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Evaluation of correlation between NF-κB mediated PAI-1 gene and sepsis

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Abstract. – OBJECTIVE: The present study is aimed to explore the correlation between PAI-1 gene mediated by NF-κB signaling pathway and human sepsis.

PATIENTS AND METHODS: In this study, we used 74 cases of sepsis patients preserved by the laboratory as the observation group, and 68 cases of healthy people served the control group. Further methods like fluorescence quantitative PCR, enzyme-linked immunosorbent assay, Western-blotting were used to determine NF-B expression, NF-κB gene mRNA analyses and protein expression on different research subjects. Further, the positive expression rates of PAI-1 gene in the observation group and the control group were determined by immunohistochemistry.

RESULTS: The expression levels of NF-κB and PAI-1 gene mRNA in the blood of the observation group significantly increased in comparison to control group ($X^2 = 3.24$, $p < 0.05$; $X^2 = 2.81$, $p < 0.05$). Also, NF-κF and PAI-1 gene protein expressions (0.14 ug/l, 0.32 ug/l) were significantly higher in the observation group in comparison to control group ($p < 0.05$). The results of blood glucose measurement showed that the fasting blood glucose (14.3 mmol/l) in the observation group was significantly higher than that in the control group (4.6 mmol/l). Immunohistochemical were also in sync with above results.

CONCLUSIONS: The present study concludes that PAI-1 gene expression gets significantly increased via NF-κF signaling pathway during sepsis.

Key Words:
NF-kappa B signaling pathway, PAI-1 gene, Sepsis, Correlation, Promotion.

Introduction

Sepsis is a major threat to the rehabilitation of patients with severe trauma, burns, and postoperative infection, as a result of a series of severe inflammatory reactions caused by infection[1,2]. Further, nearly 18 million people are infected by sepsis each year, and about 24.9% of them die[3]. The main clinical manifestations are fever, abnormal changes in the number of white blood cells in the blood, increased blood flow dynamics and increased cardiac output, higher blood glucose levels, etc.[4]. At present, severe shock, multiple organ failures and other diseases caused by sepsis have become one of the most common causes of death in intensive care unit[5]. Although sepsis poses a great threat to postoperative rehabilitation, its pathogenesis is still not clear, hence no effective drug treatment for sepsis[6]. A recent report revealed that sepsis might get triggered by a number of factors, including genetic factors and environmental factors[7]. Moreover, it has been observed that various external factors that lead to sepsis are bacterial, viral infections, and a series of immune system diseases[8]. However, there is still paucity of information about the molecular mechanisms of sepsis. Plasminogen activator inhibitor-1, PAI-1 as the main regulatory factor of the fibrinolytic system, could be combined with the plasminogen activator (tPA) in the human body, which causes the loss of its activity. The results showed that[9] PAI-1 could also inhibit fibrin degradation in the human body for the maintenance of the dynamic balance of PAI-1 system and blood coagulation system[10]. Although there is a close relationship among PAI-1 and blood coagulation factors, there is little research on the correlation between PAI-1 and sepsis. In the present study, we studied for the first time the correlation between the NF-κB signaling PAI-1 gene during sepsis.

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Patients and Methods

Patients

In the present study, we selected 74 patients with sepsis as the study subjects, their average age was (34.5 ± 22.4) years old and 68 healthy people were selected for the control group. 5 ml of blood samples were taken from the elbow veins of the subjects from both the groups. Blood samples were then centrifuged at 3000 rpm/min for 10 min and the pellets were preserved in the cryopreservation solution for subsequent experiment. At the same time, the tissue samples with sepsis were excised from both the groups by operation method.

Reagents and Kits

The commonly used molecular reagents used in this study were purchased from TAKARA Company (Dalian, China). RNA extraction kit and reverse transcription kit were also purchased from TAKARA (Dalian, China) Company. Immunohistochemical kit was procured from Qiagen Company (Qiagen, Hilden, Germany), Further, first anti-ELISA, Western blotting and immunohistochemistry used were mouse anti-human monoclonal antibody and second antibody was HRP labeled rabbit anti, and were obtained from Thermo Scientific (Waltham, MA, USA).

