who did not consume alcohol. More than half of the participants, 2,600 (58.24%), had a history of formal schooling, whereas 1,864 (41.76%) had no schooling history. 1,658 (37.27%) participants reported high salt intake, while 2,791 (62.73%) did not. 2,448 (55.31%) participants were physically active, indicating a relatively good level of engagement in exercise. However, 1,978 (44.69%) reported no regular physical exercise.

Table 2: Clinical factors of the participants

Variable	Presence	Frequency	Percentage
Arthritis (N=3011)	Yes	236	7.84
	No	2,775	92.16
Angina (N=3,011)	Yes	61	2.03
	No	2,950	97.97
Stroke (N=3,012)	Yes	50	1.66
	No	2,962	98.34
Asthma (N=4,448)	Yes	97	2.18
	No	4,351	97.82
Depression (N=3,012)	Yes	27	0.90
	No	2,985	99.10
Lung disease (N=4,455)	Yes	20	0.45
	No	4,435	99.55
Systolic BP	Low SBP	31	0.70
(4,449)	Normal	1,756	39.47
	Pre-HTN	1,533	34.46
	Syst. HTN	1,129	25.38
Diastolic BP	Low DBP	234	5.29
(N=4,420)	Normal	2,415	54.64
	Pre-HTN	1,041	23.55
	Diast. HTN	730	16.52
Health Status	Very good	631	14.24
(N=4,431)	Good	2,389	53.92
	Moderate	1,041	23.49
	Bad	370	8.35
BMI Category	Underweight	498	11.16
	Normal wt	2,468	55.29
(N=4,464)	Overweight	960	21.51
	Obese	538	12.05

Table 3: Lifestyle-related of the participants

	N	Frequency	Percentage
Use of tobacco	4,449		
Yes		273	6.14
No		4,176	93.86
Use of alcohol	4,449		
Yes		1,306	29.35
No		3,143	70.65
Schooling History	4,464		
Yes		2,600	58.24
No		1,864	41.76
Salt intake	4,449		
Yes		1,658	37.27
No		2,791	62.73
Physical exercise	4,464		
Physically active		2,448	55.31
No Exercise		1,978	44.69

Prevalence of Obesity

Figure 1 shows the prevalence of Obesity in the study which was 12.05% (95% CI: 11.13, 13.04). Most of the population were of normal weight, 2,468 (55.29%).

Figure 2 is a stacked bar chart showing the BMI distribution by age-group. High BMI (obesity) was more in the adults (14%) and middle-aged adults (16%); while underweight (low BMI) was more among the older adults (19%) and the oldest adults (28%).

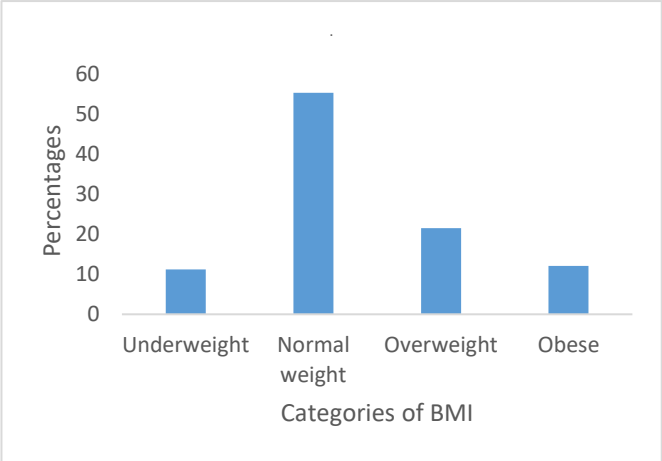

Figure 1: Prevalence of Obesity by BMI category

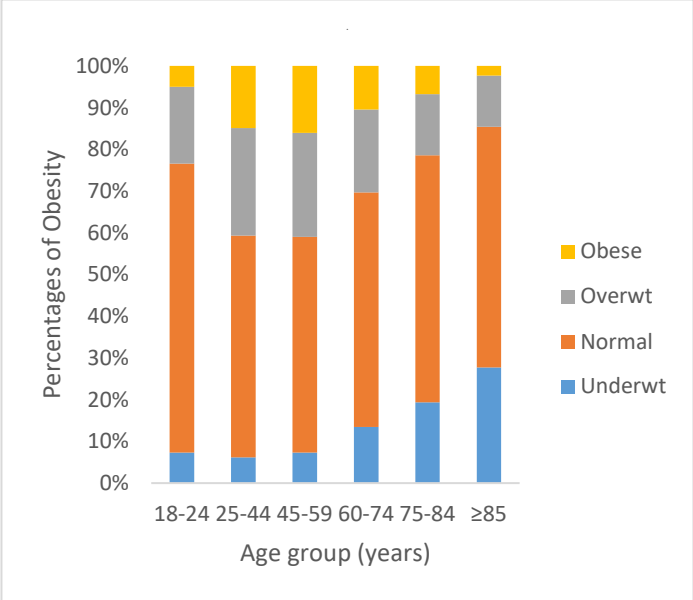

Figure 2: Distribution of Obesity by age group

Table 4 shows the Wilcoxon sign rank analysis of the relationship between the demographic, clinical and lifestyle factors and the BMI.

Sex, location, tobacco use, education, alcohol and health status had a significant relationship ($P < 0.05$)

Table 5 shows that all the continuous variables were statistically significantly related to BMI.

Age had a significant weak negative correlation with BMI (rho= -0.19, $p < 0.001$), while there are weak positive correlations with SBP (rho=0.098, $p<0.001$) and DBP (rho=0.088, $p<0.001$).

Table 6 shows the strength of the relationship using quantile linear regression model with robust estimates. Female sex, urban dwelling, and education were all found to significantly increase the BMI. The higher the age, the lower the BMI (Coefficient -0.04).

A unit rise in SBP increases the BMI by 0.03kg/m2 (β=0.03, 95% CI: 0.73-1.53). Females and urban residence significantly increase the BMI by aprox 2 (β=1.98, 95% CI: 1.54-2.2

Table 4: Association between Sociodemographic, Clinical, and lifestyle Characteristics and BMI

	N	Rank sum	Z	P
Sex				
Male	1854	3437004	-16.545	<0.001*
Female	2610	6528875		
Marital status				
Not married	1943	4335901	-0.043	0.966
Married	2521	5629978		
Location				
Urban	1821	4796341	17.273	<0.001*
Rural	2643	5169538		
Tobacco				
Yes	273	458616	-7.237	<0.001*
No	4176	9440409		
Alcohol				
No	3143	7174379	4.644	<0.001*
Yes	1306	2724646		
Education				
Yes	2600	6190547	9.091	<0.001*
No	1864	3775332		
Salt intake				
Previous	1658	3630423	1.415	0.157
Never	2791	6268602		
Physical act				
Yes	1978	4460509	0.052	1.945
No	2448	5336441		
Arthritis				
Yes	236	367805	0.966	0.334
No	2775	4166761		
Angina				
Yes	61	97209	0.795	0.427
No	2,950	4437356		
Stroke				
Yes	50	85159	1.613	0.107
No	2,962	4452419		
Asthma				
Yes	97	212985	-0.223	0.823
No	4,351	9681591		
Depression				
Yes	27	39439	-0.275	0.783
No	2,985	4498139		
Chronic lung disease				
Yes	20	37930	-1.155	0.248
No	4435	9887809		
Health status				
Very good	631	1500000	23.071 [h]	<0.001*
Good	2,389	5290000		
Moderate	1,041	2230000		
Bad	312	691930		
Very bad	58	99359		

*: significant P value: Z: Man Whitney's test. h: Kruskal Wallis test

Table 5: Spearman's test of Correlation

	Median	IQR	rho	*p* value
Age (years)	58.00	18.00	-0.192	<0.001*
SBP (mmHg)	124.33	27.33	0.098	<0.001*
DBP (mmHg)	76.67	16.33	0.088	<0.001*

*: significant P value: rho: Spearman rho.

Table 6: Independent Predictors of BMI

	β	RSE	P	Lower CI	Upper CI
Age (years**)**	-0.04	0.01	<0.001*	-0.04	-0.02
SBP (mmHg)	0.03	0.01	<0.001*	0.73	1.53
DBP (mmHg)	0.01	0.01	0.712	-0.02	0.03
Residence					
Rural	Ref				
Urban	1.98	0.19	<0.001*	1.54	2.23
Sex					
Male	Ref				
Female	1.91	0.16	<0.001*	1.52	2.25
Tobacco					
No	Ref				
Yes	-0.32	0.21	0.352	-1.08	0.38
Alcohol					
No	Ref				
Yes	0.16	0.16	0.408	-0.55	0.23
Education					
No	Ref				
Yes	0.96	0.16	<0.001*	0.59	1.34
Constant	25.06		<0.001	24.01	26.10

β: Coefficient; RSE: **Robust** Standard error; CI: Confidence interval. *: significant;

Discussion

This study examined the factors contributing to high BMI and Obesity in the Ghanaian population with interest in sociodemographic, clinical and lifestyle-related factors from a household survey conducted among Ghanaians.

The age distribution included all adults age groups from the young adults to the oldest old adults, with a median age of 58 years. Each of the middle-aged adults (45-65 years) and the young olds (60-75years) constitutes about a third of the population, thus both making two-thirds of the population. The sociodemographic profile shows the broader epidemiological transition occurring in many low- and middle-income countries (Muthuri et al., 2014b).

The shift toward older age, urbanization, and changing lifestyle factors is reflected in the rising, though still moderate, prevalence of overweight and obesity. The median BMI in the population is about 23kg/m2 which falls within the normal BMI range. However, about one-eight of the population are obese while one-fifth have above the normal BMI, both making up about a third of the population.

This study highlighted appropriate interventions for the population. This study found an inverse relationship between age and BMI, especially given the predominance of middle aged and young olds in the study. The trend in the study shows that while the obesity level tends to reduce with age group, the underweight level increases with age. Hence, the young and the middle-aged adults have more obesity while the underweight level is most among the oldest olds. This trend likely reflects a combination of physiological changes, survivorship bias, and possible undernutrition in the oldest age groups (Muthuri et al., 2014b; Ssentongo et al., 2021). Also, the RODAM study in Ghanaian adults also identified early-life factors (Danquah et al., 2019).

Other demographic factors contributing to obesity in Ghanaians include being female and urban residence. The significant influence of urban residence on BMI highlights the role of the built

environment and lifestyle changes including increased sedentary behaviour and dietary shifts toward processed foods in driving weight gain (Yussif et al., 2024).

Similarly, the strong association between female sex and higher BMI may be attributable to biological differences in fat distribution, as well as gendered patterns of physical activity, nutrition, and health-seeking behaviours (Amoah, 2003).

High systolic and diastolic blood pressures and health status are the clinical states found to relate with BMI on bivariate test. Dwomoh and colleagues support that improving the quality of life can help the hypertensive patients in Ghana (Dwomoh et al., 2025a), which along with education and awareness in Ghana can control the BMI (Sanuade et al., 2018).

Thus, the lack of prediction by chronic diseases such as stroke, arthritis, and depression may suggest some protective factors at play in this cohort. However, as the population continues to age and urbanize, these factors may become more prominent, potentially driving up rates of obesity and associated non-communicable diseases (Vos et al., 2017).

Health status stands out as a robust correlate, those reporting "very good" health have significantly higher BMI rank sums, suggesting a nonlinear relationship between perceived health and BMI. This finding may reflect the "obesity paradox" where moderate BMI is associated with better outcomes in older populations (Amugsi et al., 2017; Doku & Neupane, 2015a; Freedman et al., 2012a).

Alcohol is the major lifestyle factor predicting a high BMI in this study, and with tobacco, they were both significant on bivariate did not significantly predict BMI on multivariable analysis. This may suggest confounding effects from other factors in the population. On the other hand, Physical activity was found in this survey to reduce BMI which supports the effect of exercise on BMI reduction as evidenced by the WHO STEPwise surveillance on non-communicable diseases and events such as obesity (Riley et al., 2016).

Limitations

The cross-sectional design limits the ability to establish causality between demographic, clinical, or lifestyle factors and BMI. Self-reported data on clinical history and lifestyle factors may introduce recall bias. Additionally, unmeasured confounders, such as dietary patterns beyond salt intake, were not fully explored. Also, the high proportion of rural and older participants may limit generalizability to younger or urban populations. The study is however strengthened by being a National survey, exploring several relevant sociodemographic, clinical and lifestyle-related factors.

Conclusion and Recommendation

This study shows that female gender, hypertension, alcohol intake, middle-age and urban residence are some factors predicting high BMI and obesity while physical activity among other factors predicts low BMI. The obesity level in this population is not exceptionally high compared with global trends, however given the established risks in this study, there is a need for attention.

The findings underscore the need for interventions targeted especially to the middle-aged adults towards preventing obesity, and conversely, nutrition-improving interventions at the older adults to address their underweight. To address the obesity, there is a need for primordial and primary prevention in the population and secondary and tertiary prevention in the affected which include screening, early diagnosis, public health education.

Also, epidemiological surveillance and longitudinal research are essential to clarify causal pathways and to inform the development of effective, culturally tailored prevention and management strategies for obesity and related chronic diseases.

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Original Article

Sociodemographic Correlates of Substance Use Disorders among Undergraduates at the Federal University of Agriculture, Abeokuta

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Abstract

Background: Substance use disorders (SUDs) are a major public health concern among young adults and university students because of their adverse effects on physical and mental health, academic performance, and social functioning. Although substance use among Nigerian undergraduates has been widely studied, evidence on clinically diagnosed substance use disorders and their sociodemographic correlates remains limited.

Objective: To determine the prevalence and sociodemographic correlates of substance use disorders among undergraduate students at the Federal University of Agriculture, Abeokuta, Nigeria.

Methods: This analytical cross-sectional study was conducted among 413 undergraduate students aged 18 to 29 years selected using a multistage sampling technique. Data were collected using an interviewer administered sociodemographic questionnaire and the Substance Use Disorder modules of the Mini International Neuropsychiatric Interview version 7.0.2 based on the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition criteria. Descriptive statistics were used to summarise the data. Associations between sociodemographic variables and substance use disorders were assessed using Chi square and Fisher's exact tests where appropriate. Independent predictors were identified using binary logistic regression at a significance level of $p < 0.05$.

Results: The mean age of respondents was 23.3 ± 2.6 years, and 69.3% were male. Overall, 104 respondents met the diagnostic criteria for a substance use disorder, giving a prevalence of 25.2% (95% CI: 21.2% to 29.6%). Cannabis was the most commonly used substance (60.6%), followed by opioids (22.1%), methamphetamine (18.3%), and benzodiazepines (10.6%). Bivariate analysis demonstrated significant associations between substance use disorders and age, sex, level of study, college of study, marital status, family type, religious affiliation, level of spiritual activity, and parental history of substance use (all $p < 0.05$). Multivariable logistic regression showed that increasing age (AOR = 1.25, 95% CI: 1.10 to 1.41), male sex (AOR = 4.70, 95% CI: 2.04 to 10.84), polygamous family structure (AOR = 2.97, 95% CI: 1.61 to 5.47), and parental history of substance use (AOR = 4.37, 95% CI: 2.34 to 8.20) independently increased the odds of substance use disorder. Christianity and Islam were associated with significantly lower odds of substance use disorder compared with no religious affiliation.

Conclusion: One in four undergraduates met the diagnostic criteria for a substance use disorder. Older age, male sex, polygamous family structure, and parental history of substance use were independent predictors. These findings highlight the need for targeted screening, prevention, and early intervention programmes focusing on high-risk student populations within Nigerian universities.

Keywords: "Nigeria, prevalence, sociodemographic correlates, Substance use disorder, undergraduates"

Introduction

Substance use disorders (SUDs) constitute a major public health challenge worldwide, particularly among adolescents and young adults. According to the United Nations Office on Drugs and Crime, over 316 million people aged 15 to 64 years used psychoactive substances in 2023, with approximately one in five users meeting criteria for a substance use disorder(United Nations Office on Drugs and Crime (UNODC), 2025). Young adults remain the age group with the highest prevalence of substance use disorders because this developmental period is characterised by increased experimentation, sensation seeking, peer influence, and greater independence. The consequences of substance use during this period extend beyond physical and mental health, affecting academic performance, interpersonal relationships, productivity, and overall quality of life.

University students represent a particularly vulnerable population because of the unique social and environmental circumstances associated with higher education. Transitioning from parental supervision to a relatively independent lifestyle exposes students to new peer networks, academic pressures, financial challenges, and greater accessibility to psychoactive substances. These factors may facilitate the initiation and progression of substance use, with some students developing harmful patterns of use that satisfy diagnostic criteria for substance use disorders. Substance use disorders among undergraduates have been associated with poor academic achievement, risky sexual behaviours, interpersonal violence, road traffic injuries, psychiatric comorbidity, and school dropout, making them an important public health concern within university communities.

Globally, studies have consistently demonstrated substantial levels of substance use among university students, although prevalence estimates vary across regions because of differences in study populations, sociocultural contexts, and assessment methods. In the United States, the National Survey on Drug Use and Health reported that approximately one-fifth of college students met criteria for a substance use disorder, with alcohol and cannabis use disorders being the most common(Center for Behavioral Health Statistics, 2021). Similarly, studies conducted in Africa have documented high levels of substance use among university students, with alcohol, cannabis, tobacco, and prescription medications accounting for most substances consumed(Babalola et al., 2014; Makanjuola et al., 2007; Olashore et al., 2018). These findings underscore the growing burden of substance-related problems among young adults in tertiary institutions.

Nigeria has witnessed a steady rise in substance use among young people over the past two decades. The National Drug Use Survey reported that 14.4% of Nigerians aged 15 to 64 years had used a psychoactive substance within the preceding year, a prevalence considerably higher than the global average. Cannabis remains the most commonly used illicit substance, while the misuse of prescription opioids, including tramadol and codeine containing preparations, has become increasingly common(United Nations Office on Drugs and Crime (UNODC), 2018). Among university students, studies from different regions of the country have reported high lifetime prevalence of substance use, ranging from about 45% to over 80%, with alcohol, tobacco, cannabis, and stimulants being the most frequently used substances(Olanrewaju et al., 2022; Onofa Lucky Umukoro, 2016). However, most Nigerian studies have focused primarily on substance use rather than clinically significant substance use disorders and have relied on screening instruments instead of diagnostic assessments.

The occurrence of substance use disorders is influenced by a complex interaction of biological, psychological, social, and environmental factors. Sociodemographic characteristics have consistently been identified as important determinants of substance use behaviours and substance-related disorders. Variables such as age, sex, socioeconomic status, level of education, family structure, living arrangements, religion, ethnicity, and place of residence may influence an individual's exposure to psychoactive substances, perception of risk, access to substances, and likelihood of developing problematic patterns of use. Identifying these correlates is important because they facilitate early identification of high-risk groups and support the development of targeted prevention and intervention programmes within university settings.

Evidence regarding the association between sociodemographic characteristics and substance use disorders among Nigerian undergraduates remains inconsistent. While some studies have reported significant associations between male gender, older age, financial independence, and substance use, others have found weak or no significant relationships after adjusting for potential confounding factors. Furthermore, most available Nigerian studies have examined substance use rather than substance use disorders, limiting their clinical relevance. Few studies have employed diagnostic criteria to determine the prevalence of substance use disorders and their sociodemographic correlates among university students.

The Federal University of Agriculture, Abeokuta, is one of Nigeria's leading universities with a diverse undergraduate population drawn from different geographical, ethnic, religious, and socioeconomic backgrounds. Understanding the sociodemographic factors associated with substance use disorders in this population is essential for designing evidence-based preventive strategies, strengthening campus mental health services, and informing institutional policies aimed at reducing the burden of substance-related disorders among students.

This study therefore aimed to determine the sociodemographic correlates of substance use disorders among undergraduate students at the Federal University of Agriculture, Abeokuta, Nigeria. The findings are expected to contribute to the growing body of evidence on substance use disorders among Nigerian undergraduates and provide useful information for clinicians, university administrators, policymakers, and public health practitioners involved in the prevention and management of substance-related disorders among young adults.

Methodology

Study design and setting

This analytical cross-sectional study was conducted among undergraduate students at the Federal University of Agriculture, Abeokuta (FUNAAB), Ogun State, Nigeria. FUNAAB is a federal public university located in Abeokuta, Southwestern Nigeria, with an undergraduate population of approximately 19,721 students during the 2024/2025 academic session. The university comprises ten colleges offering diverse academic programmes and admits students from all geopolitical zones of Nigeria.

Study population

The study population consisted of registered undergraduate students of FUNAAB aged 18 to 29 years. Students who gave written informed consent were eligible to participate. Students with severe mental health conditions that could impair their ability to complete the interview reliably and those who declined or withdrew consent were excluded from the study.

Sample size and sampling technique

The minimum sample size was calculated using the single population proportion formula for cross-sectional studies. After adjusting for nonresponse, a final sample size of 413 participants was obtained.

A multistage sampling technique was employed. First, five colleges were selected from the university through simple random sampling. Subsequently, one department was selected from each participating college. The sample allocated to each department was determined using proportionate allocation based on the departmental student population. Eligible students in the selected departments were then recruited using systematic random sampling from the 300 to 500 levels. Students in the 100 and 200 levels were excluded because many were younger than 18 years, which was below the minimum age required for one of the study instruments.

Data collection

Data were collected using an interviewer-administered sociodemographic questionnaire(SDQ) and the Substance Use Disorder modules of the Mini International Neuropsychiatric Interview version 7.0.2 (MINI), a structured diagnostic interview based on the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition criteria. The sociodemographic questionnaire obtained information on participants' age, sex, religion, ethnicity, marital status, level of study, family characteristics, and other relevant demographic variables. The MINI was used to establish diagnosis of substance use disorders and to classify their severity into mild, moderate, or severe according to the number of diagnostic criteria fulfilled. The instrument has demonstrated good psychometric properties and has been previously used in Nigerian populations. The questionnaires were administered by the principal investigator and two trained research assistants following standardised training to ensure uniformity in data collection. A pilot study involving 41 undergraduate students was conducted before the commencement of the main study to assess the feasibility of the study procedures and establish interrater reliability.

