Effect of Opiorphin and Alpha-II Spectrin Breakdown Product on Insulin Resistance and Lipid Profile for Establishing the Prognostic Model predicting PE

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Abstract

Preeclampsia (PE) disorder affects a significant percentage of pregnant women and is associated with many biochemical disorders. The present study examined the levels of spectrin breakdown products 145 (SBDP145), vitamin D (VitD), and opiorphin in 90 PE women compared with 30 healthy pregnant women. The correlation of these biomarkers with cations, insulin resistance (IR) parameters, and lipid profile has been examined. The results showed an increase in the serum opiorphin and SBDP145 and a decreased VitD level in PE women compared with controls. PE women also had hypocalcemia, mild IR state, and dyslipidemia. Atherogenic indices indicated a high risk of CVD in PE women, while IR disturbances indicated a risk of glycemic disturbances in PE women. It can be concluded that VitD deficiency is a diagnostic biomarker for PE; simultaneously, SBP145 may act as a potential biomarker for the prediction of abortion in PE women. Measuring lipid profile and IR is recommended to reduce the risk of atherogenicity and glycemic disorders, respectively. SBDP145 is a new biomarker for the risk of brain injury by high blood pressure and eclampsia during labor in PE women.

Keywords: PE, Spectrin breakdown products 145, Vitamin D, opiorphin, Insulin resistance, Lipid profile, atherogenicity biomarkers

1. Introduction

Preeclampsia (PE) is when a previously normal person has high blood pressure (BP) (>140/90 mm Hg systolic/diastolic BP) with/without proteinuria, defined as urinary excretion of >300 mg/24 hours after 20 weeks of pregnancy with a return to normal BP after delivery or after giving birth.[1] PE is characterized by the development of new-onset hypertension (HT) as well as the presence of signs of end-organ dysfunction[2, 3]. Once PE develops, the only effective treatment is a prompt delivery, which results in adverse neonatal outcomes, especially when the delivery date is far from the due date (<34 weeks of gestation). [4] Geographical area, season, diet, and race/ethnicity all have a role in the prevalence of this disease, which affects around 3–8 percent of women globally [5]. According to the Global Burden of Diseases, Injuries and Risk Factors Study 2019, hypertensive disorder in pregnancy increased by over 10% between 1990 and 2019 [6]. Additionally, severe PE is characterized by liver failure, abdominal pain, vomiting, and nausea [7]. Thrombocytopenia and hemolysis are hallmark characteristics of severe PE.[8] Another common symptom of PE is impaired coagulation, which may occur as a result of thrombotic microangiopathy [8]. PE risk factors include severe cardiovascular and metabolic illnesses such as chronic hypertension, chronic renal disease, hypertension during a prior pregnancy, type 1 and type 2 diabetes, and obesity with a maternal body mass index (BMI) of more than 30 kg/m [2, 9]. advanced age (greater than 40 years) [9].Although the origin of PE is unknown, research on this subject has implicated the placenta in the pathophysiology, among other reasons, since it has been shown that the disease's signs and symptoms generally vanish after birth [10].

While the pathophysiology of PE is not completely known, it is likely a complex interaction of genetic and environmental variables and defective placentation [2]. Finally, PE also affects the unborn. Due to insufficient oxygen delivery, fetal growth restriction is frequently associated with PE [11] However, the interconnections and pathophysiological implications of maternal predisposition and placental variables are not fully defined [12]. Another theory deals with the balanced ratio of pro- and anti-inflammatory cytokines essential to regulating the maternal inflammation system throughout pregnancy [13]. It is suggested that maternal attempts to balance the concentration of both pro- and anti-inflammatory factors were not sufficient to cause a placental response, and this failure may contribute to the development of PE [14].

Effective prophylaxis with aspirin (acetylsalicylic acid) is now available [15]. Aspirin (50-150mg daily) is currently the only medication recommended for the prevention of PE in low- and high-risk women [16]. Methyldopa is the optimal treatment for hypertension in PE [17, 18]. Adipose tissue inflammation is a key factor in developing IR and T2DM in obesity, along with other factors that likely include inflammation and fat accumulation in other metabolically active tissues [19]. Even after correcting for BMI or the presence of gestational diabetes, PE is independently related to an elevated maternal future risk of DM [20].

Pregnant women were found to have a higher IR during their pregnancy [21]. IR and metabolic syndrome are also linked to endothelial dysfunction, oxidative stress (OS),

doi.org/10.31838/hiv22.02.183 Received: 22.04.22, Revised: 21.05.22, Accepted: 02.08.22

and a diminished inflammatory response during pregnancy [22]. Thus, after comparing normotensive and PE pregnancies with and without antecedent IR, it has been postulated that IR contributes to the pathogenesis of PE [23]. After omitting women with gestational diabetes, it was shown that women who had PE had a substantially higher chance of getting diabetes [24]. PE, newborn hypoglycemia, and respiratory distress syndrome are more common in women with IR [25].

