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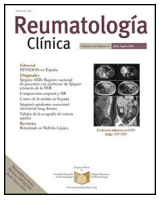

Original Article

Are the specific and nonspecific ANA staining patterns of Behçet's Disease patients important?

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ABSTRACT

Background: The autoinflammatory character of Behçet's Disease has led researchers to investigate the role of autoantibodies. However, no significant positive result has been reported for autoantibody tests for the disease.
Aims: To investigate the specific and nonspecific staining patterns of Behçet's Disease (BD) patients.
Methods: 140 patients (87 females, 53 males) with an average of 41.9 ± 3 years who were being followed up for Behçet's Disease, and a control group consisting of a total of 736 (464 females, 272 males) healthy volunteers made up of blood donors without any disease whose average age was 50.2 ± 4 years were included in the study. Peripheral venous blood was collected from the patients and the sera were separated. Patient sera were studied by indirect immunofluorescence antibody test (IFA) at a dilution of 1/40 and 1/100.
Results: A total of 140 (87 females, 53 males) Behçet's Disease patients and 736 (464 females, 272 males) healthy controls were examined. The rate of ANA positivity was 11.6% in the control group and 10.7% in the Behçet's Disease group. In general, no difference was detected between the patients and the healthy controls in terms of autoantibody positivity ($p > 0.05$). However, when examined in terms of patterns, the low detection of DFS70 and the observation of centriole staining type patterns in Behçet's Disease patients was noteworthy ($p < 0.05$).
Conclusion: Autoantibody tests, which hold an important place in classic autoimmune diseases, are not necessary for Behçet's patients, but they should be examined in terms of nonspecific patterns.

¿Son importantes los patrones de tinción ANA específicos y no específicos para los pacientes con enfermedad de Behçet?

Palabras clave:
Patrones de tinción del método ANA
Autoanticuerpos
Enfermedad de Behçet

RESUMEN

Antecedentes: El carácter autoinflamatorio de la enfermedad de Behçet (EB) ha llevado a los investigadores a estudiar el rol de los autoanticuerpos. Sin embargo, no se ha reportado un resultado positivo significativo para las pruebas de autoanticuerpos.
Objetivo: Investigar los patrones de tinción específicos y no específicos de los pacientes con EB.
Métodos: Se incluyó en el estudio a 140 pacientes (87 mujeres y 53 varones) con una edad media de 41,9 ± 3 años con seguimiento por EB, y un grupo control que incluyó a un total de 736 voluntarios sanos (464 mujeres y 272 varones) integrados por donantes de sangre sin enfermedad alguna, con una edad media de 50,2 ± 4 años. Se extrajo sangre de vena periférica a todos los pacientes, separándose el suero, que se estudió mediante el test de anticuerpos por inmunofluorescencia directa (IFA) a un factor de dilución de 1/40 y 1/100.

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Resultados: Se examinó a un total de 140 pacientes (87 mujeres y 53 varones) con EB y 736 controles sanos (464 mujeres y 272 varones). La tasa de positividad de ANA fue del 11,6% en el grupo control y del 10,7% en el grupo de EB. En general no se detectó diferencia entre los pacientes y los controles sanos en términos de positividad de autoanticuerpos ($p > 0,05$). Sin embargo, al realizarse el examen en términos de patrones, fue destacable la baja detección de DFS70 y la observación de los patrones tipo tinción de centriolos en los pacientes con EB ($p < 0,05$).
Conclusión: Los test de autoanticuerpos, que ocupan una posición importante en las enfermedades autoinmunes clásicas, no son necesarios para los pacientes con EB, aunque deberían examinarse en términos de patrones no específicos.

Introduction

Behçet's disease is a chronic autoinflammatory disease characterized by recurrent attacks. Although the precise role is unknown, certain changes that occur in immune system homeostasis are believed to play a role, and there may be an increased tendency for autoimmunity although this is arguable. Various studies carried out until now have suggested that antinuclear antibody screening tests, which provide guidance for the diagnosis of autoimmune diseases, do not point to a significant positivity in these patients. However, certain specific and nonspecific staining patterns or cell organelle staining associated with certain diseases have been identified in ANA screenings in recent years. The aim of this study was to reveal these patterns in more detail and to investigate whether they could be related, even to a minor extent, to the yet unexplained etiopathogenesis of the disease. With this in mind, we aimed to investigate autoantibodies in active and inactive period Behçet's patients in this study.

