

Immunological Profile: CD4, CD8, HIV Cofactors and Viral Load in HIV Discordant Couples when Compared with Concordant Couples

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Abstract

To assess if the differences between discordant couples were due to the differences of CD4, CD8; the absolute number and proportions of these T cells were determined using a flow cytometer and other HIV Co-factors and viral load was also measured and compared with concordant and AIDS patients. Discordant negative partners had adequate amount of CD4 equivalent to healthy subjects and highly significantly ($P < 0.001$) different from discordant positives and CD4 and CD8 ratio was also high. Discordant positive partners had a significantly ($P < 0.05$) different number of CD4 cells when compared to concordant couples. The CD4 number of discordant positive subjects was closer but slightly higher than the normal boundary count.

The CD8 number of discordant positives was very similar to discordant negatives and there was no significant difference ($p > 0.05$). Increased CD8 number was associated with decreased viral load and in some subjects even to the level of below detection level. Viral load in discordant positives was 60X lower than concordant couples. Concordant couples showed elevated viral load and decreased CD8 T cells number while discordant positives showed elevated CD8 and very low viral load. The same result of viral load (slightly decreased) and CD4 and CD8 count was also obtained after one and half year when samples were collected from some of the previously studied subjects. Syphilis was a known risk factor for HIV transmission as it was diagnosed in many of discordant positives and concordant couples.

Keywords: T-cells; Syphilis; HIV Co-factors; Viral load; HIV transmission; Molecular assays; Immunoglobulin; Natural killer cells; Coreceptors

Introduction

It is a very established fact that the clinical course and outcome of HIV infection are highly variable. Some are rapid progressors and develop AIDS after a time, while others are none or slow progressors [1]. Still others are resistant to HIV even in a permanent relationship with HIV positive partners. The latter are discordant couples, where one partner is positive and the other partner is negative by all standard serological and molecular assays [2,3]. Some of these may of course seroconvert, while many remain negative despite a long time of unprotected sexual intercourse. This shows that variability in susceptibility to HIV infection exists as in all other diseases.

Discordance in HIV serostatus, which was expected to be very rare originally, is now becoming more widespread [4]. Resistant individuals are different from slow or rapid progressors [2]. Resistance to HIV mainly involves host immunological factors [5,6], although other factors may also play important roles. Stable and high CD4 count [1]; high, efficient and strong cytotoxic T lymphocytes (CTLs) [7]; HIV-neutralizing immunoglobulin A (IgA) [5] all involving HIV specific humoral and cellular immunity have been implicated in discordant couples.

Ability to attack CD4 bearing cells and the possession of some unique enzymes (Reverse transcriptase) had immensely contributed for the success of HIV in causing immunodeficiency in many subjects, although in many resistant discordant couples both CD4 and CD8 T

cells are potent and never surrender to the destructive role of HIV. Specifically, HIV-specific CTLs bearing CD8 markers have been observed in several settings [7-9] in individuals who remained HIV negative despite frequent exposure to the virus. CTLs were also known in protecting HIV by the release of HIV suppressive factors [10], which may also be true in resistant discordant couples.

Although the major damage caused by HIV is due to the ability of the virus to destroy and clear CD4 lymphocytes even at an immature stage, for productive infection and multiplication other coreceptors are also important [11,12]. These coreceptors, CCR5 and CXCR4, in addition to helping productive infection of HIV, are known to determine disease progression and viral load in infected subjects. Individuals with viruses using the CCR5 coreceptors, for example, have a slower rate of progression and viral load [13]. In a similar way, the presence of the other coreceptor, CXCR4 and CCR5/CXCR4 (mixed/dual-tropic) using

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viruses are associated with lower CD4 count and higher viral load [13] and hence rapid progression.

The amount of HIV RNA also differs significantly between progressors and long term asymptomatic persons [14]. A similar mechanism holds true for HIV positive discordant partners. Co-factors such as sexually transmitted diseases and environmental factors inducing immune activation are known in facilitating HIV transmission between couples and in increasing transient rebounds in seminal viral loads [15]. The more viruses infected partners carried, the more likely they were able to infect their sexual partners [16].

Both in humans and monkeys (Rhesus macaques), HIV and SIV, respectively, increase rates of cell division in CD4+ and CD8+ T cells, B cells and natural killer cells [17]. This phenomenon, known as immune activation, is known in Ethiopia and other sub-Saharan countries to drive progressive decline of CD4 and disease progression [18-21]. It was also shown that Ethiopians had lower CD4 and higher CD8 count when compared with others [18]. Thus, it is possible that decreased immune activation and increased CD4 and CD8 count may offer resistance to discordant couples in Ethiopia.

Several studies were carried out about discordant couples in many countries. But there were little or no study carried out on discordant couples separately by comparing with concordant, AIDS patients and healthy controls. This study investigated the immunological and other host factors of discordant partners separately by comparing with concordant couples and healthy controls.

Material and Method

Study area

The study was carried out on HIV discordant, concordant and HIV-seronegative (as a healthy control) couples from January 2009- January 2012 in four Administrative Regions and Addis Ababa, the capital city of Ethiopia. The study was all in all carried out in government health center and hospitals. The subjects were all on follow up for a long time in their respective health centers and were discordant in their HIV sero-status for a long time. Samples obtained from these areas were analyzed in Ethiopian health and nutrition research institute (EHNRI).

