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Comparison of total white blood cells and lymphocytes between pregnant and non-pregnant women infected with human immunodeficiency virus.

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ABSTRACT

To determine and compare WBC and lymphocyte distribution between pregnant and non-pregnant women with HIV infection.Thirty-six pregnant HIV infected adult women not on antiretroviral therapy (ART) were compared with equal number of non-pregnant HIV infected women of similar characteristics. WBC and lymphocyte counts were obtained using a Coulter AcT5 five-part differential analyzer while lymphocytes cell subsets were done using a flow-cytometer. Statistical evaluations and comparisons between variables were by non-parametric computerized measures. Significance of the results was set at $P<0.05$. No statistically significant differences were found in the socio-economic and demographic characteristics of the study participants ($P>0.299$). Significant difference were observed in the total WBC ($P=0.001$), percentage lymphocyte counts, absolute and percent CD4 cell counts ($P=0.004$ and $P=0.005$ respectively) and CD4/CD8 ratio. Immune status of Pregnant HIV infected women is characterized of leucocytosis with lymhocytopenia and stable CD4 counts compared to non-pregnant HIV infected women. Larger Studies with more robust designs to confirm the present study findings.

INTRODUCTION

Pregnant women are not immune-suppressed in the classic sense, but physiological changes of pregnancy induce a state of relative immune-suppression in cellular immune response [1, 2]. In normal human pregnancy, the sex hormones produced during pregnancy contributes to local suppression of cell-mediated immunity [3-5]. In the early two decades of the HIV epidemic, the relationship of pregnancy to immune alterations gave rise to concerns about a possible adverse effect of pregnancy on the natural history of HIV disease [6-9]. There is difficulty in identifying cofactors, which are unique to affecting immunity in HIV infection and it has not been clear if pregnancy is a cofactor in determining the immunity of HIV infected person. Immunologic alterations occur during pregnancy and the effect of pregnancy on the altered immune system of the HIV infected mother is controversial [10-14]. Little information is available about the patterns of alterations of immunological indicators of immune function during pregnancy and HIV infections. To date the effects of pregnancy on the trend of total leucocytes and the characterization of lymphocytes among the HIV infected and pregnant women has not been well studied, and therefore remains unclear. Laboratory measurements, using total leucocytes counts, lymphocyte distribution are helpful in determining the immune status during HIV disease [15]. White blood cells especially lymphocytes independently predict and remain associated with the immune status of the HIV infection have clear usefulness in clinical and therapeutical management [16]. Thus, the study set to investigate the resultant immune status by analyzing immunological markers that are affected by pregnancy as well as HIV infections and can lead to a better understanding of the immunological relationship of HIV and pregnancy. The study has contributed to an increase of knowledge of pregnancies in HIV infections and may be important to all women before they decide to become pregnant. The study results also lay a foundation for future follow-up studies to confirm and provide more needed information on immunological outcomes in pregnancy and HIV infections.

MATERIALS AND METHODS

The study evaluated seventy-two adult HIV-infected women, newly diagnosed, not on ART. Informed consent was sought and obtained from each study participants, and anonymity was assured. The sample population was drawn from the HIV care

outpatient clinics at the Academic Model for providing access to Health care (AMPATH) centre- outpatient clinics and Mother Child Health Clinics (MCH) at Moi Teaching and Referral Hospital (MTRH). All participants were asymptomatic and seemed to be healthy. Non-pregnant HIV infected women with similar characteristics, were also included in the study. A face-to-face interview guided by a standard questionnaire was used to enable exclusion and inclusion of the subjects and to obtain the social-economic demographic data. A consecutive sampling technique was used to recruit those who met the inclusion criteria until equal study subjects, thirty-six pregnant and thirty-six non-pregnant HIV infected women, were enrolled in the study. Each sample was collected in tubes containing ethylenediamine tetraacetic acid (EDTA), 1 mg/ml, for cell counts. Laboratory analyses were performed at the AMPATH Modular and Reference Laboratory, which are fully equipped to perform diagnostics in support of HIV care and management. The laboratory maintains quality of its results by running controls daily and participating in the laboratory external proficiency testing panels by the United Kingdom National Quality Control Assurance Service and College of American Pathologist for hematology and clinical chemistry.

