The Egyptian Journal of Hospital Medicine (2006)Vol., 24: 548 – 555

Serum level of IL13 and expression of BCL2 in Behcet's disease

Hanan.M.A Darwish*, Sabila Gomaa Mousa Noha Hamdy , and Sahar Zalam.**

Departments of Dermatology and Venereology*, General Mmedicine** and Clinical Pathology. Faculty of Medicine for girls Al-Azhar University.

Abstract

Background BD: BCL2 family is a large family of apoptosis regulating proteins consisting of both blockers and promoters of cell death. Immunological processes and a variety of cytokines may play a role in pathophysiological process. Defective regulation of programmed cell death (apoptosis) also play a role in development of Behcet's disease

Objective: To investigate the level of BCL2 and IL13in BD and to determine their to relation monitory disease activity.

Patients and methods: This study was conducted on thirty patients (15 active and 15 inactive) and 15-health control, the activity of BD was evaluated according to international study group for BD disease, using ELISA technique for IL 13 and flow cytometry forBCL2.

Results: Elevated serum levels of IL13 in patient with active BD than inactive and both had elevated levels than control($P< 0.01$) and also the serum levels of Bcl2 was elevated in patient with active BD than inactive and control($P< 0.01$).

Concolusion: The data suggested that IL13 and BCL2 could be involved in the pathogenesis of BD and its serum levels can be used as marker to monitor disease activity.

Introduction

Behcet's disease (BD) is an inflammatory multisystem disorder chara-cterized by recurrent oral genital and aphthous ulcers , arthritis,uveitis and therombophlepitis that can involve several organs (Emmi *et al.,* 1997).

Hulusi Behcet, is Turkish dermatologist ,described the recurrent orogenital ulceration and uveitis in1937. The prevalence of BD is highest in Japan, south Asia ,the middle east and southern Europe .The disease is rare in northern Europe and the United state (Jorizzo,1999).Although the etiology and the pathogenesis of BD still remain uncertain, it has been suggested, that three major pathophosiologic changes, neutrophil hyperfunction, vasculitis and autoimmune response may be involved in its pathogenesis (Sakane *et al*.,1997)

Recent studies have indicated that cytokine- producing cells play an important role in the immunopathogenesis of inflammation in BD (Sugi *et al*., 1998). In particular, a divergent cytokine production profile of TH1/TH2 cell type is very important in the immune response occurring in BD (Raziuddin *et al*.,1998) .

Programmed cell death (apoptosis) is important in down-modulation immune response after activation and proliferation of inflammatory cells. It has been suggested that dysregulated apoptosis of lymphocytes may be linked to the development of autoimmune disease as Sjogrens syndrome (Ichikawa *et al.*,1995), systemic lupus erythematosus (SLE)(Aringer *et al*.,1991) and rheumatoid arthritis(RA) (Isomaki *et al.*,1996). Defective regulation of programmed cell death (apoptosis) may play a role in the development of BD and the protoncogene Bcl-2 is involved in the control of apoptosis in immunocompetent cells (Hamzaoui *et al.,* 1999).

The diagnostic criteria established by the international study group for Behcet's disease requires the presence of recurrent oral ulceration and two of the following: recurrent genital ulceration, eye lesions,

skin lesions and/or a pathergy test. Pathergy respone is anoduleor pustule, 3-10mm, appearing 24-48 hours after puncture of the forearm Srin with sterile 20 gauge needle. (Table 1)

Patients and methods

Patient group:

Thirty patients with BD were studied (15 active and15 inactive).The active group,the sex (M/F 13/2) ,aged (30-41)the mean (40.40 ± 587).Inactive group 15 patients (M/F 12/3) aged (32-44) The mean (41.80 ± 5.89) the activity was evaluated according to international study group of Behects disease. We collected the patients from out patient clinic of dermatology ,internal medicine and ophthalmology Al Zahraa hospital, Al-Azhar university and otherhospitals .

Control group:

15 healthy subjects (M/F 9/6) aged (22-45) years, the mean (35.93 ± 1.31) years were served as control group.

All patients and control group were subjected to the following:

Full history, thorough clinical examination, complete blood picture, eryth-rocyte sedimentation rate (ESR), fundus examination ,serum IL 13 and Bcl_2.

Sample collection & storage:

A fresh peripheral blood sample is collected by veni-puncture and divided into two tubes, one empty tube for serum sample and the other with heparin for lymphocyte separation.

Measurement of IL-13:

Serum sample was used for estimation of IL–13 using ELISA technique (Bio-source international).