Study design

The study design was a prospective cross sectional study involving comparisons of immunological, and other host factors contributing to resistance or susceptibility to HIV infection in discordant couples. After one and half year, samples were obtained from 50 discordant couples and analyzed for some critical parameters.

Study population and sample size

A total of 46 discordant couples, 46 concordant couples and 4 healthy control couples were investigated for the study. The age range was between 30 and 50 years. The majorities were less than 50 years of age [22-26]. The marriage relationship was permanent and lasted from 3-14 years. The inclusion criteria involved:

- Willingness to provide individual informed consent, on the part of either or both members of the couples;
- Acceptance of couples counseling on the part of one or both partners regarding HIV results;
- Age greater than 18. Children are not included in this study since it specifically focuses on permanent sexual relationships;

- Having permanent monogamous (marriage) relationships for more than one year;
- Being HIV serodiscordant or seroconcordant couples; and
- Being treatment (ARV) naive.

The subjects were counseled, tested and registered as HIV discordant or concordant couples and were on follow up by the respective health institutions (health centers and hospitals). That is, they were identified, counseled, tested and registered as discordant or concordant couples by the nurses and doctors of the respective health centers and/or hospitals.

The study was conducted after obtaining the national ethical clearance from the then Ethiopian Science and Technology Commission (ESTC) and the now Science and Technology and the institutional clearance from Ethiopian Health and Nutrition Research Institute (EHNRI) and Addis Ababa University (AAU). Participation in the study was voluntary. Detailed information about the study was made available for all patients in their language. Only patients who gave informed consent were included in the study. The consent form was completed only after the patient had understood the points enumerated in the information sheet. All study participants were able to withdraw from the study at any point without any consequence to his/her care and clinical management.

Data on HIV status was dealt with due care for respect of anonymity. This was achieved by identifying blood samples and test results by code, not by name, with no personal identifier to link the samples to the client.

Sample collection, transportation and analysis

After the patients were identified and their willingness to participate in the research was approved, patients were asked to give samples (blood). Blood was collected by trained and experienced nurses. Twenty milliliter whole blood was collected from each study subject in vacuoliner tubes in EDTA and transported to the laboratory on the same day it was collected for analysis. Blood samples were always collected at the same time starting early in the mornings from 8:00 AM to 11:30 AM and was analyzed within 24 hours.

The blood sample was rejected if it was haemolysed, turbid or had not been stored and transported properly, didn't carry appropriate label, and the container had leaked. Laboratory analysis was carried out at EHNRI. Three samples were rejected due to haemolysis and being turbid.

Data analysis

The collected data was entered and analyzed using SPSS version 13 software. Mean, median, mode and standard deviation were collected for many parameters in the study. Results were compared in discordant, concordant and negative control. When the comparisons involved two groups, non-parametric (Mann-Whitney U-test) method was used. But when comparisons were made between three groups or more groups, the level of significance (α) was adjusted using Bonferroni corrections ($\alpha=0.033$). This association between several parameters was determined using a multivariate regression analysis. Correlation coefficients were calculated by the spearman's test.

HIV-testing

HIV testing was performed by using a combination of HIV rapid assays (according to the National HIV testing algorithm) using Determine (Abbott, Japan), Capillus (Biotech, Ireland) and Uni-

gold (Biotech, Ireland) and enzyme-linked immunosorbent assay (Vironostica, HIV Uniform Ag/Ab, Boxtel, The Netherlands). The testing involved serial testing algorithm and this was done to re-test subjects who were already tested in their respective health institutions to prove whether the subjects were truly HIV positive or not and hence truly discordant or concordant couples. The enzyme-linked immunosorbent assay was carried out first and samples which were both positive and negative were re-tested by serial testing algorithm and categorized as positive and negative after the completion of serial testing algorithm. Results were interpreted as positive when the test was positive by ELISA and by two successive tests of serial algorithm and negative when it was negative by ELISA and the two successive serial algorithm tests. Thus, the results were confirmed by many times testing and re-testing.

Syphilis serology

Syphilis serology was performed by *Treponema palladium* particle agglutination assay (TPPA) (Serodia-TPPA, Fujirebio, Japan) and rapid plasma reagin assay (RPR) (RPR-nosticon II; Organnon Teknika, Boxtel, The Netherlands), according to the manufacturers instruction. Serum samples isolated and kept frozen at -80°C were tested with RPR (according to the manufacturers' instruction) and samples which were positive for RPR were re-tested by TPPA and results were accepted only when were found positive by TPPA and rejected when found negative by TPPA.

Peripheral blood mononuclear cell isolation

Venous blood was collected from the study subjects in EDTA vacutainer tubes and plasma and blood cells were separated by centrifugation. The plasma was separated and stored at -80°C until further analysis was carried out. The remaining blood cells were diluted with PBS and layered over Ficoll-Hypaque. After density gradient centrifugation on Ficoll-Hypaque, PBMC was collected and viable frozen in liquid nitrogen until further analysis was carried out.

