Research Article

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Serum NF-κBp65, TLR4 as biomarker for diagnosis of preeclampsia

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Abstract: The aim of this study was to evaluate the serum NF-κBp65, TLR4 (Toll-like receptor 4) expression in patients of preeclampsia and its diagnostic value as biomarkers.

Methods: Thirty patients with preeclampsia (case group) and 30 normal pregnant women (control group) were included in this study. The serum level of NF-κBp65 and TLR4 were examined by enzyme linked immunosorbent assay (ELISA), and compared between the two groups. The diagnostic sensitivity, specificity and area under the receiver operating characteristic (ROC) curve were calculated by STATA11.0 statistical software.

Results: The expression level of TLR4 and NF-κBp65 in serum of preeclampsia patient group was 3.76±1.07ng/ml and 183.20±49.19ng/ml, whereas that in the serum of the normal pregnant group was 2.43±0.69ng/ml and 98.68±29.80ng/ml. The expression of TLR4 and NF-κBp65 in serum of preeclampsia patient group was significantly higher than that of the normal pregnant group (P<0.05); The Pearson correlation test showed that the TLR4 expression in the serum of preeclampsia patients and normal pregnant women was positively correlated with their NF-κBp65 expression [$r_{preeclampsia}$=0.46, (P<0.05), r_{normal}=0.48, (P<0.05)]. When TLR4 and NF-κBp65 were selected as the reference indexes, the diagnostic sensitivity of preeclampsia was 86.67% (95%CI:69.28%-96.24%) and 90.33% (95%CI:73.47%-97.89%), and the specificity was 70.00% (95%CI:50.60%-85.27%) and 83.33% (95%CI:65.28%-94.36%). The area under the ROC curve was 0.84 and 0.89.

Conclusion: Serum levels of TLR4 and NF-κBp65 was significantly higher in patients with preeclampsia which may involve in the pathogenesis of preeclampsia, and can be used as biomarker for predicting preeclampsia.

Keywords: Preeclampsia; TLR4; NF-κBp65; Diagnosis

1 Introduction

Recent researches showed that Toll-like receptor 4 (TLR4) and NF-κB was highly expressed in the placenta of patients who suffered from preeclampsia, but the expression of TLR4 and NF-κB was low in second trimester of pregnancy of normal pregnant women [1, 2]. This implies the possibility of TLR4 and NF-κB involvement in the pathogenesis and development of preeclampsia [3]. As early as 1994, Faas et al [4] found that infusion of low-dose lipopolysaccharide (LPS) into animals could lead to the typical symptoms of preeclampsia, hypertension, proteinuria, and glomerular endothelial disease, and they also verified the correlation of LPS with the inflammatory reaction induced by TLR4. Some literatures also reported that NF-κB, a network of cytokines and oxidation enzyme system, participates in the pathogenic course of preeclampsia [5]. However, few studies have been made on the expression level of TLR4 and NF-κB in the serum of patients who suffer from preeclampsia. Our present study checked for any difference in the expression level of TLR4 and NF-κB in the serum between normal pregnant women and those who suffer from preeclampsia using the enzyme-linked immunosorbent assay (ELISA) method and explore their effect in the pathogenic course of preeclampsia and their feasibility to work as the serum marker for diagnosis of preeclampsia.

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2 Materials and methods

2.1 Patients

Thirty preeclampsia patients admitted into Zhangqiu maternity and childcare hospital and 30 normal pregnant women were selected as the subjects of the study. The preeclampsia was diagnosed based on Obstetrics and Gynecology edited by Sarabatnam Arulkumaran. The average age of the normal pregnant group was 27.61±5.36 years old, their average gestational age was 39.2±0.8 weeks, and their average neonatal weight was 3.28±0.41 kg. The average age of the preeclampsia group was 31.33±3.89 years old, their average gestational age was 38.75±1.04 weeks, and their average neonatal weight was 3.31±0.37kg.

Ethical approval: The research related to human use has been complied with all the relevant national regulations, institutional policies and in accordance the tenets of the Helsinki Declaration, and has been approved by the authors' institutional review board or equivalent committee.

Informed consent: Informed consent has been obtained from all individuals included in this study.

2.2 Serum TLR4 and NF-κBp65 examination

A total of 2mLfasting blood was drawn from the peripheral elbow vein of the participants from both groups in the morning before delivery, kept still, centrifuged at 1,000r/min for 10min to separate the serum, placed into the high-pressure-treated 1.5mL EP tubes, and kept in the refrigerator at -80°C for further use in tests. The ELISA method was adopted to test the protein concentration of TLR4 and NF-κBp65 in the serum, and the test was carried out according to the manufacturer's instruction (Germany, IBL company).

