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PLASMA LEVELS OF SERUM METALLOPROTEINASES MMP-9, MMP-2 AND TISSUE INHIBITORS TIMP-2 IN NEWBORNS WITH NECROTIZING ENTEROCOLITIS

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ABSTRACT — The analysis of the balance of elastase (MMP-2, MMP-9) and TIMP-4 was performed in order to determine its involvement in the pathogenesis of NEC. A progressive duration of NEC with sepsis is accompanied by increased serum concentrations of MMP-9, MMP-2 and TIMP-4. Increases in concentrations of MMP-2>503 ng/ml, MMP-9>812 ng/ml TIMP-4>1404 ng/ml can be regarded as statistically significant predictors of fatal outcome of NEC. The proposed method for determination of the outcomes of NEC in newborns is characterized by high sensitivity (94%) and specificity (87%).

KEYWORDS — necrotizing enterocolitis (NEC), newborns, sepsis, matrix metalloproteinase MMP-9, matrix metalloproteinase MMP-2, tissue inhibitors of /matrix metalloproteinase TIMP-2.

INTRODUCTION

Necrotizing enterocolitis (NEC) is a devastating disease found primarily in premature infants. NEC is characterized by rapid coagulation necrosis of distal ileum resulting in severe cases of intestinal perforation. The spectrum of clinical manifestations is presented by reversible intestinal disorders to fulminant forms, accompanied by gangrene of the intestinal wall perforation, development of abdominal sepsis and septic shock [1, 2]. One of the primary directions in the study of the pathogenesis of NEC is a search for reliable molecular markers, which help identifying the severity of the disease and help to determine the prognosis and selection of individual treatment strategies [3]. Currently, the prognosis of the duration of the disease is based on the use of standard criteria including clinical symptoms, laboratory parameters and morphological characteristics of the disease. Matrix metalloproteinases (MMPs), zinc endopeptidase are synthesized in a latent form and activated by proteolytic cleavage of the amino-terminal domain or conformational changes induced by oxidative stress. They take a part in the degradation of collagen type IV, which is the main component of the basal membrane as it contributes to the destruction of gastrointestinal perforation [4, 5].

Aim of research

To study the role of matrix metalloproteinases MMP-9, MMP-2 and TIMP-2 tissue inhibitors in newborns with NEC.

MATERIALS AND METHODS

The study was approved by the local medical ethics committee. The patients were divided into two groups. Group I (n=25) was defined as premature infants (≤ 37 completed weeks) without NEC, sepsis, septic shock, or systemic inflammatory response syndrome. Group II (control group, n=30) included infants with proven NEC (Bell's stage III) who were treated in the intensive care unit of the University Medical Center (Volgograd, Russia) between September 2012 and June 2019. The distribution of gestational age, birth weight, and gender was similar in patients with NEC and control groups. Concentrations of elastase (MMP-9, MMP-2) and tissue inhibitors of metalloproteinases (TIMP-2) in blood plasma were determined twice in Group II: during the first clinical presentation and 7 days after the surgery. In Group I, concentrations of elastase (MMP-9, MMP-2) and tissue inhibitors of metalloproteinases (TIMP-2) in blood plasma were determined once during the first clinical presentation. Blood was sampled by venipuncture and serum harvested by centrifugation and stored at -20° C. Plasma enzyme concentration was quantified via kits commercially used for ELISA (Human Human MMP-9 Quantikine ELISA Kit, Human Human MMP-9 Quantikine ELISA Kit Human Human TIMR-1 Quantikine ELISA Kit, R & D Systems, USA) by the ELISA analyzer ANTHOS 2020 (Austria). The methods of correlation and discriminant analysis were used to perform statistical analysis via SPSS 17.0 software. All data were presented as mean ± SD. Links between continuous variables were examined using Spearman rank correlation. Statistical

differences between the two groups were evaluated using unpaired Student's t-test or Kruskal-Wallis test. Differences were considered statistically significant at $P < 0.05$.

