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High Prevalence of Isolated Hepatitis B Core Antibody among Adults in Oyo State, Nigeria: The Need for Review of Diagnostic and Vaccination Guidelines

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Authors' contributions

This work was carried out by the two authors. Author OGO did the study design, statistical analysis, literature searches and manuscript writing while sample analyses were done by author ECO. Both authors read and approved the final manuscript.

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ABSTRACT

Background: Ten years of neonatal immunization with hepatitis B vaccine has not had any significant effect on the prevalence of the disease in Nigeria.
Aim: To investigate hepatitis B virus (HBV) markers as potential risk factors for viral transmission.
Study Design: A pilot prevalence study.
Place and Duration of Study: Community centers in Ibadan and Ogbomosho, between July 2014 and September 2014.
Methodology: One hundred and ninety-two apparently healthy volunteers (61 men, 131 women:

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age range 11-88 years) were investigated for HBV serological/immunological markers using enzyme linked immunoassays with 99 - 99.7% and 98.4 - 100% for specificity and sensitivity respectively (Diagnostic Automation/Cortez Diagnostics, Inc., Calabasas, CA, USA). Also data were processed with the statistical package for the social sciences version 20.0 (SPSS Inc., USA).
Results: A prevalence of 22.9%, 14.6%, 19.3%, 90%, 53.6%, 6.8% and 22.9% was obtained for HBsAg, HBsAb, HBcAb-IgM, HBcAb-Total, isolated HBcAb, HBeAg, and HBeAb, respectively. Analysis of the lifetime exposure of 90% among participants showed that 44 and 103 have markers for present (HBsAg) and past (isolated HBcAb) infections respectively, while 26 recovered with evidence of surface antigen neutralizing antibodies (HBsAb) in their serum. Seropositivity for HBsAg was highest among males and the 21-30 years old, while the female gender and persons aged between 30 and 40 years accounted for the largest percentage of isolated HBcAb carriers. Although history of exposure to risk factors like scarification, tattooing, injection from quacks, surgery, blood transfusion, sharing of sharp instrument/tools and injection drug use was common among participants, only 15.6% took hepatitis B immunization. On the overall, the risk of HBV infection was significantly associated with scarification and sharing of sharp instruments and tools.
Conclusion: The study highlights potential risk associated with the current diagnostic guideline for hepatitis B status and advocates the need for immunization among susceptible unvaccinated population.

Keywords: Hepatitis B virus; isolated HBcAb; adult population; high prevalence; Nigeria.

1. INTRODUCTION

Hepatitis B is a potentially life-threatening infection of the liver, ranked by the World Health Organization as one of the most common and serious infectious disease worldwide [1]. Presently three quarters of the world's population live in highly endemic areas, while about 2 billion are estimated to be infected [1]. Therefore the global disease burden of hepatitis B is substantial. Every year, there are over 4 million acute cases and about 25% of carriers, while 1 million people die from chronic active hepatitis, cirrhosis or primary liver cancer [1]. Hepatitis B is highly endemic in developing countries of Southern Asia and Tropical Africa, where at least 8% of the populations are chronic carriers and 70-90% becomes infected before the age of 40, showing past or present serological evidence of hepatitis B virus (HBV) infection [1]. Although most infections occur in children with little evidence of acute disease related to hepatitis B, the rates of chronic liver disease and liver cancers in adults are high [2].

The virus is transmitted majorly by exposure to infectious body fluids and sexual intercourse, while risk factors include medical/dental/surgical procedures, living with an infected person, multiple sex partners, reusing of needles and syringes, tattooing, risky sexual activities, and sharps contaminated with infected blood as well as working in healthcare facilities [3]. Hepatitis B is a vaccine preventable disease, and about 85-90% of HBV–associated death can be prevented, when given before or shortly after exposure. However, in spite of the availability of safe and effective vaccine for an upward of 20 years, worldwide infection persists [1]. Nigeria has adopted the universal neonatal vaccination through the expanded programme on immunization (EPI) and had utilized a monovalent hepatitis B vaccine since 2004. However in 2012, a pentavalent containing hepatitis B vaccine was introduced to commence at 6 weeks after the initial monovalent dose given at birth. Despite this continuous immunization exercise, the prevalence of HBV infection among Nigerians has remained high. Hence it becomes important to assess the population for viral factors with potential for the spread of the disease.

