ORIGINAL RESEARCH ARTICLE

Assessment of Serological Markers of Genital *Chlamydia trachomatis* Infection Among the Gynaecology Patients attending Babcock University Teaching Hospital, Ilishan-Remo, Ogun State, Nigeria

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Abstract

Genital *Chlamydia trachomatis* infection causes significant morbidity and mortality in women. A number of epidemiologic studies have suggested that Polymerase Chain Reaction (PCR) is more accurate as a diagnostic tool for *Chlamydia trachomatis.* However, the use of serological markers may be cost effective and practical in diagnosing and estimating the burden of the disease in resource limited countries.This study was aimed at determining the serological markers (IgG, IgM and IgA) of *Chlamydia trachomatis*, evaluate the association between *Chlamydia trachomatis* infection and the sociodemographic characteristics and clinical diagnosis of the participants. This was a cross sectional hospital-based study in which blood samples from 145 consenting participants were tested for IgG, IgM and IgA antibodies against *Chlamydia trachomatis* using enzyme linked immunosorbent assay and their clinical diagnosis, retrieved from their case notes. The cumulative prevalence of seropositivity for *Chlamydia trachomatis* (IgG, IgM, IgA) was 112 (77.2%) while 33 (22.8%) were seronegative. The overall predominant seromarker was IgG 91(62.8%) while IgM and IgA accounted for 85(58.6%) and 54(37.2%) respectively. A statistically significant association was found between *Chlamydia trachomatis* infection and PID (p value = 0.031), primary infertility (p value 0.011) and level of income (p value= (0,045). *(Afr J Reprod Health 2019; 23[4]: 54-62).*

Keywords: *Chlamydia trachomatis*, Serological markers IgG, IgM, IgA, PID

Résumé

L'infection génitale à *Chlamydia trachomatis* provoque une morbidité et une mortalité importantes chez les femmes. Un certain nombre d'études épidémiologiques ont suggéré que la réaction en chaîne par polymérase (RCP) est plus précise en tant qu'outil de diagnostic pour *Chlamydia trachomatis*. Cependant, l'utilisation des marqueurs sérologiques peut être rentable et pratique pour diagnostiquer et estimer la charge de la maladie dans les pays à ressources limitées. Cette étude visait à déterminer les marqueurs sérologiques (IgG, IgM et IgA) de *Chlamydia trachomatis*, évaluer l'association entre l'infection à *Chlamydia trachomatis* et les caractéristiques sociodémographiques et le diagnostic clinique des participantes. Il s'agissait d'une étude transversale en milieu hospitalier dans laquelle des échantillons de sang de 145 participantes consentantes ont été testés pour les anticorps IgG, IgM et IgA contre *Chlamydia trachomatis* en utilisant un dosage immuno-enzymatique et leur diagnostic clinique, extraits de leurs notes de cas. La prévalence cumulative de la séropositivité pour *Chlamydia trachomatis* (IgG, IgM, IgA) était de 112 (77,2%) tandis que 33 (22,8%) étaient séronégatives. Le séromarqueur prédominant global était l'Ig G 91 (62,8%) tandis que l'Ig M et l'IgA représentaient respectivement 85 (58,6%) et 54 (37,2%). Une association statistiquement significative a été trouvée entre l'infection à *Chlamydia trachomatis* et le PID (valeur p = 0,031), l'infertilité primaire (valeur p 0,011) et le niveau de revenu (valeur p = (0,045). *(Afr J Reprod Health 2019; 23[4]: 54-62).*

Mots-clés: *Chlamydia trachomatis*, marqueurs sérologiques IgG, IgM, IgA, PID

Introduction

Chlamydia trachomatis serovars D through K typically cause cervicitis, PID in women and non-gonococcal urethritis in men[1]. Prevalence is consistently highest among young women aged 15 to 24 years compared to others and higher among blacks than other races[2]. Chlamydial infection in females is often asymptomatic or present with less severe symptoms than other sexually transmitted diseases. The asymptomatic nature precludes early diagnosis and treatment. This ultimately leads to complications such as such as Pelvic inflammatory diseases (PID) as chromic pelvic pain sets in, ectopic pregnancy, salpingitis, recurrent abortions and infertility[1,3-4].

