

Impact of HIV/HBV Co-infection on Blood Donor CD4+ Cell Count in Jos, North-Central Nigeria

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Abstract: Background: Human Immunodeficiency Virus (HIV) is known to target CD4+ lymphocytes and other CD4 molecule-bearing cells like macrophages, monocytes and glial cells of the brain. Although, liver cells are the most readily infected, Hepatitis B Virus (HBV) also infects blood mononuclear cells like monocytes, B cells, CD4+ and CD8+ T cells, and polymorphonuclear leucocytes. We undertook this study to evaluate the effect of HIV/HBV co-infection on CD4+ cell counts of blood donors. Patients and Methods: A total of 510 prospective blood donors were screened for HIV and HBV, and classified into four groups. Group I (HIV positive), group II (HBV positive), group III (HIV+HBV co-infected) and group IV (Control). The mean CD4+ cells were analysed using Partec Cyflow machine and the differences were tested with nonparametric methods. Results: The mean CD4+ cell count for group I (HIV mono-infection) was 441.30±59.20cells/ul, group II (HBV mono infection) was 574.90±47.50 cells/ul, group III (HIV+HBV co infections) was 418.60±75.30 cells/ul and group IV (control donors) was 576.90±14.8 cells/ul. There was no significant difference in the CD+ cell count levels among all groups (p=0.302). Conclusion: We concluded that HIV/HBV co-infection does not significantly affect CD4+ cell count of blood donors. Voluntary blood donors are more likely to come with clinically early stage HIV disease. This and a possible lack of a clear role of CD+ cells in HBV immunity may explain this observed lack of effect by co-infection on the immunologic status of blood donors. More studies are advocated to further elucidate and study the role CD4+ bearing cells on HBV immunity.

Keywords: HIV/Hepatitis Co-infection, CD4, Blood donors, Jos, Nigeria

1. Introduction

HIV is known to targets CD4+ lymphocytes and other CD4 molecule-bearing cells like macrophages, monocytes and glial cells of the brain. Though liver cells are most readily infected, HBV also infects peripheral blood mononuclear cells like monocytes, B cells, CD4+ and CD8+ T cells, and polymorphonuclear leucocytes^[1]

HIV/HBV co-infections tend to accelerate viral hepatitis with consequent chronic complications^{[2]-[3]}. These patients are 6 times more likely to develop chronic hepatitis B than those without HIV. This is even more likely in patients with lower CD4+ cell^{[4]-[6]}. HBV co-infections are also associated with decreased rates of clearance of HBeAg and increased HBV replication, with higher HBV DNA viral load^{[7]-[9]}. HIV infected individuals have also been observed to easily lose previously developed protective anti-HBs antibody with resultant acute hepatitis B infection; this risk is also associated with lower CD4+ cell counts^{[10]-[11]}.

These studies therefore agreed with the observations that chronic hepatitis B infection is frequent in HIV-infected patients; co-infection is known to increase HIV and HBV replication, hepatitis flares, and increased risk of progression to chronic HBV infection, cirrhosis, and hepatocellular carcinoma. These risks were all observed to be higher in those with lower CD4+ cell counts.

Recent interest in HIV/HBV pathogenicity among other proposed implicating factors, focuses on the discovery of human Apolipoprotein B RNA-editing enzyme-catalytic polypeptide-like 3G (APOBEC3G) which is a cytidine deaminase, and the virion infectivity factor (vif) of HIV^[12].

APOBEC3G is a recently recognized human innate intracellular protein with lethal activity against HIV. APOBEC3G enzymatic activity leads to HIV DNA degradation. As a counter attack, HIV virion infectivity factor (Vif) targets APOBEC3G for proteasomal proteolysis to exclude it from budding virions. APOBEC3G is a host factor that is antiviral, capable of inhibiting the replication of both exogenous and endogenous retroviruses as well as hepatitis B. HIV vif can trigger the degradation of human APOBEC3G, thereby preventing the degradation of nascent HIV and HBV genomes. This way co-infection with these viruses enhances the replication of both viruses^[12].