Total WBC and lymphocyte counts were obtained using a Coulter AcT5 five-part differential hematology analyzer as soon as possible after specimen collection. Immunophenotyping for lymphocytes and their subsets were performed using a flow-cytometer FACSCalibur™ (BD Biosciences, San Jose, CA, USA). The blood samples were stained for color flow cytometry with panels of CD3, CD 56/16, CD19, CD4, and CD8 monoclonal antibodies. Multiset software and FACS Lyse /No wash method, acquired on a BD FACSCalibur instrument were used to process the samples. The samples were stained within 48 hours of collection and analyses within 24 hours of staining. For each sample mixed with anticoagulant EDTA, tubes were prepared. Twenty micro-liters of the antibodies were put in each tube and vortexed. Then fifty micro-liters of the blood sample were added vortexed and incubated for 15 minutes. After staining and following incubation, cells were lysed with four hundred and fifty micro-liters of BDFacLyse reagent and incubated for at least another 15 minutes. All the data were collected on a standard case report forms, a tool developed for this study.

Statistical analysis for descriptive analysis focusing on frequencies and cross-tabulations was done using the statistical package for social sciences (SPSS) version 12.0 (Norusis, SPSS, Chicago, IL, USA). Statistical evaluations of the data and comparisons between the tests were performed by non-parametric computerised measures since the data was not normally distributed**.** Significance of the results was set at $P<0.05$.

The study was given ethical approval by Institutional Research and Ethics Committee (IREC) from Moi University/Moi Teaching and Referral Hospital. The study involved human subjects and did not interfere in any manner with the routine clinical care procedures provided to HIV-infected women enrolled in the AMPATH program and MCH clinics. Prospective participants were given adequate opportunities to discuss and contemplate their participation. Subjects were given time to ask questions and to have all concerns addressed. The participants retained the right to refuse to answer individual questions or to discontinue study participation without jeopardy. The participants had privacy and all information was treated as confidential. Data was stored in a password-protected computerized database that includes only the subjects' study identification number. Only the authorized persons in the study can have access to the subject identities.

RESULTS AND DISCUSSION

The study sought answers on how pregnancy alters total WBC and lymphocyte distribution in HIV infection. Seventy-two HIV infected subjects were studied of which thirty-six (50%) were pregnant and the other half were not. The mean age of the seventy-two women included in this analysis at the time that it was performed was 30.35 years with that of the non-pregnant women being 30.75 years and pregnant ones 29.94 years. Fifty-two (72.2%) were married and fifty-six (77.8%) were unemployed or earned less than 125 US dollars per month as income. No statistically significant differences were found in the socio-economic and demographic characteristics ($P>0.299$) between the two study groups. Patient characteristics other than pregnancy are therefore unlikely to have influenced the study results.

The present study supports the view that pregnancy may not affect negatively the immunity of HIV infected women. The results of the present study and other studies have shown that pregnancy is associated with a number of changes during HIV infection that include; increased distribution of WBCs with lower lymphocyte counts, stable CD4, and lower CD8 T-cell subsets. CD4 and CD8 T cells have been reported to influence clinical progression of the disease in other previous studies [15, 17, 18]. Significant difference in the total WBC ($P=0.001$) was observed between the two groups. The median total WBC count for the HIV infected non-pregnant and pregnant women were $5.1 *10^3$ cells/□l and $6.8 *10^3$ cells/□l respectively. Studies concurrent with this study indicate that high WBC counts during pregnancies are a common phenomenon [19, 20]. Leucocytosis is achieved and remains intact to enable the mother to combat infections effectively. The changes observed could be due to re-compartmentalization with increase in granulocytes and slightly with lymphocytes, rather than an increase in susceptibility.

No significant difference was observed with regard to absolute lymphocyte count ($P=0.978$).The absolute lymphocyte counts were same for both groups. Percentage lymphocyte counts however varied significantly ($P=0.002$) with the non-pregnant study group having higher counts (median=48%) in comparison with the pregnant group (median= 37%). The present study results could be a reflection of relative leukocytosis in pregnancy creating a relative lymphocytopenia. Studies with similar results as the present observed an increase in the total leukocyte count and reductions of total lymphocyte counts in pregnancy without HIV infection [21-23]. Unlike the counts of WBC and percentage lymphocyte counts; no significant difference was observed in the lymphocyte subsets [Absolute CD3 count ($P=0.341$), $CD3^+$ %($P=0.072$), absolute CD16/56 count ($P=0.195$), CD16/56 %($P=0.108$), absolute CD19 Lymphocyte count ($P=0.371$), CD19 % Lymphocyte count ($P=0.102$)] between the two study groups. These data are as shown in table 1.

