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Heterogeneous Circulation of Salmonella Species and Plasmodium falciparum Specific Immune Complexes: Immuno - Suppressant in HIV Participants in Nigeria

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ABSTRACT

CD4+ T lymphocyte count status of HIV sero-positive participants in Nigeria was estimated using Patec Cyflow SL-3 Germany, considering high rate of Immune Complex (IC) formation due to endemicity of Salmonella species and Plasmodium falciparum, to ascertain their involvement in CD4 count depletion. 200 participants were recruited (100 HIV sero-positive and 100 HIV sero-negative). Circulating immune complexes (CICs) were precipitated from serum samples and dissociated using PEG (6000) to generate immune solution. The results were compared with that of HIV sero-negative control participants. Mean CD4+ T lymphocyte count (367±190) of 38(38%) HIV sero-positive participants with homogenous circulation of Salmonella species antigens only was significantly higher compared to CD4+ T lymphocyte count (193±117) of 62 (62%) other HIV sero-positive participants (P<0.001). Mean CD4+ T lymphocyte count (181±112) of 53(53%) HIV sero-positive participants with heterogeneous circulation of Salmonella species-Plasmodium falciparum antigens was significantly lower compared to CD4+ T lymphocyte count (346±185) of 47(47%) other HIV sero-positive participants (P<0.001). characterization of protein components of Immune complexes in HIV patients is strongly recommended as their persistence after major infection, can cause immune suppression. Thus, special care should be taken when dealing with HIV sero-positive patients in Salmonella species, Plasmodium falciparum endemic areas. Searching and detecting Malaria parasites alone is not diagnostic enough and depletion of CD4 count is not Bizarre to HIV infection in this locality.

Keywords: CD4+ T Lymphocyte Depletion, Plasmodium falciparum., Salmonella species, Immune Complexes, HIV Positive

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INTRODUCTION

Human Immunodeficiency Virus (HIV), infection has been synonymous with reduced CD4+ T Lymphocyte count- a very important thymocyte that plays important role in regulation of cell mediated and antibody mediated immune responses. Determination of CD4+ T lymphocyte count is a valuable measure for accessing the disease progress in HIV sero-positive patients, as well as monitoring of response to antiretroviral therapy. A profile of opportunistic infections has been associated with HIV/AIDS [1]. Equally concurrent infections of prevalent endemic diseases with HIV/AIDS have been reported [2]. It would be recalled that the combined effects of a low-grade persistent infection from these parasites, together with a weak antibody response, as well as liberation of partially synthesized bacterial polypeptide or viral nucleic acid antigens leads to chronic and or soluble immune complex formation [3] In this way, Salmonella and Malaria parasite antigens may continue to pose a threat to the host even after the infections have been treated.

Hence, for clinical interest, it becomes desirable to occasionally analyze circulating immune complexes especially in infectious agents' prone areas. Estimation of CD4 count as well, would enable us find the contributory pattern of CD4 count depletion by these infectious agents irrespective of HIV effect.

METHODS

Subjects: A total of 200 randomly selected subjects, (male=72, female=128) who are attending clinics at Nnamdi Azikiwe University Teaching Hospital (NAUTH), HIV Unit, Nnewi Campus, (100 screened HIV sero positive and 100 screened HIV sero negative participants) were recruited for this study. The mean age of the participants is 35 years. The participants gave informed consent, and the ethical committee of NAUTH gave approval for this study.

Blood sample collection: 10mls of blood was collected from each participant; 8mls was dispensed in vaccutainer tube for serum extraction, while 2mls was dispensed in EDTA –K2 anticoagulant container for CD4+ count (The blood samples for CD4 count were assayed within 6 hours of collection on each day). The sera extracted were treated with Polyethelene glycol of molecular weight 6000 (PEG 6000) to produce what we termed immune solution. The immune solutions were stored at 4°C and were assayed the following day.

Statistical Analysis: the variables were expressed in percentage while significant mean +/- SD was determined using One-way Anova Post Hoc Multiple Comparisons (Games-Howell) and Independent T test where applicable.

