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Original Article

Prevalence and associated risk factors for *Helicobacter pylori* infection using stool antigen test among children presenting to the outpatient clinic of a tertiary hospital in Nigeria

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ABSTRACT

Objectives: *Helicobacter pylori* is a ubiquitous Gram-negative spiral, flagellate bacillus organism that infects about 50% of individuals globally. The distribution of *H. pylori* infection is influenced by age, sex, geographical location, ethnicity, and socioeconomic factors. Due to the geographic variations, which include intra-country variations and different epidemiologic factors associated with infections with *H. pylori*, local studies are imperative to assess the prevalence and risk factors of *H. pylori* as this could assist in marking out preventive measures for the locality. There are no studies on *H. pylori* infection in children presenting to the University of Calabar Teaching Hospital (UCTH), Calabar, South-south Nigeria. This study aimed to determine the prevalence and associated factors of *H. pylori* infection in Nigerian children aged 3–18 years of age using the stool antigen test.

Material and Methods: This was a cross-sectional study of 169 children aged 3–18 years presenting to the children's outpatient clinic of the UCTH. Semi-structured questionnaire was used to obtain information on socioeconomic and demographic characteristics, source of drinking water, personal and household hygiene, and social class. Fresh stool samples were collected and analyzed using lateral flow immune-chromatographic assay for the qualitative detection of *H. pylori* antigen in a fecal specimen. Adjusted odds ratio (AOR) with its 95% confidence interval (CI) was used to assess the strength of the association. To identify the associated factors of *H. pylori*, multivariable logistic regression models were built. In the multivariable analysis, variables with $P < 0.05$ were considered statistically significant.

Results: The prevalence of *H. pylori* among the study participants was 27.2%. The infection rate increased with an increase in the age of the participants, from 25.4% among 6–10-year-olds to 30.6% among 11–15-year-old adolescents. At univariate analysis, mother's level of education ($P = 0.002$), father's level of education ($P = 0.02$), social class ($P = 0.002$), place of residence ($P = 0.009$), number of rooms in the house ($P = 0.001$), method of domestic waste disposal ($P \leq 0.001$), and past history of diarrhea ($P = 0.007$) were significantly associated with *H. pylori* infection. After multivariable logistic regression analysis, living in a house with less than three rooms (AOR 0.38, 95% CI 0.16–0.89; $P = 0.026$), domestic waste disposal in the bush (AOR 0.18, 95% CI 0.06–0.50; $P = 0.001$), residence in urban areas (AOR 4.64, 95% CI 1.47–14.65; $P = 0.009$), and past history of diarrhea (AOR 2.88, 95% CI 1.23–6.74; $P = 0.015$ were independently associated with *H. pylori* infection).

Conclusion: The study showed a high prevalence of *H. pylori* infection among children presenting to the UCTH. Living in a house with less than three rooms, poor disposal of household waste, poor source of drinking water in schools, and a past history of diarrhea were risk factors for *H. pylori* infection. Public health education and the provision of potable water in schools are therefore advocated to curb *H. pylori* infection.

Keywords: *Helicobacter pylori* infection, Prevalence, Stool antigen test, Children, Nigeria

INTRODUCTION

Helicobacter pylori is a ubiquitous Gram-negative spiral, flagellate bacillus organism infecting about 50% of individuals globally.[1,2] The distribution of *H. pylori* infection is influenced by age, sex, geographical location, ethnicity, and socioeconomic factors.[3-5] The geographical distribution of *H. pylori* shows higher prevalence in developing countries of Africa, South America, and South-east Asia when compared to the developed countries, ranging from 5% in developed countries to 80% in developing countries.[6-13] Li *et al*.,[14] in a systematic review and meta-analysis, showed a declining trend in the pooled prevalence of *H. pylori* infection, particularly in the 2011–2022 period to 43.1% compared to 58.2% in 1980–1990 period globally, with the largest decline seen in the World Health Organization African region with a prevalence of 58.3%, this region still had the second highest prevalence following the Eastern Mediterranean region with a prevalence of 59.1%. In Nigeria, the prevalence of *H. pylori* infection ranges from 20% to 69%. The variation in the prevalence rate depends on the geographic region, location of studies such as hospital or community-based studies, type of diagnostic method used for the study, and socioeconomic and environmental factors [8,9,12,15-21] Infection with *H. pylori* occurs mostly through the faeco-oral or oro-oral routes and the risk factors includes poor socioeconomic status, overcrowding in household, poor personal and environmental hygiene, infection among household members, poor water supply, and fecal waste disposal method.[22-24]

