Original Article
The role of MBL2 gene polymorphism in sepsis incidence

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Abstract: Aim: This case-control study was aimed to explore the role of mannose-binding lectin 2 (MBL2) gene rs1800450 polymorphism (codon 54 A/B, G230A) in the development of sepsis in Han Chinese. Methods: MBL2 rs1800450 polymorphism was genotyped by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). MBL serum level was detected by enzyme-linked immunosorbent assay (ELISA). Associations between rs1800450 and sepsis susceptibility was detected by Chi-square test and represented by odds ratios (ORs) and 95% confidence intervals (CIs). Correlation of rs1800450 genotypes and MBL serum level was assessed using t test. Result: Variant A allele frequency was significantly observed in cases than that in controls, indicating a significant association with the susceptibility of sepsis (OR = 1.979, 95% CI = 1.200-3.262). GA genotype also relate to the onset of sepsis (OR = 2.090, 95% CI = 1.163-3.753). MBL serum concentrations were significantly different between case and control groups (P<0.001). Meanwhile, variant allele carriers had lower serum level compared with wild homozygous (P<0.001). Conclusion: Variant A allele in MBL2 gene rs1800450 polymorphism might increase the risk of sepsis via decrease the MBL serum level.

Keywords: Sepsis, MBL2, polymorphism, PCR-RFLP, ELISA

Introduction

Sepsis is defined as the host response to infection including the infection and the systemic manifestations of infection [1]. Sepsis, which will lead to severe sepsis and septic shock, is a kind of systemic inflammatory response syndrome (SIRS). It is a category of infection complication for some diseases, disorder and severe trauma. Symptoms of sepsis mainly include abnormal body temperature, heart rate, breathing rate and the abnormal peripheral blood leukocyte. Sepsis has a high mortality [2-4], and is one of the most prevailing cause of death in intensive care unit (ICU) [5]. Although many therapy methods have made great progress in recent years, the sepsis mortality is still very high [6]. Multiple researches were carried out to detect the pathogenesis of sepsis. It was found that this disease is influenced by various genetic and environmental factors [7-9]. However, the sepsis etiology is still unclear.

As we all know, mannose-binding lectin (MBL) belong to the C-type collectin family, and is an important element in innate immunity [10]. MBL is compounded by liver and stored in plasma. It was verified that MBL may be contribute to activate complement system, facilitate opsono-phagocytosis, mediate inflammation and promote apoptosis [11]. MBL could mediate phagocytosis via directly combined with the oligose group receptors which is in the surface of pathogenic microorganisms; and activate the complement system via MBL-MASP pathway [12].

In human, MBL protein is encoded by MBL2 gene. Recent researches indicate that MBL2 gene relate to some diseases which is caused by infection including sepsis [13-17]. Recently, genetic polymorphisms play a potential role in the investigations of disease pathogenesis. These polymorphisms could alter the structure and function of the corresponding protein. MBL2 exon 1 encode the signal peptide of MBL. Many studies indicated that polymorphisms in the promoter region and exon1 of MBL2 gene contribute to the MBL serum concentration and MBL activation [18-20].
Because the polymorphism distributions are different in ethnicity and region. In this study, we explore the association between MBL2 rs1800450 polymorphism (codon 54 of exon1, A/B) and sepsis incidence, then analyzed the association of rs1800450 genotypes with serum MBL level in sepsis patients in unrelated Han Chinese.

Table 1. Genotype distributions of rs1800450 polymorphism in cases and controls

<table>
<thead>
<tr>
<th>SNP</th>
<th>Case n = 107 (%)</th>
<th>Control n = 134 (%)</th>
<th>P</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GG</td>
<td>67 (62.62)</td>
<td>105 (78.36)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>GA</td>
<td>36 (33.64)</td>
<td>27 (20.15)</td>
<td>0.013</td>
<td>2.090 (1.163-3.753)</td>
</tr>
<tr>
<td>AA</td>
<td>4 (3.74)</td>
<td>2 (1.49)</td>
<td>0.173</td>
<td>3.134 (0.559-17.588)</td>
</tr>
<tr>
<td>G</td>
<td>170 (79.44)</td>
<td>237 (88.43)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>A</td>
<td>44 (20.56)</td>
<td>31 (11.57)</td>
<td>0.007</td>
<td>1.979 (1.200-3.262)</td>
</tr>
</tbody>
</table>

Figure 1. MBL serum level in different genotypes of rs1800450 polymorphism in sepsis patients.

