Effects of purulent meningitis on the changes of matrix metalloproteinase-2 (MMP-2) and matrix metalloproteinase inhibitor-1 (TIMP-1) in cerebrospinal fluid of neonates

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Abstract: This study was aimed to investigate the changes of matrix metalloproteinase-2 (MMP-2) and matrix metalloproteinase inhibitor-1 (TIMP-1) in cerebrospinal fluid (CSF) of neonates with purulent meningitis. 195 cases (n=195) were divided into PM group (neonatal purulent meningitis), VM group (neonatal virus meningitis) and control group (healthy neonates). The expression levels of MMP-2 and TIMP-1 were detected by ELISA while the level of PCT was determined by chemiluminescence analyzer. The levels of MMP-2 and TIMP-1 in CSF and PCT in serum were compared in three groups and the correlation was discussed. The level of MMP-2 in CSF in 3 groups were statistically significant ($F=16.126$, $P<0.05$) similarly the level of TIMP-1 in CSF of 3 groups were statistically significant ($F=16.093$, $P<0.05$). The serum level of PCT in PM group was 14.73±2.14ng/l, in VM group was 9.06±1.05ng/l and in control group it was 0.37±0.12ng/l. The levels of MMP-2 and TIMP-1 in CSF were positively correlated with the serum level of PCT in both PM and VM group. The expression of MMP-2, TIMP-1 and serum PCT in CSF of newborns with purulent meningitis was increased. The findings suggest that MMP-2, TIMP-1 and PCT are involved in the occurrence and development of neonatal purulent meningitis.

Keywords: CSF, MMP-2 and TIMP-1, purulent meningitis.

INTRODUCTION

Neonatal meningitis is a common disease of central nervous system (CNS) infection in children, which is difficult to be diagnosed in early stages, with high disability and seriously affects the life and health of children. Purulent meningitis and virus meningitis are two major types of CNS infection in children (Mahmood, 2015; Srinivasan *et al*., 2016; Lee *et al*., 2015). Matrix metalloproteinases (MMPs) and inhibitors of matrix metalloproteinases (TIMPs) play an important role in the process of degradation of the extra cellular matrix (ECM) and basal membrane (BM) in relation to tumor invasiveness (Pesta *et al*., 2015). At present, the gold standard for clinical diagnosis of purulent meningitis and virus meningitis is the etiological examination of cerebrospinal fluid. However, this technique is not optimistic and there are some shortcomings, such as low positive rate of bacterial culture, time-consuming operation and so on, therefore, the clinical application is limited (Roine *et al*., 2015; Xiong *et al*., 2016). Hence, it is necessary to seek new methods for the diagnosis of purulent meningitis and virus meningitis. Matrix metalloproteinase 2 (MMP-2), matrix metalloproteinase inhibitor-1 (TIMP-1) and pro-calcitonin (PCT) are important factors to which people are paying close attention in recent years. Previous studies showed that MMP-2, TIMP-1 and PCT may be involved in the pathogenesis of CNS infection (Dano *et al*., 2016). At present, there are few reports about the clinical diagnosis and treatment of neonatal purulent meningitis about MMP-2, TIMP-1 and PCT. The purpose of this study was to investigate the significance of MMP-2 and TIMP-1 expression in cerebrospinal fluid of neonatal purulent meningitis and their correlation with the dynamic changes of serum PCT in order to provide, a theoretical reference for clinical diagnosis and treatment of neonatal purulent meningitis.

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MATERIALS AND METHODS

Study subjects

Among the PM group's cases, 45 were male, 20 were female and gestational age was 38 to 42 weeks. Admission time was 2 hours to 25 days with admission weight 2.5 to 4.3Kg. All patients conformed to the diagnosis standard of purulent meningitis and other diseases were excluded.

65 cases of neonatal virus meningitis in our hospital at the same period were served as the VM group. Among them, 44 males, 21 females, gestational age 38 to 42 weeks, admission age 1 hour to 24 days, admission weight 2.4 to 4.2Kg. All patients conformed to the diagnosis standard of viral meningitis and other diseases were excluded. Control group consisted of 44 male and 21 female. Gestational age was 38 to 42 weeks and admission time was 1 hour to 26 days. Admission weight was 2.4 to 4.2Kg.

Method

Cerebrospinal fluid and venous blood samples were

collected within few hours of admission to the hospital in the acute phase in PM and VM group, while were collected at the time of admitted to hospital for physical examination in control group. All samples were kept at -70°C refrigerator for spare.