Most infections occurring in early childhood are asymptomatic and may persist for life if left untreated.[2] However, some may present with gastrointestinal (GI) symptoms, including abdominal pain, diarrhea, gastric reflux, and peptic ulcer disease.[9,25,26] Chronic infections could lead to chronic gastritis, mucosa-associated lymphoid tissue lymphoma, gastric cancer, and gastric adenocarcinoma.[26,27]

H. pylori can be isolated using invasive and non-invasive methods.[25] The type of diagnostic method used to isolate *H. pylori* contributes to variable infection rates in children.[28] The invasive methods include an upper GI endoscopy with biopsies taken using modalities such as rapid urease test, culture, and histology.[25] In developing countries, upper GI endoscopy is expensive, the expertise and cost of the procedure is not within the reach of many.

Non-invasive methods have gained wider acceptability, and these include serology tests, urea breath tests, fecal antigen tests, and fecal polymerase chain reaction assay.[29] The sensitivities of the invasive and non-invasive tests are relevant and comparable in clinical practice.[29,30] *The H. pylori* stool antigen (HpSAg) test has high sensitivity and specificity, has gained popularity, and is almost readily available in all hospitals.

Due to the geographic variations, which include intra-country variations and different epidemiologic factors associated with infections with *H. pylori*, local studies are imperative to assess for the prevalence and risk factors of *H. pylori* as this could assist in marking out preventive measures for the locality. There were no studies on *H. pylori* infection in children using faecal antigen test presenting to the University of Calabar Teaching Hospital (UCTH), Calabar, South-south Nigeria. This study aimed to determine the prevalence and associated factors of *H. pylori* infection in Nigerian children aged 3–18 years of age using the fecal antigen test.

MATERIAL AND METHODS

Study design and setting

This was a cross-sectional study conducted at the children's outpatient clinic of the UCTH, Calabar, Cross River State, Nigeria. UCTH is the only tertiary hospital in Cross River State and serves as a referral center to other hospitals across the state and beyond. The clinic opens on weekdays and is closed on weekends and public holidays.

Study population

The study population consisted of children aged 3–18 years presenting to the children's outpatient clinic whose parents gave informed consent or assent given by the patients. Children whose parents did not give consent were excluded from participating in the study. Study participants were recruited purposively.

Sample size determination

The minimum sample size was determined to be 169 using the formula for single proportion[31] by taking 5% as the marginal error, 95% CI, and 11% prevalence from a previous study by Ugwuja and Ugwu.[12]

Data collection

An interviewer semi-structured administered questionnaire was used for data collection. Information obtained included the socioeconomic and demographic characteristics of the patient and family, the source of drinking water, and personal and household hygiene. The social class of parents was determined using the social classification proposed by Olusanya *et al*.[32]

Laboratory investigation

Fresh stool samples voided within 2 h were collected into clean, plainly labeled sample bottles. The time and date specimens were collected were indicated on each sample bottle. Specimens were voided for more than 2 hours, and watery fecal specimens were discarded. A qualitative assay was performed using onsite *H. pylori* Ag rapid test kits, a commercially available rapid lateral flow immunochromatographic assays for the qualitative detection of *H. pylori* fecal antigen manufactured by CTK (Biotech, Inc., 13855 Stowe Drive, Powey, CA 92064, U.S.A). Test was performed within 10 min of specimen collection. The membrane on the test line region of the test strip was pre-coated with HpSAg. During testing, the fecal specimen containing *H. pylori* reacts with the particle coated with HpSAg. The mixture migrates upward on the membrane chromatographically by capillary action and generates a colored line, and results are interpreted. The test result was read off at 10–15 min after the fecal specimen was applied to the sample well. The presence of this colored line in the test region indicated a positive result, while its absence indicated a negative result. To serve as a procedural control, a colored line appears in the control line region, indicating that the proper volume of membrane wicking has occurred. The HpSAg assay has manufacturer-reported specificity, sensitivity, and accuracy of 96.7%, 93.8%, and 94.9%, respectively.