Determination of BCL2:

Heparin blood sample was used for separation of lymphocyte by Ficall-Hypique density-gradient centrifugation & washed twice with PBS at $+4^{oC}$. Then add 20 Ml of Antigen Extraction Agent (AEA) for every 100 Ml of cell suspension of incubate 30 minutes on ice with the occasional vortexing then transfer extracts to micro-centrifuge tubes and centrifuge for 5 minutes. The samples are now ready for analysis by Bender Med systems human BCL-2 ELISA.

Results

Thirty patients (15 active and 15 inactive). Active patient with BD (male to female ratio 13/2 (86.87% - 13.33%) age ranged (30-41) the mean (40.4 □ 5.87), inactive BD male to female 12/3 (80%-20%) age ranged (32-44) the mean (41.80 □ 5.89) and control 15 healthy subject male to female (9-6) age (22-45) the mean (35.93 □ 7.31)table (2and 3).

Table (4) shows some clinical date of some patients with BD.

Table (5)shows that Serum levels of IL13 are higher in-patient with active BD (73.4 □ 14.41) than in patients with inactive BD (7.27 □ 3.06) and control (2.73 □ 2.05). This difference were statistically significant (P< 0.01 and P< 0.01)) respectively. Also, it was noted that serum IL13 level in inactive group was significantly higher than normal control group (p,<0.01) (figure 2)

Serum levels of BCL2 is higher in-patients with active BD than inactive and both are higher than control (73.4 □14.41), (7.27 □3.06) and (2.73 □2.05), respectively with significance difference(P< 0.01) for all difference.

On comparing BCL2 in active BD with control there were statistically significance (P<0.01). On comparing BCL2 inactive with control there was statistically significance (P< 0.01). Table (6) and figure (3).

ESR is higher in-patient with active BD than in-patient with inactive and control group. This difference was statistically significant. Table (7) and figure (1).

(Table 1)

Criteria for the diagnosis of Behcet's syndrome **International study group for Behcet's disease**
In the absence of other clinical explanation, patients must have: 1- Recurrent oral ulceration: (Aphthous or herpetiform recurring at least 3 times in 1 year period and 2 of the following: 2- Recurrent genital ulceration. 3- Eye lesion: Anterior or post-uveitis, cells in the vitreous by slit lamp examination or retinal vasculitis observed by an ophthalmologist. 4- Skin lesions: Erythema nodosum, pseudo-folliculitis, papulopustular lesions or acneiform nodules in post adolescent patients not on corticosteroids. 5- Pathergy, read at 24 – 48 hours.

(Mouts,1994)

Table (2) shows the sex in patients and control

SEX	Active group number	%	Inactive group number	%	Control group number	%
females	2	13.33	3	20	9	60
males	13	86.67	12	80	6	40
total	15	100	15	100	15	100

Table (3) shows the age in patient and control.

Age (mean □ SD)	Control group	Active group	T value	P value	
	35.93 □ 7.31	40.40 □ 5.87	1.86	> 0.05	NS
	Control	Inactive	T value	P value	
	35.93 □ 7.31	41.80 □ 5.89	2.42	> 0.05	NS
	Inactive	Active	T value	P value	
	41.80 □ 5.89	40.40 □ 5.87	0.65	> 0.05	NS

Table (4):- clinical features of some patients with active Behcet's disease

Patient	**Disease**	**Symptoms**			
1	Active	Oral	Genital	Uveitis	
2	Active	Oral	Genital	Uveitis	Arthritis
3	Active	Oral	Genital	Uveitis	EN
4	Active	Oral	Genital		Pulmonary
5	Active	Oral			
6	Partial				
7	Active	Oral	Genital		Acneiform

Table (5) shows the serum level of IL-13 **in patients and control group.**

IL-13 (mean □ SD)	Control	Active	T value	P value	Significance
	2.73 □ 2.05	73.40 □ 14.41	18.80	< 0.01	HS
	Control	Inactive	T value	P value	Significance
	2.73 □ 2.05	7.27 □ 3.06	4.77	< 0.05	S
	Inactive	Active	T value	P value	Significance
	7.27 □ 3.06	73.40 □ 14.41	17.37	< 0.01	HS

Table (6) shows the serum level of BCL2 **in patients and control** group

BCL2 (mean □ SD)	Control	Active	T value	P value	Significance
	6.47 □ 2.50	147.73 □ 31.76	17.16	< 0.01	HS
	Control	Inactive	T value	P value	Significance
	6.47 □ 2.50	29.73 □ 11.16	7.87	< 0.01	HS
	Inactive	Active	T value	P value	Significance
	29.73 □ 11.16	147.73 □ 31.76	13.57	< 0.01	HS

Table (7) shows the ESR in patient and control.

