Predictive markers of pre-eclampsia in hypertensive disorders of pregnancy

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Abstract

Objective: The aim of this work is to assess the most widespread methods currently proposed and two new markers for predicting the development of pre-eclampsia in pregnant women with hypertension. Methods: The study involved 212 pregnant Caucasian women: 104 normotensive, 68 pregnancy-induced hypertensive and 40 chronic hypertensive. Blood and urine were sampled between 28 and 30 weeks gestation. All 108 hypertensive pregnant women, at the time of sampling, demonstrated proteinuria below 0.3 g/24 h. The following laboratory tests were performed: fibronectin, antithrombin-III, α-1-microglobulin, U-N-acetyl-β-glucosaminidase, uric acid and albumin excretion rate. Student’s t-test, discriminant analysis and χ² (chi-square) test were used as statistical methods. A P value less than 0.05 was considered significant. Results: After discriminating analysis, only three of the six variables analyzed were able to discriminate patients who would develop pre-eclampsia from the remaining hypertensive pregnant women: microalbuminuria, uric acid and fibronectin (χ² = 29.122, P < 0.01). Conclusions: In agreement with previous studies, albumin excretion rate appeared to be the best predictive test for pre-eclampsia in hypertensive pregnant women, giving a higher positive predictive value and specificity (87.5 and 98.9%, respectively). © 1999 International Federation of Gynecology and Obstetrics.

Keywords: Predictive markers; Microalbuminuria; Uric acid; U-α-1-microglobulin; Pre-eclampsia

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1. Introduction

In hypertensive disorders of pregnancy, pre-eclampsia is a common and major complication causing significant morbidity and mortality in the fetus, newborn infant and mother in both developed and developing countries [1].

It has been underlined that the clinical syndrome is the manifestation of an illness which starts early in pregnancy [2]. There is evidence that impaired trophoblastic invasion into maternal spiral arterioles plays an important pathogenetic role. This incomplete remodeling of the spiral arteriolar wall occurs at 16–20 weeks gestation as a result of failure of the second wave of trophoblastic invasion [2,3].

Reduced production of prostacyclin in the endothelium [3] together with factors deriving from platelets such as thromboxane [4] and serotonin, move the balance of paracrine factors in favor of vasoconstriction. The clinical disease at this stage is characterized by the development of hypertension. If untreated, this pre-eclampsia leads to multiorgan disease involving renal, hepatic and cerebral ischemia [5].

Diagnosis of pre-eclampsia in its initial clinical stages can be difficult since the only clinical sign is hypertension. Thus many attempts have been performed to find out useful clinical and biochemical tests for an early diagnosis. Since the pathogenetic mechanisms behind pre-eclampsia are totally different from other hypertensive disorders of pregnancy, biochemical markers are generally chosen on the basis of peculiar pathophysiological aspects of the disease. Therefore, since the pathophysiology of pre-eclampsia includes endothelial damage [6], a number of potentially useful biochemical markers of endothelial damage have been proposed: fibronectin, factor VIII-von Willebrand, microalbuminuria itself. Fibronectin, a dimeric glycoprotein with a relative molecular weight of 440 Da, is thought to be a potential marker for endothelial vascular injury [7]. Fibronectin is present in both soluble form in plasma and other body fluids, and insoluble form in interstitial connective tissue. Soluble plasma fibronectin is synthesized by hepatocytes and endothelial cells. Patients with hypertension had almost normal fibronectin values in plasma [8].

ATIII reduction is the first marker of pre-eclampsia induced coagulative disorder. Therefore, previous data support the hypothesis that the activation of clotting factors in pre-eclampsia is secondary to endothelial injury [9]. Initially, ATIII inhibits the effect of thrombin and plasma concentration of ATIII may decrease without producing any change in overall coagulation test results [9].

