

The effect of depression and anxiety on expression levels of toll like receptor signaling molecules in chronic HBV infected patients

Mehdi Nikbakht Dastjerdi¹, Mohammad Momeni², Reza Bidaki³
Mehdi Khaleghinia⁴, Masoud Karimi-Googheri⁵, Mohammad Kazemi Arababadi⁶
Ahmad Shabanizadeh⁷

Received: 14 January 2014

Accepted: 28 June 2014

Published: 16 February 2015

Abstract

Background: Toll-like receptors (TLRs) play an important role in the recognition of DAMPs and PAMPs and induction of inflammation. Previous studies demonstrated that depression and anxiety can influence the expression levels of immune related molecules. Our previous study revealed that mRNA levels of IRAK4, TRAF3 and IRF7 were significantly decreased in chronic HBV infected (CHB) patients when compared to healthy controls. Therefore, the aim of this study was to evaluate the effects of depression and anxiety on the expression levels of these molecules in CHB patients.

Methods: Sixty CHB patients participated in this study and filled out the standard questionnaires; and the expression of IRAK4, TRAF3 and IRF7 were examined using Real-Time PCR techniques.

Results: The results of this study demonstrated that expression of IRAK4, TRAF3 and IRF7 did not differ between patients with various stages of depression and anxiety (all $p > 0.05$).

Conclusion: According to the results, it seems that declined expression of IRAK4, TRAF3 and IRF7 in CHB patients were not related to depression and anxiety, and other factors including genetic and immunoregulatory effects of HBV may be responsible for the declined expression of these molecules.

Keywords: Depression, Anxiety, Chronic HBV infection, IRAK4, TRAF3, IRF7.

Cite this article as: Nikbakht Dastjerdi M, Momeni M, Bidaki R, Khaleghinia M, Karimi-Googheri M, Kazemi Arababadi M, Shabanizadeh A. The effect of depression and anxiety on expression levels of toll like receptor signaling molecules in chronic HBV infected patients. *Med J Islam Repub Iran* 2015 (16 February). Vol. 29:180.

Introduction

In general, chronic hepatitis B infection is a type of liver disease in which the body is notable to completely eliminate the virus from the liver and serum (1). The main mechanism responsible for the lack of an appropriate immune response against

chronic hepatitis B infection is still unknown. It seems that the profile of the host immune, genetic and epigenetic factors is responsible for the differences between groups of people with and cured of the disease (2-5).

The main intra/extra-cellular immune cell receptors, toll-like receptors (TLRs), identi-

1. PhD in Anatomy, Department of Anatomical Sciences and Molecular Biology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran. nikbakht@med.mui.ac.ir

2. MSc in Immunology, Immunology of Infectious Diseases Research Center, Rafsanjan University of Medical Sciences, Rafsanjan, Iran. Immunology85@yahoo.com

3. MD, Department of Psychiatry, Research center for Addiction and Behavioral Sciences, Shahid Sadoughi University of Medical Sciences, Yazd, Iran. reza.bidaki111@yahoo.com

4. MD in Internal Medicine, Kerman University of Medical Sciences, Kerman, Iran. m.inf088830@yahoo.com

5. MSc in Immunology, Department of Immunology, Kerman University of Medical Sciences, Kerman, Iran. masoud7756@gmail.com

6. PhD in Immunology, Immunology of Infectious Diseases Research Center, Rafsanjan University of Medical Sciences, Rafsanjan, Iran. dr.kazemi@rums.ac.ir

7. (Corresponding author) PhD in Anatomy, Department of Anatomy, Faculty of Medicine, Rafsanjan University of Medical Sciences, Rafsanjan, Iran. shebani54@yahoo.com

fy the pathogen linked molecular pattern (PAMPs) of the microbes, including viruses and induce immune responses including nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activation (6), migration (7), phagocytosis (8) and inflammatory cytokine expression (9). TLRs accomplish the discussed topics through the myeloid differentiation primary response 88 (MYD88) and the TIR-domain-containing adapter-inducing interferon- β (TRIF) dependent pathways (10). Some intracellular signaling molecules which cover interleukin-1 receptor associated kinases (IRAKs), such as IRAK1 and IRAK4, in addition to TNF receptor associated factors (TRAFs) including TRAF3 and TRAF6, are phosphorylated and activated via convention of MYD88 and TRIF (as adaptor molecules) pathways (11).

