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Research Article

Abnormalities of Fibrinolytic Parameters in Tunisian Patients with Behçet's Disease. Correlations with the Clinical Presentation, the Activity, and the Severity of the Disease

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Abstract

Background: The thrombotic tendency in Behçet's disease (BD) has been studied with variable results. We have studied d-dimer, tissue plasminogen activator (t-PA), and active Plasminogen Activator Inhibitor-1 (PAI-1) levels in Tunisian BD patients. We analyse hereby the correlations with the clinical presentation, the activity, and the severity of the disease.

Patients and methods: A case-control prospective study of Tunisian BD patients was performed. Patients were classified as active and inactive, and evaluated according to Yazici severity score. D-dimer was determined by a quantitative assay test (VIDAS D-DIMER exclusion). T-PA and PAI-1 were determined by enzyme linked immunosorbent assay (ELISA).

Results: Fifty- seven patients, 42 men (73%) and 15 women (27%), were studied; their mean age at the onset was 33 years. D-dimer was increased in 17 patients (31%) and normal in 39 (69%). D-dimer in BD patients was significantly higher than in healthy controls (p=0.033). There was a correlation between positive d-dimer and articular involvement (p=0.025). Positive d-dimer was significantly associated to the active phase of the disease (p=0.04). T-Pa levels were lower than in healthy controls (4.85 ± 7.36 vs 5.62 ± 8.49 ng/ml, p=NS). No significant differences were noted concerning t-PA levels among the clinical manifestations, the activity, or the severity of the disease. There was a significant correlation between ocular involvement and low level of PAI-1 (p=0.03).

Conclusion: There are abnormalities in the fibrinolytic parameters in Tunisian BD patients. Due to the scarcity of publications, their implication in the pathogenesis of the disease as a cause or as a consequence is not clear.
Keywords: Behçet's disease; D-dimer; Tissue Plasminogen Activator; Active Plasminogen Activator Inhibitor-1.

Abbreviations:

BD	: Behçet's disease;
t-PA	: tissue plasminogen activator;
PAI-1	: active Plasminogen Activator Inhibitor-1;
ELISA	: enzyme linked immunosorbent assay;
u-PA	: urokinase type plasminogen activator;
ISG	: International Study Group;
TAFI	: thrombine activatable fibrinolysis inhibitor

Introduction

Behçet's disease (BD) is a chronic multisystemic inflammatory disorder characterized by recurrent oral and genital ulcers, uveitis, and skin lesions (1). BD can affect nearly all systems and organs, including the vascular system, central nervous system, gastrointestinal tract, lungs, kidneys, and joints [1]. Venous thrombosis is frequent in BD, and particularly high in Tunisia, with 33% of patients reported by Houman et al [2]. The pathogenesis of thrombosis in BD is not clear. Endothelial damage and/or defects in coagulation or fibrinolysis are thought to take a part [3]. Deficient Protein C, protein S, and anti thrombin, procoagulant mutations like factor V Leiden, prothrombin gene G20210A, methylenetetrahydrofolatereductase gene C677T, and the presence of antiphospholipid antibodies have all been implicated in the pathogenesis. However, the results of these studies have been somewhat conflicting and inconclusive [4].The synthesis of plasmin from plasminogen is an important step in the activation of the fibrinolytic system, and requires tissue plasminogen activator (t-PA) or urokinase type plasminogen activator (u-PA). Inadequate t-PA secretion from the endothelium and increases in the levels of its inhibitor plasminogen activator inhibitor (PAI-1) may result in an insufficient fibrinolytic system and may increase the risk of thrombosis. D-dimer is the degradation product of cross linked fibrin, formed by the action of plasmin and its levels increase in acute thrombosis, various prothrombotic and inflammatory states [5]. The aim of this study was to compare d-dimer, t-PA, and PAI-1 levels in Tunisian BD patients with healthy controls, and to investigate correlation with the clinical manifestations, the activity, and the severity of the disease.

Patients and Methods

Patients were recruited consecutively between January and July 2011, with an additional 6 months of follow up. The patient group included 57 subjects with BD (42 men and 15 women, median age 40 years). All BD patients fulfilled the criteria for diagnosis of Behçet's disease according to the International Study Group (ISG) [6]. According to the Yosipovitch severity scale [7], patients were divided into three subgroups: mild, moderate, and severe disease. The activity index was elaborated according to the Yazici scale [8]. We excluded all patients with physiological and pathological situations that could increase d-dimer (pregnancy, current infection, renal failure, hepatic failure…).

