Activation of complements pathways in patients with Bechcet's Disease.

Ikbal Kh.Al-Joofy*pH.D
Khalida M.Mousawy**pH.D
Raad K.Muslih ***pH.D
Jassim Al-Khafaji****pH.D
Makram M.ALWaiz*****pH.D

Summary:

Background: Behcet's disease (BD) is amultisystemic inflammatory disorder , onset is belived to be triggered by many factors with a particular immunogenetic Factor. The aim of this study is elucidate the role of C3,C4 complement components With CH50 and HLA typing as immunogenetic factor in aetiopathogenesis of this disease .

Methods : Seventy patients with Behcet's disease have been studied to assess the level of serum complement's components (C3,C4), complement total hemolytic activity (CH50), and HLA- typing using single radial immunodifusion (SRID), titration assay, and microlymphocytotoxicity methods respectively , compared with age, sex and ethic matched 30 patients control with recurred oral ulcer, 25 healthy relative , and 30 healthy volunteer.

Results : Significant elevation ($P<0.01,0.05,0.0005$) in C3, C4& CH50 levels was clearly observed in patients with BD, especially those who show to be HLA-B51(5) positive in comparison to control groups.

Conclusion : Increased level of CH50 which is reflected the total activity of the classical & lytic pathways , elevation of C3 level is an index of the disease activities which support that alternative pathway play a role in the pathogenesis of BD in Iraqi patients especially in those with HAL – B51(5) positive .

Keywords: *Behcet's disease, HLA-typing, Complement's components (C3, C4,CH50).*

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Introduction:

Behcet's diseases is considered as a chronic myti-systemic inflammatory disease, despite extensive researches , etiology of BD remained myseterious, though evidences had accumulated that genetic , environmental & hormonal factors do play a role in the development of this disease, never the less, immunological mechanisms are deeply implicated in its pathogenesis[1,2,3], among which is complement elements which shown to play a crucial role in the pathogenesis of BD, for instance, the complement system is one of the major mechanisms where by antigen recognition is converted into an effective defense against infection, particularly against extracelluar bacteria[4].

The simplest laboratory measurement of complement activity is to determine the concentration of the complement which will cause lysis of 50% of a standardized preparation of antibody–sensitized erythrocytes , this technique reflect the total activity of the classical ,and lytic pathways. On the other hand , further techniques, such as radioimmunoassay, enzyme labeled immunosorbent assay, and SRID in which using antibody specific for the protein under investigation as C3&C4[5] .

In this study, estimation of serum C3 & C4 complement components, with CH50, and HLA typing were carried out in patients with BD, in order to elucidate the role of these immunological elements in the pathogenesis of this disease in Iraqi patients.

**Department of Biology*
***Department of Microbiology*
****Department of Chemistry, College of Science, AL-Mustansiriyah university, ,*
*****Medical City, Laboratory Teaching Hospital.*
******Dept of Dermatology & Venereology, College of Medicine, Baghdad University,*

Subjects & Methods

Three study groups were interviewed

A. Patients study group

A total of 70 Arab, Iraqi patients (P.), who fulfilled the international study group criteria for diagnosis of BD. Those patients were attending the multidisciplin Behcets disease clinic at Baghdad teaching hospital .

B. Control groups

They were age, sex, ethnic matched with patients group. They were including .

- 30 Patients control group (P.C.) with only recurrent oral ulceration.
- Healthy control groups

-25 healthy relative (R.).
-30 healthy control (H.C.)

Methods

Typing for HLA class I antigens was carried out at Al-Karama hospital Tissue typing laboratory, using microlymphocytotoxicity test established by Terasaki (1964)[6] & modified by Dick (1979)& Bender (1984). Serum C3 & C4 complement components were estimated by SRID test using a specific endoplate, with incubation for 48 hr in case of C3, and 72hr for C4.

The concentration of C3 &C4 were assessed from the standard curve (reference C3 & C4 concentration versus squares of ring diameter), and expressed as mg/dl.[7]

The total complement activity was assayed in the studied groups sera according to the WHO immunological techniques.[8]

Briefly, the sera were serially diluted with PBS, and incubated with washed sensitized SRBCs (at round bottom wells of the plastic tray) at 37°C for 30 min., following over night refrigeration, the plates were inspected to determine the titer of the complement which reflects the dilution that leads to the lysis of 50% of cells in the well (CH50).[8]

Statistical analysis were analysed using students t-test.

