Serum Matrix Metalloprotienase 9 (MMP9) in patients with Behcet's Disease and its relation to disease activity and vascular affection

Thesis

For fulfillment of MD degree in internal medicine

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2012

Acknowledgment

*Before all and above all, thanks to **GOD** for all things in my whole life.*

*I would like to express my sincere thanks and gratitude to **Prof. Dr.Gaafar Mohamed Ragab**, professor of Internal Medicine, Faculty of Medicine, Cairo University, for his guidance,encouragement and valuable supervision of this work.*

*I would like also to express my deepest gratitude to **Prof. Dr. Badawy El-Kholy,** professor of Chemical pathology, Faculty of Medicine, Cairo University, for his guidance, great efforts and help in doing this work.*

*I would like as well to thank **Dr.Abir Zakaria**, Assistant professorr of Internal Medicine, Faculty of Medicine, Cairo University, for her effort, time and great help in finishing this work.*

Last but not least, I wish to express my appreciation to my directors and my colleagues in the Internal Medicine and Laboratory departments in El- Kasr El-Ainy hospital for their cooperative attitude and encouragement during the preparation of this work.

Abstract

Objectives: Basic and clinical studies had revealed a strong correlation between matrix metalloproteinases (MMPs), particularly MMP-9, and the formation of abdominal aortic aneurysms. In addition, previous studies had clearly shown that MMP-9 plays an important role in the pathogenesis of vasculitis characterized by aneurysm formation such as Kawasaki disease, temporal arteritis and Takayasu arteritis. Depending on those findings, we hypothesized that circulating MMP-9 could be useful marker to demonstrate vascular involvement in patients with BD.

Methods: Thirty patients with BD, and 20 healthy controls were enrolled in the study. We assessed the disease activity of patients according to the Leeds activity score system (BDCAF). We compared the Leeds activity scores of patients with their serum levels of MMP- 9. Patients with BD were categorized as active (total activity score $\geq$ 5) or inactive (total activity score $<$ 5). Patients were further categorized with respect to their extent of involvement as those with and those without vascular involvement. The level of MMP-9 was measured by ELISA.

Results: Serum MMP-9 level was significantly higher both in patients with active and inactive disease as compared to healthy controls ($p < 0.001$). We found positive correlation between serum level of MMP-9 and disease activity assessed by BDCAF particularly the vascular affection whether on the venous or the arterial side.

Conclusions: We concluded that serum MMP-9 levels can be used in the diagnosis and assessment of disease activity in general and vascular activity in particular in BD.

Key words:

Matrix metalloproteinases – Behcet's disease – Disease activity- BDCAF

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List of Abbreviations

AAA	Abdominal aortic aneurysm
ANA	Antinuclear antibodies
ANCA	Antineutrophil cytoplasmic antibodies
ASCA	Anti-saccharomyces cerevisiae antibodies
BCS	Budd Chiari syndrome
BD	Behcet's disease
BDCAF	Behcet's disease current activity form
CD	Cluster of differentiation
DVT	Deep venous thrombosis
ELISA	Enzyme linked immunosorbent assay
ENOS	Endothelial nitric oxide synthase
EULAR	European league against rheumatism
FMF	Familial Mediterranean fever
HIDS	Hyperimmunoglobulin D syndrome
HLA	Human leucocytic antigen
HSP	Heat shock protien
ICBD	International criteria for Behcet's disease
IL-10	Interleukin 10

ISG	International Study Group
IVC	Inferior vena cava
MMP	Matrix metalloprotienase
MWS	Muckle Wells syndrome
NK	Natural killer
NO	Nitric oxide
NOMID	Neonatal onset multisystem inflammatory disease
PAA	Pulmonary artery aneurysm
PAI-1	Plasminogen activator inhibitor-1
PAPA	pyogenic arthritis,pyoderma gangrenosa, disfiguring acne
PG	Pyoderma gangrenosum
RCTs	Randomized controlled trials
RF	Rheumatoid factor
ROS	Reactive oxygen species
SVC	Superior vena cava
TAFI	Thrombin activatable fibrinolysis inhibitor
TIMP	Tissue inhibitor of matrix metalloprotienase
TNFα	Tumor necrosis factor alpha
TRAPS	Tumor necrosis factor receptor associated periodic syndrome

