Mycobacteriology

Monocyte chemoattractant protein-1 in spinal tuberculosis: -362G/C genetic variant and protein levels in Chinese patients

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A B S T R A C T

The objective of the study is to explore the possible association of the monocyte chemoattractant protein (MCP)-1-362G/C genetic polymorphism and plasma levels of MCP-1 in patients with spinal tuberculosis (TB). The MCP-1-362G/C (rs2857656) polymorphism and blood levels of MCP-1 in patients with spinal TB and healthy subjects were evaluated and compared. Three hundred thirty-two patients and 336 healthy subjects were genotyped using polymerase chain reaction and Sanger DNA sequencing technology. MCP-1 plasma levels were measured by a solid-phase enzyme-linked immunosorbent assay. When comparisons were made between patients and controls, the frequency of the MCP-1-362G minor allele [55.4% versus 47.5%, P = 0.004, odds ratio (OR) = 1.376, 95% confidence interval (CI): 1.109–1.706] and the carriers of the MCP-1-362G allele (80.7% versus 71.4%, P = 0.005, OR = 1.657, 95% CI: 1.167–2.403) were over-represented in patients. The mean MCP-1 plasma level in spinal TB patients was significantly higher than in controls (154.44 ± 68.81 pg/mL versus 36.69 ± 21.71 pg/mL, t = −5.85, P < 0.001). The patients with the CC genotype had the highest MCP-1 level (150.63 ± 73.89 pg/mL), followed by those with the GC genotype (108.63 ± 52.09 pg/mL, t = 3.091, P = 0.003, OR = 1.706, 95% CI: 1.109–2.403) and GG (91.29 ± 54.31 pg/mL, t = 2.351, P = 0.022) and GC (91.29 ± 54.31 pg/mL, t = 2.351, P = 0.022) homozygotes.

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1. Introduction

Monocyte chemoattractant protein (MCP)-1 is a member of the CC chemokine family; it is a strong monocyte/macrophage chemotactic factor and plays an important role in immunity to tuberculosis (TB) (Deshmukh et al., 2009). When Mycobacterium tuberculosis infection occurs, the early expression of MCP-1 can be found, thus greatly affecting TB granuloma formation and survival of pathogenic bacteria (Kondratieva et al., 2010). As announced in the Global Tuberculosis Report by the World Health Organization (WHO) in 2012, there are about 8,700,000 new TB patients worldwide in 2011 (WHO, 2012). Among these patients, nearly 20% developed extrapulmonary TB, and 5–5% of them had spinal TB (Schirmer et al., 2010). Human immune response to M. tuberculosis is mainly mediated by activated T cells, macrophages, various types of white cells, and various inflammatory cytokines (Philips and Ernst, 2012; Raja, 2004). MCP-1 expression can modulate TH1 cytokine expression and down-regulate proinflammatory responses, thus affecting the onset and progression of TB (Lee et al., 2003). The MCP-1 encoding genes are located at the 17q11.2 chromosome region. Jamieson et al. (2004) have shown that this region is closely associated with susceptibility to TB. Thye et al. (2009) have further found a correlation between MCP-1-362G/C (rs2857656) genetic variant and TB susceptibility. The timely, adequate expression of MCP-1 plays a decisive role in specific immunity to spinal TB (Algood et al., 2003; Scott and Flynn, 2002; Ramsay et al., 2011). High systemic blood levels of MCP-1 have been found in patients with TB (Hasan et al., 2009; Hussain et al., 2011; Peters et al., 2001). Although it has been confirmed that spinal TB involves an independent susceptibility gene (Garq and Somvanshi, 2011; Selvaraj et al., 2004; Zhang et al., 2010), the current studies about the MCP-1 gene polymorphism and the correlation between serum expression levels and TB still focus on pulmonary TB, while there is little evidence reporting the correlation with spinal TB. This study was designed to investigate whether spinal TB in the Chinese Han population is attributed to MCP-1-362G/C genetic variant and serum expression levels.