Fluorescence Quantitative PCR

RNA Extraction

Taken about 0.3 g of sample preserved in liquid nitrogen and quickly placed it on the ice for melting. It was followed by addition of 0.35 ml RNA Plus (TAKARA). Samples were then crushed in the precooled mortar. Immediately after the RNA removed enzyme, samples were put into the Eppendorf (EP) 1.5 ml tubes, 0.15 ml RNAPlus was then used to rinse the mortar again. Samples were then transferred to the centrifuge tubes. 200 μl of chloroform was added to each EP tube. Centrifuged for 15 min at the speed of 12000 rpm at room temperature. It was followed by 4°C centrifuge for 15 min. After being taken out, transferred the supernatant to RNase and an equal volume of isopropanol was added. Then, quickly reversed and mixed samples evenly at room temperature It was again followed by centrifuge at the speed of 12000 rpm at 4°C for 10 min[6]. Discarded supernatant, added 1000 μl 75% ethanol, gently mixed, 12000 rpm 4°C centrifugal for 10 min. Discarded supernatant, cleared the residual ethanol as far as possible. The proper amount of liquid to remove RNase was then added, and the quality of the extracted RNA was determined, and the remainder was used for fluorescence quantitative PCR test.

RT-PCR

The fluorescence quantitative reverse transcription and quantitative experiments were carried out according to the TAKARA specification, fluorescence quantitative reaction system: SYBR Premix Ex Taq II (2* ROMANEx Taq tative reaction μM) 0.5 μl, PCR reverse primer (10 μM) 0.5 μl, cDNA 1 μl, dH2O 3 μl, (the object sequence is shown in Table I).

Enzyme-Linked Immune Response

In the experimental group and the control group were taken the total protein of sample extraction as the research objects. Detected the expression of NF-κB and PAI-1 gene protein in different samples, and the specific operation was performed according to ELISA Kit (Qiagen, Hilden, Germany) specification. The samples of observation group and the control group through the sterilization of PBS (pH 7.2) in accordance with the scale of 1:200 were diluted and 100 μl solution was taken to add into 96 well plates, and 50 μl detection solution was added into each hole, after 2h incubation at room temperature. The TMB color substrate was added, the absorption value was measured at 495 nm, and the expression of gene protein and concentration of NF-κB and PAI-1 in each sample was calculated according to the standard curve.

Western-Blotting

The Western-blotting experiment was performed in accordance with the "Third edition of molecular cloning experimental guide".

Table I. Fluorescence quantitative PCR primer.

Primer	Sequence
NF-κB-F	TAGCTAGTCGTAGCTAGCTCG
NF-κB-R	GTCGATGCTAGCTAGAGCTGC
PAI-1-F	AGTCGTAGGCTAGCTAGGCTAC
PAI-1-R	CGTAGTCGTAGCTAGTCGGATCG
GAPDH-F	TGACTTCAACAGCGACACCCA
GAPDH-R	CACCCTGTTGCTGTAGCCAAA

Determination of Blood Glucose

The blood glucose assay was performed with the reference to the "Chinese medicine".

Immuno-Histochemistry

The tissues of the experimental group and control group were selected, and the different samples were fixed in 10% formalin, and then embedded in paraffin ("molecular cloning experiment guide Third Edition"). (1) In the test paraffin section thickness was about 4 μM, the well-prepared slice was fixed on the slide, at 70°C baked for about 1h. (2) Through pre prepared xylene dewaxing, elution was performed by anhydrous alcohol, and then removed the residual alcohol by ultra pure water. Finally, take PBS (pH 7.2) washing (5 times, each time for 5 min). The sterilizing pot was heated for 2 min at 121°C, and then cool it, place it in PBS solution at room temperature for 30 min. (3) After the PBS solution was evaporated, the 50 μl peroxidase blocking agent was taken into the chip, at 37°C it was placed for about 10 min. It was then washed with PBS. It was then followed by removal of PBS solution, and the addition of 45 μl nonimmune animal serum, at room temperature for 10 min incubation. (4) Added, the first anti to the above sections, and then placed them at room temperature for incubation for about 2 h (or 4°C overnight). Samples were then washed again with PBS (5 times, each time for 5 min). (5) Added about 50 μl streptomyces to the above sections, at 37°C for incubation for 2 h, with PBS cleaning (5 times, each time for 5 min). (6) Added colored liquid A (100 μl) with observation under microscope. (7) After 10 min, rinsed with distilled water, and then counterstained with hematoxylin for 5 min, and rinsed again. Taken ethanol dehydration and mounting were done by neutral gum.