Determination of viral load

Viral load was determined by quantifying the amount of HIV RNA in plasma samples stored at -80°C using Nucleic Acid Sequence Based Amplification (NASBA) assay (NUCLISENS, Organon Teknika, The Netherlands). The minimum detection limit of this assay was 50 copies/ml. It was known that NASBA methodology gives quantitatively reliable results on HIV subtype C plasma samples [27,28].

Cell surface staining and analysis

Surface staining and analysis was performed using standard flow cytometry procedure by FACSCalibur (BD, San Jose, CA). All straining were carried out by monoclonal antibody (mAb) to which is conjugated three different kinds of cytochromes: Peridinin chlorophyll protein (PerCP), Flourescein Isothiocyanate (FITC) and Phycoerythrin (PE) (all from BD, San Jose, CA). Absolute CD4+ and CD8+ T-cells count was carried out by three color surface staining involving the following flouochrome conjugated monoclonal antibodies: CD3FITC-CD45RAPerCP-CD4PE, CD3FITC-CD45RAPerCP-CD8PE (BD, San Jose, CA). Whole blood samples were stained for CD3, CD4, CD8, and CD45RA monoclonal antibodies. In brief, 20 ul of CD3FITC-CD45RAPerCP-CD4PE and CD3FITC-CD45RAPerCP-CD8PE were added to two test tubes and to each 50 ul whole blood was added and incubated at room temperature in the dark for 15 minutes, After 15 minute's incubation, 450 ul of lysing solution was added to each test tube and incubated for 15 min at room temperature. Finally, analysis

was performed using three colors FACSCalibur (cell quest software, BD). In the lymphocyte gate, 50,000 to 100,000 events were acquired and results were expressed in terms of absolute number [29-32]. The FAScan/FACSCalibur was calibrated with CaliBRITE fluorescent beads on weekly basis.

Result

To asses if the difference in the amount of CD4 and CD8 T cells were responsible for the susceptibility and/or resistance to HIV infection in discordant couples, absolute counts of CD4 and CD8 T cells were measured using a three color – flow cytometer. The result showed that the median average number of CD4 in discordant negative partners was 749 (95% CI 706-792) and 570 (95% CI 483-658) (Figure 1) in discordant positive partners and the difference was very highly significant ($p < 0.001$) (Figure 1). Similar result was also obtained for CD8 T cells. The median average number of CD8 T cells in discordant negative partners was 921 (95% CI 825-1017) and in discordant positive partners it was 850 (95% CI 798-904) and the difference was not significant ($p > 0.05$) (Figure 1). The ratio of CD4 to CD8 in discordant negative partners was 0.81 and 0.67 for discordant positive partners and 0.94 for the negative control (Figure 1). There was a positive correlation ($r = 0.520$) between CD4 and CD8 in discordant negative partners although this was not significant ($p > 0.05$). Comparison of the difference between CD4 and CD8 in discordant positive partners, however, showed a negative correlation ($r = 0.468$) and the difference was very highly significant ($p < 0.001$). The median average number of CD4 and CD8 for healthy control subjects (CD4 (879 (95% CI 762-996); CD8 (934 (95% CI 854-1013) was very similar to the discordant negative partners and the difference was not significant ($p > 0.05$). But there was a difference ($p < 0.05$) between the healthy control and the positive discordant partners. The ratio of CD4 to CD8 (0.94) was also very close to discordant negatives than discordant positives (Figure 1).

The median average of CD4 count for concordant couples was 261 (95% CI 278-325) and CD8 673 (95% CI 455-779) and the ratio of CD4 to CD8 was very low (0.37) (Figure 1). The difference between CD4 and CD8 in concordant couples was very highly significant ($p < 0.001$). The difference in CD4 between concordant couples and discordant partners was two-fold and significant ($p < 0.05$). Similarly, the difference in CD8 numbers between both groups was also significant ($p < 0.05$) (Figure 1).

For the majority of discordant negatives CD4 was greater than 700 (Figure 1) while CD8 was for all greater than 700. CD4 count was between 400 and 800 and CD8 was between 700 and 1000 for the majority of discordant positive partners (Figure 1). For concordant

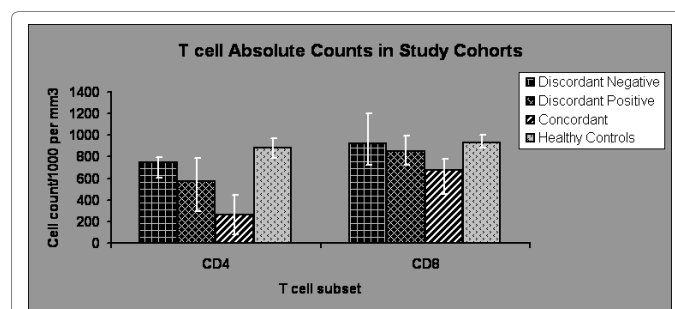


Figure 1: T cell Absolute counts in study subjects. P-values were significant ($p < 0.05$) for CD4 and CD8 count between Discordant positive (DSCP) and Healthy controls (HC); and Concordant couples. (CONC) vs. DSCP (discordant partners); very highly significant ($p < 0.001$) DSCP (discordant positive) and DSCN (discordant negative), CD4 and CD8 count in CONC.

couples, however, CD4 was between 200 and 450 and CD8 was 600 to 800 for the majority of the subjects (Figure 1). The result of the healthy controls was very similar to discordant negatives, although it was slightly higher (Figure 1).