The expression levels of MMP-2 and TIMP-1 in cerebrospinal fluid were detected by ELISA (Nanjing sailing Biotechnology Co., Ltd.), following the kit instructions strictly. The level of PCT was determined by chemiluminescence analyzer (DiaSorin Deutschland GmbH, German).

Observed indexes

The levels of MMP-2 and TIMP-1 in cerebrospinal fluid and PCT in serum were compared in three groups, and the correlation of the above indexes in neonates with purulent meningitis was discussed.

Ethical approval

This study was approved from the institutional ethical review board of Gansu Provincial Hospital, Lanzhou, Gansu, PR China. All the experiments were conducted as per Helsinki's declaration for human volunteers with Reference No.0098/ERB/GPHL/2017.

STATISTICAL ANALYSIS

Using SPSS 20 (BIM, New York, USA) statistical software, statistical analysis was carried out. The F test was performed between the three groups, and the correlation analysis was performed by Spearman correlation test.

RESULTS

Comparison of the expression of MMP-2 levels

The level of MMP-2 in cerebrospinal fluid in PM group was 138.65±25.65ng/ml, in VM group was 45.44±6.69ng/ml, in control group was 1.33±0.47ng/ml, and differences among the 3 groups were statistically significant (F=16.126, P<0.05) (fig. 1).

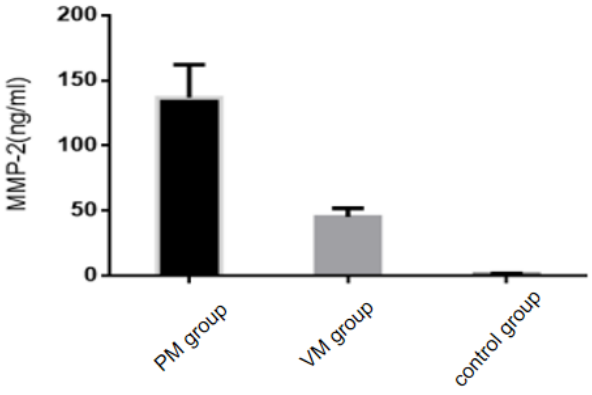

Fig. 1: Comparison of the expression levels of MMP-2 in cerebrospinal fluid in each group.

Comparison of the expression of TIMP-1 levels

The level of TIMP-1 in cerebrospinal fluid in PM group was 375.67±34.42ng/ml, in VM group was 177.54± 20.17ng/ml, in control group was 7.68±2.33ng/ml, and differences among the 3 groups were statistically significant (F=16.093, P<0.05) (fig. 2).

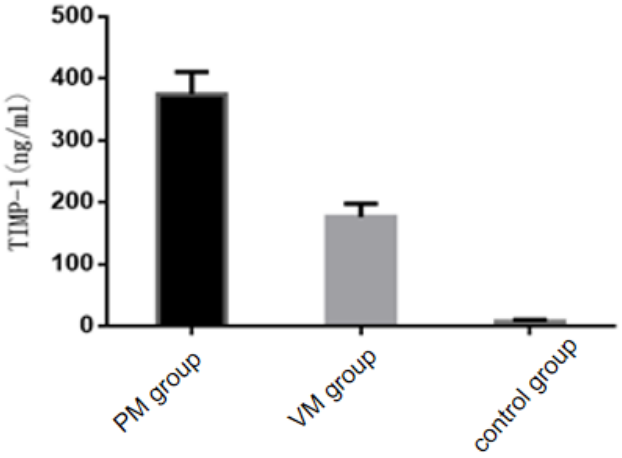

Fig. 2: Comparison of the expression levels of TIMP-1 in cerebrospinal fluid in each group.

Comparison of the serum levels of PCT

The serum level of PCT in PM group was 14.73±2.14ng/l, in VM group was 9.06±1.05ng/l, in control group was 0.37±0.12ng/l, and differences among the 3 groups were statistically significant (F=14.702, P<0.05) (fig. 3).