Statistical analysis

Data were entered and analysed using Stata version 18.0. Descriptive statistics were used to summarise participants' sociodemographic characteristics and the prevalence of substance use disorders. Continuous variables were summarised using means and standard deviations, while categorical variables were presented as frequencies and percentages. Associations between sociodemographic variables and substance use disorders were examined using the Chi-square test or Fisher's exact test, where appropriate. Variables that were statistically significant at the bivariate level were entered into a multivariable binary logistic regression model to identify independent sociodemographic predictors of substance use disorders. Adjusted odds ratios with 95% confidence intervals were reported, and statistical significance was set at a p-value less than 0.05.

Ethical considerations

Ethical approval for the study was obtained from the Health Research Ethics Committee of the Neuropsychiatric Hospital, Aro, Abeokuta, Nigeria (PRO06/24), before commencement of data collection. Written informed consent was obtained from all participants after explaining the study objectives and procedures. Participation was voluntary, confidentiality was maintained by anonymising all data, and participants were informed of their right to withdraw from the study at any stage without any consequences.

Results

A total of 413 undergraduate students participated in the study, yielding a response rate of 100%.

Sociodemographic characteristics of the respondents

The mean age of the respondents was 23.3 ± 2.6 years. Males constituted 69.3% of the study population. Most respondents were single (93.7%), from monogamous families (60.3%), and identified as either Christians (48.4%) or Muslims (49.6%). Nearly half of the participants (46.7%) reported regular participation in spiritual activities, while 40.4% participated frequently. Approximately one quarter (24.0%) reported a parental history of substance use. The respondents were fairly distributed across the five participating colleges, with the largest proportion from the College of Agriculture Management and Rural Development (23.3%). Students in the 400 level constituted the largest group (41.7%), followed by those in the 300 level (36.6%) and 500 level (21.8%) (Table 1).

Prevalence of substance use disorders

Of the 413 respondents, 104 met the diagnostic criteria for a substance use disorder, giving an overall prevalence of 25.2%. The prevalence and severity of substance use disorders is presented in Table 2 while the pattern of substance use is presented in Table 3.

Association between sociodemographic characteristics and substance use disorders

Bivariate analysis showed that several sociodemographic characteristics were significantly associated with SUDs. Respondents with SUDs were significantly older than those without the disorder (24.7 ± 2.5 years versus 22.9 ± 2.5 years, $p < 0.001$). Male respondents had a significantly higher prevalence of substance use disorder than females ($p < 0.001$). Significant associations were also observed between substance use disorder and level of study ($p = 0.031$), college of study ($p < 0.001$), marital status ($p = 0.017$), family type ($p < 0.001$), religious affiliation ($p = 0.004$), level of spiritual activity ($p < 0.001$), and parental history of substance use ($p < 0.001$) (Tables 3A and 3B).

Sociodemographic predictors of substance use disorders

Multivariable logistic regression analysis identified several independent sociodemographic predictors of SUD with higher odds of substance use disorder (adjusted odds ratio [AOR] = 1.25, 95% confidence interval [CI]: 1.10 to 1.41, $p < 0.001$). Male respondents were almost five times more likely to have a substance use disorder than females (AOR = 4.70, 95% CI: 2.04 to 10.84, $p < 0.001$). Respondents from polygamous families had significantly higher odds of substance use disorder than those from monogamous families (AOR = 2.97, 95% CI: 1.61 to 5.47, $p < 0.001$). Having a parent with a history of substance use was also an independent predictor of substance use disorder (AOR = 4.37, 95% CI: 2.34 to 8.20, $p < 0.001$).

Compared with respondents who reported no religious affiliation, those who identified as Christians or Muslims had significantly lower odds of substance use disorder. Students in the 300 level were less likely to have substance use disorder than those in the 400 level (AOR = 0.55, 95% CI: 0.32 to 0.93, $p = 0.026$). Similarly, respondents from the College of Agriculture Management and Rural Development and the College of Plant Science and Crop Production had significantly lower odds of substance use disorder compared with respondents from the College of Environmental Resources Management (Table 4A and 4B).

Table 1: Sociodemographic characteristics of the participants in the study

Characteristic	Categories	Frequency	Percentage
Age (years)	Mean	23.3	
	SD	±2.6	
Sex	Female	127	30.75
	Male	286	69.25
Level	300	151	36.56
	400	172	41.65
	500	90	21.79
College	COLERM	77	18.64
	COLANIM	90	21.79
	COLAMRUD	96	23.25
	COLFHEC	76	18.40
	COLPLANT	74	17.92
Marital Status	Single	387	93.70
	Married	23	5.57
	Cohabiting	3	0.73
Family type	Parent not married	36	8.72
	Polygamous	128	30.99
	Monogamous	249	60.29
Religious affiliation	No affiliation	8	1.94
	Christianity	200	48.43
	Islam	205	49.63
Spiritual activity	None or Rare	53	12.83
	Regular	193	46.73
	Frequent	167	40.44
Parental substance use	Yes	99	24.0
	No	314	76.0

SD: Standard Deviation.

Table 2: Prevalence and severity of alcohol use disorder and substance use disorder

Disorder and Severity	Frequency	%	95% CI	
			Lower	Upper
SUD absent	309	74.82	70.39	78.78
SUD present	104	25.18	21.22	29.61
Mild	21	5.08	3.33	7.68
Moderate	51	12.35	9.50	15.90
Severe	32	7.75	5.53	10.76

CI: **Confidence Interval; %: Percentage**

Table 3: Pattern of use of substances

Substance type	Frequency	Percentage (%)
Cannabis	63	60.57
Opioids	23	22.11
Methamphetamine	19	18.27
Benzodiazepine	11	10.58
Cocaine	4	3.85
Others	2	1.92

Others: MDMA, Steroids.

Note: The total frequency exceeds the number of respondents with substance use disorder because some respondents use multiple substances.

Table 4A: Association between Sociodemographic Characteristics and substance use disorders

	Substance Use Disorder			
Variables	**No(N=309) Freq (%)**	**Yes(N=104) Freq (%)**	χ^2 (df)	***p* value**
Age (years) (Mean (SD))	22.85 (2.50)	24.72 (2.47)	6.62[t] (411)	<.001*
Sex			19.51 (1)	<.001*
Female	113 (89.0)	14 (11.0)		
Males	196 (68.5)	90 (31.5)		
Level			6.95 (2)	.031*
300	124 (82.1)	27 (17.9)		
400	123 (71.5)	49 (28.5)		
500	62 (68.9)	28 (31.1)		
College			56.60 (4)	<.001*
COLERM	54(70.1)	23(29.9)		
COLANIM	62(68.9)	28(31.1)		
COLAMRUD	86(89.6)	10(10.4)		
COLFHEC	37(48.7)	39(51.3)		
COLPLANT	70(94.6)	4(5.4)		

Test stats: Test Statistics; χ^2: Chi-squared test; t: t-test; df: degree of freedom; *: significant; Freq: Frequency; %: Percentage; SD: Standard deviation.

Table 4B: Association between Sociodemographic characteristics and substance use disorders

	Substance Use Disorder			
Variables	**No Freq (%)**	**Yes Freq (%)**	χ^2 **(df)**	***p* val**
Marital Status			9.66[F] (2)	.017*
Single	290 (74.6)	97 (25.1)		
Married	19 (82.6)	4 (17.4)		
Cohabiting	0 (0.0)	3 (100)		
Family type			41.62 (2)	<.001*
Not married	27 (75.0)	9 (25.0)		
Polygamous	70 (54.7)	58 (45.3)		
Monogamous	212 (85.1)	37 (14.9)		
Religion			10.88 (2)	.004*
No affiliation	2 (25.0)	6 (75.0)		
Christianity	150 (75.0)	50 (25.0)		
Islam	157 (76.6)	48 (23.4)		
Spiritual activities			21.63 (2)	<.001*
None or Rare	30 (56.6)	23 (43.4)		
Regular	136 (70.4)	57 (29.6)		
Frequent	143 (85.6)	24 (14.4)		
Parental use			86.73 (1)	<.001*
Yes	39 (39.4)	60 (60.6)		
No	270 (86.0)	44(14.0)		

F: Fisher's exact test

Table 5: Multivariable logistic regression showing Sociodemographic predictors of Substance Use Disorder

	OR	S/E	p-val	Lower CI	Upper CI
Age	1.25	0.08	<.001*	1.10	1.41
Sex					
Famale	REF				
Male	4.70	2.00	<.001*	2.04	10.84
Level					
400	REF				
300	0.55	0.49	.026*	0.32	0.93
500	1.13	0.32	.658	0.65	19.23
College					
COLERM	REF				
COLANIM	1.06	1.57	.862	0.54	2.05
COLAMRUD	0.27	1.52	.002*	0.12	0.62
COLFHEC	2.48	3.60	.007*	1.27	4.80
COLPLANT	0.13	0.30	<.001*	0.04	0.41
Marital status					
Single	REF				
Married	0.22	0.18	.068	0.45	1.11
Family type					
Monogamous	REF				
Polygamous	2.97	0.93	<.001*	1.61	5.47
Affiliation					
None	REF				
Christianity	0.03	0.04	.002*	0.00	0.30
Islam	0.02	0.01	<.001*	0.00	0.15
Spiritual activities					
None/rare	REF				
Regular	1.10	0.48	.822	0.47	2.58
Frequent	0.52	0.25	.169	0.21	1.32
Parental use					
No	REF				
Yes	4.37	1.50	<.001*	2.34	8.20

OR: odds ratio**; SE:** Standard error**; CI:** Confidence interval**; *:** significant; **REF**: Reference

Discussion

This study examined the sociodemographic correlates of substance use disorders among undergraduate students at the Federal University of Agriculture, Abeokuta. Approximately one quarter of the respondents met the diagnostic criteria for a substance use disorder, with age, sex, family type, religion, level of study, college of study, and parental history of substance use emerging as important correlates. Multivariable analysis further identified increasing age, male sex, polygamous family structure, and parental history of substance use as independent predictors of substance use disorder.

The overall prevalence of substance use disorder in this study was 25.2%, indicating that one in every four undergraduate students met the diagnostic criteria for a substance use disorder(Center for Behavioral Health Statistics, 2021). This finding is lower than estimates reported in several Nigerian university-based studies that relied on screening instruments to assess substance use rather than diagnostic criteria(Olanrewaju et al., 2022; Onofa Lucky Umukoro, 2016). The relatively lower prevalence observed in the present study may be attributed to the use of the Mini International Neuropsychiatric Interview, a structured diagnostic instrument based on the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition criteria, which provides a more clinically meaningful assessment of substance-related disorders. The finding nevertheless highlights the substantial burden of substance use disorders among university students and underscores the need for routine screening and early intervention within tertiary institutions.

Increasing age was significantly associated with substance use disorder, and older students had greater odds of developing the disorder than their

younger counterparts. This finding is consistent with reports from previous Nigerian and international studies, which have demonstrated that prolonged exposure to psychoactive substances increases the likelihood of developing problematic patterns of use(Blows & Isaacs, 2022; Soremekun et al., 2021). Older students are more likely to have experienced greater autonomy, increased financial independence, and wider social networks that facilitate repeated substance use. They may also have had a longer duration of exposure to alcohol and other psychoactive substances, increasing the probability of meeting diagnostic criteria for a substance use disorder.

Male respondents were significantly more likely than females to have a substance use disorder, and male sex remained an independent predictor after controlling for other variables. This finding agrees with numerous studies conducted in Nigeria, other African countries, and high-income settings that consistently report higher rates of substance use disorders among males(United Nations Office on Drugs and Crime(UNODC), 2018, 2025). Several explanations have been proposed for this gender disparity. Cultural norms often encourage greater risk-taking behaviour among males, while substance use by females is more likely to be discouraged because of prevailing social expectations. Male students also tend to have greater peer exposure to substance-using networks and may experience less social restriction regarding alcohol and other psychoactive substance use. Biological and psychosocial factors may further contribute to the observed gender differences.

Family structure was another important determinant of substance use disorder. Respondents from polygamous families were significantly more likely to develop substance use disorders than those from monogamous families. Similar observations have been reported in previous African studies where family instability, reduced parental supervision, sibling rivalry, and economic constraints associated with larger family sizes were linked to increased substance use among adolescents and young adults(Bray et al., 2022; Fatoye, 2003). Polygamous households may present additional psychosocial stressors that predispose vulnerable individuals to maladaptive coping strategies, including psychoactive substance use. Although family structure itself may not directly cause substance use disorders, it may reflect broader family dynamics that influence behavioural outcomes.

Parental history of substance use was one of the strongest predictors identified in this study. This finding is consistent with extensive evidence demonstrating the influence of parental substance use on children's behavioural and mental health outcomes. Several mechanisms may account for this association, including genetic susceptibility, modelling of substance-using behaviours, greater accessibility to psychoactive substances within the home environment, and reduced parental monitoring. These findings emphasise the importance of family-centred preventive interventions in reducing the burden of substance use disorders among young adults.

Respondents who identified as Christians or Muslims were less likely to have substance use disorders than those reporting no religious affiliation. This finding supports previous studies suggesting that religious beliefs and participation in organised religious activities may provide protective effects against substance misuse through the promotion of moral values, social support, and behavioural regulation(Daniel et al., 2024; Falade et al., 2022; Li et al., 2022). However, the relationship between religiosity and substance use is complex and may be influenced

by the intensity of religious commitment rather than affiliation alone.

Level of study and college of study were also associated with substance use disorder. Students in higher academic levels generally demonstrated higher rates of substance use disorder than those in lower levels, although only selected categories remained significant after adjustment. Increased academic workload, prolonged exposure to university life, expanding peer networks, and greater independence may explain the higher prevalence observed among senior students. Differences across colleges may reflect variations in academic culture, stress levels, peer influence, and the social environments within different faculties. These findings suggest that preventive programmes may need to be tailored to the specific characteristics of different student populations rather than adopting a uniform institutional approach.

The findings of this study have important implications for public health and university mental health services. The identification of specific sociodemographic risk factors provides an opportunity for targeted preventive interventions directed at high-risk groups, particularly male students, older undergraduates, students from polygamous families, and those with a parental history of substance use. University authorities should strengthen campus-based mental health services by incorporating routine screening for substance use disorders into student health programmes and implementing evidence-based prevention strategies, including peer education, counselling services, and family-oriented interventions where feasible.

A major strength of this study is the use of a structured diagnostic interview to establish substance use disorder rather than relying solely on self-report screening questionnaires, thereby improving the clinical validity of the findings. In addition, the multistage sampling technique enhanced the representativeness of the study population.

However, several limitations should be acknowledged. The cross-sectional design limits causal inference between sociodemographic factors and substance use disorders. Information obtained from participants was based partly on self-report and may therefore be subject to recall and social desirability bias. Furthermore, the study was conducted in a single university, which may limit the generalisability of the findings to undergraduate populations in other tertiary institutions within Nigeria.

In conclusion, substance use disorders are common among undergraduates of the Federal University of Agriculture, Abeokuta, with important sociodemographic disparities in their distribution. Increasing age, male sex, polygamous family structure, and parental history of substance use independently increased the likelihood of substance use disorders. These findings underscore the need for targeted prevention programmes and early identification of at-risk students within Nigerian universities.

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Original Article

Hematological Inflammatory Markers Are Associated with Parkinson's Disease Severity

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Abstract

Background: Parkinson's disease (PD) is a progressive neurodegenerative disorder with increasing prevalence in Sub-Saharan Africa. Hematological biomarkers, reflecting systemic inflammation, may predict disease severity.

Objective: This study evaluates their prognostic potential in PD patients from Southwest Nigeria.

Methods: A hospital-based, cross-sectional study was conducted, involving 70 PD patients stratified into severe (stages 3–5, n=10) and non-severe (stages 1–2, n=60) groups using the Hoehn and Yahr (H&Y) scale, and 70 age- and sex-matched controls. Hematological parameters (white blood cell, red cell, platelet indices, and ratios) were analyzed using t-tests, binary logistic regression, and Receiver Operating Characteristic (ROC) curve analysis. A p-value < 0.05 was considered significant.

Results: Severe PD was observed in 14% of patients, with 76% being male. Compared to controls, PD patients had lower red blood cell counts (P=0.045), haemoglobin (P=0.014), and higher red cell distribution width-standard deviation (RDW-SD, P<0.001). Independent predictors of severity included age (P=0.010), gender (P=0.003), red blood cell count (P=0.002), haemoglobin (P=0.003), haematocrit (P=0.007), RDW-SD (P=0.001), and plateletcrit (PCT, P<0.001). ROC analysis showed excellent predictive accuracy for RDW-SD (AUC=0.959) and PCT (AUC=1.000).

Conclusion: RDW-SD and PCT are robust biomarkers for PD severity, highlighting the role of systemic inflammation. These findings support their integration into PD management and warrant further longitudinal studies.

Keywords: Parkinson's disease, hematological biomarkers, RDW-SD, PCT, inflammation, severity

Introduction

There has been increasing burden of PD in the last two decades.(Alabi et al., 2019; A. A. Ojagbemi et al., 2013; Ojo et al., 2021; Otubogun et al., 2020). Observed prevalence of PD ranges from 7/100,000 in Ethiopia to 67/100,000 in Nigeria. The most recent community based study reported a mean age at onset of 69.4 years.(A. Ojagbemi, 2013; Ojo et al., 2020; Zesiewicz, 2019) PD are the most common movement disorders and are generally perceived to have lower prevalence and incidence in Sub-Saharan Africa (SSA) because of the relative youthfulness of the SSA population, under-recognition, paucity of published reports, and cultural perception of neurologic disorders generally as being part of normal ageing. (Ojo et al., 2020, 2021)

Parkinson's disease (PD) is marked by α-synuclein accumulation and chronic neuroinflammation, contributing to neuronal loss. Inflammatory processes involve microglial activation, cytokine release, and gut microbiota changes.(Eo et al., 2024) Several anti-inflammatory agents have shown neuroprotective effects in PD models. Key inflammatory biomarkers such as NLRP3, NF-κB, α-synuclein, chemokines, and VEGFA play roles in immune regulation and disease progression. Blood-based ratios like NLR, NHR, RDW-SD, PLT, and MHR, along with cytokines (e.g., IL-6, TNF-α, hsCRP), offer promise for early detection, monitoring, and targeted therapy in PD.(Li et al., 2024a)

Reliable biomarkers are needed to improve early diagnosis, monitor disease progression, and evaluate treatment response in Parkinson's disease (PD), as current methods are limited by cost and accessibility. Indices derived from full blood count (FBC) parameters such as the red cell distribution width to platelet (RDW-/PLT), neutrophil to lymphocyte ratio (NLR), neutrophil to high-density lipoprotein ratio (NHR), and monocyte to high-density lipoprotein ratio (MHR) are emerging as valuable, cost-effective tools for assessing disease severity and progression(Kwak et al., 2024; Muñoz-Delgado, Macías-García, et al., 2023). These biomarkers indicate systemic inflammation, which plays a crucial role in various chronic conditions, including neurodegenerative diseases like PD(Muñoz-Delgado, Labrador-Espinosa, et al., 2023). However, there is a significant lack of knowledge about the usefulness of these hematological markers in prognosticating and differentiating the severity of PD (Li et al., 2024b). This study attempts to assess the relationship between hematological biomarkers, their prognostic potential, and the severity of PD. Incorporating these indices into the care of PD patients could notably improve monitoring, ultimately aiding the development of new, personalized therapeutic strategies.

Materials and Methods

2.1 Study Design

This hospital-based, descriptive, cross-sectional study was conducted at a tertiary healthcare institution serving as the principal referral centre for the state and neighbouring regions. Ethical approval was obtained from the Health Research Ethics Committee (HREC) (Ref No: OOUTH/HREC/275/2019AP). Informed consent was secured from all participants, with confidentiality maintained and the option to withdraw without affecting medical care.

2,2 Selection and Description of Participants

The study included 70 PD patients and 70 healthy controls, matched for age and sex. PD diagnosis was confirmed using the National Institutes of Health (NIH) Parkinson Disease Clinical Data Elements, requiring bradykinesia plus at least one of rest tremor, rigidity, or postural instability (11).

Supportive criteria included asymmetric onset, positive response to dopaminergic therapy, and exclusion of alternative parkinsonism causes. Exclusion criteria included atypical parkinsonism or comorbidities affecting hematological parameters. PD patients were stratified into severe (stages 3–5, n=10) and non-severe (stages 1–2, n=60) groups using the Hoehn and Yahr (H&Y) scale (Li et al., 2024b).

2.3 Data Collection

An interviewer-based questionnaire collected socio-demographic details (age, gender, education, occupation), clinical history (age of onset, symptom duration, side of onset), symptom types (tremor, rigidity, bradykinesia, postural instability), medication usage, and medical records reviewed by a neurologist. Hematological parameters were obtained from full blood counts.

2.4 Statistical Analysis

Data were entered into Microsoft Excel for cleaning and analysed using IBM SPSS version 23. Normality was confirmed using the Shapiro-Wilk test, enabling parametric tests. Descriptive statistics were calculated for demographic and clinical variables. Group comparisons used Chi-square tests for categorical data and independent t-tests for continuous variables. Binary logistic regression identified predictors of PD severity, and ROC curve analysis evaluated diagnostic performance of significant hematological parameters. A p-value < 0.05 was considered significant.

Results

3.1 Comparison of Demographic and Hematological Parameters Between Cases and Controls

Among 70 PD patients, 14% (n=10) had severe PD, with 53 (75.7%) being male and 17 (24.3%) females based on biological sex. The mean age at onset was 69.4 years. Table 1 compares demographic and hematological parameters between PD patients and controls, showing significant differences in red blood cell count (RBC, P=0.045), haemoglobin (HGB, P=0.014), mean corpuscular volume (MCV, P=0.001), mean corpuscular haemoglobin (MCH, P<0.001), RDW-SD (P<0.001), platelet distribution width (PDW, P=0.002), platelet-to-lymphocyte ratio (PLR, P<0.001), and RDW-SD/PLT (P=0.003).

3.2 Demographic and Clinical Characteristics

Age Group: Patients aged >60 years had a higher prevalence of severe PD (100% vs. 38.3%, χ^2=13.081, P<0.001).

Gender: Females showed a higher proportion of severe PD (60% vs. 18.3%, χ^2=8.093, P=0.004).

3.3 Hematological Parameters

White Blood Cell Indices: Severe PD patients had higher total white blood cell counts (7.77±5.27 vs. 5.37±2.11 $\times 10^9$/L, P=0.013). No significant differences were found for neutrophils, lymphocytes, monocytes, eosinophils, or basophils.