2. Materials and methods

2.1. Patients and controls

A total of 332 consecutive patients with spinal TB (184 male and 148 female; mean age, 39.97 ± 17.91 years), of Han ethnicity living in...
Hunan Province, China, were recruited from our hospital between December 2004 and December 2012. Preoperative imaging showed signs of spinal TB. All patients had high level of erythrocyte sedimentation rate and C-reactive protein preperation. The preoperative purified protein derivative test was positive in all patients. Preoperative pulmonary imaging revealed no apparent signs of pulmonary TB. All patients had negative sputum acid-fast stain results and lacked of clinical pulmonary manifestations. Other types of extrapulmonary TB were excluded. Postoperative histopathologic sections demonstrated TB infection, and etiological examination showed pus-positive acid-fast staining or positive results of pus isolation and culture of M. tuberculosis.

Three hundred thirty-six healthy, consecutive subjects (156 male and 180 female; mean age, 41.43 ± 12.79 years) of Han ethnicity, living in Hunan Province, were selected between December 2004 and December 2010 from the Xiangya Hospital Medical Center. Subjects with pulmonary TB, spinal TB, and other extrapulmonary tuberculosis were excluded by imaging. Subjects with spinal TB who underwent surgery were enrolled in case group.

Any of the following complications were criteria for exclusion: positive culture of drug-resistant M. tuberculosis (no matter single-drug resistance, multidrug resistance, or extensively drug resistance); immune-related disease (such as infection, trauma, tumor); accompanying disease that influenced MCP-1 expression (such as coronary heart disease and psoriasis); accompanying autoimmune disease; accompanying TB in other organs (or history); accompanying genetic diseases; previous spinal surgery; and no bacillus Calmette-Guérin vaccination. All patients and control subjects gave their informed consent to participate in the study, which was approved by the Ethical Committee of the Central South University.

2.2. Specimen collection, extraction of genomic DNA, and serum extraction

Fasting blood was extracted from the median cubital vein in the morning. Two-millilitre blood was stored in acid citrate dextrose anticoagulant tube for DNA extraction, and the other 5-mL blood was stored in a pro-coagulation tube for serum extraction. Leukocyte genomic DNA was extracted using a blood genomic DNA rapid extraction kit (Dalian Takara, Liaoning Province, China), according to the manufacturer's instructions. DNA concentration was determined with ultraviolet spectrophotometry and diluted to 100 ng/μL for storage.

2.3. Polymerase chain reaction (PCR)

The primers were synthesized as previously described; forward primer: 5′-GAG CCT GAC ATG CTT CTA TCA A′-3′; reverse primer: 5′-TTT CCA TTC ACT CTT GAG AC-3′. Amplified fragment size was 174 bp. The PCR was performed using 10-μL samples containing 1 μL of template DNA (100 ng/μL), 0.3 μL forward and reverse primers (100 ng/μL), 0.8-μL dextronucleotide triphosphate, 1 μL 10× PCR buffer (Mg2+), 0.05-μL Takara HotStart enzyme, and 6.55-μL double-distilled water. PCR reaction conditions: prenaturation at 95 °C for 5 min, 94 °C for 1 min, 58 °C for 30 s, 72 °C for 1 min for 30 cycles in total, followed by extension at 72 °C for 10 min and annealing at 4 °C.

2.4. PCR products sequencing and analysis

If the amount and specificity of the products were not satisfactory (e.g., if there were confounding bands), the PCR products were retrieved and purified as follows (50-μL PCR amplification system as example). The target fragment was transferred to a new Eppendorf tube, mixed with 500-μL absolute alcohol at −20 °C for 4 h, and centrifuged at 16 200 × g for 10 min. White precipitate was eluted with 70% alcohol, centrifuged at 540 × g at 4 °C for 15 min, dried at room temperature for 5 min, dissolved in 10-μL PCR water for 2 h, and then sequenced on an ABI3100 sequencer. Sequencing was performed by Shanghai Majorbio Pharm, China. The sequencing results were compared with the human genome sequence to verify their correctness.

