ORIGINAL REPORT

Extrinsic and Intrinsic Coagulation Pathway, Fibrinogen Serum Level and Platelet Count in HIV Positive Patients

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Abstract- Infection with human immunodeficiency virus (HIV) is a progressive condition which may cause endothelial dysfunction and liver damage leading to coagulopathy. With adventure of highly active antiretroviral therapy (HAART), life expectancy has prolonged in HIV positive patients but several acquired immunodeficiency syndrome (AIDS)-related conditions such as coagulopathies are responsible for associated morbidity and mortality. This study aimed to evaluate the extrinsic and intrinsic pathways of coagulation, serum level of fibrinogen and platelet count in HIV positive patients and compare it with negative healthy individuals. Through a case-control study, 114 HIV seropositive patients were compared with 114 seronegative samples in terms of hematological and other coagulation parameters. Mean age of study patients was 37.48 years. Intra venous drug abuse was the most common route of infection transmission with a prevalence of more than 50%. HIV route of transmission had a direct relationship with PTT abnormal levels (P<0.0001). However, this relationship was not significant for PT values. Stages of HIV disease and administration of HAART did not reveal any significant relationship with PT and PTT. There was also a statistically significant correlation between $CD4^+$< 200 and PT in case group (P=0.008). On the other hands, in control group, $CD4^+$ had a weak relationship with PTT (P=0.02) and an inverse correlation with serum fibrinogen (P=0.013). Hematological parameters and serum fibrinogen are decreased in HIV positive patients especially in direct relation with $CD4^+$ cell count<200 cell/µl. PT and PTT abnormal values are also more prevalent in this population.

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Keywords: Human Immunodeficiency Virus; Coagulation Pathway; Fibrinogen; Platelet

Introduction

Several studies have showed that risk of thrombosis increases in association with progressed stages of HIV infection (1-4). Acute inflammation due to infection is one of the important causes of coagulation disorders (5,6). Cytokines act as mediators for activation of coagulation system with more effect on extrinsic pathway than intrinsic pathway (7,8). The cytokines consist of tumor necrosis factor-α (TNF-α), interleukin-1 (IL-1), and IL-6 (9,10). Vascular endothelial cells interacting with releasing cytokines from leukocyte, adhesion molecules and growth factors play an important role in up-regulation of coagulation system. Furthermore, the effect of endothelial cells on formation and removal of fibrin during the inflammatory process is well described (11-13).

Vascular endothelium and liver are the mainstay of coagulation system (14-16). HIV infection leads to endothelial dysfunction, so activation and consumption of coagulation factors occurs resulting in coagulopathy (17). Impairment in liver function during HIV infection by reducing coagulation factors add to the compromised coagulation state (18). The primary tests for assessment of coagulopathy are prothrombin time (PT) and partial prothrombin time (PTT). Additionally, platelets (plt) have a significant role in hemostasis and due to endotoxins, cytokines and platelet activation factor

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(PAF) secreted during HIV infection, plt activities increase which in association with impaired thrombopoiesis leads to thrombocytopenia (20,21). Although treatment of HIV infection with highly active antiretroviral therapy (HAART) has decreased mortality of this disease, but increased non-AIDS related conditions such as cardiovascular diseases and coagulopathies have caused particular morbidities (22-24). The purpose of this study was to evaluate the extrinsic and intrinsic coagulation pathways, serum fibrinogen, and plt function in HIV positive patients and compare it with healthy control individuals.

Materials and Methods

Through a case-control study in central laboratory of hematology in a university hospital in Tehran, Iran, 114 HIV positive patients with confirmed diagnosis with serology, PCR or western-blot test were enrolled and compared with 114 healthy HIV negative subjects in a control group attending our laboratory for routine checkup. Patients with pregnancy, autoimmune disease, malignancy or hematological disorders were excluded from the study. Five to six milliliters (ml) of blood sample were obtained from each patient for CBC, PT, PTT and defining serum level of fibrinogen. Complete blood count was done with Sysmex-K21 (Japan) instrument on blood samples anticoagulated with EDTA. Additionally, some amount of blood was added to sodium citrate to check for PT and PTT with Sysmex-K1500 coagulometer. Serum level of fibrinogen was checked with chronometric method with a normal range from 200-400. Patients gave an informed consent before entering to the study and the institutional review board of Tehran University of Medical Sciences approved the study protocol. Data were analyzed with statistical package for social science (SPSS, version 18, Chicago, Inc) and a *P*-value less than 0.05 was considered statistically significant.

Results

Of two hundred twenty eight patients included in this study, 137 were male (61%) and 69 were female (39%) with an average age of 37.48 years. The most prevalent age group was between 20 and 30 years. IV drug abuse with common syringe was the most frequent route of HIV transmission with a frequency of more than 50%. Based on our analysis, after excluding the data of 6 patients due to clotted blood for PT test and samples of 7 patients for PTT test, patients of the control group did not reveal any disorder in coagulation parameters while in the case group, the intrinsic pathway showed abnormal PT result. In contrast, PTT test was normal showing normal function of the extrinsic pathway.