According to the World Health Organization (WHO), About 131 million people are infected with Chlamydia each year[5]. Centre for Disease Control and prevention estimated approximately 3 to 4 million new cases of Chlamydia infection in the United States each year and 75% of such new cases are diagnosed among asymptomatic women[6].

In Nigeria, the prevalence of Chlamydia cervicitis was found to be 17.6% among women with infertility[3] while another study reported a prevalence of 33% among asymptomatic women[7]. A study also reported a prevalence of 7.3% among asymptomatic infertile women in Ibadan[8].

Considering the burden of Chlamydia, the United States Centre for Disease Control and Prevention recommends annual screening of women of reproductive age to ensure early diagnosis and treatment. This has been implemented in developed countries, but is yet to be done in developing countries like Nigeria[9, 10]. Unless implemented, the infection will remain a "silent epidemic"[11].

Although advances in highly accurate and non-invasive diagnostic testing such as PCR have allowed for better estimation of the burden of the disease, particularly the asymptomatic state, however, these methods of diagnosis need specialized equipment, infrastructure and trained personnel. Thus they are neither cost effective nor readily accessible in resource limited countries like Nigeria[12].

Considering the high prevalence of 33% among asymptomatic women as reported in a study in Nigeria[7], serological diagnosis may be more practical and accessible when compared to the molecular assays for *Chlamydia trachomatis.* While most patients are likely to present when there is a complication, a positive serology result on routine screening of at risk patients may be the only indication of chlamydial involvement[13].

The higher sensitivity and specificity of qualitative third generation ELISA for *Chlamydia trachomatis* serological markers makes it more reliable than the rapid diagnostic test strips used in most laboratories. Our facility uses the rapid tests for chlamydia diagnosis; this often yields negative results which are inaccurate and misleading.
Therefore, the aim of this study is to determine the serological markers of genital *Chlamydia trachomatis* among patients presenting at the gynaecology clinic of the Babcock University teaching Hospital, Ilishan-Remo, Ogun State, Nigeria and to evaluate the association between *Chlamydia trachomatis* infection and the sociodemographic characteristics and clinical diagnosis of the participants.

Methods

Study design

This was a cross sectional hospital-based study conducted in the gynaecology clinic of Babcock University Teaching Hospital from November 2017 to July 2018.

Study population

The participants were gynaecology patients attending the clinic at this period.

Inclusion criteria

Consenting females within the reproductive age, who were willing to fill the questionnaire, allow blood collection for *Chlamydia trachomatis* testing were included in the study.

Exclusion criteria

Those excluded were females who were on antibiotics or who had used antibiotics within the

previous 6 months and those who were not willing to provide consent.

Sample size determination

A sample size of 145 was calculated based on *Chlamydia trachomatis* seroprevalence of 9.2% found among gynaecology and STI patients in Kano, Northern part of Nigeria, to give a 95% confidence level and margin of error of ±5%.

Specimen collection and processing

Blood samples were tested using qualitative third generation enzyme linked immunosorbent assay (ELISA), type-specific for IgG, IgM and IgA against polypeptide derived from *Chlamydia trachomatis* major outer-membrane antigen (MOMP) (DIAPRO Diagnostic Bioprobes Milano Italy). All the blood samples were tested separately for each of these serology markers.

Data collection procedure

Pretested interviewer- based structured questionnaires were used to obtain the sociodemographic factors of the women and clinical diagnosis retrieved from their case notes.

Data analysis

Standard descriptive and inferential statistical analysis was carried out using SPSS version 23. (SPSS Inc. Illinios, USA). Means and standard deviations were derived for quantitative variables while proportions were derived for qualitative variables. Association between categorical variables was determined using Chi-square test at statistical significance level of set 5%. Ethical approval was received from Babcock University ethical review committee.

Results

The age of the 145 participants ranged from 15 to 45 years with the mean age of 23.7 years (SD 1.483). Majority of the participants were within the age range of 15 to 20 years 70(48.3%). Majority 127 (87.6%) of them were students, 110 (75.9%) were unmarried and 119 (82.1%) had tertiary education. The socio-demographic characteristics are illustrated in Table 1.

Majority 44 (30.3%) of the participants had PID, 22 (15.2%) had Dysmenorrhea, 14 (9.7%) had primary infertility, 11 (7.6%) was diagnosed with cervicitis, 7 (4.8%) had Secondary infertility, 6 (4.1%) had recurrent abortion and 41 (28.3%) were asymptomatic women who presented for preschool medical checkup.