Statistical analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences for Windows software version 25.1. Mean and standard deviations were calculated for continuous variables, while frequencies and proportions were calculated for categorical variables. Categorical variables were compared using the Pearson Chi-squared (χ^2) test. The odds ratio with 95% confidence intervals (95% CI) was calculated to measure the degree of relationship between risk factors for *H. pylori* infection and *H. pylori* positivity. Multivariable logistic regression was used to identify factors that independently predicted *H. pylori* infection among the study participants. $P < 0.05$ or 95% CI not embracing unity was accepted as statistically significant.

RESULTS

Sociodemographic characteristics of participants

A total of 169 children, 97 females (57.4%), were recruited into the study. Participants' age ranged from 3 to 18 years, with a median age of 10 years (interquartile range = 6–13 years). Children aged 6–10 years were the most represented with 34.9% of the sample population, while adolescents aged 16 years and above were the least represented with 10.1%. Most participants' parents were educated above the secondary level of education and 87% of participants' families were of the high socioeconomic class. This is shown in Table 1.

Prevalence of *H. pylori* infection

Of the 169 children studied, 46 (27.2%) were positive for *H. pylori* fecal antigen, while 123 (72.8%) were negative.

Association between sociodemographic variables and *H. pylori* infection

As shown in Table 2, there was a significant association between *H. pylori* infection and participants' mother's level of education ($P = 0.002$), father's level of education ($P = 0.02$), social class ($P = 0.002$), and place of residence ($P = 0.009$). However, there was no significant association between *H. pylori* infection and study participants' age or sex.

Association between household variables and *H. pylori* infection

H. pylori infection was significantly associated with the number of rooms in the house ($P = 0.001$) and method of domestic waste disposal ($P \leq 0.001$). Other household variables studied were not significantly associated with *H. pylori* infection [Table 3].

Association between school, hygiene, and other variables and *H. pylori* infection

As shown in Table 4, the source of drinking water in schools was significantly associated with *H. pylori* infection ($P = 0.038$). There was no significant association with the type of school, toilet facilities in school, or handwashing facilities in school.

Table 1: Sociodemographic characteristics of study participants.

Characteristic	Frequency (*n*=169)	Percentage
Age group (years)		
3–5	33	19.5
6–10	59	34.9
11–15	62	26.7
16–18	17	10.1
Sex		
Male	72	42.6
Female	97	57.4
Mother's level of education		
No formal/primary education	4	2.4
Secondary education	36	21.3
Higher education	129	76.3
Father's level of education		
No formal/primary education	3	1.8
Secondary education	35	20.7
Higher education	131	77.5
Social class category		
Middle	22	13
High	147	87
Place of residence		
Urban	118	69.8
Semi-urban	30	17.8
Rural	21	12.4

Table 2: Association between sociodemographic variables and *H. pylori* infection.

Variable	*H. pylori* Status		*P*-value
	Positive *n* (%)	Negative *n* (%)	
Age group (years)			0.84*
3–5	9 (27.3)	24 (72.7)	
6–10	15 (25.4)	44 (74.6)	
11–15	19 (30.6)	43 (69.4)	
16–18	4 (23.5)	13 (76.5)	
Sex			0.05*
Male	14 (19.4)	58 (80.6)	
Female	32 (33)	65 (67)	
Mother's level of education			**0.002****
No formal/primary education	1 (25)	3 (75)	
Secondary education	18 (50)	18 (50)	
Higher education	27 (20.9)	102 (79.1)	
Father's level of education			**0.02****
No formal/primary education	1 (33.3)	2 (66.7)	
Secondary education	16 (45.7)	19 (54.3)	
Higher education	29 (22.1)	102 (77.9)	
Social class category			**0.002***
Middle	12 (54.5)	10 (45.5)	
High	34 (23.1)	113 (76.9)	
Place of residence			**0.009***
Urban	25 (21.2)	93 (78.8)	
Semi-urban	10 (33.3)	20 (66.7)	
Rural	11 (52.4)	10 (47.6)	

*Chi-square test. **Fisher's exact test. Figures in bold are significant. *H. pylori*: *Helicobacter pylori*

Association of *H. pylori* infection with a past history of diarrhea

The prevalence of *H. pylori* infection was higher among participants who had diarrhea in the preceding 1 year compared to those who did not (40.7% vs. 20.9%; $P = 0.007$).