Circulating Immune complex precipitation: The polyethylene glycol (PEG) precipitation technique as described by Brunner and Sigal, (2000) [4] with minor modifications was used. Briefly, 1ml of 8% (average molecular weight, 6000) PEG6000; Sigma, St. Louis in 0.1 M borate buffer (pH 8.4) was added drop wise with constant stirring to 1ml of serum collected from the participant. The tubes were vortexed and incubated at 4°C, for 3 hours, and centrifuged at

8,320 g for 15 minutes. Supernatants were carefully removed. The resultant pellet was re-suspended and washed twice with 2.0ml of 4% PEG solution in the same buffer, removing the supernatant carefully each time. After the second spin, the solutions were treated with 1ml 0.01M Phosphate Buffer Saline (pH 7.2), and kept as immune solutions in the PBS buffer at 4°C and assayed serologically the following day for Salmonella typhi antibodies O and H, Salmonella paratyphi A B C antibodies, Plasmodium falciparum antigens. (There was no discernible loss of reactivity in the immune solution after storage for one month).

Detection of Plasmodium falciparum antigens : Commercially procured Immunoblot strips containing anti-Specific Immunoglobulin (IgG) raised against Plasmodium falciparum was used to analyze the Immune solution. Thus specific Plasmodium falciparum IgGs were detected, indicating the presence of specific Plasmodium falciparum IgG in the Immune solution. Plasmodium falciparum antigens strips (CORE Diagnostics, United Kingdom) was reacted with two drops of 1:10 dilution of representative dissociated immune complex (immune solution), which revealed bands comparable to those identified by specific Monoclonal Antibody (Mbs). same process was repeated for its unprocessed serum using the same dilution from HIV/AIDs participants in separate strips. Upper and lower immuno-sensitive areas were indicated on the strip. Where band appeared only on the lower sensitive area, the result indicated absence of antigen (negative), but where bands appeared on both lower and upper sensitive areas, the result indicated presence of antigen (positive).

Detection of salmonella antigens: Detection of salmonella antigens in the immune solution was done using monoclonal antibody (MAbs) (anti-somatic and anti-flagellins IgG) (Antec diagnostics United Kingdom), raised against Salmonella typhi and Salmonella paratyphi antigens. This was reacted against the representatives of known Salmonella typhi and Salmonella paratyphi antigens (Antec diagnostics United Kingdom) as control; Salmonella typhi and Salmonella paratyphi antigens in the Immune solution and against the un processed serum in 2 fold dilutions of solution respectively (1;10, 1:20, 1;40, 1:80, 1:160), using tube agglutination method. Some anti- Salmonella typhi and Salmonella paratyphi antibodies found in Immune solution were at a higher level than those found in serum at the same dilutions.

CD4 T-LYMPHOCYTE CELLS COUNT: Flow cytometry is a method by which cells or micro particles in suspension is differentiated and counted according to the cell size and internal structure, using Cyflow SL-3. The procedure was as described by (Fryland et al., 2006) [5].

RESULTS

Mean CD4 T Cell Count Amidst Antigenic Component Of Immune Complexes Amongst HIV Sero-Positive Participants.

Mean comparison of CD4+ T lymphocyte count (191±119) of 61(61%) HIV sero-positive participants with antigenic components of Immune complexes (Plasmodium falciparum-Salmonella species 53%; Plasmodium falciparum 8%,) was significantly lower compared to