Of the 145 participants, the cumulative seroprevalence for *Chlamydia trachomatis* (IgG, IgM, IgA) was 112(77.2%) while 33 (22.8%) were seronegative. Of the 112 seropositive participants, 37 (33%) had one seromarker, 32(28.6%) had two seromarkers, while 43 (38.4%) were positive for all three markers. Of the 37 who had one seromarker, 21(56.8%) were IgG, 13(35.1%) were IgM and 3(8.1%) were IgA. Of the 32 participants positive for 2 seromarkers, 24(75%) had both IgG and IgM, 5(15.6%) had IgM and IgA, while 3(9.4%) had both IgG and IgA.

In total, 91 (62.8%) participants were positive for IgG making it the predominant seromarker, 85(58.6%) were positive for IgM and 54 (37.2%) for IgA. The overall combined prevalence of *Chlamydia trachomatis* IgG and IgA was 46(31.7%), IgM and IgA was 48 (33.1%), IgG and IgM was 67 (46.2%). This is further illustrated in Table 2.

The relationship between *Chlamydia trachomatis* serological markers and the sociodemographic characteristics as well as clinical diagnosis of the participants are shown in tables 3 and 4 respectively. Table 5 shows the association between *Chlamydia trachomatis* infection and the sociodemographic and clinical diagnosis of the participants.

Discussion

Molecular diagnostic tools for *Chlamydia trachomatis* infection such as PCR has allowed for more accurate diagnosis and estimation of the disease burden but in resource limited countries such as ours, molecular diagnosis is expensive and not routinely found in most laboratories. Readily available screening tools such as serological markers are more practicable for determination of *Chlamydia trachomatis* infection.

Table1: Sociodemographic characteristics of the participants (N=145)

Variables	Frequency	Percentage
Age		
15-20	70	48.3
21-25	35	24.1
26-30	15	10.3
31-35	8	5.5
36-40	10	6.9
41-45	7	4.8
Marital status		
Single	110	75.9
Married	32	22.1
Widowed	2	1.4
Separated	1	0.7
Level of Education		
No schooling	3	2.1
Primary	3	2.1
Secondary	20	13.8
Tertiary	119	82.1
Type of housing		
Personal	88	60.7
Rented(≥ 2bedrooms)	29	20.0
Self-contained	16	11.0
Single room	7	4.8
Others	5	3.4
Level of income (Naira)		
< 18000	75	51.8
≥18000	70	48.2
Occupation		
Students	127	87.6
Professional	4	2.8
Artisan	4	2.8
Non-professional	10	6.9

In the index study, the cumulative prevalence of *chlamydia trachomatis* serologic markers was 77.2%. This is similar to the cumulative prevalence of 68.5% reported by Joyee AG et al in Indian where the three serology markers of *chlamydia trachomatis* were utilized as well as prevalence of 70.8% reported by Moses *et al in* Ilorin and 74% among subfertile women in Port Harcourt both in Nigeria [13-14]. While it is higher than rates reported in other regions in Nigeria such as 56.1% among gynaecology patients in Jos by Mawak et al[16] and 51% among pregnant women in Lagos state by Okoror et al[17], it is at variance with other studies in that reported much lower prevalence rates[18-19]. These variances in the prevalence rates across the country can be attributed to varying study population, sensitivity and specificity of laboratory diagnostic tool used.

The highest prevalence of *Chlamydia trachomatis* was observed among women in the 15- 25 age group 105 (72.4%), students 127 (87.6%), and those with tertiary level education 119 (82.1%). This is in line with previous studies in Nigeria and Argentina where a high prevalence rate was reported among young people3, 20, 21.These findings may suggest high sexual activity, lack or inconsistent use of condom, inadequate sexual health education and multiple sex partners among young women[22]. Furthermore, chlamydia infection was significantly higher amongst the unmarried women compared to the married ones. This is in agreement with previous finding reported in Enugu [18] but in contrast to data reported in Jos[16] both in Nigeria. This comparison however, should be done with caution as majority of the participants in our study were unmarried.

