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SEROPREVALENCE OF *BARTONELLA HENSELAE* IgG AND IgM ANTIBODIES AMONG HIV INFECTED INDIVIDUALS ATTENDING HEART-TO-HEART CLINIC AT FEDERAL TEACHING HOSPITAL IDO-EKITI, NIGERIA

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ABSTRACT

Bartonella henselae (Bh) is a fastidious, zoonotic, Gram-negative bacteria pathogen known to infect both immunocompetent and immunocompromised individuals. It is known to cause a range of clinical symptoms with bacillary angiomatosis and bacillary peliosis hepatitis being the most common complication in HIV patients. The clinical diagnosis of bacillary angiomatosis is readily missed for Kaposi's sarcoma. The paucity of empirical data of its burden has led to its neglect, thus this cross-sectional study was conducted to evaluate *Bartonella henselae* infection burden among HIV subjects attending Heart-to-Heart clinic at Federal Teaching Hospital Ido-Ekiti (FETHI). One hundred and eighty-four HIV infected subjects were evaluated for *Bartonella henselae* IgG and IgM using enzyme-linked immunosorbent assay (ELISA). $CD4^+$ count was collected from the information management system of the facility. The prevalence of *Bartonella henselae* IgG and IgM was 51% and 38% respectively. The seropositivity rate of *Bartonella henselae* IgG was higher among females (52%) and subjects who are yet to commence highly active antiretroviral therapy (HAART) [62%]. Prevalence rate of *Bartonella henselae* IgM was uniform across groups. Prevalence was not significantly influenced by sex, HAART and $CD4^+$ count ($p > 0.05$). There is a high infection burden of *Bartonella henselae* among HIV infected patients attending the clinic at Federal Teaching Hospital Ido-Ekiti requiring attention in the management of these patients.

KEYWORDS: *Bartonella henselae,* Bacillary angiomatosis, seroprevalence, Cat scratch disease, HIV, $CD4^+$, HAART.

1.0 INTRODUCTION

Bartonella henselae is a zoonotic, fastidious, facultative intracellular, pleomorphic Gram-negative pathogen known to cause persistent bacteremia in humans.[1-8] It is one of the ten *Bartonella species* known to cause Bartonellosis in humans which causes a range of disease conditions depending on the infecting species. *Bartonella henselae* causes an expanded spectrum of clinical symptoms in human patients which ranges from cat scratch disease (CSD), bacillary angiomatosis.[9,10] bacillary peliosis hepatitis, uveitis, retinal artery and vein occlusions, focal retinal phlebitis, endocarditis, and relapsing bacteremia. Occasionally neurologic sequelae such as neuronitis, retinal and neovascularization, encephalopathy, myelopathy, meningitis, cerebral arteritis, optic neuritis and radiculopathy may ensue.[11-15]

CSD was first reported in 1950 in France but its etiology was only linked to *Bartonella henselae* in 1990.[16] The cat (*Felis catus*) is its natural reservoir with an 81% prevalence among young cats less than one-year-old Worldwide, [17,19] while humans and dogs are accidental hosts. Transmission is usually by a cat scratch or bite and occasionally by vectored by cat flea (Ctenocephalides felis) and ticks.[4,20,42]

Bartonella henselae establishes persistent bacteremia by taking advantage of the immune system and strategically remaining within potential sanctuary sites following infection: (a) extracellular matrix following infection, where high local bacterial replication may be quietly achieved.[21] (b) bone-marrow stem cells such as

erythroid progenitor cells where $CD34^+$ cells in the bone marrow may be infected thus releasing infected erythrocytes into the bloodstream or endothelial progenitor cells which may also get infected in the bone marrow before their mobilization into circulation for endothelial repair mature endothelial cells.[22] (c) mature endothelial cells. It then evades the immune system with the help of its characteristic lipopolysaccharide (LPS) which is uniquely composed of Lipid A and long-chain fatty acids.[23] The inability of the Toll-like receptor 4 (TLR-4) of the dendritic cells and macrophages to recognize the surface molecule of *Bartonella* evade the innate immunity while its LPS does not induce tumor necrotic factor-alpha (TNF- α),[24,25] and has a reduced stimulation for TLR-2 thus a reduced endotoxicity.[25,26] These favors the establishment of persistent bacteremia. They also can lysosomal fusion and acidification after the bacteria invades phagocytes such as endothelial cells and macrophages.[16]

