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CLINICAL STUDY

Immunologic and psychosocial status in chronic fatigue syndrome

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Abstract: *Objective:* The aim of the study was to investigate the immunologic functions and psychosocial status in patients with chronic fatigue syndrome (CFS).
Methods: Twenty-five patients with CFS diagnosed by the international CFS definition criteria and 20 age- and gender-matched healthy controls were recruited. Depression was assessed by Beck Depression Inventory (BDI) and health status was assessed by Nottingham Health Profile (NHP).Monoclonal antibodies (MAbs) were measured to identify the following NK cell subsets: CD3, CD4, CD8 and CD56 and cytokine measurements were performed for IL2r, IL6 and IL8 in both patients and control subjects.
*Results:*The BDI and NHP scores of CFS group were found to be significantly higher than in the control group. The absolute numbers of CD56 cell were also significantly decreased in the patients with CFS compared with the healthy controls. There were no other significant differences of NK cell activity (CD3, CD4 and CD8) and there were significant differences in IL6 and IL2r levels between patients and controls. There were significant correlations between serum IL-6 level and sleep, social isolation and physical ability NHP subscores, and betweenCD56 NK cell activity and emotional reaction NHP sub score in CFS patients.
Conclusion: Significantly higher ratios of psychological and physical disturbances were found in patients with CFS. Decreased CD56 NK cell activity and increased IL2r levels seem to be important immunopathologic changes in CFS. IL-6 and CD 56 NK cell activity may play an important role in sleep, physical, social, and physicological manifestations of CFS *(Tab. 3, Fig. 1, Ref. 36).* Full Text in free PDF *www.bmj.sk.*
Key words: chronic fatigue syndrome, psychosocial status, CD56, cytokines.

Chronic fatigue syndrome has been defined as an illness of unknown etiology associated with severe debilitating fatigue lasting more than 6 months and a constellation of signs and symptoms often including chronic and recurrent low-grade fever, myalgia, migratory arthralgia, pharyngitis, adenopathy, sleep disturbances, and difficulties in cognition and temperament (1–4).

Although the etiology and pathogenesis of CFS are unknown, several studies have found a variety of immunologic abnormalities, including decreased NK cell cytotoxicity (5–7), reduced mitogenic response of lymphocytes (5, 6, 8, 9), increased expression of activation markers (6, 7, 10), and altered cytokine production (5, 6, 9, 11). However, since most of the changes are rather subtle, the pathophysiological significance and the relation with clinical symptoms or disease severity remain unclear (5).

IL-6 is a proinflammatory cytokine whose pleiotropic activities and role in the pathophysiology of various human diseases has been recently reviewed (12, 13). In the central nervous system, IL-6 is produced by a number of central loci and is a potent stimulator of the hypothalamic-pituitary axis (HPA). Overproduction of IL-6 may contribute to several inflammatory diseases and illnesses during aging and chronic stress. Administration of IL-6 results in fever, anorexia, and fatigue, some of the symptoms shared by CFS patients (12).

The relation between psychological and health status and immune response is not fully clear. Immune cells and their functions may be affected by psychological disturbances (4, 5, 14, 15).

In this study, we investigated the immunological functions and psychosocial status in patients with chronic fatigue syndrome.

Methods

Twenty-five patients diagnosed as CFS, according to the international CFS definition criteria, and 20 healthy controls were recruited from the outpatient clinic of Physical Medicine and Rehabilitation Department of Dicle University for this study. Fatigue assessment was done according to CDC criteria (16). Fatigue characteristics are persistent or recurrent lasting at least 6 months; recent and/or well defined onset; not secondary to excessive physical activity, or any organic or psychiatric disorder; not resolved by rest; and inducing important reduction of previous levels of physical and mental activities. All patients underwent medical screening that included physical examina-

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Tab. 1. Psycho and health status responses.

	Control (n=20)	CFS (n=25)	p
BDI	5.85± 7.132	18.49±11.87	,000
Energy level (Fatigue)	30.22±24.10	59.61±33.77	,002
Pain	26.33±22.12	55.13±28.55	,001
Emotional reaction	11.33± 4.17	23.59±15.77	,002
Sleep	19.26± 16.76	49.64±24.97	,000
Social isolation	16.43±9.06	46.39±23.61	,000
Physical abilities	14.79±14.52	32.14±18.07	,001

Tab. 2. Serum levels of soluble immune mediators, NK Cell Activity and Proportions of T Cell Subsets in CFS and control patients.

