

ORIGINAL ARTICLE

Differential Pattern of Cytokine Production by Depressed Medical Students; Evidence for Involvement of Cytokine Network in Pathology of Depression

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SUMMARY

Background: Previous studies revealed that the immune responses of depressed patients can be affected by alteration of immune system factors; however, the immune genes mainly influenced are yet to be fully understood. Therefore, the main aim of present study was to identify serum levels of drastic inflammatory cytokines including IL-17A, IL-12, and IL-6 as well as anti-inflammatory cytokines, IL-10 and TGF- β , amongst Iranian depressed medical students.

Methods: Peripheral blood specimens were collected from 38 Iranian medical student patients with moderate and severe depression along with 43 healthy students as control subjects. The serum levels of IL-17A, IL-12, IL-6, IL-10, and TGF- β were assessed using the ELISA technique.

Results: Our results showed that the serum IL-10 level was significantly ($p = 0.011$) decreased in depressed patients (2.8 ± 0.41 pg/mL) compared to healthy controls (4.3 ± 0.4 pg/mL). The results also revealed that serum levels of TGF- β were significantly increased in severely (12.75 ± 5.22) compared to moderately (5.3 ± 0.7) depressed patients ($p = 0.045$).

Conclusions: According to the results of the present study, the decreased IL-10 level in the depressed patients may be responsible for the induction of inflammation in Iranian depressed patients. Additionally, increased serum levels of TGF- β in severely compared to moderately depressed patients may be related to normal immune responses against inflammation in severely depressed patients.

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KEY WORDS

depression, inflammation, IL-17A, IL-12, IL-6, IL-10, TGF- β

LIST OF ABBREVIATIONS

IL - interleukin
TGF - tumor growth factor
HBV - Hepatitis B virus
HCV - Hepatitis C virus
Th - T helper
HRP - horse radish peroxidase
TMB - 3,3',5,5'-tetramethylbenzidine
TNF - tumor necrosis factor

IFN - interferon
 TLR - toll like receptor
 DAMP - damage associated molecular pattern
 NF- κ B - nuclear factor kappa-light-chain-enhancer of activated B cells
 AP-1 - activator protein 1
 IRF3 - interferon responding factor 3
 IRF7 - interferon responding factor

INTRODUCTION

Sufferers of depression present with altered immune response profiles varying from suppressed immune responses to induced risk of inflammation [1]. Chronic infections are frequently reported within depressed patients [2,3]. Several immune related diseases including asthma, diabetes, multiple sclerosis, and cancers are associated with depression [4]; hence, it appears that depression can affect several arms of immune responses. The main responsible mechanism(s) leading altered immune related molecule expressions during depression has yet to be clarified.

Previous studies evidenced that cytokines play pivotal roles in the regulation of immune responses as well as the pathogenesis of immune related diseases [5]. Altered patterns of immune responses are observed among depressed patients [6], and this is mediated by cytokines as the main components of immune system which may be involved in the pathogenesis of depression.

IL-10 and TGF- β are described as the main anti-inflammatory cytokines [7], which play crucial roles in the regulation of immune responses during various immune functions such as self tolerance, inflammation, homeostasis, and following microbe elimination [8]. In contrast, IL-17A is a major member of early immune responses against fungal and bacterial infections and is also strongly associated with autoimmune and inflammatory diseases [9]. IL-17A is also associated with the immunopathogenesis of several chronic inflammatory states including fibrosis as well as cirrhosis following chronic HBV and HCV infections [10]. Therefore, increased expression of IL-17 subsequent to immune related diseases including depression can be considered as a biological marker for affected immune responses. Additionally, IL-6 not only is involved in the development of Th17 (the main source of IL-17A) but also induces inflammation directly by mediating increased expression of addressing molecules on the endothelial cells and their corresponding homing molecules on the immune cells [11]. IL-12 is widely produced by innate immune cells such as macrophages and dendritic cells and leads to Th1 lymphocyte activation [12]. According to the crucial roles played by IL-10 and TGF- β in immune regulation as well as inflammatory effects of IL-17A, IL-12, and IL-6, we hypothesized that the cytokines probably play important roles in the pathogenesis of depression. Furthermore, due to the discrepancies regarding serum levels of pro/anti-inflammatory cyto-

kines in depression, and due to the fact that medical students are an at risk population in which depression is prevalent based on the pressure of studying, various examinations, and work conditions, the main aim of this study was to evaluate the serum levels of IL-10, IL-6, IL-12, IL-17A, and TGF- β in depressed medical students (patients).