Statistical Analysis

The statistical analyses were performed on data by statistical software SPSS 20.0 (SPSS, Inc., Chicago, IL, USA). The experimental data in research were expressed by `xe. The single factor analysis method was used to analyze the data between different groups. $p < 0.05$ indicated significant difference.

Results

The Measurement of mRNA Expression of NF-κB and PAI-1 Gene in Normal Population and Patients With Sepsis

The total RNA extracted from the control group and the observation groups were studied. The expression difference of NF-κB and PAI-1 gene in different samples was measured by fluorescence quantitative PCR, and the results are shown in Figure 1. Through Figure 1A can be seen that compared with the normal human NF-κB mRNA expression, the mRNA expression of NF-κB in the blood of patients with sepsis significantly increased. Further, PAI-1 gene expression (Figure 1B) in sepsis blood was significantly higher than the expression of PAI-1 mRNA in normal and between the two there was significant difference ($p < 0.05$).

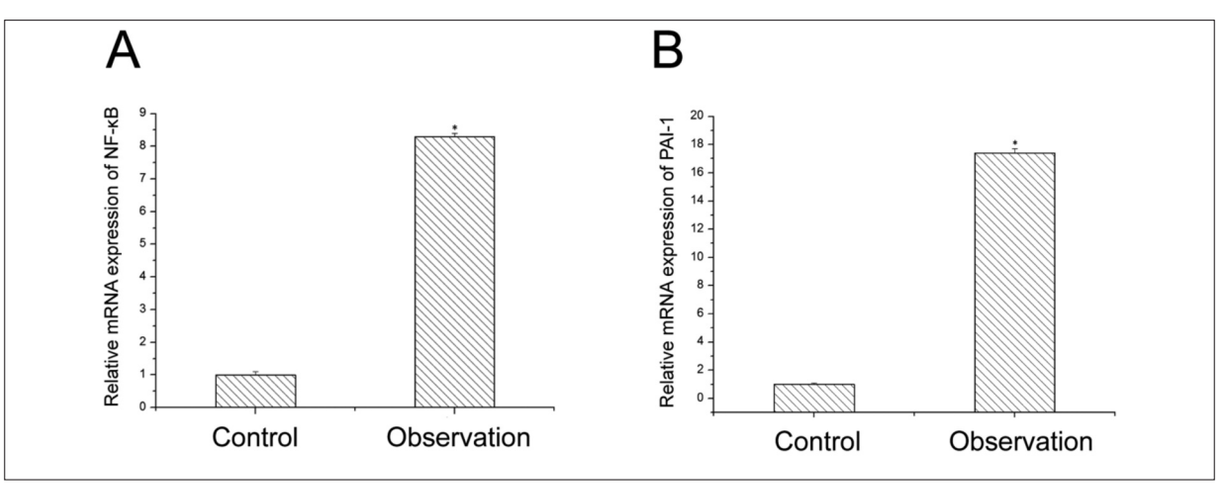

Figure 1. The measurement of mRNA expression of NF-κB and PAI-1 gene in normal population and patients with sepsis. *Represents a significant difference between groups.