2.3 Statistical analysis

The statistical analysis was made with STATA11.0 statistical software (http://www.stata.com), the measurement data were expressed with $\bar{x} \pm s$ and the comparison between groups was made based on the t-test of the sample mean. The enumeration data were expressed with a relative number, and the comparison between groups was made based on the c2 test. The correlation between TLR4 and NF-κBp65 level was calculated by Pearson correlation test. Regarding the diagnosis test, sensitivity, specificity, and the area under the ROC curve were calculated according to the Bayes' theorem. P<0.05 meant a statistical difference.

3 Results

3.1 NF-κBp65 and TLR4expression in serum

The expression level of TLR4 and NF-κBp65 in serum of preeclampsia patient group was 3.76±1.07ng/ml

Table 1: NF-κBp65 and TLR4 level in serum of the two groups (ng/ml)

Group	n	TLR4	NF-κB
Control	30	2.43±0.69	98.68±29.80
Case	30	3.76±1.07*	183.20±49.19*

*Compared with control group, P<0.05

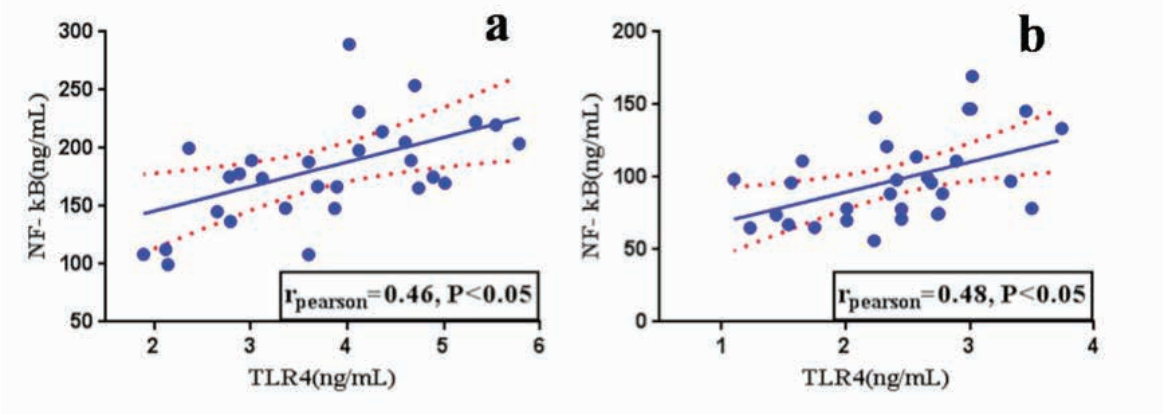

Figure 1: Pearson correlation analysis of TLR4 and NF-κBp65.(a: for preeclampsia patients; b: for normal pregnant women)

and 183.20±49.19ng/ml, whereas that in the serum of the normal pregnant group was 2.43±0.69ng/ml and 98.68±29.80ng/ml. The expression of TLR4 and NF-κBp65 in serum of preeclampsia patient group were significantly higher than that of the normal pregnant group (P<0.05), Table 1.

3.2 Correlation between serum TLR4 and NF-κBp65

The Pearson correlation test showed that the TLR4 expression in the serum of preeclampsia patients and normal pregnant women was positively correlated with their NF-κBp65 expression [$r_{preeclampsia}$=0.46, (P<0.05), r_{normal}=0.48, (P<0.05)]. With the increase of TLR4 expression, NF-κBp65 expression rose significantly, as shown in Figure.1.

3.3 Serum TLR4 and NF-κBp65 in the diagnosis of preeclampsia

When TLR4 and NF-κBp65were selected as the reference indexes, the diagnostic sensitivity of preeclampsia was 86.67% (95%CI:69.28%-96.24%) and 90.33% (95%CI:73.47%-97.89%), and the specificity was 70.00% (95%CI:50.60%-85.27%) and 83.33% (95%CI:65.28%-94.36%). The area under the ROC curve was 0.84 and 0.89, as shown in Figure.2.

4 Discussion

Preeclampsia refers to symptoms of hypertension and proteinuria that happens after 20 weeks of gestation to pregnant women whose blood pressure is normal before pregnancy [6, 7]. Clinical epidemiological studies have shown that the incidence of preeclampsia was approximately 3% in all pregnant women [8, 9]. However, the causes and exact mechanism of preeclampsia remain unclear [10, 11]. Published researches demonstrated that the probable reason was that maternal inflammatory overreaction to gestation led to immune disorder on the maternal–fetal interface and gave rise to vascular lesion of the placenta, vascular endothelial injury, restricted migration of cytotrophoblast cells, and shallow implantation [12-14]. The pathogenesis, including vasospasm, endothelial cell activation, and increased pressure response, prostaglandin, nitric oxide, and endothelinwererelated to the angiogenesis and antiangiogenesis proteins [15].

