

Determination of Plasma Osteopontin and Serum Inhibin B in Pregnant Women with Preeclampsia

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Abstract

Pre-eclampsia is a multisystem disorder of pregnancy, which complicates 3%-5% of pregnancies in the world. The present study deals with estimation of levels of Osteopontin, Inhibin B, protein, albumin, globulin and Alb./Glob. ratio in forty five Pregnant Women with Preeclampsia in comparison with thirty healthy Pregnant Women. In Pregnant Women with Preeclampsia, the level of Osteopontin and Inhibin B were significantly higher as compared to the normal subjects [$p < 0.01$]. In Pregnant Women with Preeclampsia the levels of S. calcium S. Protein and albumin were significantly decreased as compared to the normal subjects [$p < 0.05$, $p < 0.05$ and $p < 0.01$] respectively. Based on findings of the present study, it can be concluded that Patients with Preeclampsia had significantly higher serum inhibin B and Osteopontin concentrations compared to healthy pregnant women. In addition, the current study could not determine any links between Osteopontin levels and other biochemical markers in this study.

Keyword: Preeclampsia, Osteopontin, Inhibin B, protein, Albumin and Globulin.

Introduction

Preeclampsia is one of the hypertensive pregnancy conditions, which affects from 3 to 5% of pregnant women. It is the most main cause of maternal morbidity and perinatal mortality [1]. Preeclampsia is developed after 20 weeks of gestation and is categorized by hypertension and proteinuria [2]. Though, the pathophysiology of Preeclampsia remains incompletely explained [3]. In developing nations, the incidence of the disease is reported to be 4-18%, [4] with hypertensive disorders being the second most common obstetric cause of stillbirths and early neonatal deaths in these countries.[5]

A diversity of biochemical parameters have been proposed for determination of predicting the development of preeclampsia. Inhibin, one of the parameter, is a glycoprotein hormone that belongs to the transforming growth factor-superfamily, consisting of $\alpha\beta A$ (Inhibin A) and $\alpha\beta B$ (Inhibin B). Inhibin is a peptide that acts to inhibit follicle-stimulating hormonal secretion from the pituitary gland [6].

Osteopontin (OPN) is a secreted glycoprotein that was originally isolated from bone. Osteopontin was a highly acidic

calcium-binding glycosylated phosphoprotein secreted by many cell types, including macrophages, activated T cells, osteoblasts, kidney tubule cells, activated T cells, and vascular smooth muscle cells. Its molecular weights have been reported in the range of 66 kDa to 44 kDa depending on glycosylation and phosphorylation [7]. Osteopontin (OPN) is an extracellular matrix cell adhesion protein which is abundant in bone and which is synthesized by preosteoblasts, osteoblasts and osteoclastic cells that are localized in the mineralized phase of bone matrix. It is an acidic, phosphorylated, sialic acid-rich Ca^{2+} binding protein. Osteopontin contains a signal sequence and is a secreted protein. It is involved in recruiting and stimulating macrophages and lymphocytes as part of a nonspecific response to microbial infections. [8,9].

The aim of the present study was to invention biochemical factors, which could contribute in the pathogenetic processes of preeclampsia, and which probably could serve as biomarkers of the disease, also investigates if the inhibin B and Osteopontin levels are significantly correlating with the other

biochemical parameters severity in pregnant women of preeclampsia.

Materials and Methods

Forty five pregnant women with preeclampsia and 30 normal pregnant women who were admitted to the pregnancy care centers located in many areas of the city of Baghdad. Preeclampsia was diagnosed according to the National High Blood Pressure Education Program Working Group on High Blood Pressure in Pregnancy (NHBPEP). Early onset PE was diagnosed if hypertension and proteinuria started before the 34th completed gestational week [10]. The subjects with diabetes mellitus under medication and untreated diabetes, Severely anaemic (<7.0 gm% of Hb) and those Suffering from any other systemic disorder were excluded from the study. Investigation of variance followed by taking 5ml of blood have been collected into two tubes, one containing EDTA for measurement of Osteopontin. The plasma was separated by centrifugation at 3000 rpm for 15 minutes, then transferred immediately to a clean dry plain tube and anticoagulant tube for measuring Osteopontin by Enzyme Linked Immunosorbent Assay (ELISA) [IBL Co., Ltd.-Japan].