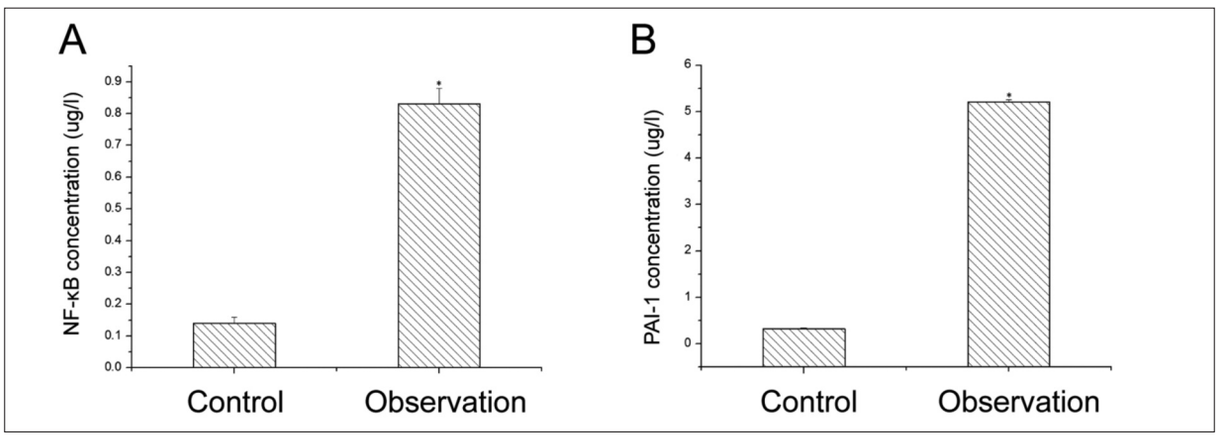

Figure 2. Protein expression of NF-κB and PAI-1 gene detected by Elisa assay in normal population and patients with sepsis. *Represents a significant difference between groups.

The Measurement of Protein Expression Differences of NF-κB and PAI-1 Gene in Normal Population and Patients with Sepsis by ELISA Method

The total RNA extracted from the control group and the observation groups were studied. NF-κB and PAI-1 protein expressions in different samples were determined by ELISA method. The results (Figure 2) showed that NF-κB and PAI-1 protein expressions (0.14 μg/l 0.32 μg/l) were significantly lower in control group than that in the blood of patients with sepsis (0.83 μg/l, 5.21 μg/l).

The Measurement of Protein Expression Difference of NF-κB and PAI-1 Gene in Normal Population and Patients with Sepsis by Western-Blotting Method

The results of Western blotting are shown in Figure 3. In the blood of patients with sepsis, NF-κB and PAI-1 gene protein expression were significantly higher than the expression of NF-κB and PAI-1 protein gene in the normal population.

Determination of Blood Glucose in the Blood of Healthy Controls and Patients with Sepsis

The blood glucose levels (14.3 mmol/l) (Figure 4a) in patients with sepsis were significantly higher than those in the normal group (4.6 mmol/l). The correlation detection of PAI-1 gene expression and blood sugar content (Figure 4b), showed that there was a significant correlation between the two, and the human blood sugar content was also higher for the people with higher PAI-1 gene expression.

Immunohistochemical Detection of PAI-1 Gene

The expression of PAI-1 gene in different samples was measured by immunohistochemistry, and the results were shown in Figure 5A, through Figure 5A it can be seen that the PAI-1 gene expression in the lesions of patients with sepsis was higher, while the PAI-1 gene expression in normal tissue was lower. Based on positive cell counting results of PAI-1 gene in different sam-