Red Cell Indices: Severe PD patients had lower RBC (3.76±0.29 vs. 4.85±0.63 $\times 10^{12}$/L, P<0.001), HGB (10.71±1.80 vs. 12.38±1.34 g/dL, P=0.001), haematocrit (HCT, 32.64±4.65 vs. 37.32±4.40%, P=0.003), and higher RDW-SD (55.65±9.63 vs. 39.73±4.98 fL, P<0.001). MCV (P=0.001) and MCH (P=0.012) differed significantly.

Platelet Indices: Plateletcrit (PCT) was higher in severe PD (0.32±0.00 vs. 0.22±0.04%, P<0.001). No differences were found for platelet count (PLT), mean platelet volume (MPV), or PDW.

Ratios: NLR (1.92±1.52 vs. 1.12±0.97, *P*=0.031), RDW-CV/PLT (0.16±0.14 vs. 0.07±0.019, *P*<0.001), and RDW-SD/PLT (0.50±0.39 vs. 0.20±0.062, *P*<0.001) were higher in severe PD.

3.4 Regression Analysis

Independent predictors of severe PD included age (p=0.010), gender (p=0.003), RBC (p=0.002), HGB (*P*=0.003), HCT (*P*=0.007), RDW-SD (*P*=0.001), and PCT (*P*<0.001).

3.5 ROC Analysis

ROC results, which showed excellent predictive accuracy for RDW-SD (AUC=0.959), PCT (AUC=1.000), Age group (AUC=0.857), Gender (AUC=0.888).

Discussion

This study investigated the association between full blood count-derived hematological biomarkers and the severity of Parkinson's Disease (PD), with the goal of identifying accessible and cost-effective biomarkers. The results reveal that red cell distribution width-standard deviation (RDW-SD) and plateletcrit (PCT) are promising biomarkers of PD severity.

Participants with severe PD had lower red blood cell (RBC) counts, hemoglobin (HGB), and hematocrit (HCT), and significantly higher RDW-SD and PCT. RDW-SD and PCT had exceptionally high predictive power, with receiver operating characteristic (ROC) area under the curve (AUC) values of 0.959 and 1.000 respectively, suggesting their strong potential as biomarkers of disease severity.

Logistic regression confirmed that age group, gender, RBC, HGB, HCT, RDW-SD, and PCT were all independent predictors of disease severity ($p < 0.05$). Older age is associated with a higher likelihood of severe PD, and females, despite being fewer in number, had disproportionately higher severity rates(Gialluisi et al., 2024). The therapeutic implication of this study with regard to hormone will need to be fully explored. These findings suggest a systemic inflammatory profile and hematological dysregulation accompanying PD progression, which aligns with emerging evidence linking chronic inflammation to neurodegeneration(Ma et al., 2024).

These results are in agreement with existing literature highlighting the role of inflammation and oxidative stress in PD (Hartai et al., 2005; Kenangil et al., 2020). Prior studies have shown elevated RDW-SD values in patients with chronic inflammatory and neurodegenerative disorders, which supports our findings of higher RDW-SD in severe PD.

Similarly, the identification of PCT as a marker of total platelet volume as a significant predictor may reflect platelet activation and vascular changes, which have been implicated in PD related neuroinflammation. While the AUC of 1.000 for PCT may appear unusually high, it suggests near-perfect discriminatory ability.

Interestingly, while the neutrophil-to-lymphocyte ratio (NLR) trended higher in severe PD, it was not statistically significant in the regression model. This was unexpected given NLR's known relationship with systemic inflammation. The small sample size or confounding variables such as medication use and comorbidities may have influenced this result.

Conclusion

RDW-SD and PCT are promising, inflammation-related biomarkers for PD severity. Their integration into clinical practice could improve patient management in resource-limited settings. Further research is warranted to establish their longitudinal utility and therapeutic implications.

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Original Article

Micronuclei Frequency and White Blood Cell Functional Indices among Patients with Leukaemia and Multiple Myeloma Attending University of Ilorin Teaching Hospital, Ilorin

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Abstract

Background: Leukaemia represents a heterogeneous group of blood cancers frequently linked to immune dysfunction and genomic instability.

Objective: This study evaluated micronuclei frequency and white blood cell functional indices among leukaemic patients attending the University of Ilorin Teaching Hospital, Ilorin, Kwara State, Nigeria**.**

Materials and Methods: An analytical case–control design was employed involving consented 43 leukaemic subjects and age-matched healthy controls recruited. Venous blood samples were analyzed for White blood cell indices using an automated haematology analyzer, and Micronuclei Frequency using the cytokinesis-block method (CBMN).

Results: White blood cell functional indices (Relative lymphocyte count) were significantly elevated in patients across all types of cancer, AML (44.50 ± 1.29) compared to control (33.83 ± 6.34), ALL (35.75 ± 1.26) Control (33.83 ± 6.34), CLL (68.38 ± 1.77) Control (33.83 ± 6.34) and Multiple Myeloma (38.00 ± 14.77) Control (33.83 ± 6.34). Micronuclei frequency parameters and proliferation index were significantly higher in ALC and ANC relative to control in all participants ($p < 0.01$).

Conclusion: Leukaemia is associated with increased genomic instability and altered immune response. Micronuclei frequency and white blood cell functional indices may serve as useful adjuncts to leukaemic cells in evaluating disease progression and prognostic potential.

Keywords: 'Leukaemia', 'Micronuclei Frequency', and 'White blood cell indices'

Introduction

Micronuclei (MN) are small extranuclear bodies containing whole chromosomes or chromosome fragments that fail to segregate into daughter nuclei during mitotic cell division (Sommer *et al.,* 2020; Zhang *et al.,* 2020). They represent a direct cytological readout of chromosomal instability, DNA damage, and whole-chromosome loss (Fenech, 2020; Di and Bakhoum, 2024). Increased micronuclei frequency is widely recognized as a validated biomarker of genetic damage and has been linked to cancer susceptibility, disease progression, treatment response, and overall prognosis (Almeida *et al.,* 2022; Rowland *et al.,* 2024).

Leukaemia and Multiple myeloma encompass a broad spectrum of malignancies originating from blood-forming tissues in the bone marrow, including leukemias and multiple myeloma (Santos *et al.,* 2023; Mulatie *et al.,* 2025). The etiology of these malignancies is multifactorial, involving genetic mutations (such as chromosomal translocations, point mutations, and epigenetic alterations), environmental exposures (ionizing radiation, benzene, cytotoxic agents), and viral infections (Coyle *et al.,* 2017; Kipps *et al.,* 2017; Pedersen *et al.,* 2018). In Nigeria, Leukaemia and Multiple myelomaaccount for approximately 2% to 10% of all diagnosed cancers (Abudu *et al.,* 2018). Hospital-based reviews at the University of Ilorin Teaching Hospital (UITH) indicate that Chronic Lymphocytic Leukemia accounts for (20-25%), Acute Leukemias (15-20%) and Multiple Myeloma (10-12%) (Akinbami *et al.,* 2014; Anifowoshe *et al.,* 2018; Babatunde *et al.,* 2020).

Although standard peripheral blood investigations such as Complete Blood Count (CBC) and differential counts reveal quantitative abnormalities (leukocytosis, lymphocytosis, neutropenia), they do not reflect underlying genomic instability or DNA damage dynamics (Kantarjian *et al.,* 2015; Terwilliger and Abdul-Hay, 2017). Evaluating micronuclei frequency alongside white cell functional indices (neutrophils, lymphocytes, monocytes) provides critical insights into cytogenetic integrity and immune status (Tan *et al.,* 2018; Fenech and Bonassi, 2019). Therefore, this study assessed white cell functional indices and micronuclei frequency in patients with Leukaemia and Multiple myeloma at the University of Ilorin Teaching Hospital, Kwara State, Nigeria.

Materials And Methods

Study Site: The study was conducted at the University of Ilorin Teaching Hospital (UITH), Oke-Oyi, Kwara State. Blood samples were equally collected at the Haematology Clinic of UITH. Laboratory culture, cytogenetic preparation, micronuclei scoring, and cellular proliferation assays were performed in the Department of Medical Laboratory Science Research Laboratories, Al-Hikmah University, Ilorin, Kwara State, Nigeria (Akinbami *et al.,* 2014).

Study Design

This study was a comparative case-control study that was carried on leukaemia and Multiple myeloma patients who met the inclusion criteria at the University of Ilorin Teaching Hospital, Ilorin.

Study Population and Selection Criteria

The study population comprised of confirmed patients presenting with various Leukaemia and Multiple myeloma (AML, ALL, CLL, and MM) attending the UITH Haematology Clinic. The controls were Age- and sex-matched apparently healthy individuals recruited from voluntary blood donors and hospital staff who gave consent were recruited.

Sample Size Determination

Sample size was calculated using the Cochrane formula $n = \frac{z^2 p (1-p)}{d^2}$

where: n = sample size, z = the standard deviation at a 95% confidence interval (1.96), P = proportion of the population with the desired factor (adopted from Haemtolyphoid neoplasm) in Ilorin = 2.9% = 0.029 as reported by (Abudu *et al.,* 2018), q = 1-p (0.971), while d is the maximum allowable error (5%). Thus, a total of 43 estimated participants were recruited for this study, 22 of which were leukaemia and Multiple myeloma, while 21 were lymphoma Patients. A corresponding group of healthy controls was recruited for comparative evaluation.

Data Collection tools

Data were collected using well-structured, self-administered questionnaires which were specifically designed to obtain information that was used to either include or exclude individuals from this study. A questionnaire was given to the participants through an interviewer-assisted administration method, which served as the major source of primary data for this study. The participants were informed of the study's goals and objectives. The consent forms were submitted with the questionnaire, and informed consent from the participants was sought. The gathered data were kept on a safe, password-protected I-drive for future reference.

Sample collection

From each participant, 7 mL of peripheral venous blood was collected aseptically via venipuncture. Three milliliters (3 mL) were dispensed into Ethylenediaminetetraacetic acid (EDTA) anticoagulant tubes for automated full blood count and leukocyte differential analysis. Four milliliters (4 mL) were collected in lithium heparinized tubes for leukocyte isolation, lymphocyte culture, and cytokinesis-block micronucleus assay.

Laboratory Procedures and Protocols

Full Blood Count and Differential Indices

Full blood counts and differential white cell parameters were performed within 2 hours of sample collection using a 5-part automated hematology analyzer (Mindray BC-5300) operating on electrical impedance, flow cytometry, and laser light scatter principles. Relative neutrophil count (RNC %), relative lymphocyte count (RLC %), relative monocyte count (RMC %), absolute neutrophil count (ANC x10^9/L), absolute lymphocyte count (ALC x10^9/L), and absolute monocyte count (AMC x10^9/L) were recorded. Peripheral blood smears were stained with Leishman stain and evaluated under oil immersion (x100 objective) for morphological verification.

Cytokinesis-Block Micronucleus (CBMN)

The CBMN assay was conducted according to the standard protocol defined by (Fenech *et al.,* 2020). with slight modifications:
Leukocyte Isolation: Equal volumes (1.5 mL) of fresh whole blood and Hank's Balanced Salt Solution (HBSS) were layered over 1.0 mL of Ficoll-Paque density gradient media and centrifuged at 2000 rpm for 30 minutes at room temperature. The leukocyte layer was aspirated, washed twice in HBSS, and resuspended in RPMI 1640 culture medium.
Culture and Cytokinesis Block: Isolated lymphocytes were cultured in RPMI 1640 supplemented with 10% Fetal Bovine Serum (FBS), 2 mM L-glutamine, 1 mM sodium pyruvate and stimulated with Phytohemagglutinin (PHA, 2.25 mg/mL). Cultures were incubated at 37°C in a humidified 5% CO2 incubator for 44

hours. At 44 hours, Cytochalasin-B (6 ug/mL) was added to block cytokinesis and arrest dividing cells at the binucleated stage, followed by an additional incubation of 28 hours (total culture duration = 72 hours).

Harvesting and Slide Preparation: Cultured cells were harvested by gentle centrifugation, fixed with cold methanol, and smeared onto clean, grease-free glass slides. Slides were air-dried, stained with Giemsa stain, and cover slipped with DPX. Scoring was conducted under high-power microscopy (x1000 magnification). Cytome endpoints mononucleated cells, binucleated (BN) cells, acentric fragments/micronuclei per 1000 cells, nucleoplasmic bridges, and nuclear buds were scored according to strict international criteria (Fenech *et al.,* 2020).

Spectrophotometric Cellular Proliferation Assay

Cellular proliferation was measured using a modified spectrophotometric approach (Akinbami *et al.,* 2014). Following 28 hours of cytochalasin-B incubation, cell culture turbidity and optical density (OD) were measured at 550 nm wavelength using a calibrated spectrophotometer to quantify cellular proliferation capacity.

Statistical Analysis

Data were entered, cleaned, and analyzed using IBM SPSS Statistics for Windows, Version 27.0 (IBM Corp., Armonk, NY). Descriptive statistics were expressed as frequencies and percentages for categorical variables, and as Mean ± Standard Deviation (SD) for continuous parameters. Independent sample t-tests and Analysis of Variance (ANOVA) with post-hoc tests were used to evaluate statistical differences between patient subgroups and healthy controls. Pearson correlation analysis was conducted to establish bivariate relationships between white cell functional indices and micronuclei cytome endpoints. Statistical significance was set at $p < 0.05$.

Ethical Considerations

Ethical approval for the study was granted by the Ethical Review Committee of the University of Ilorin Teaching Hospital (Approval Protocol Number: ERC PAN/2025/07/0618). Written informed consent was obtained from all adult participants or guardians of pediatric participants prior to recruitment. All procedures strictly adhered to the ethical standards outlined in the Declaration of Helsinki. Confidentiality and data security were strictly maintained.

Results

Table 1 shows the socio-demographic characteristics of the participants. A total of 43 participants with Leukaemia and Multiple myelomawere evaluated. As detailed in Table 1, the majority of the participants belonged to the 50–69 years age bracket (n = 24, 55.8%), followed by those under 30 years (n = 9, 20.9%), 30–49 years (n = 5, 11.6%), and >=70 years (n = 5, 11.6%). Females represented 53.5% (n = 23) of the cohort, while males constituted 46.5% (n = 20). Most participants were married (79.1%), engaged in business/trading (67.4%). All participants were undergoing active systemic chemotherapy regimens. Hypertension was present as a co-morbidity in 9.3% (n = 4) of patients. More than half of the study population (n = 24, 55.8%) had a history of prior blood transfusions.

Fig 1 shows the classification of haematological malignancy in the study population. Chronic Lymphocytic Leukemia (CLL) was the most prevalent malignancy (n = 8, 18.6%), followed by Multiple Myeloma (MM, n = 6, 14.0%) Acute Myeloid Leukemia (AML, n = 4, 9.3%). Acute Lymphoblastic Leukemia (ALL, n = 4, 9.3%).

Table 1: Socio-demographic and Clinical Characteristics of the study Participants

Parameter	Frequency	Percentage
Age		
< 30 years	9	20.9%
30–49 years	5	11.6%
50–69 years	24	55.8%
>= 70 years	5	11.6%
Sex		
Female	23	53.5%
Male	20	46.5%
Marital Status		
Married	34	79.1%
Single	9	20.9%
Occupation		
Civil Servant	2	4.7%
Business	29	67.4%
Unemployed	12	27.9%
Treatment Regimen		
Chemotherapy	22	100.0%
Co-morbidity Status		
None	39	90.7%
Hypertension	4	9.3%
Blood Transfusion		
Previously Transfused	24	55.8%
Never Transfused	19	44.2%

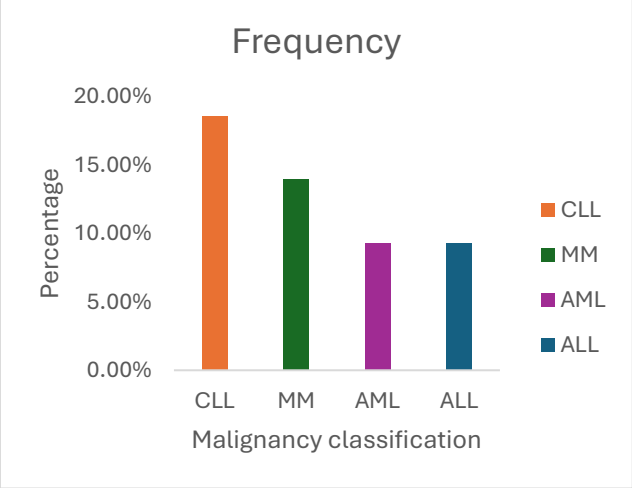

Fig 1: Prevalence of haematological malignancies in the study participants.

Table 2 shows the association between white blood cell functional indices and micronuclei frequency (mononucleated cells, binucleated cells, acentric fragments, and cellular proliferation) in acute myeloid leukaemia patients and healthy controls. Mononucleated cells (345.00±12.91 vs 186.67±18.62, p = 0.000) and acentric fragments (97.50±17.08 vs 28.33±9.83, p = 0.000) were significantly elevated compared to controls, indicating severe genomic fragmentation.

Table 3 shows the Association between white blood cell functional indices and Micronuclei frequency in Acute Lymphoblastic Leukemia (ALL) and healthy controls. Mononucleated cells exhibited a massive and highly significant elevation (705.00±12.91 vs 186.67±18.62, p = 0.000), reflecting intense cell division and arrest in the mononucleated stage.

Table 4 shows the association between white blood cell functional indices and Micronuclei frequency in Chronic Lymphocytic Leukemia (CLL) and healthy controls. Patients demonstrated statistically significant elevations in Absolute Lymphocyte Count (5.14±1.06 vs 1.83±0.74 x10^9/L, p = 0.001), Absolute Monocyte Count (0.96±0.19 vs 0.39±0.12 x10^9/L, p = 0.000), Mononucleated cells (p = 0.000), Binucleated cells with micronuclei (58.75±18.17 vs 0.00±0.00, p = 0.000), and Acentric fragments (76.25±12.82 vs 28.33±9.83, p = 0.000).

Table 5 shows the association between white blood cell functional indices and Micronuclei frequency in Multiple Myeloma (MM). Micronuclei frequency is significantly higher in MM at a p-value ≤ 0.05

Table 2: Association between white blood cell functional indices and Micronuclei frequency in Acute Myeloid Leukemia (AML)

White Cell / Cytome Index	Control (Mean±SD)	AML (Mean±SD)	Mean Diff.	p-value
Relative Neutrophil Count (RNC %)	57.50 ± 7.12	44.00 ± 1.41	13.50	1.000
Relative Lymphocyte Count (RLC %)	33.83 ± 6.34	44.50 ± 1.29	-10.67	1.000
Relative Monocyte Count (RMC %)	6.67 ± 1.51	8.75 ± 0.96	-2.08	1.000
Absolute Neutrophil Count (ANC x10^9/L)	3.06 ± 0.73	2.48 ± 0.03	0.57	1.000
Absolute Lymphocyte Count (ALC x10^9/L)	1.83 ± 0.74	2.29 ± 0.26	-0.46	1.000
Absolute Monocyte Count (AMC x10^9/L)	0.39 ± 0.12	0.49 ± 0.01	-1.10	1.000
Mononucleated Cells (/1000 cells)	186.67 ± 18.62	345.00 ± 12.91	-158.33	0.000*
Binucleated Cells (/1000 cells)	0.00 ± 0.00	20.00 ± 8.17	-20.00	0.351
Acentric Fragments (/1000 cells)	28.33 ± 9.83	97.50 ± 17.08	-69.17	0.000*
Cellular Proliferation (OD 550nm)	182.50 ± 21.90	190.25 ± 2.22	-7.75	1.000

Table 3: Association between white blood cell functional indices and Micronuclei frequency in Acute Lymphoblastic Leukemia (ALL)

White Cell / Cytome Index	Control (Mean±SD)	ALL (Mean±SD)	Mean Diff.	p-value
Relative Neutrophil Count (RNC %)	57.50 ± 7.12	54.50 ± 1.73	3.00	1.000
Relative Lymphocyte Count (RLC %)	33.83 ± 6.34	35.75 ± 1.26	-1.92	1.000
Relative Monocyte Count (RMC %)	6.67 ± 1.51	7.00 ± 0.82	-0.33	1.000
Absolute Neutrophil Count (ANC x10^9/L)	3.06 ± 0.73	5.62 ± 0.02	-2.57	1.000
Absolute Lymphocyte Count (ALC x10^9/L)	1.83 ± 0.74	3.80 ± 0.03	-1.97	0.439
Absolute Monocyte Count (AMC x10^9/L)	0.39 ± 0.12	0.74 ± 0.03	-0.35	1.000
Mononucleated Cells (/1000 cells)	186.67 ± 18.62	705.00±12.91	-518.33	0.000*
Binucleated Cells (/1000 cells)	0.00 ± 0.00	20.00 ± 8.17	-20.00	0.351
Acentric Fragments (/1000 cells)	28.33 ± 9.83	20.00 ± 8.17	8.33	1.000
Cellular Proliferation (OD 550nm)	182.50 ± 21.90	183.00 ± 4.97	-0.50	1.000

Table 4: Association between white blood cell functional indices and Micronuclei frequency in Chronic Lymphocytic Leukemia (CLL)

White Cell / Cytome Index	Control (Mean±SD)	CLL (Mean±SD)	Mean Diff.	p-value
Relative Neutrophil Count (RNC %)	57.50 ± 7.12	44.00 ± 2.14	13.50	0.297
Relative Lymphocyte Count (RLC %)	33.83 ± 6.34	46.13 ± 2.17	-12.30	0.198
Relative Monocyte Count (RMC %)	6.67 ± 1.51	8.63 ± 0.74	-1.96	1.000
Absolute Neutrophil Count (ANC x10^9/L)	3.06 ± 0.73	4.88 ± 1.02	-1.82	0.315
Absolute Lymphocyte Count (ALC x10^9/L)	1.83 ± 0.74	5.14 ± 1.06	-3.31	0.001*
Absolute Monocyte Count (AMC x10^9/L)	0.39 ± 0.12	0.96 ± 0.19	-0.57	0.000*
Mononucleated Cells (/1000 cells)	186.67 ± 18.62	468.75 ± 90.47	-282.08	0.000*
Binucleated Cells (/1000 cells)	0.00 ± 0.00	58.75 ± 18.17	-58.75	0.000*
Acentric Fragments (/1000 cells)	28.33 ± 9.83	76.25 ± 12.82	-47.92	0.000*
Cellular Proliferation (OD 550nm)	182.50 ± 21.90	210.00 ± 11.34	-27.50	0.720

Table 5: Association between white blood cell functional indices and Micronuclei frequency in Multiple Myeloma (MM)

White Cell / Cytome Index	Control (Mean±SD)	MM (Mean±SD)	Mean Diff.	p-value
Relative Neutrophil Count (RNC %)	57.50 ± 7.12	52.50 ± 2.14	5.00	1.000
Relative Lymphocyte Count (RLC %)	33.83 ± 6.34	39.00 ± 1.67	-5.17	1.000
Relative Monocyte Count (RMC %)	6.67 ± 1.51	7.33 ± 0.61	-0.66	1.000
Absolute Neutrophil Count (ANC x10^9/L)	3.06 ± 0.73	3.42 ± 0.14	-0.36	1.000
Absolute Lymphocyte Count (ALC x10^9/L)	1.83 ± 0.74	2.55 ± 0.12	-0.72	1.000
Absolute Monocyte Count (AMC x10^9/L)	0.39 ± 0.12	0.48 ± 0.03	-0.09	1.000
Mononucleated Cells (/1000 cells)	186.67 ± 18.62	363.33 ± 34.42	-176.66	0.002*
Binucleated Cells (/1000 cells)	0.00 ± 0.00	35.00 ± 8.06	-35.00	0.007*
Acentric Fragments (/1000 cells)	28.33 ± 9.83	76.67 ± 11.45	-48.34	0.000*
Cellular Proliferation (OD 550nm)	182.50 ± 21.90	183.33 ± 2.11	-0.83	1.000

Discussion

This study evaluated white blood cell functional indices and micronuclei frequency across diverse Leukaemia and Multiple myeloma at the University of Ilorin Teaching Hospital, Kwara State, Nigeria. The spectrum of malignancies observed in this study was dominated by Chronic Lymphocytic Leukemia (18.6%) and Multiple Myeloma (14.0%), which aligns closely with previous epidemiological reviews in North-Central Nigeria and broader Sub-Saharan Africa

(Abudu *et al.,* 2018; Anifowose *et al.,* 2018; Tan *et al.,* 2018). The peak age distribution in the 50–69 years group (55.8%) reflects the age-dependent cumulative risk of somatic mutations, DNA repair decay, and environmental genotoxic exposures in adult hematological oncogenesis (Mulatie *et al.,* 2025).

