

# Hyperhomocysteinemia in HIV-Infected Individuals: Correlation of a Frequent Prothrombotic Factor with CD4+ Cell Count

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## Abstract

**Objective:** This study was aimed at providing an analysis of the correlation between CD4/CD8 counts and some coagulation factors in HIV-Positive Iranian patients.

**Methods:** A case-control study on 58 HIV-infected patients and control group (58 healthy individuals). Patients and controls were matched for sex and age. In this study, several blood parameters were measured in 58 HIV-infected patients and the controls. Laboratory data were then measured including hemoglobin, platelets, homocysteine, serum levels of IgM and IgG antiphospholipid antibodies (aPL), IgM and IgG anticardiolipin antibodies (aCL), and CD4<sup>+</sup> and CD8<sup>+</sup> cell count.

**Results:** The HIV-infected patients, compared to healthy controls, showed a significant decline in platelets, CD4 count and CD8 count ( $p < 0.0001$ ), and an increase of homocysteine ( $p < 0.0001$ ) and IgG aPL levels ( $p < 0.0001$ ). No statistical difference was found between patients with CD4 count  $\leq 200$  and CD4 count  $> 200$  in the evaluated variables.

**Conclusion:** The results showed that thrombophilic abnormality in the form of hyperhomocysteinemia is more frequent in HIV-infected patients and should be considered by clinicians in view of an early diagnosis of the hypercoagulability state to prevent thrombotic complications.

**Keywords:** CD4/CD8 Counts; Coagulation Factors; HIV-Positive Patients.

## Introduction

Human immunodeficiency virus (HIV) infection may result in variable hematologic manifestations.<sup>1</sup> Several clinical studies have reported a higher risk of thrombotic complications among HIV-infected patients.<sup>2-4</sup> The risk of venous thrombotic events has been described 6.5 times,<sup>3</sup> and 10 times,<sup>5</sup> more prevalent in these

patients than in general population, and autopsy studies have shown high frequencies of previously undiagnosed thromboembolism among patients with AIDS.<sup>6</sup> HIV patients with CD4 counts  $< 200$  cells/ $\mu$ L have a greater risk of thrombosis than those with higher CD4 counts.<sup>7</sup> A wide variety of coagulation abnormalities could predispose to a hypercoagulable state in HIV disease, including the high levels of antiphospholipid antibodies and anticardiolipin antibodies, deficiencies of protein C, protein S,<sup>8,9</sup> antithrombin III and heparin factor II,<sup>8</sup> increased platelet activation and an increase of homocysteine levels.<sup>8,9</sup> The early diagnosis of these abnormalities could prevent potentially lethal thrombotic complications including pulmonary thromboembolism. Hence, in this study, the presence of some thrombophilic abnormalities in HIV-infected patients was evaluated and compared to HIV negative comparators. A second objective of this study was to examine the association between CD4 count and coagulation abnormalities in HIV-infected patients.

## Methods

In this case-control study investigating 58 HIV-infected patients (HIV infected cases treated naive) who visited the outpatient clinic of our teaching hospital (the HIV positives had been presented one year after they were diagnosed with their disease), Tehran University Medical Sciences, Iran.

HIV infection was documented by serology, confirmatory western blot analysis or by polymerase chain reaction (PCR). The control group included 58 healthy individuals who attended the biochemical laboratory of the hospital to undergo periodical checkup. Patients and controls were matched in age and sex. The study was approved by the hospital's ethics committee.

Whole blood, serum or plasma were collected from all subjects and eight markers were measured: Hemoglobin and platelets measured by cell counter (sysmex, KX21- Japan); Homocysteine measured by auto-analyzer (Alcion-300, Abott-USA), Turbidometric method; Anticardiolipin and Antiphospholipid antibodies (IgG, IgM) measured by ELISA method and lymphocyte CD4, CD8 counts were done by a single platform flow cytometric method.