The control group consisted of Tunisian healthy individuals, matched by age and sex. These controls were recruited during the study period from voluntary blood donors of the hematological department. We excluded all controls with family and personal history of oral and genital ulcers, and other symptoms related to BD.

Blood samples were drawn in the morning hours by veinpuncture from the antecubital vein and without veinoocclusion; they were then collected in tubes containing 3.2% trisodium citrate, which were centrifuged within one hour with the velocity of 2500 g. For d-dimer, t-PA and PAI-1 determinations, 9 ml of blood were transferred through a 19 G needle to polypropylente tubes containing 1 ml 0.109 M tri sodium citrate. Plasma was obtained by centrifugation at 4°C for 10 minutes, at 3000 g, and stored at – 80°C until tested.

Levels of d-dimer were determined by a quantitative assay test which consisted of immunosorbent determination of degradation products of fibrin (VIDAS D-DIMER exclusion). The cut-off value for d-dimer was ≥ 0.5µg/ml. Plasma concentrations of t-PA and PAI-1 were determined by enzyme linked immunosorbent assay (ELISA), using the commercially available kits containing mouse monoclonal antibodies (Asserchrom® t-PA, DiagnosticaStago, France Ref 00948 and Asserchrom PAI-1, DiagnosticaStago, France, Ref 00807).

Statistics analyses were performed with SPSS 11.5 version. Percentage comparison was performed chi-2 test, average comparison with t Student test. Correlations were compared by parametric Pearson and non-parametric Spearman tests. Data were expressed as the mean and standard deviations (±DS). $P< 0.05$ was considered statistically significant.

Results

We included in our study 57 BD patients (sex ratio M/F: 42/15, median age: 40 years). Fifty-five patients had oral ulcers and 35 had genital ulcers. Ocular involvement was present in 42% (24/57); 36% (20/57) had posterior uveitis, 25% (14/57) retinal vasculitis, 21% (12/57) anterior uveitis, and 18% (10/57) panuveitis. Vascular involvement was present in 35% (20/57), venous thrombosis in 75% (15/20), and arterial in 9% (5/57). Neurological involvement was diagnosed in 10.7% (6/57), and articular involvement in 5% (3/57). Eight patients were in the active phase of the disease, and 49 were inactive. According to Yosipovitch severity scale, 34% had mild form, 18% moderate and 48% had severe disease. The control group consisted of 48 healthy subjects (38 male and 10 female, median age: 37 years). All patients and controls gave their written consent for this study. Before analyzing d-dimer variations, we noticed that one patient had a high level of d-dimer. He was a 24-year old male patient in whom BD was retained for bifocal ulcers and deep venous thrombosis; BD was complicated by an acute myocardial infarction 2 years after time of diagnosis and treated with thrombolytic agents and immunosuppressive agents. He was completely asymptomatic the day he had the blood test. He was on oral anticoagulant and his INR was within the therapeutic range. D-dimer was 10µg/ml and 4 months later he was diagnosed with proximal deep venous thrombosis. We excluded this patient which result diverges from our statisti-

cal analysis. Our analysis was realized on 56 patients. Patients were divided in 2 subgroups according to the positivity of d-dimer (d-dimer≥0.5µg/ml). Table 1 summarizes the variation of d-dimer positivity according to demographic characteristics, clinical features, activity scale, and severity.

	Positive d-dimer n(%)	Negative d-dimer n (%)	p
Number of patients	17	39	**0.033**
Mean age (years)	36.5	32.3	NS
Sex ratio (M/F)	14/3	27/12	NS
Oral ulcers	17(100%)	38(97%)	NS
Genital ulcers	9(52%)	26(66%)	NS
Ocular involvement	8(47%)	15(38%)	NS
Neurological involvement	3(17%)	3(7%)	NS
Vascular involvement	6(35%)	10(25%)	NS
Articular involvement	3(17%)	0(0%)	**0.025**
Active disease	6(35%)	2(5%)	0.04
Inactive disease	11(23%)	37(71%)	NS
Severe forms	11(65%)	22(57%)	NS
Moderate forms	4(23%)	2(5%)	NS
Mild forms	2(12%)	15(38%)	NS

Table 1. Variations of d-dimer's positivity according to demographic characteristics, clinical features, activity scale, and severity in BD patients.