The pathophysiology of pre-eclampsia also includes renal ischemia and microinfarction, which could induce renal tubular cells to release enzymes into the urine (i.e. NAG) or impair the reabsorption of small proteins freely filtering through the glomerulus, such as α1-microglobulin. In physiological conditions this protein is rapidly filtered by the glomerulus and completely reabsorbed by proximal tubular cells [10]. Human α1-microglobulin is a glycosylated protein with a molecular mass smaller than 50,000 Da, synthesized by liver cells and readily associating with immunoglobulin A [11].

NAG is a ubiquitous lysosomal hydrolase with a high molecular mass (136,000 Da). The plasma molecule, due to the high molecular mass, cannot be filtered by the glomerulus and is rapidly cleared from circulation by the liver. Urinary concentration of the lysosomal enzyme U-NAG is considered a reliable and sensitive means for detecting renal tubular damage [12,13]. There is evidence that the epithelium of proximal tubules is the part of the renal parenchyma most sensitive to anoxia, and damage can occur rapidly even in cases of mild hypoxia [14].

There have been many theories concerning the cause of increased serum uric acid concentration in pre-eclampsia, most recent works point to an alteration in renal handling of urate, in particular reduced tubular uric acid secretion [15].

Finally, a previous report confirmed the assumption that microalbuminuria preceded clinical proteinuria and other hypertensive complications [16].

Early recognition of pre-eclampsia would make it possible to identify high risk patients who should be followed up carefully clinically, with laboratory test and with pharmacological treatment to modu-
late prostacyclin and thromboxane unbalance [17,18].

The aim of this work is to assess the most widespread parameters currently proposed and two new markers for predicting the development of pre-eclampsia in pregnant women with hypertension at 28–30 weeks gestation.

2. Materials and methods

In our clinic we define disorders in pregnancy as follows: chronic hypertension, pregnancy-induced hypertension and pre-eclampsia. Chronic hypertension is defined as preexisting hypertension before 20 weeks or before pregnancy. Pregnancy-induced hypertension: diastolic blood pressure raised above 90 mmHg on two occasions 6 h apart, proteinuria below 0.3 g/24 h and return to normotension after delivery. Pre-eclampsia: raised diastolic blood pressure above 90 mmHg on two occasions 6 h apart with proteinuria above 0.3 g/24 h and return to normotension after delivery. Superimposed pre-eclampsia: the development of pre-eclampsia in patients with chronic hypertension [19].

The study involved 212 women who attended our clinic for antenatal care between June 1995 and June 1998 between 28 and 30 weeks gestation. Our hospital is a referral clinic for hypertension in the community. All demonstrated proteinuria below 0.3 g/24 h at the time of sampling. One hundred and four women were normotensive and 108 hypertensive. Of the 108 hypertensive women, 68 pregnancy-induced hypertensive and 40 chronic hypertensive. In the normotensive group, six cases developed pre-eclampsia during pregnancy, and 98 cases had no adverse outcome during follow-up 10 days after. These cases were used as a control group.

Of the 108 hypertensive women demonstrating proteinuria below 0.3 g/24 h between 28 and 30 weeks gestation, 10 developed pre-eclampsia during follow-up 7 days after (three cases with chronic hypertension and seven cases with pregnancy-induced hypertension). After enrolment, pregnant women were controlled weekly until delivery.

Each participant signed a written informed consent form approved by the Human Subjects Committee of the University of Padua Health Sciences Center. The data collected from patient records included: age, parity, gestation at delivery and birthweight.

