Amniotic Fluid Selenium and Maternal Biochemical Findings among Pre-Eclamptic Women in Cairo, Egypt

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Abstract: Introduction: Pre-eclampsia (PE) is a multi-factorial and a multi-system disease. Selenium (Se) may be one of the disease causes. Objectives: The aim of this study was to find out the mean level of amniotic fluid selenium and the biochemical profile among the pre-eclamptic mothers and controls in Cairo, Egypt. Subjects and methods: A case-control, hospital based study design was used. All the cases and controls were examined laboratory. Results: The mean amniotic fluid Se level was lower among pre-eclamptic cases; total, mild and severe compared to controls with statistically significant differences (P=0.00, 0.00 and 0.00, respectively). Further, the difference was statistically significant between mild and severe cases (P=0.00). Also, the mean hemoglobin level, mean hematocrit percent and mean platelet count were lower among pre-eclamptic cases compared to controls with statistically significant differences (P=0.00 for each of them). While; the mean lipid profile (except SHDL-cholesterol), mean liver enzymes levels, mean blood urea level, mean fasting blood glucose level and indicators of infections and/or inflammatory processes, mean total leucocytic count and mean C-reactive protein level were higher among pre-eclamptic mothers compared to controls with statistically significant differences (P=0.00 for each of them). On the other hand, the mean serum createnine level was higher among pre-eclamptic cases compared to controls with a statistically insignificant difference. Further, these differences were also present between the mild and severe PE cases. Lastly, the study showed that amniotic fluid Se was negatively correlated with severe PE (P=0.01); while total leucocytic count, fasting blood glucose, C-reactive protein, SLDL- cholesterol, S. TG, S. cholesterol and ALT were positively correlated with severe PE (P=0.02, 0.02, 0.03, 0.04, 0.04, 0.04 and 0.04, respectively). Recommendations: Early ante-natal care, including health education, and treatment of pre-eclamptic women are recommended. Se supplementation may be a way to prevent PE. Population based studies are needed in different areas in Egypt on large number of pregnant women to determine their biochemical profile and to find out other possible trace element deficiencies that might be risk factors for PE.
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1. Introduction:

Pre-eclampsia (PE) is an inflammatory state characterized by maternal endothelial dysfunction and leukocyte activation (Raijmakers et al., 2004a). It is pregnancy-specific condition and it is major complication of pregnancy (Roberts, 1998a). PE has a multi-systemic nature (Roberts, 2000 and Roberts et al., 2003). Also, PE can progress rapidly; putting mother at severe risk if there is no good management (Basso et al., 2006).

In spite of importance of PE, its etiology is unknown (López-Jaramillo, 2000). The proposed "2-stage model" (Roberts and Cooper, 2001) in which reduced placental perfusion leads to release of vasoactive factors and occurrence of the maternal syndrome; is likely to provide a simplified, yet largely accurate, description of the origin of severe early-onset disease, but may be less relevant for later-onset milder disease (Redman and Sargent, 2000). The proposed role of the placenta in the pathology of PE is also strongly supported by the rapid resolution of symptoms after delivery. Although there is clearly a focal role for placental dysfunction in PE, a number of theories are proposed to explain how this may be associated with the maternal syndrome (Hubel, 1999 and Levine et al., 2004).

PE is much more than hypertension and proteinuria. Increased attention to the multi-systemic nature of PE, activation of coagulation and increased sensitivity to pressor agents has expanded its understanding (Roberts, 2000 and Roberts et al., 2003). A basic feature of PE pathophysiology is reduced perfusion of virtually all organs, which is due to vasoconstriction, microthrombi formation and reduced circulating plasma volume. The vasoconstriction is secondary to an increased sensitivity of the vasculature to pressor agent. Activation of the coagulation cascade produces microthrombi. The reduced plasma volume, reflecting an endothelial leak with fluid loss from the

intravascular compartment, further compromises perfusion. These abnormalities precede clinically evident disease by weeks to months and have led to the suggestion that a primary target in PE is the vascular endothelium (Roberts et al., 1990 and Roberts, 1998b).

PE is a complex disorder caused by a series of genetic, nutritional and environmental factors that lead to the creation of an imbalance between the free radicals; nitric oxide (NO), superoxide (O_2^-) and peroxynitrate in the vascular endothelium (López-Jaramillo, 2000). The placenta appears to be the principal source of free radical synthesis. Deleterious effects of free radicals include initiation of lipid peroxidation, oxidative damage of biomolecules, and cellular dysfunction. It is proposed that these may initiate maternal vascular endothelial dysfunction and leukocyte activation, recognized features of PE (Raijmakers et al., 2004a).