More than half of the patient cohort (55.8%) had a history of prior blood transfusions. Chronic blood transfusion introduces foreign cellular antigens, minor histocompatibility mismatches, and residual donor leukocytes into the recipient, driving immune activation, alloimmunization, and oxidative stress (Kareem *et al.,* 2024). Repetitive transfusion therapy can elevate systemic inflammatory signaling, which further exacerbates genomic instability in multi-transfused oncological patients (Nava *et al.,* 2019).

A central finding of this investigation is the significant elevation of micronuclei cytome endpoints, specifically mononucleated cells, binucleated (BN) cells containing micronuclei, and acentric chromosome fragments across all groups relative to healthy controls. The Cytokinesis-Block Micronucleus (CBMN) assay provides a comprehensive cytome evaluation of chromosomal breakage, chromosome loss, and mitotic failure (Nava *et al.,* 2019).

Micronuclei originate from acentric fragments or whole lagging chromosomes that fail to integrate into daughter nuclei during anaphase due to defects in double-strand break repair (such as non-homologous end joining and homologous recombination) or mitotic spindle checkpoints (Sommer *et al.,* 2020: Zhang *et al.,* 2020).

In this study, Chronic Lymphocytic Leukemia (CLL) demonstrated the highest frequency of binucleated cells with micronuclei (58.75±18.17 per 1000 cells) and acentric fragments (76.25±12.82 per 1000 cells). These findings are consistent with international studies reporting profound genomic instability, deletion of 13q14, TP53 aberrations, and complex karyotypic alterations in mature B-cell malignancies.

Furthermore, Multiple Myeloma (MM) exhibited significantly elevated binucleated and micronucleated cells and acentric fragments compared to controls ($p < 0.01$). In Multiple Myeloma, ongoing DNA damage driven by immunoglobulin gene hypermutation, genomic translocations involving the IGH locus, and bone marrow microenvironmental stress contribute to elevated micronuclei formation. In Acute Myeloid Leukemia (AML) and Acute Lymphoblastic Leukemia (ALL), mononucleated cell counts were markedly elevated (345.00 ± 12.91 and 705.00 ± 12.91, respectively), reflecting rapid blast proliferation and mitotic arrest. All participants in this cohort were undergoing active chemotherapy regimens. Cytotoxic chemotherapeutic agents, such as alkylating agents, antimetabolites, and anthracyclines, kill malignant cells primarily by inducing DNA double-strand breaks, cross-linking, or spindle poisoning. Consequently, while chemotherapy targets neoplastic clones, it simultaneously induces systemic genotoxicity in non-neoplastic lymphocytes, compounding the baseline micronuclei burden.

Conclusion

This study concludes that leukaemic patients exhibited profound genomic instability, characterized by significantly elevated micronuclei frequency, acentric chromosome fragments, and altered white cell functional indices. The Cytokinesis-Block Micronucleus assay serves as a highly reliable, accessible, and cost-effective biomarker for genotoxicity assessment and therapeutic monitoring in resource-limited clinical settings.

Recommendations

1. Institutional Integration of CBMN Assay: Diagnostic haematology laboratories in tertiary hospitals should adopt the CBMN assay as a routine, low-cost adjunctive test for monitoring baseline genomic instability and chemotherapy-induced genotoxicity in cancer patients.
2. Multi-Center Longitudinal Studies: Multi-center longitudinal studies across regional tertiary centers should be commissioned to evaluate dynamic micronuclei fluctuations before, during, and after specific chemotherapy and immunotherapy regimens.
3. Enhanced Blood Transfusion Compatibility: For multi-transfused leukaemic patients, advanced extended antigen matching and leukoreduced blood products should be prioritized to minimize immune alloimmunization and oxidative stress.

Contributions To Knowledge

1. Validation of CBMN as a Cost-Effective Genotoxic Biomarker: This study establishes the CBMN assay as a practical, highly informative biomarker for evaluating chromosomal damage in African leukaemic patients, providing a viable alternative to high-cost molecular cytogenetics in resource-constrained settings.
2. Baseline Epidemiological Data for North-Central Nigeria: The study provides comprehensive baseline clinical, sociodemographic, and cytogenetic data on leukaemic patients in Kwara State, supporting future regional oncology research and health policy planning.

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Review Article

Health-System Determinants of Gender Disparities in HIV Infection Among Adolescent Girls and Young Women in Sokoto, North-Western Nigeria: Policy and Service Delivery Implications

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Abstract

Background: Adolescent girls and young women remain disproportionately affected by HIV in sub-Saharan Africa. In north-western Nigeria, lower overall HIV prevalence does not eliminate concentrated vulnerability created by gender inequality, early marriage, limited education, economic dependence, low sexual autonomy, HIV-related stigma, and barriers to confidential health services. This review examines how biological and socio-cultural risks are intensified or mitigated by health-system design in Sokoto State.

Methods: An expanded narrative review was undertaken using the submitted conference abstract as the conceptual foundation. Targeted searches of PubMed and authoritative reports from UNAIDS, the World Health Organization, the National Agency for the Control of AIDS, UNFPA, and the World Bank were conducted for evidence available up to July 2026. The literature was organised using social-ecological and health-systems perspectives, with emphasis on service accessibility, acceptability, referral, confidentiality, workforce capacity, commodities, data, and the meaningful engagement of adolescent girls and young women.

Results: Gender disparities in HIV vulnerability arise from interacting biological, interpersonal, economic, socio-cultural, and health-system determinants. Biological susceptibility and sexually transmitted infections operate within relationships shaped by age-disparate partnerships, early marriage, limited condom negotiation, and economic dependence. Health-system barriers include fear of stigma and disclosure, judgemental provider attitudes, insufficient privacy, restrictive service hours, weak referral pathways, fragmented HIV and sexual and reproductive health services, inadequate commodities, and limited youth participation. Nigeria has developed relevant strategies, including the National HIV and AIDS Strategic Plan and the Generation Negative strategy. Programmes such as the Adolescent Girls Initiative for Learning and Empowerment can address educational and economic determinants, while Operation Triple Zero offers a youth-engagement model for adolescents living with HIV. However, coverage, institutionalisation, local coordination, and evaluation remain uneven. Evidence supports combination approaches that integrate youth-friendly services, HIV testing and self-testing, pre-exposure prophylaxis choice, condoms, sexually transmitted infection care, gender-based violence response, peer navigation, education, and social protection.

Conclusion: Reducing HIV disparities among adolescent girls and young women in Sokoto requires more than risk communication. It requires a gender-responsive health system that protects confidentiality, brings services closer to young people, connects clinics with schools and communities, strengthens referrals and commodity security, and gives adolescent girls and young women meaningful roles in programme design and accountability. Education and economic empowerment should be treated as core HIV prevention interventions rather than peripheral social activities.

Keywords: adolescent girls and young women; HIV prevention; gender disparity; health systems; youth-friendly services; Sokoto; Nigeria; PrEP; empowerment

1. Introduction

HIV remains a major public health and social justice challenge despite substantial progress in testing, treatment, and prevention. The burden is not evenly distributed. Adolescents and young adults face barriers associated with developmental stage, dependence on families, limited income, stigma, legal and policy constraints, and health services that are frequently designed around the needs of older adults (Barr-DiChiara *et al.,* 2021; Velloza *et al.,* 2020). Within this population, adolescent girls and young women experience a distinct intersection of biological susceptibility and gendered social disadvantage (Celum *et al.,* 2019; Iwelunmor *et al.,* 2020). UNAIDS estimated that 210,000 adolescent girls and young women aged 15-24 acquired HIV in 2023, equivalent to approximately 4,000 new infections every week, with more than three-quarters occurring in sub-Saharan Africa. Updated global estimates for 2024 continued to show approximately 4,000 infections per week among adolescent girls and young women, with the great majority occurring in the region. Nigeria's HIV epidemic is heterogeneous across states, age groups, sexes, and social networks. Although national prevalence is lower than that of several countries in eastern and southern Africa, Nigeria's large population means that the country continues to carry a substantial HIV burden. National evidence has demonstrated pronounced gender differences among young people. The 2021-2025 investment case for adolescents and young people reported that HIV prevalence among females aged 15-19 was approximately three times that of males in the same age group, while prevalence among women aged 20-24 was more than four times that of their male counterparts (Iwelunmor *et al.,* 2020). These findings demonstrate that a low average prevalence can coexist with substantial gender inequality.

Sokoto State presents a distinctive context. It is in north-western Nigeria and includes urban, peri-urban, rural, and hard-to-reach communities. Social and economic conditions relevant to adolescent health include poverty, low female educational attainment in some communities, early marriage, gendered decision-making, and barriers to independent mobility and health-seeking (Zeleke *et al.,* 2024). These conditions are not uniform across all families or communities and should not be interpreted through a deficit-based or culturally dismissive lens. Rather, they point to the need for programmes that work respectfully with adolescents, parents, spouses, religious leaders, traditional leaders, schools, community organisations, and health workers to protect health and rights. The term adolescent girls and young women usually refer to females aged 15-24 years, although adolescence itself is defined by the World Health Organization as the period between 10 and 19 years of age. These age bands include individuals with substantially different needs. A 15-year-old who depends on parents and remains enrolled in school differs from a married 23-year-old woman who may be pregnant, breastfeeding, or economically responsible for children. Health systems that treat adolescent girls and young women as a homogeneous category risk overlooking important differences in legal status, marital status, schooling, fertility intentions, sexual exposure, partner dynamics, and service preferences (Barr-DiChiara *et al.,* 2021; Celum *et al.,* 2019).

Gender disparities in HIV are commonly described through biological and socio-cultural determinants. Biological susceptibility may be shaped by the larger mucosal surface exposed during heterosexual intercourse, genital inflammation, mucosal injury, and untreated sexually transmitted infections. Socio-cultural determinants include gender norms, relationship power imbalances, age-disparate relationships, violence, early marriage, constrained condom negotiation, poverty, and educational exclusion (Celum *et al.,* 2019; Iwelunmor *et al.,* 2020). These factors are important, but they do not fully explain why some young women receive effective prevention services while others do not. The health system determines whether information becomes access, whether testing is confidential, whether a referral is completed, whether commodities are available, whether a provider is respectful, and whether a young person returns for follow-up. Evidence on HIV testing, PrEP,

community-based care, and self-testing demonstrates that service organisation, confidentiality, provider attitudes, and linkage mechanisms significantly affect utilisation among young people (Barr-DiChiara *et al.,* 2021; Ngcobo *et al.,* 2022). Health-system determinants therefore occupy a central position between vulnerability and health outcomes. A service can technically exist but remain inaccessible because it is distant, expensive, open only during school hours, or known to breach confidentiality. A health worker may offer HIV testing but discourage uptake through judgemental language. A facility may diagnose a sexually transmitted infection but fail to provide PrEP, contraception, or gender-based violence support. Similarly, a community programme may generate demand but lose clients through weak referral and follow-up systems (Ngcobo *et al.,* 2022; Zeleke *et al.,* 2024). These failures are not simply matters of individual behaviour; they represent deficiencies in service design, governance, coordination, and implementation (Celum *et al.,* 2019). Nigeria has adopted strategic frameworks relevant to adolescents and young people. The National HIV and AIDS Strategic Plan 2023-2027 provide overarching direction for prevention, testing, treatment, and health-system strengthening, while the Generation Negative strategy seeks to empower adolescents and young people as agents of change and broaden prevention beyond narrow risk messaging. Education-sector initiatives such as the Adolescent Girls Initiative for Learning and Empowerment address school access, life skills, and economic barriers that are structurally linked to HIV vulnerability (Celum *et al.,* 2019). Operation Triple Zero, although primarily designed for adolescents and young people living with HIV, demonstrates the potential value of peer support, self-management, and youth-responsive service delivery (Zeleke *et al.,* 2024).

2. Conceptual Framework

This review applies a social-ecological framework combined with a health-systems perspective. The social-ecological approach recognises that HIV vulnerability is produced at multiple levels, including individual, interpersonal, community, institutional, and policy levels. The health-systems perspective focuses on governance, financing, the health workforce, information systems, commodities, service delivery, and community participation. Gender is treated as a cross-cutting structure that shapes exposure, agency, service access, and the distribution of programme benefits. Within this framework, biological susceptibility does not operate in isolation. Its effects are mediated by relationship power, social norms, material resources, and service availability. Early marriage may increase the duration or frequency of sexual exposure, but the resulting risk depends on the partner's HIV status, condom use, access to testing, availability of PrEP, and the young woman's ability to seek care without violence, stigma, or other adverse consequences (Celum *et al.,* 2019). Poverty may contribute to school dropout, early marriage, economic dependence, or transactional relationships, but social protection, continued education, mentoring, and economic empowerment can alter these pathways (Iwelunmor *et al.,* 2020). Stigma may discourage testing or PrEP uptake, whereas confidential self-testing, peer support, and discreet service delivery can lower access barriers (Velloza *et al.,* 2020; Zeleke *et al.,* 2024). The framework therefore directs attention towards modifiable system conditions rather than attributing infection or limited-service uptake to individual failure.

3. Methods

3.1 Information Sources and Search Approach

Targeted searches were conducted for literature available up to July 2026. PubMed was searched for peer-reviewed studies and reviews on HIV vulnerability among adolescent girls and young women, youth-friendly sexual and reproductive health services, HIV self-testing, PrEP, stigma, peer interventions, economic empowerment, and integrated service delivery in sub-Saharan Africa. Authoritative policy and epidemiological documents were identified from UNAIDS, the World Health Organization, the National Agency for the Control of AIDS, UNFPA, and the World Bank. Nigeria-specific and Sokoto-relevant

sources were prioritised where available. Search terms were combined in different ways and included adolescent girls, young women, HIV, Nigeria, Sokoto, gender disparity, early marriage, youth-friendly services, stigma, health systems, PrEP, HIV self-testing, peer navigation, economic empowerment, education, AGILE, and Operation Triple Zero. Reference lists of key reports and reviews were examined to identify additional evidence. Recent sources were prioritised, while foundational or older sources were retained where they remained scientifically or policy relevant.

3.3 Eligibility and Thematic Synthesis

Sources were considered relevant if they addressed adolescent girls and young women or adolescents and young people, HIV prevention or care, gendered vulnerability, service access, health-system performance, or interventions applicable to sub-Saharan Africa or Nigeria. Studies focusing exclusively on populations or contexts with limited relevance to Sokoto were used cautiously to identify transferable principles rather than to generate direct local estimates. Evidence was organised into the following themes: Biological and individual determinants; Interpersonal and socio-cultural determinants; Economic and educational determinants; Health-system barriers; Programme responses; and Policy and service-delivery implications.

3.4 Limitations of the Evidence Base

Direct empirical literature on HIV vulnerability and youth-friendly service delivery among adolescent girls and young women in Sokoto is limited. Much of the detailed intervention evidence comes from eastern and southern Africa, where HIV incidence and health-system arrangements may differ considerably from those of north-western Nigeria (Celum *et al.,* 2019; Garcia *et al.,* 2022). Transferability therefore requires careful local adaptation. National programme documents may aggregate diverse state contexts and may not capture informal barriers such as family permission, transport costs, partner opposition, or provider behaviour. The review distinguishes documented evidence from policy inference and avoids assigning numerical weights to determinants where local data are unavailable.

4. Epidemiological and Policy Context

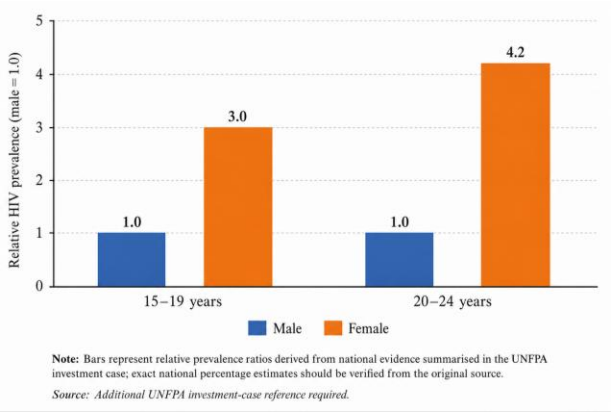
4.1 Global and Regional Context

The disproportionate burden of HIV among adolescent girls and young women is a defining feature of the epidemic in sub-Saharan Africa. The pattern has been associated with interacting biological, socio-economic, interpersonal, and structural factors, including poverty, gender inequality, violence, age-disparate relationships, limited control over sexual and reproductive decisions, and restricted access to prevention services (Iwelunmor *et al.,* 2020). Although new infections among adolescent girls and young women have declined since 2010, progress remains below global targets (Celum *et al.,* 2019). The persistence of thousands of new infections every week indicates that effective biomedical tools have not yet been delivered at sufficient scale or in forms that young women can consistently access and use (Nair *et al.,* 2023). Regional averages can conceal considerable diversity. HIV risk is higher in some settings, locations, and sexual networks than in others. Young women in age-disparate partnerships, those experiencing violence, young women who have left school, mobile populations, pregnant and breastfeeding women, young sex workers, and other marginalised subgroups may face elevated vulnerability.

4.2 Nigeria

Nigeria's national strategic documents recognise adolescents and young people as a priority population but also acknowledge that prevention investment has historically been limited relative to expenditure on testing and treatment. The Generation Negative strategy advocates a broader approach that empowers young people and addresses the social and institutional contexts in which HIV vulnerability is produced. National data indicate that female HIV prevalence rises substantially from mid-adolescence into young adulthood and exceeds male prevalence. Marriage is an important marker of sexual exposure among

young Nigerians, making marital status, antenatal care, family planning, and reproductive health services essential components of HIV prevention.



4.3 Sokoto State

Sokoto contributes a smaller share of Nigeria's HIV burden than several higher-prevalence states, but this should not lead to complacency. Prevention is likely to be most effective when it is strengthened before prevalence increases (Ojo, 2022). The state's large rural population, poverty, low female educational participation in some areas, early marriage, and limited availability of specialised adolescent services may create conditions in which infections remain undiagnosed, and prevention options are underused (Ngcobo *et al.,* 2022). Distance, transportation difficulties, insecurity along some routes, shortages of skilled health personnel, and weak digital connectivity may further affect service continuity. Sokoto also possesses important community and institutional assets. Religious and traditional institutions can support health promotion when they are meaningfully engaged. Primary health-care networks provide potential platforms for integrated HIV and reproductive health services. Schools, women's groups, youth organisations, community health workers, and peer networks can extend service reach. Community-based providers and peer structures can improve access when they receive adequate training, supervision, referral support, and integration with health facilities (Garcia *et al.,* 2022).

5. Determinants of Gender Disparities in HIV

5.1 Biological and Reproductive-Health Determinants

Biological differences contribute to heterosexual HIV acquisition risk. The female genital tract provides a larger mucosal surface that may be exposed during sexual intercourse, while genital inflammation, mucosal injury, and untreated sexually transmitted infections can increase susceptibility to HIV infection. These biological mechanisms do not make infection inevitable; their importance depends on viral exposure, partner characteristics, relationship dynamics, and access to effective prevention (Celum *et al.,* 2019). Adolescence and early reproductive life may coincide with cervical ectopy, hormonal changes, and limited experience in negotiating safer sex or seeking sexual and reproductive health care. Pregnancy and breastfeeding create additional contact with the health system but can also alter actual or perceived HIV risk. Integrating repeat HIV testing, partner services, PrEP assessment, and sexually transmitted infection management into antenatal, delivery, and postnatal care can transform these contacts into prevention opportunities (Nair *et al.,* 2023). Female-controlled prevention technologies are especially relevant in situations where condom negotiation is constrained. Oral PrEP and the dapivirine vaginal ring, where approved and available, can reduce dependence on partner cooperation (Celum *et al.,* 2019). Trials among African adolescent girls and young women have demonstrated substantial interest in prevention choice, although adherence and persistence can vary across products, individuals, and periods of risk (Nair *et al.,* 2023). Product availability alone is insufficient. Counselling must address side effects, disclosure, stigma, partner opposition, fertility intentions, convenience, and changing perceptions of risk. Programmes should also support informed switching between prevention methods rather than treating discontinuation of one product as programme failure (Velloza *et al.,* 2020).