When syphilis plasma antibody was tested to determine co-factor effect of STDs, 34 % (79 out of 232) were positive for syphilis. This included 8.3% discordant negatives, 44.4% discordant positives and 33.3% concordant couples (Figure 2). This result was greater than what has been reported in the report of the history of STDs during the behavioral study (Publication in progress). The average number of viral RNA count/ml of blood was 4733 (130- 32000) c/ml for discordant positives and 272480 (200 000- 290 000) c/ml for concordant couples (Figure 3).

There was about 60- fold difference in viral load count between discordant positives and concordant couples (Figure 3). The difference between viral load of discordant positives and concordant couples was also very highly significant ($p < 0.001$) (Figure 3).

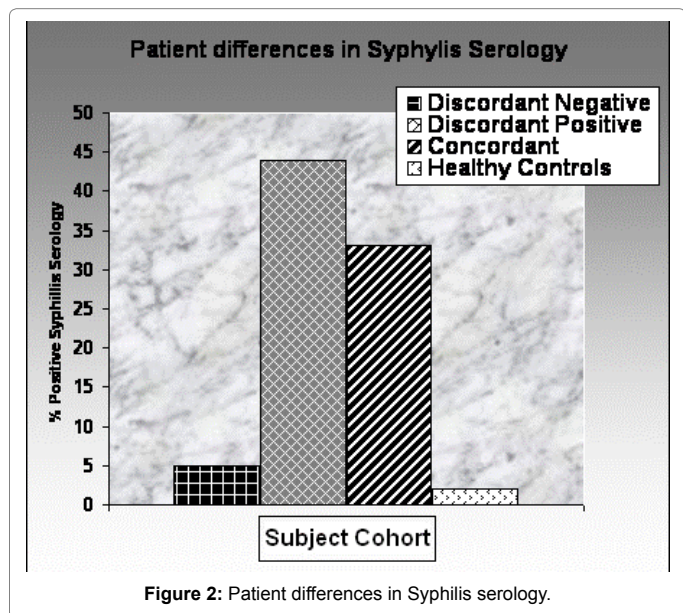


Figure 2: Patient differences in Syphilis serology.

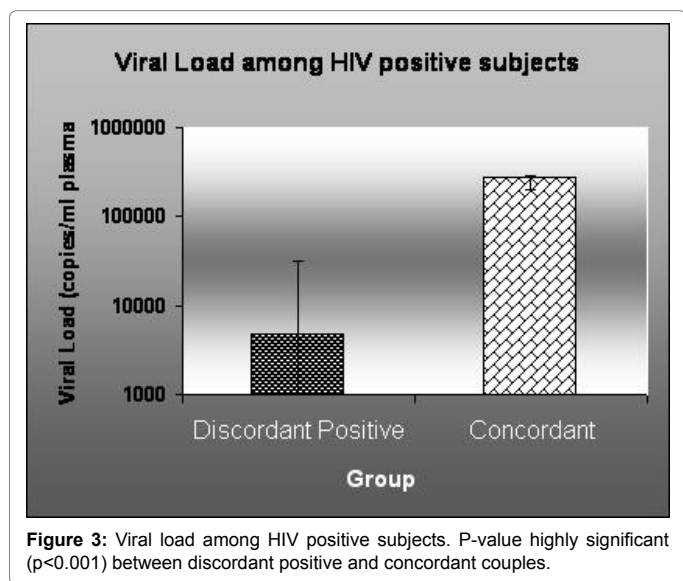


Figure 3: Viral load among HIV positive subjects. P-value highly significant ($p < 0.001$) between discordant positive and concordant couples.

