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# The HLA Profile of Ethiopian Discordant and Concordant Couples: In Comparison with AIDS Patients

**Meseret Y[1]*, Mengistu Y[2], Howe RC[3], Messele T[4] and Wolday D[5]**

[1]Kotebe University College, Addis Ababa, Ethiopia
[2]Mauricio's, Botswana
[3]Armour Hansen Research Institute (AHRI), Addis Ababa, Ethiopia
[4]African Society for Laboratory Medicine (ASLM), Addis Ababa, Ethiopia
[5]Medical Biotech Laboratory, Addis Ababa, Ethiopia

## Abstract

In order to analyze the effect of host HLA types on resistance or susceptibility to HIV infection and to study the HLA profile Of Ethiopian HIV/AIDS subjects DNA based HLA typing was carried out on 239 discordant, concordant couples and full blown HIV AIDS subjects. Our study showed that there was a clear difference between discordant positives and discordant negative couples, and discordant positives and concordant couples in their genetic profiles. Ethiopian AIDS patients were different from Ethiopian concordant couples in their very significant to significant association with HLA-A*29, *18, and *41; HLA-B*0705, *1517, *4101, *5001, *7301 and *18; HLA-C*0501, *0701, and *0740. AIDS patients were also very significantly different from discordant positives in their associations with HLA-A*68, HLA-B*39 and HLA- DR*11. AIDS patient were also different from discordant negatives in their very highly significant to highly significant association with HLA-*0801, *1817, *352001 and *4901; HLA-C*7 and HLA-DR*40301. Discordant positive subjects were found to be more heterozygous at all loci (HLA-A, B, C and HLA-DR) when compared with concordant couples and HIV/AIDS subjects.

**Keywords:** HLA Profile; HIV/AIDS; Ethiopia; Genotype; Discordant couples

## Introduction

Discordant couples (couples with different HIV-serostatus) and concordant couples (couples with similar HIV positive serostatus) are good models for comparison of HIV genetic studies. In this study, we compared HLA profile of these two groups with patients showing full blown or typical AIDS characteristic. Concordant couples and discordant positive subjects were HIV positive but did not show typical AIDS characteristics.

CD8+ cytotoxic T lymphocytes (CTL), which are the major antiviral adaptations, are also known against retroviral infections [1-4]. If CTLs are important in controlling HIV infection, HLA class I type should play a major role in determining disease progression. HLA class I molecules have a direct and special connection to viruses. This is particularly true in that they play a central role in the task of alerting CTLs to cells that have been breached by virus [4]. An individual's HLA genotype is also predictive of whether HIV is likely to kill quickly or slowly. For example, there is a direct association between HLA class I types and rates of HIV disease progression [5], as was shown that heterozygosity for HLA-A, B, and C is known in delaying onset of AIDS. Studies with HIV infected long term non-progressors (LTNP) also showed increased frequency of specific HLA class I [6-12]. These and other studies indicated that there is a genetic background behind resistance and/or susceptibility to HIV infection. Even heterozygosity versus homozygosity of these HLA types are known in determining susceptibility and /or resistance to HIV [7,12].

Limited studies have been conducted to determine HLA class I allelic frequency in Ethiopia. One amongst this was a study carried out on 50 HIV- positive and HIV-negative subjects [8]. Few studies, if at all were carried out, investigated HIV discordant and concordant couples separately and looked at their HIV profiles by comparing them with full-blown AIDS profile. Thus, the objective(s) of this study is to investigate the profile of HLA subtypes in discordant negatives (DSCN), discordant positive (DSCP), concordant couples (CONC) and full blown AIDS patients. This is particularly important because the study of HLA polymorphism facilitate diagnosis or prognosis of the potential AIDS patients and elucidates how susceptibility and resistance to HIV infection is related to genes.

## Materials and Methods

### Study area

The study was carried out on 239 HIV discordant, concordant and full blown AIDS subjects from January 2010-January 2012 in five Administrative Regions and Addis Ababa, the capital city of Ethiopia.

### Study design

The study design was a single spot prospective cross sectional study involving comparisons of HLA subtypes in discordant and concordant couples and full blown AIDS patients.

### Study population

Most of the subjects were counselled, 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, counselled, tested and registered as discordant or concordant couples by the nurses and doctors of the respective health centers and/or hospitals.

### Ethical considerations

The study was conducted in accordance with the ethical principles

***Corresponding author:** Yohannis Meseret, Kotebe University College, Addis Ababa, Ethiopia, Email: yohannis_meseret@yahoo.com

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stipulated in the last revised version of the Helsinki declaration, the operational guideline for ethical committees of Ethiopia. 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 Minister and the institutional clearance from Ethiopian Health and Nutrition Institute (EHNRI) now Ethiopian Public Health Institute and Addis Ababa University (AAU). An official letter of cooperation attached with ethical clearance copy from EHNRI, AAU was written to regional health Bureaus, and a similar letter was written from regional health Bureaus to the respective health centers and hospitals.

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.

### 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 vacationer 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 h.

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. Two samples were rejected on these bases. Laboratory analysis was carried out at EHNRI and Center for Clinical Immunology and Biostatistics (CCIB) research institute attached to Murdoch University, Perth, Australia.

### 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 and concordant couples. 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 Boferroni corrections (α=0.033). This association between several parameters was determined using a multivariate regression analysis. Correlation coefficients were calculated by the spearman's test.

## Methods

### 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. Using Buffy coat isolation mechanism PBMC was also isolated from the plasma separated blood. 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.

### Sequence based HLA typing

HLA-typing was determined using PCR amplification of exons 2 and 3 of the genes HLA-A, HLA-B, HLA-C and HLA-DR followed by DNA sequencing methods. Briefly, genomic DNA was extracted from Buffy coats, whole blood or plasma manually with the QIAamp DNA Blood Mini Kit (Qiagen) or the Agencourt Genfind DNA extraction kit (Beckman Coulter) with a method adapted for the Biomek FX robotic workstation. Exons two and three were amplified from each HLA gene and products were then purified using Agencourt Ampure (Beckman Coulter), sequenced with Big Dye terminator v3.1 methods (Applied Biosystems) and then cleaned up with Agencourt Cleanseq (Beckman Coulter). Finally, sample data was collected on an ABI PRISM 3730 Genetic Analyzer. Applied Biosystems 3730 Data Collection software v5.0 was used to collect electropherograms which are then analyzed with Applied Biosystems Software v5.2 and Assign v4.0.0.11 (Conexio Genomics Pty Ltd). The Assign program compared collected sample data against a database of known HLA sequences and assigned alleles accordingly.

### HLA typing

HLA genotyping has been performed by DNA sequencing of the exon 2-3 region of HLA class I and exon 2 region of HLA-DRB1. To determine HLA class I and II frequencies and to investigate if the differences in HLA types and frequencies were associated with resistance or susceptibility to HIV, DNA based (molecular) HLA typing was carried out and the results of 10 discordant negatives, 52 discordant positives, 49 concordant couples and 138 HIV/AIDS subjects HLA frequencies were obtained. Five different types of HLA-A1, 6 different types of HLA-A2, 7 different kinds of HLA-B, 5 different kinds of HLA-B2, 3 different kinds of HLA-C1 and HLA-C2, 3 different kinds of HLA-DR1 and 2 different kinds of HLA-DR2 were found in discordant negatives. In discordant positives 16 different kinds of HLA-A1, HLA-A2, HLA-B1, 13 different kinds of HLA-B2, 6 different kinds of HLA-C1, 5 different kinds of HLA-C2 and HLA-DR1 and 4 different kinds of HLA-DR2 were found. In a similar way, 12 different kinds of HLA-A1, 14 different kinds of HLA-A2, 24 different kinds of HLA-B1 and 16 different kinds of HLA-B2, 4 different kinds of HLA-C1 and C2, 3 different kinds of HLA-DR1 and 1 type of HLA-DR2 was found in concordant couples. The results for HIV/AIDS were: 25 different kinds of HLA-A1 and A2, 31 different kinds of HLA-B1, 28 different kinds of HLA-B2, 18 different kinds of HLA-C1 and HLA-DR2 and 17 different kinds of HLA-DR1. In almost all cases more different kinds of HLA-B1 and B2 followed by HLA-C1 and C2 were found among HLA class I types. Similar results were obtained for HLA class II.

