Serial evaluation of percentage of activated T-lymphocytes in peripheral blood of human immunodeficiency virus infected individuals as a prognostic marker

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ABSTRACT

Objective: Immune activation often associated with human immunodeficiency virus (HIV) infection is characterized by increasing number of peripheral blood T-lymphocytes expressing HLA-DR molecule. This study was performed to investigate the changes in the percentage of activated lymphocytes in the peripheral blood of HIV infected patients on antiretroviral therapy.

Methods: Routine flow cytometry data for peripheral blood lymphocyte subsets were analyzed in 11 HIV infected hemophilia patients (mean age 27 $\pm$ 7) at approximately 6 monthly intervals from 1996 to 2002 in the Division of Immunology, King Khalid University Hospital, Riyadh, Kingdom of Saudi Arabia. The number of data sets for each individual was variable, ranging between 5-13. Percentages of each lymphocyte subset were extracted and correlations were sort by using linear regression analysis.

Results: Proportion of activated T-lymphocytes in the peripheral blood was initially high. Over a period of 2-5 years the percentage of T-lymphocytes, expressing HLA-DR molecule was found to have decreased significantly ($P \leq 0.0001$) in all the patients most probably as a result of antiretroviral therapy. There was no statistically significant change in the proportion of any other lymphocyte subtype studied. The reduction in the percentage of HLA-DR+ T-lymphocyte population inversely correlated with CD4/CD8 ratios in 8 and for the CD4+ lymphocyte proportions with 5 out of 11 patients, whereas positive correlation for CD8+ lymphocyte proportions was noted in 4 patients.

Conclusion: These findings confirm immune activation in HIV infection with the increasing percentage of HLA-DR+ T-lymphocytes in the peripheral blood. Declining activated T-lymphocyte proportion in the peripheral blood and its inverse correlation with CD4/CD8 ratio may be more sensitive in detection of changes in CD4+ and CD8+ lymphocyte populations in HIV infection serving as a prognostic marker.

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Infection with human immunodeficiency virus (HIV) induces characteristic immunodeficiency over a variable period. The level of circulating CD4+ lymphocytes is the marker most commonly used for clinical and therapeutic purposes during HIV infection. A significant reduction in the percentage of CD4+ cells resulting in decreased CD4/CD8 lymphocyte ratio in the peripheral blood is considered a reliable indicator of future progression of the disease.[1-3] Several laboratory markers have been proposed and confirmed as clinical predictors. Some of these are related to the immune cell activation caused by HIV infection such as measurement of ß2-microglobulin, neopterin and interleukin-2 (IL-2) receptor levels in serum.[4-6] In addition to this factor,

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there is an increased expression of membrane antigens associated with cellular activation. Of these, human leukocyte antigen (HLA)-DR has been associated with the host response to HIV virus and the severity of the disease.[7,8] The presence of virus is to induce antigen stimulation leading to T-lymphocyte activation and HLA-DR molecule expression. T-cells expressing HLA-DR antigen is to support better replication of HIV compared to the cells lacking HLA-DR molecule.[9] In addition, the viral load as measured by estimation of plasma HIV RNA levels also shows a positive correlation with the host T-cell activation.[10-12] It would therefore be quite relevant to relate changes in activated T-lymphocyte population in the peripheral blood of HIV infected individuals to immune status of the host and its utility as a prognostic marker. This study evaluates changes in the percentage of activated T-cell population in peripheral blood of hemophilia patients with HIV infection.

Methods. Flow cytometry data of 11 hemophilia patients infected with HIV was analyzed for proportions of lymphocyte subsets in peripheral blood. The data was collected by examining the laboratory records at the Division of Immunology, Department of Pathology, King Khalid University Hospital, Riyadh, Kingdom of Saudi Arabia. All patients were receiving antiretroviral therapy after having been diagnosed with HIV infection 2-4 years before presentation. Therefore, the data available for the initial assessment varied for each patient as far as the duration of illness and the time of diagnosis was concerned. Flow cytometric assessment of peripheral blood lymphocyte subsets for each patient attending the outpatient clinic was being requested at an approximate interval of 6 months. There were 7 males and 4 females with the mean age of 27 ± 7 years. A variable number of data sets ranging from 5-13 were collected for each patient between 1996 and 2002. All patients except one were alive at the time of collection of data. Information regarding proportions of CD4+ lymphocytes, CD8+ lymphocytes, B cells, Natural Killer (NK) cells, activated (CD3+, HLA-DR+) T lymphocytes and CD4/CD8 ratio was extracted for each flow cytometric assessment. Assessment of peripheral blood lymphocyte subsets was performed on flourescent activated cell scan (Becton Dickinson Immunocytometry System) using relevant monoclonal antibodies. Analysis was performed on CELLQuest™ software. Since virus culture is not a routine investigation, the data regarding virus load was therefore not available. Correlations were sort by using linear regression analysis and paired t test was applied for comparison of means, P value ≤0.05 was considered significant.