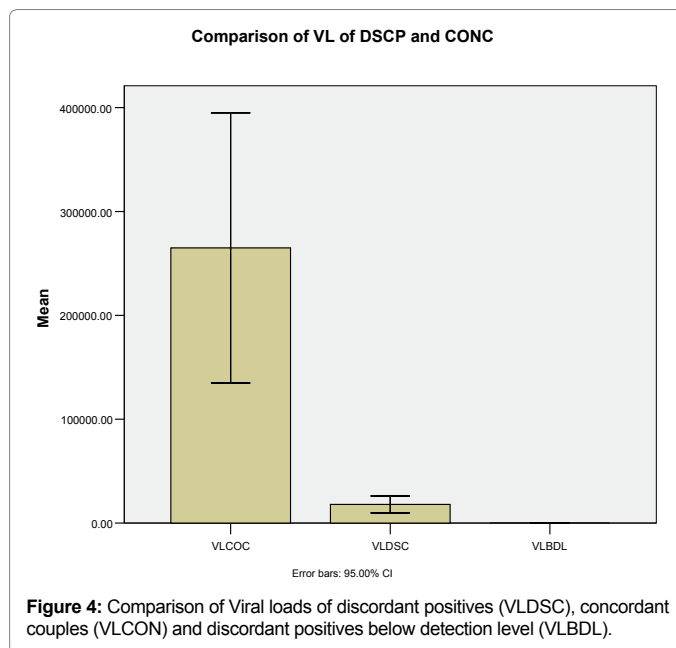


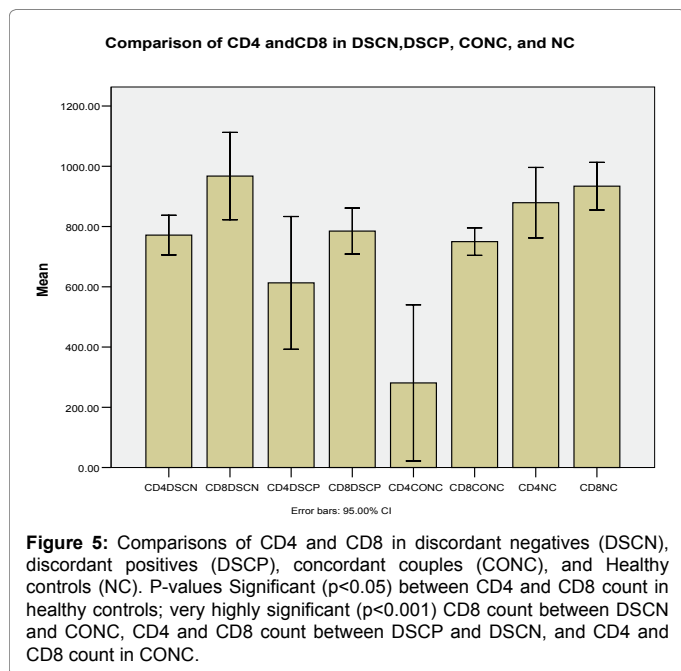
Figure 4: Comparison of Viral loads of discordant positives (VLDC), concordant couples (VLCOC) and discordant positives below detection level (VLBDL).

Among discordant positives 15 out of 61 (24.5%) were serologically positive for HIV but viral load was below detection level (VLBDL) (Figure 4). The difference between those serologically positive with detectable viral load and those who are serologically positive but without detectable viral load was also very highly significant ($P < 0.001$). Figure 4 shows the comparison in viral load count between concordant couples, discordant positives and those whose viral load is below detection level. These subjects had elevated number of both CD4 and CD8 and were negative for syphilis.

Viral load was also found to be closely associated with CD4, CD8 and serological syphilis positivity (Data not shown). There was a negative correlation ($r = -0.662$) between viral load and CD4 and a weaker negative correlation ($r = -0.244$) between viral load and CD8 in discordant positives. The relationship was not however significant ($p > 0.05$) in both cases in discordant positives. The relationship of CD4 between discordant negative partners and discordant positive partners was an inverse relationship ($r = -0.671$) ($p < 0.05$) and the difference between them was significant. There was no difference between CD4 of discordant negatives and the healthy controls ($p > 0.05$). However, there was a weak direct relationship between CD8 of discordant negatives and discordant positives ($r = 0.432$) ($p > 0.05$) and their difference was not significant. But the difference in the number of CD8 T cells between discordant negatives and concordant couples was highly significant ($p < 0.001$).

But there was no difference in the number of CD8 T cells between discordant negatives and the healthy control. Figure 5 summarizes the relationship between CD4 negative control and CD8 in discordant positives, discordant negative, concordant couples.

The relationship between CD4 and CD8 was also variable. There was a direct correlation ($r = 0.552$) between CD4 and CD8 in discordant negatives and there was also a significant difference between them ($p < 0.001$). But there was a weak negative correlation ($r = -0.443$) between CD4 and CD8 of discordant positives and negatives, although it was not statistically significant ($p > 0.05$). The difference between CD4 and CD8 of discordant positives and negatives was however very significant ($p < 0.001$). The difference between CD4 and CD8 of concordant couples



Parameters	DSCP	DSCN	CD4 : CD8
CD4	577(458-897)* 570 (297-790)** CD4 Vs VL** $p < .01$ $r = -0.73$	749 (603-792)* 837(728-941)**	0.81* 0.88**
CD8	789(671-968)* 850(720-997)** CD8 Vs VL** $p > .05$ $r = 0.33$	921(720-1200)* 945(749-1045)**	
VL	4562(1900-7690)* 4533(130-32000)**		

*First study; ** Second study; P-value > 0.05 not significant, < 0.01 highly significant

Table 1: Median average of CD4, CD8 count and viral load (VL) of discordant couples after one and half year. DSCP (discordant positive), DSCN (discordant negative), VL (viral load) and r (correlation).

was also very highly significant ($P < 0.001$). The difference between CD4 and CD8 of the healthy controls was however not significant ($p > 0.05$).

To study the change in the number of CD4, CD8, viral load and possibilities of seroconversion over time, samples were obtained and analyzed from 50 previously examined discordant couples after one and half year. No seroconversion was observed after one and half year in these subjects and the change in CD4, CD8 and viral load were as shown in Table 1.

CD4 was decreased by seven after one and half year but the difference was not significant ($p > 0.05$) when compared with the first study in discordant positive subjects (Table 1). But CD8 was increased by 88 in discordant positives after one and half year. The difference between the results of the first study and the second one was however not significant. The ration of CD4 to CD8 increased from 0.67 to 0.68 in the second study in discordant positive subjects. There was a strong negative correlation ($r = 0.73$, $p < 0.01$) between CD4 and viral load, although there was a weak negative correlation ($r = -.33$, $p > 0.05$) which was not significant between CD8 and viral load in discordant positives. Viral load was decreased by 11 in the second study when compared with the first study in discordant couples.