## Result

HLA genotyping has been performed by DNA sequencing of the exon 2-3 region of HLA class I and exon 2 region of HLA-DRB1. To determine HLA class I and II frequencies and to investigate if the differences in HLA types and frequencies were associated with resistance or susceptibility to HIV, DNA based (molecular) HLA typing was carried out and the results of 10 discordant negatives, 52 discordant positives, 49 concordant couples and 138 HIV/AIDS subjects HLA frequencies were obtained. Five different types of HLA-A1, 6 different types of HLA-A2, 7 different kinds of HLA-B, 5 different kinds of HLA-B2, 3 different kinds of HLA-C1 and HLA-C2, 3 different kinds of HLA-DR1 and 2 different kinds of HLA-DR2 were found in discordant negatives ( Tables 1 and 2). In discordant positives 16 different kinds of HLA-A1, HLA-A2, HLA-B1, 13 different kinds of

HLA-B2, 6 different kinds of HLA-C1, 5 different kinds of HLA-C2 and HLA-DR1 and 4 different kinds of HLA-DR2 were found. In a similar way, 12 different kinds of HLA-A1, 14 different kinds of HLA-A2, 24 different kinds of HLA-B1 and 16 different kinds of HLA-B2, 4 different kinds of HLA-C1 and C2, 3 different kinds of HLA-DR1 and 1 type of HLA-DR2 was found in concordant couples (Table 1). The result for HIV/AIDS was: 25 different kinds of HLA-A1 and A2, 31 different kinds of HLA-B1, 28 different kinds of HLA-B2, 18 different kinds of HLA-C1 and HLA-DR2 and 17 different kinds of HLA-DR1. In almost all cases more different kinds of HLA-B1 and B2 followed by HLA-C1 and C2 were found among HLA class I types. Similar results were obtained for HLA class II (Table 1).

(HLA-A1=HLA-A allele1, HLA-A2=HLA-A allele 2, HLA-B1=HLA-B allele1, HLA-B2=HLA-B allele2, HLA-C1=HLA-C allele1, HLA-C2=HLA-C allele2, HLA-DR1=HLA-DR allele1, HLA-DR2=HLA-DR allele 2).

DSCN=Discordant negative, DSCP=Discordant positive, CONC=Concordant couples, AIDS=AIDS patients.

The HLA alleles were then pooled out to determine the frequency, proportions and $X^2$ –based p-value to see the associations between the different groups. Frequencies and proportions were compared between AIDS, concordant couples, discordant negatives and discordant positives. The results were as (Table 2).

For the subjects in which 20% of the expected count was less than 5, p-value was calculated by Fishers Exact Test method and the result was as shown (Table 3).

HLA-B*49 ($p<0.01$), HLA-A*68 ($p<0.01$), and HLA-B41 ($p<0.001$)

| | HLA-A1 | HLA-A2 | HLA-B1 | HLA-B2 | HLA-C1 | HLA-C2 | HLA-DR1 | HLA-DR2 |
|---|---|---|---|---|---|---|---|---|
| DSCN | 5 | 6 | 7 | 5 | 3 | 3 | 3 | 2 |
| DSCP | 16 | 16 | 16 | 13 | 6 | 5 | 5 | 4 |
| CONC | 12 | 14 | 24 | 16 | 4 | 4 | 3 | 1 |
| AIDS | 25 | 25 | 31 | 28 | 18 | 13 | 17 | 18 |

**Table 1:** The different kinds of HLA class I and II types in discordant negatives, discordant positives, concordant couples and HIV/AIDS subjects.

| HLA-A | AIDS | | | CONC | | | DSCN | | | DSCP | | | p-value |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| HST | N | P | % | N | P | % | N | P | % | N | P | % | |
| 1 | 99 | 32 | 14 | 40 | 7 | 3 | 7 | 2 | 1 | 34 | 6 | 3 | 0.39 |
| 2 | 89 | 42 | 19 | 27 | 20 | 9 | 4 | 5 | 2 | 27 | 13 | 6 | 0.34 |
| 3 | 102 | 29 | 13 | 36 | 11 | 5 | 7 | 2 | 1 | 26 | 14 | 6 | 0.44 |
| 26 | 130 | 1 | 0.4 | 46 | 1 | 0 | 9 | 0 | 0 | 38 | 2 | 1 | 0.4 |
| 29 | 125 | 6 | 2.6 | 47 | 0 | 0 | 9 | 0 | 0 | 40 | 0 | 0 | 0.08 |
| 30 | 88 | 43 | 19 | 33 | 14 | 6 | 8 | 1 | 0 | 30 | 10 | 4 | 0.42 |
| 34 | 128 | 3 | 1.3 | 46 | 1 | 0 | 8 | 1 | 0 | 39 | 1 | 0 | 0.67 |
| 66 | 117 | 14 | 6.2 | 44 | 3 | 1 | 9 | 0 | 0 | 36 | 4 | 2 | 0.45 |
| 68 | 111 | 20 | 8.8 | 34 | 13 | 6 | 5 | 4 | 2 | 27 | 13 | 6 | 0.02 |
| HLA-B | AIDS | | | CONC | | | DSCN | | | DSCP | | | p-value |
| HST | N | P | % | N | P | % | N | P | % | N | P | % | |
| 7 | 122 | 20 | 8.5 | 38 | 7 | 3 | 7 | 3 | 1 | 32 | 7 | 3 | 0.63 |
| 14 | 121 | 21 | 8.9 | 40 | 5 | 2 | 9 | 1 | 0 | 31 | 8 | 3 | 0.64 |
| 15 | 106 | 37 | 16 | 37 | 8 | 3 | 8 | 2 | 1 | 28 | 11 | 5 | 0.62 |
| 18 | 139 | 3 | 1.3 | 40 | 5 | 2 | 8 | 2 | 1 | 38 | 1 | 0 | 0.02 |
| 27 | 137 | 5 | 2.1 | 45 | 0 | 0 | 10 | 0 | 0 | 37 | 2 | 1 | 0.26 |
| 35 | 137 | 5 | 2.1 | 44 | 1 | 0 | 9 | 1 | 0 | 38 | 1 | 0 | 0.75 |
| 39 | 130 | 12 | 5.1 | 40 | 5 | 2 | 10 | 0 | 0 | 39 | 0 | 0 | 0.03 |
| 41 | 115 | 27 | 11 | 44 | 1 | 0 | 9 | 1 | 0 | 34 | 5 | 2 | 0.01 |
| 44 | 130 | 12 | 5.1 | 40 | 5 | 2 | 9 | 1 | 0 | 35 | 4 | 2 | 0.95 |
| 51 | 136 | 7 | 3 | 42 | 3 | 1 | 10 | 0 | 0 | 37 | 2 | 1 | 0.73 |
| 57 | 115 | 27 | 11 | 35 | 10 | 4 | 8 | 2 | 1 | 30 | 9 | 4 | 0.93 |
| HLA-C | AIDS | | | CONC | | | DSCN | | | DSCP | | | p-value |
| HST | N | P | % | N | P | % | N | P | % | N | P | % | |
| 3 | 127 | 6 | 3.2 | 22 | 2 | 1 | 4 | 0 | 0 | 23 | 3 | 2 | 0.49 |
| 4 | 99 | 34 | 18 | 19 | 15 | 3 | 3 | 1 | 1 | 20 | 6 | 2 | 0.96 |
| 7 | 51 | 82 | 44 | 14 | 10 | 5 | 0 | 4 | 2 | 9 | 17 | 9 | 0.05 |
| 8 | 125 | 8 | 4.3 | 21 | 3 | 1 | 4 | 0 | 0 | 24 | 4 | 1 | 0.63 |
| HLA-DR | AIDS | | | CONC | | | DSCN | | | DSCP | | | p-value |
| HST | N | P | % | N | P | % | N | P | % | N | P | % | |
| 1 | 110 | 20 | 11 | 19 | 5 | 3 | 5 | 0 | 0 | 22 | 4 | 2 | 0.53 |
| 4 | 104 | 26 | 14 | 21 | 3 | 2 | 4 | 1 | 1 | 24 | 2 | 1 | 0.37 |
| 11 | 117 | 13 | 7 | 23 | 1 | 1 | 4 | 1 | 1 | 26 | 0 | 0 | 0.09 |
| 13 | 116 | 14 | 7.6 | 21 | 3 | 2 | 5 | 0 | 0 | 22 | 4 | 2 | 0.64 |
| 15 | 108 | 22 | 12 | 16 | 8 | 4 | 4 | 1 | 1 | 18 | 8 | 4 | 0.19 |

**Table 2:** Comparison of HLA-A, HLA-B, HLA-C and HLA-DR aggregate subtypes in AIDS, DSCP (discordant positive), DSCN (discordant negative), and CONC (concordant couples) subjects. HST (HLA subtypes).

| HLA/subtype | AIDS *vs.* CONC | AIDS *vs.* DSCP | AIDS *vs.* DCSN | CONC *vs.* DSCN | CONC *vs.* DSCP | DSCN *vs.* DSCP |
|---|---|---|---|---|---|---|
| HLA-A | 29[C] | 68[B] | | | | |
| | 18[B] | | | | | |
| | 41[A] | | | | | |
| HLA-B | | | | | | |
| | 18[B] | 39[B] | | | | 39[B] |
| | 41[A] | | | | | 41[C] |
| HLA-C | | | | | | |
| | | | 7[B] | | | |
| HLA-DR | | 11[B] | | | | 11[C] |