The blood in the second part was allowed to clot for at least 10-15 min. at room temperature, centrifuged for (10) min. at (4000xg). Serum was removed for the measurement of biochemical factors. Serum glucose, calcium, protein and albumin levels were measured by spectrophotometric methods supplied by Human, Germany. Serum globulin concentration was determined mathematically from the subtraction the value of albumin concentration from that of total protein. The concentration of albumin divided by the concentration of globulin was expressed as albumin to globulin ratio. The serum Inhibin B was measured by Enzyme Linked Immunosorbent Assay (ELISA) [RayBiotech, Inc.

All statistical analyses in studies were performed using SPSS version 17.0 for Windows (Statistical Package for Social Science, Inc., Chicago, IL, USA). Descriptive analysis was used to show the mean and standard deviation of variables. The

significance of difference between mean values was estimated by Student T-Test. The probability $P < 0.05$ = significant, $P > 0.05$ = non-significant. Correlation analysis was used to test the linear relationship between parameters.

Results and Discussion

The clinical characteristics of the present study participants are described in Table (1). There were no statistically significant differences age and gestational age between the two study groups. There were A significant increase ($p < 0.001$) in Systolic blood pressure and Diastolic blood pressure [mm Hg] in group A when compared to group B.

*Table (1)
The mean and standard deviation of Age, weight and Gestation age in group A [pregnant women with preeclampsia], group B [healthy pregnant women].*

Characteristic	Group A[n=45]	Group B[n=30]	P Value
Age[year] Mean ±SD	32.23±4.16	29.12±2.35	NS
Weight[Kg] Mean ±SD	80.07±8.99	75.30±9.78	<0.01
Systolic blood pressure [mm Hg]	174.07±7.43	115.80±5.78	<0.001
Diastolic blood pressure[mm Hg]	105.55 ±9.67	78.07±5.00	<0.001
Gestation age [Week] Mean ±SD	34.21±0.55	33.80±0.75	NS

**Significant at 0.05 level of significance*

The mean levels of sera protein, globulin, Alb/Glb ratio and blood glucose showed no significant change in group A [pregnant women with Preeclampsia] comparing to group B [healthy pregnant women], while albumin showed a significant decrease in group A when compared with group B ($P < 0.01$) as shown in Table (1). In pregnancy, albumin decreases and variations over time [11]. This is may be due to an increase in plasma and interstitial volume, and possibly by an increase in albumin metabolism. Furthermore, albumin is lower in women

with preeclampsia than in healthy pregnant women [12]. Improved capillary penetrability secondary to endothelial destruction seems to be partly responsible for this result [13]. There were a significant increase in mean levels of Inhibin B and Osteopontin, in group A [pregnant women with preeclampsia] comparing to group B [healthy pregnant women] ($P < 0.01$) as shown in Table (2).

A significant increase in mean levels of Osteopontin in the present study agreement with Stenczer B et al [14] while another study showed no significant difference in plasma Osteopontin concentrations between the preeclamptic and the control group [15]. The extravillous trophoblasts of the human placenta also express Osteopontin, which regulates the invasiveness of these cells [16] Osteopontin may be released from the vessel wall into the peripheral circulation in cases of extensive endothelial injury, and may be used as a marker of endothelial injury in patient with preeclampsia.

Table (2)

The mean and standard deviation of S. glucose, rotein, albumin, globulin, Alb/Glb ratio, Inhibin B- and Osteopontin in group A [pregnant women with preeclampsia], group B healthy pregnant women.