5.2 Early Marriage, Age-Disparate Partnerships, and Limited Sexual Autonomy

Early marriage may increase the frequency and duration of sexual exposure while reducing an adolescent girl's ability to insist on condom use or request joint HIV testing. Married adolescents may also be missed by programmes that focus primarily on unmarried, school-going girls (Iwelunmor *et al.,* 2020). Service design should therefore include confidential and acceptable approaches for married adolescents and young women without framing marriage itself as a pathology. Age-disparate relationships can create inequalities in income, social authority, sexual decision-making, and previous exposure to HIV (Velloza *et al.,* 2020). Older male partners may have had more previous sexual partnerships and may have a higher probability of HIV exposure than adolescent boys. Economic dependence and unequal relationship power may make refusal of sex or negotiation of condom use more difficult (Celum *et al.,* 2019). Couples testing and male engagement can be helpful, but safeguards are required to ensure that these services do not expose adolescent girls and young women to coercion, involuntary disclosure, abandonment, or violence. Consent policies and practices must also recognise the rights, evolving capacities, and protection needs of adolescents (Barr-DiChiara *et al.,* 2021). Sexual autonomy is shaped by more than individual knowledge. A young woman may understand HIV prevention but lack permission to travel, money for transport, confidence that confidentiality will be protected, or power to use a product discreetly. Programmes that measure knowledge without assessing effective access and agency may therefore overestimate a young woman's ability to act on health information (Velloza *et al.,* 2020).

5.3 Poverty, Education, and Economic Dependence

Poverty influences HIV vulnerability through multiple pathways. It can contribute to school interruption, early marriage, dependence on partners, inability to pay for transportation, and difficulty prioritising preventive care. Food insecurity and urgent household needs may also increase reliance on transactional relationships. At the facility level, nominally free services may still impose indirect costs through transportation, waiting time, laboratory fees, missed school or work, and repeated visits (Iwelunmor *et al.,* 2020). Education can be protective through delayed marriage, improved health literacy, stronger social networks, and expanded future economic opportunities. School attendance also creates a platform for life-skills education, safeguarding, and referral to health services. The Adolescent Girls Initiative for Learning and Empowerment is therefore potentially relevant to HIV prevention, even though it is not primarily an HIV programme (Barr-DiChiara *et al.,* 2021). By improving girls' access to secondary education, scholarships, safe schools, and life skills, the programme may reduce structural vulnerabilities. To maximise health impact, education programmes should establish clear referral links to confidential adolescent health services. These services should also include out-of-school, married, pregnant, and parenting adolescents who may not be reached through school-based interventions. Economic empowerment interventions show promise but are not automatically effective. Cash transfers, savings groups, vocational skills, and livelihood support may improve agency, but programme designs must account for safety, household dynamics, implementation fidelity, sustainability, and local labour markets (Iwelunmor *et al.,* 2020). Evidence suggests that economic interventions may be most effective when combined with gender-transformative education, mentoring, social support, and accessible biomedical prevention rather than delivered as isolated income-generating projects (Iwelunmor *et al.,* 2020).

5.4 Gender Norms, Stigma, and Violence

Gender norms influence expectations regarding obedience, sexuality, mobility, and disclosure. Girls may be expected to remain uninformed about sexual health, while seeking condoms, HIV testing, or PrEP may be interpreted as evidence of sexual activity. These norms can discourage prevention even when services are physically available. Community dialogue should therefore address the social meaning attached to service use rather than merely advertising clinic locations. HIV-related stigma operates at multiple levels.

Anticipated stigma may deter testing or PrEP uptake; experienced stigma may follow disclosure; and internalised stigma can affect self-worth, prevention adherence, and continued engagement in care (Velloza *et al.,* 2020). Stigma may also attach to PrEP because other people may assume that the medication is antiretroviral treatment or evidence of promiscuity (Velloza *et al.,* 2020). Private service spaces, discreet packaging, trained providers, supportive counselling, and peer support can reduce these barriers. However, confidentiality must be embedded in facility procedures and not left solely to the discretion of individual health workers (Barr-DiChiara *et al.,* 2021) Gender-based violence increases HIV vulnerability directly through forced sex and genital traum. HIV services should therefore include safe enquiry, first-line support, and functional referral pathways to medical, psychosocial, legal, and protection services. Screening for violence without an effective and safe response pathway may be unethical and can expose young women to further harm (Barr-DiChiara *et al.,* 2021).

6. Health-System Determinants

6.1 Availability and Geographic Access

Many health facilities provide some HIV services, but adolescent-friendly care is often concentrated in urban or higher-volume facilities. Rural adolescent girls and young women may face long travel distances, transport costs, limited service hours, and dependence on adults or partners for mobility. Outreach, community-based testing, mobile services, task sharing, and integration into primary health care can improve access (Ngcobo *et al.,* 2022; Zeleke *et al.,* 2024). However, outreach must maintain privacy and avoid publicly identifying individuals as being sexually active or at risk of HIV. Community-based approaches require confidential procedures, trained personnel, secure documentation, and reliable referral links to health facilities (Ngcobo *et al.,* 2022).

6.2 Acceptability, Confidentiality, and Provider Behaviour

Youth-friendly care requires more than the establishment of a signboard or designated youth corner. Provider attitudes, privacy, confidentiality, waiting time, consent requirements, opening hours, and perceived judgement can strongly influence whether young people use available services (Zeleke *et al.,* 2024). Adolescents need clear explanations of confidentiality and its legal or safeguarding limits. Consultation rooms should prevent conversations from being overheard, records should be securely protected, and providers should avoid requiring unnecessary parental or spousal permission. National policies concerning adolescents' independent access to HIV testing vary widely across sub-Saharan Africa, and restrictive or unclear consent requirements can create major barriers (Barr-DiChiara *et al.,* 2021). Provider training should be reinforced through mentorship, supportive supervision, client feedback, and accountability. A single disrespectful or confidentiality-breaching encounter can undermine trust not only for one adolescent but also across her peer network (Barr-DiChiara *et al.,* 2021).

6.3 Fragmentation of HIV and Sexual and Reproductive Health Services

Adolescent girls and young women may require HIV testing, contraception, pregnancy testing, sexually transmitted infection diagnosis, PrEP, post-violence care, and mental-health support at the same time (Ngcobo *et al.,* 2022). Fragmented services require multiple queues, providers, referrals, and visits, increasing financial cost, waiting time, stigma, and loss to follow-up. Integrated service delivery can improve convenience and reduce stigma by making HIV prevention part of routine reproductive and primary health care. Integration should include clear clinical algorithms, task sharing, provider competencies, commodity planning, and referral tracking rather than merely locating services in the same building (Celum *et al.,* 2019).

6.4 Weak Referral and Continuity Systems

A referral is successful only when a client reaches the designated service and receives the required intervention. Peer navigators, community health

workers, and appropriate digital follow-up can improve continuity, provided confidentiality and safeguarding are protected (Garcia *et al.,* 2022). Referral directories should be regularly updated, and facilities should establish closed-loop communication so that the originating provider knows whether the client received the recommended care (Ngcobo *et al.,* 2022). Newly diagnosed or otherwise high-priority clients require rapid linkage to treatment and support. Young women initiating PrEP require early follow-up, adherence support, flexible refill options, and opportunities to discontinue or restart PrEP as their circumstances and periods of risk change (Nair *et al.,* 2023).

6.5 Workforce Capacity and Workload

Shortages of trained health workers can contribute to long waiting times, rushed consultations, weak counselling, and limited provision of preventive services. Providers may prioritise acute clinical tasks over preventive conversations, particularly in understaffed facilities (Ngcobo *et al.,* 2022). Task sharing with nurses, medical laboratory personnel, community health workers, and trained peer educators can expand service capacity. However, each cadre requires a clearly defined scope of practice, appropriate training, supportive supervision, job aids, and access to functional referral systems (Garcia *et al.,* 2022). Youth-friendly services should be incorporated into routine performance review and quality-improvement systems rather than depending on a single enthusiastic focal person. Overdependence on one trained worker can cause the service to collapse when that person is transferred, unavailable, or overburdened (Ngcobo *et al.,* 2022).

6.6 Commodities, Diagnostics, and Prevention Choice

Stock-outs of HIV test kits, condoms, sexually transmitted infection medicines, pregnancy tests, or PrEP can rapidly damage community confidence and discourage future service use. Commodity security requires accurate forecasting, timely reporting, redistribution mechanisms, buffer stocks, and clear accountability (Celum *et al.,* 2019). Prevention choice should be expanded as national guidelines, regulatory approvals, and resources permit. HIV self-testing may appeal to young people because it offers privacy, autonomy, and convenience, but distribution must be linked to confirmatory testing, counselling, prevention services, and treatment (Zeleke *et al.,* 2024). PrEP delivery should recognise changing periods of risk and support informed choice rather than coercive risk labelling. Evidence from African adolescent girls and young women demonstrates the importance of offering product choice and adapting support to the user's preferences and circumstances (Nair *et al.,* 2023).

6.7 Information Systems and Data Use

Age- and sex-disaggregated data are essential for identifying gender and service-delivery gaps. However, routine systems may aggregate adolescents with older adults or fail to record outcomes across multiple referral points. Data systems should capture HIV testing, positivity, linkage, PrEP initiation and continuation, sexually transmitted infection services, and violence referrals by age, sex, marital status, and location while maintaining privacy (Celum *et al.,* 2019). Qualitative feedback from adolescent girls and young women is also necessary because numerical service uptake does not reveal disrespectful treatment, fear of disclosure, hidden financial costs, partner opposition, or the reasons for PrEP discontinuation (Velloza *et al.,* 2020).

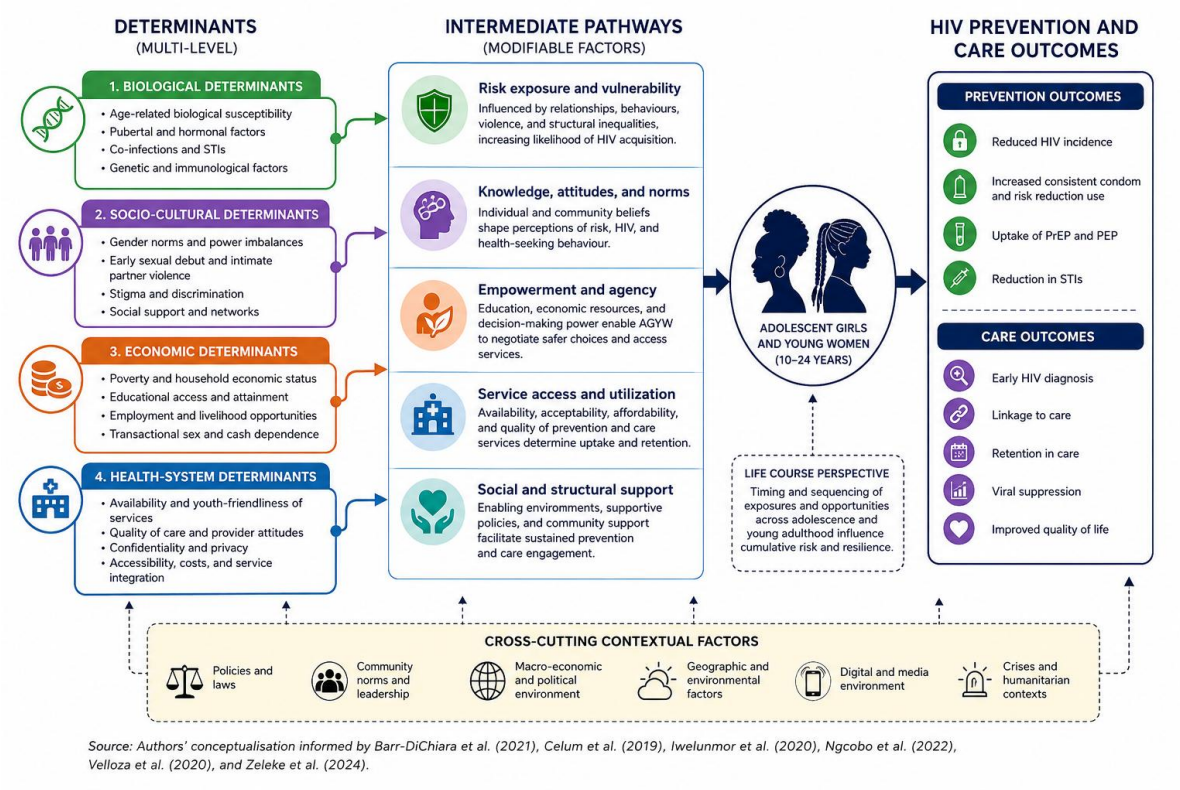
6.8 Governance, Financing, and Youth Participation

Underinvestment in primary HIV prevention is a national concern (Garcia *et al.,* 2022. State and local plans should include defined budgets for youth-friendly services, community engagement, commodities, outreach, referral systems, peer navigation, and quality improvement. Coordination among the health, education, women's affairs, youth, social protection, and community sectors is essential. Peer-led models can strengthen trust and demand creation when peers are properly selected, trained, supervised, protected, and remunerated (Garcia *et al.,* 2022).

Table 1; Health-System Barriers and Corresponding Service-Delivery Responses

System domain	Common barrier	Priority response
Geographic access	Long distance, transport costs, and dependence on others for mobility	Integrate services at primary health-care level; use outreach, mobile services, and community delivery
Service hours	Clinics operating during school or working hours	Introduce flexible hours, scheduled appointments, and fast-track services
Acceptability	Judgemental language and fear of being labelled sexually active	Provide adolescent-centred communication training, mentorship, and client-feedback systems
Privacy and confidentiality	Consultations being overheard or information disclosed	Establish private consultation spaces, secure records, and clear confidentiality procedures
Consent	Unclear or unnecessary parental or spousal permission	Provide practical guidance consistent with national law and adolescent safeguarding principles
Service integration	Separate HIV, reproductive health, STI, and violence-response services	Provide coordinated or one-stop services using clear clinical algorithms
Referral	Paper referrals without follow-up	Introduce peer navigation, referral tracking, feedback, and transportation support where required
Workforce	Inadequate staffing and high provider workload	Use task sharing, mentorship, job aids, and routine quality improvement
Commodities	Stock-outs of test kits, condoms, STI medicines, and PrEP	Strengthen forecasting, reporting, redistribution, buffer stocks, and accountability
Prevention choice	Limited availability of confidential or female-controlled options	Expand HIV self-testing, oral PrEP, and other approved prevention choices
Information systems	Poor age and sex disaggregation	Develop AGYW-sensitive indicators while protecting individual privacy
Governance	Fragmented sectors and donor-dependent activities	Establish state-led multisectoral coordination, recurrent financing, and youth representation

Note. AGYW = adolescent girls and young women; PHC = primary health care; SRH = sexual and reproductive health; STI = sexually transmitted infection; GBV = gender-based violence; PrEP = pre-exposure prophylaxis; HIVST = HIV self-testing. The table was developed from evidence presented by Barr-DiChiara *et al.* (2021)



Source: Authors' conceptualisation informed by Barr-DiChiara et al. (2021), Celum et al. (2019), Iwelunmor et al. (2020), Ngcobo et al. (2022), Velloza et al. (2020), and Zeleke et al. (2024).

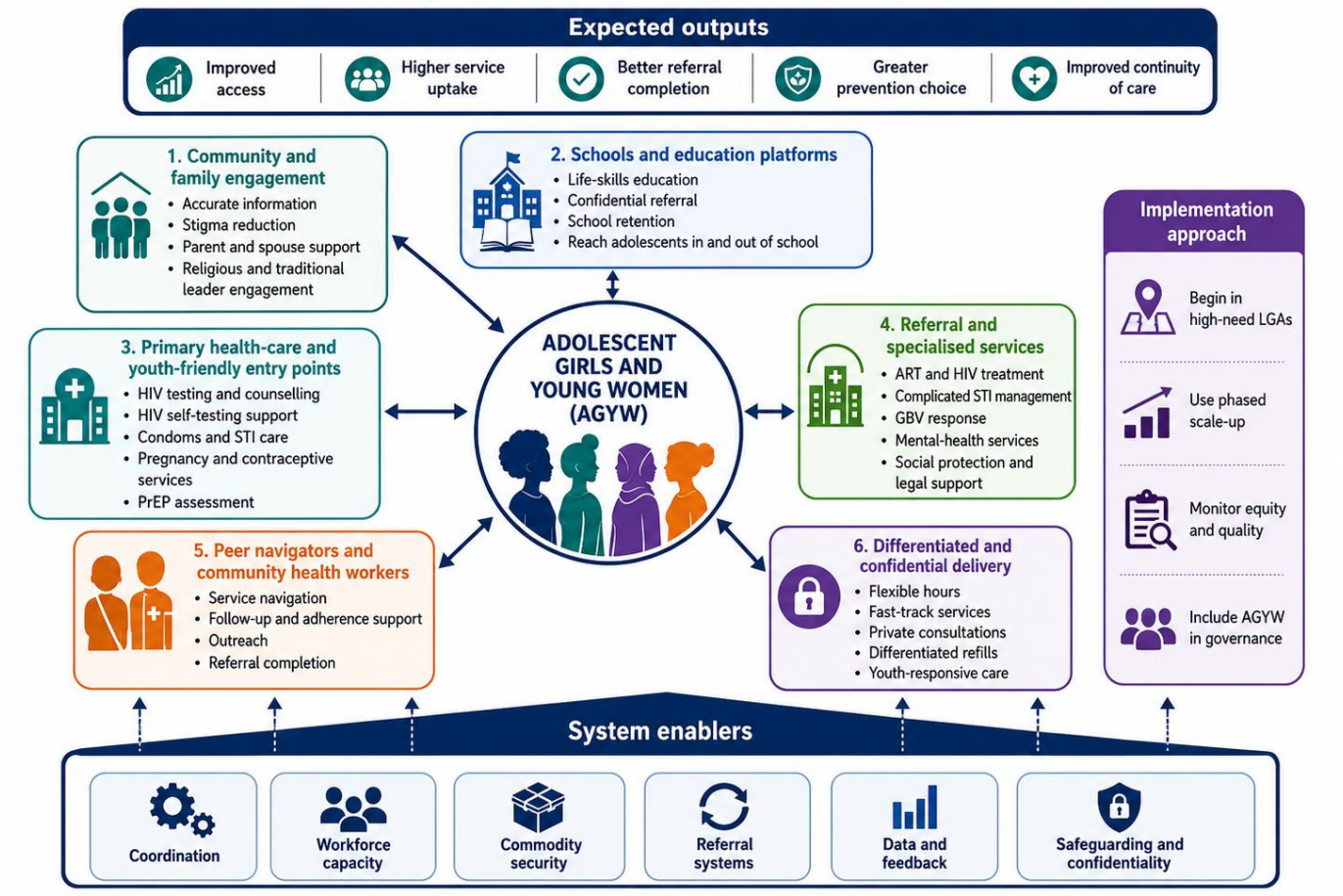


Figure 3. Proposed integrated and AGYW-centred service-delivery model for Sokoto State. Source: Authors' conceptualisation informed by Celum *et al.* (2019).

7. Existing and Emerging Programme Responses

7.1 Operation Triple Zero

Operation Triple Zero promotes zero missed appointments, zero missed medication doses, and zero unsuppressed viral load among adolescents and young people living with HIV (Ngcobo *et al.,* 2022). Its primary purpose is to improve treatment adherence and viral suppression rather than prevent HIV acquisition among HIV-negative adolescent girls and young women. Nevertheless, its youth-driven structure, peer support, and emphasis on self-management may provide useful lessons for prevention programming. A Sokoto platform for adolescent girls and young women could adapt these principles to promote regular testing when indicated, sustained PrEP use during periods of vulnerability, completion of referrals, and active participation in service-quality monitoring. The distinction between programme adaptation and direct evidence of prevention effectiveness should be maintained.

7.2 AGILE and the Education Platform

The Adolescent Girls Initiative for Learning and Empowerment seeks to increase access to secondary education and address demand- and supply-side barriers in northern Nigeria, including Sokoto (Ngcobo *et al.,* 2022). Education, scholarships, life skills, and safer schools may influence HIV vulnerability through delayed marriage, improved agency, stronger health literacy, and expanded livelihood opportunities. Health-sector collaboration can strengthen referral from schools to confidential services.

However, school-based activities must protect privacy and must not exclude out-of-school, married, pregnant, parenting, or otherwise marginalised adolescents.

7.3 Peer-Led and Community-Based Interventions

Peer educators and ambassadors can improve trust, prevention literacy, demand creation, and service navigation. Evidence from a PrEP ambassador field test in Zimbabwe showed that trained adolescent girls and young women could promote PrEP awareness and engagement within their communities (Garcia *et al.,* 2022). Peer programmes require careful selection, adequate remuneration, supervision, confidentiality training, safeguarding, and functional referral tools. Unpaid volunteer models may be difficult to sustain and may reproduce existing economic inequities. Community health workers can extend HIV education, testing, follow-up, and linkage, particularly in underserved communities. However, they require updated training, supportive supervision, adequate supplies, recognition within the health system, and strong connections with facilities (Ngcobo *et al.,* 2022).

7.4 HIV Self-Testing and Differentiated Testing

HIV self-testing is acceptable to many young people and can reach individuals who avoid facility-based testing because of stigma, inconvenience, or confidentiality concerns (Zeleke *et al.,* 2024). Distribution options may include pharmacies, community agents, health facilities, antenatal services, and appropriately designed educational platforms. Safeguards should include clear instructions, access to assistance, confirmatory testing, violence-sensitive counselling, and confidential linkage to prevention or treatment. Testing strategies should be guided by epidemiology, informed consent, individual preference, and potential benefit rather than indiscriminate mass testing or stigmatising risk profiling (Barr-DiChiara *et al.,* 2021; Zeleke *et al.,* 2024).

7.5 PrEP and Prevention Choice

Oral PrEP is effective when taken as prescribed, but persistence among adolescent girls and young women may be affected by side effects, changing risk perception, partner opposition, HIV-related stigma, disclosure concerns, and frequent clinic visits (Celum *et al.,* 2019; Velloza *et al.,* 2020). Evidence from trials of oral PrEP and the dapivirine vaginal ring demonstrates the importance of prevention choice and user-centred delivery (Nair *et al.,* 2023). In Sokoto, implementation should include provider readiness, reliable commodity systems, simple eligibility assessment, pregnancy and breastfeeding guidance, and community education that clearly distinguishes PrEP from antiretroviral treatment. Peer support, differentiated refill arrangements, discreet packaging, and flexible service-delivery models may improve continuation and acceptability (Celum *et al.,* 2019) (Velloza *et al.,* 2020).

