COMPARISON BY SERUM HUMANIN BETWEEN WOMEN WITH PREECLAMPSIA AND WOMEN WITH UNCOMPLICATED PREGNANCIES

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ABSTRACT

Background: Preeclampsia is thought to be a low-grade inflammatory condition with oxidative stress and endothelial dysfunction. Humanin is suggested to give protection against the development of endothelial dysfunction and atherosclerosis through the inhibition of apoptosis and oxidative stress.

Objective: To evaluate the role of serum Humanin in the diagnosis and prediction of severity of preeclampsia.

Methods: A case-control study conducted in the Department of Obstetrics and Gynecology at AL-Yarmouk Teaching Hospital / Baghdad, Iraq during a period from the 1st of July 2017 till the 1st of July 2018. It included 45 pregnant women diagnosed as preeclamptic (case group) and 45 women with uncomplicated pregnancy who were selected after matching for age and gestational age of another one in the case group (Control group). All the participants were submitted to blood sampling for biochemical/hormonal assays (urea, creatinine, serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), uric acid, platelets, urinary protein, Humanin).

Results: There was no significant difference (P value ≥ 0.05) between women with preeclampsia and control regarding their age, body mass index, and gestational age. 57.8% of case group diagnosed as severe preeclampsia. The mean of serum Humanin concentration was significantly higher among women with preeclampsia than that in control group (473.1 versus 298.6 pg/ml P value 0.001); also it was significantly higher among women with severe preeclampsia than that in women with mild to moderate preeclampsia (491.6 versus 454.6 pg/ml, P value 0.001).

Conclusion: Maternal serum Humanin was significantly higher in preeclamptic pregnant women than women with uncomplicated pregnancy. Maternal serum Humanin was significantly higher in pregnant women with severe preeclampsia than those with mild to moderate preeclampsia.

INTRODUCTION

Preeclampsia

Definition of preeclampsia:

It is a multiorgan disease process of unknown etiology characterized by de novo development of hypertension and proteinuria after 20 weeks of gestation, sometimes progressing into a multiorgan cluster of varying clinical features. It is a pregnancy-specific syndrome that can affect virtually any organ system (1).

Incidence: The incidence varies between countries, but it is believed that worldwide, 3–5% of pregnant women are affected (2).

Classifications of preeclampsia (3):

Non severe: Where BP < 160/110, proteinuria none to positive, absence of (visual disturbance, upper abdominal pain, oliguria, eclampsia, fetal growth restriction, pulmonary edema, thrombocytopenia), minimal elevation of serum transaminase and normal serum creatinine.

Severe: Where BP equal or >160/110, proteinuria none to positive, presence of (headache, visual disturbance, upper abdominal pain, oliguria, eclampsia, thrombocytopenia, pulmonary edema), obvious fetal growth restriction, marked serum transaminase elevation, elevated serum creatinine.

Mortality / Morbidity:

Preeclampsia is a multisystem, highly variable disorder unique to pregnancy and a leading cause of maternal and fetal/neonatal morbidity and mortality. The evidence suggests that preeclampsia accounts for approximately
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15.9% of all maternal deaths in the United States and is a major cause of perinatal morbidity and death (4).

Pathophysiology:
Four main classes of dysregulation accompany preeclampsia (7):
1- Abnormal trophoblast invasion of the placenta.
2- Anti-angiogenic responses.
3- Oxidative stress.
4- Inflammation.

Humanin

Humanin (HN) is a recently identified endogenous peptide that protects cells against cytotoxicity induced by various stimuli (6). It may represent the first peptide of a new class of mitochondrial-derived peptides (MDP). Humanin is a small, secreted, 24 or 21 amino acid peptide, depending on cytoplasmic or mitochondrial translation respectively. Humanin is an open, reading frame (ORF) found within the 16s rRNA gene. Like a Russian nesting doll, Humanin is a gene within a gene within a genome of an organelle within cell (7). Humanin discovered firstly in 2001 by the Nishimoto lab, which found Humanin while looking for possible proteins that could protect cells from amyloid beta, a major component of Alzheimer disease (9). Humanin is secreted from cells and found in circulation, as well as bound to cellular membranes (9). From 2001, the anti-apoptotic effect of Humanin was studied in multiple medical conditions other than Alzheimer disease (10).