Results. Analysis of the data revealed that the percentage of activated lymphocytes in the peripheral blood decreased in all patients. **Figure 1** shows the serial evaluation of declining percentages of HLA-DR T-lymphocytes in peripheral blood of HIV infected individuals. Although there was a variable individual response but in all patients with significant reduction (P=0.0001) in the percentage of activated cells in the peripheral blood was noted between 2-5 years of follow up. **Figure 2** shows changes in CD4+ lymphocyte proportions for the same period of assessment, increase in the proportions of CD4+ cells in the peripheral blood was evident in 3 individuals, however, a general upward trend of rising CD4+ lymphocyte proportions in the peripheral blood was noted. No change in the percentage of CD8+ lymphocytes in the peripheral blood was observed during the study period (**Figure 3**). It was, however, interesting to note that in the absence of any statistically significant changes in the proportions of CD4+ and CD8+ lymphocytes the declining HLA-DR+ T-lymphocyte proportions were found to be inversely correlated to the changes occurring in the CD4/CD8 ratios in 8 out of the 11 patients (**Table 1**). Similarly, inverse correlations were also noted for CD4+ lymphocyte population in 5 out of 11 patients. CD8+ lymphocytes, however, correlated positively with the declining activated lymphocytes only in 4 patients. No significant changes in the percentages of B cells and NK cells were noted during the study period (data not shown).

Discussion. In addition to providing valuable information to assist in patient management, follow up studies of biological markers of risk of progression of disease in HIV infected patients can play a significant role in understanding pathogenic process induced by HIV. This study involves evaluation of lymphocyte subsets in hemophilia patients infected with HIV. Since all the patients were receiving antiretroviral therapy the percentage of activated lymphocytes declined when serial evaluation of data was performed. This finding is in agreement with the previous reports where antiretroviral therapy has been shown to correlate with reduced number of activated lymphocytes.[13-16] The reduction in activated lymphocytes in these studies was also shown to correlate with the viral load indicating that the percentage of activated lymphocytes in peripheral blood may be an indirect measure of the viral load in HIV infection. Serial assessment of activated lymphocytes in the peripheral blood of HIV infected patients may therefore be useful in monitoring disease activity. Immune activation is blamed for low CD4+ counts in HIV infection. All the established markers of disease activity such as low CD4 counts, decreased CD4/CD8 ratio, increased CD8 counts and serum neopterin levels in HIV infection have been shown to have a strong correlation with immune activation.[17-19] The percentage of CD4+ lymphocytes in this study failed to show a significant increase despite antiretroviral therapy. Consistent with the findings of this study, immune reconstitution consequent to initiation of antiretroviral therapy appears not only a slow process but a great deal of individual variation is