Bartonella henselae infects both immune-competent and immune-compromised humans. In the immune-compromised (e.g. HIV patients) and immune-suppressed (e.g. post-transplant patients on immune suppressive therapy), the most common sequelae are bacillary angiomatosis characterized by vasoproliferative lesion.[10] *Bartonella henselae* induces a localized bacterial replication which is facilitated by an antiapoptotic state in endothelial cells induced by secretion of effector proteins (BepA and BepA2) which bind to the endothelial membrane receptor.[27] The ensuing transmembrane signal transduction results in high cytoplasmic cAMP levels which in turn upregulate cAMP-responsive genes and induce an antiapoptotic state in the endothelial cells, resulting in their proliferation.[28] BepA has also been shown to be able to inhibit cytotoxic T- mediated apoptosis of endothelial cells infected by *Bartonella henselae* and *Bartonella quintana*. *Bartonella henselae* has been associated with the development of AIDS-related neurological disease and HIV-associated dementia.[28]

Seroprevalence of *Bartonella Henselae* among HIV infected patients is estimated to be 17.3 – 47.5% globally,[29] and 3.6 – 6.0% in healthy individuals. Prevalence among healthy people is higher among the veterinarians (7 – 15%),[30] children (8.5 – 61.6% in Italy),[31] and cat owners.[32] The annual estimate in the US is about 22000 – 24000 with about 2000 cases[30] requiring hospitalization. Seroprevalence has been reported to be lower both in humans and cats from the northern latitudes, increasing in warmer climates.[17]

Bacillary angiomatosis occurs primarily in immunocompromised persons. Though treatable and curable, It may be life-threatening if not treated [9, 10]. This most often than not goes untreated due to missed diagnosis because it has a similar clinical manifestation with Kaposi's sarcoma which is also an angioproliferative opportunistic infection caused by human herpesvirus 8 seen in HIV infected patients with low $CD4^+$ count.[31] This is further compounded by the fact that *Bartonella henselae* is fastidious and does not readily yield growth in a blood culture notwithstanding bacteremia. Once blood culture results yield negative, coupled with clinical presentations that overlap with that of Kaposi's sarcoma, diagnosis is usually concluded in favor of Kaposi sarcoma resulting in a false diagnosis consequently a missed treatment. Hence the study was designed to evaluate the prevalence of *Bartonella henselae* among HIV subjects which is imperative to guide health professionals in the management of HIV patience.

2.0. MATERIALS AND METHODS

2.1 Study area

The cross-sectional study was conducted at Federal Teaching Hospital Ido-Ekiti (FETHI), Ekiti State, among human immunodeficiency virus (HIV) infected patients attending Heart-to-Heart clinic in the hospital. Federal Teaching Hospital Ido-Ekiti is a referral hospital for Ado-Ekiti and some towns in neighboring States such as Kogi, Kwara and Osun State. It is located in Ido-Osi Local Government Area of Ekiti State Nigeria, on latitude 7.843093 and 5.182314 (Find Coordinates.https://www.distancesto.com/coordinates/ng /ido-ekiti-latitude-longitude/history/759 44.htmllast accessed 04 April 2020).

Laboratory analysis was carried out in the Medical Laboratories of the Department of Medical Laboratory Science, Afe Babalola University, Ado-Ekiti (ABUAD), Ekiti State. Ado-Ekiti is a city in southwestern Nigeria and lies on latitude 7^o 35 and 7^o38 north of the equator and longitude 5^o10 and 5^o15 east of the Greenwich Meridian [32]. Afe Babalola University is a private institution with its campus located at Km. 8.5, Afe Babalola way, opposite Federal Polytechnic, Ado-Ekiti.

2.2 Ethical Consideration

Ethical approval was sought for and obtained from the Ethics and Research Committee, Federal Teaching Hospital, Ido-Ekiti, Ekiti State (Protocol No: ERC/2019/03/13/198B). The study participants were informed about the purpose of the study and written consent was obtained from each participant before sample collection.