In discordant negative subjects CD4 was increased by 88 and CD8

by 24 and the ratio of CD4 to CD8 was improved from 0.81 to 0.88 showing increased number of both CD4 and CD8 and better resistance to HIV (Table 1).

Over all, CD4, CD8 and viral load were stable when compared with the first study and was as shown in the following Figures 6A-6C. For the majority discordant positives CD4 count was above 500 and stable. Similarly, CD8 count was above 760 and viral load was relatively lower than was in the first study and below 5000 C/ml.

Discussion

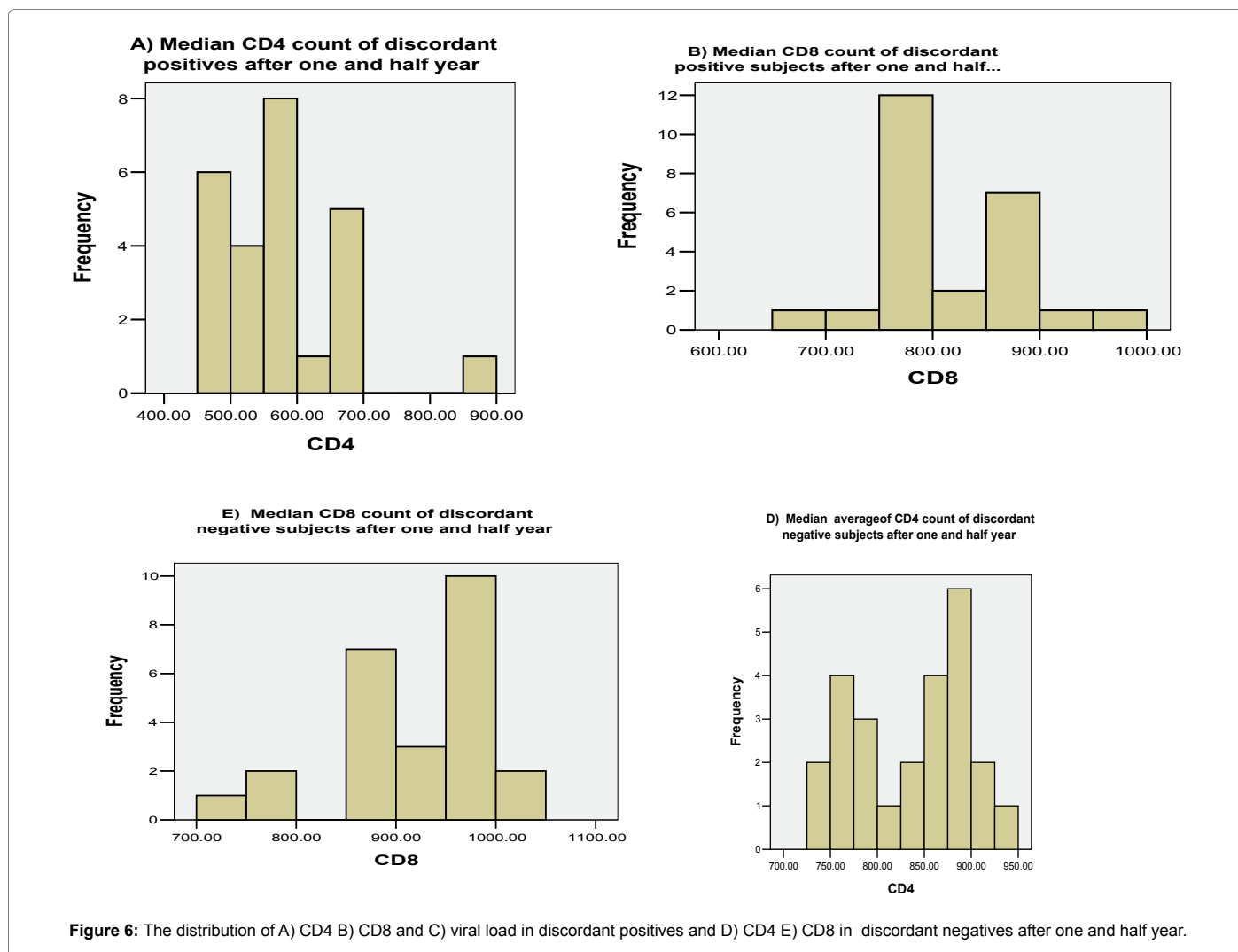
Cellular immune responses are critical part of the host's defense against viral infections. Both CD4 and CD8 T cells play a very important role in immunity to viral infections. The major damage caused by HIV to the immune system is a depletion of CD4 T cells. CD4 T cells loss leads to an irreversible breakage or weakening of the immune system and to an inevitable AIDS and finally to the demise of the infected person. On the other hand, strong immunity including an appropriate help provided by CD4 T cells is the major contributor of an aborted HIV infections and a delayed progression to AIDS [22].