Introduction

Behçet disease (BD) is a complex, multisystemic disease that includes involvement of the mucocutaneous, ocular, cardiovascular, renal, gastrointestinal, pulmonary, urologic, and central nervous systems and the joints, blood vessels, and lungs. It is most prevalent and more virulent in the Mediterranean region, Middle East, and Far East ***(Marjan Yousefi et al.,2009).***

BD gives rise to a chronic relapsing systemic vasculitis involving arteries and veins of various sizes. The vascular involvement may consist of thrombophlebitis, deep vein thrombosis, and arterial obstruction. Aneurysms, particularly of the pulmonary arteries, may also develop ***(Yazici et al., 1998***).

In 1994 a workshop was held in **Leeds (UK)** to arrive at a consensus view "The Behçet's Disease Current Activity Form (BDCAF)" which depends on accurate history of clinical features present during the month prior to the date of assessment.

Many laboratory markers had been studied regarding its relationship to BD activity like soluble E-selectin ***(Sari RA et al.,2005)***, leptin ***(Evereklioglu C et al., 2002)***, Nitric oxide ***(Turkoz Y et al.,2002)*** homocystiene ***(Sarican T et al., 2007)***, vascular endothelial growth factor ***(Cekmen M et al.,2003)***, galectin 3 ***(Lee YJ et al.,2007)*** and others but all of them were non conclusive.

Matrix metalloproteinase 9 is a zinc dependent endopeptidase that degrades most components of the extracellular matrix and basement membrane and plays an important role in the pathogenesis of vasculitis characterized by aneurysm formation such as Kawasaki disease, temporal arteritis and Takayasu arteritis ***(Pay S et al., 2007).*** Depending on these findings, we may use circulating MMP-9 as a useful marker to demonstrate vascular involvement in patients with BD.

Aim of the work:

The aim of the work is to study the level of serum matrix metalloproteinase 9 (MMP9) in patients with Behçet's disease and its relation to disease activity and vascular affection.

In 1937 Huluci Behcet, a Turkish dermatologist, described three patients with oral and genital ulceration and hypopyon uveitis ***(Behcet, 1937).*** Three years later he reported four similar cases and named this constellation of symptoms as "triple symptom complex" ***(Behcet, 1940).*** However, the first evidence of acquisition to this symptomatology returns to the age of Hippocrates in the fifth century BC ***(Feigenbaum, 1956).***

In Greece, the disease is named Adamantiades - Behcet's syndrome because Adamantiades presented a case of recurrent hypopyon, irits, phlebitis, oral and genital ulcerations and knee arthritis six years before Behcet's paper ***(Adamantiades ,1930).***

Lie term "Adamantiades-Behcet's disease" honors both men who first described the several manifestations which constitute an autonomous disease.

Behcet's disease (BD) was given many names such as:

•Triple symptom complex ***(Behcet, 1940).***

•Multiple symptom complex of Behcet ***(Berlin, 1944).***

•Complex aphthosis ***(Jorizzo et al., 1985).***

•Silk route disease ***(Ohno, 1986).***

Since BD has many manifestations including musculoskeletal, gastrointestinal, urogenital, cardiac, cutaneous and neurological manifestations, symptoms were added to "triple symptom complex" as BD is designated to a discrete clinical entity. The use of the name 'Behcet's syndrome', rather than 'Behcet's disease', is preferred by many of those interested in the condition since it may present with considerable variation in manifestations and severity between patients, and indeed in the prevalence of individual manifestations in different parts of the world ***(Barnes, 2006).***

Epidemiology

BD is more prevalent in Far East (Japan, Korea); Middle East (Iran, Iraq, Israel, Saudi Arabia, Kuwait, Syria) and countries around Mediterranean sea (Turkey, Italy, Egypt, Greece, Morocco, Algeria, Tunis), therefore it occurs most commonly in the countries along the ancient "silk road" ***(Borhani 2004)***.