Characteristic	Group A[n=45]	Group B[n=30]	P Value
R.S. Glucose[mg/dl]	82.18 ± 18.53	89.03 ± 12.34	NS
S. Protein[g/dl]	6.18 ± 0.65	6.55 ± 0.66	<0.05
S. Albumin [g/dl]	3.93±0.59	4.35±0.18	<0.01
S. Globulin [g/dl]	2.14±0.60	2.17±0.44	NS
Alb./Glb. ratio	2.03 ±0.83	2.01±0.46	NS
S.Inhibin B [pg/ml]	128.63± 35.22	11 . 41± 21.35	<0.01
Plasma Osteopontin [ng/ml]	9.26 ± 0.83	6.50 ± 0.37	<0.01
S. Calcium (mg/dl)	7.45 ±0.59	8.78 ±0.59	<0.05

*Significant at 0.05 level of significance.

Serum inhibin B levels increase during the third trimester of pregnancy, showing the

highest levels at term. This finding is in agreement with the gestational-related increase of inhibin a and b subunit mRNA expression in trophoblast tissue [17]. Placenta and related tissues are also submitted as bases for the other inhibin-related proteins measured in maternal circulation, *i.e.* inhibin A, activin A, and follistatin [18]. In pregnant women without Preeclampsia, levels of serum of these proteins increase through the three trimesters of pregnancy, with the maximum values at term [19].

Several studies [20,21] showed significantly decreased plasma calcium content in preeclampsia compared to healthy pregnant women and the present results also agreed with that studies. This can be due to the reduction was attributed to the expanded intravascular space happening during pregnancy which was revealed by the reduction of serum albumin as Calcium bound to plasma protein mostly Albumin represents about 30-45% of total Ca.[22].

The present study showed no significant correlation between Osteopontin and inhibin B in group 1 and group 2

Further studies are required to implicate use inhibin b and Osteopontin in early pregnancy could be in predicting preeclampsia, and fetal growth restriction in the first and/or second trimester before the onset of the clinical symptoms.

Conclusion

Patients with Preeclampsia had significantly higher serum Inhibin b and Osteopontin concentrations compared to healthy pregnant women. The results suggest that an increase in the serum concentration of inhibin seen in Patients with Preeclampsia, together with inhibin b, might be a cause of reduced placental blood flow. In addition, the current study could not determine any links between Osteopontin levels and other biochemical markers in this study. Decrease in levels of serum calcium through pregnancy may be probable providers in etiology of preeclampsia, and supplementation of these elements to diet may be of value to avoid preeclampsia.

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الخلاصة

تسم الحمل هو اضطراب في انظمة متعددة اثناء الحمل، مما يعقد ٣٪ - ٥٪ من حالات الحمل في العالم. تضمنت الدراسة الحالية تقدير مستوى Osteopontin و Inhibin B والبروتين والألبومين والكلويولين وكذلك نسبة الالبومين الى الكلويولين في ٤٥ من النساء الحوامل المصابات بتسم الحمل بالمقارنة مع نساء حوامل لا يعانين من المرض. اشارت الدراسة الى زيادة معنوية في مستوى Osteopontin و Inhibin B عند النساء الحوامل المصابات بتسم الحمل بالمقارنة مع مجموعة الضبط [p < 0.01]. لوحظ في الدراسة الحالية انخفاض معنوي في مستويات الكالسيوم والبروتين والألبومين في النساء الحوامل المصابات بتسم الحمل بالمقارنة مع مجموعة الضبط [p<0. 01, p<0.05, p<0.05] على التوالي. استنادا إلى النتائج التي توصلت إليها الدراسة الحالية، يمكن الاستنتاج إلى أن المرضى الذين يعانون من تسم الحمل كان مستوى Osteopontin و Inhibin B أعلى بكثير مقارنة بمجموعة الضبط. بالإضافة إلى ذلك لم تتوصل الدراسة الحالية الى وجود علاقة بين مستويات Osteopontin والدوال البيوكيميائية الأخرى في هذه الدراسة.