

Para-Clinical and Immunological Evaluation in Buerger's Disease as a Suspected Autoimmune Disease: Case Series

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Abstract

Background: Autoimmunity causes the loss of normal immune homeostasis and involves the presence of autoantibodies and inflammation. Thromboangiitis obliterans or Buerger's disease (BD) refers to a type of vascular obstructive syndrome, with tobacco exposure accounting for disease formation and progression. However, the current understanding of autoimmunity is unclear in the context of BD, and the scientific findings are not enough to support autoimmune mechanisms. This study was aimed at investigating autoimmunity factors in patients with BD.

Methods: Clinical and experimental examinations were performed on 80 patients with BD. The diagnostic work-up for autoimmunity was composed of IgM rheumatoid factor (RF), anti-nuclear antibodies (ANA), The erythrocyte sedimentation rate (ESR), anti-cyclic citrullinated peptide (CCP) antibodies, Antiphospholipid antibodies (APA), Anti-cardiolipin antibodies (ACLA), anti-double-stranded DNA (ds-DNA), and extractable nuclear antigen (ENA) profile. Immunomarkers were detected using the quantitative enzyme-linked immunosorbent assay (ELISA).

Results: Raynaud's phenomenon (84.93%), cold sensitivity (76.25%), and claudication (73.75%) were the most common symptoms in the BD patients. Also, 64.29% represented with high ANA levels and positive RF, while 42.11% were found with increased ANA and ESR levels. The ANA/RF positive BD patients had ESR > 15 mm/hr and a high prevalence of cold sensitivity, claudication, and Raynaud's phenomenon ($p > 0.05$).

Conclusions: There is a possibility of a non-specific autoimmune disposition among BD patients. RF and ANA could be considered for predicting disease progression.

Keywords: Antibodies, Autoimmunity, Buerger's Disease, Immune System.

Introduction

Thromboangiitis obliterans, also known as Buerger's disease, is a type of vascular obstructive syndrome that is usually associated with the use of tobacco derivatives and cigarettes (1, 2). The reason of this obstruction is frequent and progressive inflammation, and a blood clotting in the vessels (1, 3, 4). The clinical signs of BD are intermittent pain in the fingers, and pain at the tip of the fingers and toes. This disease seems to have

no definitive treatment, and the only way to prevent its rapid progression is a cessation of cigarette and tobacco products (3, 5, 6).

Buerger's disease is included in the list of differential diagnoses of all younger patients, who are referred with lower extremity ischemia. This diagnosis is strengthened in case of some clinical manifestations, such as rest pain, wounds, and gangrene (1-3). The presence of certain factors in

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the patient's records also makes this diagnosis more probable. Compared to patients with atherosclerosis, claudication is one of the rare manifestations of BD. Ischemic pain is also observed during rest or ulceration (3, 7). Multi organs are typically involved in BD, with Raynaud's phenomenon and thrombophlebitis occurring in 40% of these patients (7, 8). During the checkup, the positive result in Allen's test is useful for distinguishing the disease from atherosclerosis (9). Lupus erythematosus, rheumatoid vasculitis, antiphospholipid antibody syndrome, and mixed connective tissue disease are among the relevant differential diagnoses of BD (10, 11).

Despite the decreasing trend in BD in North America and Western Europe, its prevalence is on the rise in Central Asia, the Far East, and the Mediterranean (3, 5). So far, the leading causes of the BD have not been identified; however, smoking plays an active role in its development (4, 5). Interestingly, there are some pieces of evidence in the related literature about patients who had no history of smoking (12, 13). With this in mind, other factors may exist that affect the etiology of BD. Some studies have discovered viral and bacterial impacts, narcotics, and environmental and genetic conditions (12, 13).

Additionally, there are studies reporting a group of genes, which have contributions to autoimmune diseases and are widely known as the HLA complex (14-16). One of the arguable aspects of the BD etiology is autoimmunity. The probable association between the immune system and BD has been investigated to some extent in previous studies (1, 12, 17, 18).

The objective of the present study was to assess the presence of possible autoimmune phenomena in BD patients and the potential role of anti-nuclear antibodies (ANA)/IgM rheumatoid factor (RF) in developing the most common clinical symptoms.

Materials and Methods

Ethical statement

This study was approved by the Medical Research Committee of Mashhad University of Medical Sciences (MUMS), Mashhad, Iran.

Patient selection

This was a cross-sectional study, where 91 participants were initially recruited according to a call from the Vascular and Endovascular Research Center in 2012. They were then examined based on Shionoya's clinical criteria (5, 19). Also, those patients who were reluctant to participate or share additional information about their autoimmune diseases, malignancy, viral diseases, and allergies were removed from the study. In total, 80 male patients were confirmed with a score of 4 and 5 for further assessments.