7.6 Combination Prevention and Social Protection

The available evidence supports combination approaches that address biomedical, behavioural, interpersonal, and structural determinants together. Economic empowerment interventions show potential, although effectiveness depends on implementation quality, reach, context, and integration with other services (Iwelunmor *et al.,* 2020). DREAMS evaluations have reported improvements in testing, HIV status awareness, and some sexual-health outcomes, with evidence of reduced incidence in particular settings. For Sokoto, a practical combination package would connect HIV and reproductive health services with school retention, livelihood support, violence response, mental-health services, peer networks, and community norm change (Garcia *et al.,* 2022).

8. Proposed Integrated Service-Delivery Model for Sokoto

A Sokoto service-delivery model should be built around multiple entry points connected by a common referral, safeguarding, and quality-improvement system. Community and family engagement should create supportive norms and improve access to accurate information. Schools

and education programmes should provide age-appropriate life skills and confidential referral options (Garcia *et al.,* 2022). Primary health-care facilities and youth-friendly entry points should provide HIV testing, support for self-testing, condoms, sexually transmitted infection services, pregnancy-related care, and PrEP assessment. Higher-level facilities and specialised partners should receive referrals for antiretroviral therapy, complicated sexually transmitted infections, gender-based violence, mental-health conditions, and social protection. Peer navigators and community health workers can help adolescent girls and young women move between these services, if they receive adequate training, supervision, safeguarding, and referral support (Ngcobo *et al.,* 2022).

The model requires a coordination platform involving the Sokoto State Ministry of Health, the State Agency for the Control of AIDS, education authorities, ministries responsible for women and youth, local government authorities, implementing partners, community leaders, and representatives of adolescent girls and young women. Core functions should include joint microplanning, commodity monitoring, maintenance of updated referral directories, data review, safeguarding, provider mentorship, and community feedback. A phased approach could begin in high-volume or high-need local government areas and expand following implementation evaluation.

9. Policy and Service-Delivery Implications

First, adolescent-friendly care should be institutionalised as a quality standard across relevant primary and secondary health facilities. This requires private consultation spaces, trained personnel, flexible service hours, confidential records, clear consent procedures, and routine quality monitoring (Velloza *et al.,* 2020). A standalone youth corner may be useful in some facilities but should not become the only place where young people receive respectful care. Second, HIV prevention should be integrated with sexual and reproductive health services. Adolescent girls and young women should be able to receive HIV testing, condoms, contraception, sexually transmitted infection care, pregnancy services, PrEP counselling, and violence-sensitive support through coordinated pathways. Integration can reduce repeated visits and minimise the stigma associated with attending a visibly HIV-labelled service (Barr-DiChiara *et al.,* 2021). Third, the state should treat girls' education and economic empowerment as HIV prevention investments. Health authorities should collaborate with AGILE and other education and social-protection initiatives to establish referral, safeguarding, and outcome indicators. Economic programmes should be linked with mentoring, gender-responsive education, and access to biomedical prevention (Iwelunmor *et al.,* 2020). Programmes must also include out-of-school and married young women rather than focusing only on students. Fourth, service delivery should offer prevention choice. HIV self-testing, provider-administered testing, oral PrEP, and future long-acting options should be introduced in accordance with national policy, regulatory approval, local capacity, and informed preference. Choice increases the likelihood that a young woman can identify an option compatible with her relationships, privacy needs, reproductive intentions, and daily activities (Nair *et al.,* 2023; Zeleke *et al.,* 2024). Fifth, community engagement should move beyond one-way sensitisation. Parents, husbands, religious leaders, and traditional leaders can contribute to stigma reduction and timely care, but the autonomy, safety, and confidentiality of adolescent girls and young women must remain central. Dialogue should frame HIV services as components of general health, responsible prevention, family well-being, and human dignity (Barr-DiChiara *et al.,* 2021). Sixth, local data should drive action. Facilities and programmes should review age- and sex-disaggregated indicators, referral completion, PrEP continuation, and experience-of-care measures. Data should be interpreted carefully in low-prevalence settings, where small numbers may fluctuate considerably. Qualitative information should be used to identify hidden barriers before they become visible in coverage indicators. Seventh, financing should correspond with stated policy priorities. Youth-friendly services, outreach, peer navigators, commodities,

supervision, and quality improvement require recurrent budgets. Dependence on short-term donor projects can produce repeated cycles of expansion and collapse. State ownership and integration into routine operational plans are therefore necessary for sustainability.

10. Discussion

The central argument of this review is that gender disparity in HIV infection among adolescent girls and young women cannot be reduced through individual behaviour change alone. Biological susceptibility and relationship-level risks are important, but the health system determines whether young women can convert knowledge into effective protection. Services that are distant, judgemental, fragmented, or unreliable can reproduce vulnerability, whereas respectful and integrated care can strengthen agency even when broader social inequalities persist (Barr-DiChiara *et al.,* 2021; Celum *et al.,* 2019).

11. Recommendations

11.1 Recommendations for the Sokoto State Government and Public Health Institutions

Adopt and operationalise state-level standards for confidential, respectful, and integrated adolescent- and youth-friendly HIV and sexual and reproductive health services. Map the availability of services for adolescent girls and young women and establish referral networks linking primary health-care facilities, hospitals, schools, community platforms, gender-based violence services, and social-protection programmes. Create a multisectoral coordination mechanism for HIV prevention among adolescent girls and young women, with meaningful youth representation and a dedicated implementation budget. Strengthen forecasting, reporting, redistribution, and buffer-stock arrangements for HIV test kits, condoms, sexually transmitted infection commodities, pregnancy tests, and PrEP. Include age-, sex-, marital-status-, and location-disaggregated prevention indicators and experience-of-care measures in routine performance reviews. Partner with religious and traditional institutions using culturally respectful messages that promote prevention, confidentiality, informed decision-making, and timely care.

11.2 Recommendations for Health Facilities and Providers

Protect privacy, clearly explain confidentiality, and minimise unnecessary parental, partner, or spousal permission requirements, consistent with applicable laws and safeguarding standards (Barr-DiChiara *et al.,* 2021). Offer integrated HIV testing, sexually transmitted infection care, reproductive health services, violence-sensitive support, and PrEP assessment at convenient service entry points. Use flexible hours, fast-track visits, community delivery, and differentiated refill models for students, married adolescents, and working young women. Establish closed-loop referral systems and use trained peer navigators or community health workers for high-priority clients (Garcia *et al.,* 2022; Ngcobo *et al.,* 2022). Conduct routine provider mentorship, confidential client-feedback exercises, and quality-improvement cycles focused on adolescent experience and confidentiality.

11.3 Recommendations for Implementing Partners and Civil Society

Co-design programmes with diverse groups of adolescent girls and young women, including rural, married, out-of-school, pregnant, breastfeeding, and disabled young women. Avoid duplicative vertical projects and align investments with state systems, national strategies, and sustainability plans. Support peer-led demand creation, HIV self-testing linkage, PrEP literacy, and economic empowerment with adequate safeguarding, supervision, and remuneration (Garcia *et al.,* 2022). Generate implementation evidence on programme reach, equity, referral completion, service quality, cost, and sustainability in north-western Nigeria.

11.4 Research Priorities

Conduct mixed-methods studies on HIV knowledge, testing preferences, service barriers, and prevention needs among adolescent girls and young women in Sokoto. Disaggregate findings by age, marital status, educational status, rurality, pregnancy status, disability, and other relevant characteristics. Evaluate integrated youth-friendly service models using implementation outcomes such as acceptability, feasibility, fidelity, reach, equity, sustainability, and cost. Assess how educational and economic empowerment programmes influence HIV-related agency, relationship power, and service uptake. Document the effectiveness and transferability of peer-support models.

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Case Report

Penile Telescoping, Dermatological and Multisystem Adverse Effects of Lithium Therapy in a Middle-aged Man: A Case Report in a Resource-Limited Setting

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Abstract

Background: Lithium is the gold standard in the management of bipolar affective disorder (BAD). This case is unique because it involves multiple lithium adverse effects in a patient on long-term lithium therapy, showcasing a rare dermatological and genital complication. It has longitudinal laboratory monitoring of lithium assays and describes the complex interplay of factors that led to the rare genital complication.

Method: Information was derived from his medical records, clinical interview, in-patient and out-patient records, multidisciplinary management, and serial lithium levels assessed at intervals of not more than 3 months. Also, electrocardiograms, thyroid function tests, renal function tests, and other investigations spanning across 8-10 years of follow-up were conducted. WHOQOL-BREF assessed his quality of life. This was followed by correlating temporal changes in serum lithium levels and clinical findings to assess patterns of lithium toxicity. Specialist evaluations from general medical practice, urology, cardiology, dermatology, neurology, and nephrology were extrapolated to characterize the multisystemic manifestations and rare complications. Informed consent was obtained from the patient for the publication of this report including the patient's perspective, while his personal information has been anonymized and de-identified. This report was prepared following the Case Report (CARE) guidelines.

Results: Genital findings include marked scarring of the penile shaft and penile telescoping, while dermatological examination revealed psoriatic plaques in the genital area, umbilical region, and prominent silvery scaling on both elbows. Metabolic effects include truncal obesity, subclinical hypothyroidism, and Blood pressure changes. There was evidence of chronic kidney disease with an eGFR of 56ml/min/1.73m². The chronic burden of these multisystem side effects led to a reduced quality of life. However, serum lithium levels showed values within the therapeutic range, despite these adverse effects.

Conclusion: This report demonstrates that chronic lithium therapy exposure influences morbidity, with the patient developing progressive multi-organ effects despite serum lithium levels within therapeutic range. This underscores the need for proactive clinical inquiry about the multiple systems during lithium therapy, irrespective of the tolerated therapeutic dose.

Keywords: "adverse effect", "Bipolar disorder", "case report", "lithium therapy", " penile telescoping"

Introduction

A particularly unique aspect of this case is the presence of a rare and underreported genital complication, that is, acquired penile telescoping secondary to glans scarring and abnormal adiposity. There hasn't been any description of a direct relationship between this complication and lithium therapy in current literature, making this a potentially novel finding. While lithium has been associated with cutaneous manifestations such as psoriasis (Cuniberti et al., 2024) and weight gain that may lead to altered body habitus, the progression to significant urogenital impairment with associated urinary and sexual dysfunction is highly unusual. (Eskandari., 2025)

This finding broadens the scope of possible adverse effects from lithium therapy and suggests a need for greater clinical vigilance and holistic physical examination of systems, including those the patient may feel reluctant to report (Yi OS., 2022). It also demonstrates extensive multisystemic toxicity associated with chronic lithium therapy in a single patient being managed for Bipolar Disorder, despite serum lithium levels largely remaining within or near the therapeutic range. Although there have been reports of lithium causing neurological, endocrine, dermatological and renal adverse effects, they are often reported individually or in limited combinations. In contrast, this patient exhibited multiple organ systems involvement occurring simultaneously, emphasizing the concept of cumulative toxicity rather than acute, dose-dependent toxicity. This highlights the need for comprehensive physical examination and laboratory monitoring of systemic lithium toxicity other than just serum lithium levels alone. (Gitlin., 2016)

Patient Information

A 58year old married African business man with a long standing history (over 3 decades) of mood disorder, consistent with Bipolar Affective Disorder presenting for routine outpatient follow up. The patient had been on long -term lithium therapy with sustained use for approximately 10-15 years alongside other adjunct medications.

His Premorbid personality had been characterized by being calm, introverted, religious, and socially well-adjusted. He resides in a resource-limited area, where access to medications, laboratory monitoring, and specialist care have been largely inconsistent due to cost, industrial actions, healthcare constraints, and logistics sometimes necessitating external sourcing, including importation of his drugs. He has experienced multiple episodes of the illness characterized by predominantly manic symptoms with periods of remission in between episodes. His treatment course had been marked by unorthodox approaches and intermittent medication non-adherence largely due to inconsistent access to medications and financial constraints Baseline Weight at presentation: 65 kg, height 1.63 metres, BMI 24.5kg/m², and Waist circumference 98cm.

There was difficulty maintaining Psychiatric stability without lithium therapy having failed to respond to other mood stabilizers. Presenting in the 6th episode of mental illness with 2 months history of Hyperactivity, Talkativeness, Undue irritability, Hearing voices of unseen persons in clear consciousness, making false claims about his identity. He was admitted on the 5th of August, 2017 to the male psychiatric ward of the hospital, spent 2 months on admission, and was ,managed as a case of Bipolar Affective Disorder, Current Episode Manic With Mood Congruent Psychotic Features. Index episode was preceded by failure to comply with medication and erratic availability of his lithium therapy.

Also, there was financial constraint affecting medication adherence and toxicity monitoring. He was noted to have verbalized financial constraints in procuring the lithium carbonate tablets and other medications aggravated by scarcity of lithium carbonate in Nigeria's pharmacies which led to subsequent medication non-adherence. At the time, a pack of lithium cost ₦50,400, which

will last him about 6 weeks. And he couldn't get at all the pharmaceutical stores in his state for a couple of weeks. He currently imports his latest stock of lithium carbonate from the United Kingdom. The patient provided written informed consent for the publication of this case report and accompanying images.

Management:

Psychopharmacological treatment was commenced, routine investigations (Full blood count, Electrolytes, Urea and Creatinine, Urinalysis) were requested; medications reinstated; Tabs Chlorpromazine 200mg AM, 300mg ON, Tabs Benzhexol 5mg AM and 5mg 6PM, Tabs lithium 800mg daily; long-acting IM zuclopenthixol 200mg stat which led to substantial clinical improvement.

By four months on lithium, his wife reported slurred speech, neck stiffness, and body weakness, though the patient said the symptoms had subsided. At 27 months, his weight had increased to 77 kg. By August 2022, after about six years of treatment, he presented with worsening tremors of the lips and both hands; mental state examination confirmed fine bilateral hand tremors.

At 67 months, he developed loose watery stool occurring at least three times daily for one week, without mucus, blood, fever, abdominal pain, or body weakness. Symptoms improved with oral rehydration therapy and antibiotics, while bilateral hand tremors remained the only positive physical finding. At 71 months, he reported worsening skin rashes and persistent mouth and hand tremors. At 79 months, he complained of right knee pain and excoriations affecting both elbows, the umbilical region, and perineum; examination showed fine bilateral hand tremors and hypopigmented skin patches.

At 82 months, he again reported recurrent loose watery stool, two episodes daily for one week, with similar past episodes resolving after lime or tea. Serum lithium assay, thyroid function test, electrolytes/urea/creatinine, and liver function tests were requested, but he declined because of financial constraints despite counselling on the importance of monitoring for lithium toxicity. At 84 months, he returned with investigation results. His wife noted worsening slurred speech during prolonged talking, and persistently elevated blood pressure over six months was documented. Consequently, lithium carbonate was reduced to 600 mg daily, while other medications were unchanged.

By 101 months on lithium therapy, he presented with investigation results and a one-year history of progressive penile shrinkage, with complete retraction of the glans into the shaft. This was associated with difficulty voiding urine and inability to have sexual intercourse. He reported needing to squat to avoid urine dribbling and splitting, and his last intercourse with his wife was one year earlier.

Table 1 illustrates the summary of the pattern of presentation over the last 10 years, while figure 1 shows their progression, and figure 2 summarises the lithium levels during these periods

Figures 3 and 4 show psoriatic patches on dorsal surface of both hands. Figure 5 shows similar psoriasis plaques on both elbows, about 4 by 4 cm, Figure 6 shows a 2cm scaly, hyperpigmented lesion on the umbilicus with truncal obesity.

Figures 7 and 8 show urogenital examination findings with retraction of the glans penis into the penile shaft, hypopigmented scaly skin rashes on the shaft of the penis, surrounding erythema around the corona, whitish discharge deposit around the glans.

Table 1: Pattern of Presentation and Interventions

Case Report Timeline: Multisystem Adverse Effects of Long-term Lithium Therapy

Time/Year	Clinical Events & Symptoms	Investigations	Interventions/Outcomes
1983 (Age 24)	First episode – mania	–	Unorthodox care → remission
1984	Second episode	–	Unorthodox care → remission
2001	Third episode	–	Private psychiatric care → remission
2006	Fourth episode	–	Admission, lithium introduced
2008	Fifth episode (non-adherence)	–	Admission → remission
2017	Index episode: mania with psychosis	Baseline labs	Lithium + antipsychotics
1–4 months	Skin rash, tremors, slurred speech	Lithium ~1.2–1.25	Monitoring, dose adjustment
5 months	Persistent tremors	TFT abnormal	Subclinical hypothyroidism
7–12 months	Stable mood, tremors persist	Lithium 0.8–1.0	Continue treatment
30 months	Hypomania	Lithium 0.98	Dose adjustment
54 months	Tardive dyskinesia	Lithium 0.8	Pyridoxine added
67 months	Diarrhea	Lithium 0.72	Dose reduced
71–75 months	Psoriasis worsens	Creatinine ↑	Further dose reduction
~100 months	Penile telescoping	TFT abnormal, BP ↑	Specialist referral
105 months	CKD stage G3a	Lithium 1.3, eGFR 56	Lithium reduced, multidisciplinary care

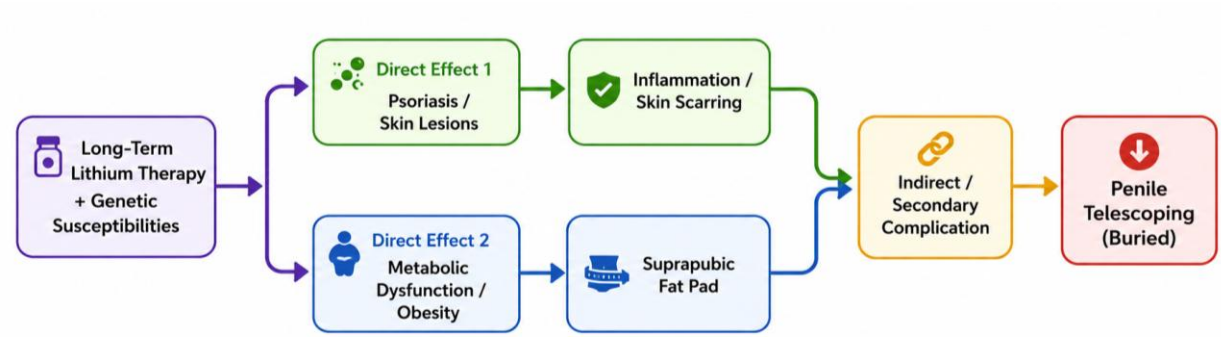

Figure 2

Figure 3:

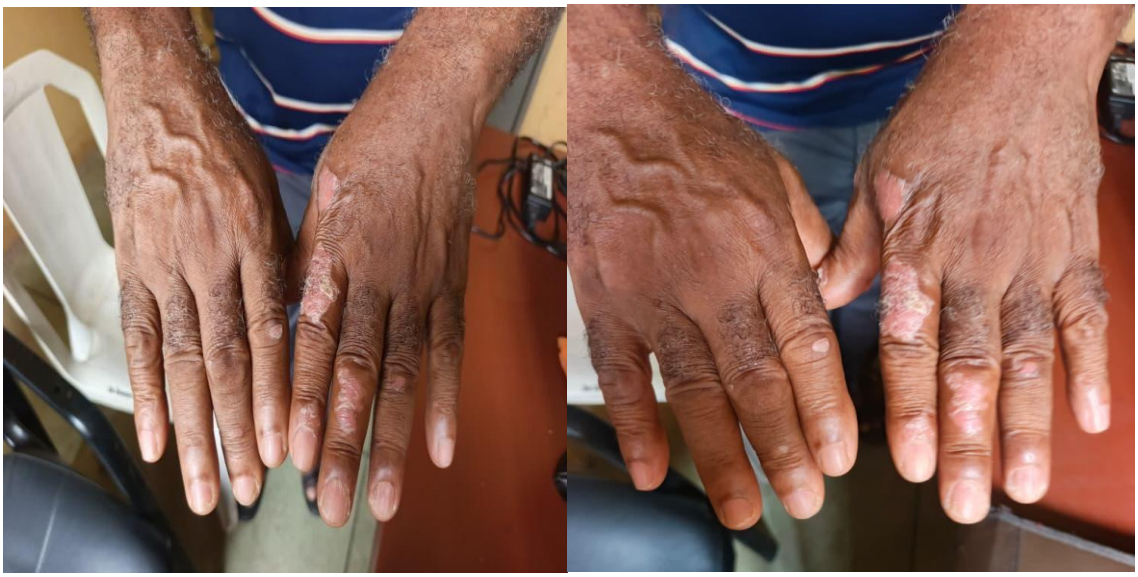

Figure 3 and Figure 4: Psoriatic plaques and patches at the dorsum

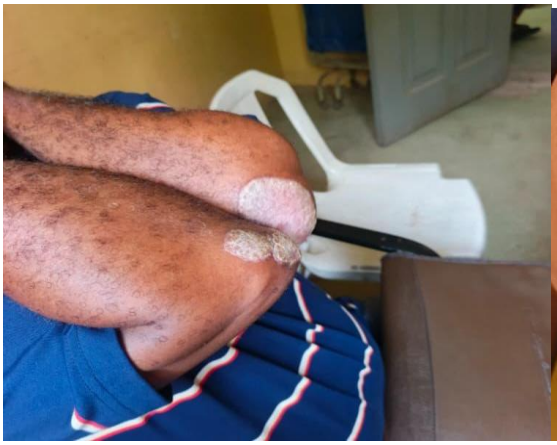

Figure 5: Plaque at the elbows.