Information is scanty on the HIV/HBV co-infection and the CD4 cell immunologic effect among blood donors from our centre. This study was therefore aimed at determining the effects of HBV co-infection with HIV on the CD4+ cell count of prospective blood donors.

2. Methods

This study was carried out at blood banks of Jos University Teaching Hospital, Plateau Specialist Hospital, and the zonal centre of the National Blood Transfusion Service (NBTS) all in Jos, Nigeria. The study period was from May 2011 to October, 2014.

The study population comprised of 510 adult blood donors between the ages of 18-65 years. Ethical approval was obtained from the Ethical Review Committee of Jos University Teaching Hospital (JUTH). The nature and objectives of the study were explained to the donors. Written informed consent was obtained from all participants after detailed explanation was given to them. The study was at no

cost to the participants, while the option to opt out of the study at any time was left open to them without any prejudice. The control group were blood donors negative for both HIV and HBsAg.

The bio-data, including age, sex, weight, blood pressure, haemoglobin levels and all other relevant pre-transfusion screening information were collected with the aid of a pretested self-administered questionnaire to the consenting prospective blood donors, who met the inclusion criteria. The participants were screened for HIV and HBV infections, and divided into four groups. Group I (HIV positive), group II (HBV positive), group III (HIV+HBV co-infected) and group IV (Control).

3. Sample Collection

Five milliliters (5mls) of whole blood was collected aseptically from each donor, 2mls and 3mls were emptied into sterile labeled EDTA and plain vacutainer tubes respectively. The blood in the plain vacutainer was allowed to clot, and the serum collected into a plain bottle.

Detection of HbsAg and HIV infections

The serum was used to test for hepatitis B surface antigen (HbsAg) and HIV-1 & 2, using third generation enzyme-linked immunosorbent assays. Monolisa HBsAg ULTRA, a one step enzyme immunoassay was used to detect HbsAg, while the Genscreen™ ULTRA HIV Ag-Ab, a qualitative enzyme immunoassay kit for the detection of HIV p24 antigen and antibodies to HIV-1 (groups M and O) and HIV-2 in human serum or plasma was used for the HIV. The test was done following manufacturer's instructions and the results interpreted and documented accordingly.

Absolute CD4+ count Determination

The whole blood in the EDTA vacutainer tube was placed on a mixer to get a homogenous mixture. Monoclonal antibody, 20ul was pipetted into a tube; 20 ul of the patient's blood sample was added. The mixture was thoroughly mixed and incubated in the dark for 15 minutes. 800ul of CD4 Easy Count No Lyse Buffer was added and mixed gently and the mixture connected to a cyflow counter (Partec, Germany) for analysis. Observing peaks generated on the screen, the CD4+ count value was read directly on the machine and recorded. Based on a preset dilution factor in counts / ul, absolute CD4+ T-Lymphocyte count was expressed in cells/ul.

Statistical analysis

The data collected was analyzed using Graphpad Prism 6, Minitab 14 and Genstat Discovery Edition 4. Mean and Standard error (SE) determined were used to describe the continuous variables and proportions for the categorical data. The mean CD4+ cell count for Control, HIV, HBV and HIV/HBV co-infections were calculated for each group and the differences in their mean CD4+ cell count levels were tested and compared with nonparametric methods. The significance of the difference among the groups was accepted at $p \leq 0.05$. The results are presented in tables.

Interpretation of Result of Immunologic Status

The CD4+count of healthy (immune competent, HIV and HBV negative) adult Nigerians lie between 365 - 1,571 cells/ μ l^[3]. A CD4+ Count lower than the lower limit of this range may suggest immune compromise

4. Results

Of the 510 participants, majority were males, 405 (79.4%). The age group (25-31) years, constituted the vast majority 171 (33.5%). The gender and age distribution of the participants are shown in Table 1.