TLR4 is a key protein molecule involved in nonspecific immunity and is also a bridge between nonspecific and specific immunity. TLR4 is a single transmembrane noncatalytic protein that could be used to identify the molecules with conservative structure from microorganisms. Recent studies showed that the inflammatory reaction induced by TLR4 was closely related to preeclampsia [16]. Zhao et al. [17] examined the expression of TLR4 in the placenta of60 preeclampsia patients and normal pregnant women using the immunohistochemical method. Their study indicated that TLR4 expression in placenta tissue of preeclampsia patients was higher than that of the normal pregnant women in the late trimester. Their study also believed that the increased TLR4 expression was closely related to the occurrence of preeclampsia and might be one part of the pathogenesis of preeclampsia. In the present study, we found that the TLR4 expression in the serum of preeclampsia patients was significantly higher than that of the normal pregnant women and could be used as the serum marker for preeclampsia diagnosis.

As the nuclear transcription factor, NF-κB is involved in the in vivo cytokine network and participates in the

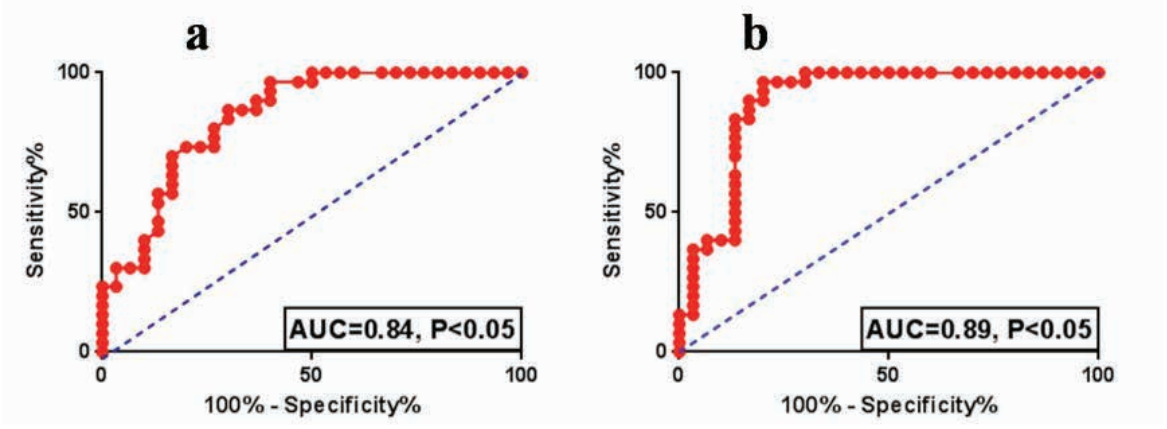

Figure 2: The area under the ROC curve (a: AUC for TLR4; b: AUC for NF-κBp65)

pathogenic course of preeclampsia together with the oxidase system. Wang et al [18] examined the expression of NF-κB in the placenta tissue of 50 preeclampsia patients using immunohistochemical and RT-PCR methods. Their findings indicated that the expression of both NF-κB and mRNA in the placenta tissue of preeclampsia patients was significantly higher than that of the normal late pregnant women. As the preeclampsia worsened, NF-κB expression increased. Their study implied that the inflammatory factor induced by NF-κB might be one of the key reasons for the occurrence of preeclampsia. In the present study, the author also found that NF-κBp65 expression in serum of preeclampsia patients increased significantly, and this was consistent with the findings of Wang et al [18]. Moreover, the level of NF-κBp65 expression in serum could also work as the reference index for preeclampsia diagnosis.

The expressions of both NF-κBp65 and TLR4 are high in the serum of preeclampsia patients; thus, NF-κBp65 and TLR4arespeculated to be involved in the pathogenic course of preeclampsia. Furthermore, the differential expression of NF-κB and TLR4 in the serum between preeclampsia patients and normal pregnant women has also affirmed their role as the serum marker for diagnosis of preeclampsia.

However, there are only 30 patients included in each group, the sample size is small and more patents are need for further evaluating the clinical efficacy of serum NF-κBp65, TLR4 as biomarker for diagnosis of preeclampsia.

Conflict of interest statement: Authors state no conflict of interest.

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