The lipid profile is described as the connection between total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDLc), and low-density lipoprotein cholesterol (LDLc) concentrations in the blood [26]. They are increasingly recognized as valuable predictors of CVD risk, [27] The pooled standardized mean difference of serum lipid profiles such as TC, TG, LDLc, and VLDLc were significantly higher in PE women than normotensive pregnant women, but HDLc was lower in PE women. It is concluded that dyslipidemia could play certain roles in the pathogenesis of PE [28]. PE women exhibit changed serum lipid profiles in the third trimester of pregnancy compared to normotensive pregnant women. Thus, early identification of these indicators will assist in improving PE care, which is critical for improving maternal and fetal outcomes.

Alpha II-spectrin is a 280-kDa neuronal cytoskeleton protein found in the central nervous system's axons and presynaptic terminals [29]. Alpha II-spectrin is required for brain cell integrity because it connects the cytoskeleton and the plasma membrane [30]. Notably, the present data are in line with suggestions that alpha II-spectrin might be a promising biomarker of brain injuries in infants following cardiac operations [31] and pediatric traumatic brain injuries [32]. Cytoskeletal proteins are released when there is cellular damage or death, which may serve as markers of brain damage. Myelin damage has been correlated with white matter injuries and epilepsy [33]. The epilepsy episodes may present in severe forms of PE.

Opiorphin is a naturally produced pentapeptide (H-Gln-Arg-Phe-Ser-Arg-OH) secreted into the human saliva [34] and blood, [35] endowed with a strong analgesic and antidepressant effect, even superior to that of morphine [36]. Additionally, opiorphin is a key regulator of the hypoxic response, activating pathways involved in enhanced blood flow and angiogenesis [37]. Opiorphin is upregulated in smooth muscle cells in response to hypoxia [37]. Opiorphins have previously been shown to be directly involved in regulating blood flow to tissues through their modulation of smooth muscle tone [38]. Opiorphin raised the serum level of angiotensin II, mean arterial pressure, and BP [39]. Therefore, we hypothesized that opiorphin might act as a marker for PE or the severity of the mood disorders.

Vitamin D can also be met through the synthesis in the skin by exposure to ultraviolet radiation in sunlight to convert 7- Dehydrocholesterol which is present in the skin is converted into the active form in the liver [40] and kidney [41] VitD stimulates intestinal absorption of calcium [42] and has a beneficial effect on physical fitness, healthy bone structure and skeletal muscle health [43]. Among the many physiological processes influenced by vitamin D, a critical role in reproductive physiology has been suggested [44]. Available data have correlated circulating VitD levels with reproductive success, such as achieving pregnancy using assisted reproductive technology [45].

In the United States, 20%–90% of reproductive-aged women are VitD deficient despite prenatal vitamin intake [46]. VitD deficiency is associated with obstetrical and reproductive complications, including recurrent pregnancy loss, small-for-gestational-age babies, abnormal puberty, and infertility [47]. However, little is known about the processes through which VitD insufficiency impacts reproductive health and function. It has recently been demonstrated that VitD levels in serum [48] and follicular fluid [49] were highly positively correlated and that patients who achieved clinical pregnancies after in vitro fertilization (IVF) had significantly higher VitD in follicular fluid and serum [49]. During pregnancy, vitamin D insufficiency is prevalent, ranging from 8–70 percent depending on skin pigmentation and sun exposure [50]. Compared to women with replete VitD levels, women with deficient VitD levels had a greater prevalence of PE [51] More recently, researchers have looked into the possibility of a link between VitD levels during pregnancy and the risk of PE. There are reports about the strong links between VitD insufficiency and an increased risk of PE, [52, 53] whereas others have found no link between maternal VitD deficiency and the rate of PE [54]. Early identification of vitamin D insufficiency in early or mid-pregnancy may help to avoid later obstetric difficulties [54]. Furthermore, VitD has a protective effect against recurrent PE [55].

Micronutrients and trace elements play vital roles in metabolism and preserving tissue functions [56]. Several elemental micronutrient abnormalities like calcium, magnesium, zinc and copper have been suggested to play contributing roles in PE [57, 58]. In some research, calcium supplementation may reduce the incidence of PE and pregnant HT [59]. There is a report regarding a significant association between low dietary calcium intake and low serum calcium levels with PE [53, 60, 61]. Magnesium is one of the most important elements since it serves as a cofactor for various enzyme systems [62, 63]. A great number of studied pregnant women had hypomagnesemia. However, oral magnesium supplementation seems not to have contributed to preventing PE in these women [64]. There is a significant association between PE and serum levels of calcium and magnesium [65]. The hypomagnesemia state was reported in PE women that correlated inversely with the severity and presence of complications [66]. Serum Mg level during the first trimester of pregnancy is a significant prediction tool for PE and could also play an important role in predicting newborns' week of gestational outcome and birth weight [67]. In pregnant women, serum albumin has an inverse relationship with OS but has a positive relationship with endothelial function [68]. Several researchers have found a drop in serum albumin levels during a typical pregnancy. In individuals suffering from severe toxemia during pregnancy, all of the distinct

fractions of plasma protein decrease in concentration [69, 70]. In women with the illness condition, hypoalbuminemia is frequent, but macroalbuminuria is a risk factor for poor pregnancy outcomes in the general population [71]. Therefore, we aimed to investigate the serum level of SBDP145 in the sera of PE women and investigate the correlation of SBDP145 with other clinical and biochemical parameters.