A pivotal role of enhanced placental superoxide generation leading to oxidative stress (OS) is increasingly recognized (Hubel, 1999 and Raijmakers et al., 2004b). Placental OS has been shown to be a key feature in the pathogenesis of PE. OS is an imbalance between the cellular generation of reactive oxygen species and the capacity of antioxidants to prevent oxidative damage. The expression and activity of important antioxidant proteins are decreased in placental tissues from pre-eclamptic women, resulting in an imbalance between prooxidants and antioxidants leading to OS. Two of these antioxidant proteins are glutathione peroxidase (GSH-Px) and thioredoxin reductase enzymes that have selenocysteine within their active site and are selenium (Se)-dependent for activity (Walsh, 1998; Hubel, 1999 and Redman & Sargent, 2000). In a suitable maternal environment, OS and subsequent endothelial activation and injury result (Roberts and Hubel, 1999). This endothelial dysfunction initiates the coagulation cascade and ensuing multi-system sequelae (Surratt, 1993).

The trace element Se level lowers during pregnancy. Further, lower Se concentration and GSH-Px activity have been noticed in pregnant women compared to pre-pregnancy status (Sibai et al., 2000) and non pregnant women (Ferrer et al., 1999). Se is capable of limiting adverse endothelial effects. Se removes the products of attack by reactive oxygen species (hydro-peroxides and oxidized lipoproteins), which can break down to further reactive free radicals and cytotoxic agents (Rayman, 2000).

Considered risk factors of PE are nutrition deficiencies (Roberts et al., 2003), obesity (Roberts and Cooper, 2001), diabetes (Funai et al., 2005) and genitourinary infection and inflammatory processes (López-Jaramillo et al., 2001).

Study Objectives

1- To find out the mean level of amniotic fluid selenium among the pre-eclamptic mothers in Cairo, Egypt.
2- To determine the biochemical profile of the pre-eclamptic mothers in Cairo, Egypt.
3- To find out the correlation between PE severity and amniotic fluid Se & biochemical results of the pre-eclamptic mothers in Cairo, Egypt.

2. Subjects and methods

I- Study Questions:

Is there amniotic fluid selenium deficiency in the pre-eclamptic women? Is there a biochemical profile disturbance in the pre-eclamptic women? Is there correlation between amniotic fluid Se and biochemical markers, and severity of PE?

II- Study Design:

A case-control, hospital based design was used to investigate the current research problem.

III- Study Setting:

This study was conducted in the Obstetrics and Gynecology Department, Al-Hussein Hospital, Al-Azhar University.

IV- Study Sample:

According to sample size equation the sample was 86 cases, and to guard against sample size bias we increased the sample to be 100 PE cases. So, all the cases of PE attending the Obstetrics and Gynecology Department, Al-Hussein Hospital, Al-Azhar University were included in the study till sample reached the required number; 100. For each pre-eclamptic patient a healthy pregnant woman was chosen randomly. So, a control group of 100 healthy pregnant women was recruited.

All the PE patients must be fulfilling the following inclusion criteria: 1) Age of patients up to 30 years, 2) Gestational age ≥33 weeks, 3) Have a definite specific diagnosis of PE. Also, all patients recruited in this study have fulfilled the following specific exclusion criteria: 1) Essential hypertension, 2) Pregnancy induced hypertension without proteinuria, 3) Intake of vitamins/antioxidants during the current pregnancy, 4) Blood diseases, 5) Kidney disease, 6) Liver disease, 7) Intrauterine fetal death, and 8) Ante-partum hemorrhage.

The controls enrolled in the study have fulfilled the following inclusion criteria: 1) Age up to 30 years, 2) Have no history of PE in the current or previous pregnancy, and 3) Gestational age ≥33 weeks. Also, the controls have fulfilled the same specific exclusion criteria used for the patients group.

V- Ethical Consideration:

The purpose of the study and procedures to be performed were explained to the cases and controls, an oral consent to participate in the study was taken accordingly. All patients were managed properly to control PE. All the cases and controls were delivered spontaneously in the normal vertex position or by Caesarian section according to the condition of each case.

VI- Study Tools and Methods:

1- Diagnosis of PE:

All patients must be fulfilling the following inclusion criteria: 1) Hypertension: Blood pressure (BP) ≥140/90 mmHg, 2) Proteinuria: Trace or more by dipstick method, and 3) Bilateral lower limb edema: ≥+1.

Pregnancy-induced hypertension is defined as BP ≥140/90 mmHg (mild PE) and BP ≥160/110 mmHg (severe PE) (National Education program Working Group on High Blood Pressure, 2000). Proteinuria, trace to +1 (mild PE) and ≥+2 (severe PE). Two random midstream urine specimens, collected ≥4 hours apart taken from each woman to avoid error due to false positive tests, were used for detection of proteinuria. The two results must be positive to diagnose significant proteinuria **(**MacGillivary, 1983). Edema is a common feature of pregnancy, but edema of PE is pathological (≥+1) and not just dependant; it usually involves the face, hands and persists even after arising (Cunningham et al., 2010).

