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ORIGINAL ARTICLE

Combination of NGAL and Cystatin C for Prediction of Preeclampsia at 10-14 Weeks of Gestation

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SUMMARY

Background: **Preeclampsia (PE) is a severe pregnancy complication and is an important cause for maternal and child death, premature delivery, and limited intrauterine growth and development. The aim of this study was to investigate the role of NGAL and cystatin C, alone and in combination, for early prediction of PE at 10 - 14 weeks of gestation.**

Methods: **Serum levels of NGAL and cystatin C were assessed in women at 10 - 14 weeks of gestation who subsequently developed PE (n = 128) and normal pregnancy outcome (n = 183). Comparison of clinical characteristics, NGAL, and cystatin C levels between normal pregnancy and PE groups were analyzed using Mann-Whitney test. The receiver operating characteristic curve (ROC curve) was used to analyze the value of serum NGAL and cystatin C levels in predicting PE.**

Results: **The levels of cystatin C and NGAL in the serum were significantly higher in the PE group [0.64 mg/L (0.52 - 0.78)] and [34.9 ng/mL (24.4 - 55.2), respectively] than in the normal pregnancy group [0.56 mg/L (0.49 - 0.65)] and [20.2 ng/mL (13.8 - 26.9), respectively]. ROC curve analysis showed that serum NGAL levels predicted the area under the curve in the PE period 0.739 (95% CI: 0.618 to 0.860). Serum cystatin C levels predicted the area under the curve in the PE period 0.722 (95% CI: 0.592 to 0.853). The combination of serum NGAL and cystatin C levels predicted the area under the curve in the PE period 0.877 (95% CI: 0.811 to 0.943).**

Conclusions: **NGAL and cystatin C levels in serum appear to be ideal biomarkers for PE prediction at 10 - 14 weeks. The combination of NGAL and cystatin C will also be more valuable in discriminating patients at risk of developing PE from other pregnancy complications early in gestation.**

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INTRODUCTION

Preeclampsia (PE) is one of the leading causes for maternal and perinatal death, which accounts for 5% - 7% of pregnant women [1,2]. The clinical diagnostic criteria include new hypertension (systolic blood pressure ≥ 140 mmHg or diastolic blood pressure ≥ 90 mmHg) and urine protein (≥ 300 mg/24 h) after 20 weeks of gestation. In some severe cases, acute renal failure, convulsions (eclampsia), pulmonary edema, and even HELLP (hemolysis, elevated liver enzymes and low

platelets, HELLP) may occur, characterized by hemolysis, elevated liver enzymes, and thrombocytopenia. Recent studies have shown that early pregnancy screening for basic risks and indicators in pregnant women including MAP, uterine artery, Doppler parameters and different serological indicators (pregnancy-associated plasma protein-A and placental growth factor, etc.) are important for early prediction of the occurrence of PE [3-5]. Thus, positive exploration for sensitive indicators to predict PE has become the focus of clinical research. Neutrophil gelatinase-associated lipocalin (NGAL) is a new adipocytokine and an inflammatory factor belonging to the lipocalin family. NGAL was accidentally discovered by Danish scientist Kjeldsen when electrophoresed under the condition of reducing metal matrix proteinase 9 (MMP-9) in 1993 [6]. NGAL is a secreted glycoprotein consisting of 178 amino acid residues and mainly expresses in neutrophils, mononuclear macrophages, and adipocytes in the body [7]. In recent years, studies have found that NGAL is significantly associated with pregnancy-induced hypertension, gestational diabetes, preterm birth due to infection, and PE [8-11]. Studies also found that the content of NGAL in cord blood could be used as a newborn biological marker of asphyxia during life [12].

Cystatin C is one of the cysteine protease inhibitors. It was first isolated and purified by Nastasi et al. in 1983. It is composed of 122 amino acids and is a low molecular weight, non-glycosylated basic protein. It has no histological specificity and widely exists in nucleated cells and body fluids [13,14]. The studies found that cystatin C is a good indicator of renal dysfunction [15,16], and a higher level of serum cystatin C is associated with increasing cardiovascular disease and mortality [17-19]. Serum cystatin C increased in PE at an early stage and can closely reflect the renal functional changes [20]. Some studies manifested that cystatin C also could be used to predict the occurrence and subsequent development of PE, but more studies are required to validate these findings [21,22].

In the present research, we investigated the role of NGAL and cystatin C, alone and in combination, for prediction of PE at 10 - 14 weeks of gestation. These results would help early identification of women at risk of developing PE and give the patients better therapeutic management.

