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Full Length Research Paper

Increased incidences of *Salmonella, Plasmodium falciparum* and hepatitis C viral specific circulating immune complexes in participants from malaria endemic and HIV prevalent area of Nigeria

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The present study used dissociated circulating immune complexes (CIC) to identity the burden of exposure to certain infectious agents. The participants were divided into HIV seropositive group (n=100) and HIV seronegative group (n=100). Polyethylene glycol (PEG) 6000 and phosphate buffer techniques were used for precipitation and dissociation of CIC in sera. The dissociated CIC were tested for *Salmonella typhi* antibody, Plasmodium falciparum histidine rich protein (Pf-hrp)- 2 antigen and HCV antibody using commercially available kits. Result showed that Salmonella typhi antibody was detected in 76 (76%) of the HIV seropositive participants; Plasmodium falciparum histidine rich protein-2 (Pf-hrp-2) antigen was detected in 48 (48%) of HIV seropositive participants while Hepatitis C viral antibodies was detected in 20 (20%) of the HIV seropositive participants. Similarly, Salmonella typhi antibody was detected in 24(24%) of the HIV seronegative participants, Pf- hrp-2 antigen was detected in 47(47%) of the participants while Hepatitis C viral antibody was detected in 5(5%) of the HIV seronegative participants. There were significant differences between the number of HIV seropositive and seronegative participants with positive Salmonella typhi (P<0.05) and HCV antibody (P<0.05). The rates of homogeniuty and heterogeniuty of CIC in HIV seropositive participants was; 26 (34%) and 50 (66%) for Salmonella typhi antibody, 3 (6%) and 45 (94%) for Pf-hrp- 2 antigen and 0 (0%) and 20 (100%) for HCV antibody, respectively. While the rates of homogeniuty and heterogeniuty of CIC in HIV seronegative participants was 1 (4.2%) and 23 (95.8%) for Salmonella typhi antibody; 25 (53%) and 22 (47%) for Pf-hrp-2 antigen and 3 (60%) and 2 (40%) for HCV antibody respectively in all cases. The finding of the present study suggest that HIV infection may enhance susceptibilty to both salmonella typhi and HCV infection but not Plasmodium falciparum. The study thus revealed that Salmonella and HCV infections may constitute the major secondary infection in HIV infected patients and could be a cause for concern as HIV progressed to AIDS.

Key words: Antigen, antibody, complexes, HIV, malaria HCV and participants.

INTRODUCTION

The prevalence of HIV infection is on the increase in sub-Saharan Africa (Abu-Raddad et al., 2006). Unfortunately,

sub-Saharan Africa is also endemic to malaria. Cases of HIV-malaria co-infection have been reported in Sub-Saharan Africa (Onyenekwe et al., 2007) and several parasitic, bacterial and viral infections popularly called tropical diseases have been reported in sub-Saharan Africa (Agbakoba and Oseni, 2007; Das et al., 2007). The development of acquired immunodeficiency syndrome in HIV infected individuals gives an open door for opportunistic infections and changes in epidemiological patterns of some if not all common infections resident in this environment. Exposures to some of these infectious agents would result in generation of specific antibodies thereby forming circulating immune complexes. The more the antigens, the greater the reaction and formation of CIC. Raised serum levels of circulating immune complexes have been reported in HIV/AIDS subjects (Onyenekwe et al., 2006), in malaria infection (Arinola et al., 2005; Mibei et al., 2005), in liver diseases (Tsai et al., 1996; Akinlade et al., 2004) in diabetic mellitus (Akinlade et al., 2004) and in other physiologic conditions (Tanyigna et al., 2004; Semple et al., 2004) . Most report on CIC in Nigeria had been to either confirm its presence or absence, none have attempted to identify the antigens responsible for such raised CIC and possible epidemiologic implications. Therefore, the present study sets out to determine the specific antigen / specific antibody responsible for the formation of CIC in HIV infected subjects. Performing this analysis may help us learn more about possible infections associated with HIV immunosuppresion.

MATERIALS AND METHODS

Subjects

200 participants were randomly recruited for this study after undergoing HIV screening. The participants were divided into 2 groups: HIV seropositive group (n=100) and control HIV seronegative group (n=100). Serum of blood samples collected from the participants was used for the detection of CIC by a precipitation method using polyethylene glycol (PEG) 6000 and the CICs were dissociated into antigens and antibodies using specific buffers and pH. The dissociated CICs were screened for salmonella antigens, *Plasmodium falciparum* histidine rich protein-2 (Pf-hrp-2) antigen and hepatitis C viral antigens. The study designed was approved by our ethical committee and the participants gave informed consent.