ESR (mean □ SD)	Control	Active	T value	P value	
	13.07 □	82.67 □	20.19	< 0.01	HS
	Control	Inactive	T value	P value	
	13.07 □	23.67 □	6.66	< 0.01	HS
	Inactive	Active	T value	P value	
	23.67 □	82.67 □	16.23	< 0.01	HS

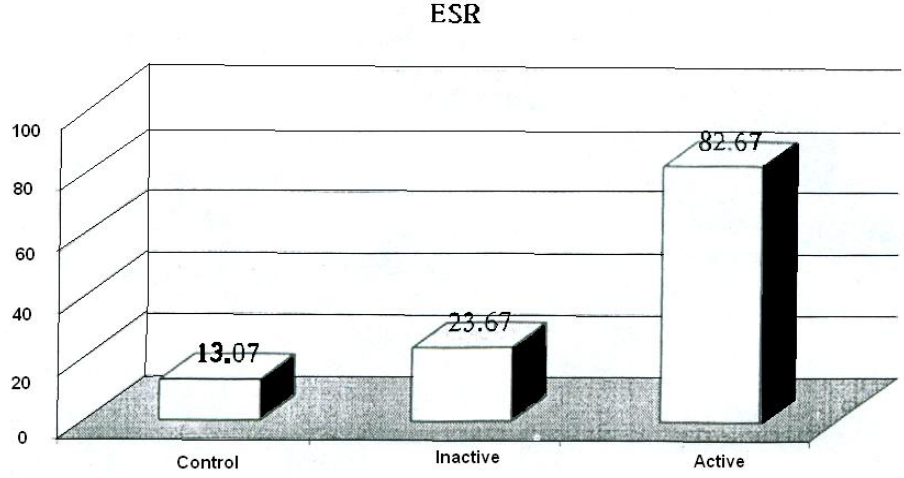

Fig (1) Comparison of ESR level in control , inactive and active groups

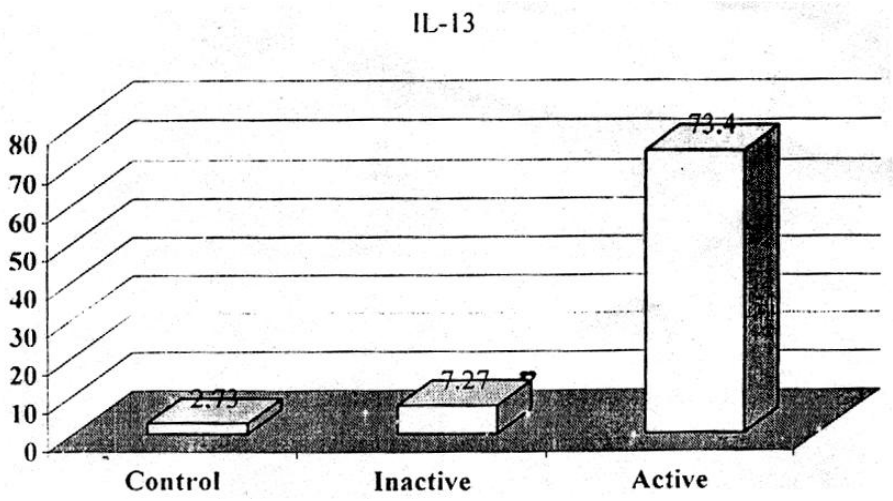

Fig (2) Comparison IL-13 in control , inactive and active groups

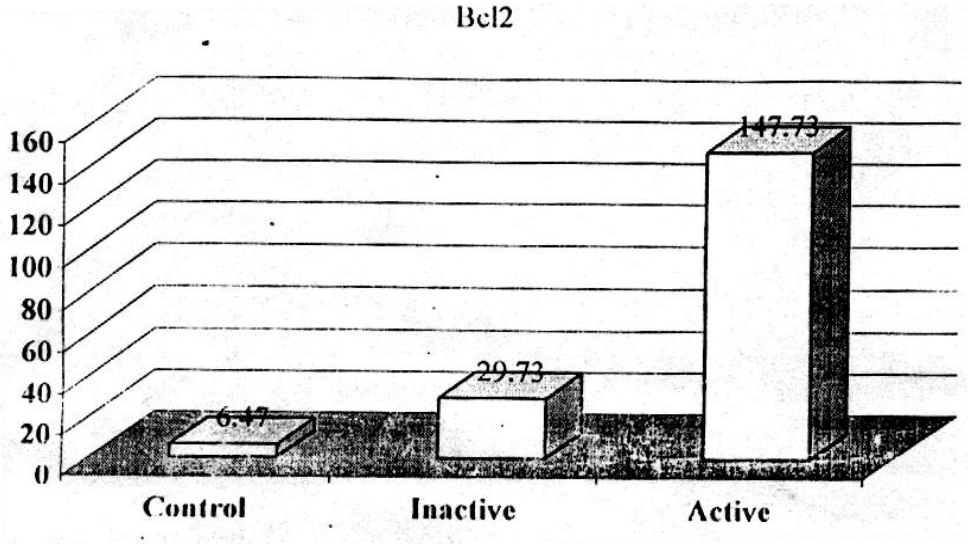

Fig (3) Comparison of BCl 2 level in control , inactive and active groups

Discussion

Bechet's is a polysymptomatic disease, as a result of recurrent systemic vasculitis with choronic course and unknown acetiology (Emmi *et al.,* 1997). Several organs or systems can be involved and the symptomatology and severity depend on the system affected, The main clinical features are: oral aphthous, genital ulcers, ocular lesion,skin lesion, arthritis,central nervous system affection, vasculitis and other less frequent findings such as pulmonary manifestation (Jorizzo,1999). Increasing evidences indicate that immunological processes and a variety of cytokines may contribute to the pathophysiological process in BD (Sakane *et al*.,1997).

Recently it was reported that patients with BD have a dysregulation of programmed cell death (Hamzoui *et al*.,1998). BCL2 is a proto-oncogene that regulates apoptosis of several cell types, BCL2 plays a role in the maintenance of the immune system, since inactivation of BCL2 in mice leads to the disappearance of the lymphoid system (Nakayama and Neghishi,1993). Lymphocyte activation in active BD was increased, in particular in the inflammatory sites (Hamzaoui *et al*.,1999). So it is possible that the expression of apoptosis-regulating proteins, like BCL2, is dysregulated in these lymphocytes.

In our study we found that the serum level of IL13 was significantly higher in active BD than inactive and both had higher level than control group .