Immunity to HBV develops only in persons who are vaccinated or exposed to transient infection. Therefore, the prevalence of hepatitis B surface antigen (HBsAg) alone may be an underestimation of the current hepatitis B status in any population. Although most publications from Nigeria investigated the surface antigen prevalence [4-7]; data from other parts of the world have shown that this is not sufficient to predict latent infections. The recovery of HBV for many years in the blood of infected persons following clinical recovery and the induction of HBV-specific antibodies and cytotoxic T lymphocytes corroborated this fact [8]. Also HBV transmissions have been reported from surface antigen negative but hepatitis B core antibody (HBcAb) positive healthy individuals under

various circumstances [9-12]. Therefore, HBsAg seronegativity does not exclude the existence of HBV DNA in the blood and liver tissue of individuals with previous exposure to the virus. In this study, we have investigated a cohort of apparently healthy individuals for HBV markers. This is necessary to generate data to propose recommendations for limiting the spread and reducing the prevalence of the disease in the nearest future.

2. MATERIALS AND METHODS

2.1 Study Population

Public educational programs were organized bi-monthly at the community centers to give information on hepatitis B disease, risk factors, prevention, and control to participants. The study was conducted for 3 months (July - September 2014) in two cities (Ogbomosho and Ibadan) in Oyo State, Nigeria. However only consenting participants (older than 10 years) who were apparently healthy, with unknown hepatitis B status and have not had hepatitis B related symptom in the past were recruited for this study. The apparent health status of participants was evaluated from normal vital signs measurement and the absence of a medical complaint.

2.1.1 Data collection

Relevant information describing the demography, as well as past and present behavioural risks and exposure was obtained from recruited individuals. These include age, sex, occupation, marital status, scarification, transfusion, surgery, tattooing, indiscriminate injection from non-professional healthcare practitioners, drug abuse, multiple sex partners, sharing of sharp objects, and hepatitis B immunization status.

2.1.2 Sample collection

Three millilitres (3 mL) of venous blood specimen was collected from each participant. Samples were kept in upright position at room temperature for 45 minutes to allow clotting. Samples were spun for 15 minutes at 1,500 rpm at 4℃. The supernatant (sera) were aliquoted in 0.5 mL labelled cryovials and stored at -40℃ until used.

2.2 Sample Analysis

Assessment of recruited persons for HBV markers was done using some sets of antigen-antibody systems. These are the: the surface antigen-antibody system (HBsAg, HBsAb), envelope antigen-antibody system (HbeAg, HBeAb), core antibody system (HBcAb-IgM, HBcAb-Total). The ELISA kits (HBsAg, HBsAb, HBcAb, HBcAb IgM, HBeAg, HBeAb) were used according to the manufacturer's instructions (Diagnostic Automation/Cortez Diagnostic Inc., Calabasas, California, USA).

2.2.1 Detection of HBsAg and HBeAg

The analysis uses an antibody sandwich ELISA technique in which polystyrene microwell plates were pre-coated with monoclonal antibodies specific to HBsAg. Fifty microliters (50 µl) of test sera were added to plates together with 50 µl of a second antibody conjugated to the enzyme horse radish peroxidase (HRP-conjugate) directed against a different epitope of HBsAg. The incubation at 37℃ for 60 minutes allowed the formation of a specific immunocomplex which was captured on a solid phase. After washing 5 times with diluted wash buffer, chromogen solution containing tetramethyl benzidine (50 µl) and urea peroxide (50 µl) were added to the wells and was followed by incubation at 37℃ for 15 mins in the dark. The enzymatic reaction between the chromogen solutions and the HRP-conjugate produces blue coloured product in HBsAg positive wells. Finally intense yellow colour developed in positive wells following the addition of 50 µl stop solution. Absorbances were read with a dual filter reader at 450 nm/630 nm, and specimen giving an absorbance equal to or greater than the cut-off value was considered reactive.