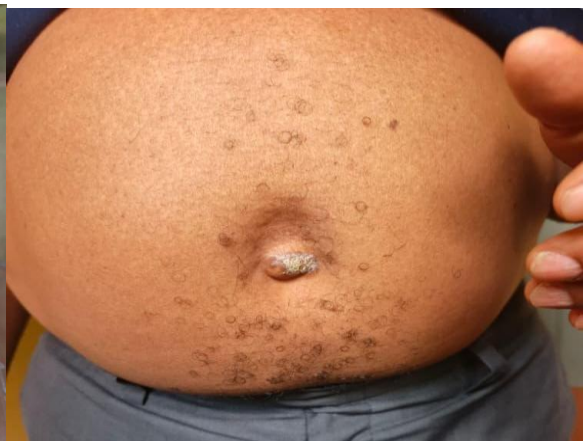

Figure 6: Umbilical plaque and obesity

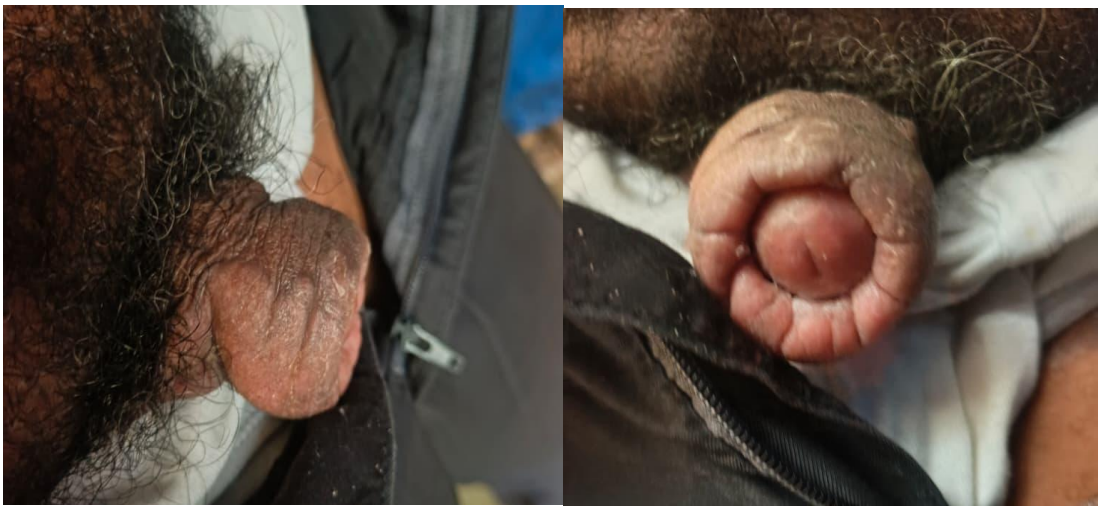

Figure 7 and Figure 8: Penile telescoping

Multi-disciplinary interventions

The management plan involved coordinated pharmacological, psychological, dermatological, genito-urinary, surgical, psychosocial, monitoring, and relapse-prevention interventions.

The use of the lowest effective dose with close monitoring remains the core strategy in reducing morbidity. A risk vs benefit assessment should be done to reassess need for lithium, while considering a gradual switch or augmentation if risks outweighs benefits, alternatives considered in this case include olanzapine. He was counselled on lifestyle modifications including diet and exercise and the drugs adjusted to abate the reported complaints. The dose of Chlorpromazine was reduced from 300mg ON to 200mg ON, Lithium carbonate, Depot Zuclopenthixol decanoate dose however remained unvaried. During the 91 months on lithium therapy, the dose of chlorpromazine was reviewed downward to 100mg ON, and atypical antipsychotic mood stabilizer Tabs Olanzapine 20mg ON was added, Tabs Benzhexol was also reviewed to 5mg BD, Lithium remained unvaried at 600mg daily, Pyridoxine phosphate 100mg Bd. The dose of lithium carbonate was reviewed downwards on different occasions following reports of adverse effects, laboratory findings suggestive of toxicity and upward trend of serum lithium from 800 mg > 600 mg > 400 mg daily.

Dermatological care included topical corticosteroids and retinoids, follow-up for suspected lithium-induced psoriasis, assessment for alternative dermatoses such as lichen sclerosis, and possible biopsy to exclude chronic inflammatory or premalignant conditions. Genito-urinary care addressed obesity through lifestyle modification, restoration of skin integrity, hygiene counselling, and treatment of secondary infections. Urology review was recommended for possible penile shaft reconstruction, suprapubic lipectomy, skin grafting, urethral evaluation, and management of glans scarring if voiding symptoms persisted. Psychosocial care included couple counselling, support for body image concerns, occupational therapy for tremors, and structured sexual function assessment using IIEF-5. Ongoing monitoring should include lithium levels, renal and thyroid function, weight, BMI, and blood pressure, with multidisciplinary specialist review. Relapse prevention emphasized psychoeducation on medication side effects, lithium toxicity, early relapse signs, adherence, follow-up, and diagnostics including uroflowmetry, PVR, and cystoscopy.

Patient's Perspective:

"I have had this illness for many years, and I must acknowledge that the medications, especially lithium, have helped me maintain mental stability most of the time. However, I have had to cope with many bodily changes over the years which is quite distressing. The tremors in my hands are always there and make it hard for me to do simple things. I also developed skin problems that itch and change how my skin looks which worries me when people around me notice.

The most troubling issue is a problem with my private part, which has gradually decreased in size. I now find it difficult to urinate unless I squat. Intimacy with my wife has also been affected, which has reduced my self-esteem as a man. The specialists I was referred to suggested that most of my problems may be due to my medication. This puts me in a difficult situation because the same drug that helps my mental health is also causing serious side effects. They also mentioned that this might explain my abnormal kidney function tests, blood pressure, and other health problems I have developed. Despite these challenges, including the cost of treatment and tests, I would like to

remain mentally stable while hoping for a way to reduce these side effects and enjoy my life better.

Prognosis

He has demonstrated Psychiatric stability with good response to lithium therapy, the need for dose reduction due to toxicity however predisposes him to risk of future relapse, particularly given his recurrent history of relapses following medication non-adherence.

The medical prognosis is also more concerning, in the presence of chronic kidney disease (stage 3a, Subclinical hypothyroidism, persistent neurological symptoms (tremors, dyskinesia), and dermatological complications. Renal impairment is likely to progress over time with continued lithium exposure, although dose reduction may slow down progression.

The genito-urinary complications (acquired penile telescoping) may greatly impact a patient's quality of life, as reversibility is uncertain without surgical interventions.The functional impairment related to voiding and sexual dysfunction are likely to persist without specific treatment approaches.

Contextual factors such as financial constraints, inconsistent access to medications and laboratory monitoring,and health system disruptions further influence the prognosis, increasing risk of delayed complications detection, suboptimal treatment adjustments, and overall poorer outcome.

Discussion

Lithium has been shown to demonstrate multisystemic toxicity. The renal system is a major target of this assault. Reduction in glomerular filtration rate and urine concentrating ability has been reported in systematic review. Hypothyroidism, hyperparathyroidism leading to hypercalcaemia, and weight gain were also reported in the same review. A population-based cohort study of patients with a mean duration of 55 months on lithium therapy however did not demonstrate a consistent effect of therapeutic lithium levels on rate of change in estimated glomerular filtration rate (eGFR) over time suggesting renal function affectation results from acute lithium toxication rather than prolonged use in the stable maintenance dose. Age, baseline eGFR, concomitant use of nephrotoxic medications, comorbidities and history of lithium toxicity rather than duration of exposure to lithium and average serum lithium levels were found to be predictors of reduction in eGFR(Clos et al., 2015).

Polyuria (urine output greater than 3000ml/24hrs) and polydipsia (excessive thirst) are common side effects of lithium therapy occurring in up to 70% of chronic use. Polydipsia is thought to result from reduction in the ability of the kidneys to produce concentrated urine Risk factors for these symptoms include increased treatment duration, raised serum lithium levels, multiple lithium intoxication, use of antipsychotics.

Lithium is notable for its narrow therapeutic window, with the implication that there is a tight margin of safety with small dosage changes capable of causing toxicity. Toxicity even within therapeutic serum levels is a real possibility with the use of the medication as blood lithium levels are not always an accurate reflection of intracellular toxicity. Chronic lithium toxicity is potentially life threatening due to the risk of lithium accumulation in the intracellular spaces. This is an important reason for lithium prescription hesitancy among psychiatrists despite it being widely recognized as the gold standard medication in the management of bipolar affective disorder (Gitlin, 2016).

Preventing lithium intoxication, regular serum lithium measurement with aim of achieving

efficacy at lowest possible concentration, annual serum creatinine monitoring and restricting lithium use to once daily dosing have been proposed to reduce the potential for nephrotoxicity from lithium use. (Gitlin, 2012)

The antithyroid effects of lithium are well recognized. Possible mechanisms include blockade of thyroid hormone release from the thyroid gland, reduced iodine storage in the thyroid and inhibition of thyroid hormone production. Some of these effects are even exploited for the treatment of hyperthyroidism (Kibirige et al., 2013; Lazarus, 2009).

Regarding neurological effects, tremor is a common side effect occurring in up to 25% of patients on lithium.(Gitlin, 2016) Lithium-induced tremor could occur at any point in treatment, especially in the early stage with potential for improvement after long-term treatment. Tremors were more frequent at serum lithium levels above 0.7mmol/l. Advanced age, use of antipsychotics and antidepressants, and increased serum lithium levels have been seen to correlate with higher risk of tremor.(Vestergaard et al., 1988). More serious neurological complications of lithium toxicity include cerebellar dysfunction manifesting as ataxia, dysarthria and dysmetria.

Though neurological symptoms of lithium use are usually reversible, permanent irreversible symptoms have been reported. A serious neurological condition termed “The syndrome of irreversible lithium effectuated neurotoxicity (SILENT) syndrome” has been described in 1987 following a review of 55 cases of persistent complications of lithium therapy. The neurological sequelae consisted of cerebellar signs though atypical presentations included optic neuritis, papilloedema, isolated downbeat nystagmus, peripheral neuropathy and myopathy. Widespread peripheral nerve demyelination was found on biopsy with the suggestion that similar demyelination may have also occurred in the central nervous system. (Adityanjee, 1987). Risk factors include fever, high serum lithium in the context of intoxication and co-administration of antipsychotics or antidepressants. The syndrome can also occur within therapeutic serum lithium concentrations and symptoms have been noted to persist for several months following lithium discontinuation highlighting the need to focus on prevention by addressing risk factors. (Farouji et al., 2023)

Weight gain has been shown to occur in the first 2 years of lithium treatment (average weight gain of 4kg) before weight is seen to stabilize. Pre-treatment body weight and concomitant antidepressant therapy were found to be contributory to weight gain.(Vestergaard et al., 1988). Postulated mechanisms of weight gain include increased thirst leading to intake of calorie-heavy drinks, undetected hypothyroidism and oedema.

Dermatological side effects including adverse cutaneous reactions have been reported from lithium therapy. The first report linking lithium use to adverse skin reactions was in 1968 with five patients on treatment for mania developing pruritic maculopapular rashes and cutaneous ulcers within the first 21 days of treatment. The lesions resolved upon dosage reduction or treatment with steroids and antihistamines without recurring with subsequent increase in the dose of the medication. Emphasizing the potential toxicity of lithium even within therapeutic levels, four of the five patients experienced the cutaneous reactions at serum lithium levels significantly lower than expected toxic levels. (Callaway et al., 1968). Acne and psoriatic lesions have also been reported with lithium therapy. Psoriasis was commoner in men possibly due to the effect of testosterone (Chan et al., 2000; Pinna et al., 2017)

In addition to the highlighted multisystem effects, lithium has been associated with a range of sexual side effects including erectile dysfunction, reduced libido, orgasmic dysfunction and premature ejaculation with mechanisms such as reduction in testosterone and impaired nitric oxide function (leading to impaired penile smooth muscle relaxation) implicated (Sheibani et al., 2022)(Almeida et al., 2023; Ferensztajn-Rochowiak & Rybakowski, 2023). Despite an extensive search of the literature, it was difficult to find published cases of penile telescoping in the context of treatment with lithium. To our knowledge, reports linking chronic lithium therapy with adult acquired buried penis are extremely limited, making this case clinically noteworthy.

Penile telescoping is the clinical manifestation of adult acquired buried penis, a condition in which a normal-sized penis progressively becomes concealed beneath suprapubic skin, subcutaneous tissue and fat. (Ho & Gelman, 2018)

Possible causes include obesity, connective tissue defects, penile scarring from lichen sclerosis or chronic balanitis.

Altered appearance and penile hygiene as well as voiding and sexual dysfunction may result. Spraying and dribbling of urine are possible outcomes as seen in the case being discussed. This has significant psychosocial implications with shame, stigma and practical difficulties with daily life as experienced in the limitation of sexual intercourse (patient's inability to have conjugal relations with his spouse). He also has difficulties passing urine normally. Obesity which is an important cause of the anatomical changes in the condition was also noted in this patient with a progressive increase in weight over the course of treatment. In the condition, the normal sized phallus appears shorter due to the reduction in the visible penile area.(Ho & Gelman, 2018) This is a potential cause for distress in men who may have come to associate penile length with virility and manliness, shaping perceptions of masculine competence sexual attractiveness and authority(Aich et al., 2026). This patient as noted below in the patient's perspective verbalized distress at the apparent shortening of the penis; this was compounded by the practical difficulties experienced in voiding and sexual intercourse.

Weight gain results in selective fat deposition in the suprapubic area. As the suprapubic fat pad surrounds the penis circumferentially, the patient is forced to sit to void due to dribbling of urine.(Ho & Gelman, 2018) This was voiced as a great source of distress in this case. The changes in the skin-penis structure provides a fertile ground for microbial growth as a result of persistent pooling of moisture around the penis leading to recurrent bacterial or fungal infections. This case also had suboptimal hygiene and developed a scaly rash. Chronic infection can result in inflammatory skin contracture and a phimotic scar ring which could then lead to further invagination of the penile shaft skin, further enveloping the penis.

The observed multiple organ system involvement in toxic effects is most plausibly attributable to chronic lithium therapy. A causal relationship is supported by a temporal relationship between the neurological, renal, endocrine, dermatological and metabolic features which clearly developed after prolonged use of lithium. These findings are biologically plausible, as each has been well described among the recognized adverse effects of long-term lithium treatment. The progressive clinical deterioration despite therapeutic serum lithium concentrations is in line with the body of evidence that chronic toxicity may occur through cumulative tissue exposure rather than raised serum levels alone

A partial improvement following lithiu.m dose reduction further supports a treatment-related effect, although established chronic complications may not be completely reversible. Alternative explanations, including concomitant chlorpromazine .and olanzapine therapy, obesity and ageing, were considered. While these factors may have contributed to specific issues such as weight gain, they do not fully explain the combination of renal dysfunction, neurological features, worsening psoriasis and the temporal progression observed over long-term follow-up.

The patient's acquired buried penis (penile telescoping) is unlikely to represent a direct adverse effect of lithium. Rather, it is more plausibly an indirect consequence of lithium-associated weight gain, with obesity promoting suprapubic fat deposition and progressive concealment of the penile shaft. This proposed mechanism is coherent with current understanding of adult acquired buried penis, in which obesity is recognized as the commonest underlying cause. Chronic psoriasis and inflammatory skin changes may also have contributed to local tissue changes, making the overall process multifactorial.

Although causality cannot be conclusively established from a single observational case, the temporal relationship, biological plausibility, multisystem involvement, longitudinal clinical course, partial improvement following lithium dose reduction and exclusion of more likely alternative explanations strongly support chronic lithium exposure as the principal contributor to the patient's adverse clinical outcomes. The acquired buried penis is most plausibly explained as an indirect consequence of lithium-associated obesity rather than a direct pharmacological effect of lithium itself.

The primary “take-away” lessons of this case report

Therapeutic serum lithium concentrations should not be regarded as absolute guarantees of safety, as chronic toxicity may occur despite levels within the therapeutic range. This case highlights the potential for cumulative multisystem adverse effects involving the renal, neurological, endocrine, dermatological and metabolic systems, with possible indirect consequences such as adult acquired buried penis secondary to lithium-associated weight gain. It underscores the importance of regular clinical and laboratory monitoring, early recognition of chronic toxicity, and multidisciplinary management to minimize morbidity, particularly in resource-limited settings where access to specialist care and monitoring may be constrained.

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Original Article

Differential Modulation of IRF1, IRF3 and IRF7 Gene Expression by an n-Hexane Fraction of a Polyherbal Formulation in Human Peripheral Blood Mononuclear Cells: An In-Vitro Study

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Abstract

Background: Interferon regulatory factors (IRFs) coordinate antiviral gene programmes, but the effects of non-polar polyherbal fractions on individual IRF pathways in primary human immune cells remain poorly defined. This exploratory study evaluated the effect of an n-hexane fraction of a polyherbal formulation on IRF1, IRF3 and IRF7 expression in peripheral blood mononuclear cells (PBMCs) from HIV-negative donors.

Methods: PHA-stimulated PBMCs were cultured without treatment or with 50 µg/mL of the n-hexane fraction. Total RNA was extracted, reverse-transcribed and analysed by quantitative reverse-transcription PCR, using GAPDH as the reference gene and the 2^-ΔΔCt method for relative expression. The available dataset comprised four observations per group. Independent-samples t-tests were used, and Welch's result was prioritised when variance homogeneity was violated.

Results: IRF1 expression decreased from 1.06 ± 0.05-fold in untreated cells to 0.50 ± 0.09-fold after treatment (52.7% reduction; $p < 0.001$). IRF3 increased from 1.06 ± 0.05-fold to 2.05 ± 0.63-fold (93.5% increase). The pooled-variance test yielded $p = 0.021$, but the variance-robust Welch test yielded $p = 0.052$. IRF7 changed from 1.06 ± 0.05-fold to 0.96 ± 0.62-fold and was not significant (Welch $p = 0.763$).

Conclusion: The n-hexane fraction produced a gene-specific transcriptional pattern characterised by marked IRF1 suppression, a large but statistically uncertain increase in IRF3 and no clear change in IRF7. These findings identify candidate immunomodulatory activity but do not establish antiviral efficacy. Larger donor-matched, dose-response and time-course studies with MIQE-compliant qPCR reporting, protein-level validation, cytotoxicity assessment and functional viral challenge are required.

Keywords: interferon regulatory factor; IRF1; IRF3; IRF7; innate antiviral immunity; PBMCs; polyherbal formulation; n-hexane fraction; RT-qPCR

Introduction

1. Introduction

Innate antiviral immunity provides the first coordinated cellular response to viral invasion. Recognition of viral RNA or DNA by pattern-recognition receptors, including RIG-I-like receptors, Toll-like receptors and cGAS-STING, activates adaptor proteins and kinases that converge on nuclear factor-κB and interferon regulatory factors (Al Hamrashdi & Brady, 2022). The resulting programme induces type I interferons and interferon-stimulated genes that restrict multiple stages of viral replication and shape subsequent adaptive immunity. (Lazear *et al.,* 2019). IRF1, IRF3 and IRF7 occupy distinct but overlapping positions within this network. IRF3 is constitutively expressed in many cell types and is activated rapidly by TBK1- or IKKε-mediated phosphorylation, dimerisation and nuclear translocation (Wang & Fish, 2019). IRF7 is generally expressed at lower basal levels but amplifies type I interferon production through positive-feedback signalling (Schwanke *et al.,* 2020). IRF1 contributes to constitutive and inducible antimicrobial defence, antigen presentation, apoptosis and inflammatory transcription (Schwanke *et al.,* 2020). Consequently, a compound that increases one IRF while suppressing another should be interpreted as a selective pathway modulator rather than a uniformly stimulatory or suppressive agent (Feng *et al.,* 2021; Ikushima *et al.,* 2013).

Peripheral blood mononuclear cells are useful for exploratory immunomodulation studies because they contain lymphocytes, monocytes and smaller dendritic-cell populations. Their cellular diversity improves physiological relevance compared with a single transformed cell line, but it also introduces donor variability and the possibility that bulk-RNA changes reflect altered cell survival, activation or subset composition. Experimental interpretation therefore depends on careful control of culture conditions, viability, biological replication and reference-gene stability (Ikushima *et al.,* 2013; Mogensen, 2019). Polyherbal formulations contain chemically diverse constituents that may act on multiple host and microbial targets. n-Hexane preferentially extracts lipophilic compounds, including some terpenoids, steroids, fatty acids and other non-polar metabolites (Al Hamrashdi & Brady, 2022). Plant-derived immunomodulators may alter oxidative stress, receptor-proximal kinases, transcriptional activity or cytokine production; however, their composition and activity can vary with species, plant part, geography, processing and extraction conditions. Botanical authentication and chemical standardisation are therefore essential for reproducibility (Balasubramaniam *et al.,* 2024; Song *et al.,* 2025).

The present experiment investigated baseline host-cell modulation in PBMCs obtained from HIV-negative donors. It did not expose cells to HIV-1 and therefore cannot demonstrate anti-HIV activity. This distinction is important because HIV-1 can evade innate sensing, and type I interferon responses may be protective early in infection yet contribute to chronic immune activation during established disease. A gene-expression change in uninfected PBMCs should therefore be regarded as a mechanistic signal that requires confirmation in functional infection models (Chen *et al.,* 2021). This study aimed to determine whether exposure to 50 µg/mL of the n-hexane fraction alters IRF1, IRF3 and IRF7 gene expression in HIV-negative human PBMCs. We hypothesised that the fraction would differentially modulate at least one of the selected IRF genes.

2. Materials and Methods

2.1 Study design

An exploratory in vitro comparative study was conducted using HIV-negative human PBMCs cultured under two conditions: untreated control and treatment with 50 µg/mL of the n-hexane fraction of a polyherbal formulation. The available statistical output contained four observations per condition. The conference abstract treated these observations as independent groups. The biological relationship between conditions must be confirmed because aliquots from the same donors would constitute a paired design.

2.2 Ethical Approval

Ethical approval was obtained from the Human and Biomedical Research Ethics Committee (HBREC) of Usmanu Danfodiyo University, Sokoto (Approval No: HBREC/25/025). The study complied with the Declaration of Helsinki on ethical principles for medical research involving human subjects.

Written informed consent was obtained from all participants, including permission for genetic testing and blood sample use. Participants were assured of confidentiality, and data were anonymized.

All HIV-related work was performed in a certified Biosafety Level 2 (BSL-2) facility with Class II biosafety cabinets. Staff underwent training in handling infectious materials.

2.3 Informed Consent

Written informed consent was be obtained from the all the study participants which include permission for genetic testing relevant to the research. They were made aware that all the information obtained will be treated with confidentiality and would be used for research purposes only and the research will be published.

2.4 Source of n-hexane fraction

The n-hexane fraction was sourced from the Department of Immunology, Usmanu Danfodiyo University, Sokoto, where it had been previously extracted and stored at 5°C in an airtight container. However, the PHF consists of five medicinal plants: *Acacia polyacantha* Willd. (bark), *Bauhinia rufescens* Lam. (bark), *Acacia senegal* (bark), *Adansonia digitata* (leaves), and Allium sativum (bulb).

