Defective NF-kB Transcription Factor as the Mediator of Inflammatory Responses: A Study on Depressed Iranian Medical Students

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SUMMARY

Background: NF-kB is a transcription factor that is a downstream target of several cell signaling systems including TLRs. Defective expression of the molecule can lead to inappropriate immune responses. Previous studies revealed that depression can affect immune responses, but its molecular mechanisms are yet to be fully understood. Thus, the main aim of this study was to identify if mRNA levels of NF-kB are changed in the PBMCs isolated from Iranian depressed medical students.

Methods: This cross-sectional study was done on 38 Iranian depressed medical students and 43 healthy students as a control group. The mRNA levels of NF-kB were assessed in parallel with beta-actin (as the housekeeping gene) using Real-Time PCR technique.

Results: Our results showed that mRNA levels of NF-kB were significantly decreased in isolated PBMCs from depressed patients compared to healthy controls.

Conclusions: According to the results obtained in the present study, it seems that depressed patients are unable to appropriately express NF-kB at mRNA levels which may in turn lead to defective molecule expression.


KEY WORDS

depression, NF-kB, Real-Time PCR

INTRODUCTION

Patients showing depression behavior exhibit altered immune responses from inducing inflammation to suppressed immune responses [1]. Incidences of chronic infections were reported repeatedly in depressed patients [2,3]. Furthermore, several autoimmune diseases such as multiple sclerosis as well as cancers are associated with depression [4] and, hence, it has been proposed that depression can affect several types of immune responses. The main responsible mechanism(s) in alteration of immune responses as well as affected immune related molecules during depression have yet to be fully understood.

Toll like receptors (TLRs) as the main intra/extra-cellular innate immune cell receptors, recognize pathogen associated molecular patterns (PAMPs) and damage associated molecular patterns (DAMPs) [5]. The active interaction of TLRs with their corresponding ligands re-
sults in activation of intra-cytoplasmic and nuclear signaling molecules to induce several immune cell functions including expression of inflammatory cytokines [6], migration [5], NADPH oxidase activation [7], and phagocytosis [8]. TIR-domain-containing adapter-inducing interferon-β (TRIF), myeloid differentiation primary response (MYD88), interleukin-1 receptor associated kinase-1 (IRAK1) and tumor necrosis factor receptor associated factor (TRAF6) are amongst the main TLR intracellular signaling pathway molecules that induce nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) activation [9]. NF-κB, as the known pro-inflammatory transcription factor, recognizes consensus elements present in the regulatory region of inflammatory mediators such as cytokines and co-stimulatory molecules [9]. Therefore, defective NF-κB expression may lead to altered immune responses. According to the crucial roles played by NF-κB in the induction of immune responses, it may be hypothesized that it may also play important roles in the pathogenesis of depression. Therefore, the main aim of this study was to evaluate the mRNA levels of NF-κB in the peripheral blood mononuclear cells (PBMCs) of depressed medical students (patients).

MATERIALS AND METHODS

Subjects
Four hundred undergraduate students from Rafsanjan University of Medical Sciences participated in the study and filled out the standard questionnaire. Depression was diagnosed in 38 students by an expert psychologist based on the scores obtained by questionnaire and also clinical symptoms. Peripheral blood samples were collected from the depressed students (38 cases) and 43 healthy controls in 5.5 mL tubes which were previously pre-coated by EDTA as anti-coagulant. Controls also were selected from the undergraduate students with the same age and gender. All of the controls were also visited by psychologists and followed up for a duration of six months to exclude the presence of either psychological symptoms and past history including criteria for entering to the study. The excluding criteria for the controls and patients were smoking, alcohol abuse, immune system related diseases and also immune system influencing drug consumption (e.g., corticosteroids). RNA was extracted from the samples immediately following entrance to the laboratory. This study was approved by the regional ethical committee of the Rafsanjan University of Medical Sciences, Rafsanjan-Iran. A written informed consent was obtained from all participants prior to sample collection.

RNA extraction and reverse transcription
Total RNA content of PBMCs was extracted using a RNA extraction kit from Cinnaclon Company (Tehran, Iran). The RNA quality was determined by both electrophoresis on ethidium bromide pre-treated formalin/formaldehyde gel and measurement of absorption at 260/280 nm by spectrophotometric analysis. DNase-I was used to remove possible genomic DNA contaminations before cDNA synthesis. cDNA was synthesized using a cDNA synthesis kit (Parstous, Iran) using both oligo (dT) and random hexamer primers (Aryatous, Iran). The following program was set on the Bio-Rad system model CA1000 for reverse transcription: 70°C for 10 minutes (without reverse transcription enzymes), -20°C for 1 minute (cooling), 42°C for 60 minutes (added reverse transcription enzymes), and 95°C for 10 minutes (reverse transcription enzyme inactivation).

