Introduction

An estimated 2 billion people worldwide are infected with the hepatitis B virus (HBV), and 350 million people are chronically infected[1]. HBV infection results in approximately 280 thousand deaths per year, caused by chronic hepatitis, cirrhosis, and hepatocellular carcinoma, which are associated with heavy psychological stress, enormous medical-care costs, and emotional and economic burden on those afflicted and/or their families[2]. HBV infection is a major life stressor, with up to 90% of those subjects reporting significant stress since the diagnosis of HBV infection[3]. Accumulating evidence has linked stress to the initiation, course and outcome of liver diseases[4]. Emotional stress, such as that induced by hypnagogic fear and anxiety, significantly decreased the hepatic blood flow. Moreover, the type I personality has been associated with the severity of chronic hepatitis C[5]. Stress has been reported to aggravate alpha-galactosylceramide induced hepatitis and carbon tetrachloride induced liver injury[6]. Recently, stress has been implicated in the hepatitis B antibody production in healthy participants[7], however little is known about the role of stress in the course of hepatitis B.

It has been reported that the stress is associated with a suppression of NK cell cytotoxicity[8], lymphocyte proliferation, and immune responses[9]. Despite high prevalence of stress among patients with hepatitis B virus (HBV) infection, little is known about whether and how stress exerts an effect on the course of hepatitis B.

Background:

Psychological and physical stress have been demonstrated to have an impact on health through modulation of immune function. Despite high prevalence of stress among patients with hepatitis B virus (HBV) infection, little is known about whether and how stress exerts an effect on the course of hepatitis B.

Methods:

Eighty patients with chronic hepatitis B (CHB) completed the Perceived Stress Scale-10 (PSS-10) and State-Trait Anxiety Inventory (STAI). Fresh whole blood was subject to flow cytometry for lymphocytes count. Plasma samples frozen at −80°C were thawed for cytokines, alanine aminotransferase (ALT), and virus load. These patients were grouped into high or low perceived stress, state anxiety and trait anxiety groups according to the scale score. Sociodemographic, disease-specific characteristics, lymphocytes count and cytokines were compared.

Results:

Firstly, a negative association between ALT and stress (t = −4.308; p = .000), state anxiety (t = −3.085; p = .003) and trait anxiety (t = −4.925; p = .000) were found. As ALT is a surrogate marker of hepatocytes injury, and liver injury is a consequence of immune responses. Next, we tested the relationship between stress/anxiety and lymphocytes. No statistical significance were found with respect to counts of total T cells, CD4+ T cell, CD8+ T cell, NK cell, and B cell count between high and low stress group. Type-2 cytokine interleukin-10 (IL-10) level was significantly higher in high stress group relative to lower counterpart (t = 6.538; p = .000), and type-1 cytokine interferon-gamma (IFN-γ) level shown a decreased tendency in high stress group (t = −1.702; p = .093). Finally, INF-γ/IL-10 ratio displayed significant decrease in high perceived stress (t = −4.606; p = .000), state anxiety (t = −5.126; p = .000) and trait anxiety (t = −4.670; p = .000) groups relative to low counterparts.

Conclusion:

Our data show stress is not related to the lymphocyte cells count in CHB patients, however, stress induces a shift in the type-1/type-2 cytokine balance towards a type-2 response, which implicated a role of psychological stress in the course of HBV related immune-pathogenesis.
Perceived stress

To obtain information about subjects’ stress, we used the 10-item Perceived Stress scale (PSS); an instrument was designed to measure the degree to which situations in one’s life are appraised as stressful. This scale assessed the amount of stress in one’s life rather than in response to a specific stressor and has been used widely. This scale has good internal consistency (α = 0.80–0.85) and test-retest reliability (r = 0.73–0.85) in Chinese people based on our data. Subjects rated each item from 0 (never) to 4 (very often).

Anxiety

The State-Trait Anxiety Inventory (STAI) provided information on the degree of anxiety. The scales of both State anxiety and Trait anxiety consist of 20 self-report items, with each item running from 1 to 4, for a full score of 80 and a lower score reflecting a better psychological status.

Statistical analyses

In the present study, we dichotomized chronic hepatitis B subjects into high versus low perceived stress and anxiety score (n = 40/group) on the basis of a median split of the distribution of stress and anxiety score. Based on the scores obtained from these patients, subjects with perceived stress score ≥17 were assigned to the high stress group (PH), while subjects with perceived stress score <17 were assigned to the low perceived stress (PL); state anxiety ≥ 41 to the high state anxiety group (SH), and <41 to the low state anxiety group (SL); trait anxiety score ≥ 42 to the high trait anxiety (TH), and <42 to the low trait anxiety (TL).