Materials and method

140 patients (87 females, 53 males) with an average of 41.9 ± 3 years who were being followed up for Behçet's Disease, and a control group consisting of a total of 736 (464 females, 272 males) healthy volunteers made up of blood donors without any disease whose average age was 50.2 ± 4 years were included in the study. In compliance with international criteria, patients with signs and symptoms of oral or genital aphthae, pathergy positivity, etc. were regarded as active.[1,2] According to this, 33 patients who were being followed up for oral aphthae but had not been diagnosed with Behçet's Disease. After the study, it was confirmed that these patients were diagnosed with Behçet. The remaining 107 patients had Behçet's Disease. Peripheral venous blood was collected from the patients and the sera were separated. The sera were left to wait at $-20\,^{\circ}C$ for indirect immunofluorescence antibody testing (IFA). 1/40 and 1/100 dilution were done for the sera to be studied. Dilutions of 1/100 and above were regarded as positive. HEp-2 IIF tests from the company EUROIMMUN (IIFT Mosaic: HEp-20-10/Liver Monkey Germany Lot: F111125DG) were prepared in accordance with the recommended procedure.[3] All the slides were examined by same researcher under a fluorescent microscope (Zeiss-Eurostar, Germany) at 20x and 40x magnifications. Positivities were classified according to fluorescence intensity and nuclear and cytoplasmic stainings were examined. Where necessary, dilutions were made, and the test was repeated and positivities were graded. Because the number of subjects in certain cells was less than five in the data frame, the Fisher-Freeman-Halton Exact Test, which is Fisher's Exact Test generalized for RXC tables, was used. Comparison of the data was done using the SPSS 11.0 statistics software on a computer. $p < 0.05$ was used as the significance interval.

Results

An ANA positivity rate of 8.3% was found in the total of 736 healthy controls we studied. ANA patterns observed in healthy controls; 7 cases (1%) Homogeneous pattern, 17 cases (2.3%) Spotted pattern, 15 cases (2%) Nucleolar pattern, 5 cases (0.6%) DFS-70 pattern, 1 case (0.1%) Sentriol pattern, 1 case (0.1%) Sentromer pattern, 5 cases (0.7%) Midbody pattern. This rate was 10.7% in 107 Behçet's patients. ANA patterns in patients with Behçet's diagnosis; 1 case (0.7%) Spotted pattern, 1 case (0.7%) Nucleolar pattern, 3 cases (2%) DFS-70 pattern, 2 cases (1.9%) Sentriol pattern, 3 cases (2%, 9) Midbody pattern. It was rate 9.09% in 33 patients who were followed up with oral aphthae and later diagnosed as Behçet. ANA patterns in patients with oral aphthae; 1 case (3%) DFS-70 pattern, 1 case (3.03%) Midbody pattern. There was no significant difference between the groups in terms of autoantibody positivity ($p > 0.05$). When observed in terms of staining patterns, there was no difference between the controls and Behçet's patients ($p > 0.05$) (Table 1).

Discussion

Behçet's Disease is defined as a typical systemic vasculitic disease accompanied by recurrent oral and genital ulcers and uveitis. However, the etiopathogenesis of the disease is uncertain. Genetic predisposition and environmental factors are usually blamed. An autoimmunity process by abnormal T and B cell reactions and autoantigens is being focused on. While inflammation in which Th1 type cytokine response and neutrophils play a role is detected in Behçet's Disease, the hallmarks are no indication of the classic autoimmune disease, such as predominance of the female sex and autoantibody positivity. B cell numbers are normal but overall B cell activation is increased.[4] However, no autoantibody tests or specific autoantigen have been identified in patients. The anti-endothelial cell antibody has been identified and alpha-enolase has been reported to be a possible antigen. In addition, kinetin, which is an endoplasmic reticulum-dependent integral membrane protein, has been reported as a possible autoantigen in Behçet's Disease.[5]

Antinuclear antibodies (ANA) can be detected in several autoimmune diseases, such as SLE (Systemic Lupus Erythematosus), Scleroderma, and Sjogren's Syndrome in particular, and lead to a diagnosis. ANA tests can assist diagnosis by detecting antibodies that target various proteins found in the cell structure and proteins, receptors, and enzymes associated with mitosis as well as the cell nucleus. While the increased tendency for autoimmunity in Behçet's Disease has led researchers to the detection of autoantibodies, the presence of a specific autoantibody associated with Behçet's Disease has not been reported until now. In one study, it is stated that ANA prevalence was found high among Behçet's patients, that a significant increase was observed compared to the control group, and that this can be linked to polyclonal B cell activation.[6] In the study by Chun et al., it is reported that

Table 1
Dyeing patterns detected in patients and controls at 1/100 dilution.