The mean CD4+ cell count for group I (HIV mono-infection) was 441.30 ± 59.20 cells/ul, group II (HBV mono-infection) was 574.90 ± 47.50 cells/ul, group III (HIV+HBV) co-infections was 418.60 ± 75.30 cells/ul, and the mean CD4+ cell count for group IV (Control) donors was 576.90 ± 14.8 cells/ul. (Table 2)

A comparison was made between the CD+ cell counts of those with HIV mono-infection and control. Even though, the value for HIV mono-infection appeared lower than for control, this was not significant (Table 3). The CD4+ cell counts of those with HBV mono-infection and HIV+HBV co-infection were also compared with control (Tables 4 & 5). The value for co-infection appeared lower than the value for single infections, but again, this was not significant. All infection groups (mono-infections and co-infection) were also compared with control group, this also showed that there is no significant difference in the CD+ cell count levels among all groups $\{ (P=0.302) \}$ (Table 6).

Table 1: Age and Sex distribution of blood donors

| Age Group | n (%) | Sex | n (%) |
|-----------|------------|--------|------------|
| 18 -24 | 110(21.6) | Male | 405(79.4) |
| 25 -31 | 171(33.5) | Female | 105(20.6) |
| 32 -38 | 133(26.1) | | |
| 39 -45 | 48(9.4) | | |
| 46 -52 | 26(5.1) | | |
| 53 -59 | 22(4.3) | | |
| Total | 510(100.0) | Total | 510(100.0) |

Table 2: Donor groups and CD4+ cell count

| Group | n | Mean CD4+(cells/ul) |
|----------------|-----|---------------------|
| I.(HIV Mono) | 42 | 441.30 ± 59.20 |
| II.(HBV Mono) | 85 | 574.90 ± 47.50 |
| III.(HIV +HBV) | 14 | 418.60 ± 75.30 |
| IV.(Control) | 369 | 576.90 ± 14.80 |
| Total | 510 | |

Table 3: Mean CD4+ Cell Count in HIV Mono and control

| Group | n | Mean | p-value |
|--------------|-----|--------------------|----------------------|
| I.(HIVMono) | 42 | 441.30 ± 59.20 | 0.1168 ^{ns} |
| IV.(Control) | 369 | 576.90 ± 14.80 | |

($P > 0.05$)

Table 4: Mean CD+ cell count in HBV Mono and control

| Group | n | Mean | p-value |
|--------------|-----|-------------------|----------------------|
| II.(HBV) | 85 | 574.90 ± 47.0 | 0.9748 ^{ns} |
| IV.(Control) | 369 | 576.90 ± 14.0 | |

($P > 0.05$)

Table 5: Mean CD⁺ cell count in HIV+HBV co-infection and control

| Group | n | Mean | p-value |
|---------------|-----|--------------|----------------------|
| III.(HIV+HBV) | 14 | 418.60±75.30 | 0.2861 ^{ns} |
| IV.(Control) | 369 | 576.90±14.80 | |

($P > 0.05$)

Table 6: Infection status and CD4⁺ cell counts

| Group | Mean CD4 ⁺ (cells/ul) |
|------------------|----------------------------------|
| I. (HIV Mono) | 441.30±59.20 |
| II. (HBV Mono) | 574.90±47.50 |
| III. (HIV + HBV) | 418.60±75.30 |
| IV. (Control) | 576.90±14.80 |
| p-value | 0.302^{ns} |

No Significant difference among mean CD4 cell counts for all infection groups and control ($P > 0.05$)

5. Discussion

The control CD4⁺ cell count was observed to be 576.90±14.8 cells/ul. This value falls within the 365 - 1,571 cells/ul reference range for Nigerians reported by Oladepo *et al*^[13] in Jos, and 381-1565 cells/ul reference range for Indian males, reported by *Shete et al*^[14] in India.