MATERIALS AND METHODS

Study population

From January 2016 to December 2017, 311 pregnant women who were scheduled to complete the check-up and delivery at Nantong Maternity and Child Health Hospital were selected as subjects for this study. Then, the pregnancy outcomes were followed up. According to the pregnancy outcomes, 128 PE patients were included into the PE group and 183 cases were into the normal pregnancy group. Gestational age was calculated from the last menstrual period and confirmed by ultrasound at 10 - 14 weeks of gestation. The criteria for inclusion in the study were as follows: single pregnancy, pregnant women aging from 20 to 35 years, blood collection time from 10 to 14 weeks, no history of special medication during pregnancy, study subjects informed about the project research and signed informed consent. This study excluded patients with chronic hypertension, heart disease, kidney disease, diabetes, hyperthyroidism, connective tissue disease, blood disease, and other chronic diseases before pregnancy. Patient characteristics such as age, body mass index (BMI), GA at sampling, blood pressure, proteinuria, HELLP syndrome, delivery and birth weight were obtained during their hospital visit. The study was approved by the hospital ethics committee and all subjects signed informed consent.

Subjects were enrolled and 5 mL of elbow venous blood was collected into a non-coagulated glass test tube between 10 and 14 weeks of gestation. After natural coagulation for 30 minutes, the samples were centrifuged at 4°C, 3,000 rpm/min, for 8 minutes. The serum was separated and stored at -80°C until determination of NGAL and cystatin C levels. The concentration of NGAL and cystatin C in the serum were detected by ELISA (CST, USA). Women were followed up until delivery and the outcomes of pregnancy were obtained from the database of the hospital. Women were grouped into normal pregnancy and PE groups depending on the outcome of the delivery.

Statistical analysis

SPSS 17.0 statistical analysis software was used to analyze the data. NGAL and cystatin C levels in the serum were expressed as median (interquartile range). Comparison of clinical characteristics, NGAL, and cystatin C levels between normal pregnancy and PE groups were done using Mann-Whitney test. The receiver operating characteristic curve (ROC curve) was used to analyze the value of serum NGAL and cystatin C levels in predicting PE. $p < 0.05$ is statistically significant.

RESULTS

Patient characteristics

Three hundred eleven women were included in this research and the detailed information of these women is shown in Table 1. One hundred eighty-three women with normal delivery outcome were in normal pregnancy group and 128 women subsequently developed PE. The age, GA at sampling, and blood pressure had no statistical significance between normal pregnancy and PE groups. The BMI was markedly higher in the PE group [24.5 (19.8 - 22.9)] compared with normal pregnancy group [22.4 (19.2 - 27.2)]. Moreover, the delivery time was shorter and the birth weight was lighter in the PE group compared with normal pregnancy group. Moreover, 10% of the women in PE group developed

Table 1. Clinical characteristics of control and preeclampsia groups.

Characteristics	Normal pregnancy (n = 183)	Preeclampsia (n = 128)	p-value
Maternal age - years	25.8 (21 - 30)	25.4 (22 - 31)	NS
BMI - kg/m^2	22.4 (19.2 - 27.2)	24.5 (19.8 - 22.9)	< 0.001
GA at sampling - weeks	9.8 (8.2 - 11.5)	9.7 (8.5 - 10.4)	NS
Blood pressure - mmHg			
Systolic at 1st trimester	117 (102 - 129)	115 (101 - 132)	NS
Diastolic at 1st trimester	73 (63 - 82)	72 (62 - 83)	NS
Proteinuria - g/24 h	ND	1.8 (0.6 - 3.1)	
HELLP - n (%)	0	10 (7.8)	
Delivery - weeks	39.2 (37.8 - 40.6)	36.6 (35.2 - 38.3)	< 0.001
Birth weight - g	3,316 (3,154 - 3,586)	2,690 (2,469 - 2,977)	< 0.001

Table 2. ROC analysis showing area under curve (AUC) of NGAL and cystatin C, alone and in combination, for the prediction of preeclampsia.

Marker	AUC	95% Confidence Interval
NGAL	0.739	0.618 - 0.860
Cystatin C	0.722	0.592 - 0.853
Cystatin C, NGAL	0.877	0.811 - 0.943

Table 3. ROC analysis showing area under curve (AUC) of NGAL or cystatin C for the prediction of mild and severe preeclampsia.

Marker	AUC	95% Confidence Interval
NGAL (mild)	0.720	0.519 - 0.802
NGAL (severe)	0.802	0.647 - 0.957
Cystatin C (mild)	0.696	0.491 - 0.799
Cystatin C (severe)	0.781	0.617 - 0.946

HELLP syndrome.