2.2.2 Detection of HBsAb

The kit employed an antigen sandwich ELISA technique with recombinant HBsAg pre-coated on polystyrene microwells. Test sera (50 µl) and the horse radish peroxidase-conjugate (50 µl) were then added to labelled wells and plates incubated at 37℃ for 60 mins. The presence of anti-HBs initiated the binding of pre-coated and conjugated antigens to the two variable domains of the antibody, to form a specific immunocomplex captured on the solid phase during incubation. Following the removal of unbound HRP-conjugates by washing, 100 µl of chromogen solutions were added to the wells. The chromogens were hydrolysed by the bound HRP-conjugate during incubation for 15 mins at 37℃ in the dark to a blue coloured product which turned yellow after stopping the reaction

with 50 µl sulphuric acid. Colour intensity was read at 450 nm/630 nm and specimens with values greater than or equal to the cut-off are considered reactive.

2.2.3 Detection of IgM HBcAb

The principle of the IgM HBcAb ELISA was based on two-step incubation, solid phase antibody capture ELISA. To polystyrene microwells pre-coated with antibodies intended for human immunoglobulin M proteins were added 100 µl of test sera and were incubated at 37℃ for 30 min. This was followed by washing to remove IgG-class antibodies and other sample components. The specific anti-HBc IgM captured on the solid phase was then identified by the addition of purified HBcAg which is marked with anti-HBc monoclonal antibody conjugated to horseradish peroxidase (HRP). At the time of second incubation (37℃ for 30 min), the HRP-conjugated antigens reacted with anti-HBc IgM antibodies. Then, 100 µl of chromogen solutions were added after washing, and the content of the well reactive to HBcAb IgM turned from blue to yellow by the addition of a stop solution. Plates were read with a dual filter at 450 nm/630 nm and values greater than or equal to the cut-off were positive.

2.2.4 Detection of total HBcAb and HBeAb

The ELISA test used is based on the solid phase, competitive assay with one step incubation. Test sera were added to plates and incubated at 37℃ for 60 min. During incubation, the antibody present in the test sera competed with monoclonal antibodies conjugated to horseradish peroxidase for a fixed amount of purified antigen pre-coated in the wells. Plates were washed, followed by the addition of 100 µl chromogens which turned blue when the colourless chromogens were hydrolysed by the bound HRP-conjugate. Reaction was stopped with 50 µl of the stop solution to give a yellow colouration. Absorbance's were read with a dual filter instrument, and values less than or equal to the cut-off were considered positive.

2.3 Data Analysis

The data collected and ELISA results on HBV markers were processed with the statistical package for the social sciences Version 20.0 (SPSS Inc., USA). A significant association between outcome and predictor variables was calculated using multivariate logistic regression at 95% confidence interval and $P<0.05$ was considered significant.

3. RESULTS

3.1 Demographic Characteristics of Participants

During the 3-month study period from July to September 2014, 192 consented volunteers were recruited for this pilot study. Participants included 31.8% (61) males and 68.2% (131) females. The larger number of women in attendance at the educational programs was responsible for the inclusion of more women in the study. Also 125 persons (65.1%) were married, while 45 (23.4%) had more than one sex partner (Table 1). Recruited individuals were evenly spread through the 21-30, 31-40 and 41-50 age groups with 49, 56 and 44 individuals respectively, while the >11-20 and >50 years had 22 and 21.

Table 1. Demography and exposure to risk factors among apparently healthy participants in Ibadan and Ogbomosho, Oyo State, Nigeria

Variable	Total participants	Percentage
Demography		
Age categories		
>11-20	22	11.5
21-30	49	25.5
31-40	56	29.2
41-50	44	22.9
>50	21	10.9
Sex		
Female	131	68.2
Male	61	31.8
Risk factors		
Scarification	98	51.0
Blood transfusion	32	16.7
Tattooing	31	16.1
Surgery	33	17.2
Injection from quacks	65	33.9
Multiple sex partner	45	23.4
Injection drug use	8	4.2
Sharing of sharp Instruments/tools	140	72.9

The analysis of past exposure to risk factors showed that 51% and 16.1% had scarification and tattooing on their bodies, while 17.2% and 16.7% had undergone surgical procedure and took blood transfusion respectively (Table 1). Although sharing of sharp objects like knives,

razor, etc. was a common practice and reported by 140 (72.9%) participants, only 65 (33.9%) took injection from quacks, while 8 participants (4.2%) reported administration of unknown injectable provided by friends a few times. On the overall, only 30 (15.6%) participants took preventive Hepatitis B vaccine.