MATERIALS AND METHODS

Subjects

This cross sectional study was initially performed on four hundred undergraduate students studying at Rafsanjan University of Medical Sciences, Rafsanjan, Iran. All of the participating students filled out the standard questionnaire and the event of depression and its staging were diagnosed in 38 students based on the scores obtained by the Beck depression inventory (BDI) test questionnaire [13] and, of course, clinical symptoms determined by a psychologist. None of the patients had received antidepressants or immunomodulator drugs and no depressive episodes were reported regarding the participants. Peripheral blood samples were harvested from the 38 depressed students and 43 healthy controls in 5.5 mL tubes. A group of age and gender matched controls were also selected within undergraduate students. Smoking behavior, alcohol abuse, immune system related diseases, and also immune system influencing drug consumption (e.g., corticosteroids) were considered as excluding criteria for the controls and patients. Serum samples were isolated from whole blood as soon as samples entered the laboratory and stored at -20°C for future use. The protocol of this study was approved by the local ethical committee of the Rafsanjan University of Medical Sciences, Rafsanjan, Iran. The participants also filled out the informed consent prior to sample collection.

Detection of cytokines

The ELISA technique was used for the determination of TGF- β , IL-17A, IL-12, IL-6, and IL-10 using commercial kits (eBiosciences, Esp) according to the manufacturer's guidelines. Briefly, serum and corresponding standards were added to the anti-cytokine antibody pre-coated plates and after 2 hours incubation at room temperature the plates were washed. Biotin-conjugated anti-cytokine antibodies were added in the next stage and after appropriate incubation, the plates were washed, then HRP-conjugated Avidin was added and washed after 2 hours. TMB in association with H_2O_2 was used as substrate and the reaction was stopped after 15 minutes by H_2SO_4 2N. Then the products were analysed using an ELISA reader system model 680 (BioRad, USA). Data were only used when the inter- (the replicas of the same samples on a plate) and intra- (the replicas of the same samples on different plates) assays produced scores of CV < 14% and CV < 3%, respectively.

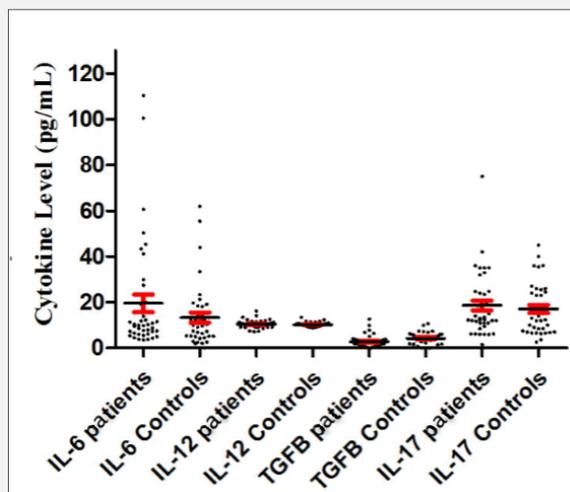


Figure 1. The serum levels of IL-6, IL-12, TGF-β, and IL-17A in depressed patients and healthy controls. The figure illustrates that the serum levels of studied cytokines did not differ between depressed patients when compared to healthy controls.

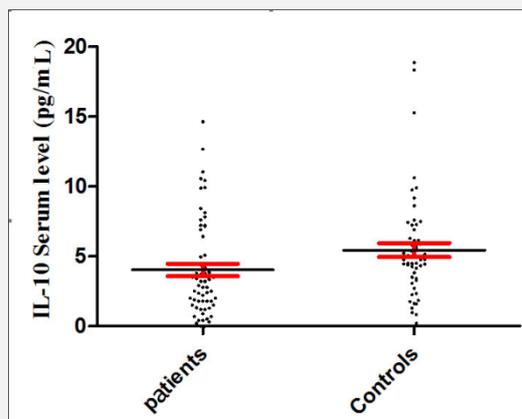


Figure 2. The serum levels of IL-10 in depressed patients and healthy controls. The figure illustrates that the serum levels of IL-10 were significantly decreased in depressed patients when compared to healthy controls.