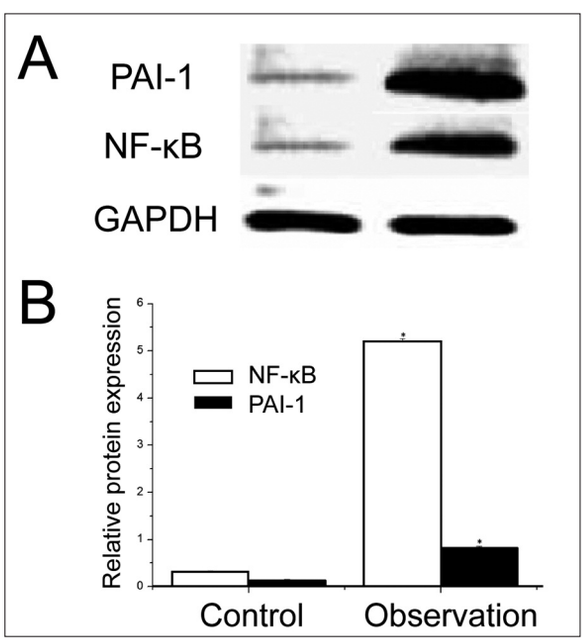

Figure 3. The measurement of protein expression difference of NF-κB and PAI-1 gene in normal population and patients with sepsis by Western-blotting method. *Represents a significant difference between groups.

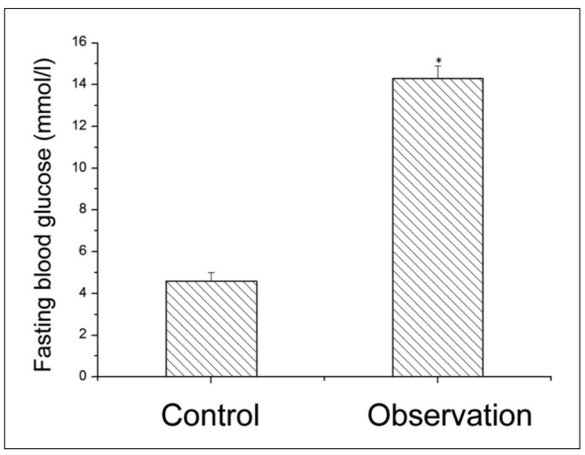

Figure 4. Determination of blood glucose in the blood of normal people and patients with sepsis and its correlation with PAI-1 gene expression. *represents a significant difference between groups.

ples (Figure 5B), it can be seen that the positive rate of PAI-1 gene in sepsis lesions was significantly higher than the positive rate of PAI-1 gene in normal tissues.

Discussion

Sepsis is a systemic inflammatory response caused by infection, usually as a complication of severe trauma, burn, and postoperative infection[11,12]. Clinical studies in the recent past showed that severe sepsis could lead to septic shock, multiple organ failure and even death[13,14]. However, due to the complex etiology of sepsis, there is no effective treatment of drugs[15], at present, the research on the pathogenesis of sepsis