**Table 3:** Aggregate HLA subtypes Fishers Exact Test calculated p-value of those in which 20% of cells have expected count less than 5 (A=p<.001(very highly significant), B=p<.01 (very highly significant), C=p<.05 ( significant) AIDS (subjects at AIDS stage, CONC (concordant couples), DSCP (discordant positive subjects), DSCN(discordant negative Subjects).

| PID | AIDS | | | CONC | | | DSCN | | | DSCP | | | $X^2$ Value | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| HST | N | P | % | N | P | % | N | P | % | N | P | % | LR | Pearson |
| 101 | 110 | 21 | 9.3 | 44 | 3 | 1 | 8 | 1 | 0.4 | 37 | 3 | 1 | 0.2 | 0.25 |
| 103 | 119 | 12 | 5.3 | 44 | 4 | 2 | 8 | 1 | 0.4 | 37 | 3 | 1 | 1 | 0.98 |
| 109 | 130 | 1 | 0.4 | 47 | 0 | 0 | 9 | 0 | 0 | 40 | 0 | 0 | 0.8 | 0.86 |
| 201 | 130 | 1 | 0.4 | 47 | 0 | 0 | 9 | 0 | 0 | 40 | 0 | 0 | 0.4 | 0.36 |
| 202 | 121 | 10 | 4.4 | 41 | 6 | 3 | 7 | 2 | 0.9 | 38 | 2 | 1 | 0.3 | 0.27 |
| 205 | 125 | 6 | 2.6 | 43 | 4 | 2 | 9 | 0 | 0 | 38 | 2 | 1 | 0.6 | 0.66 |
| 214 | 131 | 0 | 0 | 46 | 1 | 0 | 9 | 0 | 0 | 40 | 0 | 0 | 0.4 | 0.28 |
| 222 | 129 | 2 | 0.9 | 46 | 1 | 0 | 9 | 0 | 0 | 38 | 2 | 1 | 0.7 | 0.59 |
| 301 | 104 | 27 | 12 | 37 | 10 | 4 | 7 | 2 | 0.9 | 27 | 13 | 6 | 0.5 | 0.46 |
| 302 | 129 | 2 | 0.9 | 46 | 1 | 0 | 9 | 0 | 0 | 39 | 1 | 0 | 0.9 | 0.95 |
| 308 | 130 | 1 | 0.4 | 47 | 0 | 0 | 9 | 0 | 0 | 40 | 0 | 0 | 0.8 | 0.86 |
| 2301 | 123 | 8 | 3.5 | 40 | 1 | 0 | 9 | 0 | 0 | 37 | 3 | 1 | 0.4 | 0.57 |
| 2402 | 123 | 8 | 3.5 | 42 | 5 | 2 | 9 | 0 | 0 | 40 | 0 | 0 | 0.1 | 0.16 |
| 2601 | 130 | 1 | 0.4 | 47 | 0 | 0 | 9 | 0 | 0 | 40 | 0 | 0 | 0.8 | 0.86 |
| 2612 | 131 | 0 | 0 | 46 | 1 | 0 | 9 | 0 | 0 | 38 | 2 | 1 | 0.1 | 0.09 |
| 2901 | 126 | 5 | 2.2 | 47 | 0 | 0 | 9 | 0 | 0 | 40 | 0 | 0 | 0.1 | 0.29 |
| 290201 | 130 | 1 | 0.4 | 47 | 0 | 0 | 9 | 0 | 0 | 40 | 0 | 0 | 0.8 | 0.86 |
| 3001 | 110 | 21 | 9.3 | 40 | 7 | 3 | 8 | 1 | 0.4 | 37 | 3 | 1 | 0.5 | 0.59 |
| 3002 | 121 | 10 | 4.4 | 46 | 1 | 0 | 9 | 0 | 0 | 37 | 3 | 1 | 0.3 | 0.47 |
| 3004 | 120 | 11 | 4.6 | 42 | 5 | 2 | 9 | 0 | 0 | 37 | 3 | 1 | 0.6 | 0.76 |
| 3010 | 127 | 4 | 1.8 | 45 | 2 | 1 | 9 | 0 | 0 | 40 | 0 | 0 | 0.4 | 0.59 |
| 301102 | 131 | 0 | 0 | 47 | 0 | 0 | 9 | 0 | 0 | 39 | 1 | 0 | 0.3 | 0.19 |
| 3104 | 129 | 2 | 0.9 | 46 | 1 | 0 | 9 | 0 | 0 | 40 | 0 | 0 | 0.7 | 0.82 |
| 3202 | 118 | 13 | 5.7 | 45 | 2 | 1 | 9 | 0 | 0 | 39 | 1 | 0 | 0.2 | 0.24 |
| 330301 | 128 | 3 | 1.3 | 44 | 3 | 1 | 9 | 0 | 0 | 39 | 1 | 0 | 0.5 | 0.51 |
| 3402 | 128 | 3 | 1.3 | 46 | 1 | 0 | 8 | 1 | 0.4 | 40 | 0 | 0 | 0.3 | 0.24 |
| 3404 | 131 | 0 | 0 | 47 | 0 | 0 | 9 | 0 | 0 | 39 | 1 | 0 | 0.3 | 0.19 |
| 6601 | 120 | 11 | 4.6 | 44 | 3 | 1 | 9 | 0 | 0 | 37 | 3 | 1 | 0.7 | 0.81 |
| 6603 | 128 | 3 | 1.3 | 47 | 0 | 0 | 9 | 0 | 0 | 39 | 1 | 0 | 0.5 | 0.72 |
| 680101 | 124 | 7 | 3.1 | 43 | 4 | 2 | 7 | 2 | 0.9 | 35 | 5 | 2 | 0.2 | 0.18 |
| 6802 | 117 | 14 | 6.7 | 38 | 9 | 4 | 7 | 2 | 0.9 | 31 | 9 | 4 | 0.2 | 0.2 |
| 7401 | 123 | 8 | 3.5 | 46 | 1 | 0 | 9 | 0 | 0 | 40 | 0 | 0 | 0.1 | 0.26 |

**Table 4:** Proportions and $X^2$ values (likelihood ratios and Pearson's p-values) of HLA-A subtypes in AIDS, concordant (CONC), discordant negative (DSCN) and discordant positive (DSCP) study subjects. HST (HLA subtypes).

(Table 4) were found strongly associated with AIDS patients when compared with all others. The strongest association was observed for HLA-B*41(p<0.001) in AIDS patients. When AIDS patients were compared with concordant couples, HLA-A*41 (p<0.001), HLA-A*18 (p<0.01), HLA-A*29 (p<0.05) were found to be significantly associated with AIDS patients. HLA-B*41 (p<0.001) and HLA-B*18 (p<0.01) were also found to be strongly associated with AIDS patients (Table 3).

When AIDS patients were compared with discordant positive subjects, three HLA subtypes: HLA-A*68 (p<0.01), HLA-B*39 (p<0.01) and HLA-DR*11 (p<0.01) were found to be very strongly associated with AIDS subjects, showing that discordant positive subjects were different from AIDS subjects. When AIDS subjects were compared with discordant negatives, the only HLA type found to be associated with AIDS subjects was HLA-C*7 (p<0.01) (Table 3).

Comparisons of associations of HLA subtypes between discordant negative and discordant positive showed that three HLA subtypes were strongly associated with discordant negative subjects. These were HLA-B*39 (p<0.01), HLA-B*41 and HLA-DR*11 (p<0.05) (Table 4).

The subtypes which were found associated with resistance to HIV in other studies, were not observed in our study. The frequency and the prevalence of the different kind of HLA subtypes associated with different clinical status are summarized in the following figure (Figure 1).

