Detection of Epstein–Barr virus seropositivity in Behçet's disease

Thesis

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✍Hamdy Mohamed Abdelaziz Ahmed

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Abbreviations

1- ^{18}F-FDG PET/CT: Positron emission tomography with 2-deoxy-2-[fluorine-18] fluoro- D-glucose integrated with computed tomography.
2- 5-ASA: 5-aminosalicylic acid.
3- AA: amyloid A.
4- ABD: AdamantiadesBehçet's disease.
5- aCL: anticardiolipin antibodies.
6- AIDs: Autoinflammatory diseases
7- ALT: Alanine transaminase
8- AOPPs: advanced oxidation protein products.
9- APCR: activated protein C resistance.
10- APCs: antigen presenting cells.
11- aPL: antiphospholipid.
12- AST: Aspartate transaminase
13- BCS: Budd-Chiari syndrome.
14- BD: Behçet's disease.
15- BDCAF: Behçet's disease current activity form.
16- BML NB: promyelocytic leukemia nuclear body.
17- BSAS: Behcet's syndrome activity score.
18- CART: Classification and Regression Tree.
19- CCR1: (chemokine (C-C motif) receptor 1.
20- CD: Cluster of differentiation.
21- CINCA: Chronic infantile neurologic, cutaneous and articular syndrome.
22- CME: cystoid macular edema.
23- cmm: cubic milli-meter.
24- CMRO: Chronic multifocal recurrent osteomyelitis.
25- CMV: cytomegalovirus.
26- CPPD: calcium pyrophosphate dehydrate.
27- CRP: C- reactive protein.
28- CSF: Cerebrospinal fluid.
29- CTLA4: Cytotoxic T lymphocyte antigen 4.
30- CVT: Cerebral venous thrombosis.
31- DIF: direct immunofluorescence.
32- EBER: EBV encoded RNA's
33- EBNA: EBV nuclear antigen.
34- EBNA-LP: EBNA leader protein.
35- EBV: Epstein-Barr virus.

36- EGF: epidermal growth factor.
37- EMC: Essential mixed cryoglobulinemia.
38- EN: erythema nodosum.
39- ENL: erythema nudosum like lesions.
40- ERAP1: endoplasmic reticulum aminopeptidase 1.
41- ESR: Erythrocyte sedimentation rate.
42- FCAS: Familial cold autoinflammatory syndrome.
43- FMF: Familial Mediterranean fever.
44- GBS:Guillian-Barre Syndrome.
45- gm/dl: grams per deciliter.
46- GM-CSF: granulocyte-macrophage colony stimulating factor.
47- Gp: glycoprotein.
48- GPA: Granulomatosis with polyangiitis.
49- HB: Hemoglobin.
50- HCMV: Human cytomegalovirus.
51- HCV: hepatitis C virus.
52- HLA: Human leucocytic antigen.
53- HSP: Heat shock protein.
54- HSV-1: herpes simplex virus-1.
55- IBD: inflammatory bowel disease.
56- IFN- α: interferon alpha.
57- IFN-γ: interferon gamma.
58- Ig: Immunoglobulin.
59- IL: interleukin.
60- IL1RN: interleukin 1 receptor antagonist.
61- ISG: International Study Group.
62- JCV: John Cunningham virus.
63- KLRC4 gene: killer cell lectin-like receptor subfamily C, member 4.
64- LTB4: leukotriene B4.
65- Mac-1: Macrophage-1 antigen.
66- MALT: mucosa-associated lymphoid tissue.
67- MEFV: Mediterranean fever gene.
68- mg/dl: milli- grams per deciliter.
69- MHC: major histocompatibility complex.
70- MIC-A: MHC class I chain-related gene A.
71- MIF: macrophage migration inhibitory factor.
72- MIP-1a: macrophage inflammatory protein 1-a.
73- MIP-1b: macrophage inflammatory protein 1-b.
74- mm/hr: milli meter/hour.
75- MPO: Myeloperoxidase.
76- MS: Multiple Sclerosis.

77- MSU: monosodium urate.
78- MWS: Muckle-Wells syndrome.
79- N.B.D: Non Behçet's disease.
80- NK: Natural killer.
81- NLRP3: NLR family, pyrin domain containing 3.
82- NOD: nucleotide-binding and oligomerization domain.
83- NOMID: Neonatal-onset multisystem inflammatory disease.
84- NPC: Nasopharyngeal carcinoma.
85- PAA: pulmonary artery aneurysm.
86- PAI: plasminogen activator inhibitor.
87- PBMCs: peripheral blood mononuclear cells.
88- PDC: plasmacytoid dendritic cell.
89- PDGF: platelet-derived-growth factor.
90- PG: prostaglandin.
91- PRP: pattern-recognition receptors.
92- PTLD: post-transplant lymphoproliferative disorder.
93- RAS: Recurrent aphthous stomatitis.
94- RIG 1:Retenoic acid inducible gene 1.
95- SCID: subacute combined immune deficiency.
96- SMCs: Smooth muscle cells.
97- SPT: Skin prick testing.
98- STAT4 gene: Signal transducer and activator of transcription 4.
99- SVT: Superficial venous thrombophlebitis.
100- TCR: T cell receptors.
101- Th1: T helper 1 cells.
102- TLC: Total leukocytic count.
103- TLR: Toll-like receptor.
104- TLR: Toll-like receptors.
105- TMB: tetra-methylbenzedine.
106- TNF: Tumor necrosis factor.
107- TRAPs: TNF receptor-associated periodic syndrome.
108- U/l: unit/litre.
109- UV-B: Ultraviolet rays B.
110- VCA: viral capsid antigen.
111- VCAM-1: Vascular cell adhesion molecule-1.
112- VIL-10: Virally encoded IL-10.
113- vWF: von Willebrand factor.
114- VZV: Varicella zoster virus.
115- XLP: X-linked lymphoproliferative syndrome.