2.3. Eligibility Criteria

HIV infected subjects whose status has been confirmed and are duly registered with the Heart-to-Heart Center FTH Ido-Ekiti, who consented to the study, were eligible for sampling. Patients whose HIV status has not been confirmed and registered at the Heart-to-Heart Center FETHI were exempted.

2.4. Sample size

One hundred and eighty-four HIV subjects who consented to the study were enlisted.

2.5. Collection of $CD4^+$ data and anti-retroviral status

The result for $CD4^+$ count on the same day that samples were collected for each consenting subject and information on antiretroviral therapy was obtained from the information management system of the facility with due permission.

2.6. Sample collection

Five (5) ml of venous blood was collected from the cubital fossa of each of the consenting HIV infected subjects using safety vacutainer tubes and needles. Serum was obtained by centrifuging each blood sample at 1000 rpm (revolution per minute) for 10 minutes. Sera were harvested into Eppendorf tubes and stored at -20°C in the Medical Microbiology Laboratory of the Department of Medical Laboratory Science, ABUAD until ready for assay.[33]

2.7. Sample analysis

Analysis of the sample was carried out using pre-coated *Bartonella henselae* IgG and IgM ELISA kit [IgG CAT NO: EKHU-1984 and IgM CAT NO: EKHU-1985 (MELSIN MEDICAL CO., LIMITED, CHINA)].

2.7.1. ELISA Assay principle

Into the appropriate microliter plate wells pre-coated with the antigen, samples, positive control, and negative control were added and incubated. After incubation, it is washed to remove the uncombined antibodies, enzyme labeled (conjugate) anti-human antibody was added and incubated, washed again to remove unbound conjugates. Chromogen solution A and B was added changing the color of the liquid to blue after incubation. At the effect of a weak acid, the color becomes yellow. The color change is measured spectrophotometrically at a wavelength of 450 nm. The presence or absence of *Bartonella henselae* IgG or *Bartonella henselae* IgM antibodies in the samples is determined by comparing the optical density (O.D) of the samples with the cutoff value.[34]

2.7.2. Test procedure for IgG/IgM

The manufacturer's procedure was strictly followed. The reagents provided were allowed to attain room temperature for 15 minutes before use. The 20X wash buffer was diluted with distilled water using a ratio of 1:20 before use. The micro-titre plate template was set up with 1 well as blank, 2 wells as negative control and 2 wells as the positive control. 10µl of sera sample and 40µl sample diluent were dispensed into the respective wells except for the blank well, negative control well and positive control well. 50 µl of the negative and positive controls were dispensed into their wells respectively. The content was mixed by vibrating the plate gently. The microplate was covered with a sealing paper and incubated in a microplate incubator (MARVOTECH PLATE INCUBATOR, CHINA) at 37^{o}C for 60 minutes.[35] After incubation, the microplate was washed five times using wash buffer. 100 µl of Horseradish peroxidase enzyme (HRP) conjugate was added to each well except the blank; the microplate was covered with a sealing paper and also incubated in a microplate incubator at 37^{o}C for 15 minutes. After incubation, the microplate was washed five times with the diluted wash buffer in an automatic plate washer (MARVOTECH PLATE WASHER, China).[35] 50 µl of substrate solution A and B were added to each well respectively and were mixed; the plate was covered and incubated at 37^{o}C for 15 minutes. 50 µl of stop solution was added to each well and mixed. The absorbance was read in an ELISA reader machine (MARVOTECH ELISA READER, China) at a wavelength of 450 nm.[35]

2.7.3. Interpretation of *Bartonella henselae* IgG/IgM result

If the mean negative control O.D $\leq$ 0.15 and the mean positive control O.D $\geq$ 1.0, the test is valid. Cut-off O.D = the mean O.D value of the negative control × 2.1
Positive results: Sample O.D $\geq$ cut-off O.D
Negative results: Sample O.D < cut-off O.D

2.8. Statistical analysis

Collected data were analyzed using statistical package for social science (SPSS) version 17. Chi- square was used to compare the prevalence, antiretroviral status, age and $CD4^+$ count at 0.05 level of significance.