mean CD4+ T lymphocyte count (364±189) of 39 (39%) (Salmonella species 38%, no antigen 1%)) HIV sero-positive participants (P<0.001). Mean comparison of CD4+ T lymphocyte count (262±178) of total 91 HIV sero-positive participants with antigenic components of Immune complexes (Plasmodium falciparum-Salmonella species 53%; Salmonella species antigens 38%), showed no significant difference compared to CD4+ T lymphocyte count (227±84) of 9 % HIV Plasmodium falciparum 8%; no antigen 1%) sero-positive participants who do not have salmonella species antigens at all (P>0.5). Mean CD4+ T lymphocyte count (261±144) of 8 HIV sero-positive participants with homogeneous circulation of Plasmodium falciparum antigen was high compared to CD4+ T lymphocyte count (258±174) of 92 (Plasmodium falciparum-Salmonella species 53%; Salmonella species antigens 38%; no antigen 1%) HIV sero-positive participants (P>0.1). Mean CD4+ T lymphocyte count (367±190) of 38(38%) HIV sero-positive participants with homogenous circulation of Salmonella species antigens only was significantly higher compared to CD4+ T lymphocyte count (193±117) of 62 (62%) (Plasmodium falciparum-Salmonella species 53%; Plasmodium falciparum 8%; no antigen 1%) HIV sero-positive participants (P<0.001). Mean CD4+ T lymphocyte count (181±112) of 53(53%) HIV sero-positive participants with heterogeneous circulation of Salmonella species-Plasmodium falciparum antigens was significantly lower compared to CD4+ T lymphocyte count (346±185) of 47(47%) (Salmonella species only 38%; Plasmodium falciparum antigens only 8%; no antigen 1%) HIV sero-positive participants (P<0.001). Mean CD4+ T lymphocyte count (457±178) of 15 HIV sero-positive participants with homogeneous circulation of Salmonella paratyphi antigens only was significantly higher compared to the CD4+ T lymphocyte count (258±172) of 85(85%) other HIV sero-positive participants P<0.001. However, the CD4 count (142±59) of 11(11%) HIV sero-positive participants with heterogeneous Salmonella paratyphi-Plasmodium falciparum antigens was significantly lower compared to that of (273±159) 89 other HIV sero-positive participants (P<0.02). But the mean CD4 count (259±226) of 4(4%) HIV sero-positive participants with heterogeneous Salmonella typhi-Plasmodium falciparum antigens, showed no significant difference compared to that (259±174) of 96(96%) other HIV sero-positive participants (P>0.1). See table 1

Mean CD4 T Cell Count Amidst Antigenic Component Of Immune Complexes Between HIV Sero-Positive And Sero-Negative Participants

Mean comparison of CD4+ T lymphocyte count (191±119) of total 61(61%) (Plasmodium falciparum-Salmonella species 53%; Plasmodium falciparum antigens 8%) HIV sero-positive participants was significantly lower compared to CD4+ T lymphocyte count (473±191) of 47(47%) (Plasmodium falciparum-Salmonella species 22%; Plasmodium falciparum only 25%) HIV sero-negative participants (P<0.01). Mean comparison of CD4+ T lymphocyte count (262±178) of 91(91%) (Plasmodium falciparum-Salmonella species 53%; Salmonella species only 38%) HIV sero-positive participants was significantly lower compared to CD4+ T lymphocyte count (569±234) of 41(41%) (Plasmodium falciparum-Salmonella species 22%; Salmonella species only 19%) HIV sero-negative (control) participants who have salmonella species antigens (P<0.01). Mean CD4+ T lymphocyte count (261±144) of 8(8%) HIV sero-positive participants with homogeneous circulation of Plasmodium falciparum antigen was significantly

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lower compared to CD4+ T lymphocyte count (489±183) of 25 HIV sero-negative (control) participants with the same pattern of CICs (P<0.02). Mean CD4+ T lymphocyte count (367±190) of 38 HIV sero-positive participants with homogenous circulation of Salmonella species antigens only was significantly lower compared to CD4+ T lymphocyte count (716±181) of 19 HIV sero-negative participants with the same pattern of CICs (P<0.01). Mean CD4+ T lymphocyte count (181±112) of 53(53%) HIV sero-positive participants with heterogeneous circulation of Salmonella species-Plasmodium falciparum antigens was significantly lower compared to CD4+ T lymphocyte count (569±234) of 22 HIV sero-negative participants with the same pattern of CICs (P<0.01).

Table 1: Comparative data of mean CD4 T cell count of disparate possessions of antigenic component of Immune complexes due to Salmonella species and malaria parasites amongst HIV sero-positive participants.

