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Detection of microbial antigenic components of circulating immune complexes in HIV patients: Involvement in CD4$^+$ T lymphocyte count depletion

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ABSTRACT

Objective: To investigate the prevalence of microbial antigenic components of circulating immune complexes amongst grades of CD4 T lymphocyte counts in HIV sero positive and sero-negative participants. **Methods:** Polyethelene glycol (PEG-600) and buffering methods of precipitation and dissociation of immune complexes was used to generate immune solution from sera of 100 HIV sero-positive and 100 HIV sero-negative participants. These were categorized into 3 grades based on CD4 count: > 500 cell/mm^3, 200-499 cell/mm^3 and <200 cell/mm^3. The immune solutions were assayed using membrane based immunoassay and antibody titration, along side its unprocessed serum for detection of various microbial antigens and or antibodies. CD4 T cell counts were estimated using Patec Cyflow SL-3 Germany. **Results:** Antigenic component of immune complexes of various infectious agents was detected in 99 and 70 HIV sero-positive and HIV sero-negative participants, respectively. In group A, there were 10 HIV positive participants, including 4 (40.0%) had circulating immune complexes(CICs) due to *Salmonella* species only; 1(10.0%) due to *Salmonella-Plasmodium falciparum (P. falciparum)*, *Salmonella-P. falciparum*-HCV and *P. falciparum* antigens, respectively. In group B, 45(45.4%) HIV sero-positive participants with CICs had CD4 T lymphocyte count between 200-499 cells/mm^3. Out of these, 20(44.4%) had CICs due to *Salmonella* species only; 9(20%) due to *Salmonella-P. falciparum*. In group C, there were 44(44.4%) HIV sero-positive participants, including 3(6.8%) due to *Salmonella* species only; 24(54.4%) due to *Salmonella-P. falciparum;* 2(4.5%) due to *P. falciparum* only. **Conclusions:** In HIV sero-positive participants, presence of heterogeneity of *Salmonella* species-*P. falciparum* antigens was highly incriminated in CD4 count depletion but not homogeneity of malaria parasites antigens. Malaria parasites antigens only were incriminated in CD4$^+$ count depletion amongst HIV sero-negative participants. Before taking any decision on the management of HIV-1-positive individuals, their malaria and *Salmonella paratyphi* status should be assessed, but not malaria status alone.

1. Introduction

Despite vigorous researches and prophylactic measures on HIV/AID, the infection and the disease conditions continue to threaten the life of millions of people in Africa. The continued surge of HIV/AIDS in Africa can be attributed to the influence of so many other factors on pathogenesis of the disease condition. Considering the fact that many infectious agents are endemic in sub-Saharan Africa, many immune complexes are formed and the presence of circulating immune complexes (CICs) in normal individuals stimulate

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many immuno pathological conditions ranging from chronic inflammatory responses to organ tissue damage[1]. Based on this, one may begin to fathom the faith of HIV sero-positive participants who are already exposed to debilitating disease and immunocompromised. It suggested that soluble immune complex diseases arising after infections may result from the liberation of partially synthesized bacterial 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 result in significant soluble complex formation *in vitro* when reacted with antisera from many individuals[2,3].

Before now no critical studies have been done on the clinical roles of immune complexes formed by infectious agents in HIV sero-positive patients especially in Africa where many microbial agents can be found. Moreover it remains an aberration that the most incriminated infectious agents in immune complex formation constitute greater burden amongst HIV sero-positive participants in Nigeria and sub-Saharan Africa. Earlier work has shown that CICs are highly retained in HIV/AIDS sero-positive subjects compared to HIV sero-negative subjects[4,5].

In the present study, we are able to look into the condition of CD4 T lymphocyte count in the presence of this antigen-antibody as CICs, and the number of HIV sero-positive participants retaining these immune complexes. We decided to characterize the microbial antigenic components of CICs to ascertain the prevailing microbial agents or opportunistic infections in formation of CICs which may contribute great deal a burden to the immune-compromised HIV/AIDS hosts, and to find out the immunological influence of these antigens on CD4 T lymphocyte counts in these participants. In the present study, the characterized antigens were viewed as collection of possible existing clinical or subclinical HIV associated immuno-suppressive agents especially by Th-2 type immune response.

