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Comparison of Anti-Saccharomyces Cerevisiae Antibodies (ASCA) in Behcet's Disease Patients with Three Groups of Oral Aphthosis, other Rheumatologic Diseases and Healthy Volunteers

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ABSTRACT

Introduction: Behcet's Disease (BD) is a general and progressive vasculitis and involves various organs. Its main etiology is not yet understood; however, immunologic and infectious causes and genetic predisposition have been proposed. Saccharomyces Cerevisiae is a type of yeast which is used in the bread and wine industries. Antibodies against this yeast have a well-proven role in inflammatory bowel diseases. The aim of the present study was to assess the frequency of Anti-Saccharomyces Cerevisiae Antibody (ASCA) and its relation to clinical symptoms and disease activity index in patients afflicted by BD.

Materials and Methods: Serum ASCA levels, determined by ELISA, were Studied in Behcet's disease along with oral aphthosis, other rheumatologic diseases and healthy volunteers (n=30 in each group). In the BD group the disease activity index and different clinical symptoms were recorded during the study course.

Results: Serum level of ASCA in the four studied groups of BD, oral aphthosis, other rheumatologic disease and healthy volunteers was 9.18±9.69, 10.90±10.40, 11.29±17.96 and 8.86±5.31IU/ml, respectively; indicating no meaningful difference (p=0.811). The ASCA titer was not related to Behcet's disease severity (p=0.399). Serum level of ASCA in BD patients with oral aphthosis or with gastrointestinal symptoms was significantly higher than the other Behcet's Disease patients (p=0.012, p=0.014).

Conclusion: ASCS is not a valuable test for distinguishing BD from recurrent oral aphthosis or other connective tissue disorders. It also cannot be used for determining disease severity. However, it has a higher level in BD patients with oral aphthous ulcers and gastrointestinal symptoms.

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Introduction

Behcet's Disease (BD) is a chronic systemic inflammatory relapsing vasculitis that affects nearly all organs and systems and is characterized by four major symptoms consisting of oral aphthous ulcers, ocular lesions, skin lesions, and genital ulcerations (1-2).

It occurs in the third decade of life and is rarely seen in individuals over 50 years of age or before adolescence. It affects both genders equally while men and youth experience a more severe disease course (3).

The highest prevalence has been recorded along the ancient Silk Road including the Middle East and Far East (4). The etiopathogenesis of BD remains unknown (1, 5); However, epidemiologic findings suggest that it is due to an autoimmune process which is triggered by an environmental agent in a genetically predisposed individual (5). Moreover, association with HLA-B51 is known as the strongest genetic susceptibility factor for BD (6). In a meta-analysis conducted by de Menthon, HLA-B51 was associated with an increased risk of Behcet's disease (odds ratio: 5.9) (7). Clinical diagnosis of Behcet's Disease (BD) is not simple due to it multisystemic nature and the lack of any pathognomonic symptoms or laboratory findings.

Therefore, its diagnosis is mainly based on a cluster of clinical manifestations (oral, genital, skin or ocular lesions) (2, 8).

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Considering the severe morbidity and considerable mortality related to BD, early diagnosis and treatment is important. The introduction of biologic agents in recent years have well profited and even made new promises in the prognosis and treatment of this disorder (9).

Saccharomyces cerevisiae is a type of yeast which is commonly used in the bread and wine industries. It is believed that this yeast was initially obtained from the skin of grapes and plums (10).

The antibody produced against a part of the yeast membrane is ELISA termed as Anti-Saccharomyces Cerevisiae Antibody (ASCA) (11). This antibody is found in various diseases such as Behcet's disease (12, 13); ASCA is more frequently found in Crohn's Disease (CD) patients (50%-80%) compared to patients with ulcerative colitis (2%-14%) (14), besides having been reported in 8% of healthy individuals (15). ASCA has two types of IgA and IgG antibodies present in the blood.

In the present study we aimed at investigating the serum titer of ASCA in Behcet's disease patients and its comparison with three other groups of oral aphthosis, other rheumatologic diseases and healthy volunteers. Also to investigate its relation to clinical symptoms and disease activity index in patients afflicted by BD.

Materials and Methods

In this cross-sectional study 120 patients being referred to the Rheumatology clinic of Emam Reza Hospital, Mashhad, Iran from Feb. 2013 to Nov. 2014 were enrolled. They included 30 patients recently diagnosed with Behcet's disease having not yet received any type of drugs, 30 patients with recurrent oral aphthosis, and another 30 with other types of rheumatologic diseases along with 30 healthy individuals.

