Article

The International Journal of
Psychiatry in Medicine
2016, Vol. 51(5) 467–475

DOI: 10.1177/0091217416680199
ijp.sagepub.com

Anxiety leads to up-regulation of CD36 on the monocytes of chronic hepatitis B-infected patients

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Abstract

Introduction: It has been hypothesized that mental disorders including depression and anxiety can affect immune responses. The study was done to evaluate the relation between depression and anxiety and expression levels of CD36, CD68, and CD9 on peripheral blood monocytes of chronic hepatitis B (CHB) patients.

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Methods: Sixty CHB patients were selected with various ranges of depression and anxiety. Depression and anxiety were evaluated using a standard questionnaire by an expert psychiatrist according to BECK's Depression Inventory II and Hamilton Anxiety Rating Scale, respectively. The levels of CD36, CD68, and CD9 on the peripheral blood monocytes have been performed using flow cytometry technique. **Results:** The results demonstrated that levels of CD36 were significantly increased on the peripheral blood monocytes of CHB patients when compared with CHB patients with no anxiety. Expression levels of CD68 and CD9 were not significantly altered on the CHB patients with various ranges of anxiety. Expression levels of CD36, CD68, and CD9 were also not significantly altered on the CHB patients with various ranges of depression. **Discussion:** It seems that anxiety induces inflammation in the CHB patients by induction of alteration in several molecules including up-regulation of CD36. CD36 plays important roles in the induction of tissue damage; hence, it may be hypothesized that anxiety may participate in the induction of some hepatitis B complications.

Keywords
CD36, CD68, CD9, depression, anxiety

Introduction

Behavioral disorders including depression and anxiety are the prevalent diseases which lead to several complications.[1] It has been documented that depression and anxiety are associated with several malfunctions of immune system.[2] Accordingly, a hypothesis has been aroused regarding the roles of depression and anxiety on the immune responses including innate immunity. Based on the fact that depression and anxiety are prevalent among chronic hepatitis B(CHB)-infected patients,[3] it may be concluded that the mental disorders may affect immune responses in these patients. Previous studies reported that CHB patients are unable to clear hepatitis B virus completely.[4,5] Several mechanisms in these patients are considered as the responsible factors for dysfunctions of immune responses in the patients. It has been proposed that depression and anxiety as the psychological diseases can affect immune responses.[1] Our previous investigations revealed that depression was associated with decreased expression of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), a transcription factor in toll-like receptors signaling pathway.[1] The transcription factor is the most known factor for transcription from immune-related molecules including scavenger receptors as well as tetraspanins.[6] Therefore, it has been hypothesized that CHB patients with depression and anxiety may suffer from decreased expression of scavenger receptors and tetraspanins. Scavenger receptors are the important molecules on the innate immune cells such as monocytes.[7] CD36 and CD68 are the important scavenger receptors which are expressed

on the monocytes and macrophages which participate in phagocytosis of microbes and oxidized low-density lipoproteins.[7] Thus, altered expression of these molecules may be associated with dysfunction of monocytes. CD9, as a tetraspanin, plays key roles in the functions of innate immune cells like monocytes.[8] Additionally, it has been reported that CD9 can interfere with scavenger receptor,[9] so its expression may be associated with expression of scavenger receptors. Due to the information presented here, the aim of this project was to evaluate expression levels of CD9, CD36, and CD68 on the peripheral blood monocytes of CHB patients suffering from depression and anxiety.

Material and methods

Subjects

In this cross-sectional study, 60 inactive CHB patients have been enrolled. Inclusion criteria: patients who have not been infected with microbial agents and were not pregnant as well as breastfeeding. Patients addicted to smoking and opium and with a history of transplantation, alcoholic liver disease, autoimmune diseases, allergy, hepatocellular carcinoma (HCC), and users of immunosuppressive drugs were excluded from the project. The CHB patients who were older than 18 or younger than 55 years were included to the study. Chronic hepatitis was diagnosed by an expert medical doctor in the infectious diseases using previous clinical and experimental reports according to the Guide of Prevention and Treatment in Viral Hepatitis[10] criteria. Depression and anxiety has been diagnosed by an expert psychiatrist according to BECK's Depression Inventory II and Hamilton Anxiety Rating Scale (HAM-D17), respectively.[11]

Peripheral blood samples were collected from CHB patients with various ranges of depression and anxiety in pre-anticoagulant reagents and used for florescent-conjugated antibodies staining.

The protocol of this investigation was set by the ethical board of the Rafsanjan University of Medical Sciences. The participants filled out the written informed consent before blood donation.