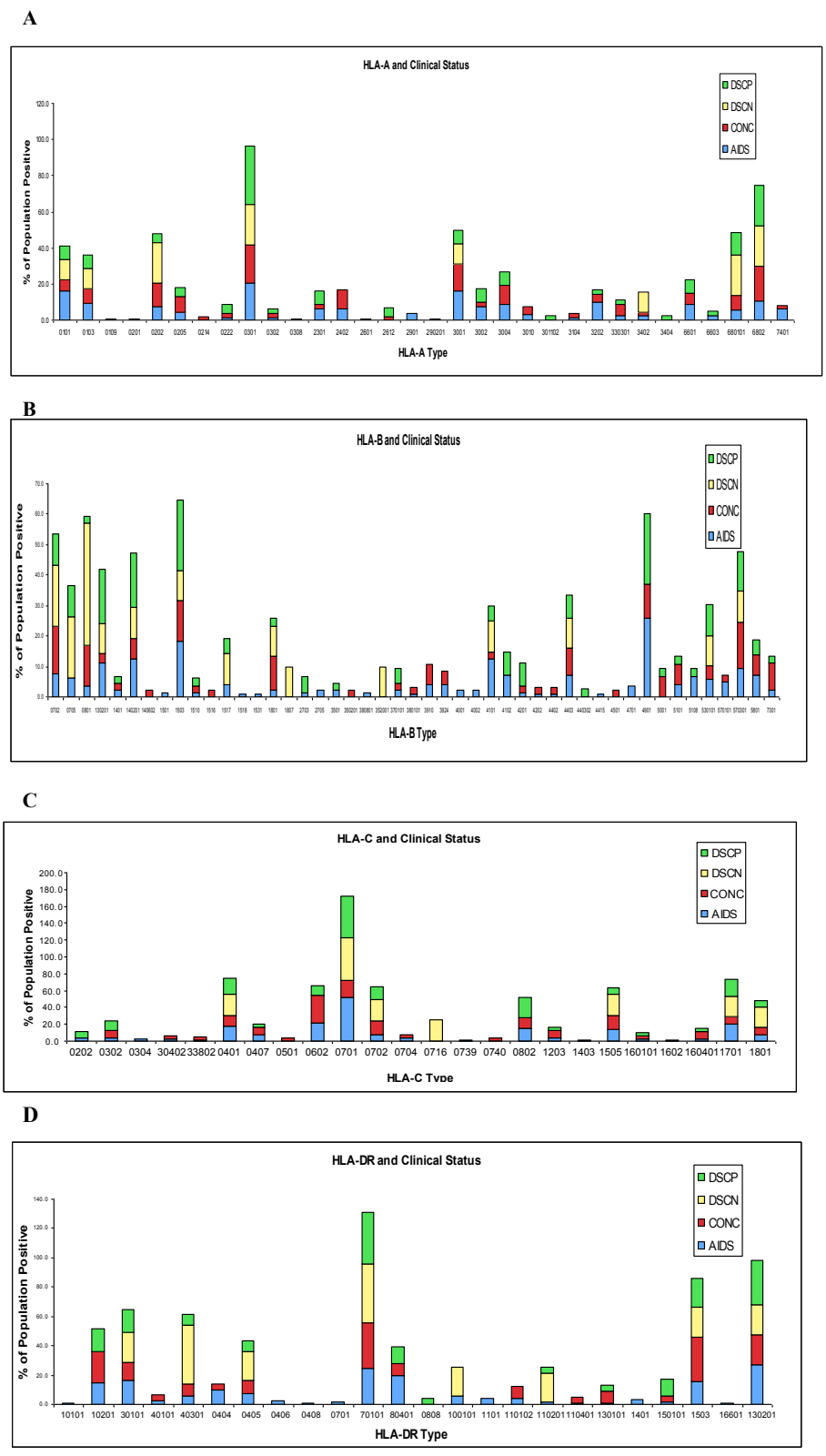


**Figure 1:** The different kinds of HLA and their clinical status's: A) HLA-A and clinical status B) HLA-B and clinical status C) HLA-C and clinical status, D) HLA-DR and clinical status.

To investigate the frequency and proportions of the different forms within the subtypes and their relationships with the different groups, the different forms of the subtypes were analyzed and the result was as shown Table 4 (HLA-A), Table 5 (HLA-B), Table 6 (HLA-C), and Table 7 (HLA-DR).

To look at the associations of the different forms of the subtypes to AIDS, concordant couples, discordant positive and discordant negative subjects, p-values were calculated from their $X^2$s and for those in which 20% cell count was less than 5, p-values were calculated by correcting with Fishers Exact Test. The values for these associations were shown (Table 8).

HLA-A*0101(9.3%), *3001(9.3%) and HLA-A*0301(11.9%) were found in higher frequency and percentage in AIDS subjects (Table 4). In concordant couples, discordant positives and discordant negative subjects the percentage and frequency of the different HLA-A subtypes was less than 5%.

Relatively, higher frequencies of HLA-B subtypes were observed

| | AIDS | | | CONC | | | DSCN | | | DSCP | | | $X^2$ values | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| HST | N | P | % | N | P | % | N | P | % | N | P | % | LR | Pearson's p-value |
| 0702 | 131 | 11 | 4.7 | 38 | 7 | 3 | 8 | 7 | 0.85 | 35 | 4 | 1.7 | 0.38 | 0.33 |
| 0705 | 133 | 9 | 3.8 | 45 | 0 | 0 | 8 | 2 | 0.85 | 35 | 4 | 1.7 | 0.11 | 0.33 |
| 0801 | 137 | 5 | 2.1 | 39 | 6 | 2.5 | 6 | 4 | 1.7 | 38 | 1 | 0.4 | 0.001 | .0001 |
| 130201 | 126 | 16 | 6.8 | 35 | 10 | 4.2 | 9 | 1 | 0.4 | 32 | 7 | 3.0 | 0.29 | 0.27 |
| 1401 | 139 | 3 | 1.3 | 44 | 1 | 0.4 | 10 | 0 | 0 | 38 | 1 | 0.4 | 0.92 | 0.97 |
| 140201 | 124 | 18 | 7.3 | 42 | 3 | 1.3 | 9 | 1 | 0.4 | 32 | 7 | 3.0 | 0.45 | 0.47 |
| 140602 | 142 | 0 | 0 | 44 | 1 | 0.42 | 10 | 0 | 0 | 39 | 0 | 0 | 0.34 | 0.23 |
| 1501 | 140 | 2 | 0.9 | 45 | 0 | 0 | 10 | 0 | 0 | 39 | 0 | 0 | 0.56 | 0.72 |
| 1503 | 116 | 26 | 11 | 39 | 6 | 2.5 | 9 | 1 | 0.4 | 30 | 9 | 3.8 | 0.60 | 0.61 |
| 1510 | 140 | 2 | 0.9 | 44 | 1 | 0.4 | 10 | 0 | 0 | 38 | 1 | 0.4 | 0.9 | 0.9 |
| 1516 | 142 | 0 | 0 | 44 | 1 | 0.4 | 10 | 0 | 0 | 39 | 0 | 0 | 0.32 | 0.23 |
| 1517 | 136 | 6 | 2.5 | 45 | 0 | 0 | 9 | 1 | 0.4 | 37 | 2 | 0.9 | 0.21 | 0.38 |
| 1518 | 141 | 1 | 0.4 | 45 | 0 | 0 | 10 | 0 | 0 | 39 | 0 | 0 | 0.80 | 0.88 |
| 1531 | 141 | 1 | 0.4 | 45 | 0 | 0 | 10 | 0 | 0 | 39 | 0 | 0 | 0.80 | 0.88 |
| 1801 | 139 | 3 | 1.3 | 40 | 5 | 2.1 | 9 | 1 | 0.42 | 38 | 1 | 0.4 | 0. 90 | .05 |
| 1807 | 142 | 0 | 0 | 45 | 0 | 0 | 9 | 1 | 0.4 | 39 | 0 | 0 | 0.09 | 0.0001 |
| 2703 | 140 | 2 | 09 | 45 | 0 | 0 | 10 | 0 | 0 | 37 | 2 | 0.9 | 0.29 | 0.29 |
| 2705 | 139 | 3 | 1.3 | 45 | 0 | 0 | 10 | 0 | 0 | 39 | 0 | 0 | 0.38 | 0.57 |
| 3501 | 139 | 3 | 1.3 | 45 | 0 | 0 | 10 | 0 | 0 | 38 | 1 | 0.4 | 0.35 | 0.73 |
| 350201 | 142 | 0 | 0 | 44 | 1 | 0.4 | 10 | 0 | 0 | 39 | 0 | 0 | 0.34 | 0.23 |
| 380801 | 140 | 2 | 0.9 | 45 | 0 | 0 | 10 | 0 | 0 | 39 | 0 | 0 | 0.56 | 0.72 |
| 352001 | 142 | 0 | 0 | 45 | 0 | 0 | 9 | 1 | 0.4 | 39 | 0 | 0 | 0093 | .0001 |
| 370101 | 139 | 3 | 1.3 | 44 | 1 | 0.4 | 10 | 0 | 0 | 37 | 2 | 0.9 | 0.69 | 0.69 |
| 380101 | 141 | 1 | 0.4 | 44 | 1 | 0.4 | 10 | 0 | 0 | 39 | 0 | 0 | 0.66 | 0.69 |
| 3910 | 136 | 6 | 2.54 | 42 | 3 | 1.3 | 10 | 0 | 0 | 39 | 0 | 0 | 0.19 | 0.39 |
| 3924 | 136 | 6 | 2.5 | 43 | 2 | 0.9 | 10 | 0 | 0 | 39 | 0 | 0 | 0.28 | 0.54 |
| 4001 | 139 | 3 | 1.3 | 45 | 0 | 0 | 10 | 0 | 0 | 39 | 0 | 0 | 0.38 | 0.57 |
| 4002 | 139 | 3 | 1.3 | 45 | 0 | 0 | 10 | 0 | 0 | 39 | 0 | 0 | 0.38 | 0.57 |
| 4101 | 124 | 18 | 7.63 | 44 | 1 | 0.4 | 9 | 1 | 0.4 | 37 | 0.2 | 0.9 | 0.38 | 0.57 |
| 4102 | 132 | 10 | 4.2 | 45 | 0 | 0 | 10 | 0 | 0 | 36 | 3 | 1.3 | 0.07 | 0.15 |
| 4201 | 140 | 2 | 0.9 | 44 | 1 | 0.4 | 10 | 0 | 0 | 36 | 3 | 1.3 | 0.07 | 0.24 |
| 4202 | 141 | 1 | 0.4 | 44 | 1 | 0.4 | 10 | 0 | 0 | 39 | 0 | 0 | 0.67 | 0.81 |
| 4402 | 141 | 1 | 0.4 | 44 | 1 | 0.4 | 10 | 0 | 0 | 39 | 0 | 0 | 0.67 | 0.69 |
| 4403 | 132 | 10 | 4.2 | 41 | 4 | 1.7 | 9 | 1 | 0.4 | 36 | 3 | 1.3 | 0.97 | 0.97 |
| 440302 | 142 | 0 | 0 | 45 | 0 | 0 | 10 | 0 | 0 | 38 | 1 | 0.4 | 0.30 | 0.17 |
| 4415 | 141 | 1 | 0.4 | 45 | 0 | 0 | 10 | 0 | 0 | 39 | 0 | 0 | 0.79 | 0.88 |
| 4501 | 142 | 0 | 0 | 44 | 1 | 0.4 | 10 | 0 | 0 | 39 | 0 | 0 | 0.34 | 0.23 |
| 4701 | 137 | 5 | 2.1 | 45 | 0 | 0 | 10 | 0 | 0 | 39 | 0 | 0 | 0.16 | 0.33 |
| 4901 | 105 | 37 | 15.7 | 40 | 5 | 2.1 | 10 | 0 | 0 | 30 | 9 | 3.8 | 0.02 | .06 |
| 5001 | 142 | 0 | 0 | 42 | 3 | 1.3 | 10 | 0 | 0 | 38 | 1 | 0.4 | 0.02 | 0.03 |
| 5101 | 136 | 6 | 2.5 | 42 | 3 | 1.3 | 10 | 0 | 0 | 38 | 1 | 0.4 | 0.63 | 0.71 |
| 5108 | 141 | 10 | 0.4 | 45 | 0 | 0 | 10 | 0 | 0 | 38 | 1 | 0.4 | 0.60 | 0.59 |
| 530101 | 134 | 8 | 3.4 | 43 | 2 | 0.9 | 9 | 1 | 0.4 | 35 | 4 | 1.7 | 0.69 | 0.65 |
| 570101 | 135 | 7 | 3.0 | 41 | 1 | 0.4 | 10 | 0 | 0 | 39 | 0 | 0 | 0.21 | 0.40 |
| 570301 | 129 | 13 | 5.5 | 38 | 7 | 3 | 9 | 1 | 0.4 | 34 | 5 | 2.1 | 0.68 | 0.66 |
| 5801 | 132 | 10 | 4.2 | 42 | 3 | 1.3 | 10 | 0 | 0 | 37 | 2 | 0.9 | 0/67 | 0.82 |
| 7301 | 139 | 3 | 1.3 | 41 | 4 | 1.7 | 10 | 0 | 0 | 38 | 1 | 0.4 | 0.21 | 0.15 |