2- Laboratory investigations:

Laboratory examinations were done for the cases and controls. Fasting venous blood samples, 10 ml, were taken for laboratory examinations. These examinations were complete blood count (CBC), renal function tests; serum (S) urea and S. creatinine, total S. cholesterol, S. triglycerides (TG), S. high and low-density lipoprotein cholesterol (SHDL-& SLDL-cholesterol), and liver enzymes; alanine amino-transferase (ALT) and aspartate amino-transferase (AST).

CBC was estimated by ABX MICROS, MEDONIC MING 60 cell counter. C-reactive protein (CRP, mg/L) was determined quantitatively using slide agglutination test. Total S. cholesterol (mg/dl) was determined by an enzymatic technique according to Richmond (1973). While, S. TG (mg/dl) was determined according to Esders and Michrina (1979). HDL-cholesterol (mg/dl) was precipitated by the addition of phosphotungestic acid in presence of magnesium ions. The supernatant obtained contains HDL, from which cholesterol was determined enzymatically according to Steele et al. (1976). LDL-cholesterol was calculated according to the Friedwald's equation (Friedwald et al., 1972). Transaminases activity was measured by continuous monitoring method (Bergmeyer et al., 1978). Creatinine was measured by Jaffe method (Spencer, 1986), while, urea was measured by enzymatic method (Titez, 2004). Fasting blood glucose was measured by glucose oxidase method (Gochman and Schmitz, 1972).

A sample of 2 cc amniotic fluid was taken from each woman at time of the delivery to determine Se level (µg/L) by flameless atomic absorption spectro-photometer model 460-graphete 2000 according to Thompson and Allen (1988). We decided to determine Se level in maternal amniotic fluid not in serum or plasma as the levels of Se in maternal serum and plasma might be decreased, partly, because increase blood volume and therefore increase hemodilution during pregnancy (Rayman, 2002). On the other hand, level of Se in amniotic fluid suggests a real level of maternal Se during pregnancy. This might be because Se deposition in placenta and fetal tissues (Mistry et al., 2008).

VII- Statistical Analysis:

Unpaired t student test and correlation co-efficient were used as tests of significance. The significance level was accepted if the P-value <0.05.

3. Results

As regard the results of amniotic fluid examinations among the PE cases and control group (table 1), mean value levels of amniotic fluid Se in total PE cases and controls were 9.11±0.93 and 11.71±1.63 µg/L, respectively with a statistically significant difference (P=0.000). Also, mean value levels of amniotic fluid Se in mild PE cases and controls were 10.64±1.45 and 11.71±1.63 µg/L, respectively with a statistically significant difference (P=0.000). Lastly, mean value levels of amniotic fluid Se in severe PE cases and controls were 6.98±0.79 and 11.71±1.63 µg/L, respectively with a statistically significant difference (P= 0.000).

Regarding the results of amniotic fluid examinations among the PE cases according to severity (table 2), mean value levels of amniotic fluid Se in mild and severe PE cases were 10.64±1.45 and 6.98±0.79 µg/L, respectively with a statistically significant difference (P=0.000).

With respect to the results of laboratory examinations among the PE cases and control group (table 3), mean and standard deviation values of hemoglobin and percent of hematocrit values of the patients and controls were 9.72±0.64, 11.05±0.59 g/dl and 29.11±2.41, 34.25±1.43%, respectively with statistically significant differences (P=0.000 for each of them). Also, mean and standard deviation value of

fasting blood glucose of the patients and controls were 137.31±46.18 and 94.27±26.15 mg/dl, respectively with a statistically significant difference (P=0.000). Regarding mean platelet count of the patients and controls, they were 172.22±29.98 and 231.91±31.22 thousand/cmm, respectively with a statistically significant difference (P=0.000). As regard laboratory results indicating infection, mean and standard deviation of leucocytic count and C-reactive protein values of the patients and controls were 12.80±3.47, 8.72±2.36 thousand/cmm and 9.53±2.82, 6.01±1.29 mg/L; respectively with statistically significant differences (P=0.000 for each of them). Regarding laboratory results of lipid profile, mean and standard deviation values of serum triglyceride, cholesterol and HDL-& LDL-cholesterol of the patients and controls were 258.82±86.11, 182.17±62.37 mg/dl; 246.57±56.92, 189.58±53.29 mg/dl; 41.27±9.16, 49.86±7.65 mg/dl and 167.96±49.38, 129.29±34.13 mg/dl, respectively, with statistically significant differences (P=0.000 for each of them). As regard laboratory results of renal function tests, mean and standard deviation values of S. urea of the patients and controls were 32.52±2.85 and 23.41±3.88 mg/dl, respectively with a statistically significant difference (P=0.000). While, mean and standard deviation values of S. creatinine was 0.85±0.27 mg/dl for patients and 0.81±0.16 mg/dl for controls with a statistically insignificant difference (P=0.2). Regarding laboratory results of liver enzymes, mean and standard deviation value levels of ALT and AST of the patients and controls were 38.20±11.32, 27.90±6.12 and 33.75±8.53, 24.87±4.81 U/L, respectively with statistically significant differences (P=0.000 for each of them).