2.5. Determination of serum MCP-1 concentration

All volunteers' (332 cases and 336 controls) MCP-1 concentration was detected by using the MCP-1 enzyme-linked immunosorbent assay (ELISA) kit (Shanghai ExCell Biology, Shanghai, China). The MCP-1 levels were obtained using the ExCell kit according to manufacturer's recommendations, and the optical density was measured at 450 nm using a microplate reader (Beckman Coulter, Inc, Brea, CA, USA); a standard curve was plotted, and the sample concentration was calculated.

2.6. Statistical analysis

The database was established using SPSS 13.0 statistical software. General information about the subjects in the 2 groups was compared using the t test and χ2 test. The concentration of serum MCP-1 was compared between the 2 groups using the t test and was also compared among different genotypes using 1-way analysis of variance (ANOVA). The normal variable was expressed as mean ± SD. The odds ratio (OR) at 95% confidence interval (CI) and Pearson’s P value were calculated. A P value <0.05 was considered to be significant.

3. Results

3.1. Distribution of the MCP-1-362G/C polymorphism in patients and controls

The distribution frequency of GG, GC, and CC genotypes is shown in Table 1. The genotype distributions in the 2 groups met the Hardy-Weinberg law of genetic equilibrium (P > 0.05). The allele and phenotype frequencies of the genetic variant in the spinal TB patients and controls are shown in Table 1.

The MCP-1-362°C minor allele was significantly more frequent in the spinal TB patients than the controls (55.4% versus 47.5%, P = 0.004, OR = 1.376, 95% CI: 1.109–1.706). Carriers of the MCP-1-

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>MCP-1-362G/C Polymorphism (n = 332)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GG</td>
<td>64 (19.3%)</td>
</tr>
<tr>
<td>GC</td>
<td>168 (50.6%)</td>
</tr>
<tr>
<td>CC</td>
<td>100 (30.1%)</td>
</tr>
<tr>
<td>Alleles</td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>296 (88.8%)</td>
</tr>
<tr>
<td>C</td>
<td>368 (55.4%)</td>
</tr>
<tr>
<td>Carriers</td>
<td>268 (80.7%)</td>
</tr>
</tbody>
</table>

In the event that the amount and specificity of products were not satisfactory (e.g., if there were confounding bands), the PCR products were retrieved and purified as follows (50-μL PCR amplification system as example). The target fragment was transferred under a Vitalight lamp, placed at room temperature for 5 h, ground using a large tip, mixed with 600-μL eluting buffer, mixed overnight on a shaking table at 37 °C, and centrifuged at 16 200 × g at 4 °C for 30 min. The supernatant was transferred to a new Eppendorf tube, mixed with 500-μL absolute alcohol at −20 °C for 4 h, and centrifuged at 16 200 × g for 10 min. White precipitate was eluted with 70% alcohol, centrifuged at 540 × g at 4 °C for 15 min, dried at room temperature for 5 min, dissolved in 10-μL PCR water for 2 h, and then sequenced on an ABI3100 sequencer. Sequencing was performed by Shanghai Majorbio Pharm, China. The sequencing results were compared with the human genome sequence to verify their correctness.
362°C allele were over-represented in the patients compared with the controls (80.7% versus 71.4%, \( P = 0.005, \) OR = 1.657, 95% CI: 1.167–2.403).

3.2. Comparison of serum MCP-1 concentration in patients and controls

The t test showed that the serum MCP-1 concentration in spinal TB patients was significantly higher than that in normal controls (154.44 ± 68.81 pg/ml versus 36.69 ± 21.71 pg/ml, \( t = -5.85, \) \( P < 0.001)\).

3.3. Comparison of serum MCP-1 concentration among subjects with different genotypes

One-way ANOVA showed that there were significant differences in serum MCP-1 concentration among all subjects (both cases and controls) with different MCP-1-362 genotypes (\( F = 4.982, P = 0.01 \)). The serum MCP-1 concentration in subjects with CC genotype (\( n = 179 \)) was significantly higher than that of those with GC genotype (\( n = 329 \)) (150.63 ± 73.89 pg/ml versus 108.63 ± 52.09 pg/ml, \( t = 2.351, P = 0.022 \)) and GG genotype (\( n = 160 \)) (150.63 ± 73.89 pg/ml versus 91.29 ± 54.31 pg/ml, \( t = 3.091, P = 0.003 \)), but no difference was found between GC and GG genotypes (\( t = 1.036, P = 0.304 \)).