In HIV discordant couples the major reason why HIV was not transmitted to HIV negative partners is due to strong cellular immunity. In subjects who have been living together as a husband and wife for more than three years and up to 14 years with a different HIV serostatus, a CD4 count similar to healthy uninfected people was observed in discordant negative partners. There was no difference between healthy uninfected subjects ($p > 0.05$) and HIV negative discordant partners. There was not only normal number of CD4 cells, but also the number of CD8 T cells was also normal and similar to healthy controls ($p > 0.05$). The ratio of CD4 to CD8 was higher (0.81) in discordant negatives when compared to the ratio of discordant positive partners (0.61) and was very similar to healthy controls (0.94), indicating that CD4 and CD8 T cells were as potent as in healthy individuals in discordant negatives. Healthy CD4 and CD8 count with higher CD4 to CD8 ratio is a characteristic of resistant individuals [23]. This had also been observed in discordant couple's studies in other countries [5,6]. Thus, our result is in agreement with other studies, as higher CD4 count is known in reducing or completely clearing HIV from the body when one is infected. CD4 count is an indicator of efficient immunity and lower CD4 count is associated with progression of disease [1].

The difference in the number of CD4 T cells between discordant negative partners and positive partners was very highly significant ($p < 0.001$), although the difference in the number of CD8 T cells between these partners was not significant ($p > 0.05$). The relationship between CD4 and CD8 in discordant negative partners was a direct relationship, which is another sign of a healthy immune system, and there was an inverse relationship between CD4 and CD8 T cells in discordant positive partners. Although the inverse relationship was weaker ($r = -0.468$, $p < 0.001$), the relationship was very highly significant indicating a different relationship between CD4 and CD8 in discordant positives when compared to discordant negatives.

The number of CD4 T cells of discordant negative partners was more than 3-fold higher than CD4 of concordant couples and was significant ($p < 0.05$). The difference in CD8 number between discordant negative and concordant couples was also significant, although it was not many-fold difference. For the majority of the discordant negative partners CD4 count was greater than 700 and CD8 count was greater than 700 for all, indicating similar pattern in all subjects.

A clear pattern of lower counts of CD4 and CD8 was also observed



for discordant positive partners and concordant couples, consecutively. The higher CD4 to CD8 ratio; the close similarity of CD4 and CD8 to healthy subjects; the big difference between discordant negatives and discordant positives and concordant couples, clearly showed that discordant negative partners had a normal and potent CD4 and CD8 T cells. This may be one of the reasons why discordant negative partners were protected from HIV despite frequent exposure to HIV.

The CD4 count of Discordant positives, although significantly different from discordant negative partners, was not below the normal range (>500) of healthy CD4 count; and it was very highly significantly ($p < 0.001$) different from concordant couples with a higher CD4 to CD8 ratio (0.61 vs. 0.37). The observation that the CD4 count was between 400 and 800 for the majority of these subjects also indicate that it was not abnormally low, although it was on the boundary between healthy and HIV infected individuals. This cannot also occur by chance as it involved many subjects.

However, the CD8 count of discordant positive partners was not significantly different from discordant negative partners but was very highly significantly ($p < 0.001$) different from concordant couples. This indicated that CD8 T cells which are potent antiviral agents may be keeping the viral load lower and preventing abrupt decline of CD4 T cells. This was clearly seen when viral load of discordant positive

partners was compared with concordant couples. The viral load of concordant couples was 60-fold higher than discordant positive partners and the difference was very highly significant ($p < 0.001$). CD4 count of discordant positive partners was more than 2-fold higher than concordant couples; and CD8 count of discordant positives were also very highly significantly ($p < 0.001$) different from concordant couples.

There was also an inverse relationship between CD4 and CD8 in both discordant positives and concordant couples, although this was much weaker in concordant couples. Discordant positives showed the characteristics of long term non progressors [24]. CD4 count did not decline drastically and was capable of providing help to CD8 T cells. CD8 T cells are strong antiviral agents and this could maintain the viral load at lower level [25]. Our results thus were not different from the study of long- term- non- progressors.

This was also supported by the evidence from the presence of certain kinds of T cell subpopulations and HLA subtypes (publication in progress). The fact that both CD4 and CD8 count and the ratio was different from concordant couple also clearly showed that discordant positives were different from concordant positives.

Some of the discordant couple, who were serologically positive for HIV, had no detectable viral load. These subjects had higher CD4 and

CD8 (particularly CD8 T cells) than others with detectable viral load. Since the only difference we observed was number of CD4 and CD8 T cells, it could be explained that potent cytotoxic T lymphocytes in these subjects might have controlled the viral load to the level undetectable in the blood. These may also be HIV-specific cytotoxic T lymphocytes capable of clearing HIV from the body tissues.

The stronger negative correlation between viral load and CD4 ($r=-0.66$) also indicated that CD4 played an important role in reducing viral load such as by providing appropriate help to CD8 or by other mechanisms. The fact that there was a potent immune response involving CD4 and CD8 (may also include others) can further be substantiated by the fact that this was not observed in concordant couples.