Variables	HIV positive	HIV Positive	P Value
	With CIC	No CIC	
Mp only	261±144	258±174	>0.1
	8 (8%)	92(92%)	
MP mixed	191±119	364±189	<0.001
	62(62%)	38(38%)	
Salmonella only	367±190	193±117	<0.001
	38(3%)	62(62%)	
Sal mixed	262±178	227±84	>0.5
	91(91%)	9(9%)	
Sal/mp	181±112	346±185	<0.001
	53(53%)	47(47%)	
S. paratyphi only	457±178	224±145	<0.001
	15(15%)	85(85%)	
S. Typhi only	287±000	258±172	>0.5
	1(1%)	99(99%)	
MP-paratyphi	142±59	273±159	<0.02
	11(11%)	89(89%)	
MP-typhi	259±226	259±174	>0.5
	4(4%)	96(96%)	

Showing CD4+ T lymphocyte count depletion amongst HIV sero-positive participants with Salmonella species-malaria parasite co-infection and specifically in Salmonella paratyphi-malaria parasite co-infection

Mean CD4+ T lymphocyte count (457±178) of 15(15%) HIV sero-positive participants with homogeneous circulation of Salmonella paratyphi antigens only was significantly lower

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compared to the CD4+ T lymphocyte count (672±222) 14(14%) HIV sero-negative participants with the same pattern of CICs (P<0.03). The mean CD4 count of (142±59) 11(11%) HIV sero-positive participants with heterogeneous Salmonella paratyphi-Plasmodium falciparum antigens was significantly lower compared to that of (469±205) 20 HIV sero-negative participants with the same pattern of CICs (P=0.01). See table 2

Table 2: Comparative data of mean CD4 T cell count of disparate possessions of antigenic component of Immune complexes due to Salmonella species and malaria parasites, between HIV sero-positive and sero-negative participants.

Variables	HIV positive	HIV Negative	P Value
Mp only	261±144	489±183	<0.02
	8(8%)	25(25%)	
MP mixed	191±119	473±191	<0.001
	61(61%)	47(47%)	
Salmonella only	367±190	716±181	<0.001
	38(38%)	19(19%)	
Sal mixed	262±178	569±234	<0.001
	91(91%)	42(42%)	
Sal/mp	181±112	455±203	<0.001
	53(53%)	22(22%)	
S. paratyphi only	457±178	672±222	<0.03
	15(15%)	14(14%)	
S. Typhi only	287±000	0	
	1(1%)	0	
MP-paratyphi	142±59	469±205	<0.01
	11(11%)	20(20%)	
MP-typhi	259±226	0	
	4(4%)	0	

Showing prevailing low CD4+ T lymphocyte counts amongst HIV sero-positive participants compared to HIV sero-negative participants

Mean CD4 T Cell Count Amidst Antigenic Component Of Immune Complexes In HIV Sero-Negative Participants.

Mean comparison of CD4+ T lymphocyte count (473±191) of total 47 (Plasmodium falciparum-Salmonella species 22%; Plasmodium falciparum only 25%) HIV sero-negative participants was significantly lower compared to CD4+ T lymphocyte count (660±208) 53(53%)