2. Materials and Methods

Subjects

The present case-control study was conducted in Rizgari Teaching Hospital and some private clinics from October 2021 till February 2022. The current study included 90 PE patients aged 30.96±5.266 years old as well as 30 age- and gestational age-matched healthy pregnant controls. The American College of Obstetricians and Gynecologists criteria were used to diagnose definite PE. [2] Pregnant women would be considered PE when they have a BP of systolic ≥140 mmHg or diastolic ≥ 90 mmHg after 20 weeks of gestation and proteinuria. The women should have previously normal BP. The patients in the present study followed these criteria and all patients had positive proteinuria in the dipstick test. They were overnight fasting and on treatment with methyl-dopa (Aldomet®). The Gestational age of pregnancy is calculated from the last normal menstrual period, and for those women who did not recall their last menstrual period, fundal height and/or ultrasound result was used. Gravidity was recorded as the total number of pregnancies, including abortion, ectopic pregnancy and any other pregnancies documented on the chart. Parity is defined as the number of deliveries after 28 weeks of gestation, including intrauterine fetal demise (IUFD) and stillbirth.

The study was approved by the ethical approval committee (IRB) of the Hawler Medical University, Erbil, Iraq (Document number 103/2022). Thirty pregnant women (gestational age >20 weeks) with no apparent abnormalities were selected as a control group. Their age was comparable to those of patients (29.97±6.573 years old). Their BP was within the normal range (around 120/80 mmHg).

Methods. Ten milliliters of venous blood samples were drowned by utilizing disposable needles and plastic syringes from each patient and control. The samples were transferred into a new plain tube. The blood was left at room temperature for 30 minutes for clotting, centrifuged at 3000 rpm for 15 minutes, and then serum was separated and transported into new disposable Eppendorf tubes. Estimations of SBDP145, opiorphin, insulin, VitD, were performed using enzyme-linked immunosorbent assay (ELISA), while blood estimation was done using an enzymatic technique. Insulin resistance (IR) parameters, including insulin resistance (HOMA2IR), beta-cell activity (HOMA%B) and insulin sensitivity (HOMA%S), were calculated from fasting insulin and fasting blood glucose (FBG) using HOMA2 calculator. Measuring of Ca, Mg, albumin, cholesterol, triglyceride, HDLc were done using colorimetric technique, LDLc was calculated using Friedewald formula [72]

3. Results

The results of demographic and clinical data on healthy controls (HC) and PE patients are presented in (Table 1). There is no significant difference between PE patients and the control group in terms of age, BMI, education, smoking, gravidity, gestational age, and Nullipara/multipara ratio. The results also showed a significant increase in the number of previous abortions, Cesarian deliveries, and systolic and diastolic blood pressure in PE women compared with healthy pregnant women. At the same time, there is a decrease in the parity numbers and an increase in the height in PE patients compared with the control group.

The results of serum SBDP145 in healthy controls (HC) and PE patients showed a significant increase ($p<0.001$) in SBDP145 concentration in PE patients (8.636(7.212-14.180) ng/ml) in comparing with the control group (5.852(4.170-7.143)) ng/ml.

The results of serum Opiorphin in healthy controls (HC) and PE patients showed a significant increase ($p=0.018$) in Opiorphin concentration in PE patients (4.725(4.020-5.299) ng/ml) in comparing with the control group (3.614(2.511-4.719) ng/ml). The results of serum VitD in healthy controls (HC) and PE patients showed a significant decrease ($p<0.001$) in VitD concentration in PE patients (8.137±1.977 ng/ml) in comparing with the control group (11.529±2.327 ng/ml).

The results of IR parameters in healthy controls (HC) and PE patients are presented in (Table 2). The results showed a significant increase in serum glucose ($p=0.004$), insulin ($p<0.001$), I/G ratio ($p<0.001$), and HOMA2IR ($p<0.001$) in PE patients compared with the control group. While there is a significant decrease in HOMA2%S ($p<0.001$) in PE women as compared with the healthy pregnant women. HOMA2%B values are not significantly different ($p=0.309$) between the study groups. The overall results indicated a state of IR in PE patients.

The lipid profile parameters and the calculated atherogenic indices in PE women and healthy control groups are presented in (Table 3). The results showed a significant increase in TG ($p<0.001$), TC ($p=0.006$), VLDLc ($p<0.001$), and LDLc ($p=0.001$) in PE patients in comparison with healthy pregnant women. Mean HDLc level has been decreased ($p<0.001$) in the patients compared with controls. All atherogenic indices (AC, AIP, CRI-I, and CRI-II) were significantly higher ($p<0.001$) in PE patients than in the control groups. These results indicated that PE women have dyslipidemia and they are at higher risk of atherosclerosis and cardiovascular diseases than the healthy pregnant group.