The levels of positive d-dimer were significantly higher in patients with BD than in healthy controls (17 (30%) vs 8 (13.8%) p=0.03). There were no significant correlations between positive d-dimer cutaneous manifestations, ocular, neurological, and vascular involvement. There was a correlation between positive d-dimer and articular involvement (p=0.025). Positive d-dimer was significantly associated to the active phase of the disease (p=0.04). There were no differences in d-dimer values according to the severity.

Financial limitations allowed us to realize t-Pa determination with only 42 patients and 44 healthy controls. The levels of t-PA with patients were lower than with healthy controls (4.85 ± 7.36ng/ml vs 5.62 ± 8.49, p=NS) and lower with males than with females (4.58±7.84 vs 5.52±6.26, p=NS). Due to the same reasons, PAI-1 was assessed with only 12 patients. They were 9 males and 3 females and were chosen among patients with positive d-dimer. Table 2 summarizes t-PA and PAI-1 variations according to clinical manifestations, activity scale, and severity.

	T-Pa variations			**PAI-1 variations**		
Clinical presentation	**n (%)**	**t-PA (ng/ml)**	**p**	**n (%)**	**PAI-1 (ng/ml)**	**P**
Oral ulcers +	41(97%)	4.48	NS	0		
Oral ulcers -	1(3%)	20		0		
Genital ulcers+	25(60%)	4.15	NS	6(50%)	17.95	NS
Genital ulcers -	17(40%)	17		6(50%)	3.45	
Ocular involvement+	19(45%)	5.55	NS	6(50%)	3.45	0.03
Ocular involvement-	23(55%)	4.26		6(50%)	17.95	
Neurological involvement+	4(10%)	7.75	NS	1(8%)	45	NS
Neurological involvement -	38(90%)	4.54		11(92%)	7.59	
Vascular involvement +	12(28%)	5.15	NS	4(33%)	16.4	NS
Vascular involvement -	30(72%)	4.58		8(67%)	7.84	
Articular involvement+	3(7%)	2.90	NS	3(25%)	3.33	NS
Articular involvement-	39(93%)	5.0		9(75%)	13.16	
Active phase	7(20%)	6.5	NS	4(33%)	17.6	NS
Inactive phase	35(80%)	4.5		8(66%)	7.2	
Severe forms	22(52%)	5.8		6(50%)	9.16	
Moderate forms	6(14%)	5.8	NS	4(30%)	16.43	NS
Mild forms	14(34%)	2.90		2(20%)	3.87	

+ : present, -: absent

Table 2. Variations of t-PA and PAI-1 according to clinical features, activity scale, and severity in BD patients.

No significant differences were noted according to t-PA levels among clinical manifestations, disease activity or severity. There was a significant correlation between ocular involvement and low level of PAI-1 (p=0.03).