2. Materials and methods

A total of 200 (100 HIV sero positive participants and 100 HIV sero negative control) were randomly selected from subjects who attended clinics at Nnamdi Azikiwe University Teaching Hospital, (HIV Unit) Nnewi Campus. The mean age of the participants was 35 years. The participants were grouped into 3 based on Center for Diseases Control (CDC) classification of HIV/AIDS with emphasis on $CD4^+$ T lymphocyte counts. Group A: (CD4 count>500 cells/mm^3); Group B: (CD4 count 201-499 cells/mm^3) and Group C (CD4 count<200 cells/mm^3)[6]. The participants were given by informed consent. The ethical committee of the Nnamdi Azikiwe University Teaching Hospital Nnewi gave approval for the work. Ten mL of blood was collected from each participant, 8 mL was dispensed in vaccutainer tube for serum extraction, while 2 mL was dispensed in EDTA -K2 anticoagulant container for $CD4^+$ count (The blood samples for CD4 count were assayed within 6 hours of collection 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 ℃ and were assayed the following day.

2.1. CICs precipitation

The polyethylene glycol (PEG) precipitation technique as described by Adriana *et al*[7], with minor modifications was used. Briefly, 1 mL 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 1 mL of serum collected from the participant. The tubes were vortexed and incubated at 4 ℃, 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 mL of 4% PEG solution in the same buffer, removing the supernatant carefully each time. After the second spin, the solutions were treated with 1 mL 0.01M phosphate buffer saline (PBS) (pH 7.2), and kept as immune solutions in the PBS buffer at 4 ℃ and assayed serologically the following day for *Salmonella typhi* antibodies O and H, *Salmonella paratyphi* A, B, C antibodies, *Plasmodium falciparum (P. falciparum)* antigens, Hepatitis B surface antigen (HbsAg), Hepatitis C virus (HCV), *Mycobacterium tuberculosis* antigens, *Helicobacter pylori* (*H. pylori)* antigens and *Treponema pallidum* antigens.

2.2. Detection of microbial antigens

The colourless clear immune solution containing dissociated antigens and antibodies of various microbial origins was assayed using specific membrane based immunoassay for separate detection of *P. falciparum* antigens, HbsAg antibodies, HCV antibodies, *Mycobacterium tuberculosis* antibodies, *H. pylori* antibodies and *Treponema pallidum* antibodies. The method was as described by the manufacturer of the immunoassay kits (Acon Incorporated USA).

2.3. 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 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. Presence of agglutination in each tube is an indication of presence of *Salmonella typhi* or *Salmonella paratyphi* antibodies in the immune solution and unprocessed sera, respectively. The

highest dilution with agglutination, was taken as the titre of antibody in the immune solutions or unprocessed sera, respectively.

2.4. Statistical analysis

One-Way ANOVA was applied using Post Hoc Multiple Comparisons (Game-Howell), with significance level of 0.05 at 95% confidence interval.

3. Result

The band colour on the strip was deeper using immune solution than that of the results obtained using unprocessed serum. Higher antibody titre was obtained from the immune solution than the unprocessed serum.

A total of 99 (99.0%) HIV sero-positive participants and 70 (70.0%) HIV sero-negative participants had various microbial antigens-antibodies as CICs. The CD4 of HIV sero-positive participants was significantly reduced compared to CD4 of HIV sero-negative participants [(248±159) cells/mm^3 vs (541 ±215) cells/mm^3] (P<0.001). And CD4$^+$ count of HIV sero-negative participants without CICs was significantly higher than that of HIV sero-negative participants with CICs [(646± 219) cells/mm^3 vs (248±159) cells/mm^3] (P<0.05).