The results of serum cations and albumin data in healthy controls (HC) and PE patients are presented in (Table 4). The results showed a significant decrease in serum total ($p<0.001$) and ionized calcium ($p=0.001$) in comparison with the control group. No significant difference was noticed in the total and ionized magnesium between the study groups. Also, the cations ratios and albumin showed no significant difference between PE patients and controls.

The correlations between the measured biomarkers

SBDP145, Opiorphin, and VitD and the demographic and clinical parameters are presented in (Table 5). The results in (Table 5) showed significant correlations between SBDP145 with Age of onset, systolic and diastolic blood pressure, duration of symptoms and disease, and history of cesarean delivery. Opiorphin showed significant correlations with the age of onset, systolic blood pressure, duration of disease, parity and nullipara/multipara ratio. In contrast, vitamin D has a significant inverse correlation with the age of onset, BMI, systolic and diastolic blood pressure, duration of symptoms and disease, history of cesarean delivery and abortion, and parity.

The correlations between the measured biomarkers SBDP145, Opiorphin, and VitD and the IR parameters are presented in (Table 6). The results showed only a significant inverse correlation (ρ=-0.219, p<0.05) between VitD and insulin. Opiorphin and SBDP145 showed no significant correlation with all the IR parameters.

The results of the correlations between the measured biomarkers SBDP145, Opiorphin, and VitD and the lipid profile parameters and the atherogenic indices are presented in (Table 7). There are significant correlations between SBDP145 and the TG, VLDLc, AC, AIP, CRI-I, and CRI-II. While there is a significant negative correlation between SBDP145 and the HDLc. Opiorphin has no significant correlation with any lipid profile parameter and atherogenic index. Vitamin D showed significant inverse correlation with the TG, VLDLc, AC, AIP, CRI-I, and CRI-II. At the same time, a significant negative correlation was found between VitD and HDLc.

The results of the correlations between the measured biomarkers SBDP145, Opiorphin, and VitD and the cations and albumin are presented in (Table 8). There are significant negative correlations between SBDP145 and the total and ionized forms of calcium and magnesium. No significant correlation between SBDP145 and any cation and albumin. VitD showed a significant correlation with the total and ionized calcium.

The results of the correlations among the measured biomarkers are presented in (Table 9). The results showed a significant negative correlation between SBDP145 with VitD. While no significant correlation between Opiorphin and any SPDP145, and VitD.

The multiple regression analysis was used to find out the effect of the cofounders on all measured parameters. Then, the most significant factor that affected the biomarkers (diagnosis: the presence of PE in a subject) was used as an explanatory factor to calculate the effect of each biomarker after adjusting for other cofounders by using the between-subjects analysis to estimate the effect size of each biomarker by the diagnosis (presence of PE in a subject). These tests are an important addition in comparing with the ordinary correlation coefficients because they removed, automatically, any effect of the cofounders on the biomarkers values before making an association analysis. The results of both analyses are presented in (Table 10). The results showed that the diagnosis (presence of PE in a subject) is the only cofounder that significantly affects (p<0.001) the level of the measured biomarkers with a high size of the effect (Partial η^2=0.631). Therefore, we used the diagnosis as an explanatory factor to explain its effect on the measured biomarkers.

Tests for between-subjects effects show that all IR parameters (except insulin and HOMA2%B) are significantly affected by the presence of PE in a subject (diagnosis). Also, all lipid profile parameters and the calculated atherogenic indices (except TC and LDLc) are significantly affected by the presence of PE in a subject. Regarding cation, only total calcium and ionized serum calcium are significantly affected by the diagnosis. Test for between-subjects effect shows that VitD is significantly affected by the presence of PE in a subject (diagnosis). Multivariate analysis showed that SBDP145 and Opiorphin had not significantly affected by the PE after adjustment for all the entered biomarkers. The test of between-subjects effects indicating the top six effect sizes were observed for AIP (Partial η^2=0.245), followed by HOMA2%S (Partial η^2=0.202), TG and VLDLc (Partial η^2=0.153), insulin (Partial η^2=0.136), and VitD (Partial η^2=0.135). These six biomarkers have been affected largely by the presence of disease in the group.

To determine the diagnostic sensitivity and specificity of the measured biomarkers for the diagnosis of RA, an analysis of receiver operating characteristics (ROC) was performed. The ROC curves of the analysis are plotted in Figure 1 While, the coordinates of the ROC results and the cut-off of the concentration that produce the best sensitivities and specificities are presented in (Table 11). The results showed in (Table 11) that the decrease in VitD lower than the cut-off value (9.877ng/ml) leads to diagnosis of a subject as has PE (p<0.001) with a sensitivity and specificity of 83.3% and 83.3%, respectively. VitD curve covers a high AUC (0.903, 95%CI=0.861-0.955) with a high negative Youdin J statistic (-0.74). The increase in SBDP145 higher than the cut-off value (7.111 ng/ml) indicates that the subjects may have a PE in a sensitivity and specificity of 77.8% and 76.7%, respectively. The diagnostic characteristics of Opiorphin were sensitivity=66.7%, specificity= 66.7%, at a cut-off value of 4.386 ng/ml. These results are further confirmed by the values of AUC, Youdin J statistics, and p-values of both SBDP145 and Opiorphin. All the three biomarkers have a statistically significant difference (p<0.01) between PE patients and controls when their serum level exceeds their serum cut-off values.