Results were expressed as mean (m)±standard deviation (S.D.)., a P.values that considered statically significant were 0.05,0.01,0.001, and 0.0005.[9]

Results & Discussion

It was interestingly notice that all studied groups (total BD patients, P. HLA-B51(5)+ve, P. HLA-B51(5)-ve, R., and P.C. groups) had a remarkable significant elevation ($P < 0.0005, 0.01$) in C3 & C4 levels as compared with healthy control groups. (Tables 1 & 2, Fig. 1 & 2)

Comparison between the study groups shown significant differences ($P<0.001$, 0.0005,0.001) in C3 level between patients groups versus R. & P.C. groups (Table 1)

Also significant differences ($P<0.05$, 0.001), were clearly observed regarding C4 level in P.HLA-B51(5) +ve & P.HLA B51(5) –ve versus R. & P.C. groups. (Table 2)

On the other hand, statistically significant elevation ($P<0.0005$, 0.01) in CH50 titer in P. HLP-B51(5) +ve, P.C. groups in comparison with H.C. group as shown in table 3, Fig.3. Comparison between studied groups showed significant differences ($P<0.001$, 0.001, 0.05) between P. HLA-B51(5)+ve, vs. P. HLA-B51(5)-ve, R., and P.C. groups.

In contrast non-significant difference ($P>0.05$) was found between P.HLA-B51(5)-ve, group vs. R., and P.C. group as shown in table 3.

Results of this work is comparable to what have been reported abroad10,11, these finding may serve as an additional risk factor for precipitating of this disease, though in general quantitation of complement components in serum may provides a useful information for the evaluation of certain disease states[13], and increased levels of these elements are associated with a wide variety of inflammatory disorders as part of the cute phase plasma protein response [13,14].

Whereas interesting finding demonstrated that there is a significant correlation between complement (C3), and polymorph nuclear neutrophils infiltration, suggesting that alternative complement pathway might be activated in BD, however this pathway is well known to be activated by non immunological elements such as lipoplysacharide, peptidoglycan, virus, and low heat shock protein , which might exacerbate BD.[15,16]

On the other hand, association of HLA-B51(5) with the disease susceptibility is well studied abroad & locally (sporadic & familial study).[1,2,16] Never the less B51(5) molecule could be the disease associated allele. [1,2,16,17]

We can conclude that increased level of CH50 which is reflected the total activity of the classical & lytic pathways, also C3 is work as pivotal factor in both classical & alternative pathways, though elevation of its level (C3) is an index of the disease activities which support that alternative pathway plays a role in the pathogenesis of this disease in Iraqi patients especially in those with HLA-B51(5), positive, this indicate that immunogenetic factors are deeply implicated in aetio- pathogenesis of this puzzling disease.

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Table 1: Concentration of C3(mg/dl) in the study groups

Complement component C3(mg/dl)	Study Groups					
	Total BD patients No.=70	Patients with HLA-B51(5)+ve N0.=39	Patients with HLA-B51(5) - ve No.=31	Relative No.=25	Patients control No.=30	Healthy control No.=30
Mean±S.D	193±33.73	196.16±35.39	189.68±31.01	177.57±25.35	161.97±29.62	135.52±12.38
t-value	9.05	8.94	8.89	8.18	4.58	-
P<	0.0005	0.0005	0.0005	0.0005	0.0005	-

C3(mg/dl)

Comparison between the study groups

P. HLA-B51(5)+ve vs. P.HLA-B51(5)-ve N.S.
P. HLA-B51(5)+ve vs. R. Sig. ($p<0.05$)
P. HLA-B51(5)+ve vs. P.C. H.S. ($p<0.001$)
P. HLA-B51(5)-ve vs. R. N.S.
P. HLA-B51(5)-ve vs. P.C. H.S. (0.001)
R vs. P.C. Sig. ($p<0.05$)

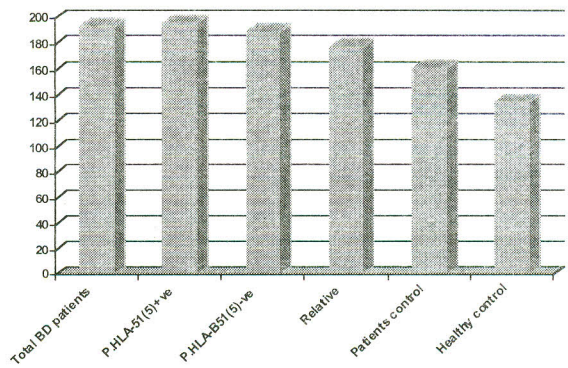

Fig. 1: levels of C3 in the study groups.