3.2 Detection of HBV Markers

A total of 44 (22.9%) participants were positive for HBsAg. Thirty seven of them had acute (HBcAbIgM+) and seven were chronic infection. The prevalence for HBSAg was higher among males (54.5%) while the 21-30 years category accounted for 36.4% of infected persons (Table 2). However HBsAg detection rate was lowest at the two extreme age categories representing 11.4% and 4.5% for 11-20 and >50 age groups respectively. Core antibody (HBcAbTotal) was found in 173 (90%) with 103 (53.6 %) representing isolated anti-HBc. The protective immunity to HBV (anti-HBs) was present only in 28 (14.6%) individuals; while a total of 17 (9%) had no serologic marker for HBV. Further investigation of hepatitis B status showed that 13 of the HBsAg positive cases were highly infectious; being HBeAg reactive, while 27 had seroconverted with anti-HBe in their blood. In addition, 10 participants with isolated anti-HBc and 7 with HBsAb were reactive for anti-HBe (Table 3).

3.3 Risk Factors Associated with HBV Positivity

One hundred and forty-seven (76.6%) individuals were presently seropositive for HBV infection without the neutralizing surface antibodies (HBsAb), this consists of 44 for current infection (HBsAg+) and 103 for past infection (isolated HBcAb+) (Table 3). The sex distribution of prevalence of 96 and 51 for female and male, respectively, was not statistically significant, while the seropositive individuals were found in all age groups.

Table 2. Distribution of Hepatitis B markers among apparently healthy participants in Ibadan and Ogbomosho, Oyo State, Nigeria

Variable	Total	HBsAg- HBcAb+ HBsAb+	HBsAg- HBcAb+ HBsAb-	HBsAg- HBcAb- HBsAb+	HBsAg+ HBcAb- HBsAb-	HBsAg+ HBcAb+ HBsAb-	HBsAg- HBcAb- HBsAb-
Number studied	192 (100%)	26 (13.5%)	103(53.6%)	2 (1.04%)	1 (0.52%)	43 (22.4%)	17 (9%)
Sex							
Female	131(68.2%)	22 (84.6%)	76 (73.8%)	1 (50%)	1 (100%)	19 (44.2%)	12(70.6%)
Male	61 (31.8%)	4 (15.4%)	27 (26.2%)	1 (50%)		24 (55.8%)	5 (29.4%)
Age groups (years)							
> 11-20	22 (11.5%)	1(3.8%)	11(10.7%)	1(50%)		5 (11.6%)	4 (23.5%)
21-30	49 (25.5%)	4 (15.4%)	24 (23.3%)			16 (37.2%)	5 (29.4%)
31-40	56 (29.2%)	11 (42.3%)	28 (27.2%)	1(50%)	1 (100%)	13 (30.2%)	2 (11.8%)
41-50	44 (22.9%)	7 (26.9%)	26 (25.2%)			7 (16.3%)	4 (23.5%)
> 50	21 (10.9%)	3 (11.5%)	14 (13.6%)			2 (4.7%)	2 (11.8%)

Abbreviations: HBsAg+/-, Hepatitis B surface antigen positive/negative; HBsAb+/-, Hepatitis B surface antibody positive/negative; HBcAb+/-, Hepatitis B core antibody positive/negative

Table 3. Prevalence of HBeAg and HBeAb among apparently healthy participants in Ibadan and Ogbomosho, Oyo State, Nigeria

Variable	HBsAg+	HBsAb+	Isolated HBcAb+
HBeAg+	13	0	0
HBeAg-	31	28	103
Total	44	28	103
HBeAb+	27	7	10
HBeAb-	17	21	93
Total	44	28	103

Abbreviations: Isolated HBcAb, Hepatitis B core antibody positive as the only marker of infection; HBeAg+/-, Hepatitis B e antigen positive/negative; HBeAb+/-, Hepatitis B e antibody positive/negative

Table 4. Multivariate logistic regression analysis of risk factors potentially associated with HBsAg positivity among apparently healthy participants in Ibadan and Ogbomosho, Oyo State, Nigeria