Methods

Dissociation of CIC

The polyethylene glycol (PEG) precipitation technique as described borate buffer (pH 8.4) was added drop-wise with constant stirring to 2 ml of serum collected from the participants. The tubes were left at 4°C for 3 h, and centrifuged at 3000 rpm (2,580xg) for 15 min. Supernatants were carefully removed with a sterile pasture pipette. The resultant pellet was re-suspended and washed twice with 2.0ml of 2% PEG solution in the same buffer, removing the supernatant carefully each time by means of aspiration using pasture pipette. After the second spin, samples were dissociated in 1 ml 0.01 M phosphate buffer saline (PBS). Dissociated immune complexes were kept in the PBS buffer (pH 7.2) at 4°C and assayed serologically the following day for *Salmonella typhi* antigens O and H, *Plasmodium falciparum* hrp-2 antigen and hepatitis C viral antigen.

Detection of salmonella antibodies

The procedure for the assay was as described by the manufacturer of the kit (Antec Diagnostics). In brief, 50 ul of dissociated immune complexes in solution was placed on a clean, grease free, white tile while 50 ul of the salmonella (H and O) antigens were added respectively to the solutions of dissociated immune complexes. The mixture was shaked for 2 min to enable the antigen and antibody to react. *Salmonella* antibodies if present in the separated components of the immune complexes will result in formation of a positive agglutination reaction. However, if there are no salmonella antibodies in the dissociated immune complexes, there will be no agglutination reaction.

Detection of hepatitis C viral antibodies

The procedure is as described by the manufacturer (CORE Diagnostics United Kingdom). In brief, 100 ul of the dissociated immune complexes solution was added into appropriately labeled sample wells of the rapid test device for the hepatitis C vial test. This was allowed to incubate for 10 min for complete reaction between the pre-coated anti- HCV antibodies within the test kit and the HCV antibodies from the dissociated immune complexes. If the HCV antibodies are present in the dissociated immune complexes, it will result in development of a red coloured line at the test region of the kit. The test kit is validated by the occurence of another red line at the control region of the test kit. However, absence of HCV antibodies will not result in development of a red coloured line at the test region but a result validating red line at the control region will be seen.

Detection of Plasmodium falciparum (hrp) antigen

The procedure was as described by the manufacturer (Global Device USA). In brief, 10 µl of the`dissociated immune complexes in solution was transferred into appropriately labeled specimen cassettes of the Pf-hrp-2 test kit, after which 30 ul of the buffer supplied by the manufacturers was added into the specimen well where the separated immune complexes solution had been added. After 15 min the results were read. The test device has inherent quality control that validates the result. The presence of two pink lines at the region of the control and test sample signifies presence of *P. falciparum* antigen while the presence of only 1 pink line in the control region signifies absence of *P. falciparum* antigen.

Statistical analysis

The variables were expressed as percentages and a non-parametric Chi-square test was used to assess the level of significance. P<0.05 was considered significant.

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RESULTS

Results of analysis of CIC from HIV seropositive participants

Analysis of specificity of the dissociated CIC against *S. typhi* antigen showed that 76 (76%) of the participants had a positive test result while 24 (24%) of the participants had a negative result. Similarly, analysis of specificity of the dissociated CIC against Pf-hrp-2 antigen showed that 48 (48%) of the participants had positive test results while 52 (52%) had negative test results. In addition, analysis of specificty of the dissociated CIC againt anti- HCV antibody showed that 20 (20%) of the participants had positive test results while 80 (80%) had negative test results. 26 (34%) of the participants with positive salmonella typhi result presented with only salmonella positive CIC. In regard to participants with positive CIC results for salmonella typhi, 33(43%)* presented with positive heterogenous (mixed reactive) CIC result for *S. typhi* and Pf-hrp2; 9 (12%)* presented with positive heterogenous (mixed reactive) CIC result for *S. typhi,* Pf-hrp2 and anti-HCV antibody and 8 (11%)* presented with positive heterogenous (mixed reactive) CIC results for *S. typh*i and anti-HCV antibody (Tables 1 to 3).

With regard to participants with positive CIC result for Pf-hrp-2 antigen, 3 (6%) presented with only Pf-hrp-2 positive CIC, 33 $(69)^{+}$ presented with positive heterogenous (mixed reactive) CIC results for *S. typhi* and Pf-hrp-2, 9 $(19\%)^{+}$ pressented with positive heterogenous (mixed reactive) CIC results for *S. typhi,* Pf-hrp2 and anti-HCV antibody, while 3 $(6\%)^{+}$ presented with positive heterogenous (mixed reactive) CIC results for Pf-hrp2 and anti-HCV antibody (Tables 1 to 3).