In Group A, 10 HIV positive participants with CICs had CD4 T lymphocyte count above 500 cells/mm^3, including 4(40.0%) had CICs due to *Salmonella* species only; 3(30.0%) due to *Salmonella*-HCV; 1(10.0%) due to *Salmonella-P. falciparum*, *Salmonella- P. falciparum*-HCV and *P. falciparum* antigens, respectively. On the other hand, out of the 70 HIV sero-negative participants with CICs, 39(55.7%) had CD4$^+$ T cell count above 500 cell/mm^3, including 15(39.5%) had CICs due to *Salmonella* species only; 13(34.2%) due to *P. falciparum* only; 8(21.1%) due to *Salmonella-P. falciparum*; 2(5.2%) due to HCV only and 1(2.5%) due to *Salmonella-P. falciparum*-HCV antigens combined. Similarly, out of 30 HIV sero-negative participants without CICs, 18(60.0%) had CD4$^+$ T cell count above 500 cell/mm^3. The mean CD4$^+$ T cell count of HIV sero-negative participants was higher than that of HIV sero-positive participants [(692±154) cells/mm^3 vs (649± 133) cells/mm^3] (P>0.05).

In Group B, 45(45.4%) HIV sero-positive participants with CICs had CD4 T lymphocyte count between (200± 499) cells/mm^3. Out of these, 20(44.4%) had CICs due to *Salmonella* species only; 9(20.0%) due to *Salmonella-P. falciparum*; 7(15.5%) due to *Salmonella*-HCV; 2(4.4%) due to *Salmonella-P. falciparum*-HBV; 1(2.2%) due to *P. falciparum*-HCV, *P. falciparum- H. pylori*, *H. pylori* only, *Salmonella-P. falciparum*-HCV, *Salmonella*-HBV-*H. pylori, Salmonella-P. falciparum*-HCV-HBV, and *Salmonella*-HBV-HCV, respectively. The only HIV sero-positive subject without CICs, fall in this category, making a total of 46 HIV sero-positive subjects in this group. On the other hand, out of the 70 HIV sero-negative participants with CICs, 28(40.0%) had CD4 T cell count above 200 cell/mm^3, including 3(10.7%) due to *Salmonella* species only; 12(42.0%) due to *P. falciparum* only; 12(42.0%) due to *Salmonella-P. falciparum*; 1(3.5%) due to HCV only, *Salmonella-P. falciparum*-HCV and *Salmonella*-HCV, respectively. Out of the 30 HIV sero-negative subjects free from CICs, 12(40.0%) had CD4 T cell count above 200 cell/mm^3. The mean CD4$^+$ T cell count of HIV sero-negative subjects was significantly higher than that of HIV sero-positive subjects [(370±79) cells/mm^3 vs (304±73) cells/mm^3] (P<0.05). Besides, the mean CD4 count of the HIV sero-negative participants free from CICs was significantly higher than that of HIV sero-negative participants with CICs [(452±27) cells/mm^3 vs (370±79) cells/ mm^3] (P<0.01) and that of HIV sero-positive participants with CICs [(452±27) cells/mm^3 vs (304±73) cells/mm^3] (P<0.01).

In Group C, 44(44.4%) HIV sero-positive participants with CICs had CD4 T lymphocyte count below 200 cells/ mm^3, including 3(6.8%) due to *Salmonella* species only; 24(54.4%) due to *Salmonella-P. falciparum*; 7(15.9%) due to *Salmonella-P. falciparum*-HCV; 2(4.5%) due to *Salmonella-P. falciparum*-HBV; 2(4.5%) due to *Salmonella-P. falciparum-H. pylori*; 2(4.5%) due to *P. falciparum* only; 1(2.2%) due to *Salmonella*-HBV, *P. falciparum*-HCV, *P. falciparum-H. pylori* and *Salmonella-P. falciparum*-HCV-HBV, respectively. On the other hand, out of the 70 HIV sero-negative participants with CICs, 3(4.3%) had CD4 count below 200 cells/mm^3, and no had CICs due to *Salmonella* species only. One (33.3%) had CICs due to *P. falciparum* only, *Salmonella-P. falciparum* and *Salmonella-P. falciparum*-HCV, respectively. The mean CD4$^+$ T cell count of HIV sero-negative participants with CICs in this group was significantly higher than that of HIV positive with CICs [(172±12) cells/mm^3 vs (124±48) cells/ mm^3], and non was found in HIV negative without CICs.