These results are agreement with that of Razidudin *et al*., (1998), AL-dalaam et al ,1998), andAridogam *et al* (2oo3) who reported that serum IL13 level were higher in patient with active BD than inactive and control. In contrast to these results two studies were done by Lehner, (1999) and Mantas *et al*., (1999) reported that there is no statistically significance difference between BD patients and controls as regard serum level of IL13.

We have found that the serum levels of BCL2 was higher in patient with active BD than inactive and both had higher levels than control. These results agree with data done by Hamzaoui *et al*. (1996) and Hamzaoui *et al*. (1999) and also other data done by Isömak, (1996) who reported that increased expression of Bcl2 in BDand SLE. The possible explanation for the increased BcL2 expression in the peripheral blood and inflammatory sites in BD disease was the increased in production of IL2 (Hamzaoui and Ayed,1990), because IL2 has been shown to increase BcL2 expression in active T-cells (Deng and Podak,1993) .

The increased BcL2 protein expression in active BD is non specific for the disease and may be explained at least in part by the increased in vivo activation levels or presence of autoimmune vasculitis combined with in vivo inducation by aetiopathological agents. These may play an important role in the chronic inflammation in BD(Hamzaoui *et al*.,1998).

Reference:

1- **AL- Dalaan A, Al- Shaikh A, Bahabri S, Al- mayouf S and El Kholfa M (1998):** Study of HLA class 2 antigens allele distribution in Behcet's disease among small population, 8th International Congress on Behcet's Disease., 11.

2- **Aridogon BC, yildirim M, Baysal V, Inaloz HS, Baz K and Kayu S (2003):** Serum levels of IL-4, IL- 10, IL- 12, IL- 13 and IFN- Gamma in Behcet's disease. J. Dermatol., 30: 602- 607.

3- **Aringer M, WinterSherger W, Steiner CW, Kierner H, Presteri E, Jaeger U, Smolen JS and Graninger WB (1994):** High level of Bcl_2 protein in circulating T-lymphocytes but not B-lymphocytes of patients with systemic lupus erythem-atosus. Arthr Rheum., 37:1423- 1430.

4- **Deng G and podak ER (1993):** Suppression of apoptosis in cytotoxic T-cell line by interleukin 2 mediated gene

transcription and deregulated expression of the proto-oncogene Bcl_2. Proc Natl Acad Sci USA., 90: 2189-2193.