Study protocol

After sampling, informed consents were obtained from the patients. Additionally, the participant's demographic data and clinical symptoms were recorded in detail by specialists. For laboratory tests, 10 mL blood samples were collected to detect antibodies to IgM rheumatoid factor (RF), anti-nuclear antibodies (ANAs), anti-cyclic citrullinated peptide (CCP) antibodies, Antiphospholipid antibodies (APAs), Anti-cardiolipin antibodies (ACLAs), anti-double-stranded DNA (ds-DNA), and extractable nuclear antigen (ENA) profile.

The samples were screened for IgM rheumatoid factor (RF) (Euroimmun, Germany), anti-nuclear antibody (ANA) (Genesis, USA), anti-cyclic citrullinated peptide (anti-CCP) antibodies (Euroimmun, Germany), anti-phospholipid antibodies (APA) (Euroimmun, Germany), anti-cardiolipin antibodies (ACLA-G for IgG and ACLA-M for IgM) (Aesku.Diagnostics, Germany), anti-double strand DNA (dsDNA) antibodies (Euroimmun, Germany), and anti-extractable nuclear antigens (ENA) (Phadia AB, Sweden) using the enzyme-linked immunosorbent assay (ELISA). According to the manufacturers' instructions, RF < 15 IU/mL, ANA < 1 IU, CCP < 20 U/mL, APA < 20 SGU or 20 SMU, ACLA < 40 GPL or 40 MPL, ds-DNA < 30 IU/mL, and ENA profile < 1 U with corresponding ranges less than 15 IU/mL, 1 IU, 20 U/mL, 20 SGU or 20 SMU, 40 GPL or 40 MPL, 30 IU/mL, and 1 U were considered normal. The most common ENA specifications used for measurements are anti-SS-A, anti-SS-

B, anti-Sm, anti-nRNP, anti-Jo-1, and anti-Sc170 (20).

Statistical Analysis

Data were collected and fed into SPSS13 software. Descriptive statistics were presented in the form of mean, standard deviation (SD), frequency, and percentage. The data distribution was evaluated by the Kolmogorov-Smirnov test. Mann-Whitney U test was carried out to compare ESR between patients with positive or negative ANA/RF. Pearson chi-square test was used for comparing three major clinical findings between the BD groups with positive or negative ANA/RF. In case that above 20% of cells had frequencies < 5, Fisher's exact test was run for the comparison of the symptoms.

Results

Eighty male patients with BD with an average age of 42.85 ± 8.35 years participated in this study. All of these samples were tobacco users, of whom 83.75% smoked cigarettes, 5% used hookahs, and 6.25% reported both cigarettes and hookahs. It

was also found that 78 (97.5%) patients consumed opium at the same time.

Clinical findings

Table 1 shows the distribution of BD severity and symptoms. Based on the disease progress, the symptoms of the patients were classified as mild (migratory thrombophlebitis, cold sensitivity Raynaud's phenomenon, or skin discoloration), moderate (chronic ulcers, claudication, or burning pain of the feet at night), and severe (pain at rest or gangrene) (21). The moderate symptoms were the most common (n= 46, 57.5%) in this study. The prevalence of the severe symptoms was around twice as many as that of the mild symptoms. Among all the patients, the majority represented with Raynaud's phenomenon (84.93%), which was followed by cold sensitivity (76.25%) and claudication (73.75%). Almost half of the patients had burning pain of the feet at night (52.05%) and skin discoloration (46.25%). Migratory thrombophlebitis was observed in only 5 (6.25%) men.

Table 1. The clinical properties of Buerger's disease patients.

Severity of the disease	Symptoms	Frequency (%)
Mild (n= 11)	Migratory thrombophlebitis	5 (6.25)
	Cold sensitivity	61 (76.25)
	Raynaud's phenomenon	62 (84.93)
	Skin discoloration	37 (46.25)
Moderate (n= 46)	Chronic ulcers	29 (36.25)
	Claudication	59 (73.75)
	Burning pain of the feet at night	38 (52.05)
Severe (n= 23)	Pain at rest	21 (26.25)
	Spontaneous gangrene	14 (17.50)

Serum level of immunologic markers

The results of ESR and the ELISA test on serum level of antibodies are summarized in Table 2. As can be seen, there were three immunomarkers with considerably high means; the elevated level of ANA (mean: 7.32 ± 1.17 IU) was found in all patients. Serum IgM rheumatoid factor with a mean level of 34.42 ± 4.18 IU/mL was positive in around 65% of the

BD cases. The level of ESR was estimated 21.16 ± 3.08 mm/hr, being within the normal range in above half of the patients. At least 90% of the subjects were detected with normal anti-CCP, ACLA, APA, and anti-dsDNA levels. The level of antibodies produced against Sm, nRNP, SS-A, SS-B, Scl-70, and Jo-1 antigens in the ENA profile of the patients was normal.