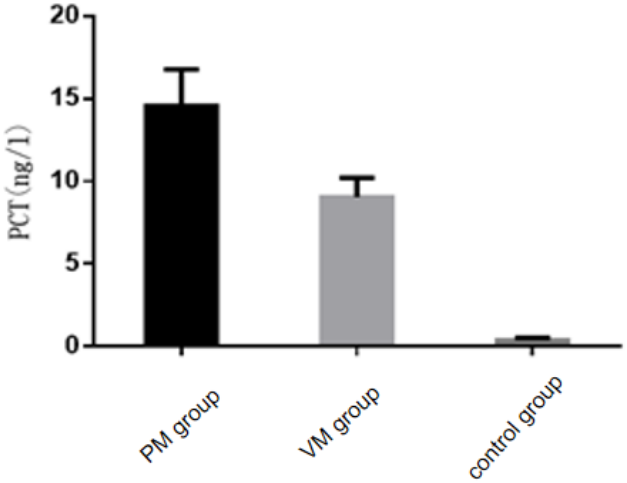

Fig. 3: Comparison of the serum levels of PCT in each group

Correlation analysis in PM group

The level of MMP-2 in cerebrospinal fluid was positively correlated with the serum level of PCT in PM group (r=0.582, P<0.05) (fig. 4). The level of TIMP-1 in cerebrospinal fluid was positively correlated with the serum level of PCT in PM group (r=0.564, P<0.05) (fig. 5).

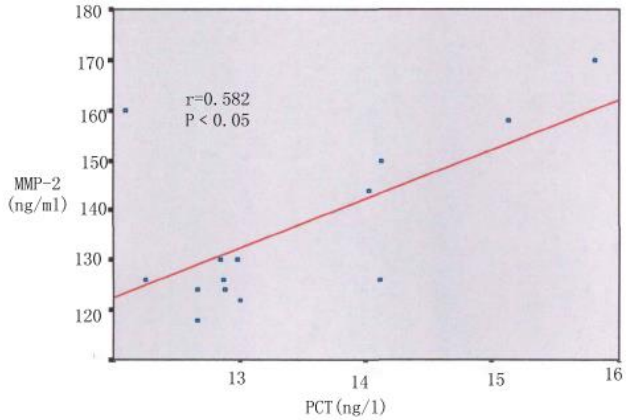

Fig. 4: The level of MMP-2 in cerebrospinal fluid with the serum level of PCT in PM group.

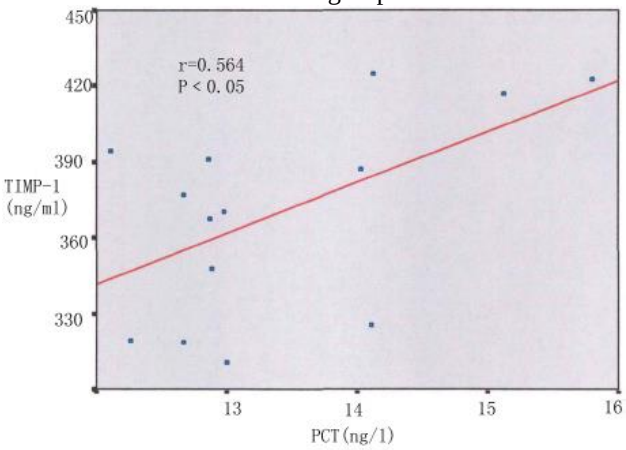

Fig. 5: The level of TIMP-1 in cerebrospinal fluid correlated with the serum level of PCT in PM group.

Correlation analysis in VM group

The level of MMP-2 in cerebrospinal fluid was positively correlated with the serum level of PCT in VM group (r=0.616, P<0.05) (fig. 6). The level of TIMP-1 in cerebrospinal fluid was positively correlated with the serum level of PCT in VM group (r=0.635, P<0.05) (fig. 7).

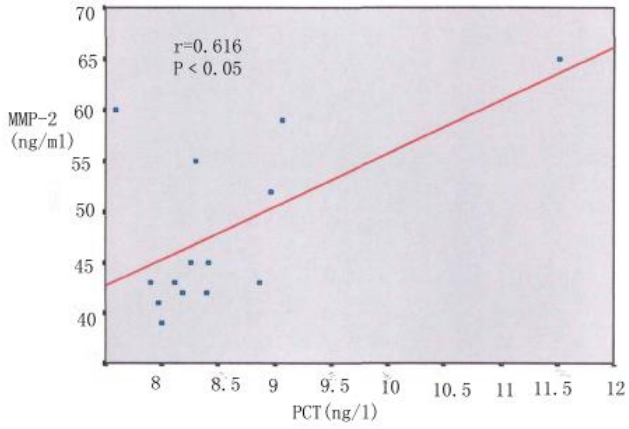

Fig. 6: The level of MMP-2 in cerebrospinal fluid correlated with the serum level of PCT in VM group