Table 2: Concentration of C4 (mg/dl) in the study groups

Complement component C4(mg/dl)	Study Group					
	Total BD patients No.=70	Patients with HLA-B51(5)+ve N0.=39	Patients with HLA-B51(5) - ve No.=31	Relative No.=25	Patients control No.=30	Healthy control No.=30
Mean±S.D.	43.69±15.22	44.78±14.99	42.09±15.25	33.9±8.21	34.96±8.97	29.66±4.21
t-value	4.96	5.53	4.32	2.5	3.09	-
P<	0.0005	0.0005	0.0005	0.01	0.005	-

C4(mg/dl)

Comparison between the study groups

P.HLA-B51(5)+ve vs. P.HLA-B51(5) N.S.
P.HLA-B51(5)+ve vs. R. sig. ($P<0.01$)
P.HLA-B51(5)+ve vs. P.C. sig. ($p<0.01$)
P.HLA-B51(5)-ve vs. R. sig. ($P<0.05$)
P.HLA-B51(5)-ve vs. P.C. sig. ($P<0.05$)
R vs..P.C. sig. ($P<0.05$)

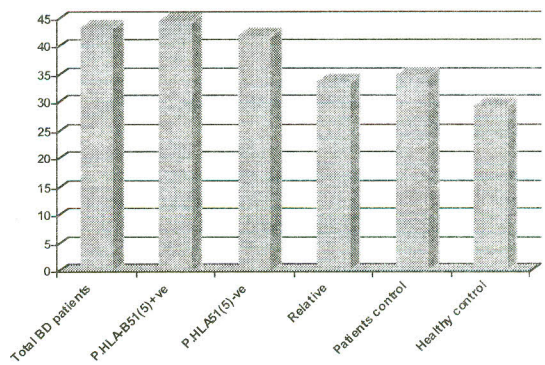

Fig. 2: levels of C4 in the study groups.

Table 3: Mean Reciprocals of CH50 Titers Among Different Study Groups

Titer of CH50	Study Groups					
	Total BD patients No.=70	Patients with HLA-B51(5)+ ve N0.=39	Patients with HLA-B51(5) - ve No.=31	Relative No.=25	Patients control No.=30	Healthy control No.=30
Mean ±S.D.	118.36±66.85	136.54±69.57	87.90±32.10	79.32±39.19	101.10±31.61	78.77±36.84
t-value	3.16	4.24	1.06	0.054	2.52	-
P<	0.005	0.0005	n.s.	n.s.	0.01	-

P.HLA-B51(5)+ve vs. P.HLA-B51(5)-ve H.S ($p<0.001$)
P.HLA-B51(5)+ve vs. R. H.S ($p<0.001$)
P.HLA-B51(5)+ve vs. P.C. sig. ($p<0.05$)
P.HLA-B51(5)-ve vs. R. N.S.
P.HLA-B51(5)-ve vs. P.C. N.S
R. vs. P.C. sig. ($P<0.05$)

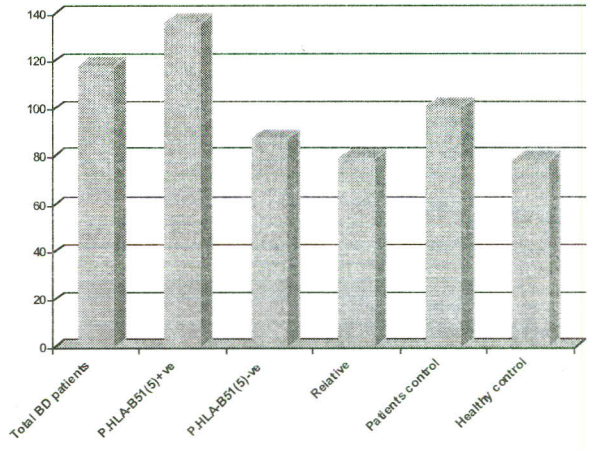

Fig. 3: Mean Reciprocals of CH50 Titers in study groups

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