As regard the biochemical results of laboratory examinations among the mild and severe PE cases (table 4), mean and standard deviation value levels of hemoglobin and mean percent of hematocrit values of the mild and severe PE patients were 10.18±0.68, 9.29±0.56 g/dl and 32.05±1.11, 27.19±2.01%, respectively with statistically significant differences (P=0.000 for each of them). Also, mean and standard deviation value of fasting blood glucose of the mild and severe PE patients were 131.21±19.05 and 161.11±26.71 mg/dl, respectively with a statistically significant difference (P=0.000). As regard platelet count values of the mild and severe PE patients, they were 191.23±31.22 and 156.21±21.18 thousand/cmm, respectively with a statistically significant difference (P=0.000). With respect to laboratory results indicating infection, mean and standard deviation of leucocytic count and C-reactive protein of the mild and severe PE patients were 8.12±2.03, 13.96±3.52 thousand/cmm and 8.21±1.02, 11.24±2.12 mg/L; respectively with statistically significant differences (P=0.000 for each of them). As regard laboratory results of lipid profile, mean and standard deviation of serum triglyceride, cholesterol and HDL-& LDL-cholesterol of the mild and severe PE patients were 214.37±42.11, 288.52±97.81 mg/dl; 218.81±31.91, 266.82±67.24 mg/dl; 45.86±9.50, 36.65±6.86 mg/dl and 149.25±46.32, 192.87±53.80 mg/dl, respectively, with statistically significant differences (P=0.000 for each of them). Regarding laboratory results of renal function tests, mean and standard deviation values of S. urea of the mild and severe PE patients were 27.81±2.24 and 36.82±3.15 mg/dl, respectively with a statistically significant difference (P=0.000). While, mean and standard deviation values of S. creatinine was 0.83±0.26 mg/dl for mild PE patients and 0.89±0.29 mg/dl for severe PE with a statistically insignificant difference (P=0.3). As regard laboratory results of liver enzymes, mean and standard deviation values of ALT and AST of the mild and severe PE patients were 35.50±8.21, 41.90±10.12 and 29.74±5.14, 38.54±8.93 U/L, respectively with statistically significant differences (P=0.002 and P=000, respectively).

Regarding the correlation co-efficient between severe PE with findings of the laboratory examinations of maternal serum and amniotic fluid Se (table 5), the study showed that amniotic fluid Se was negatively correlated significantly with severe PE (r=-0.913, P=0.01); while total leucocytic count, fasting blood glucose, C-reactive protein, SLDL-cholesterol, S. TG, S. cholesterol and ALT were positively correlated significantly with severe PE (r=1.123, P=0.02; r=0.811, P=0.02; r=1.323, P=0.03; r=0.028, P=0.04; r=0.710, P=0.04; r=0.430, P=0.04 and r=0.042, P=0.04, respectively).

Table (1): Means and standard deviations of pre-eclampsia (PE) cases and control group according to the laboratory results of amniotic fluid selenium.

Amniotic fluid selenium	Laboratory results of maternal amniotic fluid selenium M±SD		t-value	P-value
	PE cases (n=100)	Controls (n=100)		
Selenium (µg/L):				
Total cases (n=100)	9.11±0.93	11.71±1.63	-13.855	0.000
Mild PE cases (n=67)	10.64±1.45	11.71±1.63	-4.905	0.000
Severe PE cases (n=33)	6.98±0.79	11.71±1.63	-26.113	0.000

Table (2): Means and standard deviations of the laboratory results of amniotic fluid selenium in pre-eclampsia (PE) cases according to severity.

Amniotic fluid selenium	Laboratory results of maternal amniotic fluid selenium M±SD		t-value	P-value
	Mild cases (n=67)	Severe cases (n=33)		
Selenium (µg/L):	10.64±1.45	6.98±0.79	16.32	0.000

Table (3): Means and standard deviations of pre-eclampsia (PE) cases and control group according to results of the biochemical laboratory examinations