5- **Emmi L, Brugnolo F, Marchione T (1997):** pathogenesis and therapy of Behcet's disease. Ann Ital Med Int., 12:20- 25.

6- **Hamzaoui A, Hamzaoui K and Chabbou A (1996):** A high levels of Bcl_2 protein in T- lymphocytes of patients with Behcet's disease; Clin Exp Rhumatol., 14: 106- 108.

7- **Hamzaoui K and Ayed K (1990):** High affinity IL- 2 receptors on peripheral blood lymphocytes are decreased during active Behcet's disease. Clin Exp Rhumatol., 8: 100- 101.

8- **Hamzaoui K, Hamzaoui A, Zakaraoui L and Chabbou A (1998):** Fas/ APO-1 in patients with Behcet's disease. Mediators inflamm., 7:111-114.

9- **Hamzaoui K, Hamzaoui A, Zakaraoui L and Chabbou A (1999):** Expression of Bcl_2 in inflammatory sites from patients with active Behcet's disease in mediators of inflammation., 8: 101-106.

10- **Ichikawa Y, Arimori K, Yoshida M and Hoshina Y (1995):** Abnormal expression of apoptosis regulated antigens, Fas and Bcl_2 on circulating T- lymphocyte subsets in primary Sjogren syndrome. Clin Exp Rhumatol., 13: 307- 313.

11- **Isomäki P, Södersteöm KO, punnonen J, Roivainen A, Luukkainen R, Merilahti-paolo R, Nikkari S, Lassila O and Toivanen P(1996):** Br J Dermatol., 35:611- 619.

12- **Jorizzo JL (1999):** Behcet's disease. In Freedberg IM, Eisen AZ, Wolff K, Austen KF, Goldsmith LA, Katz SI, Fitzpatric TB (eds): dermatology in general medicine, McGraw- Hill, New York, pp: 2161-2165.

13- **Lehner T (1999):** Immunopathogenesis of Behcet's disease. Ann Med Interre., 150: 483-487.

14- **Mantas C, Direskeneli H, Eksioglu-Demiralp E and Akoglu T (1999):** Serum levels of Th-2 cytokines, IL- 4 and IL- 10 in Behcet's disease. J Rhumatol., 26: 510-512.

15- **Mouts poulas HM (1994):** Behcet's Syndrome. In; Isselbsacher KJ, Braun Wald E, Wilson JD, Martin JB and Fauci AS, eds. Harrison's principles of internal medicine, USA. Mc Graw. Hill Inc., 1669-1670.

16- **Nakayama K and Naghisi I (1993):** Disappearance of the lymphoid system in Bcl-2 homogenous mutant chimeric mice. Science., 261:1584- 1588.

17- **Razidudin S, Al Dalaan S, Siraj Ak and Al Sedwry S (1998):** Divergent cytokine production profile in Behcet's disease. Altered Th1/ Th2 cell cytokine pattern. J Rhumatol., 25: 329-333.

18- **Sakane T, Suzuki Nand Nagafuchi H (1997):** Etiopathology of Behcet's disease: Immunological aspects. Yonsei Med J., 38:350-358.

19- **Sugi-Ikai N, Nakazawa M, Nakkamura S, Ohano S, Minami M (1998):** Increased frequencies of interleukin-2 and interferon-gamma- producing T cells in patients with active Behcet's disease. Invest Ophthalmol Vis sci., 39:996-1004.

تحديد مستوى الإنترلوكين 13و ب س ل 2 فى مرضى بهست

د/ نهى حمدى* - د/حنان محمد على درويش * - د/ وسيلة جمعة موسى*** - د/ سحر ظلام***

قسم التحاليل الإكلينيكية* و قسم الأمراض الجلدية و التناسلية** و قسم الباطنة العامة** *

يعد مرض بهست من الأمراض الإلتهابية التى تصيب عدة أجهزة من الجسم معا و يتميز بوجود تقرحات متكررة بالفم و الأعضاء التناسلية وإلتهابات بالمفاصل و العين و قد قامت هذه الدراسة على تحديد معدل الأنيرلوكين 13و ب س ل 2 فى الدم لمرضى بهست و مدى إرتباطهم بنشاط المرض من عدمه و ذلك بعد التشخيص الإكلينيكى للمرضى و قياس سرعة الترسيب و عمل فحص قاع عين و قد توصلت الدراسة الى ارتفاع معدل الإنيرلوكين 13و ب س ل 2 فى مجموعة مرضى بهست النشط مقارنة بمجموعة مرضى بهست الغير نشط و المجموعة الضابطة.