Original Article
Value of combined detection of middle and late gestation serum markers placental growth factor, placental protein 13, β-human choionic gonadotrophin and interleukin-18 in diagnosis of preeclampsia

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Abstract: Objective: To evaluate the value of combined detection of middle and late gestation serum markers placental growth factor (PLGF), placental protein 13 (PP13), β-human chorionic gonadotrophin (β-hCG) and interleukin-18 (IL-18) in the diagnosis of pre-eclampsia. Methods: Forty-eight cases that were diagnosed as pre-eclampsia were taken as case group, among which 22 patients was mild pre-eclampsia patients and 26 cases was severe pre-eclampsia patients. Forty-eight normal gravidas were regarded as control group. Sampling analysis of their serum was conducted to test the content of placental growth factor, placental protein 13, β-human chorionic gonadotrophin and interleukin-18, at the same time, the differences between groups were evaluated, and so were the sensitivities and specificity of combined detection and area under the receiver operating characteristic curves. Results: The sensitivities of combined detection in mild pre-eclampsia and severe pre-eclampsia was 95.5% and 88.5%, the specificity was 97.9% and 100%, and the area under the receiver operating characteristic curves are 0.966 and 0.948, respectively. In addition, the results of combined detection of sensitivities, specificity and area under of receiver operating characteristic curves were higher than that of individual detection. Conclusion: The high accuracy of sensitivities and specificity for serum combined detection greatly contributed to the diagnosis and prediction of pre-eclampsia.

Keywords: Pre-eclampsia, serum markers, combined detection, receiver operating characteristic curves

Introduction
Pre-eclampsia (PE) is an angiospastic disease which belongs to hypertensive disorder in pregnancy. It has become one of the main factors to bring about maternal-child disease and death through causing multiple-organ damage, which had badly influenced the maternal-child health [1]. The incidence of PE on primipara ranged from 3% to 8% and tended to increase recently in China. The PE’s etiology is not completely clear since its pathogenesis involves many factors, which made its prevention and diagnosis difficult. Besides, the symptom of onset of PE is not obviously while some patients were already in severe phase when effected by hypertension and proteinuria, once diagnosis, the rate of incidence and mortality for gravidas and perinatal infant had a dramatic rising, and there are not effective clinical therapies except pregnancy termination. Thus it is crucial to improve maternal-child prognosis by find efficient methods for early screening, diagnosis and treatment of PE.

Placental growth factor (PLGF) belongs to vascular endothelial growth factor (VEGF) and it’s initially separated from human placenta complementary DNA (cDNA) library. In addition, PLGF was consist of placental trophoblast cells, which can regulate endothelial function and can affect the integrity and permeability of vessel wall. It has been reported that level of serum marker PLGF in pre-eclampsia patients decreased dramatically [3] but the pathogenesis of pre-eclampsia are still not clear currently. Placental protein 13 (PP13) was a new found
highly specific protein in placental and its reduction may cause shallow placentation and be associated with pre-eclampsia and other adverse pregnancy outcomes. Thus PP13 is important for the maintenance of gestation. There’s study showing that there’s a dramatical decrease of PP13 in patients with pre-eclampsia. Therefore, it can be regarded as a marker for early screening of pre-eclampsia.

β-human chorionic gonadotropin (β-hCG) tropion is a glycoprotein hormone synthesized and secreted by placental trophoblast cells. Current researches believe that biological effect of hCG is mediated by specific receptors on cell membrane. Meanwhile, there are HCG/LH receptors on endothelial cell membrane and smooth muscle cell membrane of uterine arteries and in fetal umbilical blood-flow, which indicates that biological effect of hCG plays an important role in early placentation and vascular remodeling. Cui et al. found that the level of β-hCG in pre-eclampsia patients’ serum was obviously higher than that in normal laboring group [4]. IL-18 is a new Placenta Immunomodulatory Factor which can aggravate cell apoptosis and cell toxic action (Seol HJ). At present, more and more studies show that incidence of pre-eclampsia was partial reaction of maternal systemic inflammatory response [5]. In the comparison assay of pre-eclampsia women and normal pregnant women, the level of many inflammatory factors increased markedly. Research suggested that hypertension was not only a vascular lesion but also a chronic inflammation. Moreover, inflammatory factors activation was closely involved in the incidence of hypertension [6] and IL-18 was an inflammatory factor which was inferred having strong ties to the incidence of pre-eclampsia.