At 28–30 weeks gestation blood and urine were sampled. The following laboratory tests were performed: serum fibronectin (FN), serum antithrombin-III (ATIII), albumin excretion rate, urinary (U) α1-microglobulin, U–N-acetyl-β-glucosaminidase (NAG), and serum uric acid. Urine was collected over a 24-h period and samples were stored at −20°C before assay. Maternal venous blood samples were placed in test tubes with sodium citrate (ratio 1:10) and spun at 3000 rev./min for 10 min. Plasma was separated and aliquots of 500 μl were frozen at −30°C until needed. FN was measured by nephelometer using a commercial kit (Boeringer Mannheim, IN, USA); values between 200 and 400 mg/l were considered normal. FN intra-assay and inter-assay variations were 4.2 and 4.5%, respectively. Plasma ATIII activity was measured using the ATIII kit (Immuno, Vienna, Austria); values between 80 and 120% were considered normal. FN intra-assay and inter-assay variations were 4.2 and 4.5%, respectively. Plasma ATIII activity was measured using the ATIII kit (Immuno, Vienna, Austria); values between 80 and 120% were considered normal. ATIII activity intra-assay and inter-assay variations were 7 and 6%, respectively. This assay was carried out in an autoanalyzer. Serum uric acid levels were measured using standard Auto Analyzer techniques. A range of uric acid values between 1.5 and 0.35 mmol/l was considered normal. Microalbuminuria has been measured by immunonephelometry. This method is based on the immunological reaction between rabbit antibodies against human albumin (Dade Behring, Milan, Italy) and albumin contained into urine samples. Antigen/antibody complexes increase the light scattering signal. The conversion of the signal into concentration (mg/l) is obtained by using a calibration curve originating from geometric dilutions of a standard-calibrate material (Dade Behring, Milan, Italy). The method is fully automated on a Behring nephelometer system (BN II, Dade Behring, Milan, Italy). The analytic sensitivity of this method is 8 mg/l [20]. Urine concentration of NAG and α-1-microglobulin were determined using colorimetric (FAR Diagnostic, Verona, Italy) and nephelometric methods (Behringwerke, Scopito, Italy), respectively. The U-α1-microglobulin intra- and
inter-assay variations were 20 and 44%, respectively. These were expressed relative to urinary creatinine concentration in order to compensate for variations in urine output. Values between 0.2 and 2.4 U/mmole creatine and between 0.05 and 5.50 U/mmole creatine were considered normal for U-NAG and α-1-microglobulin, respectively.

Student’s t-test, discriminant analysis and χ²-test were used as statistical methods. The χ²-test was used to predict pre-eclampsia based on the value of mean + 2 S.D. of FN, U-NAG, U-α1-microglobulin, uric acid and microalbuminuria (410 mg/l, 2.35 U/mmol creatine, 5.4 U/mmol creatine, 0.27 mmol/l, 49 mg/l, respectively). The mean − 2 S.D. is confirmed for a cut-off on ATIII (82%). A P value less than 0.05 was considered significant.

3. Results

Table 1 illustrates the clinical features of the pregnancies for the two groups.

There was no statistical difference for mean age and gestational age at admission between control and hypertensive groups. However, week of delivery and mean birth weight were significantly lower (P < 0.01) in the hypertensive group than normotensive control group.

After discriminating analysis, only three of the six variables analyzed were able to discriminate patients who would develop pre-eclampsia from the remaining pregnant women: microalbuminuria, uric acid and FN: canonical correlation = 0.677, Eigen value = 0.846, Wilks’ λ = 0.542; χ² = 29.122, P < 0.01).

Table 2 shows the predicted levels for each single variable in discriminating hypertensive pregnancies which will develop pre-eclampsia. Seven of 10 patients, who developed pre-eclampsia, manifested microalbuminuria. Albumin excretion rate turns out to be a variable with greater positive predictive value, specificity and sensibility (P < 0.01), followed by uric acid and FN, respectively (χ²-test).

4. Discussion

Since the pathogenic mechanisms behind pre-eclampsia are totally different from other hypertensive disorders of pregnancy, biochemical markers are generally chosen on the basis of peculiar pathophysiological aspects of the disease.