	Control (n=20)	CFS (n=25)	p
IL2r	525.40±157.55	638.80±170.15	,027
IL6	3.42±1.50	5.63±3.23	,007
IL8	11.95±7.99	10.40±4.78	NS
CD3	62.00±16.56	67.09± 9.12	NS
CD4	44.25±12.16	38.12±12.73	NS
CD8	25.70±8.09	26.56±8.60	NS
CD56	23.90±15.74	15.47± 9.46	,032

tion and relevant investigation, with a minimum of urine analysis, full blood count, measurement of urea, electrolytes, and erythrocyte sedimentation rate, and test for thyroid and liver function.

Subjects were excluded if they had active, unresolved, or suspected disease likely to cause fatigue; who had severe depression, schizophrenia, hypothyroidism, sleep apnea syndrome, narcolepsy, chronic viral hepatitis, malignancy and medication causing fatigue; and alcohol or other substance abuse within 2 years prior to onset of the chronic fatigue and any time afterwards; and a body mass index =45 (17).

All patients and controls were evaluated by structured psychiatric interview to exclude any additional psychiatric disorder prior to inclusion in the study. Depression rate was assessed by Beck Depression Inventory (BDI) in all patients and controls. Beck Depression Inventory is a 21-item self-reported inventory. The items consist of symptoms of depression. Some items are related to physical concerns such as sleep disturbances and loss of weight. Other items refer to emotional (hopelessness, sorrow), motivational (willingness to work), and cognitive aspects (concentration, self-evaluation). For each symptoms category, there are four sentences, and the subject chooses one according to his/her experience during the previous week. Each sentence is scored as 0, 1, 2, or 3. The sum of the points shows the level of depression. Test scores higher than 17 indicate a high level of depression (18). Health status was assessed by Nottingham Health Profile (NHP) (energy level, pain , emotional reaction , sleep, social isolation , physical abilities) 38 questions in 6 subareas, with each question in a given subarea adds up to 100 Completing questionnaire each question was answered „Yes“ or „No“, important that all questions are answered in all patients and controls. The

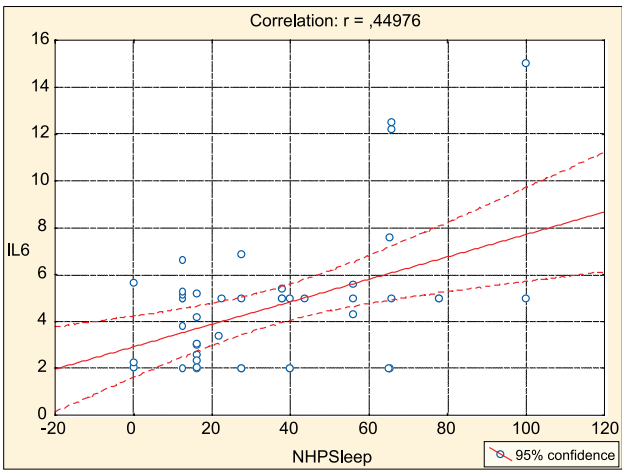

Figure 1. Correlation curve between serum level of IL6 and NHP sleep subscore in CFS Patients.

NHP is intended for primary health emotional, social and physical health problems (19). All prescription medications, included psychoactive and non-prescription medications, vitamins, and herbal remedies were tapered and stopped at least 2 weeks prior to study. All subjects and controls had no frank hypocortisolism on endocrine assessment. No patients and controls had received any oral or intraarticular corticosteroid therapy during the three months.

Venous blood (4mL) was taken in an EDTA-containing tube for white blood cell and differential counts. Heparinized blood was drawn on prepare lymphocytes immunophenotyping from whole blood for immunofluorescence measurements on optical flow cytometry with dual-color coulter immunoprep reagent system. Monoclonal antibodies (MAbs) were used to identify the following cell subsets: CD3, CD4, CD8 and CD56 (Beckman-coulter Epics-Xl flow cytometry systems, USA).

Blood samples for cytokine measurements were collected into 4-mL tubes. Cytokines were measured by IMMULITE 1000 analyzers (DPC Immulite 1000 Chemistry Analyzer, USA) for the quantitative measurement of interleukins (IL2r, IL 6 and IL 8) in serum.

Statistical analysis was done by SPSS 13 PC program. Independent t test was used to assess differences between CFS patients and the healthy control subjects. For correlation analysis, the Pearson’s test was used. A $p<0.05$ was accepted statistically significant. Data are given as mean ± SD.

Results

CFS cases (n=25) had a mean age of 33.4±8.3 (19–60) years (4 male and 21 female), while the mean age of controls (n=20) was 32.9±7.8 (18–60) years (6 male and 14 female).