**Table 5:** Proportions and $X^2$ values (likelihood ratios and Pearson's p-values) of HLA-B subtypes in AIDS, concordant (CONC), discordant negative (DSCN) and discordant positive (DSCP) study subjects. HST (HLA subtypes). HST (HLA subtypes)

| | AIDS | | | CONC | | | DSCN | | | DSCP | | | $X^2$ value | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **HST** | **N** | **P** | **%** | **N** | **P** | **%** | **N** | **P** | **%** | **N** | **P** | **%** | **LR** | **P p-value** |
| 0202 | 128 | 5 | 2.7 | 24 | 0 | 0 | 4 | 0 | 0 | 24 | 2 | 1.1 | 0.39 | 0.53 |
| 0302 | 128 | 5 | 2.7 | 22 | 2 | 1.1 | 4 | 0 | 0 | 23 | 3 | 1.6 | 0.38 | 0.35 |
| 0304 | 32 | 1 | 05 | 24 | 0 | 0 | 4 | 0 | 0 | 26 | 0 | 0 | 0.88 | 0.94 |
| 30402 | 130 | 3 | 1.6 | 23 | 1 | 0.53 | 4 | 0 | 0 | 26 | 0 | 0 | 0.64 | 0.77 |
| 33802 | 132 | 1 | 0.5 | 23 | 1 | 0.5 | 4 | 0 | 0 | 26 | 0 | 0 | 0.56 | 0.45 |
| 0401 | 109 | 24 | 12.8 | 21 | 3 | 1.6 | 3 | 1 | 0.5 | 21 | 5 | 2.7 | 0.88 | 0.88 |
| 0407 | 123 | 10 | 5.4 | 22 | 2 | 1.1 | 4 | 0 | 0 | 25 | 1 | 0.5 | 0.76 | 0.84 |
| 0501 | 133 | 0 | 0 | 23 | 1 | 0.5 | 4 | 0 | 0 | 26 | 0 | 0 | 0.25 | 0.07 |
| 0602 | 105 | 28 | 15 | 16 | 8 | 4.3 | 4 | 0 | 0 | 23 | 3 | 1.6 | 0.14 | 0.19 |
| 0701 | 64 | 69 | 36.9 | 19 | 5 | 2.7 | 2 | 2 | 1.1 | 13 | 13 | 7 | 0.04 | 0.04 |
| 0702 | 123 | 10 | 5.4 | 20 | 4 | 2.1 | 3 | 1 | 0.5 | 22 | 4 | 2.1 | 0.32 | 0.27 |
| 0704 | 128 | 5 | 2.7 | 23 | 1 | 0.5 | 4 | 0 | 0 | 26 | 0 | 0 | 0.54 | 0.75 |
| 0716 | 133 | 0 | 0 | 24 | 0 | 0 | 3 | 1 | 0.5 | 26 | 0 | 0 | 0.04 | .001 |
| 0739 | 132 | 1 | 0.5 | 24 | 0 | 0 | 4 | 0 | 0 | 26 | 0 | 0 | 0.87 | 0.93 |
| 0740 | 133 | 0 | 0 | 23 | 1 | 0.5 | 4 | 0 | 0 | 26 | 0 | 0 | 0.24 | 0.07 |
| 0802 | 112 | 21 | 11.2 | 21 | 3 | 1.6 | 4 | 0 | 0 | 20 | 6 | 3.2 | 0.47 | 0.58 |
| 1203 | 128 | 5 | 2.7 | 22 | 2 | 1.1 | 4 | 0 | 0 | 25 | 1 | 0.5 | 0.75 | 0.74 |
| 1403 | 132 | 1 | 0.5 | 24 | 0 | 0 | 4 | 0 | 0 | 26 | 0 | 0 | 0.88 | 0.93 |
| 1505 | 115 | 18 | 9.3 | 20 | 4 | 2.1 | 3 | 1 | 0.5 | 24 | 2 | 1.1 | 0.69 | 0.7 |
| 160101 | 130 | 3 | 1.6 | 23 | 1 | 0.5 | 4 | 0 | 0 | 25 | 1 | 0.5 | 0.89 | 0.91 |
| 1602 | 132 | 1 | 0.5 | 24 | 0 | 0 | 4 | 0 | 0 | 26 | 0 | 0 | 0.88 | 0.94 |
| 160401 | 129 | 4 | 2.1 | 22 | 2 | 1.1 | 4 | 0 | 0 | 25 | 1 | 0.5 | 0.67 | 0.62 |
| 1701 | 106 | 27 | 14.4 | 22 | 2 | 1.1 | 3 | 1 | 0.5 | 21 | 5 | 2.7 | 0.5 | 0.5 |
| 1801 | 123 | 10 | 5.4 | 22 | 2 | 1.1 | 3 | 1 | 0.5 | 24 | 2 | 1.1 | 0.77 | 0.65 |

**Table 6:** Proportions and $X^2$ values (likelihood ratios and Pearson's p-values) of HLA-C subtypes in AIDS, concordant (CONC), discordant negative (DSCN) and discordant positive (DSCP) study subjects. HST (HLA subtypes).