Variable	Odds Ratio (OR)	95% CI for OR		*P*-value
		Lower bound	Upper bound	
Age group				.40
Sex	1.118	.234	5.350	.89
Scarification	.081	.011	.589	.01*
Blood transfusion	2.428	.192	30.769	.49
Tattooing	42384978.86	.000		1.00
Surgery	1.442	.103	20.162	.79
Injection from quacks	1.921	.356	10.369	.45
Injection drug use	198976893.27	.000		1.00
Multiple sex partners	2.133	.331	13.762	.43
Sharing of sharp instruments/tools	1.551	.344	6.995	.57
Hepatitis B immunization	.407	.062	2.661	.35

** Significant risk factors; P<0.05*

Table 5. Multivariate logistic regression analysis of risk factors potentially associated with isolated HBcAb positivity among apparently healthy participants in Ibadan and Ogbomosho, Oyo State, Nigeria

Variable	Odds Ratio (OR)	95% CI for OR		*P*-value
		Lower bound	Upper bound	
Age group				.59
Sex	.597	.137	2.602	.49
Scarification	.278	.072	1.075	.06
Blood transfusion	.174	.018	1.714	.13
Tattooing	2.298	.274	19.239	.44
Surgery	.945	.146	6.111	.95
Injection from quacks	.561	.147	2.143	.40
Injection drug use	.000	.000		1.00
Multiple sex partners	.606	.105	3.490	.58
Sharing of sharp instruments/tools	24.051	2.420	239.078	.01*
Hepatitis B immunization	2.769	.569	13.471	.21

The association of other risk factors with infection was determined. In multivariate analysis, scarification (*P* = .01) and sharing of sharp instruments/tools (*P* = .01) were significantly and independently associated with Hepatitis B infection in the study (see above Tables 4 and 5). However, blood transfusion, indiscriminate injection from unqualified healthcare workers, previous surgery, multiple sex partners, and injection drug use were not significantly associated with HBsAg and HBcAb seropositivity.

4. DISCUSSION

The pilot study investigated the prevalence of HBV markers among apparently healthy individuals in two largest cities of Oyo state (Ibadan and Ogbomosho), with a view to provide potential evidence for viral factors in the unabated transmission of HBV in spite of over 10 years of neonatal immunization. This is necessary to generate data to appropriately target prevention/control measure for individuals born before the nationwide implementation of hepatitis B vaccine in the EPI in Nigeria in 2004.

An overall prevalence of 22.9% (44/192) was observed for active hepatitis B infection among participants. The seroprevalence of 22.9% is consistent with previous reports from Nigeria [4-7]. The reported prevalence ranges from 10.3% - 44.7%, depending on the sampling population. However, it was found to be much lower than the prevalence's among people living with HIV in Gombe (26.5%), chronic liver disease patients at a Nigerian Teaching Hospital (40%), primary school children in a rural community in Borno

state (44.7% [4-5,7]. The reason for this large difference might be because the surveys involved high risk groups. Although the number of male participants was lower than that of the females (61 vs. 131), the HBsAg seropositivity was higher among them (54.5% vs. 45.5%). Similar reports independent of the prevailing risk factors and degree of endemicity have been documented in populations randomly sampled for HBsAg seroprevalence [7,13-14]. The higher prevalence among the males has been attributed to the fast clearance of HBsAg in women [15]. Hepatitis B surface antigen carriage was found to increase with age and by 40 years; consequently, 79% have been infected. This according to El Beltagy et al. [16] may have been due to potential exposure over a long period of time, the non-immunization status of the older population as well as the lack of information on HBV infection and transmission in the past decades.

A lifetime exposure of 90% (HBcAb-Total) for HBV positivity due to past or present exposure was observed. This is in agreement with Alter (2002) that there could be 70-90% serological evidence of HBV exposure among populace in the highly endemic regions of South East Asia, sub-Saharan African and the Amazon Basin [2]. Similar studies reported 43.9% and 72.4% in China and Uganda respectively [17-18]. The endemicity of hepatitis B was revealed in the exposure pattern. This increase with age and by 50 years, 86.4% of the participants was infected.