Items of biochemical laboratory examinations	Laboratory results M±SD		t-value	P-value
	PE cases (n=100)	Controls (n=100)		
Hb (g/dl)	9.72±0.64	11.05±0.59	- 15.279	0.000
Hematocrit (%)	29.11±2.41	34.25±1.43	- 18.342	0.000
Total leucocytic count (thousand)	12.80±3.47	8.72±2.36	9.722	0.000
Platelet count (thousand)	172.22±29.98	231.91±31.22	- 13.79	0.000
Fasting blood glucose (mg/dl)	137.31±46.18	94.27±26.15	8.11	0.000
C-reactive protein (mg/L)	9.53±2.82	6.01±1.29	11.351	0.000
S. triglycerides (mg/dl)	258.82±86.11	182.17±62.37	7.209	0.000
S. cholesterol (mg/dl)	246.57±56.92	189.58±53.29	7.309	0.000
SHDL- cholesterol (mg/dl)	41.27±9.16	49.86±7.65	- 7.198	0.000
SLDL- cholesterol (mg/dl)	167.96±49.38	129.29±34.13	6.442	0.000
Urea (mg/dl)	32.52±2.85	23.41±3.88	18.923	0.000
S. creatinine (mg/dl)	0.85±0.27	0.81±0.16	1.275	0.204
ALT (U/L)	38.20±11.32	27.90±6.12	8.004	0.000
AST (U/L)	33.75±8.53	24.87±4.81	9.068	0.000

Table (4): Means and standard deviations of the biochemical laboratory results in pre- eclampsia (PE) cases according to severity.

Items of biochemical laboratory examinations	Laboratory results M±SD		t-value	P-value
	Mild cases (n=67)	Severe cases (n=33)		
Hb (g/dl)	10.18±0.68	9.29±0.56	6.949	0.000
Hematocrit (%)	32.05±1.11	27.19±2.01	12.951	0.000
Total leucocytic count (thousand)	8.12±2.03	13.96±3.52	-8.835	0.000
Platelet count (thousand)	191.23±31.22	156.21±21.18	6.602	0.000
Fasting blood glucose (mg/dl)	131.21±19.05	161.11±26.71	-5.75	0.000
C-reactive protein (mg/L)	8.21±1.02	11.24±2.12	-7.779	0.000
S. triglycerides (mg/dl)	214.37±42.11	288.52±97.81	-4.169	0.0002
S. cholesterol (mg/dl)	218.81±31.91	266.82±67.24	-3.892	0.0004
SHDL- cholesterol (mg/dl)	45.86±9.50	36.65±6.86	5.531	0.000
SLDL- cholesterol (mg/dl)	149.25±46.32	192.87±53.80	-3.986	0.0002
Urea (mg/dl)	27.81±2.24	36.82±3.15	-14.702	0.000
S. creatinine (mg/dl)	0.83±0.26	0.89±0.29	-1.006	0.318
ALT (U/L)	35.50±8.21	41.90±10.12	-3.157	0.002
AST (U/L)	29.74±5.14	38.54±8.93	-13.57	0.000

Table (5): Correlation co-efficient between severe pre-eclampsia (PE) with findings of the laboratory examinations of maternal serum and amniotic fluid Se according to the significant correlations.

Items of biochemical laboratory examinations	r-value	P-value
Amniotic fluid selenium	-0.913	0.01
Total leucocytic count	1.123	0.02
Fasting blood glucose	0.811	0.02
C-reactive protein	1.323	0.03
SLDL- cholesterol	0.028	0.04
S. triglycerides	0.710	0.04
S. cholesterol	0.430	0.04
ALT	0.042	0.04

4. Discussion

PE syndrome is recognized to be a multi-system disease of the pregnant mother (Roberts et al., 2003). It results from the interaction between economic, psychosocial, nutritional, environmental and genetic factors (López-Jaramillo, 2000). Further, PE is associated with an imbalance of increased lipid peroxides and decreased antioxidants (Ziaei et al., 2006).

The trace element Se has a biological role as catalysts for endogenous antioxidant enzymes. Also, Se for glutathione peroxide enzymes is component of numerous metalloenzymes and co-factors for super dismutase enzyme (Mahomed et al., 2000). An increased incidence of pregnancy-induced hypertension in selenium-deficient regions was reported (Lu, 1990). Selenium depletion leads to loss of glutathione peroxidase (GSH-Px) and thioredoxin reductase activity, although no one has examined the placental expression of these proteins during PE and related this to Se status **(**Vanderlelie et al., 2004). The suggested importance of the deficiencies of trace elements in PE relates to the fact that they are present in superoxide dismisses (copper, selenium and zinc) and GSH-Px (Se). Also, messenger RNA expression for copper-zinc-superoxide dismisses and glutathione peroxidase is lower in pre-eclamptic placenta (Rayman et al., 1996; Wang & Walsh, 1996; Mahomed et al., 2000 and Rayman, 2000). Biomarker information on Se is conflicting. Whole blood and plasma levels of selenium are lower in pregnant compared to non-pregnant women (Ferrer et al., 1999; Mihailovic et al., 2000 and Atmar et al., 2005) and decrease more as gestation proceeds (Zachara et al., 1993 and Sibai et al., 2000). Further, erythrocyte (Zachara et al., 1993); hair (Guvenc et al., 1995) and amniotic fluid (Mihailovic et al., 2000 and Rayman, 2000) levels of Se are lower in pregnant compared to non-pregnant women. The decreased levels of Se in maternal serum and plasma might be, partly, because increase blood volume and therefore increase hemodilution during pregnancy (Rayman, 2002). However, the decreased levels of Se in erythrocyte and amniotic fluid suggest a real decrease in maternal Se during pregnancy and this might be because of Se deposition in placenta and fetal tissues. Also, there was significant reductions in serum selenium concentrations and plasma GSH-Px activity in pregnancy per se compared to non pregnant controls. Moreover, these levels were further decreased in the pre-eclamptic mothers and babies compared to normal pregnancies. Oxidative stress associated with PE may be a consequence of reduced antioxidant defense pathways specifically involving GSH-Pxs, perhaps linked to reduce Se availability. Reduced GSH-Pxs could be associated with increased generation of toxic lipid peroxides contributing to the endothelial dysfunction and hypertension of PE (Mistry et al., 2008). In a study on 32 pre-eclamptic women and 28 healthy pregnant women, serum level of Se was measured by atomic absorption spectrometry. The study found that mean serum Se concentration in PE is 60.68±6.42 mg/dl and in healthy pregnant is 87.50±10.96 mg/dl. The difference between both groups was statistically significant ($P<0.01$) (Atmar et al., 2005). Se concentrations in maternal and umbilical cord blood, and amniotic fluid were determined in normotensive and hypertensive women in relation to their smoking status. In the group of normotensive and hypertensive women, significantly lower Se concentrations in blood of smokers were observed than in nonsmokers. Umbilical cord blood Se concentrations in both normotensive and hypertensive smokers were significantly lower than in nonsmokers as well. In the group of normotensive women, significant differences in Se concentrations in amniotic fluid were observed between smokers and nonsmokers