(Salmonella species only 19%; no antigen 34%) HIV sero-negative participants who do not have malaria antigens (P<0.001). Mean comparison of CD4+ T lymphocyte count (569±234) of total 41 (Plasmodium falciparum-Salmonella species 22%; Salmonella species only 19%) HIV sero-negative participants showed no significant difference compared to CD4+ T lymphocyte count (575±212) of 59 (Plasmodium falciparum antigens only 25%; no antigens 34%) HIV sero-negative participants without Salmonella antigen at all (P>0.5). Mean CD4+ T lymphocyte count of (489±183) 25(25%) HIV sero-negative participants with homogeneous circulation of Plasmodium falciparum antigen only was significantly lower compared to CD4+ T lymphocyte count (600±226) of 75 (75%) (Plasmodium falciparum- Salmonella species 22%; Salmonella species only 19%; no antigens 34%) HIV sero-negative participants (P<0.02). Mean CD4+ T lymphocyte count (716±181) of 19 HIV sero-negative participants with homogenous circulation of Salmonella species antigens only was significantly higher compared to CD4+ T lymphocyte count (539±216) of 81 other (Plasmodium falciparum-Salmonella species 22%; Plasmodium falciparum only 25%; no antigens 34%) HIV sero-negative participants (P<0.004). Mean CD4+ T lymphocyte count (455±203) of 22 HIV sero-negative participants with heterogeneous circulation of Salmonella species-Plasmodium faciparum antigens was significantly lower compared to CD4+ T lymphocyte count (605±215) of 78 (Salmonella species only 19%; Plasmodium falciparum antigens only 25%; no antigen 34%) other HIV sero-negative participants (P=0.02). Mean CD4+ T lymphocyte count (672±222) of 14 HIV sero-positive participants with homogeneous circulation of Salmonella paratyphi antigens only was high compared to the CD4+ T lymphocyte count (556±217) 86 other HIV sero-negative participants P>0.5. But the mean CD4 count (469±205) of 20 HIV sero-negative participants with heterogeneous Salmonella paratyphi-Plasmodium falciparum antigens was significantly lower compared to that of (598±218) 80 other HIV sero-negative participants without Salmonella paratyphi-Plasmodium falciparum antigens (P<0.02). However no heterogeneous circulation of salmonella sero var typhi and Plasmodium falciparum antigens was detected in HIV sero-negative participants. See table 3

DISCUSSION

Our analysis has unfolded a different pattern of immuno-pathological consequences resulting from infectious agents in HIV participants. We hypothesize that existence of heterogeneity of Salmonella antigens and Malaria parasite (Plasmodium falciparum) antigens together in a particular HIV host causes more CD4 count depletion and such host is more liable to develop AIDs faster than HIV hosts who have homogeneity of Malaria parasite antigens or Salmonella antigens only see table 1. Additionally, we also hypothesize that prevalence of homogeneity of Malaria parasite antigens is prevalent amongst HIV sero-negative participants than amongst HIV sero-positive participants, and that the presence of homogeneity of malaria antigens as components of immune complexes will cause depletion of CD4 more in HIV sero-negative participants than in HIV sero-positive participants see tables 1 and 3. Immune complexes due to other possible infectious agents such as *Hepatitis B virus*, *Hepatitis C virus*, *Mycobacterium tuberculosis* and *Treponema palladium*, were ruled out. This is in line with the earlier research which reported that in HIV sero-negative participants, leukocytes counts in P. falciparum infected patients was lower than that in controls (P = 0.015). Also that absolute

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counts of $CD4^+$, $CD8^+$, B, and $CD3^+$ cells and total lymphocytes were decreased very significantly during both P. falciparum ($P < 0.0001$) and P. vivax ($P < 0.0001$) infections. The report concluded that acute malaria infection causes a depletion of lymphocyte populations in the peripheral blood. Thus, special steps should be taken in dealing with malaria patients, including enumeration of peripheral lymphocyte cells for diagnostic purposes and research on peripheral blood to evaluate the immune status of patients [6].

Table 3: Comparative data of mean CD4 T cell count of disparate possessions of antigenic component of Immune complexes due to Salmonella species and malaria parasites, between HIV sero-positive and sero-negative participants.

Variables	HIV Negative	HIV NEG	P Value
CICs	with CIC	No CIC	
P.f only	489±183	600±226	<0.02
	25(25%)	75(75%)	
P.f mixed	473±191	660±208	<0.001
	47(47%)	53(53%)	
Salmonella only	716±181	539±216	<0.004
	19(19%)	81(81%)	
Sal mixed	569±234	575±212	>0.5
	42(42%)	58(58%)	
Sal/mp	455±203	605±215	<0.02
	22(22%)	78(78%)	
S. paratyphi only	672±222	556±217	>0.5
	14(14%)	86(86%)	
S. Typhi only	0	0	
	0	0	
MP-paratyphi	469±205	598±218	<0.02
	20(20%)	80(80%)	
MP-typhi	0	0	
	0	0	

Showing prevalence of homogenous infection of Malaria parasite and low CD4 count in the presence of heterogeneous infection of Salmonella species-malaria parasite in HIV sero-negative.