In this study, the several indexes’ expression in case group and control group were analyzed to investigate their correlation with PE, such as placental growth factor (PLGF), placental protein 13 (PP13), β-human choionic gonadoto-

### Methods and subjects
Forty-eight PE patients from the obstetrical department of Zhengzhou People’s Hospital who have parturition during January, 2013 to January, 2015 were enrolled as case group, among which were 22 cases in mild phase and other 26 cases in severe. The diagnostic standard refers to Obstetrics and Gynecology which was written by Dr. Le Jie. The age of patients in case group ranged from 22 to 36 years old, and patients in mild phase have an average age of 27±6.4 with 38.25±1.11 weeks of average gestational period, and that of the severe-phase patients was 29±5.2 years old and 38.26±1.23 weeks respectively. Other 48 normal gravidas who don’t have pregnancy complications and syndrome after clinical and pathological test and delivered at the same time and place were regarded as control group. Their age were within 20 to 38 years old and have an average age of 28±5.9, average gestational period of 38.15±0.98 weeks. The differences between the compared data of average age and gestational period of gravidas from case group and control group has no statistical significance (P>0.05).

Inclusion criteria: singleton pregnancy, except for such abnormal situations as chronic hypertension, heart attack, Diabetes mellitaries, disease of the kidney and liver, cardiovascular disease, connective tissue disease, infection, hyperthyroidism, premature birth, fetal growth restriction, gestational diabetes mellitus, intrahepatic cholestasis of pregnancy and placenta previa, and pregnancy complications and surgical complications, and gravidas don’t have special medical history. All gravidas were of no smoking and drinking and no special living and eating habits. No pregnant women were parturient or had premature rupture of membrane. Gravidas in control group were followed up until their delivery found that none of them had pregnancy complications. Subjects in the study

### Table 1. Comparison Of clinical data among the three groups with re-eclampsia

<table>
<thead>
<tr>
<th>Clinical features</th>
<th>Control group</th>
<th>Mild pre-eclampsia group</th>
<th>Severe pre-eclampsia group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of cases (n)</td>
<td>48</td>
<td>22</td>
<td>26</td>
</tr>
<tr>
<td>Age (year)</td>
<td>28±5.9</td>
<td>28±5.9</td>
<td>29±5.2</td>
</tr>
<tr>
<td>Gestational weeks (week)</td>
<td>38.15±0.98</td>
<td>38.25±1.11</td>
<td>38.26±1.23</td>
</tr>
</tbody>
</table>
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were all informed of and signed informed consent. And all data in this study were approved by the medical ethics council of our hospital.

Sampling

When selected gravidas were in second (20 to 24 weeks) or third trimester of pregnancy (30 to 34 weeks), 3 ml venous blood from their elbow should be collected, and kept it still under room temperature for 30 minutes, after that, the blood was centrifuged for 10 minutes in speed of 3000 r/min, and then the supernatant was collected, preserved in refrigerator at -80°C. Next, the level of PLGF, PP13, β-hCG and IL-18 in sampled serum were tested by enzyme-linked immunosorbent assay (ELISA), the experiment process employed 550-type Microplate Reader labelled BIORAD (America), and reagent kit which was brought from R&D Systems Company, including zymolyte, detection antibody, binding antibody, diluents, terminator, etc. All the specimens were detected simultaneously in two wells, and the final density is the mean value of it. All the steps were performed strictly as introductions.

Statistical analyses

Measurement data were written as x±s, and the statistical analysis were run via statistics software SPSS19.0, then differences among groups was compared by One-Way ANOVA analysis and the t test was used to do the comparison between groups. In addition, using the software MedCalc to draw receiver operating characteristic (ROC) curves and the value of combined and single detection of these indexes were calculated, such as sensitivity, specificity and the area under curve (AUC) to the diagnosis of pre-eclampsia. The building, training and validation of support vector machine model and were all based on programming through tool of MATLAB. P value is less than 0.05 means that the differences had statistical significance.

Results

Comparison of the clinical data among three study objects

Through statistical analysis, there was no statistical significance in average age and average gestational weeks of each group of gravidas. The result is shown in Table 1.

Comparison of the level of gravidas’ serum markers PLGF, PP13, β-hCG and IL-18 in middle-and-late pregnancy

The comparison results of the level of gravidas’ serum markers PLGF, PP13, β-hCG and IL-18 in all groups

<table>
<thead>
<tr>
<th>Group</th>
<th>PLGF (ng/L)</th>
<th>PP13 (ng/L)</th>
<th>β-hCG (μg/L)</th>
<th>IL-18 (ng/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>123.4±27.42</td>
<td>125.3±23.64</td>
<td>20.6±8.74</td>
<td>8.9±3.09</td>
</tr>
<tr>
<td>Mild pre-eclampsia group</td>
<td>80.1±39.92</td>
<td>76.1±22.48</td>
<td>33.2±9.98</td>
<td>15.0±5.73</td>
</tr>
<tr>
<td>Severe pre-eclampsia group</td>
<td>66.2±29.93</td>
<td>51.0±13.57</td>
<td>42.6±13.95</td>
<td>27.3±10.11</td>
</tr>
</tbody>
</table>

P value: 0.0001 0.0002 0.1291 0.0000 0.0000 0.0003 0.0000 0.0000 0.0404

Note: 1 means the comparison between control group and mild pre-eclampsia group, 2 means the comparison between control group and severe pre-eclampsia group, 3 means the comparison between mild pre-eclampsia group and severe pre-eclampsia group.