Abstract

Behçet's disease (BD) is a multisystem inflammatory disorder,BD etiology remains unknown, but epidemiologic findings suggest an autoimmune process that is triggered by an infectious or environmental agent in a genetically predisposed individual. Several viral agents including cytomegalovirus, Epstein-Barr virus, and varicella zoster virus, may also have some role.

Objectives: Try to assess the prevalence of EBV in Behçet's disease, and its relation to disease activity (assesed by BDCAF).

Methods: 75 Egyptian patients with Behçet's disease as well as 75 (age and sex matched) apparently healthy Egyptians control.All our patients were subjected to full history taking and thorough clinical examination.Disesase activity of our patients was determined using the BDCAF.Laboratory investigations in the form of CBC, ESR, CRP, liver and kidney functions were done to all our patients. The levels of EBV IgM and IgG were measured by ELISA and alsoEBV Ig M and Ig G indices were calculated to all our patients and controls.

Results: No significant correlation between presence of EBV IgG, IgM in our Behçet's cases and controls and also between active and non-active Behçet's cases using the BDCAF published by Leed'sUniversityUK 2006. Also there is no significant correlation between the presence of EBV IgG, IgMand various presentations except for genital ulcer presentation in our Behçet's cases.

Conclusion: The positivity of EBV IgG andIgM in our cases was not diffrent from those of the controls and there was no significant correlation between them, BDCAF and various presentations of Behçet's disease except between EBV IgG index and genital ulcers presentation thatmay raises the possibility ofassociation between EBV infection and genital ulcer presentation.

Introduction and aim of work

Behçet's disease is a multisystem inflammatory disorder characterized by recurrent oral aphthous ulcers, genital ulcers, uveitis, and skin lesions and generally presents with remissions and exacerbations. It can frequently involve the joints, gastrointestinal tract, and central nervous system (**Marshall, 2004**).

The cause of Behçet's disease remains unknown, but epidemiologic findings suggest that an autoimmune process is triggered by an infectious or environmental agent (possibly local to a geographic region) in a genetically predisposed individual (**Kulaber et al., 2007**). Whatever the stimulus is, the target tissue seems to be the small blood vessels, with various consequences of either vasculitis and/or thrombosis in many organ systems (**Scopus et al., 2007**).

A viral cause was first postulated by Behçet in 1937 **(Behçet, 1937).**Evidence of ongoing infection with a variety of viral agents has been sought. However, often there is only a history of previous infection and/or seropositivity(**Marshall, 2004**). Although herpetiform ulcers are unusual, herpes simplex virus-1 (HSV-1) is currently the most common virus associated with Behçet's disease. HSV DNA and serum antibodies against the virus have been found in a higher proportion of patients with Behçet's disease than in controls, and circulating immune complexes with the HSV-1 antigen have been reported. HSV DNA has been demonstrated in the genital and intestinal ulcers, but not in oral ulcers. However, anti-HSV immunity is also common in normal subjects, and results about therapeutic effects of antiviral treatment in Behçet's disease are scarce and controversial (**Direskeneli et al., 2001**). Several other viral agents, including hepatitis C virus, parvovirus B19, cytomegalovirus, Epstein-Barr virus, and varicella zoster virus, may also have some role (**Akdeniz et al., 2003).**

The Epstein–Barrvirus(EBV)orhumanherpesvirus4(HHV-4), is consideredoneofthemostcommonvirusesinhumans(**Maeda E. et al., 2009**). It is transmitted by the oral route through contamination with infected saliva and genital secretions (**Cherry G. et al., 2003**). Infection with EBV is endemic worldwide. It

is estimated that over 90% of the world's population is infected with the virus. EBV is associated with several diseases whose incidence differs dramatically in different parts of the world (**Rickinson et al., 2007**). There is evidence that infection with the virus is associated with a higher risk ofcertainautoimmunediseases(**DreyfusDHetal., 2011**)especiallydermatomyositis,systemiclupuserythematosus, rheumatoidarthritis, Sjögren'ssyndrome(**Pender MP et al., 2012; Ascherio A. et al., 2010**),andmultiplesclerosis(**Amon F. et al., 2004**).