Anticardiolipin antibodies (aCLs) (IgG, IgM) were measured using ELISA (ORGENTEC Diagnostika GmbH, Germany kits). Normal values were  $< 10$  U/ml for IgG Ab and  $< 7$  U/ml

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for IgM Ab. The lower detection limit for Anticardiolipin IgG was determined at 1 GPL U/ml. Anticardiolipin IgM yielded a sensitivity of 0.5 MPL U/ml. Antiphospholipid antibodies (IgG, IgM) were measured by (ELISA) ORGENTEC Diagnostika GmbH, Germany Kits. Normal values were <10 U/ml for IgG, IgM Abs. The lower detection limit for Antiphospholipid screen IgG and IgM were determined at 0.5 U/ml.

Serum Homocysteine was measured by Diazyme Laboratories, USA Kit. Normal value for Homocysteine was 5-18  $\mu\text{mol/L}$ . Anemia was defined as a hemoglobin level <12 g/dL, based on standard published guidelines. Platelet number <150  $\times 10^3/\mu\text{L}$  was considered as thrombocytopenia.

The SPSS-for-Windows V 16.0 was used for data analysis. The distribution of the quantitative data was examined by One-Sample Kolmogorow-Smirnov Test. In order to analyze the association between quantitative variables, independent samples t-test and the Mann-Whitney U test were used, depending on the normality of data. Chi-square test and Fisher's exact test were used for the qualitative data. Pearson's correlation coefficient obtained to evaluate the correlation between the coagulation factors and CD4 count. A two-tailed p-value of less than 0.05 was considered statistically significant.

## Results

In this case-control comparative study, 38 (65.5%) men and 20 (34.5%) women were enrolled in each group. From our data, CD4+ cell count was  $327.55 \pm 196.54$  in HIV infected patients compared to  $920.26 \pm 168.42$  in the controls ( $p=0.00010$ ). In addition, CD8+ cells count was  $803.93 \pm 332.98$  in HIV infected cases compared to  $965.09 \pm 111.54$  in the controls ( $p=0.00011$ ), which were both significantly different. The serum levels of all classes of antibodies including IgG and IgM aPL, and IgG and IgM aCL were  $3.17 \pm 1.46$  and  $2.62 \pm 1.68$ , and  $1.99 \pm 0.84$  and  $1.56 \pm 1.03$ , respectively in patients, all of which were within the normal range. However, the mean levels of Ig classes were as follows:  $1.92 \pm 1.04$  for IG aPL;  $2.23 \pm 1.37$  for IgM aPL;  $1.92 \pm 1.23$  for IgM aCL; and  $1.91 \pm 1.14$  for IgG aCL; among these, only IgG aPL exhibited a statistically significant difference ( $p=0.00010$ ). There was no significant difference in the mean values of IgM aPL, IgG aCL and IgM aCL between cases and comparators. Clinical characteristics of the subjects evaluated in this study are shown in Table 1.

Hyperhomocysteinemia was found in 53 (91.4%) of 58 patients compared with the controls with normal homocysteine and the levels of homocysteine were significantly higher in patients compared with the controls. A platelet count below  $150 \times 10^3/\text{L}$  was detected in 13 patients (22.4%) vs. 1 (1.7%) of the comparators and the mean platelets was significantly lower in patients compared to the controls ( $p=0.00010$ ).

Anemia was detected in 5 patients (8.6%) vs. none of controls. There was no significant difference in the mean values of

hemoglobin between cases and controls. Patients with CD4 count  $\leq 200$  and CD4 count  $>200$  did not show significant difference in any variables. (Table 2)

**Table 1:** Clinical characteristics of HIV-infected patients and controls.

Parameter	HIV-infected patients	Controls	p value*
	(n = 58)	(n = 58)	
Hemoglobin (g/dL)	14.43 ( $\pm$ 1.95)	14.35 ( $\pm$ 0.99)	.769
Platelets ( $\times 10^3/\mu\text{L}$ )	189.81 ( $\pm$ 70.87)	268.03 ( $\pm$ 51.02)	.000
Homocysteine ( $\mu\text{mol/L}$ )	27.09 ( $\pm$ 10.14)	12.60 ( $\pm$ 1.07)	.000
CD4 count (cells/mL)	327.55 ( $\pm$ 196.54)	920.26 ( $\pm$ 168.42)	.000
CD8 count (cells/mL)	803.93 ( $\pm$ 332.98)	965.09 ( $\pm$ 111.54)	.001
IgG aCL (U/ml)	1.99 ( $\pm$ 0.84)	1.91 ( $\pm$ 1.14)	.672
IgM aCL (U/ml)	1.56 ( $\pm$ 1.03)	1.92 ( $\pm$ 1.23)	.203**
IgG aPL (U/ml)	3.17 ( $\pm$ 1.46)	1.92 ( $\pm$ 1.04)	.000
IgM aPL (U/ml)	2.62 ( $\pm$ 1.68)	2.23 ( $\pm$ 1.37)	.300**