	Homogeneus	Speckled	Nucleolar	DFS-70	Centrioles	Centromere	Midbody
Control	7 (%1)	17 (2.3%)	15 (2%)	5 (0.6%)	1 (0.1%)	1 (0.1%)	5(0.7%)
Behçet's	– (0%)	1 (0.7%)	1 (0.7%)	3 (2%)	2 (1.9%)	–	3 (2.9%)
Oral aphthae	– (0%)	–	–	1 (3%)	–	–	1 (3.03%)

the rate of patients in whom ANA positivity was detected in a series of 554 cases was 8.3%, but it is emphasized that the results are not related to the severity of the disease.[7] In our series, the rate of ANA-positive patients was 10.7%. When the staining patterns of these patients are examined, nonspecific patterns other than the homogenous, speckle-type patterns observed frequently in autoimmune rheumatic diseases, while few in numbers, stand out. Detected patterns include midbody, centriole, nucleolar, and speckled staining and anti-DFS70 (Anti-dense fine speckles 70) staining.

In midbody staining, intercellular bridge-like staining occurs in cells in anaphase or telophase. In cells in metaphase, granular staining in the chromosomal region can be noticed. Until now, it has not been associated with a specific autoimmune disease. Autoantibody positivity is commonly found in low titers in healthy individuals and in diseases other than rheumatic diseases. However, such autoantibody positivity can sometimes also be a prodrome of a rheumatic disease that may be detected in the future. Centrioles are at the center of the net-like structure (spindle) which is formed by microtubule and observed in mitosis. Anti-centriole antibodies have been rarely detected in scleroderma spectrum diseases, systemic sclerosis and Raynaud's phenomenon. Hayakawa et al. have reported finding them in a patient with systemic sclerosis and pulmonary hypertension. In conclusion, they report that mitosis-related antigens like centrioles can be associated with vascular damage.[8] It is noteworthy that this pattern, which was not detected in the healthy controls, was detected in Behçet's patients in our series, and that the disease, in essence, is a form of vasculitis. Nucleolar staining and speckled staining, on the other hand, are usually detected in mixed connective tissue diseases such as scleroderma, polymyositis, rheumatoid arthritis, and systemic lupus, and have no known association with Behçet's Disease.

Anti DFS70 was first identified by Ochs et al. in 1994 in the form of a thin speckled staining in HEp-2 cells and a positive reaction in the chromosomal region in mitotic cells and was given this name because it formed a band of about 70 kDa when immunoblotting was done.[9] The target antigen of anti-DFS70 is identified as Lens Epithelium-Derived Growth Factor (LEDGF/p75) and as transcription coactivator p75. The antigen targeted by this antibody has important biological functions, such as sustaining cell life and increasing the cell's resistance to cellular stress.[10] The anti-DFS70 antibody can be detected in a variety of chronic inflammatory conditions, in cancer patients, and in a significant portion of the healthy population.[11,12] Behçet's Disease, which forms the basis of our study group, is an autoinflammatory disease as it is well known and anti-DFS70 positivity was found at a rate of 2.1% in our series of Behçet's patients and at a rate of 0.7% in the healthy controls, and interestingly, all of these patients were female. In Japan, DFS70 is reported at a rate of approximately 10% among healthy people,[11] but in our series, this rate is much lower than in Japanese society. The recognition of a DFS70 pattern in ANA testing is very important. DFS70 can be found alongside anti-p80 coilin antibodies or SS-A positivity in rheumatoid diseases. In their study on patients with Vogt-Koyanagi-Harada (VKH) Disease and Behçet's patients with panuveitis, Yamada et al. investigated LEDGF and found the anti lgG and anti-LEDGF detection rate as 66.7% in this group, as 21.6% in healthy controls, as 34% in Behçet's patients with panuveitis, and as 25.0% in sarcoidosis patients.[13] LEDGF is a protein linked to the stress reaction and for this reason, can increase in inflammatory lesions and break immune tolerance, but the fact that it can be detected in healthy controls decreases the likelihood of its direct association with the disease.

The inflammatory process in Behçet's patients may trigger DFS70 positivity, and centriole staining may be associated with vasculitis. However, our results also support the general belief that there is no highly specific autoantibody associated with Behçet's disease. It is noteworthy, however, that ANA staining patterns, the exact disease associations of which have not yet been revealed, can be found more in these patients, considering that chronic inflammatory reactions can lead to the breakdown of immune tolerance. The treatment of the disease with immunosuppressants is related to the ability to suppress with these treatments the inflammation that occurs due to immune response and endothelial dysfunction. There are still many unanswered questions regarding the pathogenesis of the disease. The detection of nonspecific patterns in our results suggests an indirect relationship with the disease, and the unknowns should continue to be investigated.

Conflict of interest

The authors declare that they have no conflicts of interest.

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