Majority of our study participants presented with PID 44(30.3%) and 91% were positive for at least one of the sero markers (IgG, IgM, IgA). Our finding is consistent with other studies carried out in India and America[13,23]. This observation suggests that serological studies may be useful in identifying *Chlamydia trachomatis* in ascending upper genital tract infection. Moreover, some studies have demonstrated that serology has been helpful in cases where direct test such as PCR, has failed to detect the presence of *Chlamydia trachomatis* in PID patients[13,23-24]. Indeed, serology prevents the morbidity associated with invasive procedures such as laparoscopy and hysterosalphingography (HSG) in women presenting with *Chamydia trachomatis* sequeale[24].

Type specific distribution shows that IgG seromarker was the most predominant (62.8%) and its presence indicates chronic infection or previous exposure. Seromarker IgM (58.6%), the second predominant marker in our study, signifies the presence of acute infection. However a previous study observed that antibodies produced during *chlamydia trachomatis* acute genital infection are usually long lived and cannot distinguish between current and previous infection[25]. Seromarker IgA (37.2%), the least predominant in our study has been reported as a better indicator of active infection because it has a half live of 5-7 days[26-27], thus useful for monitoring active infection after

Table 2: Cumulative prevalence of *chlamydia trachomatis* serological markers among the participants (N=145)

	***Chlamydia trachomatis* IgM**		
***Chlamydia trachomatis* IgG**	Positive (%)	Negative (%)	Total (%)
Positive	67 (46.2%)	24 (16.6%)	91 (62.8%)
Negative	18 (12.4%)	36 (24.8%)	54 (37.2%)
Total	85 (58.6%)	60 (41.4%)	145 (100%)
	***Chlamydia trachomatis* IgA**		
***Chlamydia trachomatis* IgG**	Positive (%)	Negative (%)	Total (%)
Positive	46 (31.7%)	45 (31.1%)	91 (62.8%)
Negative	8 (5.5%)	46 (31.7%)	54 (37.2%)
Total	54 (37.2%)	91 (62.8%)	145 (100%)
	***Chlamydia trachomatis* IgA**		
***Chlamydia trachomatis* IgM**	Positive (%)	Negative (%)	Total (%)
Positive	48 (33.1%)	37 (25.6%)	85 (58.6%)
Negative	6 (4.1%)	54 (37.2%)	60 (41.4%)
Total	54 (7.2%)	91 (62.8%)	145 (100%)

Table 3: Relationship between *Chlamydia trachomatis* serological markers and Sociodemographic factors of the Participants

Variables	***Chlamydia trachomatis* IgG**		***Chlamydia trachomatis* IgM**		***Chlamydia trachomatis* IgA**	
	Positive	%	Positive	%	Positive	%
Age						
15-20	40	43.9	33	38.8	22	40.7
21-25	27	29.7	24	28.2	12	22.2
26-30	8	8.8	10	11.8	8	14.8
30-35	4	4.4	6	7.1	4	7.4
36-40	8	8.8	8	9.4	5	9.3
40-45	4	4.4	4	4.7	3	5.6
Total	91	100	85	100.0	54	100.0
Marital status						
Single	70	76.9	61	71.8	37	68.5
Married	19	20.9	22	25.9	16	29.6
Widowed	1	1.1	1	1.2	1	1.9
Separated	1	1.1	1	1.2	0	0.0
Total	91	100.0	85	100.1	54	100
Occupation						
Student	80	87.9	77	90.6	48	88.9
Professional	2	2.2	2	2.4	1	1.9
Artisan	2	2.2	2	2.4	1	1.9
Non- Professional	7	7.7	4	4.7	4	7.4
Total	91	100	85	100	54	100
Level of Education						
No schooling	2	2.3	2	2,4	2	3.7
Primary	3	3.3	1	1.2	0	0
Secondary	15	16.5	15	17.7	10	18.5
Tertiary	71	78.0	67	78.8	42	77.8
Total	91	100	85	100	54	100
Type of housing						
Personal	54	59.3	48	56.5	29	53.7
Rented	19	20.9	19	22.4	14	25.9
Self- contained	11	12.1	10	11.8	7	12.9
Single room	6	6.6	5	5.9	3	5.6
Others	1	1.1	3	3.5	1	1.9
Total	91	100	85	100	54	100

