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Original article

Evaluation of Maternal Serum Homocysteine in Pre eclampsia

Maha Azeez Tawfeeq [1*], Khalidah Amin [2]

[1] Obstetrics and Gynecology, Iraqi Board for Medical Specializations

[2] Assistant Professor, Obstetrics and Gynecology, University of Kirkuk, College of Medicine.

[*] Corresponding author, e-mail: maha.azeez.tawfeeq.saqi@gmail.com

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Abstract:

- ***Background***: Pre-eclampsia affects about 2–8% of all pregnancies. It is one of the leading causes of maternal and perinatal morbidity and mortality hyperhomocysteinemia is considered as a risk factor for endothelial dysfunction and vascular disease. The vascular changes induced by homocysteine are similar to those with pre-eclampsia. The aim of the study is to identify the relationship between maternal serum homocysteine level and the development of pre-eclampsia.
- ***Method and patient:*** A case control study that was carried out in the Department of Obstetrics and Gynecology at Azadi Teaching Hospital in Kirkuk-Iraq, during the period of eight months from 1st of Feb. till 1st of October 2021. It included 80 pregnant women with singleton viable pregnancy, gestational age of > 20 weeks. They were divided into two groups: Case group included 40 pregnant women who had diagnosed as pre eclampsia (mild and severe) and control group included 40 healthy normotensive pregnant women. Patients with multiple pregnancy, history of medical disease or tacking treatment for hypertension were excluded from the study. Blood sample was taken from each woman for biochemical study, serum homocysteine level was measured using ELISA technique.
- ***Result:*** In this study, pregnant women with pre-eclampsia had significantly higher mean of serum homocysteine compared with controls. Homocysteine level > 8.95 µmol/l is a predictor for pre-eclampsia. Out of 40 women with pre-eclampsia, 42.5% were mild, while the remaining 57.5% were severe. Pregnant women with severe pre eclampsia had significantly higher mean of serum homocysteine compared to those with mild pre-eclampsia. The level of tested biomarkers and homocysteine was tested between the two study groups to assess their rule in pre eclampsia.
- ***Conclusions:*** Maternal serum homocysteine biomarker represents an early non-invasive marker for diagnosis of pre eclampsia and to name the disease according to the severity.
- **Keywords:** Hypertension, pre eclampsia, homocysteine, pregnancy.

INTRODUCTION

Pre eclampsia is an idiopathic disorder of pregnancy characterized by proteinuric hypertension, recent estimates indicates that over 30000 women die worldwide each year because of pre eclampsia and its complications, with 98% of these occurring in the developing countries. Globally, pre eclampsia has been estimated to cause between 10 and 25% of perinatal loss. Up to 5% of women will develop pre-eclampsia in their first pregnancy and although the overwhelming majority of these will have successful pregnancy outcomes, the condition can give rise to sever multisystem complications including cerebral haemorrhage, hepatic and renal dysfunction and respiratory compromise. The development of strategies to prevent and treat the disorders has been challenging due to an incomplete understanding of the underlying pathogenesis [(1).] New biomarkers could be significantly aiding the diagnosis of women suspected of having pre eclampsia. experts have long sought biomarkers for pre eclampsia that are reliable and can be measured with precision, and that can allow for an objective assessment of the disease, furthermore, because the presence of increased urinary protein is usually a late manifestation of the disease, it is hoped that the use of biomarkers would enable earlier detection or even prediction of the disease , some studies reported that high plasma homocysteine may be associated with adverse pregnancy outcomes, such as pre eclampsia [(2).]
Pre-eclampsia is defined as a new onset hypertension with significant proteinuria arising de novo after the 20th weeks of pregnancy in previously normotensive women and resolving completely by the sixth post-partum week [(3).] Significant hypertension defined as at least 140/90 mmHg recorded blood pressure on at least two separate occasions and at least four hours apart, significant proteinuria defined as a urinary protein/creatinine ratio greater than 30 mg/mmol or a validated 24-hour urine collection results showing greater than 300mg protein [(4,5)]
Pre eclampsia can originate in the placenta and may occur in the absence of fetal tissue (as in molar pregnancy) and its manifestations will only resolve following delivery of the placenta. The blueprint for establishing PE is determined at the outset of pregnancy when placental trophoblast invades the maternal uterine spiral arteries at the time of implantation .In pregnancies destined to be complicated by PE, transformation of the spiral arteries is impaired, with suboptimal remodeling of small-capacitance constricted vessels into dilated large-capacitance conduits , the prevailing theory has been that the subsequent relative placental ischemia causes release of vasoactive factors which then give rise to endothelial-mediated end-organ damage and clinical manifestations of PE [(1)].
Maternal characteristics and history provide strong clues to which women are at more risk of developing pre-eclampsia than others, particularly: Prior pre-eclampsia, chronic hypertension. ,multiple gestation ,pregestational diabetes mellitus, maternal BMI > 30 kg/m2 ,antiphospholipid syndrome/SLE ,and, assisted reproductive therapies [(6)].Other factors less

strongly associated with pre-eclampsia include, but are not limited to :advanced maternal age, family history of pre-eclampsia, short duration of sexual relationship(< 6 months) before the pregnancy, primigravida (although pre-eclampsia may occur in subsequent pregnancies even in the absence of pre-eclampsia in the first), Prim paternity , both changed paternity and an interpregnancy interval >5years have been associated with an increased risk for pre-eclampsia, chronic kidney disease, and, connective tissue disease [7].