A receiver operating characteristic (ROC) was done to determine the diagnostic ability of the measured biomarkers for predicting abortion. Figure 2 shows the ROC curve of the study. While (Table 12) shows the concentration cut-offs that generate the best sensitivities and specificities of the identified biomarkers. Opiorphin has no significant ability to predict abortion because all its results are not significant (p=0.194). The increase in SBDP145 higher than the cut-off value (7.817 ng/ml) indicates that the subjects may have an abortion with a sensitivity and specificity of 61.4% and 60.3%, respectively. The diagnostic characteristics of VitD were very low, even though it is slightly significant (p=0.043). At a serum level below the cut-off value (8.791), the diagnostic values were sensitivity=42.1% and specificity=42.9%. These values were very low in clinical

and research applications and cannot be used for diagnosis even if they are statistically significant.

4. Discussion

The lack of significant difference in the age, weight, BMI, education, smoking, gravidity, gestational age, and Nullipara/multipara ratio between PE patients and the control group in (Table 1) produce a strong conclusion and explanations of the results. In the present work, the control women have been chosen according to their similarity as much as possible to the characteristics of the PE women to reduce the cofounders that may affect the results. So, the biomarkers' possible changes would be due exclusively to the presence of PE and not due to other factors such as age, BMI, gestational age, etc.

The difference in the BP, seen in Table 1, is expected and widely reported as HT characterizes PE disease as the main symptom [73]. Higher diastolic BP were associated with more adverse outcomes [74]. unless malnutrition is present. The higher serum SBDP145 in PE patients compared to the control group is reported here for the first time. Therefore, there is no simple explanation for this finding. However, we can suggest some mechanisms for the elevation of SBDP145 depending on its activities and the clinical characteristics of PE. SBDP145 provides a highly sensitive measure of calpain activation and indicates apoptotic cell death [75] and acts as a marker of necrosis [76]. Also, SBDPs are considered highly specific for neuronal damage, including neurodegeneration disorders [77] and traumatic brain injury [78].

It has been shown that poor placentation, especially aberrant remodeling of spiral arteries, results in an ischemic placenta, which is the fundamental cause of PE [79]. Studies on human placental tissue have shown that OS and mitochondrial malfunction [80] have a role in the development of PE. Recently, it has been found that diseased placenta (as seen in PE) release molecules from their apoptotic neuronal cells [81] Factors released from the placenta may play a key role in mediating the effects of gestational hypoxia on neurodevelopment [82, 83] These molecules may damage cortical cells and causes neurodevelopmental disorders, leading to SBDP145 and increasing its serum level in PE.

The moderate significant increase ($p=0.018$) in serum Opiorphin in PE women compared with the healthy pregnant group is reported for the first time to our best knowledge. In human blood, the opiorphin physiological concentrations were dependent on the hormonal status as it was lower in six-month pregnant women than in non-pregnant volunteers [84] The low rates of circulating opiorphin levels in the blood of pregnant women and the low rates of urinary opiorphin in females compared to males suggest that the secretion of opiorphin in the female systemic compartment responds to its physiological hormonal status, notably during the menstrual cycle and gestation [84]. The strong analgesic and antidepressant effect, even superior to that of morphine and morphine-like drugs, may interact with the symptoms of PE that usually include pain [85].

When pain and depression symptoms in PE women exaggerated, the Opiorphin level increased. Healthy pregnant women normally do not suffer from severe pain or depression, and hence they normally present low Opiorphin in pregnant women. The AngII-dependent pathway may be influenced by opiorphin to modulate BP. VitD supplementation during pregnancy was related to a reduced rate of PE [86].

It is revealed that VitD has a favorable function in endothelial repair and angiogenesis [53] and that VitD deficiency is related to the etiology of cardiovascular illnesses and arterial hypertension [87]. Therefore, VitD may have a role in augmenting endothelial repair and angiogenesis and controlling BP in PE [55]. Elevated VitD reduces the composite outcome of PE and small-for-gestational-age birth [88]. In previous studies, the 10nM increase in VitD leads to a 38% reduction in the odds of developing severe PE [89].