The compared screening results of combined and individual detection in serum markers to mild phase pre-eclampsia

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>AUC</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLGF</td>
<td>59.1</td>
<td>91.7</td>
<td>0.783</td>
</tr>
<tr>
<td>PP13</td>
<td>77.3</td>
<td>91.7</td>
<td>0.892</td>
</tr>
<tr>
<td>β-hCG</td>
<td>77.3</td>
<td>83.3</td>
<td>0.818</td>
</tr>
<tr>
<td>IL-18</td>
<td>77.3</td>
<td>93.7</td>
<td>0.870</td>
</tr>
<tr>
<td>Serum markers combined detection</td>
<td>95.5</td>
<td>97.9</td>
<td>0.966</td>
</tr>
</tbody>
</table>

The compared screening results of combined and single detection in serum markers to severe phase pre-eclampsia

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>AUC</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLGF</td>
<td>80.8</td>
<td>91.7</td>
<td>0.893</td>
</tr>
<tr>
<td>PP13</td>
<td>88.5</td>
<td>100</td>
<td>0.929</td>
</tr>
<tr>
<td>β-hCG</td>
<td>76.9</td>
<td>91.7</td>
<td>0.882</td>
</tr>
<tr>
<td>IL-18</td>
<td>84.6</td>
<td>100</td>
<td>0.942</td>
</tr>
<tr>
<td>Serum markers combined detection</td>
<td>88.5</td>
<td>100</td>
<td>0.948</td>
</tr>
</tbody>
</table>
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middle-and-late pregnancy is in Table 2. From the Table 2, it is obviously that the level of PLGF and PP13 of patients in mild phase and severe phase was distinctly lower than that of the control group while the level of β-hCG and IL-18 was obviously higher than that of the control group.

The level of PLGF in serum: The compared differences within groups of PLGF level in serum between control group and mild phase group, control group and severe phase group were statistical significance (P=0.000), the compared differences between mild phase group and severe phase group were not significant (P=0.129).

The level of PP13 in serum: The compared differences within groups of PP13 level in serum between control group and mild phase group, control group and severe phase group were statistical significance (P=0.000), so were the compared differences between mild phase group and severe phase group (P=0.000).

The level of β-hCG in serum: The compared differences within groups of β-hCG level in serum between control group and mild phase group, control group and severe phase group were statistical significance (P=0.000), so were the compared differences between mild phase group and severe phase group (P=0.003).

The level of IL-18 in serum: The compared differences within groups of β-hCG level in serum between control group and mild phase group, control group and severe phase group were statistical significance (P=0.000), so were the compared differences between mild phase group and severe phase group (P=0.004).

The value of combined and individual detection in serum markers to the pre-eclampsia diagnosis

The results which evaluated the value of serum markers PLGF, PP13, β-hCG and IL-18 to diagnosis of Pre-eclampsia were in the Tables 3 and 4. Tables 3 and 4 demonstrate that the results of pre-eclampsia diagnosing by serum markers PLGF, PP13, β-hCG and IL-18 were as followed: after individual marker was used to screen the patients in mild phase and severe phase pre-eclampsia, it turns out that the sensitivity of PLGF for two groups was 59.1% and 80.8%, respectively, and its specificity was same in two groups, 91.7%, and the AUC was 0.783 and 0.893; the sensitivity of PP13 was 77.3% and 88.5% severally, and its specificity was 91.7% and 100%, and the AUC was respectively 0.892 and 0.929; then comes to the sensitivity of β-hCG with respective value of 77.3% and 76.9%, and its specificity was 83.3% and 91.7% severally, AUC was 0.818 and 0.882; at the end, the sensitivity of IL-18 was severally 77.3% and 84.6%, and specificity was 93.7% and 100%, AUC was 0.870 and 0.942. While in the combined detection the sensitivity was 95.5% and 88.5% respectively, and specificity was 97.9% and 100%, AUC was 0.966 and 0.948. It was apparently that the sensitivity, specificity and the AUC of combined detection were all higher than that of in individual detection.

Validating the accuracy rate through support vector machine

Ninety six patients’ data were dealt using waveform normalization in which normal group was
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Table 5. Results of SVM model accuracy rate in serum combined detection of severe pre-eclampsia

![Graph of actual and predicted classification of the test set](image)

marked as 0, mild group as 1 and severe group as 2. Thirty eight cases were selected from the normal group, 11 cases from mild group and 17 cases from severe group, which was regarded as training sample. And they formed a matrix of $96\times4$ and then were put into support vector machine to be trained. In addition, 10 cases were picked up from the rest of the groups to be decided as testing samples. Corresponding discriminate results were obtained from Support vector machine network after being put in the machine and after the comparison with target, accuracy rate was obtained. The test results is as shown in Table 5 in which circle represented actual output and "*" represented predicted output of SVM. Seeing from the chart, the accuracy rate of this diagnostic model was 86.67%.