Inflammatory transcription factors including nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B), interferon regulatory transcription factor 7 (IRF7), IRF3 and activator protein 1 (AP-1) are activated by the effect of these signaling molecules (12). Then, different number of inflammatory cytokine genes, for instance IL-12 and IL-6, are transcribed as a downstream response (12). Therefore, undoubtedly, the devastated expression of these molecules causes inefficient immune responses against viral infection. Patients with sadness and concern show a changed immune response, and it is clear that inflammatory effects on the immune systems are widespread ranging from the excitation of inflammation to the reduction of immune responses (13).

It is revealed that people with sadness and nervousness have a higher level of several chronic infections and cancers (14). Based on the previous studies, nervousness and anxiety can affect the performance of the immune system. Thus, we can conclude that anxiety and depression may cause a malfunction of the immune response in CHB patients. Therefore, we decided to examine the expression levels of IRAK4, TRAF3 and IRF7 in CHB patients with dif-

ferent stages of depression compared to healthy controls.

Methods

Subjects: We obtained peripheral blood samples from 60 CHB patients and 60 healthy controls in 5.5 mL tubes with and without anti-coagulant for mRNA extraction and separation of serums.

Those CHB patients who had other diseases such as HIV and HCV, cytomegalovirus, hepatitis A, C, D, and E viruses and Epstein-Barr virus co-infection, patients who were pregnant or breast feeding, and those patients younger than 18 or older than 55 years were excluded from the study. Moreover, patients with features indicative of other concurrent liver diseases including previous liver transplantation, alcoholic liver disease, autoimmune liver diseases, cirrhosis, Wilson disease, evidence of hepatocellular carcinoma and antiviral and immunosuppressive drugs users were also excluded from the study.

We diagnosed CHB patients using previous clinical and experimental reports and the Guide of Prevention and Treatment in Viral Hepatitis (15). An expert psychologist diagnosed depressed and anxious patients according to standard questionnaires (16). Depression and anxiety were evaluated according to BECK's Depression Inventory II (BDI II) and Hamilton Anxiety Rating Scale (HAM-D17), respectively. BDI II is a self-administered questionnaire which is commonly used to assess the severity of depression, while HAM-D17 is a clinician administered instrument which is used to measure these verities of anxiety.

The results were analyzed and appropriate CHB patients were selected by a psychologist expert. The method of this study was set by the ethical board of the Rafsanjan University of Medical Sciences, and all the participants filled out the written informed consent prior to sample collection.

HBV-DNA Extraction and Real-time PCR Condition: We used 200 μ L of plasma for HBV-DNA purification using a commercial

kit (Cinnaclon, Tehran, Iran) in accordance with the manufacturer's information. A commercial kit from the Primer Design Company (London, UK) was used for HBV-DNA quantification. The kit has efficiencies of $> 95\%$ and can detect less than 100 copies of HBV-DNA.

RNA Extraction, Reverse Transcription and Quantitative Real-Time PCR: A RNX extraction kit (Cinnaclon, Tehran, Iran) was used for Total RNA purification from PBMCs. The quality of obtained RNA was analyzed by measuring absorption at 260/280 nm. The protocols for cDNA synthesis and Real-time PCR amplification has been described previously (17).

Measurement of HBs Ag

HBs Ag has been measured using a commercial ELISA kit (Behring, Marburg, Germany) according to the manufacture's guidelines.

Data Analysis and Statistical Methods

We used the SPSS software version 18 for Chi square test for data analysis and a P value less than 0.05 was set as significant.

Results

The results revealed that all the CHB patients were HBs Ag and HBV-DNA positive. Our results demonstrated that of the 60 CHB patients, 38 (63.3%) did not have depression and 13 (21.7%) and 9 (15%) had mild and moderate depression, respectively. The statistical analysis revealed that the differences among CHB patients with various depression levels with respect to the expression of IRAK4 ($p > 0.05$), TRAF3 ($p > 0.05$) and IRF7 ($p > 0.05$) were not significant (Fig. 1).