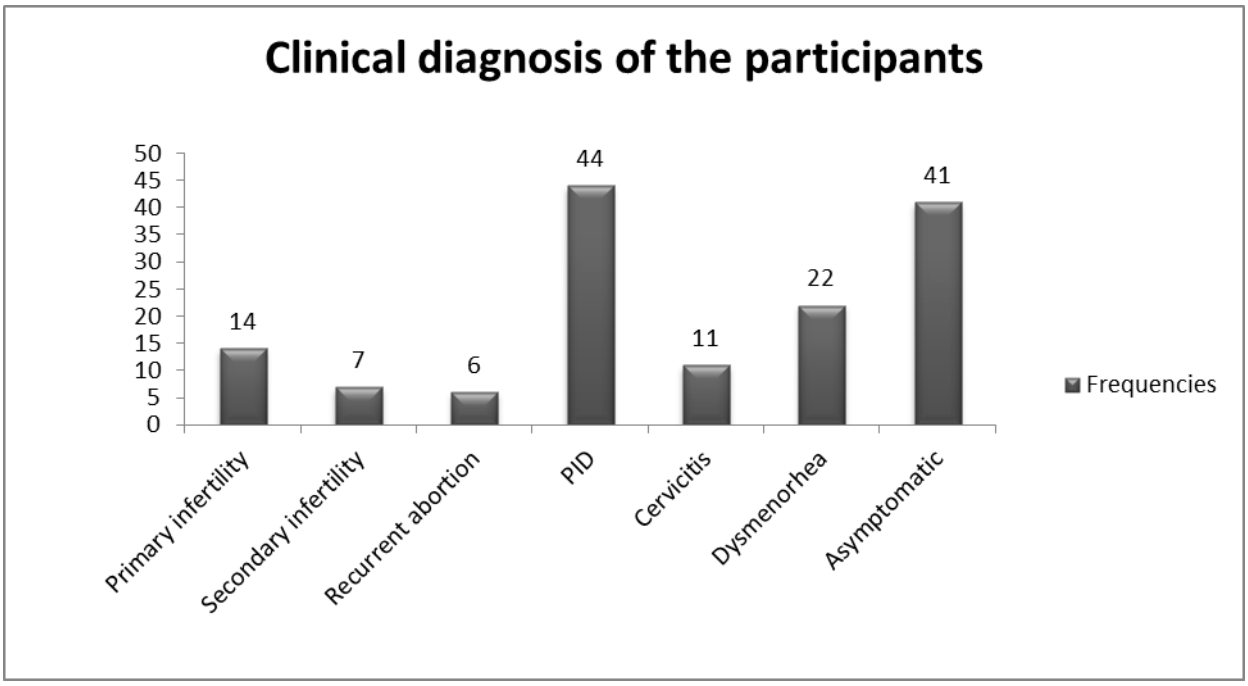

Figure 1: Clinical diagnosis of the participants N=145

Table 4: Relationship between Chlamydia trachomatis serological markers and clinical diagnosis of the participants (N=145)

Variables	*Chlamydia trachomatis* IgG		*Chlamydia trachomatis* IgM		*Chlamydia trachomatis* IgA	
	Positive	%	Positive	%	Positive	%
Clinical Diagnosis						
PID	32	35.2	33	38.8	14	25.9
Primary infertility	7	7.7	5	5.9	6	11.1
Secondary infertility	3	3.3	3	3.5	3	5.6
Recurrent abortion	4	4.4	3	3.5	2	3.7
Cervicitis	7	7.7	7	8.2	5	9.3
Dysmenorrhea	12	13.2	10	11.8	9	16.7
Assymptomatic	26	28.6	24	28.3	15	27.8
Total	91	100.	85	100	54	100.

treatment[26] In resource limited countries where access to molecular tests such as PCR is limited, seromarkers may be the only indicator that provides evidence of patients contact with *Chlamydia trachomatis.*

Some studies has suggested that the presence of IgG and IgA is a better indicator of chronic or persistent infection[25,28]. Our study shows the overall prevalence of chronic/ persistent infection was 46 (31.7%) based on the presence of *Chlamydia trachomatis* IgG and IgA altogether. This finding suggest that a high percentage of these women have chronic persistent chlamydia infection which can be transferred to their sexual partners or if /when pregnant, to their newborn thereby worsening the silent epidemic.