A stock solution of the n-hexane fraction was prepared in dimethyl sulfoxide (DMSO) to concentrate. The stock was diluted in RPMI-1640 medium supplemented with 10% foetal bovine serum (FBS) and 20 U/mL interleukin-2 (IL-2) to achieve working concentrations of 25 µg/mL and 50 µg/mL, based on preliminary cytotoxicity assays and literature (Dispinseri *et al.,* 2014).

2.5 Blood sample collection

Six millilitres (6 mL) of whole blood were collected from the volunteers; a HIV-negative healthy volunteer, aseptically using EDTA Vacutainer. All collected samples were promptly transported on ice to the Centre for Advanced Medical Research and Training (CAMRET) at Usmanu Danfodiyo University, Sokoto, for PBMCs and HIV isolation.

2.6 PBMCs Isolation Method

The PBMCs were isolated using the Ficoll density gradient method; the procedure was used to isolate PBMCs from HIV negative volunteers. It was carried out according to the manufacturer's instructions as well as described previously by Hamid *et al.* (2021). Three millilitres (3 mL) of Histopaque-1077 (Sigma-Aldrich® Co. UK) were added to a 15 mL Falcon tube and brought to room temperature. Three millilitres (3 mL) of the 6 mL blood were carefully layered onto the Histopaque-1077. Afterwards, it was centrifuged at 400 × g for 30 min at room temperature. The middle layer, which contains the PBMCs, was aspirated carefully with a Pasteur pipette to within 0.5 cm of the opaque interface containing mononuclear cells and was carefully transferred with a Pasteur pipette into a clean conical centrifuge tube. The cells were washed by adding 10 mL of isotonic phosphate-buffered saline (PBS) solution using a Pasteur pipette and mixed by gently drawing in and out of a Pasteur pipette. The cells were washed by centrifugation at 250 × g for 10 min, and the supernatant was discarded. The cell pellets were suspended in 5 mL of isotonic PBS solution with a Pasteur pipette and were mixed by gently drawing in and out of the pipette. The cells were then centrifuged at 250 × g for 10 minutes. The step was repeated two times; the cell pellets were resuspended in 2 mL of RPMI medium and treated with 10% heat-inactivated foetal bovine serum (FBS) and 10% penicillin streptomycin antibiotics.

2.7 Pre-Treatment Cell Counts

The procedure was conducted strictly according to the manufacturer's instructions and as described

by Hamid *et al* (2021). Ten microliters (10 µL) of 0.4% Trypan blue (Sigma-Aldrich® Co. UK) solution (w/v) were combined with 10 µL of the PBMCs suspension in a cryovial tube (dilution factor = 2) and mixed. It was allowed to stand for 5 minutes in the dark. While the coverslip is in place, 10 µL of the Trypan blue-PBMCS suspension mixture was transferred to both chambers of the improved Neubauer counting chamber using a pipette. The viable and non-viable cells were counted under a light microscope. Non-viable cells appear blue, while viable cells remain colourless. The percentage of viable cells was then calculated using the formula below.

Calculations of percentage yield of viable cells

Cells/ml = the average count per square x dilution factor x 10^4 (Viable)

Total Cells = cells per ml x the original volume of fluid from which the cell sample was removed (Viable) (Appendix VII).

$$\text{Viable cells\%} = \frac{\text{Total number of viable cells}}{\text{Total number of cells (viable and non – viable)}} \times 100$$

2.8 Treatment of PBMCs with n-Hexane Fraction of the PHF

2.8.1 Cell Seeding and Treatment

HIV-infected PBMCs containing 1 × 10^5 cells were suspended in RPMI-1640 medium supplemented with 10% foetal bovine serum (FBS), and 190 µL of the cell suspension was dispensed into each well of a sterile 48-well microtiter plate (Appendix IX). Thereafter, 10 µL of phytohemagglutinin (PHA; 10 µg/mL) was added to each well to serve as a mitogen for PBMC activation. The cultures were incubated at 37°C in a humidified incubator containing 5% CO_2 for 24 hours to activate the PBMCs. After activation, treatments were added in 10 µL volumes according to the experimental grouping to achieve the required final concentrations. The experimental groups were arranged as follows:

Group A: Uninfected PBMCs without treatment (normal control).

Group B: Uninfected PBMCs treated with 10 µL of 50 µg/mL n-hexane fraction.

All experimental groups were set up in quadruplicate to ensure reproducibility and statistical reliability. The plates were subsequently incubated for an additional 24 hours at 37°C in a humidified atmosphere containing 5% CO_2. At the end of the incubation period, the treated PBMCs and controls were harvested aseptically for downstream molecular and protein analyses.

2.9 Determination of Gene Expression

2.9.1 Primer design

Primers for the target genes were designed using Primer-BLAST, with in silico validation for specificity using BLAST. The RNA sequences for the IRF1, IRF3, IRF7 and APOBEC3G genes was retrieved from the National Centre for Biotechnology Information (NCBI) database. Primer efficiency was assessed using a standard curve, and acceptable efficiencies fall within 90–110% as described by Rossi *et al.* (2019).

2.9.2 Extraction of mRNA

The mRNA extraction was carried out according to the procedure described by the manufacturer (Hunan Run Mei Gene Technology Co., Ltd, China). Two hundred microlitre (200 µL) of PBMC suspension was taken and placed in a clean Eppendorf tube. Then 200 µL of Lysis Solution was added to the tube, vortexed and shaken for 10 seconds. Afterwards, the tube was left to stand at room temperature for 10 minutes. Then, 200 µL of absolute ethanol was added to the tube, followed by vortexing and shaking for another 10 seconds. Afterwards, the Adsorption Column was inserted into a collection Tube. All the liquid from the centrifuge tube was transferred to the Adsorption Column, which was centrifuged at 8000 rpm for 1

minute. The filtrate was discarded. Afterwards, 600 µL of Washing Buffer I (with absolute ethanol added) was added to the Adsorption Column. It was then centrifuged at 8000 rpm for 1 minute, and the filtrate was discarded. Afterwards, 600 µL of Washing Buffer II (with absolute ethanol added) was added to the Adsorption Column. It was centrifuged at 8000 rpm for 1 minute, and the filtrate was discarded. Afterwards, the Adsorption Column was returned to the Collection Tube and centrifuged at 8000 rpm for 2 minutes. Afterwards, the filtrate and the Collection Tube were discarded. Afterwards, the adsorption Column was placed into an RNase-free 1.5 mL centrifuge tube, and 50 µL of Elution Solution was added to the centre of the membrane and left at room temperature for 1 minute. The column was centrifuged at 8000 rpm for 1 minute, then discarded. The nucleic acids were collected in the 1.5 mL centrifuge tube and stored at -20 °C as described by Rossi *et al.* (2019).

2.9.3 Determination of RNA Concentration and Purity

The concentration and purity of the extracted mRNA were determined using a NanoDrop spectrophotometer (Thermo Fisher Scientific, USA) according to the manufacturer's instructions. Briefly, the instrument was initialized and blanked using 2 µL of RNase-free water. Thereafter, 1 µL of the extracted RNA sample was carefully loaded onto the measurement pedestal, and the absorbance was measured at wavelengths of 280 nm. The RNA concentration was automatically calculated by the instrument and expressed in nanograms per microlitre (ng/µL) based on the absorbance at 280 nm. The purity of the extracted RNA was assessed using the A260/A280 absorbance ratio. Samples with A260/A280 ratios between 1.8 and 2.1 were of acceptable purity for downstream molecular applications. Samples exhibiting acceptable purity ratios were selected for subsequent complementary DNA (cDNA) synthesis and gene expression analysis. The quantified RNA samples were stored at -80°C until further use as described by Rossi *et al.* (2019).

2.9.4 Synthesis of cDNA and Relative Quantitative RT-PCR

The cDNA synthesis was performed using TranScript® Green One-step qRT-PCR Super Mix (SYBR Green) (TransGen Biotech, Beijing, China) according to the manufacturer's instructions. Quantitative RT-PCR was conducted using gene-specific primers for IRF1, IRF3, IRF7, and GAPDH. Fold change was calculated using the $2^{-\Delta\Delta Ct}$ method s described by Rossi *et al.* (2019).

2.9.5 The cDNA synthesis and quantitative RT-PCR analysis.

In an Eppendorf tube, qPCR reaction components were mixed to make the recommended 20 µL reaction system cocktail. The components of the reaction mixture included mRNA template from each fraction (1 pg), forward primer (0.4 µL), reverse primer (0.4 µL), TransScript® Green One Step-RT/RI Enzyme Mix (0.4 µL), 2× PerfectStart™ Green One-step qPCR SuperMix (10 µL), and RNase-free water (8 µL). The mixture was transferred into 0.2 mL PCR tubes. The tube was placed in a PCR machine to run (Rossi *et al.,* 2019).

2.10 Outcome measures and statistical analysis

The primary outcomes were relative fold-expression values for IRF1, IRF3 and IRF7. Data were summarised as mean ± standard deviation (SD). The submitted analysis used independent-samples t-tests and Levene's test for equality of variances, with two-sided $\alpha = 0.05$. When Levene's test was significant, the Welch unequal-variance result was treated as primary. Percentage change and Hedges' g were calculated to describe magnitude. No multiplicity adjustment was applied because this was an exploratory analysis. Raw Ct values and donor identifiers were unavailable; therefore, normality, outliers, technical replicate variability, reference-gene stability and donor pairing could not be reassessed.

Table 1: List of primer sequences used

Gene product mRNA		Primer sequence	Sequence position		GC (%)	Tm	Amplicon size (bp)
			Start	Stop			
IRF 1	F	5' CAAGGCCAAGAGGAAGTCAT 3'	633	653	50	62	112
	R	5' CATGTAGCCTGGAACTGTGTAG 3'	723	745	50	62	
IRF 3	F	5' TATGTTCCCGGGAGGGATAAG 3'	425	446	52.4	63	89
	R	5' GCTAAACGCAACCCTTCTTTG 3'	493	514	47.6	62	
IRF 7	F	5' ACACACACATGCTGGACTC 3'	883	902	52.6	62	148
	R	5' CTTGGTTGGGACTGGATCTG 3'	1011	1031	55	62	
GAPDH	F	5'CGGATTTGG TCGTATTGG 3'	343	361	50	58	97
	R	5'GTTGAGGTCAATGAAGGG 3'	422	440	50	57	

3. Results

3.1 Overall expression pattern

Untreated cultures had a reported mean relative expression of 1.0575-fold for each gene (SD 0.0506). After treatment, IRF1 decreased to 0.5006 ± 0.0854-fold, IRF3 increased to 2.0462 ± 0.6325-fold and IRF7 was 0.9551 ± 0.6188-fold. The pattern therefore comprised strong IRF1 downregulation, a large upward shift in IRF3 and little overall change in IRF7 (Table 2 and Figure 1).

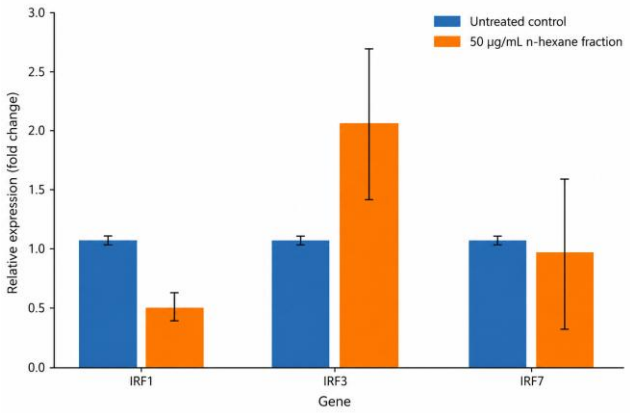

Figure 1: Relative expression of IRF1, IRF3 and IRF7 in untreated PBMCs and PBMCs treated with 50 µg/mL n-hexane fraction. Bars show mean ± SD.

3.2 Gene-specific comparisons

IRF1 expression was 0.5569-fold lower in treated than untreated cultures. Variance homogeneity was not rejected (Levene p = 0.425), and the pooled t-test showed a statistically significant difference (t = 11.22 for control minus treatment, df = 6, p < 0.001). The pooled 95% confidence interval for treated minus control was approximately −0.678 to −0.435. The estimated Hedges' g was −6.90, although this unusually large, standardised effect is inflated by the very low control-group variance.

IRF3 expression was 0.9887-fold higher after treatment. Variability was substantially greater in treated cultures, and Levene's test was significant (p = 0.011). The pooled test gave p = 0.021, whereas Welch's test gave t = −3.12, df = 3.04 and p = 0.052. The Welch 95% confidence interval for treated minus control ranged from −0.014 to 1.991. Hedges' g was 1.92. The result therefore represents a large, biologically notable but statistically uncertain upward signal. IRF7 expression was 0.1024-fold lower after treatment. Levene's test indicated unequal variances (p = 0.045), and Welch's test showed no evidence of a difference (t = 0.33, df = 3.04, p = 0.763).

Table 2: Relative IRF gene expression in untreated and treated HIV-negative PBMCs

Gene	Untreated control, mean ± SD	50 µg/mL n-hexane fraction, mean ± SD	Percentage change	Levene's *p* value	Primary *p* value
IRF1	1.0575 ± 0.0506	0.5006 ± 0.0854	−52.7%	0.425	<0.001[a]
IRF3	1.0575 ± 0.0506	2.0462 ± 0.6325	+93.5%	0.011	0.052[b]
IRF7	1.0575 ± 0.0506	0.9551 ± 0.6188	−9.7%	0.045	0.763[b]

Note: n = 4 observations per group. Welch's test is primary for IRF3 and IRF7 because Levene's test indicated unequal variances. The conference abstract reported IRF3 p = 0.021 from the pooled-variance row.

Table 3: Effect estimates for IRF gene expression in n-hexane fraction-treated versus untreated HIV-negative PBMCs

Gene	Mean Difference Treated − Control	Statistical test	*t* (df)	95% CI for mean difference	Hedges' *g*
IRF1	−0.5569	Student's *t*-test[a]	−11.22 (6.00)	−0.678 to −0.435	−6.90
IRF3	+0.9887	Welch's *t*-test[b]	+3.12 (3.04)	−0.014 to 1.991	+1.92
IRF7	−0.1024	Welch's *t*-test[b]	−0.33 (3.04)	−1.083 to 0.878	−0.20

Note: CI, confidence interval; df, degrees of freedom. All differences and effect-size signs are expressed as treated minus untreated control. Positive values indicate higher expression following treatment, negative values indicate lower expression. [a] Equal variances assumed because Levene's test was not significant. [b] Equal variances were not assumed because Levene's test indicated heterogeneity of variance. Hedges' *g* estimates should be interpreted cautiously because each group contained only 4 observations and the untreated-control variance was unusually low.

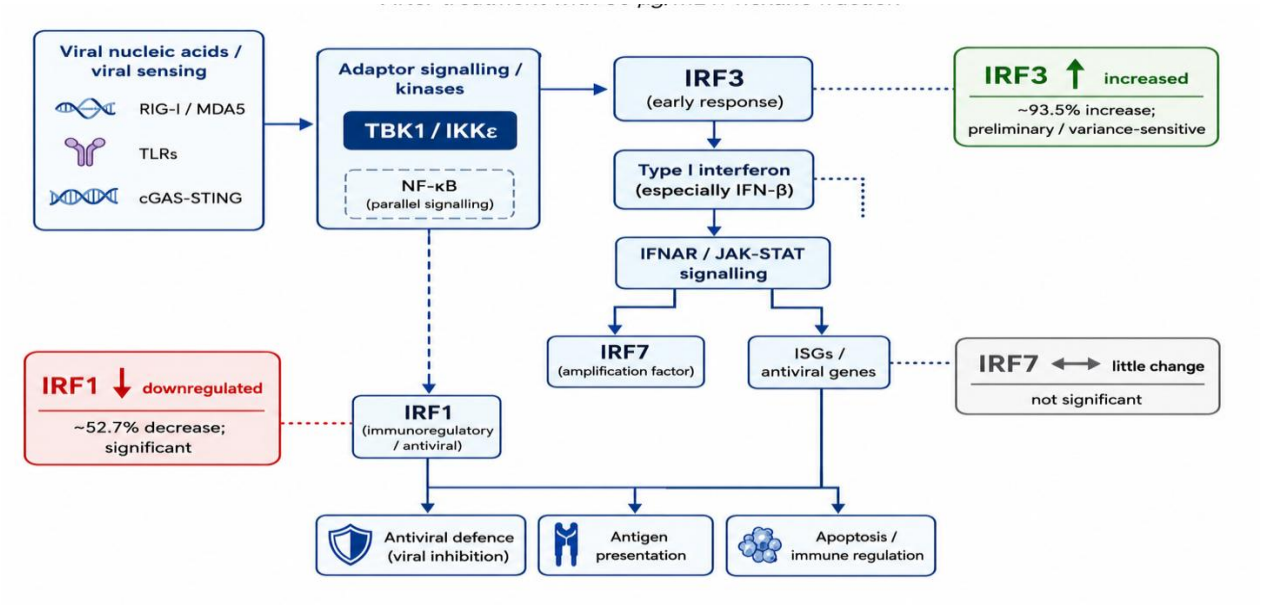

Figure 2: Simplified relationship of IRF1, IRF3 and IRF7 within antiviral signalling and the preliminary transcriptional pattern observed after treatment.

4. Discussion

This exploratory study found that the n-hexane fraction did not produce uniform activation of the three interferon regulatory factors examined. IRF1 was strongly suppressed, IRF3 showed a large but variance-sensitive increase and IRF7 remained broadly unchanged. The findings are therefore most consistent with selective transcriptional modulation rather than general stimulation of the type I interferon system.

The upward shift in IRF3 is potentially relevant because IRF3 is a sensor-proximal regulator of early antiviral signalling. Nevertheless, increased IRF3 mRNA does not demonstrate pathway activation. IRF3 activity depends on phosphorylation, dimerisation, nuclear translocation and interaction with transcriptional co-activators. Confirmation requires measurement of total and phosphorylated IRF3, nuclear localisation, IFN-β secretion and downstream interferon-stimulated genes such as MX1, OAS1, ISG15 and IFITM3 (Al Hamrashdi & Brady, 2022; Schwanke *et al.,* 2020).

The statistical interpretation of IRF3 is important. Although the pooled-variance row yielded $p = 0.021$, the treated-group SD was more than twelve times the control SD and Levene's test rejected variance homogeneity. Welch's $p = 0.052$ is therefore the more defensible primary result. This does not eliminate the observed biological signal; rather, it shows that the estimate is imprecise in a dataset of only four observations per condition. Reporting the effect estimate, confidence interval and individual biological replicates is more informative than classifying the result solely by a 0.05 threshold.

IRF1 decreased by approximately half and remained statistically significant in the supplied analysis. IRF1 supports antimicrobial defence, antigen processing and presentation, apoptosis and inflammatory transcription. Its suppression could weaken some protective responses, but it could also reflect regulatory or cytoprotective activity under a specific activation state. Without downstream gene, protein and functional measurements, the direction of biological benefit cannot be assigned (Feng *et al.,* 2021; Mogensen, 2019).

The absence of a clear IRF7 response may represent genuine selectivity, inadequate power or inappropriate sampling time. IRF7 often participates in a later positive-feedback phase of type I interferon induction; a single endpoint may therefore miss transient or delayed changes. Time-course sampling and multiple concentrations are needed to determine whether the fraction induces an early IRF3-centred response without subsequent IRF7 amplification (Ma *et al.,* 2023).

Changes in bulk PBMC RNA may also arise from altered cell viability, activation or subset composition. PHA promotes T-cell activation and proliferation, while an n-hexane fraction may affect lymphocyte and monocyte populations differently. Flow-cytometric immunophenotyping and formal cytotoxicity assays are required to distinguish direct transcriptional regulation from selective survival or expansion of PBMC subsets.

Chemical complexity is another major consideration. A non-polar fraction may contain multiple constituents with synergistic, additive or opposing effects. Bioassay-guided fractionation, chromatographic fingerprinting and testing of purified candidate compounds will be required to identify the active components and ensure batch consistency. Botanical authentication and extraction details are not optional reporting elements; they are necessary for scientific reproducibility and translational development (Balasubramaniam *et al.,* 2024; Song *et al.,* 2025).

The use of HIV-negative PBMCs establishes baseline host-cell effects but does not support claims of anti-HIV activity. Future experiments should expose donor-matched cultures to a controlled viral challenge or validated viral mimetic and measure viral replication alongside cellular viability. In an HIV model, relevant endpoints could include p24 antigen, viral RNA, proviral DNA and host restriction factors such as APOBEC3G, MX2, tetherin and SAMHD1. Appropriate antiretroviral and vehicle controls should be included.

RT-qPCR reporting must also be strengthened. GAPDH is frequently used as a reference gene, but activation and phytochemical exposure may change metabolic transcription. MIQE and MIQE 2.0 recommend validating reference-gene stability under the exact experimental conditions, reporting assay efficiency and specificity, distinguishing biological from technical replication and providing sufficient raw-data quality information for reproducibility (Bustin *et al.,* 2009, 2025; Livak & Schmittgen, 2001).

The principal limitation is the very small number of observations. Variance estimates are unstable, confidence intervals are wide and a single extreme value could alter the result substantially. The identical control means and SDs for all three genes should be checked against the raw dataset. In addition, uncertainty about donor pairing may affect the statistical model; if each donor supplied both untreated and treated aliquots, paired analysis is required. The single dose, single time point, absence of protein validation, lack of viability and PBMC-subset data, incomplete formulation characterisation and no adjustment for testing three genes further limit inference.

Despite these constraints, the use of primary human PBMCs and the gene-specific pattern provide a clear basis for follow-up. The most testable hypothesis is that one or more lipophilic constituents preferentially influence an IRF3-associated early signalling programme while suppressing IRF1-dependent transcription and leaving IRF7 amplification relatively unaffected. This hypothesis should be evaluated using a larger donor-matched design with chemical, cellular, molecular and functional endpoints.

5. Conclusion

Exposure of HIV-negative human PBMCs to 50 µg/mL of an n-hexane fraction of a polyherbal formulation produced differential IRF gene expression. IRF1 was strongly downregulated, IRF3 showed a large but statistically uncertain increase when unequal variance was considered, and IRF7 was not materially altered. The findings identify preliminary immunomodulatory activity but do not demonstrate antiviral efficacy. Confirmation requires larger donor-matched studies, multiple doses and time points, complete botanical and chemical standardisation, MIQE-compliant RT-qPCR, viability and immunophenotyping assays, protein-level pathway measurements and functional viral challenge experiments.

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