\* p value were computed using Independent Sample t-test

\*\* p value were computed using Mann-Whitney Test

**Table 2:** Mean values of evaluated parameters in CD4  $\leq 200$  and CD4  $>200$  among HIV-infected patients.

Parameter	CD4+count $\leq 200$	CD4+ count $>200$	p value*
	Hemoglobin (g/dL)	13.7	
Platelets ( $\times 10^3/\mu\text{L}$ )	178.6	194.1	0.556
Homocysteine ( $\mu\text{mol/L}$ )	25.4	27.8	0.578
IgG aCL (U/ml)	2.1	1.1	0.537
IgM aCL (U/ml)	1.5	1.5	0.998**
IgG aPL (U/ml)	3.3	3.1	0.703
IgM aPL (U/ml)	2.4	2.8	0.276**

\* p value were computed using Independent Sample t-test

\*\* p value were computed using Mann-Whitney Test

Two parameters were shown to be correlated with CD4 count among HIV-infected patients. There was a moderately strong positive correlation between hemoglobin and CD4 count ( $r=0.31$ ;  $p=0.018$ ) and between platelet count and CD4 count ( $r=0.27$ ;  $p=0.041$ ). No statistical correlation was observed between CD4 count and the levels of homocysteine, IgG aCL, IgM aCL, IgG aPL and IgM aPL in the studied patients.

## Discussion

Various hematological abnormalities could predispose to a hypercoagulable state and an increased incidence of thrombotic events in HIV infection. Several studies have proposed the participation of antiphospholipid antibodies (aPL) in the progression of thrombotic complications in HIV patients.<sup>8-10</sup>

These antibodies are highly prevalent in HIV infection, reported in 82% to 92% of patients with AIDS.<sup>11</sup> Both types of aPL have been found in HIV patients.<sup>12</sup> Although, IgG aPL were more prevalent (41%) in HIV infection than IgM aPL (7%).<sup>12</sup> All of our patients had normal values of IgG aPL (1.3 - 7.3 U/mL) and IgM aPL (0.6 - 9.8 U/mL), although the mean values of IgG aPL levels in patients were significantly higher than the controls ( $p=0.00010$ ), confirming previous studies on the matter. From the current data, it appears that there is no correlation between the severity of the disease (presented as CD4+ cells count) and an increase in the levels of aPL.

Anticardiolipin antibodies (aCL) have been detected in almost 45% to 50% of HIV-infected patients.<sup>11,12</sup> Maclean et al. found aCL in a large number of these patients and the levels were significantly higher than among the controls.<sup>13</sup> Sorice and colleagues reported a high prevalence of IgG aCL in HIV-infected patients (77.1% of 35 patients) that was significantly higher than HIV negative controls ( $p<0.0001$ ).<sup>14</sup> Guerine et al. observed the increased levels of aCL in 88% of 30 HIV patients.<sup>15</sup> In contrast to these findings, we found no significant difference in the levels of aCL between patients and controls. This discrepancy may be due to the small number of sample population, methodological reasons, laboratory methods or genetic differences of study population. Moreover, we observed no correlation between aPL and CD4 count. In 2003, a case-control study by Chretien and colleagues showed that IgG and IgM aCL levels were statistically more frequent in 105 HIV patients than 100 sex- and age-matched healthy controls. They found no correlation between these antibodies and CD4 count.<sup>16</sup> In 2007, a relatively high frequency of aCL antibodies in HIV (17.8% in 90 patients) with 16.7% positive for IgG aCL and 3.3% for IgM aCL reported by Galrão et al. In their study, no association was observed between CD4 count  $<200$  and aCLs.<sup>17</sup> The recent findings of two previous studies were in agreement with our results.