Data analysis and statistical methods

The *t*-test under SPSS software version 18 was used for data analysis and the differences were considered significant when *p* values were less than 0.05.

RESULTS

Present results demonstrated that 9.5% of the examined students (38 out of 400) were suffering from depression (26 moderately and 12 severely depressed patients). We also observed that the serum levels of IL-6 (*p* = 0.542), IL-12 (*p* = 0.273), IL-17A (*p* = 0.563), and TGF-β (*p* = 0.49) were not different in patients when compared to

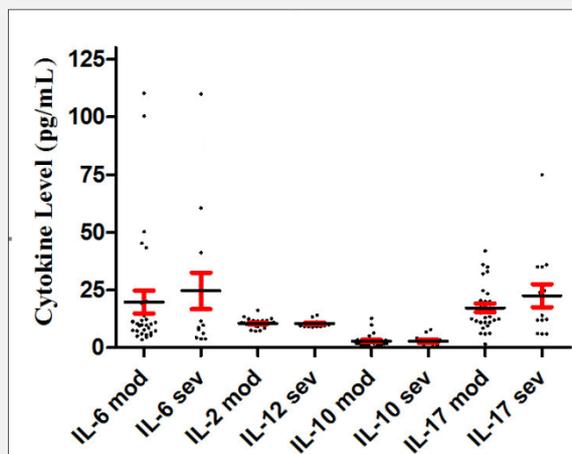


Figure 3. The serum levels of IL-6, IL-12, IL-17A, and IL-10 in severely (sev) and moderately (mod) depressed patients. The figure illustrates that the serum levels of the cytokines did not differ between groups.

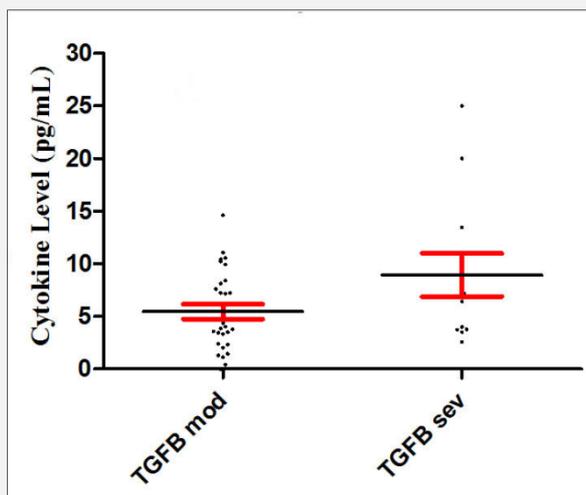


Figure 4. The serum levels of TGF- β in severely (sev) and moderately (mod) depressed patients. The figure illustrates that serum levels of TGF- β were significantly increased in severely (sev) when compared to moderately (mod) depressed patients.

controls (Figure 1). Our results also showed that serum levels of IL-10 were significantly decreased in depressed patients compared to healthy controls ($p = 0.011$, Figure 2). Results also indicated that serum levels of IL-6 ($p = 0.393$), IL-12 ($p = 0.458$), IL-17A ($p = 0.529$), and IL-10 ($p = 0.917$) were not different between moderately and severely depressed students (Fig-

ure 3), while serum levels of TGF- β were increased by approximately two fold in severely compared to moderately depressed patients ($p = 0.045$) (Figure 4). Our results demonstrated that depressed patients and healthy controls were 24 ± 3 and 25 ± 3 years old, respectively, which was not significant ($p = 0.98$). The results also revealed that 17 (44.7%) and 21 (55.3%) of the patients

and 22 (51.16%) and 21 (48.84%) of healthy controls were male and female, respectively. The statistical analysis showed that the groups were successfully matched ($p = 1.0$). The results also showed that serum levels of IL-6, IL-12, IL-17A, and TGF- β were 23.1 ± 4.8 , 9.2 ± 0.36 , 20.5 ± 3.12 , and 8.9 ± 1.02 pg/mL in female patients and 26.1 ± 6.8 , 11.2 ± 0.27 , 17.9 ± 2.24 , and 6.7 ± 1.35 pg/mL in male patients, respectively. The statistical analysis showed that the difference between female and male patients was not significant ($p = 0.891$).