The patients who fulfilled the criteria of Behcet's Disease International Study Group (16) confirmed by a single rheumatologist were selected as the Behcet's Disease group. Individuals with no other underlying disease, no positive criteria for Behcet's Disease but with at least 3 recurrences of oral aphthosis per year were selected as the recurrent oral aphthosis group. The 3rd group consisted of patients with other rheumatologic diseases (lupus erythematosus, rheumatoid arthritis, systemic scleroderma, etc) based on their medical records and also confirmed by the same rheumatologist.

Behcet's disease cases with a history of recent corticosteroid or anti-inflammatory drugs consumption were excluded from the study. The healthy controls were selected from the colleagues and hospital staff with no history of underlying malignancy, autoimmune disease or allergy.

The study protocol was fully approved by the Ethics committee of Mashhad University of Medical Sciences and an informed consent was obtained from each participant prior to study entrance.

For all patients with Behcet's disease following a through physical examination, the Behcet's Disease Current Activity/2006 form was filled for determining the disease activity index. Recent clinical symptoms were scored from 0 to 12 by the same rheumatologist: 0-3 referred to as mild disease, 4-8 as moderate and 9-12 as severe disease.

A 5cc venous blood sample was collected from each participant and then diluted by 1:100. The samples were then injected into specific antigen-covered microplates. After maintaining these plates at 20-32ºc for 90 min, serum antibodies were attached to the plates' antigens and the formed complexes were studied by ELISA (AESKULISA kit, GmBH, Germany).

For data analysis IBM SPSS Statistics for Windows version16 was used. According to the AESKULISA kit used, a serum ASCA level ≤16IU/ml was considered as negative, 16-20IU/ml as intermediate range and above 24IU/ml as a positive result. One-way ANOVA was used for intergroup comparisons whereas Independent samples T-test was applied to evaluate the association between ASCA titers and other quantitative variables.

The statistical significance level was set at $p<0.05$.

Results

The mean age of the studied cases was 34.8±7.73 year ranging from 19 to 56 years. Fifty percent were female and 45% male. The four groups showed no meaningful difference regarding sex (p=0.53).

The mean ASCA level was 10.06±11.65 IU/ml while the highest and lowest ASCA serum levels were 100 and 1.10 IU/ml, respectively. In general the ASCA titer was normal in 100 (83%), intermediate in 14 (12%) and high in 6 (5%) participants. The ASCA titer in the four groups of BD, oral aphthosis, other rheumatologic diseases and healthy volunteers was 9.18±9.69, 10.90±10.40, 11.29±17.96 and 8.86±5.31 IU/ml, respectively. Accordingly the ASCA level was highest in the other rheumatologic diseases group, yet showing no significant difference. In other words the ASCA titer was similar in the four studied groups (P=0.811).

Furthermore, the mean ASCA serum level showed no significant difference among males and females (10.12±10.12 vs 10.01±12.85 IU/ml; p=0.959).

In our study 90% of the patients with Behcet's disease had oral aphthosis whereas 13.3% had genital Aphthous ulcer. Ocular, skin, vascular, cerebral and Gastrointestinal (GI) involvement were diagnosed in 73.3%, 26.7%, 46.7%, 93.3% and 70%, respectively.

Moreover, in 96.7% of the studied patients other organs were also involved. The serum ASCA level in subgroups with various organ involvements has been displayed in Table 1.

Table1: The serum ASCA level in subgroups with various sorgan involvement

Involved organs	Serum ASCA* level (IU/ml)			Mean±SD	P
	Normal	Intermediate	High		
Oral aphthosis (n=27)	23	2	2	20.17±19.13	0.012
Genital aphthosis (n=4)	4	0	0	3.17±0.93	0.186
Ocular involvement (n=22)	19	2	1	7.87±6.99	0.226
Skin involvement (n=8)	6	1	1	10.32±9.19	0.705
Vascular Involvement (n=14)	13	0	1	10.59±11.45	0.466
Cerebral involvement (n=28)	24	2	2	9.45±9.96	0.587
GI involvement (n=21)**	18	1	2	14.73±13.40	0.014

*ASCA: Anti-Saccharomyces cerevisiae antibody
**GI: Gastrointestinal

As demonstrated and based on Independent samples T-test oral aphthosis and GI involvement were accompanied with a significantly higher ASCA titer (p=0.012 and 0.014, respectively).

Finally, Pearson's correlation coefficient was performed to determine the association between ASCA titer and the BD Activity Index; it showed no meaningful correlation between the two variables (p=0.399).