Chi-square tests, Student’s t-test, as well as correlation analysis were used for comparing the difference between high and low perceived stress group, high and low state anxiety group and high and low trait anxiety group. SPSS 13.0 statistical software was used for statistical analysis. Reported p values were two-sided, and p values <0.05 were considered statistically significant.

Results

Perceived stress

The Perceived Stress Scale (PSS) is one of most widely used instruments to measure a global level of perceived stress in clinical and research settings[15]. It was reported that 10-item PSS be superior to 14-item PSS[16]. Since this is the first study designed to evaluate the stresses by using PSS-10 for patients with hepatitis B in Chinese population, the reliability of PSS-10 was assessed. The Cronbach’s alpha was 0.88 for the whole scale, the two-week test-retest reliability of PSS-10 was 0.72, suggesting PSS-10 is suitable for evaluating stress in Chinese hepatitis B patients. During the preparation of this manuscript, the translated Chinese version of PSS-10, has been tested in Chinese policewomen with good internal consistency (α = 0.86) and test-retest reliability (r = 0.68) [17].

Psychological stress lead to emotional reaction of anxiety when an individual perceive stress, therefore the extent of anxiety is associated with the stress level. The State-Trait Anxiety Inventory (STAI) provided information on the degree of anxiety. The scales of Table 1. Sociodemographic, disease-specific characteristics comparison between high and low perceived stress and anxiety patients with chronic hepatitis B.

Both state anxiety and trait anxiety consist of 20 self-report items, with each item running from 1 to 4, for a full score of 80 and a lower score reflecting a better psychological status. Higher scores are positively associated with higher levels of anxiety. To further test the validity of PSS-10, all the participants were encouraged to
| Variables\(^a\) | \(P_H (n = 40)\) | \(P_L (n = 40)\) | \(p\) value | \(S_H (n = 40)\) | \(S_L (n = 40)\) | \(p\) value | \(T_H (n = 40)\) | \(T_L (n = 40)\) | \(p\) value |
|---|---|---|---|---|---|---|---|---|---|
| Gender\((F/M)\) | 8/32 | 10/30 | 0.79 | 8/32 | 10/30 | 0.79 | 10/30 | 8/32 | 0.79 |
| Age, years | 31.15 (8.85) | 26.4 (6.01) | 0.06 | 31.20 (8.21) | 26.35 (6.85) | 0.08 | 30.80 (8.41) | 26.75 (6.88) | 0.06 |
| Education | 0.005 | 0.050 | 0.043 |
| < college | 32 | 20 | 0.241 |
| \(\geq\) college | 8 | 20 | 0.241 |
| Marital | 0.241 | 0.241 | 0.055 |
| Married | 28 | 24 | 28 | 24 | 0.28 |
| Single | 12 | 16 | 12 | 16 | 0.28 |
| Economic status\(^b\) | 0.070 | 0.459 | 0.754 |
| \(\geq\) average | 0 | 4 | 2 | 4 | 2 | 4 |
| common | 12 | 16 | 10 | 18 | 14 | 14 |
| hard | 26 | 20 | 28 | 18 | 24 | 24 |
| Disease duration\(^c\) | 8.25 | 7.80 | 0.080 | 6.75 | 9.30 | 0.06 | 7.15 | 3.50 | 0.100 |
| mean(S.D.) | (6.3) | (5.8) | (6.08) | (5.8) | (5.8) | | (6.1) | (6.1) |
| ALT(U/L) | 98.60 | 138.45 | 0.000 | 95.45 | 132.60 | 0.003 | 102.5 | 137.5 | 0.000 |
| mean(S.D.) | (44.33) | (55.93) | (47.00) | (53.67) | (43.48) | | (56.76) | (56.76) |
| Virus Load\(^d\) | 6.38 | 6.24 | 0.18 | 6.31 | 6.32 | 0.28 | 6.37 | 6.27 | 0.26 |
| mean(S.D.) | (1.1) | (1.13) | (1.1) | (1.1) | (1.2) | | (1.1) | (1.1) |

\(^a\) PH, high stress group; PL, low perceived stress; SH, high state anxiety group; SL, low state anxiety group; TH, trait anxiety score; TL, low trait anxiety; \(^b\) Self reported economic status; \(^c\) Years; \(^d\) Log\(_{10}\)(copies/ml).

doi:10.1371/journal.pone.0105530.t001
complete the STAI forms immediately after the completion of PSS-10. Consistency with the conception of psychological stress lead to emotional reaction of anxiety, we do found, a positive correlation between PSS-10 score and state anxiety ($r = 0.811$, $p < 0.01$), and trait anxiety ($r = 0.782$, $p < 0.01$). These results also indicate that psychological stress is associated with mental health issues.