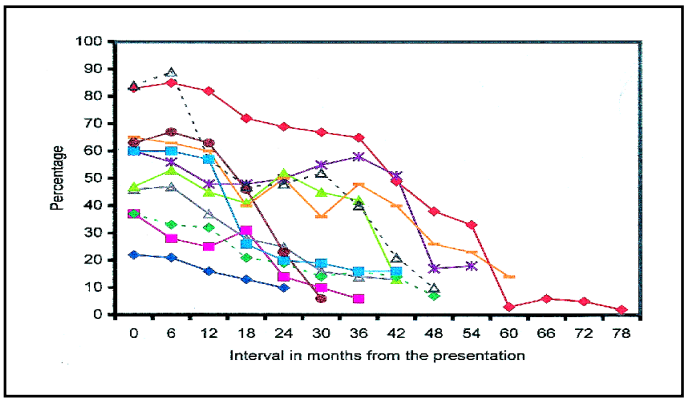

Figure 1

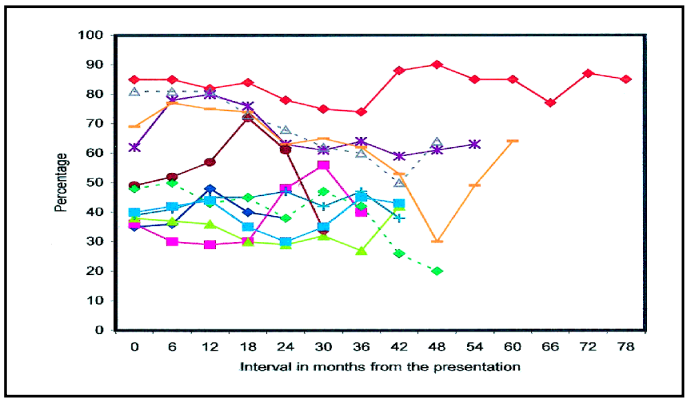

Figure 3

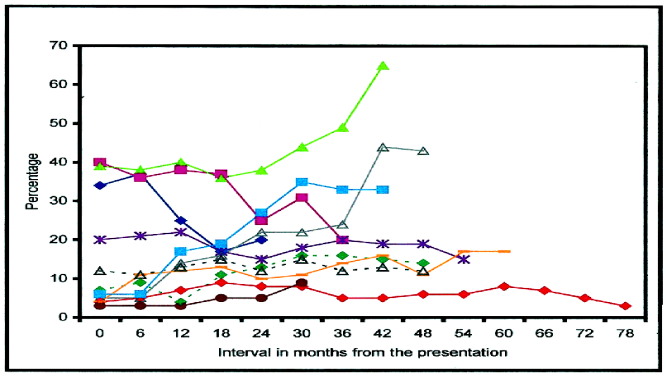

Figure 2

Figure 1 - Serial evaluation of percentage of activated T-lymphocytes (HLA-DR+) in peripheral blood of patients with HIV infection receiving antiretroviral therapy.

Figure 2 - Serial evaluation of percentage of CD4+ lymphocytes in peripheral blood of patients infected with HIV receiving antiretroviral treatment.

Figure 3 - Serial evaluation of percentage of CD8+ lymphocytes in peripheral blood of patients infected with HIV receiving antiretroviral therapy.

Table 1 - Correlation of changes in percentage of activated lymphocytes in peripheral blood with other parameters in HIV infected patients on antiretroviral therapy.

Patients	CD4+ lymphocytes		CD8+ lymphocytes		Helper/suppressor ratio		B cells	
	r	p value	r	p value	r	p value	r	p value
1	0.944	≤0.017	ns	ns	-0.904	≤0.04	ns	ns
2	ns	ns	ns	ns	-0.899	≤0.006	ns	ns
3	-0.967	≤0.0003	ns	ns	-0.749	≤0.03	ns	ns
4	ns	ns	0.863	≤0.01	ns	ns	ns	ns
5	ns	ns	ns	ns	ns	ns	ns	ns
6	-0.934	≤0.007	ns	ns	-0.943	≤0.004	ns	ns
7	ns	ns	ns	ns	ns	ns	ns	ns
8	-0.877	≤0.004	0.714	≤0.03	-0.761	≤0.02	ns	ns
9	-0.68	≤0.02	0.651	≤0.02	-0.705	≤0.01	ns	ns
10	ns	ns	0.869	≤0.01	-0.871	≤0.001	0.682	≤0.04
11	-0.920	≤0.004	ns	ns	-0.938	≤0.0003	ns	ns