The oldest and probably most often studied laboratory test, other than urinary protein determination, in the investigation of pre-eclampsia is the serum concentration of uric acid [21]. Whether the cause of increased serum uric acid concentration in pre-eclampsia is secondary to a true tubular damage because of the renal vasoconstriction and ischemia, or to a pure functional adaptation due to the well known hypovolemia existing in this disease, is not known. Despite the fairly large number of reports dealing with uric acid in patients with diagnosed pre-eclampsia, few data are available with regard to the predictive value of uric acid serum concentration for this

<table>
<thead>
<tr>
<th>Table 1 Clinical features of pregnancy</th>
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<tr>
<td><strong>Control (n = 98)</strong></td>
</tr>
<tr>
<td>Age (year)</td>
</tr>
<tr>
<td>Gestational age at admission (weeks)</td>
</tr>
<tr>
<td>No. of nullipara (n)</td>
</tr>
<tr>
<td>Duration of pregnancy (weeks)</td>
</tr>
<tr>
<td>Neonatal weight (g)</td>
</tr>
<tr>
<td>Preterm delivery (n)</td>
</tr>
<tr>
<td>IUGR (n)</td>
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<td>Cesarean delivery (n)</td>
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</tbody>
</table>

*Mean ± SD.

*P < 0.05; control group vs. hypertension group; Student’s t-test.
Table 2
Predictive value of laboratory tests for pre-eclampsia in pregnant women with hypertension

<table>
<thead>
<tr>
<th>Laboratory test</th>
<th>No. of patients with values &gt; mean + 2 S.D.</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>Predictive value</th>
<th>Ratio risk</th>
<th>$\chi^2$-test</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin excretion rate (mg/d)</td>
<td>8</td>
<td>70</td>
<td>98.9</td>
<td>87.5</td>
<td>97</td>
<td>15</td>
<td>46.5</td>
</tr>
<tr>
<td>Uric acid (mmol/l)</td>
<td>19</td>
<td>60</td>
<td>86.7</td>
<td>31.5</td>
<td>95.5</td>
<td>5.3</td>
<td>14.9</td>
</tr>
<tr>
<td>Fibronectin (mg/l)</td>
<td>43</td>
<td>90</td>
<td>65.3</td>
<td>20.9</td>
<td>98.5</td>
<td>16.7</td>
<td>14.6</td>
</tr>
<tr>
<td>Antithrombin (%)</td>
<td>14</td>
<td>30</td>
<td>88.8</td>
<td>21.4</td>
<td>92.5</td>
<td>2.8</td>
<td>3.1</td>
</tr>
<tr>
<td>U-NAG (U/mmol creatine)</td>
<td>89</td>
<td>90</td>
<td>18.4</td>
<td>10.1</td>
<td>94.7</td>
<td>3.7</td>
<td>1.5</td>
</tr>
<tr>
<td>$\alpha$1-Microglobulin (U/mmol creatine)</td>
<td>31</td>
<td>30</td>
<td>71.4</td>
<td>9.6</td>
<td>90.9</td>
<td>1.1</td>
<td>0.09</td>
</tr>
</tbody>
</table>

* $\chi^2$-test.

disease. Redman et al. [22] have suggested that patients who subsequently develop pre-eclampsia have significantly higher levels of uric acid starting from 28 weeks gestation. Other findings suggest that uric acid rises significantly only in the week before delivery in patients who develop pre-eclampsia. Finally it has been reported that clinical proteinuria (exceeding 300 mg/24 h), a late sign in the evolution of pre-eclampsia associated with poor perinatal prognosis, is usually preceded by reduced uric acid clearance and rising maternal plasma urate concentration [16].

In our study, the weight of evidence supports uric acid serum concentration as a useful predictive marker to exclude pre-eclampsia, because of its high negative predictive value and specificity (95.5 and 86.7%, respectively).