Keys

Sal/mp = Salmonella/Malaria parasite

MP = Malaria parasite

Persistence of Salmonella species and malaria parasite antigenic components of immune complexes and their effect on CD4 T cell count in both HIV sero-positive and negative participants was our focus, the direct effects of the presence of the parasites on the host were not considered. This is in line with the work of [7], who worked on the immuno-pathological effect of malaria parasite or its antigens on the host. Before now, little or no work has been done to characterize the antigenic components of these immune complexes especially in this sub Saharan Africa where many microbial agents are endemic, so as to ascertain the prevailing microbes that contribute in formation of immune complexes (ICs), instead many work have been done regarding the direct effects of the parasites . More so, there is paucity of data on the effect of Salmonella and malaria parasite antigenic components of CICs on CD4 + T Lymphocyte count. This work upholds the hypothesis that presence of immune complexes can suppress immune response by reducing the T helper cells (Th2) sub set of T lymphocytes in both HIV infected and HIV free participants. With regards to earlier works, researchers gave evidence that HIV induces $CD4^+$ depletion in part by the formation of immune complexes (IC) that attach to $CD4^+$ blood lymphocytes. In their studies, they examined the relationship of immune complexes-coated $CD4^+$ blood cells with retroviral replication in highly active antiretroviral therapy (HAART) treated patients. Patients with detectable viral replication and without ICs on $CD4^+$ blood lymphocytes had a lower viral load (4100 versus 21000 HIV-1 mRNA copies/ml; P=0.079) and higher $CD4^+$ cell counts (310/µl versus 161/µl; P=0.035) than patients with ICs on circulating $CD4^+$ lymphocytes. Among patients with <80 HIV-1 mRNA copies/ml, IC^- individuals had slightly higher $CD4^+$ lymphocyte counts than IC^+ patients (384/µl versus 316/µl) [8]. However, There is paucity of clarification in the involvement of co-infectious agents in CD4 count depletion. The work of [8], did not mention if the HIV positive participants have malaria parasites or Salmonella species as co-infections. Although, many research works have shown that malaria parasite infection can increase HIV1 viral load and deplete CD4 count [9, 10], there is little information on tendencies of many other patterns of infection that may contribute immensely in immune complex formation and subsequent CD4 count depletion, special regards on homogeneity and heterogeneity of infection. Induction of immune complexes is not peculiar to HIV infection. It is known that low-affinity antibody is more likely to lead to immune complex formation [11]. Steward & Voller, 1973 [12] have shown that malaria infection (Plasmodium falciparum) can lower the affinity of antibody produced against unrelated antigens. The immune complex formation therefore is a good source of retention of microbial antigens and as such a relevant epidemiological source of microbial infection contributing to subclinical and immunological consequences.

Plasmodium falciparum malaria is a major source of morbidity and mortality, especially in sub-Saharan Africa. The complexity of the parasite life cycle and high diversity of strains has limited progress in understanding the immuno-pathogenesis of this disease [13]. Also Typhoid fever is a systemic infection with the bacterium Salmonella enterica serotype typhi and paratyphi. These highly adapted, human-specific pathogen have evolved remarkable mechanisms for persistence in their hosts that help to ensure immunological suppression, survival and transmission. Prevalence of Salmonella sero var typhi, paratyphi and Malaria parasite (plasmodium falciparum) infections amongst HIV/AIDS sero-positive participants and