Reports from several studies have implicated several risk factors in the transmission and acquisition of HBV. These include low educational level, used needles and syringes, family history of hepatitis B, scarification, dental procedure, sexually transmitted infections, sharing of multiple sex partners and lack of immunization with HepatitB vaccine [7,16,19-20]. However, tattooing, percutaneous injection drug use and high-risk sexual activity have accounted for most cases of newly acquired hepatitis B in the developed countries [21-23]. In the present study, scarification (P = .01) and sharing of sharp instruments/tools (P = .01) were significantly associated with the prevalence of HBsAg and HBcAb respectively. Scarification has been an age long cultural practice among various tribes in Africa for defining identity, beauty, strength, and rites of passage. This is usually done by traditional practitioners using blades and knives soaked in cleansing liquids. However, this practice has been identified as a significant factor in the spread of HBV in the region [7,24-25]. Sharing of sharp instruments and tools was found to be independently associated with the prevalence of HBcAb in this study. These include grooming items (razor, toothbrushes, and shaving/hair clippers) as well as tools for daily work tasks; they can serve as effective inanimate vehicles especially during percutaneous exposure to source positive for HBeAg. Instrument and tool sharing is a common practice within households and work places and has been documented as a significant source of HBV transmission [24,26].

Fifty-four percent (103/192) of the participants had isolated HBcAb. The prevalence of 54% is higher than previous report from Nigeria [27,28]. This may not be unconnected with the fact that the present study included a wider population range compared to the cohorts of blood donors and HIV patients on anti-retroviral therapy (ART) in previous studies. The presence of isolated HBcAb has been an issue of great concern with regard to HBV transmission. Often it represents a remote resolved infection, a low-level chronic infection, or the presence of a"window phase" between the disappearance of HBsAg and the appearance of HBsAb [29]. The potential for active hepatitis B infection in isolated HBcAb was demonstrated by HBV transmission from blood and organ donors who had isolated HBcAb [9-12]. Several studies from India, a developing country endemic for HBV infection documented HBV DNA detection (12.4% - 27.2%) in persons with isolated anti-HBc [30-32]. This poses a great risk to recipients of blood products and organs from anti-HBc individuals. Also these HBsAg seronegative DNA carriers are at risk for hepatitis B reactivation, following immunosuppressive therapy. In light of the above, therefore, Anti-core antibody screening should be implemented in the screening guideline for blood/organ donation as well as hepatitis B diagnosis in Nigeria. A decline in new infection of HBV occurred in several endemic countries following the introduction of anti-core screening [29,33-34] while nucleic acid testing (NAT) was recently implemented in low prevalence countries (Ireland, 2002; Germany, 2002; Canada, 2005). According to Pereira [35] and Jackson et al. [36], this had a small health benefit in the European Union and the USA at a very high cost relative to other blood safety measure. However it has been predicted that there would be a greater benefit in countries with high hepatitis B prevalence. Therefore participants with isolated HBcAb positive in this study shall be investigated further using HBV

vaccination and HBV DNA testing to rule out chronic infection. However those found infected would be referred for appropriate treatment and follow-up.

Although only 15.6% of the participants were vaccinated, vaccination appears not to be a significant factor in disease prevention (P>0.05). The result from this study showed that only 6.7% (2/30) of the vaccinees had protective immunity. This is highly suggestive of incomplete vaccination, although the study failed to include the number of hepatitis B vaccine doses taken in the data collection. Also this may not be unconnected with the fact that only small percentage of the participants was vaccinated. The incidence of hepatitis B in the developed countries has been reduced greatly by active infant vaccination, while a more comprehensive and established infrastructure for vaccine delivery to adolescents and adults has been implemented with considerable success in the USA [37-38]. Hence, the need for increased public awareness campaign for vaccination and possibly vaccine availability free of charge for adults in Nigeria. This as a suggested strategy for combating HBV spread caters for the adult population as they enter into the ages of greatest incidence of infection.

5. CONCLUSION

The study highlights potential risks associated with the current diagnostic guideline for hepatitis B status and advocates the need for adult population vaccination. Vaccination is the most effective strategy to halt HBV infection and until aggressive vaccination of the adults is embarked upon, the risk for hepatitis B infection and associated chronic liver diseases will continue unabated for many years to come.

6. LIMITATIONS OF THE STUDY

This study has some limitations. First, the self-reporting nature of risk factors may be subject to recall bias. Second, the sample size of 384 was halved due to the financial implication of the ELISA kits used for the analysis. However our findings show a new and immediate challenge to the policy makers.

ETHICAL APPROVAL

The study was approved by the University of Ibadan/University College Hospital ethics committee and its participants provided written or oral informed consent.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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