| | AIDS | | | CONC | | | DSCN | | | DSCP | | | $X^2$ value | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **HST** | **N** | **P** | **%** | **N** | **P** | **%** | **N** | **P** | **%** | **N** | **P** | **%** | **LR** | **P- value** |
| 1102 | 130 | 0 | 0 | 24 | 0 | 0 | 5 | 0 | 0 | 25 | 1 | 1 | 0.3 | 0.1 |
| 80401 | 130 | 0 | 23 | 1 | 1 | 5 | 0 | 0 | 25 | 1 | 1 | 1 | 0.2 | 0.14 |
| 10101 | 129 | 1 | 0.5 | 24 | 0 | 0 | 5 | 0 | 0 | 26 | 0 | 0 | 0.9 | 0.93 |
| 10201 | 111 | 19 | 10 | 18 | 5 | 3 | 5 | 0 | 0 | 22 | 4 | 2 | 0.4 | 0.21 |
| 30101 | 107 | 21 | 11 | 21 | 3 | 2 | 4 | 1 | 1 | 21 | 4 | 3 | 0.9 | 0.95 |
| 40101 | 127 | 3 | 1.6 | 23 | 1 | 1 | 5 | 0 | 0 | 26 | 0 | 0 | 0.6 | 0.76 |
| 40301 | 123 | 7 | 3.8 | 22 | 2 | 1 | 23 | 2 | 1 | 24 | 2 | 1 | 0.2 | 0.03 |
| 404 | 117 | 13 | 7 | 23 | 1 | 1 | 5 | 0 | 0 | 26 | 0 | 0 | 0.1 | 0.26 |
| 405 | 120 | 10 | 5.4 | 22 | 2 | 1 | 4 | 1 | 1 | 24 | 2 | 1 | 0.9 | 0.8 |
| 406 | 127 | 3 | 1.6 | 24 | 0 | 0 | 5 | 0 | 0 | 26 | 0 | 0 | 0.5 | 0.73 |
| 408 | 129 | 1 | 0.5 | 24 | 0 | 0 | 5 | 0 | 0 | 26 | 0 | 0 | 0.9 | 0.93 |
| 701 | 128 | 2 | 1.1 | 24 | 0 | 0 | 5 | 0 | 0 | 26 | 0 | 0 | 0.7 | 0.84 |
| 70101 | 94 | 30 | 16 | 15 | 7 | 4 | 3 | 2 | 1 | 17 | 9 | 5 | 0.5 | 0.63 |
| 80401 | 103 | 25 | 14 | 23 | 1 | 1 | 5 | 0 | 0 | 23 | 2 | 1 | 0.2 | 0.29 |
| 808 | 130 | 0 | 0 | 24 | 0 | 0 | 5 | 0 | 0 | 25 | 1 | 1 | 0.3 | 0.1 |
| 100101 | 123 | 7 | 3.8 | 24 | 0 | 0 | 4 | 1 | 1 | 26 | 0 | 0 | 0.1 | 0.13 |
| 1101 | 125 | 5 | 2.7 | 23 | 1 | 1 | 5 | 0 | 0 | 26 | 0 | 0 | 0.5 | 0.74 |
| 110102 | 125 | 5 | 2.7 | 24 | 0 | 0 | 5 | 0 | 0 | 26 | 0 | 0 | 0.3 | 0.53 |
| 110201 | 128 | 2 | 1.1 | 24 | 0 | 0 | 4 | 1 | 1 | 26 | 0 | 0 | 0.2 | 0.001 |
| 110401 | 129 | 1 | 0.5 | 24 | 0 | 0 | 5 | 0 | 0 | 26 | 0 | 0 | 0.9 | 0.93 |
| 130101 | 129 | 1 | 0.5 | 22 | 2 | 1 | 5 | 0 | 0 | 25 | 1 | 1 | 0.2 | 0.11 |
| 1401 | 126 | 4 | 2.2 | 24 | 0 | 0 | 5 | 0 | 0 | 26 | 0 | 0 | 0.4 | 0.63 |
| 150101 | 128 | 2 | 1.1 | 23 | 1 | 1 | 5 | 0 | 0 | 23 | 3 | 2 | 0.1 | 0.06 |
| 1503 | 108 | 20 | 11 | 16 | 7 | 4 | 4 | 1 | 1 | 20 | 5 | 3 | 0.7 | 0.67 |
| 16601 | 129 | 1 | 0.5 | 24 | 0 | 0 | 5 | 0 | 0 | 26 | 0 | 0 | 0.9 | 0.93 |
| 130201 | 93 | 34 | 18 | 19 | 5 | 3 | 4 | 1 | 1 | 18 | 8 | 4 | 0.8 | 0.91 |

**Table 7:** Proportions and $X^2$ values (likelihood ratios and Pearson's p-values) of HLA-DR subtypes in AIDS, concordant (CONC), discordant negative (DSCN) and discordant positive (DSCP) study subjects. HST (HLA subtypes).

in AIDS and concordant couples (Table 5). HLA-B*4901 (15.7%), HLA-B*1503(11%) and HLA-B*2703(9%) were found in higher proportions when compared with other subtypes in AIDS subjects. In concordant couples, HLA-B*0702(3%), *5703(3%) were found in highest proportions among the members of the group.

Among the subtypes analyzed the highest proportions and

| HLA/subtype | AIDS *vs.* CONC | AIDS *vs.* DSCN | AIDS *vs.* DCSP | CONC *vs.* DSCN | CONC *vs.* DSCP | CONC *vs.* DSCP |
|---|---|---|---|---|---|---|
| HLA-A | | | | | | |
| HLA-B | 0705[B] | 0801[A] | | | 0705[B] | 0801[A] |
| | 1517[C] | 1817[B] | | | 0801[C] | 4901[C] |
| | 4101[B] | 352001[B] | | | 3910[C] | |
| | 5001[C] | 4901[B] | | | | |
| | 7301[C] | | | | | |
| HLA-C | 0501[C] | | | | | 0716[C] |
| | 0701[A] | | | | | |
| | 0740[C] | | | | | |
| HLA-DR | | 40301[B] | | 100101[C] | | 100101[C] |
| | | | | 110201[C] | | 110201[C] |

**Table 8:** HLA subtypes Fisher's Exact Test calculated p-value of those in which 20% of cells have expected count less than 5 (A=p<.001 (very highly significant), B=p<.01 (very significant), C=p<.05 (significant)).

frequencies were observed In HLA-C subtypes. HLA-C*0701(36.9%), *1701(14.4%), *0401(12.8%) and *1701(14.4%) (Table 6) were observed in higher proportions in AIDS subjects. HLA-C *0602(4.3%), and HLA-C *0701(2.7%) were relatively found in higher proportions in concordant couples. In discordant positive subjects, HLA-C*0802(3.2%) and HLA-C*0401(2.7%) were also observed in highest proportions among the group members.

In AIDS patients HLA-DR*130201(18.4%), HLA-DR*70101(16.2%), *80401(13.5%), *30101

(11.4%), *10201(10.3%) and *1503(10.8%) (Table 7) were observed in greater than 10% when compared with other subtypes. In concordant couples, HLA-DR*70101(3.8%), *1503(3.8%) and HLA-DR*10201(2.7%) were observed in higher proportion. In discordant positives HLA-DR*70101(4.9%), HLA-DR*130201(4.2%) and HLA-DR*30101(2.7%) and *1503(2.7%) were observed in higher proportions (Table 7).

HLA-A subtypes were not found to be significantly associated with any of the clinical group. But when AIDS subjects were compared with concordant couples (Table 8), HLA-B*0705($p<0.05$) and HLA-B*4101($p<0.01$) to be significantly associated with AIDS subjects. A similar comparison also showed that HLA-B*1517, *5001, *7301($p<0.05$) were found to be significantly associated with AIDS subjects (Table 8).

Similarly, HLA-C*0701($p<0.01$), *0501, *0740($p<0.05$) were found to be associated with AIDS subjects when compared with concordant subjects (Table 8). No HLA–DR subtype was observed to be associated with AIDS. Comparison of AIDS subjects with discordant negative subjects showed that HLA-B*0801($p<0.001$), HLA-B*1817, *352001($p<0.01$), *4901($p<0.01$) were strongly associated with AIDS subjects. HLA-DR*40301 ($p<0.01$) was also found to be very highly significantly with AIDS subjects when compared with discordant negatives (Table 8).

When concordant couples and discordant negative subjects were compared, only HLA-DR*100101 and HLA-DR*110201 ($p<0.05$) were found to be significantly associated with concordant couples (Table 8). Similar comparisons between concordant and discordant positive subjects showed that HLA-B*0705($p<0.01$), *0801($p<0.05$) and *3910($p<0.05$) were found to be associated with concordant couples (Table 8).