Monoclonal antibodies

Directly conjugated antibodies against CD14, CD9, CD36, and CD68 were used as follows:

(1) Fluorescein isothiocyanate (FITC)-conjugated mouse antihuman CD9 (clone: eBioSN4 (SN4 C3-3A2), isotype: IgG1) (eBiosciences, Spain) and appropriate FITC-conjugated isotype-matched negative control (IgG1, clone; P3.6.2.8.1) (eBiosciences, Spain). (2) FITC-conjugated mouse antihuman CD36 (clone: eBioNL07 (NL07), isotype: mouse IgM) (eBiosciences, Spain)

and appropriate FITC-conjugated isotype-matched negative control (IgM, clone; 11E10) (eBiosciences, Spain). (3) FITC-conjugated mouse antihuman CD68 (clone: eBioY1/82A (Y1/82A), isotype: mouse IgG2b) (eBiosciences, Spain) and appropriate FITC-conjugated isotype-matched negative control (IgG2b, κ, clone; eBMG2b) (eBiosciences, Spain). (4) Phycoerythrin (PE)-conjugated mouse antihuman CD14 (clone: 61D3, isotype: mouse IgG1) (eBiosciences, Spain) and appropriate PE-conjugated isotype-matched negative control (IgG1, clone; P3.6.2.8.1) (eBiosciences, Spain).

Flow cytometry analysis

For the detection of CD9, CD36, and CD68 on monocytes from CHB patients, peripheral blood samples were stained with PE-conjugated CD14 monoclonal antibodies (to determine monocytes) and FITC-conjugated CD9, CD36, and CD68 along with isotype-matched negative control based on the manufacturer's instructions. The stained peripheral blood cells were analyzed using Partec

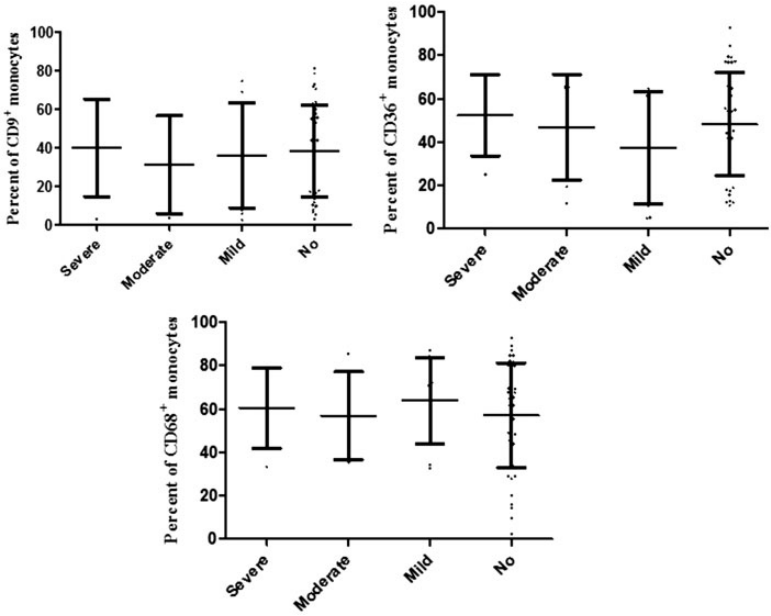

Figure 1. Percent of CD9-, CD36-, and CD68-positive monocytes in the peripheral blood of CHB patients with no, mild, moderate, and severe depression. The figure illustrates that the CHB patients with various ranges of depression have similar percent of CD9-, CD36-, and CD68-positive monocytes.

particle and cell sorting instrument with associated Flowmax software (Münster, Germany).

Statistical analysis

Data analysis was performed using SPSS software (v17; SPSS Inc., Chicago, IL, USA). The continuous variables were summarized as mean and standard deviation (SD) and chi-square test, independent t-tests, and one-way analysis of variance were used; post hoc multiple comparison by Tukey's method was also utilized. In this study, $P \leq 0.05$ was considered significant.

Results

Results of our study showed that 40, 10, 6, and 4 CHB patients were suffered from no, mild, moderate, and severe depression and 13, 19, 22, and 6 CHB patients were suffered from no, mild, moderate, and severe anxiety. The results revealed