In (Table 2), the results indicated a state of increase in serum glucose, insulin, Insulin/Glucose ratio, and HOMA2IR in PE patients compared with the control group. While there is a significant decrease in HOMA2%S in PE women compared with healthy pregnant women. IR is a diminished biological response to a given insulin dosage in the target tissue, whether endogenic or exogenous in nature (liver, muscle, or adipose tissue) [90]. IR in PE has been linked to dyslipidemia and endothelial dysfunction, although the exact etiology is unknown [91]. PE women had considerably greater insulin and IR levels than controls, and both were closely related to the severity of the PE condition in the mother [92]. When compared to non-pregnant women, pregnant women have a somewhat higher IR. PE is characterized by hyperinsulinemia and a significant rise in IR. As previously reported, PE was associated with elevated HOMA-IR and lower HOMA%S [93].

The lipid profile parameters and the calculated atherogenic indices in PE women and healthy groups in (Table 3) showed an obvious state of dyslipidemia. Having high levels of TC, LDLc, and TG and low levels of HDLc in the blood is related to an elevated risk of cardiovascular disease [94]. All atherogenic indices (AC, AIP, CRI-I, and CRI-II) were significantly higher ($p<0.001$) in PE patients than in the control groups. These results indicated that PE women have dyslipidemia and are at higher risk of atherosclerosis and cardiovascular diseases than the healthy pregnant group. While this is not consistent throughout every study, women who had a PE pregnancy tend to have higher levels of circulating TC, TG, and LDLc and lower HDL-C and higher apoB/apoA-I ratios years after giving a child [95]. Also, women with a history of PE have a lower HDLc [96]. Preliminary studies have shown that dyslipidemia is associated with PE and that the deregulations of lipid metabolism in pregnant women are connected to the pathophysiology of PE [97]. However, low calcium in women with PE is widely reported [60, 98]. Some researchers found that serum levels of calcium and magnesium were significantly lower in PE women than the healthy pregnant women [99, 100]. However, there is no significant difference in the serum levels of calcium and magnesium between PE women compared with the control group [101]. Table 4 revealed that our PE patients have a normal range, and these levels are not significantly

different from the control group due to the adequate feeding [102]. Because the most clinical states of increased SBDP145 are the traumatic brain injuries, [32, 97] besides much evidence now showing that PE is increasingly being linked to long-term cerebrovascular damage, we can suggest that there is a state of subclinical brain or nerves injuries in PE women caused by severe increase in BP in PE [103] that leads to an increase in SBDP145 with high BP.

Table 5 results also showed that Opiorphin has a significant correlation with age of onset, systolic BP, disease duration, parity and nullipara/multipara ratio. The correlation may be due to the antinociceptive activity of opiorphin,[35] which increase with increasing pain associated with high BP and other PE-related symptoms [104]. In a study, opiorphin levels were unrelated to age, gender, BMI, or hormonal condition [35].

The ROC results in (Figure 1 and Table 11) showed that the decrease in VitD lower than the cut-off value of 9.877ng/ml revealed a possibility of having PE with a sensitivity and specificity of 83.3%. The increase in SBDP145 higher than the cut-off value of 7.111 ng/ml indicates a moderate sensitivity and specificity of to have PE. The lowest sensitivity and specificity of Opiorphin indicate less accuracy for the detection of PE in a suspected woman. While Opiorphin has no significant ability to predict abortion. The increase in SBDP145 higher than the cut-off value of 7.817 ng/ml indicates that the subjects may have an abortion with a sensitivity and specificity of 61.4% and 60.3%, respectively. The results show low sensitivities and specificities (42.1% and 42.9%, respectively) of VitD to predict abortion at a serum level below 8.791ng/ml. These values were very low in clinical and research applications and cannot be used for diagnosis even if they are statistically significant.

5. Conclusions

From the results of the present study, it can be concluded that the Opiorphin and SBDP145 showed an increase in PE women compared with controls. PE women suffered from hypovitaminosis D, hypocalcemia, mild IR state, dyslipidemia, and a mild state of hypoalbuminemia. Atherogenic indices indicated a high risk of CVD in PE women. The results of IR parameters indicted a susceptibility of PE women to the glycemic disturbances that may eventually lead to diabetes mellitus. The ROC studies indicated the validity of the decrease in VitD and increase in SBDP145 for diagnosis of PE in suspected subjects with good sensitivity and specificity.

Table1: Demographic and clinical data of healthy controls (HC) and PE subjects

Parameter	Control	patient	F/χ2	p
Age yrs.	29.97±6.57	30.96±5.27	0.698	0.405
Age of Onset yrs.	-	30.87±6.36	-	-
Weight kg	84.83±12.42	85.57±15.25	0.057	0.812
Height cm	166.83±8.11	162.06±4.43	16.591	<0.001
BMI kg/m^2	30.437±3.655	32.58±5.59	3.847	0.052
Systolic B.P. mm/Hg	120.63±2.109	155.73±15.01	161.990	<0.001
Diastolic B.P. mm/Hg	80.37±2.566	91.11±9.98	33.861	<0.001
Education yrs.	5(4-6.5)	6(5-6)	MWUT	0.880
Rural/Urban	14/40	16/50	0.045	0.832
Smoking No/Yes	29/1	86/4	0.070	1.000
Gravidity (#Preg.)	3(2-5)	3(2-4)	1.976	0.133
Gestational age Wks.	29.60±4.882	30.10±4.39	0.276	0.600
Previous Abortion Yes/No	4/26	52/38	17.857	<0.001
Parity (#Deliv)	3(2-4)	2(1-3)	MWUT	0.033
Nullipara/multipara	2/28	15/75	1.85	0.174
Cesarean delivery No/Yes	21/9	37/53	7.519	0.011
Duration of PE yrs.	-	3.20±3.09	-	-
Duration Symptoms Wks.	-	8.76±4.28	-	-

Results are expressed as mean ± standard deviation for normally distributed data. Binomial data were expressed as ratios and analyzed by the Chi-squared test. BMI: body mass index, B.P.: blood pressure.