The psychological assessments also revealed that of the 60 CHB patients, 36 (60%) had no anxiety and 7 (11.6%), 7 (11.6%) and 10 (16.8%) suffered from mild, moderate and severe anxiety, respectively. The statistical analysis revealed that expression of IRAK4 ($p > 0.05$), TRAF3 ($p > 0.05$) and IRF7 ($p > 0.05$) were not dif-

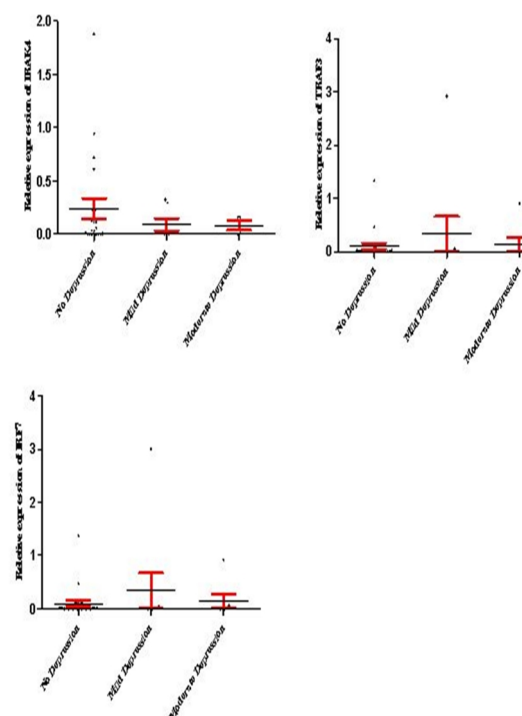


Fig. 1. Expression levels of IRAK4, TRAF3 and IRF7 in CHB patients with no depression, mild depression and moderate depression. The figure demonstrated that expression levels of the molecules were not differing among patients.

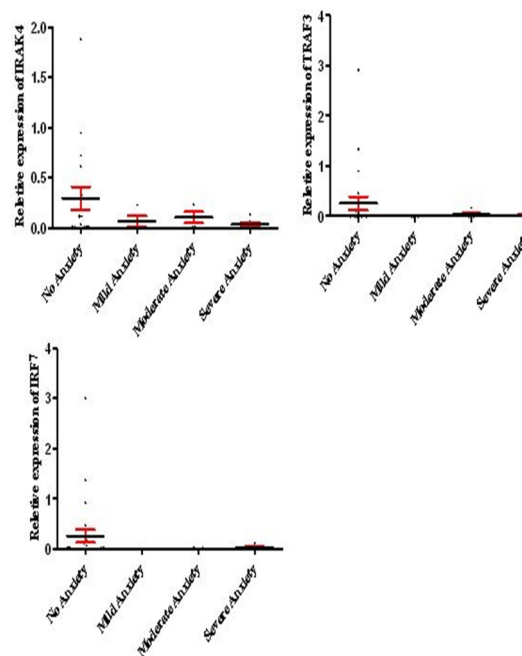


Fig. 2. Expression levels of IRAK4, TRAF3 and IRF7 in CHB patients with no, mild, moderate and severe anxiety. The figure demonstrated that expression levels of the molecules were not differing among patients.

ferent among CHB patients with various stages of anxiety (Fig. 2).

Discussion

Previous studies demonstrated that CHB patients suffer from psychiatry disorders including depression and anxiety (Keskin et al., 2012). Our results also revealed that 36.6 and 40 percent of CHB patients suffered from depression and anxiety. Based on the fact that the patients did not receive any viral therapy, it appears that the HBV infection leads to these psychiatry disorders. It has also been documented that depression and anxiety may alter the expression of immune related molecules (Eyre and Baune, 2012). Additionally, our previous study revealed that the expression levels of IRAK4, TRAF3 and IRF7 were decreased in CHB patients compared to healthy controls(17). Therefore, this hypothesis was raised that depression and anxiety may be responsible for the defective expression of these molecules. The results of the current study showed that the mRNA levels of IRAK4, TRAF3 and IRF7 did not differ between CHB patients with various stages of depression and anxiety. Therefore, it appears that the psychological state of CHB patients is not responsible for the down regulation of IRAK4, TRAF3 and IRF7. To the best of our knowledge, this study is the first to evaluate the effects of depression and anxiety on the expression of IRAK4, TRAF3 and IRF7 in CHB patients. Considering the fact that psychiatric disorders are considered as potential candidates to alter immune related molecules, it appears that conducting more studies on other immune related molecules can improve our knowledge about the relationship between psychiatric disorders, hepatitis B infection and immune responses. Additionally, it is suggested to evaluate the molecules in healthy individuals with and without depression and anxiety to improve our knowledge about the roles of behavioral disorders on the expression of immune related molecules.

Conclusion

In this study, we could not find any relations between declined expression of

IRAK4, TRAF3 and IRF7 in CHB patients with depression and anxiety; therefore, other factors including genetic and immunoregulatory effects of HBV may be responsible for the declined expression of these molecules.