With regard to participants with positive CIC result for anti-HCV antibody, none presented with only anti-HCV antibody positive CIC, 9 {45%}^ presented with positive heterogenous (mixed reactive) CIC result for *S. typhi*, Pf-hrp2 and anti-HCV antibody, 8 {40%}^ presented with positive heterogenous (mixed reactve) CIC result for S. typhi and anti-HCV antibody, while 3 {15%}^ presented with positive heterogenous (mixed reactive) CIC result for Pf-hrp2 and anti-HCV antibody (Tables 1 to 3).

Result of analysis of CIC from HIV seronegative participants (control)

Analysis of specificity of the dissociated CIC against salmonella typhi antigen showed that 22 (22%) of the participants had positive test results while 78 (78%) of the participants had negative results. Similarly, analysis of specificity of the dissociated CIC against Pf-hrp-2 antigen showed that 47 (47%) of the participants had positive test results while 53 (53%) had negative test results. In addition, analysis of specificty of the dissociated CIC againt anti-HCV antibody showed that 5 (5%) of the participants had positive test results while 95 (95%) had negative test results. 18 (82%) of the participants with positive *S. typhi* results presented with only salmonella positive CIC. In regard to participants with positive CIC results for S. typhi, 2(9%)* presented with positive heterogenous (mixed reactive) CIC results for S. typhi and Pf-hrp2; 1 (4.5%)* presented with positive heterogenous (mixed reactive) CIC results for S. typhi, Pf-hrp2 and anti-HCV antibody and 1 (4.5%)* presented with positive heterogenous (mixed reactive) CIC results for S. typhi and anti-HCV antibody (Tables 1 to 3).

With regard to participants with positive CIC results for Pf-hrp-2 antigen, 25 (53%) presented with only Pf-hrp-2 positive CIC, 2 $(4.2\%)^{+}$ presented with positive heterogenous (mixed reactive) CIC results for S. typhi and Pf-hrp-2, $1(2.1\%)^{+}$ pressented with positive heterogenous (mixed reactive) CIC results for S. typhi, Pf-hrp2 and anti-HCV antibody, while none presented with positive heterogenous (mixed reactive) CIC result for Pf-hrp2 and anti-HCV antibody. (Tables 1 to 3).

With regard to participants with positive CIC results for anti-HCV antibody, 3 {60%}^ presented with only anti-HCV antibody positive CIC, 1 {2%}^ presented with positive heterogenous (mixed reactive) CIC results for S. typhi, Pf-hrp2 and anti-HCV antibody, 1{2%}^ presented with positive heterogenous (mixed reactve) CIC results for S. typhi and anti-HCV antibody, while none presented with positive heterogenous (mixed reactive) CIC results for Pf-hrp2 and anti-HCV antibody (Table 1 to 3).

DISCUSSION

The present study was designed to use the evidence of circulating immune complexes to indicate level of exposure to certain infectious agent in a population with and without HIV infection. It is also assumed that the level or frequency of detection of specific components of the antigen-antibody reaction that formed the CIC may suggest possible disease(s) that may be present as secondary infection amongst the HIV population in the community of study. It is believed that it will also enable us to discover the burden of exposure to these infectious agents.

Circulating immune complexes are formed as a result of antigen-antibody reactions. Often times CIC is raised in several disease conditions and may be associated with certain immunopathology of such disease(s) (Arinola et al., 2005; Mibei et al., 2005; Abrass et al., 1980; Tsai et al., 1996; Akinlade et al., 2004; Tanyigna et al., 2004; Semple et al., 2004). It is a widely known that when the molecular mass of the CIC is smaller, it circulates in the circulatory system but is often filtered or engulfed by reticulo-endothelial cells. The epidemiologic strenght of using CIC may be weak when measured quantitatively without defining the contributing antigens. However, the epidemiologic strength is strong when further analysis of the CIC is done after its dissociation in order to identify

Table 1. Frequency of seroreactivity of salmonella typhi, anti-Pf-hrp-2 and anti-HCV-antibody with sample soultion of dissociated circulating immune complexes.

Seroreactivity	HIV positive subjects(n=100)		HIV negative subjects(n=100)		*Chi-square(p-value)
	Positive (%)	Negative (%)	Positive (%)	Negative (%)	
Salmonella typhi antibody	76 (76)	24 (24)	24 (42)	76 (76)	27.040 (P<0.05)
Pf-hrp-2 antigen	48 (48)	52 (52)	47 (47)	53 (53)	0.011 (P>0.1)
HCV antibodies	20 (20)	80 (80)	5 (5)	95 (95)	9.000 (P<0.05)

(%): Percentage expressed in parenthesis. *Chi-square was compared between HIV seronegative and HIV seropositive participants with positive CIC test results.

Table 2. Incidence of homogenous circulating immune complexes in HIV seropositive and HIV seronegative participants.