DISCUSSION

Our results revealed that the serum levels of IL-10 were significantly reduced in the depressed medical students compared to healthy controls. Therefore, based on our results, it can presumably be concluded that depressed (students) patients were unable to produce adequate levels of IL-10 (compared to healthy controls) and this may lead to the development of inflammation in the depressed patients.

This fact is in agreement with previous studies which identified that depressed patients suffer from chronic inflammation [14]. For example, Raison et al. reported that peripheral inflammatory biomarkers were increased in the depressed patients [1]. Several studies also reported that inflammation can induce depression [15,16]. Interestingly, an elevated Th1/Th2 ratio was reported by Li and colleagues [17]. Dhabhar et al. demonstrated that serum levels of IL-10 were decreased, while IL-6 was not changed in the depressed patients when compared to healthy controls [18]. Li et al. reported a decreased number of T regulatory lymphocytes (the main source of IL-10) in depressed patients compared to healthy controls [17]. Song and colleagues revealed that serum levels of IL-1beta were increased and, inversely, IL-10 was decreased in the depressed patients [19]. In contrast with our and the aforementioned studies, Lehto et al. demonstrated that serum levels of IL-10 were not different between depressed patients and healthy controls [20]. It appears that low sample size (14 cases) and different ethnicity of participants the study of Letho and colleagues are responsible for the different results. Therefore, it may be concluded that IL-10 plays an important role in the pathogenesis of depression.

Although serum levels of IL-10 were decreased in our patients, the serum levels of IL-12, IL-17A, and IL-6 as inflammatory cytokines were not different between patients and controls. In another experimental based study, Chen et al. revealed that the number of Th17 cells was increased in the depressed patients [21]. On the other side of this scenario, another study demonstrated that serum levels of the proinflammatory cytokines including IL-2, IL-12, and TNF-alpha were significantly increased, while IL-4 and TGF-beta1, as the members of the anti inflammatory cytokines, were increased in patients with major depression compared to healthy controls [22]. Kim and colleagues also identified that de-

pressed patients had significantly lower IFN- γ and IL-4 and higher TGF- β serum levels than healthy controls [23]. Elevated serum levels of IL-12 but not TGF- β in depressed patients also were reported by Lee KM and colleagues [24]. Decreased serum levels of TGF- β among depressed patients were reported by Musil and colleagues [25]. Interestingly, in contrast to Musil et al., our results showed that serum levels of TGF- β were increased in severe compared to moderate depression. This discrepancy might be due to the different types of depression for which we have compared serum levels of TGF- β between severely and moderately depressed patients, while Musil et al., studied it between depressed patients and healthy controls. Song et al. have reported that serum levels of IFN- γ were decreased in depressed patients [19].

Based on the controversial reports (ours and other studies) it seems that IL-10 can be considered as an important candidate where expression is disrupted in depression; however collectively, it appears that the main responsible mechanism(s) regarding the relation between depression and cytokines is yet to be determined and a definitive conclusion is very difficult.

Several possible explanations for discrepancy between the studies are as follow: 1. The participants in the aforementioned studies were of different ethnicity, 2. The patients were probably infected with occult infections which were difficult to recognize should have been excluded from the studies, hence, cytokine patterns were altered, 3. Cytokines act in network conditions, hence, alteration in a cytokine expression affect others. Additionally, the relatively small sample size of our study may be another reason for discrepancy between our and other studies.

Therefore, it may be concluded that other features of immune responses rather than cytokines deserve to be taken into account in depression.

Previous studies demonstrated that TLR/DAMP interactions lead to activation of transcription factors such as NF-kB, AP-1, IRF3, and IRF7 [26]; hence, the assessment of these transcription factors can be informative. Interestingly, we previously reported that NF-kB was decreased in depressed patients compared to healthy controls [6]. Thus, it is likely the inflammatory responses in the depressed patients might be regulated via other transcription factors. Therefore, it is possible that other transcription factors including IRF3, IRF7, and AP-1 may be elevated as well as activated in depressed patients.

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Declaration of Interest:

The authors of this manuscript have no interest in products described in this article. The authors have no conflicts of interest.

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