RESULTS

Significant differences ($p < 0.05$) were observed for MMP-2, TIMP-4 in all patients with NEC. The value of MMP-9 did not differ significantly between the groups. Average values of MMP-9 and TIMP-4 were higher among patients with a fatal outcome than among surviving patients by a factor 2-times ($p < 0.01$). Strong positive correlation between TIMP-4 and MMP-2 ($r = 0.81$; $p < 0.01$) was detected. In patients with gastrointestinal perforation, the average concentrations of MMP-2 were increased ($p < 0.01$) by 8 times, TIMP-4 by 5.8 times when compared to Group II. In patients with sepsis, production of MMP-9 and TIMP-4 was increased by 2.3-times ($p < 0.01$) when compared to patients without sepsis. The deaths of patients with signs of sepsis were accompanied by a significant ($p < 0.01$) increase in the average concentration of TIMP-4 (2085 ng/ml) and MMP-9 (1032 ng/ml). In patients without signs of lethal sepsis, average concentrations of TIMP-4 (1306 ng/ml) and MMP-9 (668 ng/ml) were elevated. After operations on recovered patients, average concentrations of MMP-2 decreased by 1.5-times ($p < 0.01$) and TIMP-4 by 1.5 times ($p < 0.05$) respectively. However, they remained significantly higher than control values. In addition, in Group II, the average concentrations of MMP-9 were still elevated before and 7 days after the start of treatment. The ROC analysis showed that predictors of mortality in newborn with NEC were the following: TIMP-4 (AUC = 0.74, 95% CI = 0.62 to 0.97; $P < 0.001$), MMP-2 (AUC = 0.95, 95% CI = 0.9 to 0.99; $P < 0.001$), MMP-9 (AUC = 0.68, 95% CI = 0.52 to 0.84; $P < 0.003$). Optimal values for each predictor of mortality were MMP-2>503 ng/ml, MMP-9> 812 ng/ml, TIMP-4>1404 ng/ml. Sensitivity of the test was 94%, specificity was 87%, which proves the high quality of our proposed method for determining the basis of NEC.

DISCUSSION

According to some investigations, in samples of removed intestinal tissue of patients with NEC, the levels of expression of MMP-2, MMP-9 and TIMP-2 remained unchanged [6]. The role of MMP-2, MMP-9 and TIMP-4 in sepsis remains unclear, but some studies indicate that MMP plays a certain role in the migration of leukocytes from the blood in inflammation by MMP-mediated proteolysis of the basement membrane [7]. High levels of MMP-9 and TIMP-4 in blood serum were found in patients with sepsis and fatal outcome, which may be important for understanding the pathophysiology of sepsis in patients with NEC. The development of endotoxemia might lead to the release of MMP-9, MMP-2 and TIMP-4, which explains the significant correlation differences between these parameters in patients with sepsis. In addition, while MMP-9 is mainly released by activated leukocytes, the observed differences cannot be explained by the presence of leukocytosis, as the values were approximately the same in surviving and deceased patients with NEC. Having analyzed the characteristics of the ROC curve, we found that serum levels of TIMP-4 and MMP-2 had high sensitivity and specificity and were good predictors for mortality in patients with NEC. From a clinical point of view, a highly sensitive test may lead to over-diagnosis, but it minimizes the risk of leaving the disease undetected, because development of complications leads to high mortality.

CONCLUSION

A progressive duration of NEC with sepsis is accompanied by increased concentrations of MMP-9, MMP-2 and TIMP-4 in blood serum. Increases in the concentrations of MMP-2>503 ng/ml, MMP-9>812 ng/ml, TIMP-4>1404 ng/ml can be regarded as statistically significant predictors of fatal outcome of NEC. The proposed method of determination of the outcomes of NEC in newborns is characterized by high sensitivity (94%) and specificity (87%).

Conflict of Interest

The authors declare no conflicts of interest.

Author Contributions

Concept and design of the study — I.N. Khvorostov; Data collection and processing — I.N. Khvorostov; Text writing — I.N. Khvorostov, I.E. Smirnov; Editing — I.N. Khvorostov, I.E. Smirnov.

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