Table 2. ESR and antibodies in the study patients.

Immunomarker		Mean (SD)	Normal level, n (%)	Abnormal level, n (%)
ESR (< 15 mm/hr)		21.16 (3.08)	44 (57.89)	32 (42.11)
Anti-CCP (< 20 U/mL)		3.15 (0.99)	74 (98.67)	1 (1.33)
ACLA	ACLA-G (< 40 GPL)	11.09 (1.08)	78 (97.50)	2 (2.50)
	ACLA-M (< 40 MPL)	2.11 (0.37)	80 (100)	0 (0)
APA	APA-G (< 20 SGU)	4.42 (1.32)	77 (96.25)	3 (3.75)
	APA-M (< 20 SGU)	2.04 (0.22)	80 (100)	0 (0)
ANA (< 1 IU)		7.32 (1.17)	0 (0)	80 (100)
Anti-ds-DNA (< 30 IU/mL)		10.29 (1.78)	68 (90.67)	7 (9.33)
RF-IgM (< 15 IU/mL)		34.42 (4.18)	20 (35.71)	36 (64.29)
ENA profile	Sm (< 1 U)	0.18 (0.01)	80 (100)	0 (0)
	nRNP (< 1 U)	0.20 (0.02)	80 (100)	0 (0)
	SS-A (< 1 U)	0.23 (0.01)	80 (100)	0 (0)
	SS-B (< 1 U)	0.17 (0.02)	80 (100)	0 (0)
	Scl-70 (< 1 U)	0.17 (0.02)	80 (100)	0 (0)
	Jo-1 (< 1 U)	0.21 (0.02)	80 (100)	0 (0)

The ANA/RF positive BD patients had an ESR level of 18.44 ± 3.53 mm/hr, which was comparable ($U= 342.00$, $p= 0.758$) to that in ANA/RF negative patients (19.90 ± 7.01 mm/hr). Also, cold sensitivity ($n= 28$, 50% vs. $n= 15$, 26.79%; Pearson chi-square test $p= 0.056$), claudication ($n= 24$, 42.86% vs. $n= 17$, 30.36%; Fisher's exact test $p= 0.120$), and Raynaud's phenomenon ($n= 29$, 51.79% vs. $n= 15$, 26.79%; Pearson chi-square test $p= 0.739$) were more common in ANA/RF positive BD patients than their counterparts with negative ANA/RF although the difference was not statistically significant.

Discussion

The main findings of this study were that about 65% of the patients with BD developed elevated ANA titers and positive RF-IgM, while above 40% had elevated ANA titers and ESR higher than 15 mm/hr.

Anti-nuclear antibodies (ANA) are composed of different antibodies acting against a high

number of macromolecules that constitute the normal cell nucleus. The ANA positivity has been considered a hallmark of autoimmune disease however these cannot be diagnostic. Because it is sometimes shown that a healthy population could develop ANA. As long as ANA are present, the specific antibody, which makes the ANA test positive, should be determined (22).

Anti-CCP antibodies are found to be reactive with synthetic peptides consisting of the amino acid citrulline (23). According to Moore and Dalrymple, many viral infections exist with RF positivity, such as hepatitis B and C. However, the affected patients do not contain antibodies to α CCP (24). In the present study, more than 98% of the patients were negative for Anti-CCP.

To define other specific antibodies, an ELISA profile is required. All the patients were evaluated to realize if there was any underlying APA or ACLA. The results did not show the presence of APA-M or ACLA-M in the BD cases, whereas APA-G and ACLA-G,

respectively, occurred in only 3.75% and 2.50%. As for antibodies to dsDNA and RF-IgM, seven patients indicated positive results for dsDNA, and 65% were found with RF-IgM positivity. On the other hand, no ENA profile in our study did contain antibodies to Sm, nRNP, SS-A, SS-B, Scl70, and Jo-1.

The erythrocyte sedimentation rate (ESR) is considered a non-specific marker of inflammation and rises in inflammatory conditions (25). In this research study, all patients revealed notable ESR levels. Further analyses showed that increased ESR among the ANA/RF positive BD patients was more likely to represent a chronic inflammatory state, leading to specific symptoms, such as cold

sensitivity, claudication, and Raynaud's phenomenon. This observation can imply that ANA/RF positive BD patients had mild-to-moderate severity.

The main strength of the present study was the selection of all the patients from a single center. Therefore, our sample represented an almost homogeneous BD population. As for the weakness, since the Vascular and Endovascular Research Center is a tertiary referral center, a bias could exist for more severe patients.

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