Discordant negative and discordant positive subjects showed strong associations with different HLA groups. HLA-B*0801($p<0.001$), *4901($p<0.01$) were strongly associated with discordant negatives than discordant positives subjects (Table 8). Among HLA-C, HLA-C*0716($p<0.05$) was found to be associated with HIV negativity. HLA-DR*100101 and *110201($p<0.05$ were also found to be significantly associated with discordant negatives (Table 8). The proportions of HLA-types in different clinical conditions were summarized (Figure 2).

To investigate whether heterozygous advantage was present in HIV discordant couples or not, homozygosity and heterozygosity was studied in the different subjects. The result indicated that discordant positive subjects were more heterozygous in HLA-A, HLA-B, HLA-C and HLA-DR loci when compared with concordant couples and AIDS subjects, showing that discordant positive subjects had clear heterozygous advantage when compared with all others.

## Discussion

Susceptibility or resistance to any infection is determined by the highly polymorphic region of the human genome (HLA). It is extremely polymorphic to cope up with ever evolving pathogenic agents, although it cannot cope up with the speed pathogenic agents evolve, which is measured in days , week and months while human HLA gene evolution requiring hundreds of years. HLA typing has practical application in identifying genes, which makes us susceptible or resistant to any infection and this is very important for vaccine design and gene therapy.

As the cause for resistance or susceptibility to any infection has its roots in HLA genes, the reasons for acting differently to HIV as in the case of HIV discordant couples may be due to the presence of different HLA subtypes offering resistance or making susceptible to infection. Thus, in order to analyze the effect of host HLA types on resistance or susceptibility to HIV infection and to study the HLA profile of Ethiopian HIV/AIDS subjects, DNA based HLA typing was carried out and result was collected from 239 subjects (discordant negative, discordant positive, concordant couples and HIV positive subjects).

HLA genes carry out their functions through the immune system. Whether the immune system is a predisposing or a protective is determined by HLA genes. Reduction in CD4 number, increased viral load and other immunological abnormalities caused by HIV are determined by host genetic factors, as resistance and /or susceptibility to an infection has host genetic regulation at its back. As CD4 T cells are the foremost targets of HIV, they are gradually lost as the disease progresses. CTLs do not have CD4 receptors; as a result, they are expected to be the major player in HIV regulation.

HIV infection and intrusion of viral particles are counteracted

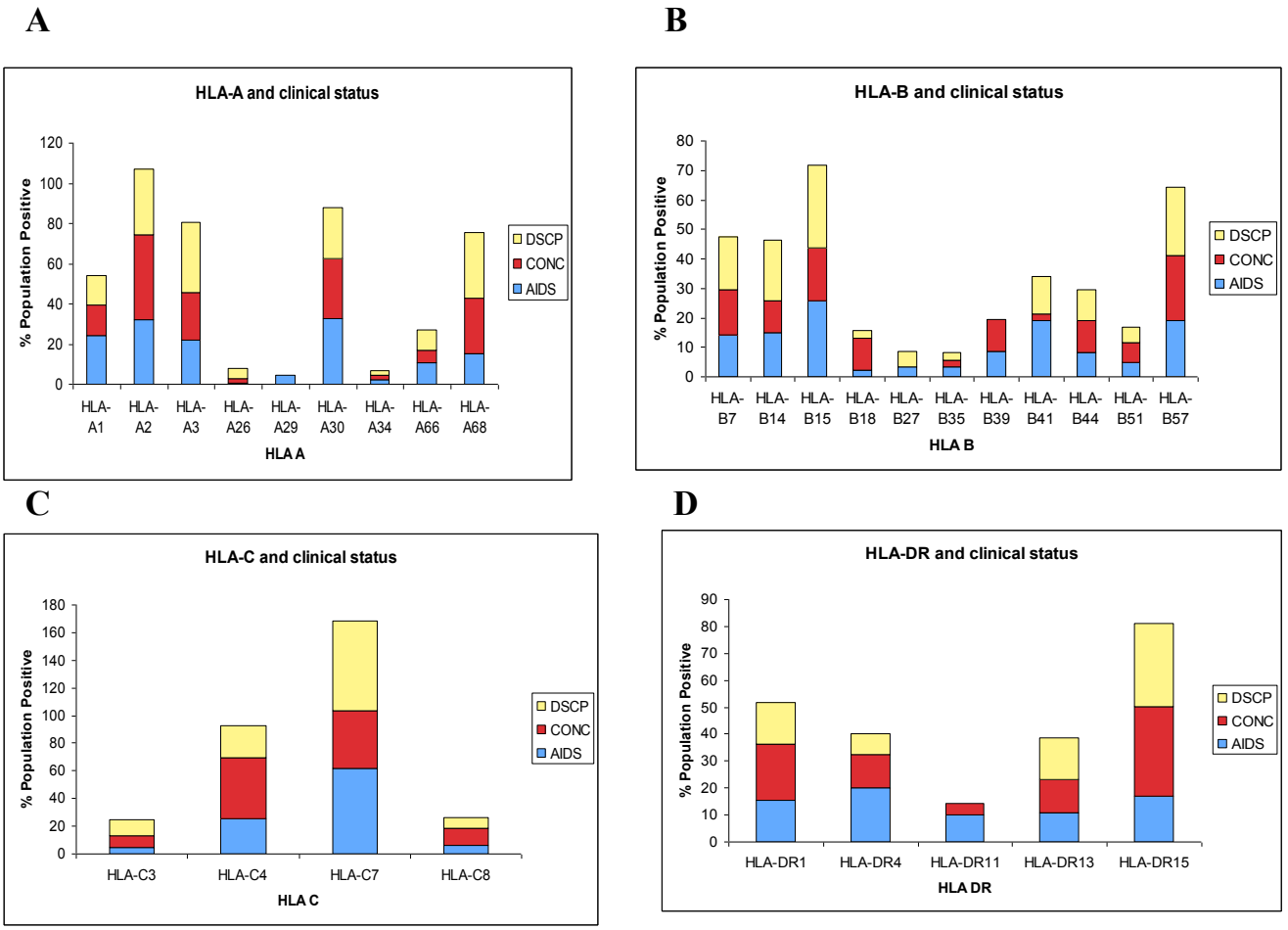


**Figure 2:** Proportions of different specific kind of HLA groups and clinical status: A) HLA-A and clinical status, B) HLA-B and clinical status, C) HLA-C and clinical status, D) HLA-DR and clinical status.

by CTL-mediated immune responses both during acute and chronic HIV infections [9], and the high concentration of CTL results from continued antigenic stimulation during chronic infection. CTLs recognize and kill HIV infected cells through the recognition of self HLA molecules on antigen presenting cells by different mechanisms. The presences of enormously large number of different HLA subtypes help to present different peptides of HIV to CTL.

Many different HLA subtypes of both classes I and II were found. The finding was proportional to the diversity of HLA class I alleles (according to IMGT-HLA database approximately 1178 HLA-B alleles compared with 767 HLA-A and 439 alleles in HLA-C (IMGT database, 2009; as cited by [10] are known). Thus, proportionally many different kinds of HLA-B alleles, followed by HLA-A and HLA-C alleles were found in all study subjects. The number of the different subtypes was highest for all classes of HLA in HIV/AIDS subjects and concordant couples. This might have been due to the higher number of sample size typed analyzed, although the diversity of HLA alleles and subtypes could not be ruled out. Relatively fewer subtypes were observed in discordant negatives and positives, the reason being similar to the above. HLA profile of Concordant couples and HIV/AIDS subject was similar as were many other similarities between them. But some subtypes were significantly associated to either of them and may not be identical.

Many different subtypes were discovered in our study when compared with previous workers [8,11], which analyzed only 50 HIV/AIDS subjects and 50 HIV negative and 36 HIV positive subjects, respectively. Moreover, their study did not include HLA class II alleles and did not involve discordant couples beyond testing and analyzing small sample size.

Our findings clearly indicated that HLA subtypes in AIDS, concordant couples, discordant positives and discordant negative subjects were not identical. Specifically, all AIDS patients, as were in other parameters, were significantly different from concordant as well as discordant couples. AIDS patients were not the same as concordant couples, as many HLA subtypes were found to be significantly associated with AIDS subjects when compared with concordant couples. Many of the subtypes significantly associated with AIDS patients when compared with concordant couples were HLA class I types. Our previous studies indicated that CD4 and CD8 T cells were significantly different in AIDS patients when compared with both concordant and discordant couples. Hence, many of the HLA subtypes such as HLA-A*29, *18 and *41; HLA-B*18 and *14 were only strongly associated with AIDS patients but not in others. These subtypes were subtypes, which made AIDS patients to succumb to AIDS quickly and were also associated with worsened and aggravated clinical conditions. Comparisons between AIDS and discordant positive subjects also indicated that AIDS subjects were significantly different in their associations with HLA subtypes. HLA-A*68, HLA-B*39 and

HLA-DR11 were very significantly associated with AIDS patients when compared with discordant positive subjects, indicating that AIDS patients were also different from discordant positives as was also observed in other difference in our previous studies. As a result, it is tempting to conclude that the difference between discordant positives and AIDS patients had genetic background. Only HLA-C*7 were significantly associated with AIDS patients when compared with discordant negative subjects among HLA-C subtypes. Many of these subtypes were also very closely associated with AIDS in many studies. Thus, it is clear that Ethiopian AIDS patients, according to our study, are rapid progressors exhibiting worsened disease conditions because of these specifically AIDS associated HLA subtypes, proving that there was a genetic background behind these scenarios.