Sex and age:

Men are affected more often, and with more severe disease, than women in some Mediterranean areas. ***(Yousefi et al., 2009)*** and according to ***Buraga et al.,(2010)***, males are more likely to develop severe presentations of the disease with pulmonary aneurysms, eye involvement, thrombophlebitis and neurologic disease are all more common in males. However, females are more likely to develop erythema nodosum-like skin lesions.

Borhani (2004) mentioned that BD is the illness of young persons with the mean age at onset of the first symptom is 20-35 years, However it may occur at any age ranging from infancy to more than 78 years.

Etiology and pathogenesis

The pathogenesis of Behçet's disease is unknown. An enhanced and dysregulated immune response has been suggested as the underlying pathology and this can be triggered by environmental agents, mainly microbes, in genetically susceptible individuals. ***(Zierhut et al., 2003).***

A) Genetic predisposition:

1) Human leukocyte antigen (HLA) genes:

Increased risk of developing BD is associated with presence of certain HLA genes. A higher than baseline prevalence of HLA-B51 has been found in patients in Italy, Germany, Middle Eastern and Far Eastern countries and of HLA-B52 in Israel ***(Sakane et al.,1999; Kotter et al.,2001).***

There may also be a genetic contribution to disease severity. Patients who have HLA-B51 allele have worse disease in general than those who do not ***(Chang et al., 2001).***

However, ***Davatchi et al.,(2008)*** mentioned that HLA-B51 cannot explain by itself the occurrence of BD as there are normal subjects with HLA-B51 and in familial forms of BD, some members with HLA-B51 may not have the disease . Therefore, another gene in the region may be involved in the susceptibility to BD.

Duftner et al., (2003) reported that BD is associated with HLA-B*51, and to a lesser extent with HLA-B*2702. There may even be a role of MICA genes in the pathogenesis of BD. Several of these MHC class I molecules can be recognized by NK receptors on NK cells independently from peptides.

2) Non- Human leukocyte antigen (HLA) genes:

Genome-Wide screening of affected families with more than one affected member had identified additional, non-HLA regions of potential interest ***(Karasneh et al., 2005b) .***

Non- HLA genes may play a role in determining susceptibility to disease. Example include associations between BD and polymorphisms in IL-10 as well as CD 28 genes ***(Krause and Weinberger, 2008)*** and polymorphism of the ENOS ***(Salvarani et al.,2002), (Karasneh et al., 2005a).***

Other genetic studies:

- Microsatellite polymorphisms of the MICA gene ***(Nishiyama et al., 2006).***
- MEFV *g*ene mutations, seen in persons with Mediterranean fever, are increased in persons with BD especially in those with vascular affection ***(Ayesh et al., 2008).***.
- Autosomal recessive inheritance in the families with pediatric BD ***(Molinari et al., 2003).***
- Tumor necrosis factor polymorphisms ***(Ahmad et al., 2003).***

B) Antigenic stimuli :

-Streptococcal antigen in the pathogenesis of BD:

Kaneko et al.,(2003) demonstrated the deposition of streptococcal related antigen at infiltrated cells which were adhering to the vascular walls in erythema nodosum like lesions in patients with BD suggesting hypersensitivy to streptococci and noticed that they were involved with chronic infectious foci, such as tonsillitis and dental caries, by nonpathogenic streptococci in the oral cavity.

-Herpes simplex virus (HSV):

Although herpitiform ulcers are not common in patients with BD, HSV is currently the only virus possibly associated with BD ***(Direskeneli, 2001).***

HSV DNA was detected in saliva, genital ulcer, and intestinal ulcers of patients with BD and BD- like symptoms were induced in mice after inoculation of HSV into the earlobe ***(Sungnack .L. et al.,2003).***