The concentrations of NGAL and cystatin C in serum

Biomarker levels in PE and normal pregnancy groups were given in Figure 1. The levels of cystatin C in the serum were significantly higher in the PE group [0.64 mg/L (0.52 - 0.78)] than in the normal pregnancy group [0.56 mg/L (0.49 - 0.65)]. Moreover, serum NGAL levels were significantly higher in the PE group [34.9 ng/mL (24.4 - 55.2)] than in the normal pregnancy group [20.2 ng/mL (13.8 - 26.9)].

Predictive value of serum NGAL and cystatin C in PE

Receiver-operating characteristics (ROC) curve analysis with positive predictive value for NGAL and cystatin C, alone and in combination, is shown in Figure 2 and Table 2. ROC curve analysis showed that serum NGAL levels predicted PE (0.739 (95% CI: 0.618 to 0.860)). The ROC analysis showed that serum cystatin C levels predicted PE (0.722 (95% CI: 0.592 to 0.853)). The ROC analysis showed that the combination of serum NGAL and cystatin C levels could predict PE (0.877 (95% CI: 0.811 to 0.943)). In addition, ROC analysis demonstrated that the AUC of NGAL and cystatin C for the prediction of mild and severe preeclampsia were

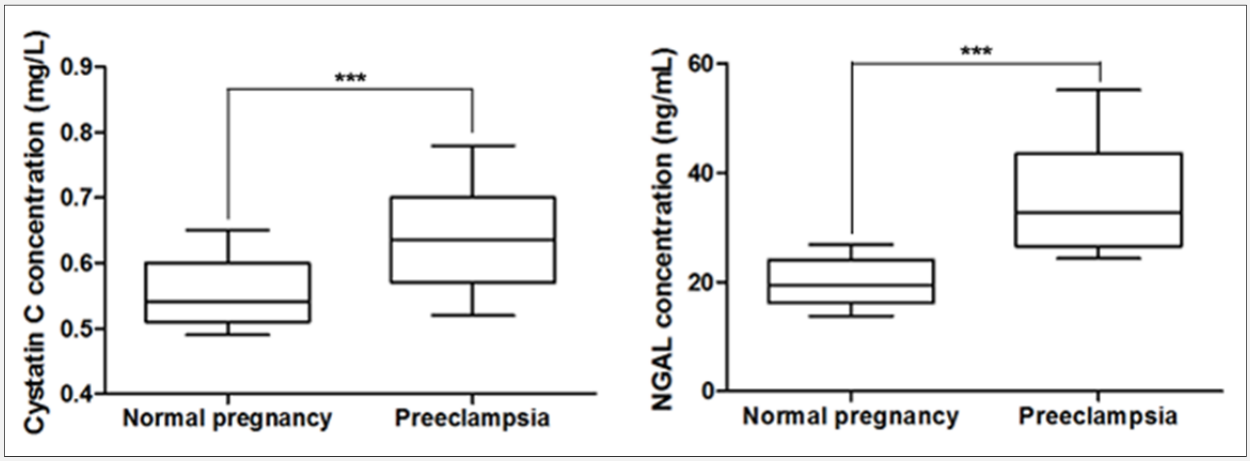

Figure 1. Serum levels of NGAL and cystatin C were detected by ELISA.

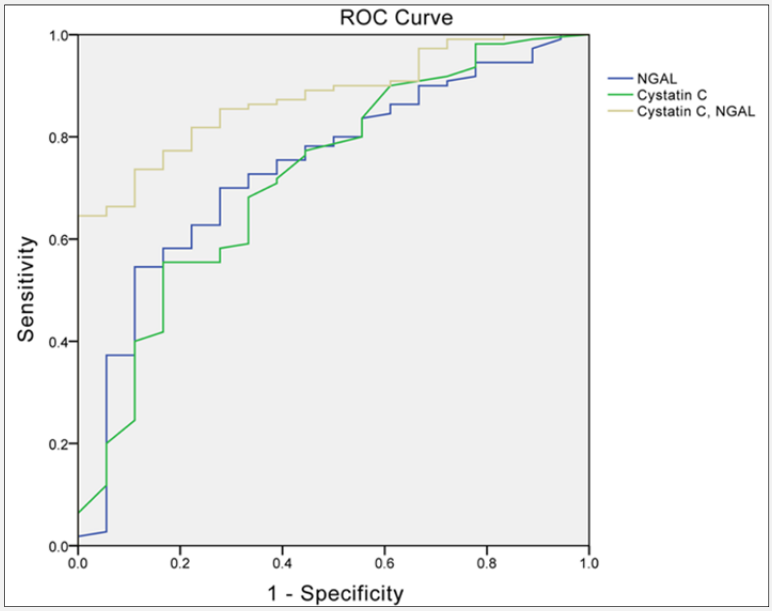

Figure 2. Receiver operating characteristic curve showing the screening characteristics of NGAL and cystatin C, alone and in combination, for prediction of PE.