Predictors of *H. pylori* infection

After multivariable logistic regression analysis, factors significantly associated with *H. pylori* infection included living in a house with less than three rooms (Adjusted odds ratio [AOR] 0.34, 95% CI 0.14–0.82; $P = 0.016$), disposal of household waste in the bush (AOR 0.17, 95% CI 0.06–0.49; $P = 0.001$), residence in urban areas (AOR 4.19, 95% CI 1.28–13.79; $P = 0.018$), and past history of diarrhea in the last 1 year (AOR 2.88, 95% CI 1.23–6.74, $P = 0.015$). This is shown in Table 5.

DISCUSSION

Our study showed an overall prevalence of 27.2% for *H. pylori* infection among children presenting to UCTH, Calabar. This finding was comparable to those reported among children in other hospital-based studies in South-south[8] and neighboring South-east[20] Nigeria, with prevalence of 30.9% and 20%, respectively. However, the prevalence rate in this study was lower than the 65.7% and 69% reported in South-west[9] and North-east[21] Nigeria, respectively. The wide variation in prevalence rates reported from studies in different regions of Nigeria could be explained by the sociodemographic, environmental, and cultural factors in different parts of Nigeria. The use of serology tests in some of these studies may also account for the higher prevalence rates, as serological methods give higher false rates compared to stool antigen tests, as the antibodies to *H. pylori* can persist for several months after eradication.[14]

The prevalence of *H. pylori* in this study was comparable to the 24.3% reported in a hospital-based study in Ugandan children aged 1–15 years using a similar stool antigen test.[11] It was, however, lower than the prevalence of 60.4% reported among 3–10-year-old children in Benin Republic[33] or the 65.7% among 10–19-year-old adolescents in Ethiopia.[34] The variations in *H. pylori* prevalence rates worldwide are thought to be due to differences in levels of urbanization, access to good water, sanitation and hygiene services, and socioeconomic status.

Sociodemographic characteristics are widely regarded as being associated with *H. pylori* infection status. Low

Table 3: Association between household variables and *H. pylori* infection.

Factors	*H. pylori* status		*P*-value
	Positive *n* (%)	Negative *n* (%)	
Type of housing			0.451*
Flat	26 (25)	78 (75)	
Shared compound	13 (35.1)	24 (64.9)	
Duplex	6 (31.6)	13 (68.4)	
Others	1 (11.1)	8 (88.9)	
Number of rooms			**0.001****
<3 rooms	28 (41.8)	39 (58.2)	
≥3 rooms	18 (17.6)	84 (82.4)	
Number of children in household			0.101**
≤4 children	32 (24.2)	100 (75.8)	
>4 children	14 (37.8)	23 (62.2)	
Number of persons in household			0.128**
≤5	19 (22.1)	67 (77.9)	
>5	27 (32.5)	56 (67.5)	
Bed sharing			0.64**
No	10 (24.4)	31 (75.6)	
Yes	36 (28.1)	92 (71.9)	
Source of drinking water			0.082*
Stream	3 (42.9)	4 (57.1)	
Borehole/Pipe borne	40 (29.9)	94 (70.1)	
Bottled water	1 (5.6)	17 (94.4)	
Others	2 (20)	8 (80)	
Domestic waste disposal method			**<0.001***
Bush	17 (58.6)	12 (41.4)	
Burning	11 (23.4)	36 (76.6)	
Pit	4 (66.7)	2 (33.3)	
Others	14 (16.1)	73 (83.9)	
Household sewage disposal			0.391*
Pit latrine	4 (30.8)	9 (69.2)	
VIP latrine	7 (43.8)	9 (56.3)	
Bush	0 (0)	1 (100)	
Water closet	35 (21.2)	104 (74.8)	

*Chi-square test. **Fisher's exact test. Figures in bold are significant. *H. pylori*: *Helicobacter pylori*

socioeconomic class is considered an important risk factor for the acquisition of *H. pylori* infection. This is due to its attendant consequences of poor hygiene and unsanitary conditions, which strongly predispose toward the acquisition of *H. pylori* infection. This study showed a significant inverse relationship between socioeconomic status and *H. pylori* infection. This is consistent with results from studies conducted in Nigeria and other parts of the world. In Owerri, South-east Nigeria, *H. pylori* prevalence was 14.3% and 50% in upper and lower social classes, respectively.[20] In Uyo, South-south Nigeria, higher *H. pylori* seroprevalence was associated with low social class.[8] In Japan, a cross-sectional study found that the prevalence of *H. pylori* infection linearly decreased with increasing socioeconomic status.[35]