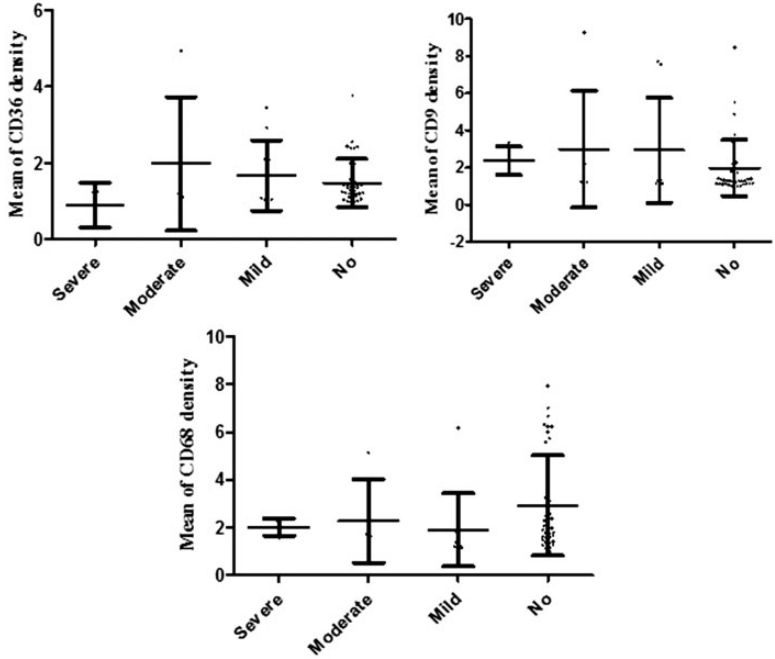

Figure 2. Expression levels of CD9, CD36, and CD68 on the monocytes of CHB patients suffering from no, mild, moderate, and severe depressions. The figure demonstrates that expression levels of CD9, CD36, and CD68 on the monocytes of CHB patients suffering from no, mild, moderate, and severe depressions were not significant.

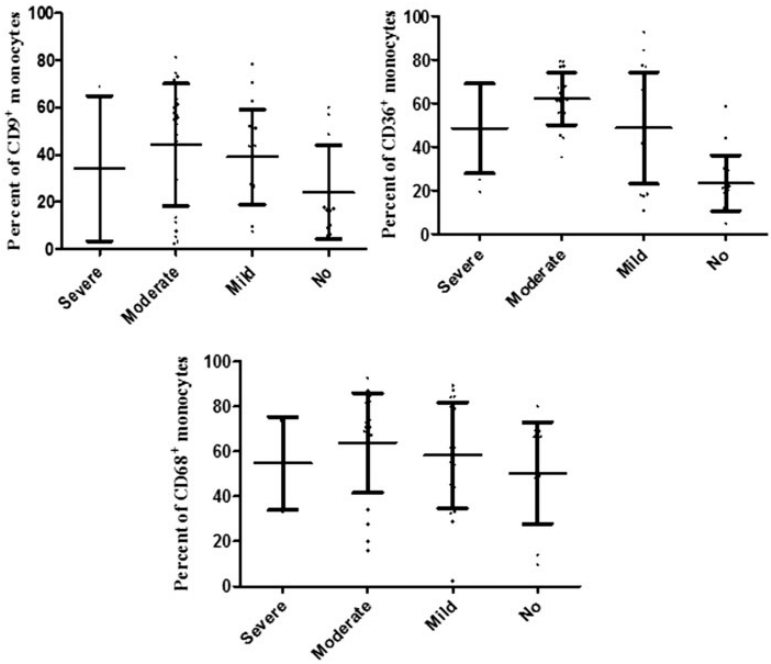

Figure 3. Percent of CD9-, CD36-, and CD68-positive monocytes in the peripheral blood of CHB patients with no, mild, moderate, and severe anxiety. The figure illustrates that the CHB patients with moderate anxiety have higher percent of CD36-positive monocytes in comparison to the patients with no anxiety. The percent of CD9- and CD68-positive monocytes were similar among CHB patients with various ranges of anxiety.

that the percent of CD9 ($P = 0.916$), CD36 ($P = 0.591$), and CD68 ($P = 0.976$) positive monocytes (CD14-positive cells) did not differ between CHB patients with various ranges of depression (Figure 1). The results also demonstrated that the differences between the groups with various ranges of depression regarding the expression levels of CD9 ($P = 0.419$), CD36 ($P = 0.228$), and CD68 ($P = 0.401$) on the monocytes were not significant (Figure 2).

Our results also revealed that the percent of CD36-positive monocytes was increased in the CHB patients suffering from moderate anxiety in comparison to the patients with no anxiety ($P = 0.026$). The percent of CD9 ($P = 0.113$) and CD68 ($P = 0.393$) positive monocytes was similar among CHB patients with various ranges of anxiety (Figure 3).

The expression levels of CD9 ($P = 0.453$), CD36 ($P = 0.556$), and CD68 ($P = 0.325$) on the monocytes were not altered significantly among the patients suffering from no, mild, moderate, and severe anxiety (Figure 4).