4. Discussion

HIV/AIDS subjects were subjected to immune complex studies, with special interest on identifying the antigenic components of the immune complexes. The occurrence of CICs is an event in any normal immune response. The half-life of such CIC is transitory in nature. Continued presence of CIC over extended periods, however, is a cause of consequence of some pathological condition or infection. Elevated levels of CIC have been found in a variety of diseases including infectious diseases, neoplasia, collagen vascular diseases[2,3]. In this study we analyzed the status of CD4 count in the presence of CICs considering the immune-clinical importance of CD4 count.

Data obtained from this work showed that the mean CD4 T cell count of HIV sero-positive participants is significantly lower than that of HIV sero-negative participants with CICs. The result has shown that more number of HIV sero-positive

participants would retain antigen-antibodies as CICs. The estimation of CD4 T lymphocyte count in HIV sero-negative participants as well, has helped us to compare the level of CD4 T lymphocyte count in both statuses in the presence of CICs. Amongst these participants in Group A, very low percentage (10%) of HIV sero-positive participants would have CD4 T lymphocyte count above 500 cell/mm^3 in the presence of CICs. While about 39(55.7%) of HIV sero-negative participants would have CD4 count above 500 cell/mm^3 in the presence of CICs. This indicates that retention of CICs soluble or insoluble, is a great burden to HIV/AIDs participants. For 70% of HIV sero-negative participants with CICs, it indicated that many patients in Africa habour CICs due to high prevalence of numerous microbial agents. This findings support the work of Tanyigna *et al*[9], that serum levels of CIC in Nigerians are elevated. one would expect that since the 70 participants do not HIV, up to 99% of them should have CD4 count above 500 cell/mm^3. However, only 55.7% have CD4 count above 500 cell/mm^3. There may be other factors other than HIV viral infections, adversely affecting CD4 count. The level of depletion of CD4 count may be determined by the type of antigen and the rate of formation of immune complex. This is in line with the report from Center for Disease Control and Prevention (CDC) stating that there are cases of Idiopathic low $CD4^+$ T Lymphocyte count. CDC defined this condition as Idiopathic CD4 lymphocytopenia with <300 CD4 cells per mm^3 or a CD4 cell count of < 20% of the total T cells on two occasion, no evidence of HIV type 1 or 2 infection and the absence of any defined immunodeficiency or therapy associated with depressed levels of CD4 cell count[6]. This study revealed that heterogeneous circulation of *Salmonella* species and *P. falciparum* antigens, is highly incriminated in CD4 count depletion in both HIV sero-positive and sero-negative participants. We also hypothesize here that malaria parasite as a single co-infection with HIV infection is not incriminated in CD4 count depletion. Instead, such pattern of infection was found to cause CD4 count depletion in HIV sero-negative than in HIV sero-positive participants. In support of this finding Chirenda[10] reported that more HIV-negative malaria cases had severely lowered CD4 counts than HIV-positive cases did. He reported that in 8 of 19 (42%) HIV-negative cases with malaria parasite had CD4 count below 200, while only 31 of 78(40%) HIV-positive cases had CD4 counts below 200. It is hypothesized that malaria reduces the CD4 count more than HIV infection[10]. In a similar development, Geertruyden et al reported that after successful antimalarial treatment, the median CD4 count at day 28 of follow-up increased from 468 to 811 cells/μL in HIV-1-negative and from 297 to 447 cells/μL in HIV-1-positive patients, and that after successful treatment, the proportion of patients with CD4 count <200/μL at day 45 decreased from 9.6% to 0% in HIV-1-negative and from 28.7% to 13.2% in HIV-1-positive malaria patients[11]. However, both Chirenda and Geertruyden *et al* focused only on malaria parasites, but our work involved heterogeneity and homogeneity pattern of infection.