Discussion

The frequency of positive d-dimer in this study was significantly higher with patients than with healthy controls; this was found by Fusegawa et al [9] and Shang et al [10] in respectively 16 and 24 BD patients compared to healthy controls. In our group of patients, we noticed the heterogeneity of d-dimer values; 30% (17/56) had positive d-dimer and 70% (39/56) negative. This variation may be explained by the patients' heterogeneity relative to disease course and treatment. In fact, we consecutively included patients newly diagnosed or followed up and treated. It may be possible that this treatment (particularly immunosuppressive agents) influences d-dimer variation. We did not find any data to consolidate our hypothesis. We did not reveal any significant difference of d-dimer levels among genders. This was also reported by Yurdakul et al [5]. We excluded from our study a patient who had a high level of d-dimer (10µg/ml). His anamnesis and physical examination at the moment of blood test were normal. There were no other causes that might increase his d-dimer levels. Four months later, he developed a deep venous thrombosis although he was still correctly treated by oral anticoagulant. Various studies reported the predictive part of d-dimer in the risk of recurrence of thrombosis of any cause, and estimated that the risk of recurrence in case of positive d-dimer varies from 4.5 to 14.4% (IC 95%), and from 2.8 to 4.8% if d-dimer were negative [11]. We did not observe any recurrent vascular event in the subgroup of positive d-dimer BD patients during the follow-up period. To our knowledge, there is no study about the importance of d-dimer in the recurrence of thrombosis in Behçet's disease, and there was no correlation between d-dimer and vascular involvement in BD [5, 10-13]. This case illustrates the inefficacity of oral anticoagulant in secondary prevention of thrombosis. These data are still a bone of contention, even if the expert group on study of Behçet's disease has already issued recommendations about the uselessness of anticoagulation in BD [14]. The study of d-dimer positivity variation according to clinical features concluded to positive correlation of articular involvement and positive d-dimer (p=0.025). To our best knowledge, there are no publications consolidating this hypothesis. However, it has been proved that there is an activation of coagulation in articular involvement in BD, which leads to fibrin formation and consequently to an activation of fibrinolytic system [15]. These data might explain our results.
In our study, there were no correlations between d-dimer values and vascular involvement. In fact, d-dimer was positive in only 35% of angiobehçet cases, which suggests its low sensitivity. Hampton et al have studied d-dimer variations among BD patients and seronegative arthritis. There was no difference between the two groups, and the comparison of d-dimer in BD patients with and without vascular involvement did not show any significant difference either [12]. Orem et al compared d-dimer in 33 BD patients and 30 healthy controls and showed no difference between the 2 groups. Moreover, there was no difference in BD patients with and without vascular involvement [13].The same conclusions were reported by Yurdakul et al in a study comparing d-dimer in BD patients to seronegative arthritis, systemic scleroderma, sepsis, thrombosis due to other causes, and healthy controls. There was no difference among the subgroups as far as BD patients with or without thrombosis [5]. Besides, there were no correlations of d-dimer with other clinical features; this was probably due to the fact that the majority of our patients had a severe form of the disease and previously received immunosuppressive agents before blood sample. There was a positive correlation between positive d-dimer and the activity of the disease (p=0.04). The same conclusion was found by Fusegawa et al [9] though not by Yurdakul et al, in a study about 65 patients [5]. These divergent results may be due to the recruitment and different techniques used for the determination of d-dimer.

T-PA levels were lower in patients with BD than in healthy controls, but the difference was not significant. Some studies analyzed t-PA levels variations in BD, and the results were contradictory; t-PA levels have been found normal [12, 16-18], high [18-20], or diminished [5,10,13,17)] Different hypotheses have been reported to explain these variations. Yurdakul et al have explained the lack of increase of t-PA in BD patients by an endothelial inflammation or an excess of production of its inhibitor, the PAI-1, resulting in impaired firbrinolytic activity [5]. Demirer et al have reported an excess of t-PA values in BD patients due to an excess of release by the damaged endothelium [19]. On the other hand, some authors have concluded that endotheliopathy leads to an elevation of fibrinolysis inhibitors, PAI-1 and thrombine activatable fibrinolysis inhibitor (TAFI), without modifying t-PA levels [20, 21]. We did not found any association between t-PA and clinical manifestations. To our knowledge, there is no published study about this correlation. In our study, there was no correlation between t-PA levels and clinical activity; this was also reported by Yurdakul et al. However, Ozoran et al concluded to a correlation between clinical activity and decreased levels of t-PA. These differences could be due to a selection bias. In fact, we did not realize a pre-selection of patients. Patients with a long course disease and on treatment have not been excluded. Besides, we have not found any correlation with the disease severity; this could be explained by the effect of treatment (steroids and immunosuppressive agents) in homogenizing the different subgroups (mild, moderate and severe).

In our study, PAI-1 level was in the normal range and there was no difference in patients with or without vascular involvement. In fact, PAI-1 has been reported to be normal [13, 16] or high [10, 12, 17, 18], and no correlations to vascular involvement was reported in 3 more different studies [5, 16, 22]. We reported negative correlation between low levels of PAI-1 and ocular involvement. To our best knowledge, no similar data were previously published. The relation between low levels of PAI-1 and ocular involvement is not clear.

Conclusion

The rarity of publications on fibrinolytic abnormalities in BD does not help us to an easy interpretation of these results and their implication in the pathogenesis of the disease as a causative agent or as a consequence, even if this seems to be most likely. Nevertheless, this is the first African and Arabian study concerned with fibrinolytic abnormalities in BD. Further studies with larger numbers as well as homogenized groups and laboratory techniques are needed in order to conclude.

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