MBL serum level (µg/L)

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Serum level (µg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GG</td>
<td>2500 (0.001)</td>
</tr>
<tr>
<td>GA</td>
<td>2000</td>
</tr>
<tr>
<td>AA</td>
<td>1500 (0.001)</td>
</tr>
</tbody>
</table>

Figure 1. MBL serum level in different genotypes of rs1800450 polymorphism in sepsis patients.

Materials and methods

Study subjects

This case-control study was approved by the ethic committee of Air Force General Hospital of PLA. All of the participants were unrelated China Han population, understand this study, and signed the informed consent form. Demographic and clinical characteristic were collected via questionnaire and interview.

107 sepsis patients (69 males, 38 females, mean age 52.47 ± 14.69) who were diagnosed by two physicians in Air Force General Hospital of PLA during 2011 to 2015 were recruited as cases. Sepsis diagnosis accorded with previous standards [21]. Inclusive criteria of sepsis patients were as follows: (1) adult patients (≥18 years); (2) had no congenital and acquired immune deficiencies, as well as diabetes mellitus; (3) did not received any immunosuppressive therapy in recent 12 months; (4) had no history of sepsis and malignancy; (5) non-pregnancy.

134 healthy individuals (86 males, 48 females, mean age 49.65 ± 14.98) who received health examination in the healthy check-up center of Air Force General Hospital of PLA during the same time were enrolled as controls. Controls matched with the cases in age, gender, ethnicity and living area.

Collection and treatment of specimens

5 mL peripheral blood was collected from every participant who had a 12 hrs fasting. These samples were anti-coagulated with EDTA, and 2000 r/min centrifuged for 10 min. Plasma and leukocytes were respectively stored in -80°C until to use.

MBL2 genotyping

Genomic DNA was extracted from leukocytes using a TIANamp Blood DNA Kit (Tiangen Biotech, China). MBL2 rs1800450 polymorphism was genotyped by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP), referring to an early study [22].

Serum MBL measurement

Plasma was utilized to detect the functional MBL via enzyme-linked immunosorbent assay (ELISA). Serum MBL was measured by a MBL human kit (Hycult Biotech, Netherlands) according to the manufacturer’s specification.

Statistical analysis

Hardy-Weinberg equilibrium (HWE) was used to examine the representativeness of the cases and controls. Genotype and allele frequencies were calculated by direct count. Associations of MBL2 rs1800450 polymorphism with sepsis incidence was evaluated by Chi-square test. MBL serum level presented by mean ± SD. Difference of MBL serum level between cases
and controls was assessed by t test. The associations were represented by odds ratios (ORs) with 95% confidence intervals (CIs). All of the calculations were performed by PASW 18.0. Statistical significance was presented when \( P<0.05 \).

Results

Association of rs1800450 polymorphism with sepsis

In order to explore the association of \( MBL2 \) gene rs1800450 polymorphism with sepsis incidence, we genotyped this polymorphism and compared them between cases and controls. We found that genotype and allele distributions in cases and controls were in accordance with the HWE test. Variant allele carriers were frequently discovered in case group than that in control group (Table 1). A allele might obviously increase the risk of sepsis (OR = 1.979, 95% CI = 1.200-3.262). Meanwhile, GA genotype significantly associated with the onset of sepsis (OR = 2.090, 95% CI = 1.163-3.753). But AA genotype had no significant association with the occurrence of sepsis.

\( MBL \) serum level

We detected the \( MBL \) serum level both in cases and controls, so as to evaluate the association of \( MBL2 \) gene and sepsis. ELISA results demonstrated that the \( MBL \) serum levels in case and control groups were respectively as 1533.44 ± 698.17 and 3017.56 ± 573.90 \( \mu \)g/L, these concentrations had a significantly difference (\( P<0.001 \)). In case group, the \( MBL \) concentrations among rs1800450 genotypes were respectively as GG = 1990.07 ± 396.50, GA = 830.58 ± 266.71, AA = 210.50 ± 125.62 \( \mu \)g/L, the differences had statistically significance (Figure 1, \( P<0.001 \)).