Lopez-Espinoza et al. [23] could find no evidence that gross proteinuria is preceded by a gradual increase of microalbuminuria, and women with mild pre-eclampsia superimposed on chronic hypertension did not differ in degree of microalbuminuria from women with chronic hypertension. On the other hand, among 41 pregnant women at risk of hypertensive complications, Bar et al. [24] detected a less favorable maternal and perinatal outcome in the cases where microalbuminuria developed in the early third trimester. In particular, they demonstrated the ability of microalbuminuria to predict the severity of hypertensive complications. Indeed, a comparison of 144 hypertensive pregnant patients with 134 non-pregnant, normal pregnant, and pregnant women at high risk for reasons other than hypertension, confirmed the assumption that microalbuminuria preceded clinical proteinuria and other hypertensive complications.

In agreement with this report, in our study, albumin excretion rate appeared to be the best predictive test for pre-eclampsia in hypertensive pregnant women, giving a higher positive predictive value and specificity (87.5 and 98.9%, respectively). Although microalbuminuria may also be of tubular origin as discussed for $\alpha$1-microglobulin, the observation that in the proposed model (Table 2) albumin excretion rate occupies the first position as a predictive test for pre-eclampsia, while $\alpha$1-microglobulin the last, supports the idea that microalbuminuria in pre-eclampsia is a marker of endothelial dysfunction. As a matter of fact, glomerular endotheliosis is the typical morphological hallmark of pre-eclampsia at the renal level, and moreover microalbuminuria is considered a cardiovascular risk factor in diabetes and hypertension since it reflects an abnormal permeability, i.e. a generalized dysfunction, of the vascular endothelium.

We speculated that by measuring plasma fibronectin (as a marker of endothelial damage) it should be possible to reveal an early preeclamptic process overlapping vasoconstriction due to chronic or gestational hypertension before activation of the coagulation cascade becomes evident.

In our study, the total FN of plasma was useful as a predictive test for identifying hypertensive patients not developing pre-eclampsia, following a high sensitivity and negative predictive value (90
and 98.5%, respectively). Although these favorable results need to be confirmed in other settings before widespread acceptance, it would appear that determination of plasma fibronectin concentration promises to be an excellent laboratory marker for ruling out pre-eclampsia. Our data confirm those by Salch et al. [9], Sen et al. [25], and Paternoster et al. [26], who found FN concentration to be more closely linked to pre-eclampsia than the measurement of ATIII. ATIII, on the contrary, seems to be a prognostic factor of pre-eclampsia, as its low levels correlate with poor maternal–neonatal outcome [27]. Interestingly ATIII had at 28–30-weeks gestation, a low positive predictive value (21.4%); thus, in agreement with previous studies, the ATIII level is not likely to be a useful predictive marker of pre-eclampsia, of which at the best may only be a useful confirmatory test.

Urinary concentration of the lysosomal enzyme U-NAG is considered a reliable and sensitive means for detecting renal tubular damage [12,13]. There is evidence that the epithelium of proximal tubules is the part of renal parenchyma most sensitive to anoxia, and damage can occur rapidly even in cases of mild hypoxia [14].

However, in our study, U-NAG always rises in all hypertensive forms during pregnancy, and cannot be considered a differential diagnostic marker in hypertensive disorders of pregnancy, given its poor positive predictive value (10.1%). Indeed, the increased urinary excretion of NAG has been reported in hypertensive patients by several groups.

Alpha-1-microglobulin tubular proteinuria may indicate ischemic damage to the proximal tubule, which is also the first to suffer [28].

In our study, this increased microproteinuria most likely indicates sub-clinical pre-preeclamptic glomerular–tubular damage and may be considered as an ancillary exclusion marker for pre-eclampsia, following a poor positive predictive value (9.6%) and a high negative predictive value (90.9%), which as a whole gave a non-statistically significant result.

If further studies confirm the predictive value and relative risk associated with microalbuminuria, serum uric acid and fibronectin, they may be useful in selecting women most likely to benefit from therapy designed to prevent or ameliorate pre-eclampsia.

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References