HIV routs of transmission had a significant and direct correlation with PTT abnormality ($P<0.0001$); however, no such a correlation was found for PT test. Moreover, stages of HIV disease and administration of HAART therapy did not have any correlation with disorders of PT and PTT. As shown in table 1, in case group there was a statistically significant correlation between $CD4^+<$ 200 and PT ($P<0.008$) while in the control group $CD4^+$ showed a weak correlation with PTT ($P<0.02$) and an inverse correlation with fibrinogen ($P<0.013$).

Table 1. Demographic characteristics of patients in case and control groups.

	Group	Prevalence
Sex	Male	61%
	Female	39%
Age	<20	3%
	20-30	20%
	30-40	46%
	40-50	20%
	50-60	7%
	>60	4%
Transmission routes	IV drug abuse with common siring	55%
	Sex	35%
	Other ways	10%

Table 2. Comparison of hematologic agents and coagulation tests between case and control groups.

	Control	Case	*P*-value
CD4	981.38±261.218	300.31±186.113	<0.0001
CD8	964.09±267.611	772.72±380.487	<0.0001
WBC	6.8810±1.29377	5.4454±1.99906	<0.0001
RBC	4.8930±0.51965	4.3498±0.86678	<0.0001
HGB	14.781±0.9103	14.110±2.0245	0.001
HCT	44.341±2.7223	40.383±5.1424	<0.0001
MCV	92.474±4.2618	94.776±12.9368	0.072
MCH	29.096±1.7191	33.173±5.3794	<0.0001
MCHC	32.904±1.8671	34.896±1.5273	<0.0001
PLT	275.39±81.174	200.58±79.344	<0.0001
PT	12.109±0.7486	11.828±0.6354	0.003
PTT	31.89±3.615	24.93±5.677	<0.0001
INR	1.0699±0.10915	1.0519±0.13289	0.274
Fibrinogen	320.51±42.314	271.59±119.230	<0.0001

Discussion

Liver disorders and endothelial abnormalities occur during HIV infection leading to disorders of coagulation (17). The correlation between age and coagulation disorder has been discussed in some studies and based on their results, HIV positive patients at the time of venous thrombosis had a mean age of 40 years and this is 20 years lower than the mean age of HIV negative patients (1,25). Similarly, the mean age of patients of the case group in this study was 37.48 years old.

In this study, $CD4^+$ and $CD8^+$ cell count had decreased in the case group compared with the control group which is similar to the results of other studies probably due to the viral attack to $CD4^+$ and $CD8^+$ lymphocytes (19).

Based on our study results and in agreement with other studies, the platelet count in HIV positive patients is lower than HIV negative patients (P<0.0001). Wolf *et al.* in their study showed that 22% of HIV positive patients had thrombocytopenia while it was not observed in their control group (19,26-29). Infection of megakaryocytes with HIV leads to disorders of thrombopoiesis. which along with production of antibody against platelets are among the suggested reasons for HIV induced thrombocytopenia (14).

PT in the case group was significantly higher than control group while such a difference was not observed between case and control group for PTT; other studies also showed that PT and PTT were significantly higher in HIV positive patients in comparison with HIV negative subjects (19).

The correlation between $CD4^+$ and $CD8^+$ cell counts and coagulation parameters including PT, PTT, platelet count, serum fibrinogen and other hematological markers indicated that $CD4^+$ cell count has a positive but weak correlation with PTT and a negative and poor correlation with serum fibrinogen in HIV positive patients while in HIV seronegative patients $CD4^+$ cell count has a negative and weak correlation with PTT. Additionally, the correlation between $CD8^+$ cell count and platelet count and fibrinogen was significantly positive (P<0.05). In contrast, one study showed that PT has a negative correlation with $CD4^+$ cell count in HIV positive patients (19).

Lijfering *et al.* demonstrated that HIV positive patients have more serum levels of fibrinogen than HIV negative individuals (32). This report is in line with our finding in which the level of fibrinogen in the case group was higher than the control group (P<0.0001). In patients at the stage of AIDS, the cytokines activating coagulation system is produced leading to increase in fibrinogen serum levels (30-32).

We also found that RBC, hemoglobin (HGB) and hematocrit (HCT) in HIV positive patients are significantly lower than control group in a way that those with decreased $CD4^+$ cell count, the amount of RBC, HGB and HCT is decreased due to the suppression of $CD4^+$ cell formation; this is also in the same direction with the results of the study by Dikshit *et al.* who showed that HGB, HCT and MCV have significant correlations with $CD4^+$ cell count (33).

Our study did not find any relationship between PTT and PT disorders and HAART. To the best of our knowledge, no study has discussed this relationship yet. In conclusion, hematological parameters and serum

fibrinogen are decreased in HIV positive patients especially in direct relation with $CD4^+$ cell count<200 cell/µl. PT and PTT abnormal values are also more prevalent in this population. Most of the patients in our study were at the middle age and male with IV addiction and usage of common syringes as the most common rout of HIV transmission. In these patients; WBC, RBC, HGB, Platelet, fibrinogen, $CD4^+$ and $CD8^+$ cell counts are decreased and PT and PTT tests are impaired. In patients with $CD4^+$ cells less than 200/µl, there was a positive correlation between $CD4^+$ cell count and PT.

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