**Sociodemographic comparison between high and low stress/anxiety patients**

In China, most of these patients did not fully covered by medical insurance, and hepatitis B virus infection has been link to substantial economic burden, stigmatization, and poor social support. As far as we know, this is the first study to test the stress of patients with hepatitis B in Chinese population. We want to know if there is association between perceived stress score and sociodemographic variable, clinical variable and virus replication variable. No significant difference was found (Table 1) between high and low stress/anxiety in sociodemographic (gender, age, marital state, and economic state, and disease duration.)

The knowledge of HBV related cirrhosis and hepatocellular carcinoma and insufficient knowledge of the modes of transmission may results in isolation[3]. Consistently with this conception, in this study, the proportion of high stress scores patients was much higher in inadequate educated participants than well-educated subjects (61.5% vs 28.5%), while the proportion of well education was much lower in high stress group than that of the low stress group (20% vs 80%), chi-square 7.912, $p = 0.009$. A positive relationship between education and state anxiety (chi-square 3.516, $p = 0.050$), and trait anxiety (chi-square 3.810, $p = 0.043$) were also observed (Table 1).

**Correlation between stress and liver inflammation**

Next, we compared virus load, virus genotypes, and ALT between these two group. As shown in table 2, no differences were observed between high and low stress anxiety group. However, a strong negative correlation (Figure 1) between ALT and stress ($t = -4.308; p = 0.000$), state anxiety ($t = -3.035; p = 0.003$) and trait anxiety ($t = -4.925; p = 0.000$) were found. ALT is a surrogate marker of hepatocytes injury. The HBV replication cycle is not directly cytotoxic toward hepatocytes. Much of the liver injury is thought to be a consequence of immune responses. Next, we analyzed the association between the subtypes of lymphocyte and stress/anxiety (t = 5.126, $p = 0.000$). As shown in table 1, no differences were found between high and low state anxiety group ($t = 1.586, p = 0.121$), and between low and high trait anxiety group ($t = 1.711, p = 0.098$).

The INF-γ:IL-10 ratio, reflecting the Th1/Th2 balance, has been implicated in psychological stress during an exam period[19]. Next, INF-γ: IL-10 ratio was calculated and then compared between high and low stress/anxiety groups. Consistent with our increased INF-γ and decreased IL-10 data in major stress/anxiety groups as shown above, the ratio displayed significantly increases in high perceived stress ($t = 4.606; p = 0.000$)(Figure 2C), state anxiety ($t = 5.126; p = 0.000$) and trait anxiety ($t = 4.670; p = 0.000$) groups as compared with the low score groups.

**Discussion**

The PSS-10 is one of most widely used instruments to measure a global level of perceived stress in clinical and research settings and has been translated into many languages including Spanish[20], Arabic[21], Greek[22], Japanese[23], Korean[24]. At the beginning of the study, for the reason that this is the first study to employ the translated Chinese version of PSS-10 and STAI inventory scale to assess Chinese population, the reliability and validity were tested. The Cronbach’s alpha was 0.88 for the whole scale, the two-week test-retest reliability of PSS-10 was 0.72, suggesting the translated Chinese version of PSS-10 is suitable for evaluating stress in Chinese hepatitis B patients. The preliminary data were submitted to 13th International Congress on Infectious Diseases as a poster[25]. During the preparation of this manuscript, an independently translated Chinese version of PSS-10, has been tested in Chinese policewomen with comparable consistency and test-retest reliability[17].

Following the validation of the reliability of PSS-10, analysis was conducted in patients with CHB to found the relationship between stress/anxiety and sociodemographic and disease-specific characteristics. Data show stress/anxiety has no association with gender, age, marital state, economic state, disease duration, HBV virus load and HBV gene type. Positively relationship between stress and education was found, Moreover, a negative correlation between stress/anxiety and ALT was established. According to our knowledge, this is the first study discovered the relationship between stress/anxiety and ALT in patients with hepatitis B. The ALT reflects injury to hepatocytes[26]. It was well documented that it’s the host immune response targeting infected hepatocytes, not the HBV replication itself in hepatocytes, led to liver injury[27–29]. To explore the role of stress related immune alteration in the course of hepatitis B, we analyzed the stress/anxiety and immune cell count and cytokines, which account for the HBV related immunity response. However, no relationship was found between stress/anxiety and T cells, T helper/inducer, T suppressor/inducer, NK, and B cells count.