Our data also showed that 30 HIV sero-negative participants that do not have CICs have mean CD4 count significantly above that of 70 others with CICs ($P<0.05$). Hence CD4 count depletion is not bizarre to HIV infection[12]. Since 99% of the HIV sero-positive participants retain antigens as components of immune complexes, virtually all HIV sero-positive participants would continue to suffer the consequences of immuno pathological effect due to presence of these antigens-antibodies bound together as immune complexes.

Out of the 100 HIV sero-positive participants, 19(19.0%) were on retroviral therapy. These 19 participants retained antigen-antibodies as CICs. This is an indication that retroviral drug reduces the viral load but does not eliminate immune complexes or prevent the formation. So the problems in suppressing HIV infection in Africa is not only on therapy but also epidemiological and secondary factors as mentioned above. With the avalanche of microbial antigens-antibodies detected in this work contributing to CICs formation and retention, this work has confidently shown that this HIV infected patients are readily exposed to many co-infectious agents ranging from bacteria, parasites and viruses. immuno-pathological effect would be continuous due to wide spread of infectious agents and their continuous infection and re-infection of HIV patients.

In group B, 45(45.4%) of 99 HIV sero-positive participants with CICs had $CD4^+$ counts above 200 cell/mm^3 while 28(40.0%) of 70 HIV sero-negative participants with CICs had CD4 count above 200 cell/mm^3. With reduced CD4 count, the number of HIV sero-negative participants with immune complexes dropped from 39 to 28 while number of HIV sero-positive participants significantly rising from 10 to 45 ($P<0.01$), with increase in number of *Salmonella*-malaria parasites co-infection. The presence of *Salmonella* antigen alone may not be a threat to CD4 count in HIV sero-negative participant but *P. falciparum* only or combination of *Salmonella-P. falciparum* antigens.

Our report has revealed higher incidence of HCV than HBV co-infection with HIV using analysis of immune complexes. Same transmission pattern of HIV, HCV and HBV co-infection is not an uncommon feature. Our report suggests that HCV is commonly associated in immune complexes in the sera of HIV-infected patients and that they may retain HCV more than HBV in patients co-infected with the HIV-HCV-HBV, or just HIV-HCV co-infection. On the other hand, this simply indicates that there is higher prevalence of HCV than HBV in HIV sero-positive participants in this locality.

CDC have rated the HIV sero-positive participants in group C as those with AIDS whose condition is critical and may remain in this condition without recovering[6]. Only 3 HIV sero-negative participants with components of immune complexes had heterogeneity of *Salmonella-P. falciparum*, homogeneity of malaria parasites infection and heterogeneity

of *Salmonella-P. falciparum*-HCV, respectively. On the other hand, 44 HIV sero-positive participants were found out of which 24 have heterogeneous infection of *Salmonella-P. falciparum*, but non had single infection of malaria parasite. The reason is not ascertained but certain immuno-pathological involvements must be the cause of immunological depression other than HIV. In support of this report, Marita and Klavs earlier suggested that if we are to intervene successfully to eradicate infections or prevent immune pathology either by vaccination or other immune intervention therapies it will be crucial to understand how co-infections with different pathogens affect the adaptive immunity and the establishment of immunological memory[13]. None of the 30 HIV sero-negative participants without CICs had CD4 count 201-499 cells/mm^3 or <200 cells/mm^3. This supports the findings in this study that the presence of immune complexes resulting from these infectious agents is a considerable factor in HIV pathogenesis and in CD4 count depletion both in HIV sero-positive and sero negative participants.