Seroreactivity	HIV positive subjects with CIC (%)	HIV Negative subjects with CIC (%)
Salmonella typhi antibody	26 (34)	1 (4.2)
Pf-hrp-2 antigen	3 (6)	25 (53)
HCV antibodies	0 (0)	3 (60)

(%): Percentage expressed in parenthesis with regard to total number of participants with positive CIC for each disease entity.

Table 3. Frequency of heterogenous (mixed) circulating immune complexes in HIV seropositive and HIV seronegative participants.

Seroreactivity	HIV positive subjects with CIC	HIV negative subjects with CIC
Salmonella typhi and Pf-hrp-2	33 (43%)*, $[69\%]^{+}$	21 (87.5%)*,$[45\%]^{+}$
Salmonella typhi and Pf-hrp-2 and HCV	9 (12%)*, $[19\%]^{+}$,{45%}^	1 (4.2%)*,$[2.1\%]^{+}$,{20%}^
Salmonella typhi and HCV	8 (11%)*, {40%}^	1 (4.2%), {20%}^
Pf-hrp-2 and HCV	3 $[6\%]^{+}$, {15%}^	0 (0%)

(%)*: Percentage in parenthesis for salmonella typhi with regard to total number of participants with positive CIC.; $[\%]^{+}$: Percentage in parenthesis for Pf-hrp-2 antigen with regard to total number of participants with positive CIC; {%}^: Percentage in parenthesis for HCV with regard to total number of participants with positive CIC.

the contributing antigen(s).

In the present study, we detected a high frequency of *S. typhi* antibodies when the antigen-antbody reaction (CIC) was dissociated. This is an indication that the participants were previously and may also be presently exposed to *S. typhi* infection. Since we did not ascertain if the salmonella specific antibodies were either IgG or IgM we may not be able to confirm if it is a prevoius or current exposure. However, the present study showed a high burden of *S. typhi* specific antigen-antibody reactions (CIC). Comparing the findings in both the HIV seropositive population and HIV seronegative population, the incidence of formation or presence of Salmonella specific antigen-antibody reactions (CIC) was about tripled in the HIV infected population. This may suggest alterations in protective immunity against *S. typhi* infection in HIV infection. It could also suggest high susceptibility to *S. typhi* infection in HIV population. Furthermore, the finding may suggest possible secondary infection with salmonella if HIV progresses to HIV/AIDS. This may also be one of the secondary infections to contend with in HIV/AIDS patients. Further analysis of the salmonella specific antigen-antibody reactions (CIC) showed the presence of mixed exposure to other infectious agents such as malaria and HCV. This may suggest the possibility of existence of co-infections of HIV-salmonella-malaria-HCV.

In addition, we detected a high frequency of Plasmodium falciparum histidine rich protein (Pf-hrp)-2 in both the HIV infected population and HIV seronegative population. There was no appreciable difference in the frequency between both groups. However, this finding may not be suprising since the study area is in a malaria endemic region but it did show that HIV may not likely enhance susceptibility of infected pateints to malaria. The assumption is based on our statement that CIC could be viewed as an indication of burden of exposure. Similarly there was evidence of heterogenous CIC involving salmonell typhi, malaria and HCV. Since the Pf-hrp-2 antigen is secreted during infection of red blood cells and could remain in circulation for 14 days post anti-malaria treatment, the finding may be an indication of current

exposure to malaria (Onyenekwe et al., 2002). This is, however, a common finding in adults residing in malaria endemic areas because of the presence of protective immunity in the presence of parasitemia (Day and Marsh, 1991; Baird, 1995).

Finally, we detected a high frequency of anti-HCV-antibody in the HIV infected population when the antigen-antibody reaction (CIC) was dissociated and analysed. The finding may equally suggest that HIV infection may predispose to HCV infection. It is also suprising that we did not observe any mixed or heterogenous CIC representing occurences of both malaria and HCV alone in the present study. The finding of 5% seroreactivity for HCV was previously reported in our study of blood donors, pregnant women and patients presenting with abnormal liver function test(Ezeani et al., 2006, 2008; Onyenekwe et al., 2008). Since our previous findings of prevalence of HCV in non HIV infected subjects was in tandem with the finding in the present study within the HIV seronegative population, the finding of the study may suggest increased exposure to HCV during HIV infection and it may be an indicator for enhanced susceptibility of HIV infected subjects to HCV.

The present study was able to showed the burden of exposure to *S. typhi*, *P. falciparum* and HCV in the studied population. The finding suggest enhance susceptibilty to S. typhi and HCV in the HIV population. This could it be breaken down in mucosal protection as may be reflected in reduced IgA concentrations in HIV infected subjects. However, susceptibility to malaria was not altered by HIV, this may suggest that HIV may not enhance susceptibilty to malaria and the protective immunity might have not been altered for malaria. We therefore conclude that *S. typhi* and HCV may pose problems of management as secondary infections in HIV infected subjects should the condition progresses to AIDS.

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