Cytotoxic T lymphocytes (CTL) have been suggested to play an important role in the control of HIV infection [9,7]. It is possible that CD8+ T cells may have the same or different roles in discordant negatives (probably by protecting HIV infection) and discordant positives (probably by delaying progression) and in concordant couples having destructive roles. It remains likely that phenotypic differences may reside in the ability to CD8+ T cells to mediate cytolysis, secrete suppressive factors, or proliferate *in vivo* [8]. The inverse relationship between viral load and CD8+ T cells indicated that CD8+ T cells could suppress HIV progression; in presence of CD8+ T cells the viral load was found to be lower. As it is known from previous studies [1,7], strong CTL could clear or suppress HIV virus completely. These may be strong HIV specific CTLs capable of clearing HIV and maintaining HIV at lower level [7]. Thus further characterization of these CTLs could explain the mechanism of actions and type of help provided by CD4 T cells to CTLs.

Syphilis as a co-factor for HIV transmission was observed in both discordant positives (44.4%) and concordant couples (33.3%) when compared with discordant negatives (8.3%), indicating that syphilis was a strong co-factor in HIV acquisition and highly related to HIV transmission. The result was much higher than that had been reported in the behavioral study report (Publication on progress), indicating that many people did not know that they had been infected with syphilis in their lives or might have under-reported due to the negative charisma associated with the disclosure of their status.

It is known that syphilis is the most common ulcerative STD in this country [26]. Syphilis can remain silent in the body for a long time. Due to this chronic nature, it might have aggravated the transmission and progression to AIDS. Since syphilis positivity was much higher in concordant couples and discordant positives than in discordant negatives, syphilis and other STDs could be the major facilitators of HIV transmission and progression. The behavioral data obtained in this study also showed the history of syphilis and other STDs, supporting our laboratory data. All these indicated the extent of exposure to risky sexual activities.

All the subjects who had undetectable viral load were negative for syphilis serology, indicating reduced co-factor effect of other sexually transmitted diseases including syphilis. There was a weak positive correlation ($r=0.085$) between syphilis and viral load, indicating the increase of viral load with an increase of syphilis positivity ($p<0.001$). It was not clear why there was a very significant association between syphilis positivity and viral load, although there was a weak positive correlation ($r=0.085$) between them. However, it was clear that the associations were weak but very highly significant in both discordant positive partners and concordant couples. This result also showed that

sexually transmission of HIV was associated and facilitated by syphilis and probably by other STDs in both discordant positive and concordant couples than discordant negative partners.

The absolute number and proportions of CD4 and CD8 T cell of discordant negative partners was normal. In Discordant positive partners, although absolute number and proportions of CD4 T cells was relatively lower than discordant negatives, it was stable and significantly different from concordant couples. The proportion and the number of CD4 T cells were also in the lower boundary of the normal count.

In their immune status, the discordant positives occupied an intermediate position between discordant negatives and concordant couples as can be deduced from both the absolute number and proportions of both CD4 and CD8 counts and the different subpopulation's counts and proportions. These clearly demonstrated that an equal balance of power where one cannot defeat or be defeated by the other, existed between the immune system and the HIV infection in discordant positives.

The evidence for this was lower viral load even to the extent of undetectability maintained by the immune system, elevated CD8 count, intermediate count of CD4, reduced activation markers and expression of efficient subpopulations of CD4+ effector/memory T cells (Publication in progress). The CD4/CD8 ratio also reflected this pattern, slightly lower than discordant negatives and about 2X of concordant couples. The proportion of CD4 and CD8 was also in favor of this notion.

The median average count of CD4 and CD8 and the viral load of discordant positive subjects were even constant over years. Significant differences were not observed in CD4, CD8 and viral load counts after one and half years and this showed what was observed in discordant couples was constant over years. Viral load was even decreased in discordant positive subjects after one and half year. Although a slight decrease in CD4 count was observed a strong correlation and highly significant association with the viral load showed CD4 was very important in decreasing viral load in discordant positive subjects. Thus stable CD4 and CD8 count in discordant negatives showed that their immune system is strong and capable resisting HIV infection. Discordant positives were also capable of maintaining a balance between their immune system and the viral infection and are even at a better position in relation to the viral pathogen as the viral load decreased after one and half year.

Conclusion

When subjects were compared immunologically, discordant negative partners had adequate amount of CD4 equivalent to healthy subjects and highly significantly different from discordant positives. CD4 and CD8 ratio was also high indicating a healthy balance and this was also similar to healthy controls. Discordant positive partners had a significantly different number of CD4 cells when compared to concordant couples. Their CD8 number was very similar to discordant negatives and there was no significant difference. Increased CD8 number was associated with decreased viral load and in some subjects even to the level of below detection level. Lower viral load in discordant positives when compared to concordant couples also indicated lower or absence of transmission to uninfected partner. CD8 T cells were responsible in decreasing viral load. The evidence for this came from the observation that concordant couples showed elevated viral load and decreased CD8 T cells number while discordant positives showed elevated CD8 and very low viral load. CD8+T cells may have different

roles in discordant positives and concordant couples as there was an inverse relationship between viral load and CD8+ T cells in discordant positives but not in concordant couples.. Their CD4 number was also closer but slightly higher than the normal boundary count and might have been capable of providing the appropriate help for CD8 cells. CD4 and CD8 of both discordant positives and discordant negatives was constant and no significant difference was observed even after one and half year showing a stable and constant immune system capable of making them resistant and keeping in check the viral load. Syphilis was a known risk factor for HIV transmission as it was diagnosed in many of discordant positives and concordant couples. This is possible because syphilis is a common STD in this country and its chronic nature might have accounted for its co-factor effect.

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