Consistent formation of immune complexes due to consistent microbial infection and re-infection of HIV sero-positive participants should be regarded as serious source of worries in Nigeria, African countries and other parts of the world where microbial agents are endemic. Almost all HIV sero-positive patients in Africa would retain antigens and antibodies as immune complexes. Persistence of these antigens in the body is a contributory factor to HIV progression and other epidemiological problems being encountered in curbing HIV menace. Many infectious agents ranging from bacteria, parasites, viruses, contribute to immune complex formation in Nigeria. Retention of *Salmonella-P. falciparum* antigens in combination, is a leading cause of low CD4 count in HIV positive and negative participants. Hence combined infections due to *Salmonella* and malaria may be a major risk factor in HIV infection and CD4 count depletion. But not infection due to *Salmonella* or malaria alone.

Conflict of interest statement

We declare that we have no conflict of interest.

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References

[1] Shmagel KV, Chereshnev VA. Molecular bases of immune complex pathology. *Biochem (Moscow)* 2009; **74**: 469-79.

[2] Cynthia JA, Nerurkar V, Karjodkar FR. Circulating immune complexes (cics) as marker for disease progress in oral cancer. *Indian J Clin Biochem* 2007; **22**(2): 114-7.

[3] Golda R,Wolski Z, Wyszomirska-Golda M, Madalinski K, Michalkiewicz J. The presence and structure of circulating immune complexes in patients with prostate tumors. *Med Sci Monit* 2004; **10**(3): 23-7.

[4] Erick KM, Alloys SSO, Jose AS. Immune complex levels in children with severe *Plasmodium falciparum* malaria. *Am J Trop Med& Hyg* 2005; **72**(5): 593-9.

[5] Onyenekwe CC, Ukaibe N, Meludu SC, Ilika A, Aboh N, Ofiaeli N, et al. Prevalence of malaria as co-infection in HIV- infected individuals in a malaria endemic area of southeastern Nigeria. *J Vec Bor Dis* 2007; **44**: 250-4.

[6] Centers for Disease Control. Guidelines for national human immunodeficiency virus case surveillance, including monitoring for human immunodeficiency virus infection and acquired immunodeficiency syndrome. *MMWR* 1999; **48**(RR13): 1-31.

[7] Adriana RM, Ronald LH, Len D, Mario TP. Detection of immune complexes is not independent of detection of antibodies in lyme disease patients and does not confirm active infection with *Borrelia burgdorferi*. *Am Soc Microbiol* 2005; **12**(9): 1036-40.

[8] Fryland M, Chaillet P, Zachariah R, Barnaba A, Bonte L, Andereassen R, et al. The Partec CyFlow Counter® could provide an option for $CD4^+$ T-cell monitoring in the context of scaling-up antiretroviral treatment at the district level in Malawi. *Trans Royal Soc Trop Med Hyg* 2006; **100**: 980-5.

[9] Tanyigna KB, Onah, JA, Bello CSS. Serum immunoglobulin and CICs in Nigeria on Jos Plateau. *Ghana Med J* 2004; **38**(1): 28-31.

[10]Chirenda J. Low CD4 count in HIV-negative malaria cases, and normal CD4 count in HIV-positive and malaria negative patients. *Cent Afr J Med* 1999; **45**(9): 248.

[11]Geertruyden, Jean-Pierre V, Mulenga M, Kasongo W, Polman K, Colebunders R, et al. CD4 T-cell count and HIV-1 infection in adults with uncomplicated malaria. *J Acquired Immune Deficiency Syndromes* 2006; **43**(3): 363-7.

[12]Rashkin S, Rouster S, Goodman ZD, Sherman KE. T-helper cells and liver fibrosis in hepatitis C virus-monoinfected patients. *J Viral Hepatitis* 2010; **17**(3): 222-6.

[13]Marita T, klavs B. Immune interaction in malaria co-infection with other endemic infectious agents implication for the development of improved disease interventions. *Microbe & Infection* 2008; **10**(9): 948-52.