No significant changes noted in natural killer cell population.
ns - not significant, r - correlates, HIV - human immunodeficiency virus

also observed.[20] Furthermore, HIV infected patients who experience substantial increases in CD4+ T-lymphocyte counts after suppression of viral replication with antiretroviral therapy have a fewer activated lymphocytes compared to those with poor CD4+ reconstitution thus emphasizing the role of activated lymphocytes as an important maker for the progression of the disease. Activation of CD8+ lymphocytes has also been related to the presence of HIV antigen.[10,11,21] In children with HIV infection higher percentage of activated (HLA-DR+) CD8+ T-lymphocytes was found in the peripheral blood of the individuals in whom the diseases progression was rapid and the viral load was higher compared to those children with lower rate of progression of the disease.[22] A separate study on children receiving antiretroviral therapy shows that the activation of CD8+ T-lymphocytes is associated with high viral load, whereas non-activated CD8+ cells are associated with lower viral load,[23,24] highlighting the role of HLA-DR molecule expression on CD8+ lymphocytes as well. Serial assessment of the expression of this molecule would therefore reflect changes in the state of immune activation induced by the viral load and the antiretroviral therapy.

Inverse correlation of activated T-lymphocytes in the peripheral blood with CD4/CD8 ratios in the majority of patients observed in this study appears to have a bearing on the events happening in CD4+ and CD8+ lymphocyte population. Although not significant, the rising CD4+ lymphocyte populations could be the result of down-regulation of immune activation, since immune activation has been directly linked with CD4+ lymphocyte depletion in HIV infection.[17] Furthermore, the inverse correlation of activated T-lymphocytes was observed in 5 out of 11 patients with regard to CD4 proportions that indicates a possible link between small but positive change taking place in this population of cells as an attempt to reconstitute CD4+ lymphocyte population. Similarly CD8+ lymphocyte proportions from 4 patients correlated positively with decreasing activated lymphocyte proportions. In contrast to these findings, declining activated lymphocyte populations correlated with CD4/CD8 ratios in a higher number (8 out of 11) of patients, suggesting an increased sensitivity for picking up changes in CD4 and CD8+ lymphocyte populations. This finding may be useful for monitoring therapeutic response before the well-established parameters in HIV infection and can provide a clearer picture.

In general, models that account for changing values of prognostic markers have occasionally been adopted in the study of the course of HIV infection[1,25,26] and deserve much greater attention. One reason for the limited use of these models is the shortcomings of the data that are available in the follow up studies of HIV infection. This study focuses on the interplay among lymphocyte subsets and an attempt was made to evaluate the changes in the percentage of activated T-lymphocyte population and its role as a prognostic marker in HIV infection. The study falls short of relating the flow cytometric findings to the clinical staging of the disease and prognosis as perceived by the treating physician. Further studies are required to investigate specificity and sensitivity of marker or markers, such as the percentage of activated lymphocytes that change over time and followed up to onset of AIDS.

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Search Word: HIV

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Title: HIV infection in Saudi Arabia. Occurrence pattern of disease and future implications
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Abstract

Between 1985 and 1990, 96 people were identified as human immunodeficiency virus (HIV)1 western blot positive. Of these, 87 were Saudi nationals and 61 attended a clinic dedicated for the management of HIV in Saudi Arabia. The demographic characteristics appear distinct from those previously described and include male predominance (male-female ratio 3:2), bimodal age distribution (childhood peak 6.4 ± 3.2 years; adult peak 29 ± 9.2 years) and a predominantly transfusion associated disease (44/71 patients). However, other well-known risk factors are represented. The sexual and maternal-fetal routes could emerge as the predominant mode of transmission in the future. Pneumocystis carinii pneumonia was the index diagnosis of aids in 9/1 7 patients (53%); kaposi's sarcoma was present in only 2/17 (12%). Oro-pharyngeal candidiasis, cmv, herpes zoster, and cerebral toxoplasmosis were common opportunistic infections, whereas tuberculosis was rare and no case of leishmania or brucella was identified. Hepatitis c antibodies were present in 19/31 patients, of which 10 (53%) had raised liver enzymes. All had acquired their HIV via blood transfusion. The point prevalence for AIDS (at mean duration of seropositivity) for blood transfusion associated HIV was 36% (6.1 ± 2.4 year), promiscuous sexual behavior/intravenous drug abuse was 58% (3.5 ± 1.7 years), hemophiliacs 4% (4.9 ± 0.9 years) and maternal-fetal acquisition 0% (3-6 ± 1.1 years). The apparent endemicity of HIV in Saudi Arabia and the important gaps in clinical attendees comprehension of the disease, highlight the need for further epidemiological studies and appropriate health education measures.