Aim of the work:

1- To assess the prevalence of EBV seropositivity in Egyptian Behçet'spatients.
2- To detect relation of EBV to Behçet's disease activity.
3- To assess the relation of EBV to various presentations in Behçet's disease.

Overview ofBehçet'sDisease

History:

BD was initiallyrecognized in the 5th century BC by Hippocrates, redescribed by the Greek Adamantiades in 1931, but in 1937 the classical triad of oral and genital ulceration with ocular inflammation was reported by the Turk HulusiBehçet. Later on, further clinical features including cutaneous, neurological, vascular, gastrointestinal, and arthritishad been identified. Until recently BD was classified as a spondyloarthropathy but now is considered to be a systemic vasculitis (**Knontogiannis et al., 2000**).

Epidemiology:

BD is most prevalent along the "Silk Road," an ancient trading route between the Mediterranean and East Asia, where it is a major cause of morbidity. In Turkey, the country with the highest incidence of the disease, the prevalence is estimated to be between 110 and 420 per 100.000, whereas that in Japan is 13–20 per 100.000, and the prevalence in the UK and USA is estimated at 1-2 per 100.000. The typical age of onset is in the third or fourth decade of life and the male-to-female ratio varies with ethnic origin (**Azizlerli et al., 2003**).

In eastern Mediterranean populations, the disease is more common in men, who alsoexperience more severe disease(**Saadoun et al., 2010**).On the other hand, women are more affected in Japan and korea (**Yazici Y et al.,2010**). BD occurs mainly between 18 to 40 years. Some pediatric onset cases are reported. After age of 55 years, onset of BD is exceptional and diagnosis has to be made very cautiously(**Akpolat et al., 2002**).

Etiology and pathogenesis

The cause of Behçet's disease remains unknown, but epidemiologic findings suggest that an autoimmune process is triggered by an infectious or environmental agent (possibly local to a geographic region) in a genetically predisposed individual (**Kulaber et al., 2007**). Whatever the stimulus is, the target tissue seems to be the small blood vessels, with various consequences of either vasculitis and/or thrombosis in many organ systems (**Scopus et al., 2007**).

1- Genetics:

In common with ankylosing spondylitis and psoriatic arthropathy, BD has MHC class I associations. A number of studies have provided evidence that HLA-B51 is strongly associated with the disease in different ethnic groups. The positive ratio of HLA-B51 in BD is approximately 60% (**GülA. et al., 2001**).

There are more than 89 different subtypes of HLA-B51, and HLA-B5101 is the major suballele associated with BD in all the populations studied. HLA-B5108 was also found to be associated with BD in the Middle Eastern, Italian, Spanish, Greek, Turkish, and German patients. Oppositely, it has been suggested that HLA-B5107 might be negatively associated with BD in the Turkish and German populations (**FiettaP., 2005; PigaM. et. al., 2011**). Recently, HLA-B5101 gene was investigated in Japanese, Turkish, Jordanian, and Iranian patients and was found that all the patients have B510101 suggesting that the susceptibility to BD was conferred by the B510101 subtype and not by any genes in linkage disequilibrium with HLA-B51 (**TakemotoY. et al., 2003**). Accordingly, Amerindian populations, known to carry a high frequency of HLA-B51 but having no BD incidence, are known to carry the highest frequencies of nonsusceptible HLA-B510201 allele (**Middleton D. et al., 2003**).

Gene associations at two other specific chromosome locations, or loci were recently identified. Each of the identified gene regions is already known to play a role in immune regulation. Among the newly identified regions, researchers found an important association between BD and a gene called *ERAP1*. *ERAP1*codes for a molecule that processes microbial proteins in white blood cells. Variants of this protein can lead to more or less efficient processing of microbial proteins before they are loaded onto HLA molecules for presentation to the immune system. The variants of *ERAP1* identified in this study increase the risk of BD, but only in those individuals with one specific HLA type, HLA-B51, which has previously been associated with Behçet's disease.The *ERAP1* variant associated with BD processes microbial proteins in such a way that they can be loaded onto the HLA-B51 molecule to trigger an abnormal immune response**(Gul et al., 2012).**

A significant association of BD with variants near the *CCR1* gene has been found. Proteins coded by this gene help migration of infection-fighting blood cells to sites of invading microorganisms. When this function is defective, the microorganisms can trigger a persistent inflammatory response, also there is an association of the disease with variants in the *KLRC4* gene and *STAT4* gene**(Evans et al., 2011).**

The MHC class I chain-related gene A (MIC-A) was also regarded as a candidate for BD genetic susceptibility. Several studies have demonstrated associations between MIC-A009, MIC-A006, MIC-A6 TM, and BD. However, these associations appear to be the result of a strong linkage disequilibrium of MIC-A with HLA-B51, so today they are not considered as the primary susceptibility genes for BD(**MizukiN. et al., 1999**; **PigaM. et. al., 2011**).