The BDI and NHP (energy level, pain, emotional reaction, sleep, social isolation and physical abilities) scores of CFS group were all signicantly higher than the corresponding data from the control group (Tab. 1).

Tab. 3. Correlations between cytokines, T Cell subsets, NK cell, BDI and NHP in CFS patients.

	BDI	IL2r	IL6	IL8	CD3	CD4	CD8	CD56	NHP -EL	NHP- P	NHP-ER	NHP -S	NHP -SI
IL2r r= P=	0,069 0,04												
IL6 r= P=	0,307* 0,040	0,567** 0,000											
IL8 r= P=	0,110 0,473	0,066 0,668	0,062 0,688										
CD3 r= P=	0,146 0,338	0,138 0,364	0,105 0,493	0,063 0,680									
CD4 r= P=	0,110 0,473	0,184 0,225	0,155 0,310	0,029 0,851	0,284 0,059								
CD8 r= P=	0,162 0,287	0,191 0,209	0,103 0,503	0,068 0,657	0,076 0,621	0,234 0,122							
CD56 r= P=	0,86 0,573	0,032 0,837	0,100 0,515	0,050 0,745	0,102 0,506	0,085 0,580	0,182 0,231						
NHP- EL r= P=	0,343* 0,021	0,143 0,348	0,067 0,660	0,133 0,384	0,040 0,794	0,077 0,616	0,100 0,513	0,149 0,327					
NHP- P r= P=	0,391** 0,008	0,160 0,294	0,281 0,062	0,002 0,987	0,061 0,690	0,156 0,306	0,208 0171	0,061 0,691	0,417* 0,004				
NHP-ER r= P=	0,198 0,192	0,159 0,298	0,058 0,703	0,0150 0,0324	0,199 0,191	0,140 0,360	0,259 0,086	0,329* 0,027	0,124 0,417	0,461** 0,001			
NHP-S r= P=	0,405** 0,006	0,403** 0,006	0,450** 0,002	0,173 0,257	0,148 0,332	0,070 0,646	0,214 0,159	0,249 0,099	0,334** 0,025	0,368* 0,013	0,142 0,351		
NHP-SI r= P=	0,308* 0,04	0,200 0,188	0,406** 0,006	0,140 0,360	0,254 0,092	0,142 0,351	0,104 0,498	0,279 0,064	0,299* 0,046	0,368* 0,013	0,303* 0,043	0,460* * 0,001	
NHP-PA r= P=	0,505** 0,000	0,171 0,261	0,407 0,005	0,027 0,862	0,019 0,90	0,192 0,206	0,295* 0,049	0,046 0,764	0,281 0,061	0,643** 0,000	0,226 0,136	0,238 0,115	0,312 * 0,037

The absolute numbers of CD56 cells were found to be significantly decreased in patients with CFS compared with the control group (p=0.032). The total numbers of CD3, CD4 and CD8 lymphocytes did not differ between CFS patients and the controls. The proportions of CD3 and CD8 are slightly increased and CD4 decreased in patients with CFS than in control group, but it did not reach a statistically significant level. Circulating concentrations of IL6 and IL2r cytokines were increased in patients with CFS in comparison to control groups; there was statistically significant difference in IL6 (p=0.007) and IL2r levels (p=0.027) (Tab. 2).

Correlation curve between serum level of IL6 and NHP sleep subscore in CFS patients is shown in Figure 1. There was a positive correlation between serum level of IL2r, IL6 and NHP sleep subscore in CFS patients (r=0.403, p=0.006, r=0,450, p=0,002, respectively). There were also negative correlations between serum level of CD56 and NHP emotional reaction subscore in CFS patients (r=0.329, p=0.027) (Tab. 3).

Discussion

Patients with CFS tend to have more frequent depression (4, 15), nearly 50 % of the patients had major depressive episodes and psychiatric histories before onset (4, 19). These studies suggest the possibility that there is an underlying psychological predisposition toward the development of CFS (20).

Psychiatric factors and immune functions of hospitalized patients with chronic fatigue have been reported (4, 21), there are no published reports on psychosocial status in patients with chronic fatigue syndrome. We investigated the immunologic functions and psychosocial status in patients with chronic fatigue syndrome. In our study, we compared the patients with the control group with regard to psychosocial status, and we found a higher depression rate in depression inventory and also higher frequencies of fatigue, pain, sleep, social isolation and physical abilities and emotional reaction scores in NHP subscales in pa-

tients with CFS as compared with that in controls. Correlation between BDI and NHP subscale (higher score pain, physical abilities, sleep and slightly scores energy level, social isolation) were statistically significant. In terms of psychosocial status responses, CFS had low physical social ability levels and higher depressive tendencies.