(Kosanovic et al., 2010). Further, toe nail Se concentrations in pre-eclamptic women were significantly lower than in their matched normal controls (Rayman et al., 2003). Measuring toe nail Se status was decided to overcome the difficulty experienced in previous studies of the PE process influencing tissue concentration of Se concerning increased plasma volume in normal pregnancy and smaller or nonexistent in blood volume in pre-eclamptic patients. In Egypt, decreased levels of Se have been observed in patients with PE (Mekkay et al., 2007). Also, third trimester amniotic fluid Se level associated with PE was lower compared to normal pregnancy (Dawson et al., 1999). So, Se supplementation could be used to lower the high incidence of PE (Lu, 1990). Further, as 15.0% of these studied pre-eclamptic group are diabetics and we reported that diabetes is risk factor for PE (El-Moselhy et al., 2011), so Se blood level is lower in gestational diabetic women (Tan et al., 2001). On the other hand, increased levels of plasma Se have been observed in patients with PE compared to controls (Mahomed et al., 1998). Also, median maternal leucocytic Se was 15.0% higher among pre-eclamptic women compared to normal pregnant (Mahomed et al., 2000). Further, the increased levels of Se were 15.0% (Ratman et al., 1996) and 18.0% (Gromadzinska et al., 1998) among PE cases compared to their normal pregnant controls. At the same time, there was no observed significant difference as regard Se levels between pre-eclamptic women and normal pregnant controls (Rayman et al., 1996). The differences in tissues and analytical techniques used to measure maternal Se status are possible explanation for why available studies concerning maternal Se status and the risk of PE are not combatable. Moreover, differences in population characteristics such as age, race, ethnicity, as well as country and region of residence may account for some of the variation in results across studies. Also, the potential limitation of some studies (as our study) must be considered as the retrospective design of the study that was unable to determine whether the observed alterations in Se concentration preceded PE or whether the alteration attributed to PE (Mahomed et al., 1998 and Mahomed et al., 2000).

The increase prevalence of anemia among the pre-eclamptic women might be attributed to their disease and low intake of nutrients (Davies et al., 1976). Also, hemoglobin serves to transport nitric oxide, as S-nitrose cysteine, from the lungs to the peripheral circulation, where it can be released. Glutathione peroxidase (GSH-Px), besides being an important antioxidant, is known to catalyze the release of nitric oxide (NO) from smaller carrier molecules, and may play a role in the distribution of NO throughout the body (Funai et al., 2002). So, an imbalance between the free radicals NO, superoxide (O_2^-) and peroxynitrate in the vascular endothelium occurs (López-Jarmillo, 2000). Also, selenium (Se) depletion leads to loss of GSH-Px activity, although no one has examined the placental expression of this protein during PE and related this to Se status (Vanderlelie et al., 2004).