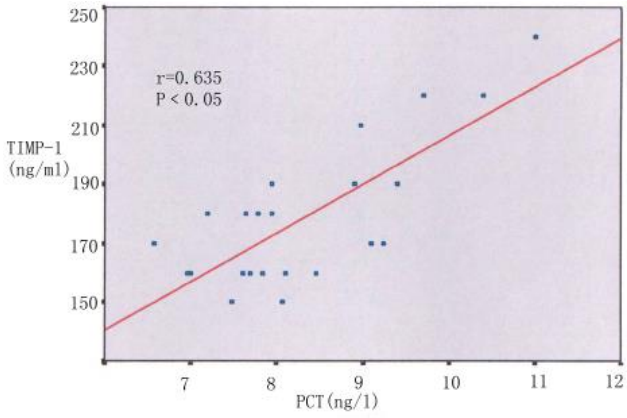

Fig. 7: The level of TIMP-1 in cerebrospinal fluid correlated with the serum level of PCT in VM group.

DISCUSSION

In the neonates with purulent meningitis, severe cases can cause death, and even the surviving neonates may have a series of sequelae, such as the slow development of the nervous system, visual impairment and hydrocephalus (Agossou *et al*., 2016; Kamoun *et al*., 2015; Softic *et al*., 2015; Masand *et al*., 2015). Neonatal purulent meningitis is a kind of inflammatory response caused by the combined action of pathogenic bacteria and inflammatory factors (De Oliveira e al., 2007). In recent years, antibiotics have been widely used in clinical practice, resulting in atypical cerebrospinal fluid examination of purulent meningitis. Especially, the clinical manifestations of neonatal purulent meningitis are more atypical (Roine *et al*., 2015; Leppert *et al*., 2000; Sellner and Leib, 2006). This also makes it difficult to distinguish the purulent meningitis from the viral meningitis.

There was close relationship between MMPs and diseases of central nervous system infection as previous study showed (Hu *et al*., 2015). This may be because MMPs induces the degradation of vascular basement membrane type IV collagen, which makes the blood brain barrier damaged. As a result, T cells pass through the barrier to arrive at central nervous system and actively participate in the inflammatory reaction. MMP-2 plays a very important role in the central nervous system infection diseases, and participates in the blood brain barrier damage and the pathogenesis of immunological pathology. In the present study, the levels of MMP-2 in cerebrospinal fluid were compared in 3 groups and the differences were statistically significant. The levels of MMP-2 in PM group were higher than that in VM group and control group. This suggested that MMP-2 was highly expressed in cerebrospinal fluid level in newborn, which could induce T lymphocytes and other inflammatory cells to pass through the blood brain barrier and enter the central nervous system, in order to induce purulent meningitis finally.

TIMP- 1 is less expressed in normal human body, but when inflammatory reaction occurs, there is high expression of TIMP-1 (Henry *et al*., 2016; Wei *et al*., 2016). Present research confirmed this point. The level of TIMP-1 in PM group were higher than that in VM group and control group, and the differences were statistically significant, which were consistent with the foreign literature (Wei *et al*., 2016).

PCT is a new inflammatory factor in recent years and is commonly used in the clinical diagnosis of infectious diseases. Under normal conditions, the level of PCT in human serum is less and is mainly produced by C cells of thyroid gland (Gaschignard *et al*., 2011; Ogunlesi, 2013; Zueter and Zaiter, 2015). However, under the condition of bacterial infection, the liver macrophages and lung lymphocytes in the body can synthesize and secrete PCT, hence PCT is highly expressed in the serum. Related study has shown that PCT has a certain value in the clinical diagnosis of neonatal sepsis (Yang *et al*., 2015). In present study, the level of serum PCT in PM group were higher than that in VM group and control group, and the differences were statistically significant. This suggested that inflammation might stimulated the synthesis and secretion of serum PCT in body and the changes of serum PCT level reflected the degree of infection and meningitis in neonates.

Further analysis showed that the level of MMP-2 in cerebrospinal fluid was positively correlated with the serum level of PCT in PM group. The level of TIMP-1 in cerebrospinal fluid was positively correlated with the serum level of PCT in PM group. The level of MMP-2 in cerebrospinal fluid was positively correlated with the serum level of PCT in VM group. The level of TIMP-1 in cerebrospinal fluid was positively correlated with the serum level of PCT in VM group.

CONCLUSION

These results indicated that MMP-2 and TIMP-1 in cerebrospinal fluid and serum PCT were involved in the occurrence and development of purulent and viral meningitis in neonates, and there was positive correlation between them. Thus, the joint detection and comprehensive judgment of MMP-2 and TIMP-1 in cerebrospinal fluid and serum PCT have important application value in differential diagnosis of purulent and viral meningitis in neonates, which are worth promoting.

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