Its actions are mediated by two different types of receptors: The seven-transmembrane, G-protein-coupled receptor formyl-peptide receptor-like-1 (FPRL1) and a trimetic receptor consisting of ciliary neurotrophic factor receptor (CNTFR), the cytokine receptor WSX-1 and the transmembrane glycoprotein gp130 (CNTFR/WSX-1/gp130) (9). In study done in 2010 revealed that HN in the endothelial cell layer of human blood vessels, and exogenious addition of HN to endothelial cell cultures was shown to be effective against oxidized low-density lipoprotein (OX-LDL) induced apoptosis. These findings suggest that HN may play a role and may have a protective effect in early atherosclerosis in humans (11).

In study done in 2011 reveals that HN may have a protective effect on endothelial function and progression of atherosclerosis by modulating oxidative stress and apoptosis in the developing plaque (12).

In study done in 2014 their results indicate that growth hormone and IGF are potent regulators of Humanin levels and that Humanin levels correlate with lifespan in mice, so this suggests that Humanin represents a circulating mitochondrial signal that participates in modulating the aging process, adding a coordinated mitochondrial element to the endocrine regulation of aging (13).

METHODS

This is a case-control study that was conducted in the Department of Obstetrics and Gynecology at AL-Yarmouk Teaching Hospital / Baghdad, Iraq during a period from first of August 2017 till first of June 2018.

The study included 90 patients who were collected from either outpatient during antenatal care or from inpatient admission to the obstetric ward.

Those patients were divided into two groups, study group (pregnant women who had been diagnosed with preeclampsia for the first time by clinical examination and laboratory investigation (45case)) and control group (women with uncomplicated pregnancy who were selected after matching for age, BMI and gestational age of another one in the case group (45 control)).

Inclusion criteria for study group were: Any pregnant woman aged 18-45 years old, diagnosed with preeclampsia clinically and by laboratory tests.

Exclusion criteria for both groups: History of chronic hypertension, history of DM, cardiac disease, neurological disorders, renal impairment, and pre labor rupture of membrane.

All patients were subjected to the following:
An informed verbal consent was taken from all participants. Information about maternal age, gestational age, parity, gravidity, previous history of preeclampsia, family history of and previous medical history.

General examination, vital signs (systolic blood pressure, diastolic blood pressure,) abdominal and obstetric examination, laboratory investigation and sonographic examination (Doppler ultrasound). When the measured blood pressure was ≥ 140/90 but ≤ 160/110 then the BP had been reassessed after four hours, if still the same reading or more then we sent the patients for urine for albumin and if the results were +ve then the patients had been diagnosed with preeclampsia and were included in the case group and then directly we took blood sample for 1- Urea, creatinine, SGOT, SGTPT, uric acid, and platelet. 2-The other part, after was been centrifuged and frozen, we took it to private lab for Humanin assay.

When the measured BP is ≥ 160/110, here without further assessment of the BP we sent the patients for urine for albumin and if the results were +ve, the patients had been diagnosed with preeclampsia and we took the blood sample and did the same procedure that mentioned above.

For selection of patients in the control group, we selected the patients matched for age and gestational age to another in a case group and after proof that she was normotensive by history, examination and investigation, then she was included in the control group and had been sent for the same all investigation that sent for case group.

Sample collection and storage

All the attendants included in the study submitted to 20 ml blood sampling, 10 ml had been sent for evaluation of platelets count, blood urea, serum creatinine, serum uric acid, SGOT & SGTPT.

The other 10 ml was used to assess the Humanin level which was done on serum , by adding the sample to serum separator tube (SST) and allow samples to clot for 30 minutes , thereafter centrifugation for 15 minutes at approximately 1000*g , the serum was removed aliquoted and stored at -80 degree centigrade. When we completed all the samples we were ready then to use the Humanin kit to assess the Humanin level in all our collected samples, we used human Humanin-like protein 1,HN1 ELISA Kit No:E12429h , 96 Tests with detection range 0.78-50.0 ng/ml.

Test principle

By enzyme-linked immunosorbent assay (ELISA). The concentration of HN1 in the samples is determined by comparing the O.D. of the samples to the standard curve. The machine that used in the study for reading the HN1 ELISA kit was Biotek FLx800 Microplate.

Statistical Analysis

The data had been analyzed using Statistical Package for Social Sciences (SPSS) version 25. Data was presented as mean, standard deviation and ranges. Categorical data presented by frequencies and percentages. Independent t-test
(two tailed) was used to compare the continuous variables among study groups accordingly. ROC curve to determine the sensitivity and specificity of serum Humanin cut off value. A level of P – value less than 0.05 was considered significant.

RESULTS

Demographic data

Study patient’s age was ranging from 18 to 45 years with a mean of 27.2 years and standard deviation (SD) of ± 8.21 years. The highest proportion of study patients in case and control group was seen in age group < 20 years (42.2%) and 46.7% respectively. Regarding BMI level, the highest proportion of study patients in case and control group was obese (55.6% and 51.1% respectively).