PE has been frequently reported as a complication of gestational diabetes (Vambergue et al., 2002). Also, diabetes is an important risk factor for PE (Roberts & Cooper, 2001; Funai et al., 2005 and El-Moselhy et al., 2011). Diabetes mellitus among PE cases and control group was 15.0% and 5.0%, respectively (OR=3.35, 95% ECL: 1.09-12.23) (El-Moselhy et al., 2011). But, the relationship between these two conditions is not well understood (Vambergue et al., 2002). In PE, there is exacerbation of physiological changes associated with pregnancy such as insulin resistance, altered immune responses and inflammatory pathway activation. These exaggerated responses seen in PE are reminiscent of metabolic syndrome, and also are evident in gestational diabetes (Scioscia et al., 2009). So, studies suggest that insulin resistance (Ostlund et al., 2004), chronic inflammation (Borzychowski et al., 2006) and endothelial dysfunction (Solomon & Seely, 2001 and Roberts & Gammill, 2006) are underlying pathophysiology. Also, common risk factors, such as elevated body mass index and advanced age have been noted for each of the two conditions (Duckitt and Harrington, 2005). Increased insulin resistance, a characteristic of gestational diabetes, has also been associated with the development of PE (Kaaja & Greer, 2005 and Parretti et al., 2006). Inositol phosphoglycan P-type (P-IPG) in PE has been extensively investigated and increased production has been demonstrated. This molecule acts as a second messenger of insulin, enhances the metabolic effects of insulin and is associated with insulin resistance (Scioscia et al., 2009). Also, abnormal endothelial function with impaired flow mediated dilatation (Dadelszen et al., 2007) and inflammatory cytokine release as C-reactive protein and E-selectin are reported in PE and diabetes (Aydin et al., 2004). Further; the post 50gm challenge glucose value at 24-28 weeks of gestation, in a case-control study of pregnant women with new-onset hypertension in late pregnancy and normotensive controls, was significantly higher among the hypertensive patients (Solomon et al., 1994). Further, hyper-insulinemia may play a role in over activity of the sympathetic nervous system associated with obesity. In rats, insulin, like leptin, causes sympatho-activation to different tissues including the kidney (Rahmouni et al., 2004). So, our results as regard

high levels of fasting blood glucose of the patients compared to controls were expected and accepted.

In PE there is marked increase in platelet aggregation and decrease of platelet count that may occur early in pregnancy. The cause of this coagulation problem in PE is either due to disturbance of the balance between the platelet aggregation effects of the thromboxane A2 and the inhibitory effect of prostacyclin (lewis et al., 1981), or formation of soluble immune complexes that become deposited in the walls of blood vessels, thus enhancing platelet aggregation and intravascular thrombosis (Burrows et al., 1987). Also, the risk factors for PE include thrombophilias (Roberts and Cooper, 2001).

A growing body of evidence links infection and inflammatory processes with PE were reported (López-Jaramillo et al., 2001). PE is an inflammatory state characterized by leukocyte activation (Raijmakers et al., 2004a). It is associated with a greater inflammatory response than observed in normal pregnancy (Sacks et al., 1998). Pre-eclamptic women had higher leucocytic count and increased total number of neutrophils compared to normal pregnant group. Also, pregnant women, who have a high risk for PE and chronic sub-clinical infection, have increased levels of CRP during the third trimester (Teran et al., 2001). These results provide further evidence of enhanced inflammation in PE. Endothelial cell dysfunction is part of more widespread intravascular inflammatory response causing clinical PE (Sacks et al., 1998). Also, placental lipid peroxidation product, tumor necrosis factor (TNF) α and syncytiotrophoblast membrane fragments are blood borne agents causing endothelial cell dysfunction (Redman et al., 1999). Lipid peroxidation predisposes to shedding of syncytiotrophoblast membrane into maternal circulation and leads to decrease fluidity of syncytiotrophoblast membrane (Morris et al., 1996) and may have a profound adverse effect on vascular endothelium (Cockell et al., 1997). Also, shedding apoptotic debris from syncytial surface is more intense in PE than in normal pregnancy and acts as inflammatory stimulus for PE (Redman and Sargent, 2003). Further, increased circulating TGs, free fatty acids, small dense LDL act as proinflammatory stimulus for PE (Redman et al., 1999). Mean plasma TGs and free fatty acids concentrations undergo near doubling in pre-eclamptic women relative to normal pregnant (Lorentzen et al., 1995 and Hubel et al., 1996). Fasting serum TGs correlates with serum malondialdehyde in pre-eclamptic women (Hubel et al., 1996). Amplification of injurious effects of placental TNFα by increased maternal free fatty acids is then possible. Free fatty acids are highly inflammatory (Toborek et al., 1996). Further, activation of maternal neutrophils during their transit through the placenta could provide a pathway for transfer of oxidative disturbances into the maternal circulation in PE (Butterworth et al., 1991). Also, pre-eclamptic women had higher incidence of asymptomatic bacteriuria than in normal pregnant women, (Hill et al., 1986). Further, the incidence of urinary tract infections in pre-eclamptic women was higher than in normotensive pregnant women (López-Jaramillo et al., 2001). Moreover, we reported that these pre-eclamptic patients had significant risk of asymptomatic bacteruria, urinary tract infection, and vaginal infection and bacterial vaginosis (ORs=3.62, 95% ECL: 1.19-13.10; 3.59, 95.0% ECL: 1.05-15.58 and 4.41, 95% ECL: 1.13-24.97, respectively) (El-Moselhy et al., 2011). On the other hand, in women with PE, it cannot be determined whether the increase in CRP and proinflammatory cytokines was a cause or a consequence of PE (Teran et al., 2001). Also, it is not possible to make a definitive statement that infection is a major risk factor for PE. However, it is proposed that chronic sub-clinical infections may increase maternal cytokines to levels high enough to affect vascular endothelial function in individuals with a predisposition to subsequent development of PE (Herrera et al., 2001). So, the role that infection and inflammation may play in the imbalance of free radicals that leads to PE needs to be studied as it may involve a fundamental change in the prevention and treatment of PE (López-Jaramillo et al., 2001).