Among all the variables assessed, high circulating IL-10 levels was the only variable strongly associated with high stress/anxiety score, low IFN-γ levels was also observed in high stress/anxiety group, however the statistical differences was not significant. IL-10
and IFN-γ are important cytokines involved in chronic HBV infection, as level of IL-10 and IFN-γ associated greatly with course of chronic hepatitis B [30–32]. IL-10, a prototype of type-2 cytokine, is considered an anti-inflammatory cytokine and has inhibitory effect on immune response, whereas IFN-γ, a type-1 cytokine, is significantly increased in patients with acute hepatitis B, which would eventually eliminated viral from hepatocytes. Dysregulation of the Th1/Th2 cytokine production is thought to be involved in the pathogenesis of HBV related liver diseases. However, the reasons for the imbalance of Th1/Th2 cytokine still poorly studied in the progression of hepatitis B. Psychological distress has been shown to induce a shift in type-1/type-2 cytokine balance toward a type-2 responses through glucocorticoids and catecholamines. Consistently, we observed a higher IFN-γ/IL-10 ratio, indicating a shift toward a Th2 response, in high stress/anxiety group.

Stress has been reported to aggravate alpha-galactosylceramide induced hepatitis and carbon tetrachloride induced liver injury, however, our data shown stress linked to low ALT level. Thus, a discrepancy between our data and literature was observed. Firstly, stressors are classified into acute, subchronic and chronic. The different duration of stress may elicit different neuroendocrine response and immune alteration. Acute stress may be associated with transient immune activation, while chronic stress seems consistently associated with immune suppression. Most of those studies, including alpha-galactosylceramide induced hepatitis and carbon tetrachloride induce liver injury in animal stress model, the duration of stress was just about weeks. In our study, the disease duration was around 8 years. Consistent with the suppressive function of chronic stress on immunity, in vivo data shown decreased liver inflammation and injury in high stress/anxiety subjects, as indicated by lower ALT level. Although ALT is a surrogated marker of liver inflammation in the context of hepatitis B and ALT has been widely clinically used, the golden diagnosis of liver inflammation is checking the necrosis of hepatocytes and infiltration of lymphocytes by liver biopsy, which call for further investigation. Secondly, consistent with the immune-suppressive notion of chronic stress, our data presented a markedly increased IL-10, a suppressive cytokine, and a decreased tendency of IFN-γ, an immune activation cytokine. Thirdly, ALT, a marker of liver

Table 2. Comparison of lymphocytes count and circulating cytokines between low and high perceived stress patients with hepatitis B.

|                      | CHB with low stress | CHB with high stress | p value  |
|----------------------|---------------------|----------------------|----------|
| CD3                  | 1728 ±812           | 1605 ±717            | t = −0.158; p = 0.875 |
| CD3/CD4              | 526±243             | 573 ±194             | t = 0.648; p = 0.519 |
| CD3/CD8              | 519±223             | 489 ±213             | t = −1.810; p = 0.074 |
| CD19                 | 198±98              | 228 ±148             | t = 0.11; p = 0.991 |
| CD16                 | 217±103             | 197 ±102             | t = −0.820; p = 0.414 |
| IFN-γ(pg/mL)         | 83.48±49.70         | 62.24±61.35          | t = −1.702; p = 0.093 |
| IL-10(pg/mL)         | 9.11±3.36           | 17.01±6.86           | t = 6.538; p = 0.000 |
| IFN-γ: IL-10         | 10.13±4.43          | 6.23±4.74            | t = −4.606; p = 0.000 |
inflammation, also an indicator of current immune competence, reflects the functional ability of body immunity system mounts a response to HB surface antigen and HB core antigen. It is reasonable that patients with major stress mount less extent of immune response relative to minor stress subjects. Finally, under the basic conception of deleterious effect of chronic stress on health, much attention has paid to the disease severity, while little paid to disease duration. Higher ALT level associated with earlier clearance of virus in both treatment naive patients and interferon treated patients, while lower or normal ALT level associated with persistent infection. In our study, profiles with low ALT, high IL-10, low IFN-γ and low IFN-γ/IL-10 ratio in major stress/anxiety patients is a typical characteristic of persistent infection, while the profiles with high ALT, low IL-10, high IFN-γ and high IFN-γ/IL-10 ratio in minor stress/anxiety patients prone to immunity activation and maybe virus clearance.

To our knowledge, this is the first study to investigate the relation among stress, immunity and hepatitis B. Ours in vivo data support the model, at least in part, that alterations in IFN-γ:IL-10 ratio, particular the IL-10 level, secondary to increased psychological stress are involved in the persistent HBV infection.

**Author Contributions**

Conceived and designed the experiments: YRZ XML. Performed the experiments: YLH HG. Analyzed the data: YLH HG. Contributed reagents/materials/analysis tools: YLH HG YRZ XML. Wrote the paper: YLH HG YRZ XML.

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