About parity, we noticed that the highest proportion of study patients was prim gravida (71.1%) while in control group; the highest proportion had at least one child (77.1%). About GA, the highest proportion of study patients in case and control group presented on sampling day with GA ≥ 35 weeks (60% and 66.7% respectively).

There were no statistically significant differences (P ≥ 0.05) between patients and controls in age, pre pregnancy BMI level, parity, and GA, (Table 3.1).

Blood pressure and investigation

In this study the means of systolic and diastolic blood pressure, B. urea, S. creatinine, SGOT, S. uric acid and protein were significantly (P < 0.05) higher among patients of case group than that of control group, while mean of platelet count was significantly higher among patients of control group than that of case group (P=0.001). No significant difference in mean of SGPT between study groups (P=0.287), (Table 3.2).

Birth weight

Women in control group delivered newborns with a significantly higher mean of weight than that of newborns delivered by women of case group (3733 ± 32.12 gms versus 2231 ± 67.11 gms, P=0.001), (Table 3.3).

Severity of Preeclampsia

The highest proportion of study patients showed severe preeclampsia (57.8%), (Figure 3.1)

Serum Humanin concentration

In this study, the mean of serum Humanin concentration was significantly higher among patients of case group than that in control group (473.1 versus 298.6 pg/ml, P=0.001), (Table 3.4).

ROC curve: represents sensitivity, specificity and cut point of S. Humanin between case and control groups. The cut point of S. Humanin was 328 pg/ml with AUC= 93.7%, sensitivity= 82.2%, specificity= 100%, accuracy= 91.1%, PPV= 100%, and NPV= 84.9%, so this mean that all patients with S. Humanin > 328 pg/ml can be diagnosed with preeclampsia as shown in figure (3.2).

Table 3.1: Distribution of study patients’ groups by general characteristics

<table>
<thead>
<tr>
<th>Variable</th>
<th>Case Group No. (%)</th>
<th>Control Group No. (%)</th>
<th>P - Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>&lt; 20</td>
<td>19 (42.2)</td>
<td>21 (46.7)</td>
</tr>
<tr>
<td></td>
<td>20 - 40</td>
<td>10 (22.2)</td>
<td>9 (20.0)</td>
</tr>
<tr>
<td></td>
<td>&gt; 40</td>
<td>16 (35.6)</td>
<td>15 (33.3)</td>
</tr>
<tr>
<td>Pre-Pregnancy BMI</td>
<td></td>
<td>(Mean ± SD) (28.6 ± 8.62)</td>
<td>(25.8 ± 7.5) (Range) (18 – 44)</td>
</tr>
<tr>
<td>Normal</td>
<td>7 (15.6)</td>
<td>6 (13.3)</td>
<td></td>
</tr>
<tr>
<td>Overweight</td>
<td>13 (28.9)</td>
<td>16 (35.6)</td>
<td></td>
</tr>
<tr>
<td>Obese</td>
<td>25 (55.5)</td>
<td>23 (51.1)</td>
<td></td>
</tr>
<tr>
<td>Parity</td>
<td></td>
<td>(Mean ± SD) (28.9 ± 5.87)</td>
<td>(27.6 ± 4.54) (Range) (21.3 – 32.2)</td>
</tr>
<tr>
<td>Prim gravida</td>
<td>32 (71.1)</td>
<td>10 (22.2)</td>
<td></td>
</tr>
<tr>
<td>&gt; 1</td>
<td>13 (28.9)</td>
<td>35 (77.1)</td>
<td></td>
</tr>
<tr>
<td>Gestational Age (Weeks)</td>
<td></td>
<td>(Mean ± SD) (3.8 ± 1.77)</td>
<td>(4.3 ± 1.83) (Range) (1 – 7)</td>
</tr>
<tr>
<td>&lt; 32</td>
<td>6 (13.3)</td>
<td>4 (8.9)</td>
<td></td>
</tr>
<tr>
<td>32 – 34</td>
<td>12 (26.7)</td>
<td>11 (24.4)</td>
<td></td>
</tr>
<tr>
<td>≥ 35</td>
<td>27 (60.0)</td>
<td>30 (66.7)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(Mean ± SD) (35.26 ± 2.33)</td>
<td>(35.89 ± 2.21) (Range) (31 – 38)</td>
<td>(30 – 39)</td>
</tr>
</tbody>
</table>

Table 3.2: Comparison between case and control groups by blood pressure and investigation