Discordant positive and discordant negative subjects were also different in their HLA subtypes, as in all other parameters. HLA-B*39, *41 and HLA-DR*11 were significantly associated with discordant negatives, indicating that resistance to HIV had a genetic background. Similar difference s was also observed between discordant negatives and AIDS patients in that HLA-C*7, had significant association with discordant negative subjects. Hence, the difference between discordant negative subjects and discordant positive, as well as AIDS patients, had a genetic background due to the significant associations of these HLA subtypes. Thus, these subtypes are resistance determining subtypes. HLA-B*39 and HLA-DR*11 were significantly associated with discordant positives when compared with AIDS patients. Although this indicated the difference of discordant positives from AIDS patients, it was not clear from this study why these subtypes differentiated discordant positives and AIDS subjects as well as discordant positives and discordant negative subjects. But it is highly likely that in discordant positive subjects these subtypes might be the subtypes determining the long-term- non-progression, maintaining both CD4 and CD8 count at normal level and providing absolute protection for discordant negatives. Other genetic and host factors might have also caused this disparity. Thus, our evidence strongly indicated that HLA-B*39, *41 and HLA-DR*11 were subtypes associated with resistance to HIV.

When these pooled subtypes were analyzed for their different forms, the different forms (as indicated in Table 9) were mainly HLA-B, C and HLA-DR subtypes. This was in agreement with our previous studies in that CD8 T cells were significantly different in different groups and were associated with different clinical out-comes. This proved that HLA class I subtypes were very important in determining the susceptibility and/or resistance of subjects to HIV infection and rapid progression to AIDS. HLA-B*0705, *4101 were very strongly ($p<0.01$) associated with AIDS patients and HLA-B*1517, *5001 and *7301 were also significantly associated with susceptibility to AIDS (Table 9).

HLA-C plays a very important role in determining the fate of HIV in many studies next to HLA-B. The strong and very significant association of HLA-C*0701 and significant association of HLA-C*0740 with AIDS could have not occurred by chance. Thus, the association of these forms with AIDS might have been responsible for the rapid progression and worsened disease conditions of these subjects.

As there were many forms associated with susceptibility to AIDS, there were many forms significantly associated with resistance in discordant negatives. These included HLA-B*0801, *4901; HLA-C*0716 among HLA class I subtypes and HLA-DR*100101 and *110201. Thus, the persistently constant and normal CD4 and CD8 count making these subjects resistant to HIV was associated with these subtypes and forms, indicating that resistance to HIV had a genetic background.

Discordant negatives were also different from AIDS subjects because of the strong associations of HLA-B*0801, *1817, *352001 and *4901 and HLA-DR*40301 with AIDS when compared with discordant negatives. Thus, in absence of these HIV susceptibility HLA subtype forms, discordant negatives would be in a better position to combat HIV as they were not naturally susceptible to HIV. As a result, the absence of these forms in discordant negatives might have also contributed to their resistance to HIV. Discordant negatives were also different from concordant couples because of the significant associations of HLA-DR*100101 and *110201 to concordant couples when compared with discordant negatives. Thus, discordant negatives were different from discordant positives, concordant couples and AIDS patient in their HLA profiles. Similarly, AIDS patients were also very significantly different from concordant couples and discordant positives. Discordant positives were also different from concordant couples in their HLA profiles. This shows that different genetic mechanism operated in all groups in determining susceptibility and resistance to HIV. Our findings are therefore in agreement with the previous behavioral, immunological and other host factor(s) differences in the different groups.

Homozygosity and heterozygosity of alleles and subtypes are known to make rapid or delay disease progression in HIV. Heterozygosity for HLA-A, B, and C is known in delaying onset to AIDS. It has been shown that homozygosity at the class I loci is associated with relatively rapid progression to disease compared with heterozygotes [12]. The heterozygote advantage probably stems from the ability of such individuals to present a wider array of virus-derived epitopes to a more diverse CTL. Hence, heterozygosity may be associated with delayed progression to AIDS.

When comparisons were made between discordant positives, concordant couples and HIV positive subjects, discordant positive subjects were found to be more heterozygous at all loci (HLA-A, B, C and HLA-DR) when compared with concordant couples and HIV/AIDS subjects. At HLA-A loci the proportion between HIV/AIDS and CONC was (98.9% *vs.* 87.9%) and between DSCP and HIV/AIDS was (98.9% *vs.* 92.7%). Similarly, the proportion between DSCP and CONC and DSCP and HIV/AIDS at HLA-B loci was (98.9% *vs.* 91.2%; 98.9% *vs.* 94.9%), respectively. At HLA-C loci this was 98.9% *vs.* 97.5%; 98.9% *vs.* 86.2%, and between DSCP and CONC and DSCP and HIV/AIDS, respectively. Similar result was also obtained when DSCPs were compared with HIV/AIDS (98.6% *vs.* 94.4%,) at HLA-DR loci. These showed that discordant positives were at an advantageous position by being more heterozygous and were capable of delaying progression to AIDS, when compared with concordant couples and HIV/AIDS subjects, which had more homozygous subtypes than discordant

| Proportions | HLA-A | | | | HLA-B | | | | HLA-C | | | | HLA-DR | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | DSCN | DSCP | CONC | AIDS | DSCN | DSCP | CONC | AIDS | DSCN | DSCP | CONC | AIDS | DSCN | DSCP | CONC | AIDS |
| n | | 10 | 41 | 138 | | 10 | 41 | 138 | | 10 | 41 | 138 | | 10 | | 138 |
| frequency | | 1 | 5 | 18 | | 1 | 4 | 7 | | 1 | 1 | 19 | | 2 | | 5 |
| HMZ% | | 1.1 | 12.1 | 7.3 | | 1.1 | 9.8 | 5.1 | | 1.1 | 2.5 | 14 | | 1.44 | | 3.6 |
| HTRZ% | | 98.9 | 87.9 | 92.7 | | 98.9 | 91.2 | 95 | | 98.9 | 97.5 | 86 | | 98.6 | | 96 |

**Table 9:** Proportions of heterozygous and homozygous HLA types in discordant negatives (DSCN), discordant positives (DSCP), concordant couples (CONC) and HIV/AIDS subjects. (HNZ=homozygosity, HTRZ=heterozygosity).

positives. The difference between concordant couples and HIV/AIDS subjects was not significantly different from each other, showing that both were equally homozygous.

As the discordant positive subjects were found to be more heterozygous at all the HLA loci, including HLA-A, B, C and HLA-DR, epigenetic mechanisms that required for the regulation of mono-allelic expression of imprinted genes in humans must be studied. This should be by genomic imprinting. Genomic imprinting refers to the mono-allelic expression of certain genes that either located on the paternal or the maternal derived allele. At imprinted loci, as the DNA methylation patterns are heterozygous and removal of this germline derived DNA methylation leads to aberrant expression of imprinted genes, it is believed that the mono-allelic expression of imprinted genes is directly controlled by imprinted DNA methylation. In this study, as the discordant positive subjects displayed heterozygous at all the HLA loci, it is interesting to investigate the status of DNA methylation that exist at these loci. In addition, since DNA methyltransferases (DNMTs) and some histone writers, such as H3K9 methyltransferases G9a and GLP, play an essential role in the depositing and maintaining of DNA methylation at imprinted loci, it is also interesting to investigate the levels of these enzymes in DSCN, DSCP, CONC as well as the patients with AIDS.

## Conclusion

Our study showed that there was a clear difference between discordant positives and discordant negative couples in their genetic profiles. There was also a clear difference between discordant positives and concordant couples and AIDS patients, in their genetic profiles. When comparisons were made between discordant positives, concordant couples and AIDS subjects, discordant positive subjects were found to be more heterozygous at all loci (HLA-A, B, C and HLA-DR) when compared with concordant couples and HIV/AIDS subjects. This showed that discordant positives better controlled HIV and maintained HIV in check and were non- progressors due to heterozygous advantage. Overall, the results for discordant positives and AIDS subjects were clear enough to show significant difference between them.

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