HIV sero-negative participants in Nigeria and sub-Saharan Africa have been widely reported [14, 15]. However the above reports boarder on the direct parasitic effects on the participants. But studies on the effect of persistence of the antigen molecules and their corresponding antibody as circulating immune complexes due to these infectious agents have not been exhausted. The formation of immune complexes by interaction of antigen with antibody is a component of the normal immune response but circumstances may exist where they are not properly removed by the Mononuclear Phagocytic System. It is suggested that soluble immune complex diseases arising after infections may result from the liberation of partially synthesized bacterial or parasitic polypeptide or viral nucleic acid antigens. These disrupted antigens will have heterogeneous molecular weights due to antigenic material which is incomplete as a result of premature termination of synthesis. Antigens of this type have been shown to result in significant soluble complex (small sized; antigen excess immune complexes) formation in vitro when reacted with antisera from many individuals and are not easily removed from the system unlike the insoluble (large sized antibody excess immune complexes) complexes which are easily removed by the mononuclear phagocytic system without development of pathological changes [3].

Immune complexes may result from microbial antigens, or autoimmune diseases, but our interest was on the antigenic components of immune complexes due to microbial agents (specifically Salmonella typhi and Salmonella paratyphi and Plasmodium falciparum). The consequences of these immune complexes circulating in the system have been widely reported to include patho-physiological and immuno-pathological effects, example [16], reported that Immune complexes have been found to be immunosuppressive in a variety of experimental systems and have been demonstrated in other parasitic diseases, such as malaria, trypanosomiasis, schistosomiasis, and onchocerciasis. Also, Soluble and insoluble immune complexes activate neutrophils by separate receptor signalling pathways. Profound changes in neutrophil responsiveness to these complexes occur after cytokine priming. It has been established that under appropriate conditions these neutrophils can actively release large quantities of reactive oxidants and discharge the contents of their granules extracellular. If such large scale release of these toxic molecules occurred in vivo, then it is likely that local antioxidants and antiproteinase would become saturated and tissue damage would ensue [17].

This work has revealed a pattern of microbial implications and involvements in Immune complexes immunosuppressive effect. Detailing our analysis, we observed that 61 HIV sero-positive participants (with Plasmodium falciparum-Salmonella species 53%; Plasmodium falciparum 8%) with malaria parasites antigens have CD4 count that is significantly lower than that of 39 others (with Salmonella species 38% no antigen 1%) without malaria parasites antigens (191±119 vs 364±189, P =0.001**).** The same level of significance was observed in HIV sero-negative participants (473±191 vs 660±208, P<0.001). But the point to note here is that amongst these 61 HIV sero-positive participants, some have malaria parasite antigens together with Salmonella antigens, while amongst the other 39 participants, there is no one with malaria parasite antigen, therefore those who have malaria parasite antigens and Salmonella antigens together are not involved amongst the 39 participants. This analysis is applicable amongst HIV

sero-negative participants. When the 9 HIV sero-positive participants (with Plasmodium falciparum 8%; no antigen 1%) were separated, we were left with 91 HIV sero-positive participants (with Plasmodium falciparum-Salmonella species 53%; Salmonella species antigens 38%) whose CD4 count was found to be low compared to that of the 9 others. This could be because those who have Malaria parasite antigen and Salmonella antigens together are involved amongst the 91 participants, forcing their CD4 count down. It is possible that those who reported depletion of CD4 count by homogeneity of Malaria parasite infection in HIV participants, did not assay for presence of Salmonella antigens as well. The cause of the strong incrimination of heterogeneous presence of Salmonella and Malaria parasites antigens in depletion of CD4 count need to be further investigated but research has shown a kind of augmentative activities in the pathogenesis of the two infectious agents and in the presence of HIV infection and other immune-suppressive diseases.