3.0 RESULTS

A total of 94/184 (51%) of subjects tested positive for *Bartonella henselae* IgG while 90/184 (49%) tested negative. For *Bartonella henselae* (*Bh*) IgM, 70/184 (38%) tested positive while 114/184 (62%) tested negative. Thirty-six percent (66 subjects) of subjects were males while 64% (118) were females. Female subjects had a slightly higher seropositivity rate (52%) of *Bh*- IgG and a low rate (36%) for *Bh*-IgM (Table 1).

Table 1: Seroprevalence of *Bartonella henselae* IgG and IgM antibody among sex.

	***Bartonella henselae* IgG**				***Bartonella henselae* IgM**			
Sex	**Positive (%)**	**Negative (%)**	χ^2	***p*-value**	**Positive (%)**	**Negative (%)**	χ^2	***p*-Value**
Male	32/66(48)	34/66(52)			28/66(42)	38/66(58)		
Female	62/118(52)	56/118(48)	14.1	0.71	42/118(36)	76/118 (64)	17.2	0.52
Total	94/184(51)	90/184(49)			70/184(38)	114/184(62)		

The age of subjects ranged from 21 to 69 years (average 41.7 years) and were grouped into three age groups; <40 years, 40-60 years and greater 60 years. The highest seropositivity rate for *Bh* –IgG was among the age group <40 years (34/58[59%]) and age group > 60 years for *Bh* IgM (14/36[39%]) this a marginal difference of 1% to other age groups. Age however, had no significance on prevalence (p= 0.29, χ^2 =3.65 and p = 0.43, χ^2 =2.32 for IgG and IgM respectively at 95% *CI*.) Table 2.

Sixty-three percent of subjects (116/184) were on Highly active antiretroviral therapy (HAART) while others had not started HAART. *Bartonella henselae* IgG was higher among those who were yet to begin HAART [42/68(62%)] but the rate was same with both groups for *Bh*-IgM. There was no significant difference between the groups (p = 0.12, χ^2 = 2.46; 0.07, $\chi^2 = 0.98$ Bh IgG and IgM respectively 95% *CI*.). Table 3.

$CD4^+$ counts of subjects ranged from 328 - 858 $Cells/mm^3$ and were divided into five (5) groups. Highest seroprevalence rate for Bh-IgM [10/20(50%)] was observed among the least $CD4^+$ count 301-400 cell/mm3 ($p < 0.64$; $\chi^2 = 2.54$). For Bh-IgG it 601-700 cell/mm3 group had the highest prevalence (63%). Table 4.

Table 2: *Bartonella henselae* IgG and IgM antibody distribution among age groups.

	***Bartonella henselae* IgG**				***Bartonella henselae* IgM**			
Age	**Positive (%)**	**Negative (%)**	χ^2	***p*-value**	**Positive (%)**	**Negative (%)**	χ^2	***p*-Value**
<40	34/58(59)	24/58(41)			22/58(38)	36/58(62)		
40-60	44/90(49)	46/90(51)			34/90(38)	56/90(62)		
>60	16/38(42)	20/38(52)	3.65	0.29	14/36(39)	22/36(61)	2.32	0.43
Total	94/184(51)	90/184(49)			70/184(38)	114/184(62)		

Table 3: *Bartonella henselae* IgG and IgM antibodies serostatus among HIV positive subjects on HAARTs and those not on HAARTS.

	***Bartonella henselae* IgG**				***Bartonella henselae* IgM**			
HAART	**Positive (%)**	**Negative (%)**	χ^2	***p*-value**	**Positive (%)**	**Negative (%)**	χ^2	***p*-Value**
HAART	52/116(45)	64/116(55)			44/116(38)	72/116(62)		
Non HAART	42/68(62)	26/68(38)	2.46	0.12	26/68 (38)	42/68(62)	0.98	0.07
Total	94/184(51)	90/184(49)			70/184(38)	114/184(62)		

Table 4: Bartonella henselae IgG and IgM antibodies serostatus among HIV positive subjects with relation to $CD4^+$ count.