0.720 (95% CI: 0.519 to 0.802), 0.802 (95% CI: 0.647 to 0.957), 0.696 (95% CI: 0.491 to 0.799) and 0.781 (95% CI: 0.617 to 0.946), respectively (Table 3). These results indicated that the combination of serum NGAL and cystatin C levels showed high predictive utility for prediction of PE.

DISCUSSION

PE is a common disease during pregnancy, which seriously affects maternal and child health [23]. Early prediction, diagnosis, and treatment of PE are of great significance in reducing maternal and child mortality. However, the pathogenesis of pre-eclampsia has not been fully elucidated so far. There are many causes including uterine spiral arterial recasting, vascular endothelial cell damage, nutritional deficiencies, genetic factors, and insulin resistance [24,25]. The more recognized mechanism is the "two-stage" doctrine [26]. The uterine spiral arteries are recast during the first 20 weeks causing the placental ischemia and hypoxia, etc. Then, the clinical manifestations gradually appear in the last 20 weeks. The placental factors are secreted into the maternal blood circulation causing the inflammatory response to be activated. Subsequently, the normal function of the vascular endothelium is impaired leading to clinical complications. Therefore, PE needs to be predicted before the occurrence of clinical symptoms in the first phase. It is necessary to explore the biomarkers or screening methods that could be applied in the first 20 weeks. In recent years, relevant biomarker studies for predicting PE have gradually shifted from about the 20th week of gestation to early pregnancy for better early prevention [27,28].

PE mostly affects the kidney, causing the increased permeability of renal glomerular capillaries, increased urine protein, and other clinical manifestations of renal dysfunction [29]. NGAL is a micro-secreted protein in neutrophils and renal tubular epithelial cells, and it can be abundantly expressed and released by renal tubular epithelial cells following renal injury. Numerous studies have confirmed that NGAL in serum and urine is a reliable marker for acute kidney injury and chronic kidney disease [30,31]. Our results showed that NGAL level in serum of pregnant women with PE was significantly higher than that of normal pregnant women. Inflammation and vascular endothelium injury in PE are the main causes for NGAL elevation. Inflammatory responses can lead to overexpression of inflammatory factors such as IL-1, IL-6, and TNF-α. Moreover, many studies have demonstrated that these factors can induce higher expression of NGAL [32]. In addition, hyperinsulinemia also can induce higher expression of NGAL, and insulin resistance can cause hyperinsulinemia in the body. It is speculated that the presence of insulin resistance in PE may also be the cause for elevated NGAL expression [33]. This study confirmed that NGAL could be used as an indicator to predict the incidence of PE. However, the role of serum NGAL in the pathogenesis of PE needs further study.

Serum cystatin C, as a sensitive indicator of early renal impairment, can accurately reflect glomerular filtration rate [34]. Cystatin C is less affected by some factors such as inflammation, age, gender, hemolysis, blood lipids, and liver disease. Cystatin C almost completely filters through the glomerular membrane and then can be reabsorbed by the renal tubules. The kidney is the sole organ that can conduct cystatin C elimination in the circulation. Thus, the glomerular filtration rate can determine the serum cystatin C concentration, which is considered to be an ideal endogenous index of glomerular filtration rate [35]. Cystatin C also can be used as a useful marker to detect early renal dysfunction in pregnancy [36,37]. A large number of studies have showed that serum cystatin C is significantly higher in pregnant women than in the normal control group, which have specific values for the early diagnosis and monitoring of PE [38,39]. Our research also confirmed that the level of cystatin C in serum in the PE group was significantly higher than that in the normal pregnancy group. Therefore, cystatin C could be used as an indicator to predict the incidence of PE.

For the complexity of the etiology and pathogenesis of PE, each serum marker may be in a state of dynamic equilibrium. Therefore, combined detection can improve the predictive value for PE, which is confirmed by many studies [27,40]. When predicting PE, ROC curve analysis showed that the AUC for serum NGAL and cystatin C was 0.739 and 0.722, respectively. After the combination of serum NGAL and cystatin C, the area under the curve was 0.877, further indicating that the combination of serum NGAL and cystatin C is more valuable in predicting PE in pregnant women.

CONCLUSION

In conclusion, this study revealed that the serum NGAL and cystatin C levels were ideal biomarkers for PE prediction at 10 - 14 weeks of gestation. The combination of NGAL and cystatin C was more valuable in discriminating patients at risk of developing PE from other pregnancy complications early in gestation.

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Declaration of Interest:
The authors declare that they have no competing interests.

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