In Salmonella infection, the total number of M cells increased in the follicle-associated epithelium as compared to uninfected mice, the average crypt depth lengthened, and the rate of enterocyte migration from the intestinal crypts increased. In addition, the numbers of CD4 cells increased and CD8 cells decreased. These results indicate that the damage elicited by invasive Salmonella induces a host response in the Peyer's patch tissue. In an experiment conducted in mice [18], showed that Salmonella hide in the lymphocytes and macrophages but to surface when the immune cells have been depleted. Upon subcutaneous infection, C3H/HeN (Ity(r)) mice showed an increase in bacterial numbers in livers and spleens, which reached a peak on day 19. After full recovery from the infection, these mice were irradiated or depleted of CD4(+) T cells. The mice displayed a secondary infection peak in livers and spleens with a course similar to that of the primary infection. They concluded that CD4(+) T cells are involved in active suppression of S. enterica serovar Typhimurium during latency. The role of CD4(+) T cells during primary infection with S. enterica serovar Typhimurium is well established [18]. Suffice it to say that depletion of CD4 count in the presence of HIV and Malaria parasites antigens, would argument the pathogenesis of Salmonella infection to cause more depletion on CD4 count. Separation of 38 (38%) HIV sero-positive participants who have Salmonella antigens alone from others, made us to observe that their CD4 count is significantly higher than that of 62 with Salmonella and Malaria parasite antigens together (370±192 vs 193±117 $P<0.001$). However, the CD4 count of the 38 HIV sero-positive participants is significantly lower than that of the 19 HIV sero-negative participants who have Salmonella antigens only as well (370±192 vs 716±181 $P<0.001$). This indicates that the rising nature of CD4 count and M cells in Salmonella infection, is adversely affected in HIV participants, due to immune cell suppressive effect of HIV and malaria parasites [18]. But in HIV sero-negative participants, the rise in CD4 count in the presence of Salmonella antigen was more appreciative while observing the CD4 count of 19 participants who have Salmonella antigen only and 81 participants with Salmonella antigens and Malaria antigens (716±181 vs 539±216 respectively, $P<0.004$). In this study, the mean CD4 count of 53(53%) HIV sero-positive participants who have Malaria antigen and Salmonella antigens together was significantly low compared to 47 others $P<.0.001$, and all the 53 participants have CD4 count below 200mm3. Also this pattern of antigenic presence is prevalent in HIV sero-positive than in HIV sero-negative $P<0.001$. This agrees with the report of

Strickland et al., (1972) [19], that more immune-pathological sequels of malaria combined with other agents will be identified in the future. Their research showed that joint infections of malaria and toxoplasmosis gave rise to particularly severe disease in mice. It is well known too that accidental contamination of animal malaria parasite strains with viruses, Eperythrozoon, Haemobartonella, or Mycoplasma can easily occur and the course of a malaria infection and its pathology can then be altered.

The role of immune-activation by co-infecting pathogens has long been postulated as a factor influencing the severity and rate of disease progression in HIV-infected individuals in developing countries [20] Circulating immune complexes that persist in absence of the parasite is entirely a different tune of pathological effect and interaction in HIV sero positive patients. The presence of Salmonella paratyphi antigen and Malaria parasite antigen together in both HIV sero-positive and HIV sero-negative participants was found to be more CD4 count depletive compared to the presence of Salmonella typhi antigen and Malaria parasite antigen together see table 2 and 3. The presence of the Immune complexes maintains continuous circulation of antigen especially when the CIC are not being removed adequately through the Mononuclear phagocytes system. This is an immunological condition that sustains chronic inflammatory responses by inducing continuous release pro-inflammatory cytokines [6].

CONCLUSIONS

Characterization of protein components of Immune complexes in HIV patients is strongly recommended as their persistence after major infection, can cause immune suppression. Persistent heterogeneous circulation of specific Immune complexes due to Salmonella and P falciparum antigens is more CD4 count depletive than P. falciparum antigen alone both in HIV sero-positive and sero-negative participants. Presence of Malaria antigens only is more CD4 count depletive in HIV sero-negative participants than in HIV sero-positive participants. Presence of Salmonella species antigens only increases CD4 count but the increase is more prominent in HIV sero-negative. HIV sero positive with Salmonella and Malaria antigens together would develop AIDs faster, and those with Salmonella paratyphi and Malaria parasite antigens together would have depletion of CD4 count but not those with S. typhi .

Competing interests: None

We declare that there is no financial or non financial competing interest following this work

Ethical Approval

The Ethical Committee of Nnamdi Azikiwe University Teaching Hospital, approved of this research work and authorized the sample collection.

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