	***Bartonella henselae* IgG**				***Bartonella henselae* IgM**			
$CD4^+$Count $Cells/mm^3$	**Positive (%)**	**Negative (%)**	χ^2	***p*-Value**	**Positive (%)**	**Negative (%)**	χ^2	***p*-Value**
301-400	10/20(50)	10/20(50)			10/20(50)	10/20(50)		
401-500	20/38(53)	18/38(47)			14/38(37)	24/38(63)		
501-600	30/64(47)	34/64(53)	2.54	0.64	18/64(28)	26/64(72)	3.99	0.07
601-700	24/38(63)	14/38 (37)			18/38(47)	20/38(53)		
Above 701	10/24(42)	14/24(58)			10/24(42)	14/24(58)		
Total	94/184(51)	90/184(49)			70/184(38)	114/184(62)		

4.0 DISCUSSION

The study recorded an overall prevalence rate of 51% for Bh-IgG and 38% Bh-IgM representing recent infections. This represents a high prevalence rate among HIV infected subjects, higher than reports from other countries like Brazil where 38.4% was reported,[2,36,37] Spain where 23% prevalence was reported,[31] and Europe where 16-41% prevalence was reported,[38] among HIV infected subjects. There is, however, a paucity of empirical data on the prevalence of *Bartonella henselae* among HIV infected subjects in Nigeria. Seroprevalence was observed to be higher among females than males contrary to the report of Pons et al.,[31] who documented a higher prevalence among males in Spain. Their variation may be due to cultural variables such as varied affinity for pets by the different gender. Evidence of recent infections suggests an epidemic of *Bartonella henselae* in the population since it has been reported to infect both healthy and immunocompromised individuals even though to a varied degree.

Pons et al.,[31] also report that age has a significant effect on prevalence which didn't agree with the findings of this present study. Prevalence increase with age (but no significant) except for the <40 age group which had an irregularly high prevalence. The prevalence rate of Bh-

IgM was uniform throughout the groups suggesting an equal incidence rate.

Subjects on HAART had a lower prevalence than those who were yet to commence HAART and seroprevalence did not take any definite pattern in relation to $CD4^+$ count of subjects. Highly active antiretroviral therapy (HAART) status and $CD4^+$ did not significantly influence seroprevalence of Bh-IgG/IgM ($p > 0.05$). There is a paucity of empirical data on the effect of HAART on the prevalence of *Bartonella henselae* in HIV infected subjects but some reports.[39,40] suggest that $CD4^+$ count is inversely proportional to *Bartonella henselae* seroprevalence. A close look at the immunity status enrolled in this present study showed that no one had a lymphocyte count of less than 200 cells/mm^3 which would have suggested immune depression. As such the effect of HAART may not be readily seen on the seroprevalence of *Bh*-IgG having that subjects were still immune-competent thus a clear impact HAART on immune status may not be felt. Pawełczyk and colleagues in their report has argued that in an immune depressed state may result in false-negative serologic reports. This is logical because once the T-helper cells are depleted, the humoral response becomes compromised and appropriate humoral immune response to *Bartonella henselae* will not be mounted. At this state, it becomes very challenging to diagnose the disease thus resulting in inappropriate management of the disease.

Disease management in HIV infected patients is usually complex most especially when fastidious etiologic agents which are difficult to isolate become involved. Often times clinical presentations are assumed to be complications of HIV infection were as if the actual diagnosis is known, management becomes easy. For example, cutaneous bacillary angiomatosis lesions are readily missed for Kaposi's sarcoma,[31] and clinically can only be distinguished by biopsy.

5.0 CONCLUSION

This study recorded 51% *Bartonella henselae* IgG antibody seroprevalence and 38% recent infection rate (as indicated by the presence of *Bartonella henselae* IgM) among HIV infected subjects. Sex, age, $CD4^+$ count and HAART status did not significantly influence seroprevalence. The study provides information on the burden of *Bartonella henselae* among HIV infected subjects attending Heart-To-Heart clinic at Federal Teaching Hospital, Ido-Ekiti, Ekiti State, Nigeria.

6.0 ACKNOWLEDGMENTS

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Disclosure of conflict of interest

All authors declare no form of conflict of interest in this study.

Statement of ethical approval

Ethical approval was sought for and obtained from the Ethics and Research Committee of Federal Teaching Hospital, Ido-Ekiti, Ekiti State. The study participants were informed about the purpose of the study and written consent was obtained from each participant before sample collection. Regarding human subject researches, the procedures are in line with the Helsink Declaration of 1975, as revised in 2000; only blood samples were required from the consented participants. Statement of informed consent was obtained from all individual participants included in the study.

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