With respect to the immune status of the study subjects, although the CD4⁺ counts of donors with co-infection were slightly lower than the values for HBV and HIV mono-infections, there was no significant association between HIV/HBV co-infection and the immune status of the donors. This finding agrees with that of Hoffman *et al*^[15] in South Africa who reported that HBV does not affect CD4⁺ cell count in people infected with HIV. Lar *et al*^[16] in Jos Nigeria also reported lower CD4⁺ cell count in pregnant women, but similarly showed no association between co-infection and their CD4⁺ cell count. Olatunji *et al*^[17] in Ilorin Nigeria reported a lower CD4⁺ cell count, in donors with HIV/HBV co-infection compared with HIV mono-infection, but this was not significant. Forbi *et al*^[18], in Keffi, North-Central Nigeria, and Idoko *et al*^[19] in Jos all reported non-significantly lower CD4⁺ cell counts among HIV/HBV co-infected individuals compared to mono-infected. Otegbayo *et al*^[20] also observed lower CD4⁺ counts in HIV/HBV co-infected pregnant women in Ibadan, Nigeria. In contrast to our finding, Olawumi *et al*^[21], in Ilorin Nigeria reported a significantly lower CD4⁺ cell count in HBV co-infected HAART naïve patients.

There is a clear paucity of literature that looked at the immune status of apparently healthy blood donors that are seropositive for HBV/HIV infections. Most of the studies were on known HIV positive patients, in various stages of HIV infection. The scanty literature may imply diminish interest in a study on this donor population for obvious reasons. Most important is the low infectivity rates observed in this study population, particularly co-infection rate. This may mean screening a much larger population, which takes much longer study period. The other reason may include the fact that the donors are more likely to be new infections, in the early stage of the infection.

These and other reasons like the nature of the study population, sample sizes, geographical and socioeconomic variations may have reflected in the findings observed in these studies.

S Stoll-Becker *et al*^[22] reported that HBV exist in peripheral blood mononuclear cells (PBMC) and are replicated by these cells which may be an extra hepatic site of viral persistence, but as to whether these and the CD4⁺ cells are important in HBV immunity is still unclear. Some of these may still remain subjects of future studies.

6. Conclusion

Against this backdrop of the small proportion of blood donors with co-infection, coupled with lack of significant difference in their CD4⁺ cell count, it is possible that CD4⁺ cells do not play significant role in HBV immunity. We must however bear in mind that the population studied were healthy blood donors in apparent good health, probably new infections in WHO clinical stage I. This may also explain the seeming lack of effect by these infections on their immunological status. Further and larger studies are recommended to properly ascertain the effects of co-infection on donor CD4 cells and the role of CD⁺ cells on HBV viral immunity.

7. Conflict of interest's disclosure

This work is an original study, and has not been published previously. The author has no other relevant affiliations. There was no form of financial support or inducement that may pose conflict of interest to this work

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References

- [1] Bouffard P, Lamelin JP, Zoulim F, *et al*. Different forms of hepatitis B virus DNA and expression of HBV antigens in peripheral blood mononuclear cells in chronic hepatitis B. *J Med Virol* 1990; 31:312-317.
- [2] Puoti M, Torti C, Bruno R, Filice G *et al*. Natural history of chronic hepatitis B inco-infected patients. *J Hepatol* 2006; 44:S65-70.
- [3] Thio, C. Hepatitis B and Human Immunodeficiency Virus Co infection. *Hepatol* 2009; 49:S138-145.
- [4] Bodsworth NJ, Cooper DA, Donovan B. The influence of human immunodeficiency virus virus type 1 infection on the development of the hepatitis B carrier state. *J Infect Dis* 1991;163;1138-40.
- [5] Gatanaga H, Yasuoka A, Kikuchi Y, *et al*. Influence of prior HIV-1 infection on the development of chronic