<table>
<thead>
<tr>
<th>Variable</th>
<th>Case Group</th>
<th>Control Group</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic BP (mmHg)</td>
<td>Mean ± SD</td>
<td>157.21 ± 9.33</td>
<td>113.65 ± 12.2</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>Mean ± SD</td>
<td>105.35 ± 7.29</td>
<td>73.86 ± 6.64</td>
</tr>
<tr>
<td>B. Urea (mg/dl)</td>
<td>Mean ± SD</td>
<td>31.44 ± 1.88</td>
<td>27.92 ± 2.04</td>
</tr>
<tr>
<td>S. Creatinine (mg/dl)</td>
<td>Mean ± SD</td>
<td>0.92 ± 0.06</td>
<td>0.87 ± 0.11</td>
</tr>
<tr>
<td>Protein (g/24h)</td>
<td>Mean ± SD</td>
<td>1.04 ± 0.12</td>
<td>0</td>
</tr>
<tr>
<td>SGOT (IU/ml)</td>
<td>Mean ± SD</td>
<td>22.3 ± 2.12</td>
<td>14.7 ± 1.32</td>
</tr>
<tr>
<td>SGPT (IU/ml)</td>
<td>Mean ± SD</td>
<td>13.92 ± 1.43</td>
<td>14.22 ± 1.22</td>
</tr>
<tr>
<td>S. Uric acid (mg/dl)</td>
<td>Mean ± SD</td>
<td>6.82 ± 0.62</td>
<td>4.11 ± 0.72</td>
</tr>
<tr>
<td>Platelets (* 1000/mm³)</td>
<td>Mean ± SD</td>
<td>234.6 ± 17.41</td>
<td>262.1 ± 19.33</td>
</tr>
</tbody>
</table>

Table 3.3: Distribution of study patients’ groups according to birth weight

<table>
<thead>
<tr>
<th>Birth Weight</th>
<th>Case Group No. (%)</th>
<th>Control Group No. (%)</th>
<th>P - Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>31 (68.9)</td>
<td>45 (100.0)</td>
<td>0.001</td>
</tr>
<tr>
<td>Low Birth Weight (&lt;2.5 kg)</td>
<td>14 (31.1)</td>
<td>0 (0)</td>
<td></td>
</tr>
</tbody>
</table>

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DISCUSSION

The early identification of patients with an increased risk for preeclampsia is one of the most important goals in obstetrics (14). Today, several markers may offer the potential to be used and one of them was serum Humanin which is an anti-apoptotic 24-amino acid peptide, the first new peptide discovered within the mitochondrial genome (mitochondria derived peptides, MDPB) since its complete sequencing in 1981 (15).

In the current study, primi gravida represented the majority among the case group and accounted for 71.1% of them, while primi gravida in control group was (22.2%). In concern to gestational age (GA), GA ≥ 35 weeks was predominant in both case and control groups (60% and 66.7% respectively). Similarly, primi gravida pregnant women were the most prevalent among other pregnant women in a local study conducted in Baghdad 2010, as they represented about the half of them (45.7%), while gestational age between 30-40 week was predominant among the case and control groups included in the study (16).

Case group in the present study had a significantly higher mean serum Humanin when compared to control group (473.1 versus 298.6 pg/ml, \( P < 0.001 \)) and when serum Humanin in case and control groups compared in regard to severity of preeclampsia, the results obtained showed that mean of serum Humanin concentration was significantly higher among those with severe preeclampsia than others with non-sever preeclampsia (491.6 versus 454.6 pg/ml, \( P < 0.001 \)), the ROC curve of our study reveal that serum Humanin can be used as a predictor for pre-eclampsia, the curve represents sensitivity 82.2%, specificity 100%, the cut point of S. Humanin was 328 pg/ml with AUC= 93.7%. Our result was in agreement with Nikolakopoulos et al in 2017 where serum Humanin included in a case-control study (case group, n = 37; control group, n = 34) conducted in Greece where they noticed that Humanin concentrations were significantly higher in women with preeclampsia than controls (422.2 ± 33.5 vs. 319.1 ± 28.1 pg/ml, \( P = 0.023 \)), the ROC curve was marginally significant with sensitivity 64.9%, specificity 47.1% and cut point 320 pg/ml (17). Another role of Humanin in pregnancy was discovered when Carla Janzen et al study in 2018 found that there was elevated HN expression with cytoplasmic localization to extra villous trophoblast on the maternal aspect of the human placenta affected by IUGR (17). Humanin provides a protective effect against apoptosis and oxidative stress, since
the rate of apoptosis was found to be increased, Humanin increases in accordance with the oxidative stress that occurs in the placenta (19).