Table 2: Insulin resistance parameters of healthy controls (HC) and PE patients.

Parameter	Control	patient	F/χ^2	p
FBG Mm	5.415±0.745	5.875±0.745	8.581	0.004
Insulin pM	61.003±9.333	86.288±20.358	43.068	<0.001
I/G fM	11.460±2.271	14.729±3.030	29.339	<0.001
HOMA2%B	93.343±26.324	98.203±21.187	1.044	0.309
HOMA2%S	88.260±12.966	63.172±13.167	82.296	<0.001
HOMA2IR	1.158±0.183	1.664±0.409	42.74	<0.001

FBG: Fasting blood glucose, HOMA2%β: homeostasis model assessment of β-cell function, HOMA2%S: homeostasis model assessment of insulin sensitivity, HOMA2IR: homeostasis model assessment of insulin resistance, and I/G: insulin/glucose ratio.

Table 3: Lipid profile parameters and the atherogenic indices in HC and PE patients

Parameter	Control	patient	F/χ^2	P
TG mM	1.160±.279	1.621±.390	35.641	<0.001
TC mM	5.016±0.405	5.388±0.694	7.698	0.006
HDL mM	1.218±0.214	0.995±0.220	23.573	<0.001
VLDL mM	0.530±0.127	0.740±0.178	35.641	<0.001
LDL mM	3.267±.435	3.652±0.553	12.016	0.001
CRI-I	4.300±1.187	5.600±1.060	31.818	<0.001
CRI-II	2.847±1.073	3.832±.960	22.307	<0.001
AC	3.300±1.187	4.600±1.060	31.818	<0.001
AIP	-0.024±0.133	0.210±0.115	85.739	<0.001

TC: Total cholesterol, TG: triglycerides, HDLc: high-density lipoprotein cholesterol, LDLc: low-density lipoprotein cholesterol, VLDLc: very-low-density lipoprotein cholesterol, CRI-I and CRI-II: Castelli's Risk index I & 2, respectively, AC: Atherogenic coefficient, AIP: atherogenic index of plasma.

Table 4: Results of cations and albumin in HC and PE patients

Biomarkers	Control	Patients	F	p-value
Albumin g/l	45.725±6.360	43.997±5.872	3.013	0.092
T.Mg mM	0.959±0.142	0.927±0.312	0.565	0.453
Ionized Mg	0.672±0.094	0.651±0.206	0.565	0.453
T. Ca mM	2.411±0.180	2.269±0.246	15.631	<0.001
Ionizad Ca mM	1.235±0.052	1.201±0.068	11.614	0.001
T. Ca/Mg	2.577±0.483	2.659±0.842	0.490	0.485
Ionized Ca/Mg	1.880±0.318	1.983±0.545	1.838	0.177

Table 5: Correlation of the biomarkers with demographic parameters

Parameter	Vitamin D	Opiorphin	SBDP145
Age	-0.066	0.038	-0.001
Age of Onset	-0.525**	0.220*	0.363**
Weight	-0.092	-0.023	-0.001
Height	0.134	-0.064	-0.182
BMI	-0.204*	-0.015	0.110
Education	0.010	0.039	-0.018
Residency	-0.016	0.149	-0.022
Systolic B.P.	-0.440**	0.225*	0.483**
Diastolic B.P.	-0.365**	0.168	0.315**
Smoking	0.005	-0.158	-0.103
Gestational age	0.037	-0.091	0.071
Duration Symptoms	-0.424**	0.194	0.344**
Duration of PE	-0.488**	0.262**	0.466**
Gravidity	0.114	-0.061	0.057
Previous Abortion	-0.195*	0.119	0.112
Parity	-0.205*	0.236**	0.141
Nullipara/multipara	-0.217*	0.222**	0.149
Cesarean delivery	-0.209*	0.180	0.330**

**. Correlation is significant at the 0.05 level (2-tailed), **. Correlation is significant at the 0.01 level (2-tailed).*

Table 6: Correlation of the biomarkers with IR parameters

Parameter	SBDP145	Opiorphin	Vitamin D
Glucose mM	-0.126-	0.067	0.048
Insulin pM	0.041	-0.078	-0.219*
I/G fM	0.062	-0.104	-0.165
HOMA2 %B	0.090	-0.081	-0.127
HOMA2 %S	-0.032	0.079	0.177
HOMA2 IR	0.032	-0.079	-0.177