The previous studies report conflicting findings on the association between *H. pylori* infection and diarrhea. This study found an association between *H. pylori* infectivity and diarrhea, with prevalence higher among children who had diarrhea in the preceding 1 year compared to those who did not. This result is in agreement with several studies which reported a positive association between *H. pylori* infection and diarrhea. A study among Gambian children found that 53% of infants with chronic diarrhea and malnutrition were infected with *H. pylori* when compared with 26% of age-matched healthy controls.[36] Similarly, a multicenter study on the incidence, etiology, and adverse clinical consequences of diarrhea among children in developing countries found a significant association between *H. pylori* and diarrhea.[37] These studies may suggest the possibility that *H. pylori* infection increases susceptibility to other enteropathogens or is simply a comorbid condition since it shares similar routes of transmission and, thus, risk factors with diarrheal pathogens. In contrast, a large population-based study in Germany found a strong inverse association between gastric carriage of

Table 4: Association between school, hygiene, and other variables and *H. pylori* infection.

Variable	*H. pylori* status		*P*-value
	Positive *n* (%)	Negative *n* (%)	
Type of school			0.139
Private	33 (24.6)	101 (75.4)	
Public	13 (37.1)	22 (62.9)	
Source of drinking water			**0.038**
Pipe borne water	12 (29.3)	29 (70.7)	
Borehole	18 (28.6)	45 (75.4)	
Bottled water	6 (60)	4 (40)	
Sachet water	6 (13.6)	38 (86.4)	
Not available	2 (22.2)	7 (77.8)	
Toilet facility in school			0.148
Water closet	34 (24.3)	106 (75.7)	
VIP latrine	6 (37.5)	10 (62.5)	
Pit latrine	6 (46.2)	7 (53.8)	
Hand Washing facility in school			0.112
Yes	34 (24.6)	104 (75.4)	
No	12 (38.7)	19 (61.3)	
Hand Washing before eating			0.463
Always	38 (27.5)	100 (72.5)	
Often	4 (19)	17 (81)	
Sometimes	4 (40)	6 (60)	
Hand Washing after defecation			0.571
Always	36 (25.7)	104 (71.3)	
Often	5 (31.3)	11 (68.8)	
Sometimes	5 (38.5)	8 (61.5)	
Eat food cooked at home			0.355
Always	39 (28.5)	98 (71.5)	
Always buy food	0 (0)	7 (100)	
Sometimes	6 (31.6)	13 (68.4)	
Seldom buys food	1 (16.7)	5 (83.3)	
Worm medicine recently			0.294
Yes	31 (30.1)	72 (69.9)	
No	15 (22.7)	51 (77.3)	

Figures in bold are significant. VIP: Ventilated improved pit, *H. pylori*: *Helicobacter pylori*

Table 5: Logistic regression model for predictors of *Helicobacter pylori* infection among study participants.

Variables	*P*-value	Odds Ratio	95% CI	
			Lower	Upper
Had diarrhea in the past 1 year	**0.015**	2.88	1.23	6.74
School drinking water	0.280	1.20	0.86	1.69
Source of home drinking water	0.796	1.08	0.59	1.98
Mother's education	0.740	0.78	0.17	3.46
Father's education	0.702	1.33	0.31	5.47
Social class category	0.211	2.55	0.59	11.03
Less than three rooms in the house	**0.016**	0.34	0.14	0.82
Disposal of household waste in the bush	**0.001**	0.17	0.06	0.49
Residence in urban areas	**0.018**	4.19	1.28	13.79

CI: Confidence interval, Figures in bold are significant

H. pylori and the occurrence of diarrhea in children.[38] The contrast in findings between studies in a developed country like Germany and those in developing countries may be explained by contrasting exposure to enteric pathogens and differing availability of water, sanitation, and hygiene services, and most diarrhea in developed countries are due to chronic inflammatory diseases and not faeco-oral diarrheal microbial agents as is the case in developing countries.[39] Further studies are required to determine the true relationship between diarrhea and *H. pylori* infection.