All normal pregnant women (control) delivered normal newborn (100%), with a mean of birth weight significantly higher than that of newborns delivered by women of case group (37.33 ± 3.12 gms versus 22.31 ± 6.71 gms, P=0.001). In Germany 2007, similar results noticed when newborn weight <2500 g was significantly higher in preeclamptic pregnant women in comparison to healthy women (66% vs. 3.0%) (19).

Age range included in current study was from 18 to 45 years with a mean and standard deviation (SD) of 27.2 ± 8.21 years. Age group <20 years was the highest in both case and control group (42.2% and 46.7%, respectively). Obese pregnant in both groups were predominant in present study in regard to pre pregnancy BMI (55.6% and 51.1% respectively).

No statistically significant differences (P ≥ 0.05) had been observed between study groups regarding all characteristics mentioned above.

Rates of pre-eclampsia in Sweden study in 2016 were higher in younger (≤24 years), as they constituted about 3.3% of the study patients and increased with maternal BMI, when they found BMI ≥ 35.0 (kg/m²) represented 7.6% of all study patients (19), similarly to the current finding although differed in the percentage.

In conclusion, maternal serum Humanin was significantly higher in preeclamptic pregnant women than women with uncomplicated pregnancy. Also, it was significantly higher in pregnant women with severe PE than those with mild to moderate PE.

We recommend, using maternal serum Humanin as it is easy, safe and acceptable to the pregnant women after 20th week of gestation to differentiate preeclamptic women from another group with uncomplicated pregnancy and to differentiate between women with severe PE from those with mild to moderate PE.

Further studies are required to detect the cost-effectiveness of widespread use of this marker, detect the cutoff values of maternal serum Humanin and its role in the pathophysiology of PE.

REFERENCES
مقارنة بواسطة مصل الامام (الهومويسين) بين النساء ذوات تسمم الحمل مع النساء ذات الحمل الطبيعي.

الم특별

الخلفية: على الصعيد العالمي، 3-5% من النساء الحوامل تتأثر بمرض مقايضات الإنجاب الذي يعاني منه حالة التهابية منخفضة الدفء مع الإجهاد النكدي والخلط البسط. ويقترح الهومويسين للعامة من تطور الحمل البطني ونسبة التراث من خلال تجربة الاستمرارية والأكية.

الهدف الدراسة: تقييم تأثير الهومويسين في النساء الحوامل مع وجود مقايضات الإنجاب.

والطريق: كانت هذه دراسة صالحة بالشكاوى في مقايضات الحمل التبسطي في مستشفى الهومويسين/ بغداد، العراق، خلال الفترة من الأول من أغسطس 2017 حتى الأول من يونيو 2018. شملت 45 حالة ملتحية يتم تشخيصهن بمقايضات الإنجاب و45 حالة ملتحية بحمال غير ملتحية، تم اختبارهن بعد مطابقة العمر والعمر الحملي معًا في مجموعة الحالات. استخدمت تقنيات متعددة لقياس في جميع الحالات التي تم مصلى منها:

بين النساء المصابات بمقايضات 
(0.001 ≤ ρ ≤ 0.05) الصيني المرضى الذين يعانون من مقايضات الإنجاب المفاجئة بالنواة الحوامل.

المؤثرة في فصول اضطرابسية: قال ضبط الممارسات الإجمالية، الهومويسين
SGOT، حمض الورك والبروتينات على نسب مضارب قيادات الأدوات. مقايمت بمرضي الحمل تبسطي، مع وجود تفاوت بين المصابين والمتالين بقائمة المصابات بمقايضات الإنجاب.

الإجراء: كان متوسط تركيز الهومويسين في الدلالة أعلى بكثير من متوسط المرضى الذين يعانون من مقايضات الإنجاب المفاجئة بالنواة الحوامل.

المتالي: كان متوسط prueba في الحالات التي تم مصلى منها:

على مثال عند وضع رنين المرضى الذين يعانون من مقايضات الإنجاب المفاجئة بالنواة الحوامل.

(0.491 ≥ ρ ≥ 0.001). الاستنتاج: كان مصل الهومويسين أعلى بكثير متوسط في النساء الحوامل المصابات بمقايضات الإنجاب من النساء الحوامل البسطيات. وكان أعلى بكثير متوسط في النساء الحوامل المصابات بمقايضات الإنجاب المفاجئة. من أقلان الذين يعانون من مقايضات الإنجاب المفاجئة المتالي. 

كلمات العناية: مرض مقايضات الإنجاب تأثير الهومويسين في المصف.