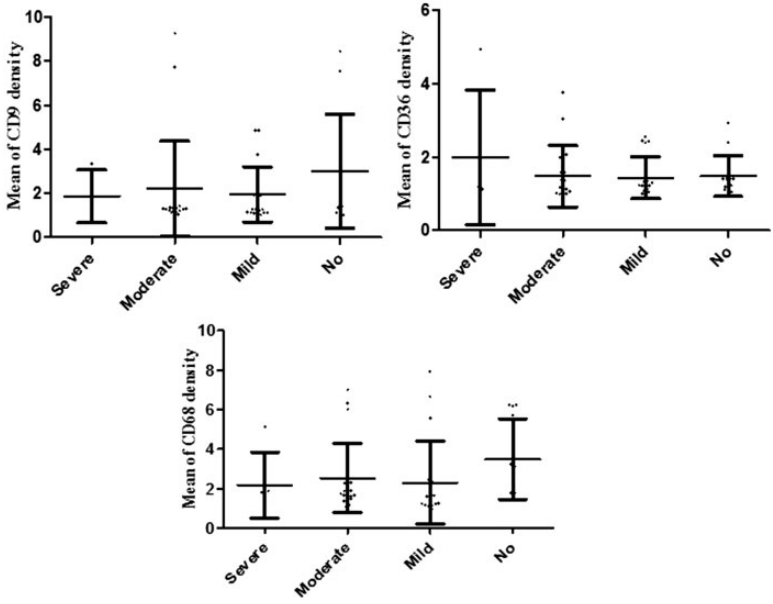

Figure 4. Expression levels of CD9, CD36, and CD68 on the monocytes of CHB patients suffering from no, mild, moderate, and severe anxieties. The figure demonstrates that expression levels of CD9, CD36, and CD68 on the monocytes of CHB patients suffering from no, mild, moderate, and severe anxieties were not significantly different.

Discussion

CHB is a prolonged infection with hepatitis B virus and the infected patients suffer from several complications.[12] Our results revealed that more than 33% and 78% of CHB patients suffered from depression and anxiety, respectively. Thus, it can be concluded that the behavioral disorders are the main complications involved in the pathogenesis of CHB. As mentioned in the introduction section, depression and anxiety can be considered as immune-modulators, hence, may affect immune responses in the CHB patients. The results demonstrated that the percent of CD36-positive monocytes has increased in the CHB patients suffering from moderate anxiety when compared with CHB patients without anxiety (Figure 3), while the percent of CD36-positive monocytes has not altered significantly in the CHB patients suffering from severe anxiety. It appears that low numbers of severe depressed CHB patients is the cause of controversy. The percent of CD68- and CD9-positive monocytes in the peripheral blood has not changed in the CHB patients with and without depression and anxiety. CD36 is a scavenger receptor which recognizes damage-associated molecular patterns, pathogen-associated molecular patterns, and

oxidized low-density lipoproteins to facilitate phagocytosis.[13] Additionally, it has been documented that CD36 significantly participates in the pathogenesis of several organs' complications including vessels.[14] Liver is a main target of CD36-positive monocytes to induce liver cirrhosis and cancer.[13] CD36-mediated phagocytosis of apoptotic cells also is the main pathway for induction of fibrosis in several tissues including kidney.[15] CD36 is also a main factor for fatty acid-induced podocyte apoptosis which results in diabetic nephropathy.[16] Based on the fact that some of the patients with CHB will be directed to cirrhosis and HCC, and according to the important roles played by CD36 in the induction of fibrosis and malignancies,[17] it may be concluded that anxiety may be considered as a potential risk factor for direction of CHB patients to cirrhosis and HCC. It appears that a cohort study on the CHB patients can shed light on this issue. The results showed that expression levels of CD36 and other molecules have not changed on the monocytes of the CHB patients with various ranges of depression and anxiety. Accordingly, it seems that increased number of CD36-positive monocytes, but not expression levels of CD36 on the monocytes, is a main complication of anxiety. Moreover, as mentioned in the Introduction section, CD9 is a tetraspanin molecule and can manipulate functions of CD36. Based on the results, it seems that the molecules have not involved in the pathogenesis of depression and anxiety. Additionally, it seems that altered expression of CD36 in the patients suffering from moderate anxiety were not associated with CD9. Thus, although CD9 can modulate expression of CD36, it is unable to change expression of CD36 in the patients suffering from anxiety and the altered CD36 expression is associated with moderate anxiety directly. To the best of our knowledge, this is the first study which has evaluated the roles of depression and anxiety on the expression of CD9, CD36, and CD68 on the monocytes and also percent of the CD9-, CD36-, and CD68-positive monocytes in the CHB patients; therefore, it seems that more studies are needed.

Acknowledgments

The authors thank the participants and Bahonar Hospital Laboratory for their help in this project.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) received no financial support for the research, authorship, and/or publication of this article.

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