- hepatitis B infection. *Eur J Clin. Microbiol Infect Dis* 2000; 19:237-9.
- [6] Hadler SC, Judson FN, O'Maeely PM *et al.* Outcome of hepatitis B virus infection in homosexual men and its relation to prior human immunodeficiency virus infection. *J Infect Dis* 1991; 163:454-9.
- [7] Colin JF, Cazals-Hatem D, Lioriot MA *et al.* Influence of human immunodeficiency virus infection on chronic hepatitis B in homosexual men. *Hepatology* 1999; 29:1306-10.
- [8] Krogsgaard K, Lindhardt BO, Nielsen JO *et al.* The influence of HTLV-III infection on the natural history of hepatitis B virus infection in male homosexual carriers. *Hepatology* 1987;7:37-41.
- [9] Gilson RJ, Hawkins AE, Beecham MR *et al.* Interactions between HIV and HBV in homosexual men: effects on the natural history of infection. *AIDS* 1997; 11:597-606.
- [10] Biggar RJ, Goedert JJ, Hoofnagle J. Accelerated loss of antibody to hepatitis B surface antigen among immunodeficient homosexual men infected with HIV. *Engl J Med* 1987; 316:360.
- [11] Laukamm-Josten U, Muller O, Bienzle U *et al.* Decline of naturally acquired antibodies to hepatitis B surface antigen in HIV-1 infected homosexual men. *AIDS* 1988;2:400-1.
- [12] Gang P, Ke JL, Wenwen J, Teresa GW, Sharon MW. Induction of APOBEC3 family proteins, a defensive maneuver underlying interferon-induced anti-HIV-1 activity. *JEM* 2006; 203, 1 41-46.
- [13] Oladepo DK, Idigbe EO, Audu RA, *et al.* Establishment of reference values of CD4 and CD8 lymphocyte subsets in healthy Nigerian Adults. *Clin Vaccine Immunol* 2009; 16(9):1374-7.
- [14] Shete A, Thakar M, Abraham PR, Paranjape R. A review on peripheral blood CD4+ T lymphocyte counts in healthy adult Indians. *Indian J Med Res* 2010; 132:667-75.
- [15] Hoffmann CJ, Charalambous S, Martin DJ, *et al.* Hepatitis B virus infection and response to antiretroviral therapy (ART) in a South African ART program. *Clin Infect Dis* 2008;47(11):1486-9
- [16] Lar, IP. M, Pam, IV. K., Christopher, IP. B. *et al.* Prevalence and Immune Status of HIV/HBV co-infected Pregnant Women. *Afr. j. Clin. Exper. Microbiol.* 14(3): 120-126.
- [17] Olatunji OP, Iseniyi OJ. Hepatitis B and C virus co-infection with HIV in infected patients at UITH, Ilorin. *Nigerian Medical Practitioner* 2008; 54 (I): 8-10.
- [18] Forbi JC, Gabadi S, Alabi R, *et al.* The role of triple infection with hepatitis B virus, hepatitis C virus, and human immunodeficiency virus (HIV) type-1 on CD4+lymphocyte levels in the highly HIV-infected population of North-Central Nigeria. *Mem Inst Oswaldo Cruz* 2007; 102:535-7.
- [19] Idoko J, Meloni S, Muazu M *et al.* Impact of Hepatitis B Virus Infection on Human Immunodeficiency Virus Response to Antiretroviral Therapy in Nigeria. *Clin Infect Dis* 2009 15; 49(8): 1268-73.
- [20] Otegbayo JA, Taiwo BO, Akingbola TS. Prevalence of Hepatitis B and C seropositivity in a Nigerian cohort of HIV-infected patients. *Ann Hepatol* 2008; 7(2):152-6.
- [21] Olawumi HO, Olanrewaju DO, Shittu AO *et al.* Effect of hepatitis-B virus co-infection on CD4 cell count and liver function of HIV infected patients. *Ghana Medical Journal* 2014; 48(2): 1-5.
- [22] S Stoll-Becker, R Repp, D Glebe, *et al.* Transcription of hepatitis B virus in peripheral blood mononuclear cells from persistently infected patients. *J Virol* 1997; 71(7): 5399-5407.