Pregnancy induced hypertension is characterized by high TG and low HDL2-cholesterol levels. Alterations in HDL profiles may contribute to endothelial dysfunction in PE (Von Versen-Hoeynck and Powers, 2007). About one-third of pre-eclamptic women develop plasma TG values above 400 mg/dl (Hubel et al., 1996) greater than normal pregnancy (Knopp et al., 1992). Further, the risk factors for PE include obesity and diabetes (Roberts & Cooper, 2001 and El-Moselhy et al., 2011). These factors are also risk factors for atherosclerosis. Also, other similarities exist between PE and atherosclerosis. In both of them, endothelial cells are important targets and the dyslipidemia predisposing to atherosclerosis occurs in pre-eclamptic pregnancies (Hubel and Roberts, 1999). Hyper triglyceridemia shifts the spectrum of LDL subclasses toward proportional increases in smaller, denser, more atherogenic LDL particles (Krauss, 1997). Small dense LDL particles more readily infiltrate into arterial tissue (the presumed site of LDL oxidation) and exhibit enhanced adhesiveness to artery intemal proteoglycans (Anber et al., 1996). Further, smaller denser LDL particles are intrinsically more susceptible to oxidation (Chait et al., 1993). Small

dense LDLs show greater capacity to provoke changes in vascular cells in culture consistent with vasoconstrective effects in vivo (Weisser et al., 1993). Acute atherosis of decidual arterioles is characterized by fibrinoid necrosis of the vessel wall, disruption of the endothelium, aggregates of platelets and accumulation of lipid-laden macrophages, a true atherosclerosis-like change (Sheppard and Bonnar, 1981). Women with PE have reversible increases of serum TGs and LDL-cholesterol and reduced HDL-cholesterol. Importantly, small dense serum LDLs are, also, increased in women with PE compared to controls having normal pregnancies (Hubel et al., 1989 and Hubel et al., 1998).

PE syndrome is now recognized to be a multi-system disease (Roberts, 2000 and Roberts et al., 2003). PE variably may affect the brain, lungs, kidney and liver (Raijmakers et al., 2004a). So, the laboratory results of high liver enzymes and renal function tests are expected and accepted. The difference in laboratory results of ALT in mild (28.6±7.6 U/L) and severe (55.5±10.4 U/L) PE was statistically significant (P<0.001). Also, the difference in laboratory results of AST in mild (26.5±6.9 U/L) and severe (57.2± 11.8 U/L) PE was statistically significant (P<0.001) (Ziaei et al., 2008). Further, when patient have liver dysfunction, thrombocytopenia and hemolysis, they are classified as having HELLP syndrome (i.e., hemolysis, elevated liver enzymes, low platelets) (Curtin and Weinstein, 1999).

The potential limitations of this study, which must be considered is the retrospective design of the study that was unable to determine whether the observed alteration in amniotic fluid trace element selenium concentration preceded PE or whether alteration attributed to PE.

Conclusion and Recommendations

In this study pre-eclamptic patients had lower mean amniotic fluid Se, higher mean levels of fasting blood glucose, low platelet mean count**,** higher mean total leucocytic count, increased mean levels of CRP, increased mean serum TGs and LDL-cholesterol and reduced mean HDL-cholesterol, higher mean liver enzymes (ALT and AST)**,** and higher mean renal function tests compared to pregnant controls. Further, these differences were also present between the mild and severe PE cases. Also, the most important significantly correlated factors for severe PE were low amniotic fluid Se (negatively correlated), and high total leucocytic count, high fasting blood glucose and CRP levels (positively correlated). The best way to prevent PE in an effective manner is the establishment of an adequate prenatal control system, whose procedures should contain an adequate Se supplementation. In addition, adequate prenatal care would allow physicians to diagnose and promptly treat symptomatic and asymptomatic urinary and vaginal infections. Finally, the role that infection and inflammation may play in the imbalance of free radicals that leads to pre-eclampsia needs to be studied in depth because it may involve a fundamental change in the prevention and treatment of PE. Also, we recommend studying the role of other nutrient trace elements and vitamins in the occurrence of PE in Egypt.

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