Acknowledgments

The authors would like to thank all the participants of the study. This project was supported by a grant from the Rafsanjan University of Medical Sciences.

Conflict of Interest

The authors declare no conflict of interest with any commercial or other associations in connection with the submitted article.

References

1. Chan HL, Jia J. Chronic hepatitis B in Asia-new insights from the past decade. *J Gastroenterol Hepatol.* 2011;26 Suppl 1:131-7.
2. Ahmadabadi BN, Hassanshahi G, Arababadi MK, Leanza C, Kennedy D. The IL-10 promoter polymorphism at position -592 is correlated with susceptibility to occult HBV infection. *Inflammation.* 2011; Article in press. DOI: 10.1007/s10753-011-9381-x.
3. Arababadi MK, Pourfathollah AA, Jafarzadeh A, Hassanshahi G, Rezvani ME. Association of exon 9 but not intron 8 VDR polymorphisms with occult HBV infection in south-eastern Iranian patients. *J Gastroenterol Hepatol.* 2009;25(1):90-3.
4. Feitelson MA. Parallel epigenetic and genetic changes in the pathogenesis of hepatitis virus-associated hepatocellular carcinoma. *Cancer Lett.* 2006;239(1):10-20.
5. Assar A, Arababadi MK, Mohit M, Ahmadabadi BN, Pumpens P, Khorramdelazad H, et al. T helper and B cell escape mutations within the HBc gene in patients with asymptomatic HBV infection: a study from the South-Eastern region of Iran. *Clin Lab.* 2012;58:53-60.
6. Bae YS, Lee JH, Choi SH, Kim S, Almazan F, Witztum JL, et al. Macrophages generate reactive oxygen species in response to minimally oxidized low-density lipoprotein: toll-like receptor 4- and spleen tyrosine kinase-dependent activation of NADPH oxidase 2. *Circ Res.* 2009;104(2):210-8, 21p following 8.
7. Nguyen-Pham TN, Lim MS, Nguyen TA, Lee YK, Jin CJ, Lee HJ, et al. Type I and II interferons enhance dendritic cell maturation and migration capacity by regulating CD38 and CD74 that have synergistic effects with TLR agonists. *Cell Mol Immunol.* 2011;8(4):341-7.

8. Hirayama T, Tamaki Y, Takakubo Y, Iwazaki K, Sasaki K, Ogino T, et al. Toll-like receptors and their adaptors are regulated in macrophages after phagocytosis of lipopolysaccharide-coated titanium particles. *J Orthop Res*. 2011;29(7):984-92.
9. Imani Fooladi AA, Mousavi SF, Seghatoleslami S, Yazdani S, Nourani MR. Toll-like receptors: role of inflammation and commensal bacteria. *Inflamm Allergy Drug Targets*. 2011;10(3):198-207.
10. Jia N, Xie Q, Lin L, Gui H, Wang H, Jiang S, et al. Common variants of the TLR9 gene influence the clinical course of HBV infection. *Mol Med Report*. 2009;2(2):277-81.
11. Takeda K, Akira S. Toll-like receptors. *Curr Protoc Immunol*. 2007;Chapter 14(14):Unit 14 2.
12. Manghera M, Douville RN. Endogenous retrovirus-K promoter: a landing strip for inflammatory transcription factors? *Retrovirology*. 2013;10(1):16-.
13. Raison CL, Miller AH. Is depression an inflammatory disorder? *Curr Psychiatry Rep*. 2011; 13(6):467-75.
14. Pratt LA, Xu F, McQuillan GM, Robitz R. The association of depression, risky sexual behaviours and herpes simplex virus type 2 in adults in NHANES, 2005-2008. *Sex Transm Infect*. 2012;88(1): 40-4. Epub 2011/11/08.
15. Liu HG, Chen WW, Fan ZP, Yang HY, Shi M, Zhang Z, et al. The high prevalence of the I27 mutant HBcAg18-27 epitope in Chinese HBV-infected patients and its cross-reactivity with the V27 prototype epitope. *Clin Immunol*. 2007; 125(3): 337-45.
16. Beck AT, Beck RW. Screening depressed patients in family practice. A rapid technic. *Postgrad Med*. 1972;52(6):81-5. Epub 1972/12/01.
17. Momeni M, Zainodini N, Bidaki R, Hassanshahi G, Daneshvar H, Khaleghinia M, et al. Decreased expression of toll like receptor signaling molecules in chronic HBV infected patients. *Hum Immun*. 2013; Article in press.