Hyperhomocysteinemia was found in 91.4% of our patients *versus* none of the controls and the plasma homocysteine levels were significantly higher in patients as compared with control subjects ( $p=0.00010$ ). The increase in serum homocysteine of HIV patients may have resulted from special antibodies in the serum or their immunity shortcomings. The association between cardiovascular risk and increased plasma homocysteine levels has been reported. The prevalence of hyperhomocysteinemia in HIV-positive patients has been reported to be between 12.3% and 35%.<sup>9</sup> In 2001, Bernasconi et al. found significantly higher fasting plasma homocysteine levels in 82 HIV patients who received highly active antiretroviral therapy in comparison with 80 healthy controls.<sup>9</sup> Such a high level of homocysteine has been correlated with nutritional deficiency and antiretroviral therapies.

Considering the high prevalence of hyperhomocysteinemia in our patients, we suggest regular examinations of cardiovascular system in HIV-infected patients to prevent atherothrombotic

vascular complications and to reduce cardiovascular risk. On the other hand, HIV-infected patients have an increased risk of hemorrhagic complications by reason of decreasing platelet count. Thrombocytopenia is one of the first clinical signs of HIV infection. As shown in other studies, thrombocytopenia developed in approximately 10-40% of HIV-infected patients.<sup>18</sup> Although the average platelets volume in both groups stands at the normal range, it is lower in HIV-infected group than in HIV-negatives.

In 2003, Palomo et al. observed thrombocytopenia in 16.2% of 37 HIV patients.<sup>19</sup> Our study showed that 21% of patients had the platelet count  $<150 \times 10^3/dL$  and the mean values of platelets in patients was significantly lower than in the controls. Moreover, we found a moderately strong positive correlation between the number of platelets and CD4 count ( $r=0.27$ ;  $p=0.041$ ), in an agreement with Elaine et al.<sup>20</sup> In a large prospective study, Kaslow and colleagues compared 1,611 HIV-positive patients in hematologic abnormalities with 2,646 controls and observed that 6.7% of patients presented thrombocytopenia. They found an inverse relationship between the intensity of thrombocytopenia and CD4 counts, confirming our findings.<sup>21</sup> However, none of the HIV infected patients in the current study was defined as thrombocytopenic nor did they show any symptoms of this disorder. By reason of the high frequency of thrombocytopenia in HIV patients as already discussed, they should be examined for platelet count even before minor surgeries to prevent hemorrhagic complications.

Anemia is a frequent complication of HIV infection. Ramezani et al. observed that mild to moderate anemia existed in 46% of 143 HIV infected subjects.<sup>22</sup> We observed no significant difference in hemoglobin levels between patients and controls ( $p=0.769$ ). This finding may be attributable to the number of subjects included or the supplementary treatment with Iron or folate-vitamin B12 complex. We found that there is a moderately strong positive correlation between the hemoglobin levels and CD4 count ( $r=0.31$ ;  $p=0.018$ ) in the current studied patients. Low CD4 counts ( $<200$  cells/ml) have been associated with an increased risk of anemia.<sup>23</sup> In a large cohort of HIV-infected women in 2001, an inverse correlation between CD4 count and the risk of anemia was found by Levine et al.<sup>24</sup> In 2007, Mildvan et al. reported a high prevalence of anemia in 9690 HIV-infected patients and a strong correlation between anemia and low CD4 count.<sup>25</sup> Recently, a prevalence of 20% was reported for anemia in 63 HIV patients by Mata-Marín and colleagues. Also, they detected a moderately strong positive correlation between CD4+ cell count and hemoglobin levels ( $r=0.595$ ;  $p<0.0001$ ) that confirms our findings.

The main limitations to the current study include its cross-sectional design, the limited number of subjects included and the lack of adequate information regarding clinical evidence of thrombotic events in patients. Further studies are needed to evaluate the association between such thrombophilic abnormalities and the occurrence of thrombotic events in HIV patients.

## Conclusion

Our results showed that some thrombophilic abnormalities are more frequent in HIV-infected patients compared to HIV-negative controls and should be considered by clinicians in view of an early diagnosis of the hypercoagulability state to prevent thrombotic complications including pulmonary thromboembolism.

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