Cofactors significantly associated with increased positivity of *Chlamydia trachomatis* infection were PID, primary infertility and low level of income. This further buttresses the need for use of cost effective diagnostic tool to routinely screen women of reproductive age

Table 5: Association of chlamydia trachomatis infection with Socio-demographic factors and clinical diagnosis

Variables	Chlamydia infection		X^2	P -value
	Positive(%)	Negative		
Sociodemographic factors				
Age (years)				
15-20	50(71.4)	20(28.6)		
21-25	28(80.0)	7 (20.0)	5.339	0.378
26-30	11(73.3)	4 (26.7)		
31-35	7 (87.5)	1 (12.5)		
36-40	10(100.0)	0 (0.0)		
41-45	6 (85.7)	1 (14.3)		
Marital status				
Single	83(75.5)	27(24.5)		
Married	27(84.4)	5 (15.6)	2.265	0.159
Widowed	1 (50.0)	1 (50.0)		
Separated	1 (100.0)	0 (0.0)		
Level of Education				
No schooling	2 (66.7)	1 (33.3)		
Primary	3 (100.0)	0 (0.0)	1.202	0.753
Secondary	16 (80.0)	4 (20.0)		
Tertiary	91 (76.5)	28 (23.5)		
Type of housing				
Personal	65 (73.9)	23(26.1)		0.690
Rented (≥ 2)	25 (86.2)	4 (13.8)	2.250	
Self-contained	12(75.0)	4 (25.0)		
Single room	6(85.7)	1(14.3)		
Others	4(80.0)	1 (20.0)		
Level of income (Naira)				
< 18000	63(84.0)	12(16.0)	4.037	0.045
≥18000	49(70.0)	21(30.0)		
Occupation				
Students	99(78.0)	28(22.0)		
Professionals	2 (50.0)	2 (50.0)	1.780	0.619
Artisans	3 (75.0)	1(25.0)		
Non- professionals	8 (80.0)	2(20.0)		
Clinical diagnosis				
PID	39 (88.6)	5(11.4)	4.666	0.031
Primary infertility	7 (50.0)	7(50,0)	6.439	0.011
Secondary infertility	3(42.9)	4(57.1)	4.947	0.26
Recurrent abortion	4(66.7)	2(33.3)	0.398	0.528
Cervicitis	9(81.8)	2(18.2)	0.142	0.706
Dysmenorrhea	17(77.3)	5(22.7)	0.000	0.997
Asymptomatic	33(80.5)	8 (19.5)	0.343	0.558
P value less than 0.05 is taken as significant				

particularly those at risk and thus aid early diagnosis, treatment and prevent morbidity associated with *Chlamydia trachomatis* infection. This will go a long way in reducing the burden of the disease and address the silent epidemic.

Conclusion

Use of serologic markers is practical and cost effective in diagnosing *Chlamydia trachomatis* infection in symptomatic and asymptomatic women and is a useful tool for routine use in screening women at risk for the infection. In view of the potential sequelae and the morbidity associated with *Chlamydia trachomatis* among females, it may be necessary to implement prevention and control strategies like screening programmes by using serological markers. This is even more pertinent in resource limited country like ours where molecular diagnostic tools like PCR might not be affordable, accessible and therefore impracticable.

Contribution of Authors

TINUADE ADESOLA AJANI contributed to this article by conception, design, acquisition, analysis and interpretation of data, drafting the article, final approval of the version to be published and general supervision of the work. CHARLES JOHN ELIKWU, VICTOR UGOCHUKWU NWADIKE, BABATUNDE TAYO, OPEOLUWA SHONEKAN, CHIKA CELEN OKANGBA, CHINENYE GLORIA ANAEDOBE, TUNDE EHIMEN THOMPSON, EJIME EBEIGBE, AZUBUIKE OMEONU, BIBITAYO FALUYI contributed to this article by the design and interpretation of the data, drafting the article and final approval of the version to be published. MUSTAPHA AKANJI AJANI, TIMOTHY OLUWASOLA AKINTOYE OLUSEGUN COKER contributed by analysis and interpretation of data, drafting the article and final approval of the version to be published while AMELIA KIKI- JOSHUA, TITILOPE KOLAWOLE, HERITAGE KRISTILERE, CHIBUIKE MARTIN MEREMIKWU, LUCKY MGBEMENA, CHIKA SOLOMON-NWAEJIKE, AYODEJI SALAMI, ANATORUN TANTUA, MAYOWA TIMOTHY, TOBENNA AKAGBUSUM contributed to the acquisition, analysis and interpretation of data and drafting the article.

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