As shown in table 2, higher CD4 and lower CD8 T cell counts in pregnancy compared to non-pregnancy were observed. In pregnant women, the median absolute CD4 and CD8 T cell counts were 442.5 cells/ ml and 902 cells/ ml and the CD4 and CD8 T cell per cent counts were 25% and 51.5% respectively. In the non-pregnant women, CD4 and CD8 T cell counts were 316 cells/ ml and 923 cells/ ml whereas the CD4 and CD8 T cell percent were 15% and 59% respectively. Absolute and percent CD4 T cell counts were significantly higher ($P=0.004$ and $P=0.005$

Table 1: Total WBC, lymphocyte and lymphocyte subsets cell counts in pregnant and non pregnant HIV infected women [Data are median (Inter-Quartile Range)]

Parameters	Pregnant women	Non-pregnant women	P Values
WBCs(counts/µl)	6.8(5.4,7.8)	5.1(4.2,6.1)	0.001*
Absolute-lymphocyte(counts/µl)	2.4(1.8,3.1)	2.4(2.0,2.8)	0.978
lymphocyte %	37.2(31.6,47.5)	48(42.9,52.7)	0.002*
Absolute T cells (CD3) (counts/µl)	1295(1070.8,1804.3)	1279(889.3,1726.3)	0.341
T-cells (CD3) %	78.5(75,82)	75(66,80)	0.072
Absolute- B-cells (CD19) (counts/µl)	161.5(120.3,224.5)	189.5(115.8,279)	0.371
CD19(B-Cells) %	8(6.3,11)	11(7,16.75)	0.102
Absolute- NK-Cells (CD56/16)	130(70.5,198.5)	105.5(68.5,136)	0.195
NK-Cells CD56/16%	7(4.3,10.8)	6(4,7)	0.108

Table 2: T-subsets cell counts in pregnant and non pregnant HIV infected women [Data are median (Inter-Quartile Range)]

Parameters	Pregnant women	Non-pregnant women	P-Values
Absolute T-helper (CD4) (counts/µl)	442.5(331.5,539.5)	316(178.5,485)	0.004*
Absolute T-cytotoxic (CD8) (counts/µl)	902(564.3,1166.75)	923(576.3,1366.5)	0.991
T-helper (CD4)%	25(21,30.75)	15(10.25,30)	0.005*
T-cytotoxic (CD8)%	51.5(41.3,58.3)	59(38,64)	0.379
CD4/CD8 ratio	0.5	0.3	0.043*

respectively) in the pregnant group as compared to the non-pregnant. The higher CD4 T cell counts in the pregnant group could be pregnancy-related and this would need to be taken into account in future studies. These results reflect limited depletion of CD4 cells in the pregnant as compared to the pregnant subjects. Thus, this may be in part an interpretation that pregnancy may not affect the immune status of HIV infected woman negatively. The pattern in CD4 T cell counts described here was also similar to that reported from a New York study[24]. This would indicate that there is likely to be little or no pregnancy-related adverse effect or even a benefit on the immune status of HIV infected woman as supported by other studies [14, 25]. The CD4 and CD8 T cell counts in pregnant HIV infected women may represent the fetal effect on immune response, despite the presence of HIV. The higher CD8 T-cells counts observed in the non-pregnant subjects may relate to the maintenance of homeostasis in the absence of T cell renewal resulting in clonal expansion of CD8 T cells, which leads to depletion of CD4 T-lymphocytes [26]. A wide variation was observed in the CD4 and CD8 T cell absolute counts in both groups. It was also observed that proportions of CD4 T cells are significantly low, relative to proportions of CD8 T cells (CD4/CD8), in the non-pregnant women compared to the pregnant women. A marked lower CD4/CD8 ratio of 0.3 was observed in non-pregnant as compared to the pregnant that had a ratio of 0.5. In the present study, CD4/CD8 ratio was observed to be high in HIV-infected pregnant women compared to non-pregnant and increased as pregnancy progresses. The ensuing CD8 T cell expansion in HIV infection may have contributed to the CD4/CD8 ratio observed that is also evident in acute HIV infection in adults[14] and precedes detection of loss of CD4 cells. An advantage of the CD4/CD8 ratio is that it does not matter whether the ratio is derived from percentages or absolute numbers of CD4 and CD8 T cell counts.

The study has contributed to an increase of knowledge on pregnancy in HIV infections and may be important to all women before they decide to become pregnant. This study provides baseline information on changes in helpful immunologic indicators during pregnancy and in HIV infection for further studies that employ control arms for confounding factors e.g. HLA genetic differences capable of affecting the immune response. The study results therefore strengthen the foundation for future studies to confirm and provide more needed information on immunological outcomes in pregnancy and HIV infections. The study recommends follow-up studies with more robust designs to confirm the present study findings and would provide much needed data and document the cause-effect relationships.

CONCLUSION

The study findings support the view that pregnancy can alter immune function with regard to WBC and lymphocyte distribution, which may not be negative to the health of HIV-positive women in any case, may be of beneficial. Immune status of Pregnant HIV infected women was shown to be characterized of leucocytosis with lymhocytopenia and stable CD4 counts compared to non-pregnant HIV infected women.

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