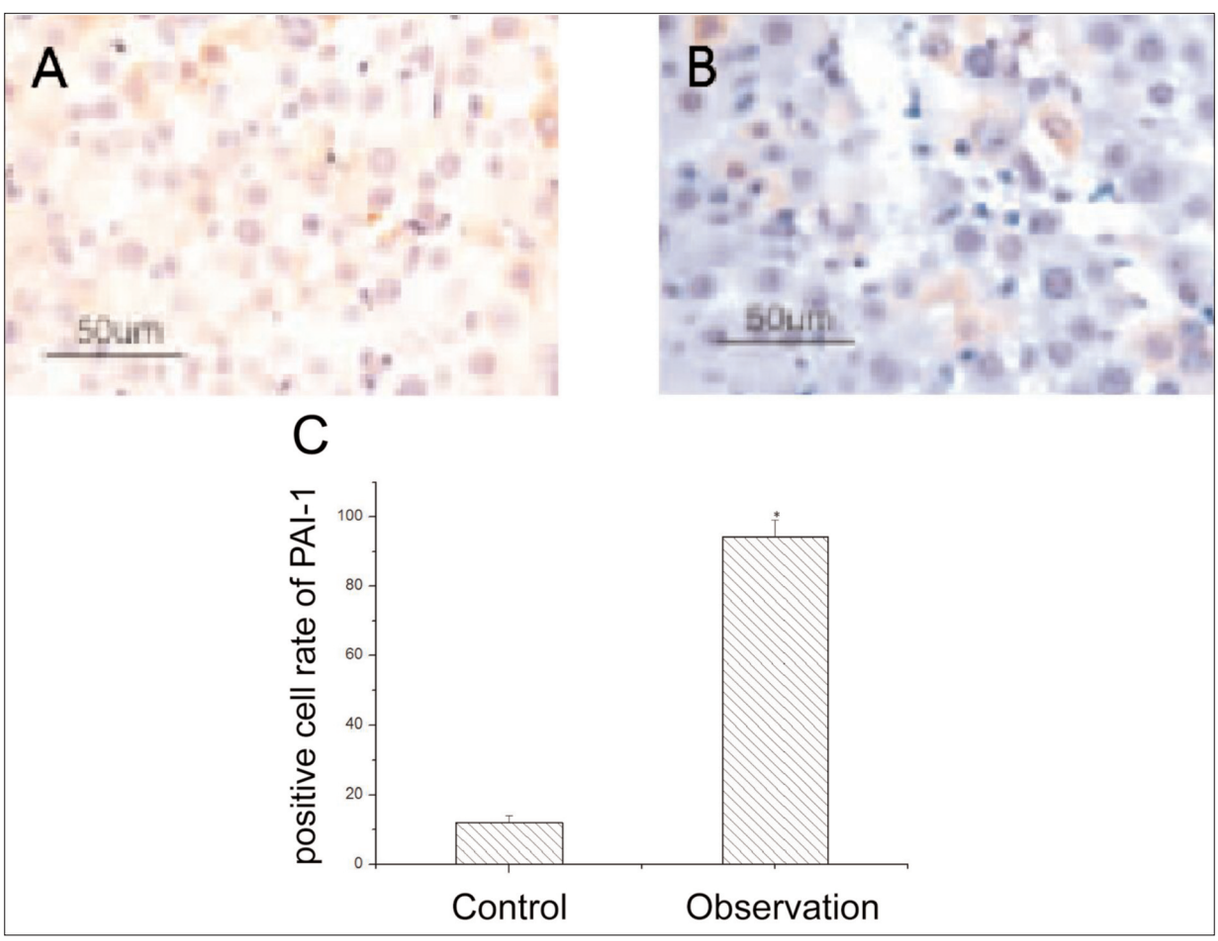

Figure 5. Immunohistochemical detection of PAI-1 gene a: Immunohistochemical results of different samples. ***A,*** PAI-1 immunohistochemistry results in normal population. ***B,*** In patients with sepsis, the results showed that the pink was PAI-1 negative cell, and the purple was PAI-1 positive cell. ***C,*** Results of positive cell count in different samples. *Represents a significant difference between groups.

has become a hot direction in the field of medical research[16]. Research results show that[17] the invasion of pathogenic microorganisms is an important cause of sepsis and other inflammation in patients with severe burn after surgery[18]. For instance, the bacterial virus intrusion could activate the body's immune system, by means of the complement system, mononuclear macrophage, and KK cell immunity that could in turn remove invading bacteria and viruses, etc.[19,20]. Further, NF-κB signaling pathway mediates many immune regulations, and in the process of promoting the immune regulation system, the expression quantity according to different causes is not the same[21]. For example, in the pathogenesis of influenza, the expression of NF-κB signaling pathway showed a trend of first increase and then decrease, while the expression of NF-κB signaling pathway was always at a high level in the pathogenesis of pneumonia. It indicated that NF-κB signaling pathway could regulate the body's immune system by regulating the level of its own expression[22]. The PAI-1 gene in the human body has been observed to be involved in the processes of coronary heart disease, type 2 diabetes and peripheral arterial occlusive disease and many other diseases. In the present study, we successfully demonstrated for the first time that the expression of PAI-1 gene in the patients with sepsis was significantly higher than that in the normal human. Moreover, immunohistochemical results also confirmed that the number of PAI-1 positive cells was significantly higher than that of normal persons. Furthermore, the NF-κB pathway detection results revealed that expression of NF-κB protein in sepsis was significantly higher than that of normal people, and because the NF-κB signaling pathway has been proved to be involved in many immune regulation processes.

Conclusions

NF-κB signaling pathway might mediate the correlation between PAI-1 gene and the incidence of sepsis. However, more detailed studies are required future for exploration of PAI-1 gene-specific regulatory mechanism working in coordination with NF-κB during sepsis.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

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