Discussion

As an important member of complement system, \( MBL \) play a potential role in innate immunity. \( MBL \) could recognize the antigen via bind to oligosaccharide group which displayed on the surface of pathogenic microorganisms, and directly serve as an opsonin [23]. Many studies indicated that the low or the lack of serum \( MBL \) content closely relate to many diseases [11, 24, 25]. Immune defensive function of the \( MBL \) is bound up with the serum level and oligomeric type of \( MBL \) [24]. Peptide chain of \( MBL \) is comprised by N-terminal segment, collagen-like region (CLR), helical coiled hinge region, and carbohydrate-recognition domain (CRD). In human plasma, the common type of \( MBL \) is hexamer, \( MBL \) molecules which less than tetramer cannot bring into play the complete physiological functions [12]. Human \( MBL \) protein is encoded by \( MBL2 \) gene.

\( MBL2 \) gene is located in 10q11.2, and included 5 exons. Variations in \( MBL2 \) gene will alter the peptide chain structure of it, influencing the formation and stability of oligomeric \( MBL \) [26-28]. Five widely studied \( MBL2 \) gene polymorphisms were reasonably related to reduced \( MBL \) serum level. These polymorphism include three structure variants, codon 52 (rs5030737C/T, A/D), 54 (rs1800450G/A, termed A/B), 57 (rs1800451G/A, termed A/C) of exon1 (wild allele termed A and the three variant alleles collectively termed O) and two promoter variants -550 (rs11003125G/C, termed H/L), -221 (rs7096206G/C, termed Y/X) [12, 29, 30]. B, C alleles could block the formation of helix structure in CLR; D allele could destroy the disulfide bond in peptide chain; and promoter polymorphisms will affect the \( MBL \) concentration in transcription level.

Sepsis, which is caused by infection, significantly relate to the immune system. It is proved by many studies that \( MBL \) could control the development of sepsis, and low \( MBL \) level maybe increase the risk of sepsis [16, 29, 30]. However, the association of \( MBL2 \) gene polymorphisms with sepsis risk had inconsistent results. Klostergaard et al. [31] found that A/O polymorphism had no significant association with sepsis risk. Huh et al. [32] indicated that A/B variant had no significant association with sepsis, but could decrease the risk of septic shock in Korean. Another study performed by Davis et al. [33] demonstrated that B allele of A/B polymorphism was a risk factor for sepsis in Turks. Therefore, the association of \( MBL2 \) gene polymorphisms with the occurrence of sepsis is still unclear. In additionally, gene polymorphism was different among regions and ethnicity. So, in this study, we explored the association between \( MBL2 \) gene rs1800450 polymorphism and sepsis occurrence, as well as the serum concentration in the different genotypes of rs1800450 in China Han population.
Current study found that GA genotype and A allele frequencies of MBL2 rs1800450 polymorphism was obviously discovered in sepsis patients. Further ELISA test demonstrated a significantly decreasing trend of MBL serum level in cases compared with controls, moreover, the same trend was observed in rs1800450 genotypes. That was according to early studies [16, 33]. We speculated that rs1800450 A allele increase the sepsis risk via decrease the MBL serum level.

In conclusion, we suggested that rs1800450 might significantly increase the risk of sepsis in Han Chinese. This finding will contribute to the diagnosis and therapy of sepsis. However, this finding was insufficient to certify the pathogenesis of MBL2 gene in sepsis because there were some limitations in our study. Firstly, the sample size was small. Secondly, sepsis is a complex disease which will be affected by various genetic and environmental factors, in this study other polymorphisms were not included. Thirdly, this result was not adjusted by confounding factors. Finally, the sepsis grade and the infections which lead to sepsis were no detailed classification. So, in order to understand the pathogenesis of sepsis, well designed further cohort studies were imperative.

Disclosure of conflict of interest

None.

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References


**MBL2** polymorphism and sepsis