**. Correlation is significant at the 0.05 level (2-tailed), **. Correlation is significant at the 0.01 level (2-tailed).*

Table 7: Correlation of the biomarkers with lipid profile parameters and the atherogenic indices

Parameters	SBDP145	Opiorphin	Vitamin D
TG	0.219*	0.095	-0.422**
TC	0.067	0.140	-0.105
HDLc	-0.350**	-0.085	0.263**
VLDLc	0.219*	0.095	-0.422**
LDLc	0.149	0.172	-0.106
CRI-I	0.370**	0.175	-0.302**
CRI-II	0.338**	0.193	-0.254**
AC	0.370**	0.175	-0.302**
AIP	0.392**	0.124	-0.448**

**. Correlation is significant at the 0.05 level, **. Correlation is significant at the 0.01 level. TC: Total cholesterol, TG: triglycerides, HDLc: high-density lipoprotein cholesterol, LDLc: low-density lipoprotein cholesterol, VLDLc: very-low-density lipoprotein cholesterol, CRI-I and CRI-II: Castelli's Risk index I & 2, respectively, AC: Atherogenic coefficient, AIP: atherogenic index of plasma.*

Table 8: Correlation of the SBDP145,Opiophin, and vitD with albumin and cations

	SBDP145	Opiorphin	Vitamin D
Albumin	-0.053	-0.080	0.062
Total Mg	-0.248**	-0.009	0.136
Ionized Mg	-0.248**	-0.009	0.136
Total Ca	-0.333**	-0.009	0.356**
Ionized Ca	-0.321**	0.008	0.348**
T. Ca/Mg	0.117	0.000	-0.009
Ionized Ca/Mg	0.183	0.004	-0.071

Table 9: Correlation among measured biomarkers

Parameter	SBDP145	Opiorphin	Vitamin D
SBDP145	1.000	0.084	-0.396**
Opiorphin	0.084	1.000	-0.095
Vitamin D	-0.396**	-0.095	1.000

Table 10: Results of the multivariate generalized linear model (GLM) analysis and the between-subjects effects of the effect of the diagnosis on the biomarkers.

Tests	Dependent Variables	Explanatory variables	F	P	Partial η2
Multivariate	Biomarkers	Diagnosis	7.323	<0.001	0.631
		BMI	1.050	0.416	0.197
		Systolic B.P	1.466	0.11	0.255
		Diastolic B.P	0.691	0.832	0.139
		Gestational age	1.550	0.081	0.266
		Abortion	0.903	0.588	0.174
		Parity	0.564	0.932	0.116
		Smoking	1.353	0.165	0.24
		Cesarean delivery	1.616	0.063	0.274
Tests for between-subject effects	Vitamin D	Diagnosis	17.196	<0.001	0.135
	SBDP145	Diagnosis	0.019	0.89	0.001
	Opiorphin	Diagnosis	2.608	0.109	0.023
	Albumin	Diagnosis	0.548	0.461	0.005
	Total Mg	Diagnosis	0.944	0.333	0.009
	Ionized Mg	Diagnosis	0.944	0.333	0.009
	Total Ca	Diagnosis	13.6	<0.001	0.110
	Ionized Ca	Diagnosis	12.071	0.001	0.099
	T.Ca/Mg	Diagnosis	0.113	0.737	0.001
	Ionized Ca/Mg	Diagnosis	0.421	0.518	0.004
	TG	Diagnosis	19.904	<0.001	0.153
	TC	Diagnosis	0.653	0.421	0.006
	HDLc	Diagnosis	7.766	0.006	0.066
	VLDLc	Diagnosis	19.904	<0.001	0.153
	LDLc	Diagnosis	0.6	0.44	0.005
	CRI-I	Diagnosis	8.063	0.005	0.068
	CRI-II	Diagnosis	4.542	0.035	0.040

	AC	Diagnosis	8.063	0.005	0.068
	AIP	Diagnosis	35.65	<0.001	0.245
	Glucose	Diagnosis	2.541	0.114	0.023
	Insulin	Diagnosis	17.246	<0.001	0.136
	HOMA2%B	Diagnosis	2.095	0.151	0.019
	HOMA2%S	Diagnosis	27.763	<0.001	0.202
	HOMA2IR	Diagnosis	16.701	<0.001	0.132
	I/G	Diagnosis	13.993	<0.001	0.113

Table 11: Receiver operating characteristic-area under curve (AUC) analysis of the measured biomarkers for the diagnosis of PE. CI: Confidence interval.

Table 12: Receiver operating characteristic-area under curve (AUC) analysis of the measured biomarkers for the diagnosis of PE. CI: Confidence interval.

Figure 4: Receiver operating characteristic curves of VitD, Opiorphin, and SBDP145 for diagnosis of PE against healthy controls.

Figure 5: Receiver operating characteristic curves of VitD, Opiorphin, and SBDP145 for diagnosis of abortion.

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