Quantitative Real-Time PCR
Quantitative Real-Time PCR was performed using a SYBR green master mix (Parstous Co. Tehran, Iran), 200 ng of produced cDNA, and 2 pg/µL of appropriate primers (Table 1). The following program was set on the BIO-RAD CFX96 system (Bio-Rad Company, USA): one cycle of 95°C for 15 minutes, 40 cycles of 95°C for 30 seconds, 60°C for 30 seconds, and 72°C for 30 seconds. Real-Time PCR was carried out in triplicate and the β-actin as the housekeeping gene was used for normalization of amplification signal of target genes. The relative amounts of PCR product were determined using the 2^(-ΔΔCt) formula. The dissociation stages, melting curves, and quantitative analyses of the data were performed using CFX manager software version 1.1. 308.111 (Bio-Rad, USA).

NB: PCR products were also electrophoresed on 1% gel containing 0.5 mg/mL ethidium bromide to visualize and check the size of the PCR product.

Real-Time PCR was performed for all of the depressed samples in one run and healthy controls in another run. Data were only used when the inter- and intra-assays produced scores of CV < 3% and CV < 0.3%, respectively. The inter-precision had a CV < 0.3%.

Data analysis and statistical methods
The parametric statistical analyses were performed using the t-test using SPSS software version 18. A p value less than 0.05 was considered significant.

RESULTS

Our results revealed that 38 out of 400 participating students (9.5%) were suffering from depression (26 moderate and 12 severe depressed patients).

Our results also revealed that the mRNA expression levels of NF-κB were significantly decreased by 5.45-fold in depressed students when compared to healthy controls (p < 0.023, Figure 1).

Current results also showed that mRNA levels of NF-κB were not significantly different between moderate and severe depressed students (p < 0.41, Figure 2).
Table 1. Primer sequences of evaluated genes.

<table>
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<tr>
<th>Target gene</th>
<th>Primer sequences</th>
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<tbody>
<tr>
<td>NF-κB</td>
<td>F: 5′-TCTCCCTGGTCACAAAGGAC-3′</td>
</tr>
<tr>
<td></td>
<td>R: 5′-TCATAGAAGCCATCCCAGGC-3′</td>
</tr>
<tr>
<td>β-Actin</td>
<td>F: 5′-GGCACCACACACATTGAAG-3′</td>
</tr>
<tr>
<td></td>
<td>R: 5′-CCGATCCACACGGAGTACTTG-3′</td>
</tr>
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</table>

Figure 1. The figure illustrates that the mRNA expression levels of NF-κB were decreased in depressed students in comparison to healthy controls.

Figure 2. The figure illustrates that the mRNA expression levels of NF-κB were significantly decreased in severe compared to moderately depressed students.

**DISCUSSION**

NF-κB is a main transcription factor involved in transcription of inflammatory cytokine and co-stimulatory genes [10,11]. Our results revealed that the mRNA levels of NF-κB were significantly reduced in the depressed medical students when compared to healthy controls. Therefore, based on our results it can be concluded that depressed subjects (student patients) express lower levels of mRNA NF-κB than healthy controls possibly.
leading to failed appropriate immune responses in these patients. To the best of our knowledge this is the first study which evaluated the mRNA levels of NF-kB in the depressed patients. Interestingly, previous studies demonstrated that depressed patients present chronic inflammation [12]. Elevated levels of peripheral inflammatory biomarkers in the depressed patients were reported by Raison et al. [1]. Further studies also demonstrated that inflammation can induce depression [13,14]. There has been also evidence that a reciprocal relation between inflammation and depression exists and studies have shown that the inflammation may also positively affect depression [15]. Therefore, it seems that there is a potential association between inflammation and depression. With regard to our results, NF-kB is decreased in the depressed patients. Thus, it seems that the inflammatory responses mediated by cytokines in the depressed patients might be regulated through other transcription factors, including interferon regulatory transcription factor 3 (IRF3) and IRF7, which alternatively regulate transcription of inflammatory genes. Therefore, it is possible that these transcription factors may be elevated and activated in depressed patients. Therefore, more studies are required to elucidate the exact role played by other related transcription factors regulating these genes’ expression. Interestingly, previous studies revealed that the NF-kB upstream signaling molecules such as MYD88 also can induce several brain disorders including anorexia and depression-like behavior [16,17]. Therefore, it is not far to conclude that NF-kB, its upstream molecules, and other transcription factors may play important roles in pathogenesis of depression.

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Declaration of Interest:
The authors of this manuscript have no invested interests in products described or used in this article. The authors have no conflicts of interest.

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