In previous study, bivariate correlation between life event stress and NK cell activity was not found significant, but self-reported psychiatric symptoms, such as anxiety and depression, were found inversely correlated with NK cell activity (4, 22). In other hand, the effects of depression on immune function may be relatively more important than effects of associated life events (4, 23). The severity of depressive symptoms was associated with an impairment of NK cell activity. (14). We found significant correlation between NK cell activity (CD56) and NHP emotional reaction subscores.

Low levels of NK activity in patients with CFS have been frequently reported in the literature (1, 23, 24). Low NK cell activity and decreased numbers of CD56 cells had been showed in a previous study (4). Several groups have reported that NK cell numbers and functions are depressed in persons with CFS (24–26). Barker et al. found decreased CD8+ and CD56+ cells with CFS, as compared with health individuals (27). Mihaylova et al reported significantly lower NK CD56 cells in CFS patients than in healthy subjects (28). Decreased CD56 cells do suggest impairment of NK cell kinetic activity (6). Masuda et al. reported that fatigue-nonCFS and CFS groups had decreased NK cell number and function, but degree of changes in these factors were slightly lower in the fatigue-non CFS group. Therefore, they suggested that fatigue-non CFS group may be an intermediate state (4). In our study, CFS patients had significantly marked decreased NK cell activity in CD56 levels compare with the control group, but there was no relationship with fatigue subscores of NHP.

There is a close relationship between IL-2r and CD56 NK cell which was shown in a recent study of daclizumab, a humanized mAb directed against the IL-2r that strongly reduced brain inflammation in multiple sclerosis patients (29). In this study, daclizumab therapy was associated with a gradual decline in circulating CD4 and CD8 T cells and significant expansion of CD56 bright natural killer (NK) cells *in vivo*, and this effect correlated strongly with the treatment response. In our study, CD56 NK cell activity was reduced and IL-2r levels were increased in CFS patients. CD56 known as Neural Cell Adhesion Molecule (NCAM) is expressed on the surface of neurons, glia, skeletal muscle and natural killer cells (30). In addition, decreased CD56 NK cell activity may be also related with virus infection. But it remains to be investigated whether this decrease occurs as a result or as an underlying mechanism. However, treatment to increase CD56 NK cell activity may have some hopeful effect in managing CFS.

A number of immunological abnormalities, including alterations in the number and function of T cells and natural killer cells, and cytokine production, have been reported in CFS (12, 28, 31, 32). It has been suggested that cytokines play a role in the pathogenesis and clinical manifestations of CFS (28, 33). Some investigators have reported conflicting results of cytokine levels in the serum and culture supernatants in CFS patients (12, 28, 31, 34). The reason for these discrepancies could be due to difference in captured antigen used for ELISA assay, and the effect of certain serum blocking factors, including soluble receptors and inhibitors of cytokines (28, 35). Since cytokines have been proposed as mediators of the symptoms of CFS, it is important to be able to detect cytokine differences between cases and controls (24).

Chao et al observed increased IL-6 with CFS (9). In the present study, there were increased IL6 levels and positive correlations between NHP sleep subscore and IL6 were detected. Gupta et al. showed increased IL-6 production by mononuclear cells in CFS as compared to controls (33). However, the relationship between cytokines, including IL-6, with clinical symptoms of CFS has not been more explored. Later, Gupta et al have shown that both spontaneous and PHA- and LPS-induced IL-6 production by lymphocytes and monocytes is increased during the stages when patients felt 'fatigued' as compared to those when they felt 'rested' (12). Because of the suggested role of cytokines in clinical manifestations of CFS, we investigated IL-6 according to depressive mood, physical and social behavior of CFS. We found that IL6 increasing in level related with higher depressive mood and physical ability, sleep and social isolation scores. This indicated that IL6 is an important effective factor. These data suggest that increased IL-6 may play a role in the CFS symptomatology, especially poor physical, social and physiological course of CFS

In conclusion, there were significantly higher ratios of psychological and physical disturbances in patients with CFS than health controls. There were significant differences in NK cell activity (CD56) and cytokine levels (IL6 and IL2r). IL-6 and CD 56 NK cell activity may play important role in the development of clinical manifestations (especially poor physical, social and physiological activity) of CFS. Decreased CD56 NK cell activity seems to be important immuno-pathophysiologic mechanism of CFS development and treatment targeting to increase CD56 NK activity may have a promising effect for CFS managing. Further biomolecular and clinical studies are needed to understand the underlying immunopathologic mechanisms of psychosocial disturbances and low general physical activity levels (energy loss) in CFS and to manage this obstinate disorder successfully.

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