The educational level of a child's parents, either independently or as a surrogate for socioeconomic status, is associated with *H. pylori* infection status. Our data showed that the prevalence of *H. pylori* infection significantly increased with a decrease in parental educational levels. This finding is consistent with data from earlier studies, which found an inverse association between the educational level of parents and *H. pylori* positivity. In China, a cross-sectional study among children aged 0–12 years found that having parents with a higher educational level was a protective factor against *H. pylori* infection.[40] Likewise, a systematic review and meta-analysis of observational population-based studies found that lower education of parents was significantly associated with a higher prevalence of *H. pylori* infection in children.[28] A higher educational level is believed to be associated with greater knowledge of good sanitation and personal hygiene practices which act to reduce the risk of *H. pylori* infection. The significant association between socioeconomic status and parental level of education with *H. pylori* infection indicates that poverty alleviation, provision of jobs, and improved access to education may be employed as strategies for *H. pylori* prevention among children in Calabar.

Household-level factors found to be significantly associated with *H. pylori* infection in this study were the location of residence, the number of rooms in the house, and the method of household waste disposal. Residence in a rural area was significantly associated with a higher prevalence of *H. pylori* than urban residence. This is in keeping with findings in a recent meta-analysis of global studies that the prevalence of *H. pylori* infection was higher in rural areas than in urban areas.[28] The higher prevalence among rural residents may be explained by the lack of access to good healthcare services, lack of potable water supply, poor sanitation and hygiene services, and low levels of education and poverty, which are common in rural areas in Nigeria. Living in a home with less than three rooms was a risk factor for *H. pylori* infection in children in this study. This may indicate that the relative lack of space in homes with less than three rooms enhances interpersonal contact, thereby facilitating the transmission of *H. pylori*. Thus, this finding provides further evidence in support of household crowding as a risk factor for *H. pylori* infection, as reported in earlier studies.[8,11,40] This study also found that disposal of household waste in open pits was associated with increased *H. pylori* prevalence compared to waste disposal in the bush or the practice of burning waste. Similarly, Etukudo *et al.*[8] found that open space disposal of household waste had a higher risk prediction for *H. pylori* infection than other waste disposal methods. This finding is presumably because refuse disposal pits are usually close to residential areas and are easily accessible to houseflies, thus increasing vector-borne transmission of *H. pylori* from fecal matter to food. There was no significant association between *H. pylori* positivity and other household variables studied, including number of persons in the house, bed-sharing, source of drinking water in the house, and method of home sewage disposal. This could be due to similarities in these characteristics among most of the study participants.

Several studies in the literature have established unclean water as a source of the spread of *H. pylori*.[8,11] Source of drinking water in schools was significantly associated with *H. pylori* infection of study participants. The provision of potable water supply in schools in Calabar would go a long way in reducing the prevalence of *H. pylori* infection among children. This study showed no association between the type of toilet in the school and *H. pylori* infection. There was also no association between hygiene-related variables such as washing hands before eating or after defecation and *H. pylori* infection status of children in Calabar. These findings of no association between some variables assessed and *H. pylori* infection status may be because most of the children studied had similar characteristics.

After multivariable logistic regression analysis, having less than three rooms in the house, disposal of household waste in the bush, residence in urban areas, and having diarrhea in the past 1 year remained significant as independent predictors of *H. pylori* infection among children in Calabar. These findings are in keeping with the predisposing factors to *H. pylori* infection, as demonstrated in other studies.[9-11]

The limitation of this present study is that the study was hospital-based; therefore, the prevalence may not be a true reflection of the prevalence of the infection in the communities.

CONCLUSION

The study showed a high prevalence of *H. pylori* infection among children presenting to the UCTH. Living in a house with less than three rooms, poor disposal of household waste, poor source of drinking water in schools, and a past history of diarrhea were risk factors for *H. pylori* infection. Public health education and the provision of potable water in schools are, therefore, advocated to curb *H. pylori* infection.

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Ethical approval

The research/study was approved by the Institutional Review Board at the University of Calabar Teaching Hospital, number UCTH/HREC/33/111, dated 15th January, 2021.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent.

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Conflicts of interest

There are no conflicts of interest.

Use of artificial intelligence (AI)-assisted technology for manuscript preparation

The authors confirm that there was no use of artificial intelligence (AI)-assisted technology for assisting in the writing or editing of the manuscript and no images were manipulated using AI.

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