Discussion

The PE could cause functional lesion in various viscera, especially its effects on gravidas' heart, brain, liver, kidneys and eyes were extremely serious, besides, the growth of fetus could be also restricted by it, and it was also liable to Placental Abruption, disseminated intravascular coagulation, cerebra edema, acute heart failure and acute renal failure, which made it the one of the risk factors lead to unhealthy gravida and fetus [3]. The onset process of pre-eclampsia was divided into two phases by lots of researchers [4], the first phase is decreased placental perfusion which caused by shallow nidation of villi and vaso-spasm, the second phase is the gravidas' all vascular endothelial cells were activated and/or damaged to initiated varying degrees of lesion in multiple system and viscera, at the same time, the gravidas had clinical symptom such as hypertension (blood pressure $\geq 140/90$ mmHg), albuminuria (the amount of urinary protein $\geq 300$ mg/d), headache, blurred vision, epigastric discomfort, etc.

The incidence of pre-eclampsia in Occident is range from 6% to 12% while that of in China is within 3% to 9%. Every year there are about 8 million gravidas suffer from this disease all around the world, and its mortality could reach
at 77 thousand death among 100 thousand cases, and the birth defects even newborn death caused by it was about 7%~8% [5, 6]. Although recently there was a trend of year by year decline in incidence of pre-eclampsia, the reports of severe cases appeared every now and then, since there isn’t effective clinical treatment, the gravidas didn’t spare from the death of pre-eclampsia. Therefore, it is significant to prevent and cure it by predicting before the attack of it and do things for efficient intervention.

PLGF, which is dimeric protein of secreting type discovered by Majlione from placental, belongs to vascular endothelial growth factor (VEGF). PLGF shares similar bioactivity with VEGF, it has such functions as adjusting cytotrophoblast cells and endothelial cells in placenta, and induces cells mitosis, proliferation and migration. Many researchers found that the level of PLGF in pre-eclampsia patients had a distinct decrease when comparing to normal gravidas, which would brought declined proliferation and infiltration to cytotrophoblast cells, and causing placental ischemia and hypoxia, lead to the risk of pre-eclampsia increased eventually [8, 9]. That is exactly the same as the results in this study: the more serious the pre-eclampsia patients are, the lower level of PLGF is. As a kind of placental protein, PP13 plays a fundamental role in the growth of cells and the construction of placental microenvironment. There are researches indicate [10-12] that the pre-eclampsia patients had obvious lower level of PP13 in their serum when comparing to normal gravidas. β-hCG, produced by syncytiotrophoblast in placenta, has a vital function in sustained pregnancy. Some of researches show that [13] before the attack of pre-eclampsia the level of β-hCG in gravidas serum had a dramatic rising, which could be regarded as a signal for predicting the onset of it. Roiz-Hernández’s research shows that [14] the gravidas who had rising level of β-hCG in the second trimester of their pregnancy would got apparently increased risk of pre-eclampsia, which is as the same as the results of this study. It was discovered by researches of recent years [1] that IL-18 had involved in the pregnancy, among which it participated in the formation of placenta at the first trimester of pregnancy, and lead to different pregnancy results for different level of it in serum at second or last trimester, such as abortion and premature delivery, etc. The research of Seol HJ [15] also shows that the level of IL-18 in serum of patients from case group is obviously higher than that in control group, considering that, they deduced that IL-18 was in the process of pregnancy and had close link to the attack of pre-eclampsia, which was manifested as its expression level had a positive correlation with the degrees of pre-eclampsia severity.

To sum up, it was possible for PLGF, PP13, β-hCG and IL-18 got involved in the nosogenesis of pre-eclampsia, which means they are important to the screening of it. Taking its complicated nosogenesis into account, the sensitivity and specificity of screening with single marker are low, and the value could be promoted through combined detection to diagnose it. In this study, the serum PLGF, PP13, β-hCG and IL-18 were combined detected to screen mild and severe phase pre-eclampsia, and their sensitivity were 95.5% and 88.5% respectively, their specificity were 97.9% and 100%, and AUC were 0.966 and 0.948. The sensitivity, specificity and AUC of combined detection were all higher than the results with single detection, which has considerable significance to the pre-eclampsia diagnosis. However, the relevance of indexes as PLGF, PP13, β-hCG and IL-18 and pre-eclampsia nosogenesis need to be further proved through plenty of forward-looking case-control study, so was the meaning of diagnosis and treatment.

Disclosure of conflict of interest
None.

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