Discussion

Behcet's disease is a chronic, inflammatory and recurrent disease which involves different organs in the body. It has no definite lab indicator for diagnosis, activity or prognosis; therefore international criteria based on clinical signs and symptoms are used for confirming its diagnosis and determining disease severity (17, 18). Thus, the presence of a relatively specific laboratory marker can substantially facilitate the diagnosis of BD, and possibly support a diagnosis before all disease manifestations have occurred (2).

The present study aimed at assessing the prevalence of ASCA in Behcet's disease patients recently diagnosed with this condition and its association with BD activity index. In addition, the probable correlation between ASCA serum level with each group of clinical symptoms in the Behcet's disease group was also assessed.

We found no meaningful relationship between the serum ASCA level in different study groups (p=0.811).

Moreover, the Disease Activity Index also revealed a non-significant association with ASCA level in the BD group (p=0.399).

Previous studies have generally shown no meaningful relationship between ASCA and BD in comparison to healthy controls. Vaiopoulos studied 58 patients with Behcet's disease ranging from 17 to 70 years with 55 healthy individuals matched for age and sex. No statistically significant difference was observed in the ASCA level between the two groups (19). In another study by Filik in Turkey, none of the 18 studied patients with BD had a positive ASCA test result (8).

One of the main findings of our study was the significant correlation between serum ASCA level with oral aphthosis and GI symptoms in patients with BD.

Moreover, the mean serum ASCA level of patients with BD who had oral aphthosis at study entrance was significantly higher than patients without this symptom (p=0.012).

A similar study was conducted in Korea from 1996 to 2003 on 65 patients with recurrent oral aphthosis and 44 BD cases. Only one case of positive ASCA was diagnosed in each group. The BD group had a higher mean serum ASCA level, yet not statistically significant (2).

In the present study the mean serum ASCA level in BD patient with GI symptoms such as nausea, vomiting, abdominal pain, diarrhea, etc was significantly higher than the patients with no such symptoms (p=0.014).

In similar studies, BD patients with GI symptoms had a higher serum ASCA level in comparison to the other Behcet's patients. Among such studies Fresko reported that the serum ASCA level was significantly higher in Crohn's disease patients in comparison to those with BD, ulcerative colitis, and ankylosing spondylitis (p=0.0001) (13). Among other groups the ASCA level showed no meaningful difference. On the other hand, among patients with BD the subgroup with GI symptoms had a significantly higher ASCA level in comparison to Behcet's patients as the mean ASCA level in the 8 patients with GI symptoms of the BD group was higher than the 77 patients with no GI symptoms (p=0.02) (13).

Their results were in accordance to our findings indicating a significantly higher mean serum ASCA level in the 21 BD patients with GI symptoms out of the total 30 BD cases (14.73 vs 5.97, p=0.014)

In the study by Krause the mean serum ASCA level in Behcet's patients was higher than the Systemic Lupus Erythematosus (SLE), recurrent oral aphthosis and the healthy control groups (p=0.01). Accordingly, the mean ASCA level in the Behcet's disease group was 20.7 IU/ml while in the other mentioned groups it was 11.8, 10.0 and 10.8 IU/ml, respectively (p<0.02, p<0.001 and p<0.03) (20). This outcome was in contrast to our study results. Moreover, no association was found between the serum ASCA level and the clinical symptoms of Behcet's disease and the presence or absence of HLA-B51 which is in accordance to our study (20).

The findings of the present study revealed that the serum level of ASCA and basically a positive or negative test result are not related to Behcet's disease

severity score. However in BD patients with oral aphthosis or GI symptoms at disease diagnosis, the ASCA titer is higher in comparison to patients without such symptoms. Altogether, this antibody cannot be used for the differential diagnosis of Behcet's disease with isolated oral aphthosis and other connective tissue disorders. Eventually, regarding the high number of patients afflicted with Behcet's disease in our country due to the passage of the ancient Silk Road, the main limitation of the current study was the small study population due to the high price of the ASCA test. The other limitation was not segregating rheumatologic diseases and not studying the subgroups independently.

Conclusion

ASCS is not a valuable test for distinguishing Behcet's disease from recurrent oral aphthosis or other connective tissue disorders. However, patients with BD who have oral aphthosis or gastrointestinal involvement may have higher levels of ASCA. This needs to be further studied in future researches on a larger sample size and with different rheumatologic disease subgroups. Finally, ASCA does not seem to cause an increased risk for a more severe disease course.

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