4. Discussion

Increasing evidence shows that MCP-1 gene polymorphisms can influence host susceptibility to TB (Buijlets et al., 2008; Flores-Villanueva et al., 2005). We found that the MCP-1-362°C minor allele is more frequent in spinal TB patients than in controls. Also, the MCP-1-362°C carriers were over-represented in patients compared with controls. Our results did not confirm the study of Velez Edwards et al. (2012), which has suggested that there is no significant association between MCP-1-362G/C polymorphism and susceptibility to TB. However, Thye et al. (2009) have shown that MCP-1-362°C is not associated with susceptibility to TB and even has a role in resistance to infection. There are 2 reasons for such a difference. Firstly, different races and living environments may lead to different results. In the study of Thye et al. (2009), they also found that MCP-1-2518G, which has a high linkage equilibrium with MCP-1-362°C, can prevent TB in West African populations (Ghana). According to a meta-analysis of Feng et al. (2012), MCP-1-2518G is highly correlated with TB susceptibility in Asian populations. Secondly, Thye et al and Velez Edwards et al mainly investigated patients with pulmonary TB, while the present study excluded this disease. Spinal TB patients have different pathogenesis from those with pulmonary TB, so the predisposing factors are also different. Further studies with the involvement of other populations/ethnic spinal TB groups will help to clarify this question.

Concerning the levels of MCP-1 protein, our findings showed higher MCP-1 plasma levels in spinal TB patients compared with controls. Our data are in accordance with those reported previously by Hussain et al. (2011). Over-high expression of MCP-1 may limit the mononuclear cell responses, inhibit interleukin (IL)-12 and interferon-\( \gamma \) expression, and then selectively inhibit Th helper (Th) cell type 1 immune response; it also reduces the severity of immune response and affects granuloma formation (Matsukawa et al., 2000). Alternatively, it can lead to receptor saturation and loss of ligand sensitivity, and the gradient change in concentration limits the monocyte chemotactic response (Charo and Ransohoff, 2006). At the same time, serum MCP-1 also can up-regulate the transcription activity of IL-4 and induce Th0 cells into Th2 cells, thus up-regulating Th2 immune responses and further restricting the Th1 immune response (Mendez et al., 2011). The above processes can lead to TB susceptibility and increase its risk of dissemination. In addition, we also noticed major differences in MCP-1 concentration in different studies, owing to the following factors: different ELISA kits, specimen preservation methods, and populations investigated and varying degrees of disease severity. Peters et al. (2001) have shown that serum MCP-1 concentrations may be associated with the severity of TB. Hasan et al. (2009) have confirmed that serum MCP-1 concentrations in Pakistani patients with TB may gradually increase with severity of illness, ranging from 31.5 to 1712 pg/ml. Our study investigated patients with severe spinal TB; therefore, the MCP-1 concentrations were relatively high.

In addition, our data revealed a difference between plasma MCP-1 levels in individuals depending on the -362G/C genotype: subjects carrying the CC genotype had significantly higher MCP-1 levels compared with those with either GC or GG genotype. This may be due to the protein-binding rate in the promoter region. Page et al. (2011) have demonstrated that genetic changes in the MCP-1 promoter region increase the transcription activator binding rates of the TALE homedomain proteins Prep1 and Pbx2 with the MCP-1 promoter, thereby increasing the transcriptional activity of the MCP-1 gene and up-regulating the expression levels of downstream serum MCP-1.

5. Conclusion

In conclusion, our results suggested that the MCP-1-362G/C genetic variant as well as increased levels of MCP-1 are associated with spinal TB. Furthermore, the -362°C minor allele seems to be a risk factor for the development of spinal TB in the Chinese population. We believe that our data on the relationship between MCP-1-362G/C polymorphism and plasma levels are the first reported in a Chinese population, and as they are limited to one relatively small cohort, we will, in the future, perform the study in larger patient groups. In accordance with the guidelines on genome-wide association studies, our findings should be replicated in other ethnic groups.

Acknowledgments

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