Homocysteine is an amino acid not supplied by the diet, its levels vary between men and women with a normal range typically between 5-15 μmol/L. Hyperhomocysteinaemia is when levels more than 15 μmol/L. Hyperhomocysteinemia, or increased circulating levels of homocysteine, is generally recognized as an independent risk factor for coronary, cerebral, and peripheral atherosclerosis. The levels of homocysteine can be increased by defective metabolism of methionine, resulting from either mutation in genes coding for the enzymes of homocysteine metabolism, or deficiencies of certain vitamin cofactors. In addition to genetic alterations, vitamin deficiencies, and several other environmental factors as increased intake of methionine, certain medications are known to contribute to variations in homocysteine levels [8].

Hyperhomocysteinemia is considered as a risk factor for endothelial dysfunction and vascular disease just as is pre eclampsia . Over the past two decades, endothelial cell activation has become the center piece in the contemporary understanding of the pathogenesis of PE. The vascular changes induced by homocysteine are similar to those associated with PE and include atherosis and endothelial dysfunction resulting in blunted vaso-relaxation mechanism. Intact endothelium has anticoagulant properties, and it blunts the response of vascular smooth muscle to agonists by releasing nitric oxide [9].

Hyperhomocysteinemia appears to cause endothelial dysfunction through direct toxic and oxidative stress mechanism. The association of hyper-homocysteinemia and pre eclampsia has been suggested some authors in 1995 who demonstrated that homocysteine was seven times more common in women with history of severe pre eclampsia. Since then, there have been multiple studies concluding the strong association of hyper-homocysteinemia and PE [1].

PATIENT and METHOD

This study is a case control study that was carried out in the department of obstetrics and gynecology at Azadi Teaching Hospital in Kirkuk-Iraq, during the period of eight months from 1st of Feb. till 1st of oct. 2021. The study protocol was approved by the scientific council of obstetrics and gynecology / Iraqi Board for Medical Specializations, it included 80 pregnant women with singleton viable pregnancy, gestational age of > 20 weeks assessed by menstrual dates and proved by first trimester ultrasound scan visiting consultancy clinic or were admitted to the obstetric ward for assessment and management ,those women classified into two groups:

Case control; Included 40 pregnant women who had diagnosed as pre eclampsia (mild or severe).

Control control: Included 40 normotensive pregnant women without any known pregnancy complications and with matched gestational age.

women included in this study were singletone pregnancy, not in active labour, no signs of infection, received folic acid supplements until 12 weeks of gestation. women excluded from this study are those with gestational age < 20 week , essential hypertension suggested by history or documentation of hypertension in pregnancy state or hypertension before 20 weeks of gestation ,history of cardiovascular disease , history of pregestational and gestational diabetes mellitus, neurological disorders (like Alzheimer's coincidental seizure in pregnancy),renal impairment , premature rapture of membrane and chorioamnionitis, hematological disorders (like megaloblastic anemia),inflammatory or infective disorders drug history (methotrexate, PPI, nitric oxide, theophylline and phenytoin), history of smoking and alcohol intake.

RESULTS

The total number of study subjects included in the statistical analysis was 80, divided into two groups 40 pregnant women with pre eclampsia and 40 pregnant women with normal pregnancy.

The comparison between PE group and controls by certain demographic features showed that there were no statistically significant differences between the two groups in terms of age (P= 0.467) and BMI (P= 0.093) as shown in (Table 1)

Table 1. distribution of certain demographic characteristics between study groups

Characteristics	Study Groups		P – Value
	PE Mean ± SD	Control Mean ± SD	
Age (Years)	30.22 ± 6.02	31.3 ± 7.08	0.467
BMI (kg/m^2)	23.73 ± 3.4	23.09 ± 3.24	0.093

Regarding obstetrical characteristics, there were no statistically significant differences between the two groups in terms of parity (P= 0.787), and GA (P= 0.867) as shown in (Table 2)

Table 2. Distribution of mean obstetrical characteristic between study groups

Characteristics	Study Groups		P - Value
	PE Mean ± SD	Control Mean ± SD	
Parity	1.52 ± 1.76	1.42 ± 1.51	0.787
GA (Weeks)	36.92 ± 3.26	37.02 ± 1.88	0.867

there was a statistically significant difference in levels of SBP and DBP between the two groups. The means of SBP and DBP were significantly higher in PE group compared with control group (146.1 mmHg versus 119.8 mmHg, P= 0.001 and 103.3 mmHg versus 75.6 mmHg, P= 0.001, respectively) (Table 3)

Table 3. Comparison in mean levels of SBP and DBP between study groups

Biomarker	Study Groups		P - Value
	PE Mean ± SD	Control Mean ± SD	
SBP (mmHg)	146.1 ± 12.42	119.8 ± 10.22	0.001
DBP (mmHg)	103.3 ± 9.69	75.6 ± 10.63	0.001

In the present study, there was a statistically significant difference in homocysteine levels between the study groups. Pregnant women with PE had significantly higher mean of serum homocysteine compared with controls (154.8 ng/ml versus 66.33 ng/ml, P= 0.001) (Table 4 and figure 1)

Table 4. Comparison in mean level of homocysteine between study groups

Homocysteine (ng/ml)	Study groups		P - Value
	PE Mean ± SD	Control Mean ± SD	
	154.8 ± 79.46	66.33 ± 33.27	0.001

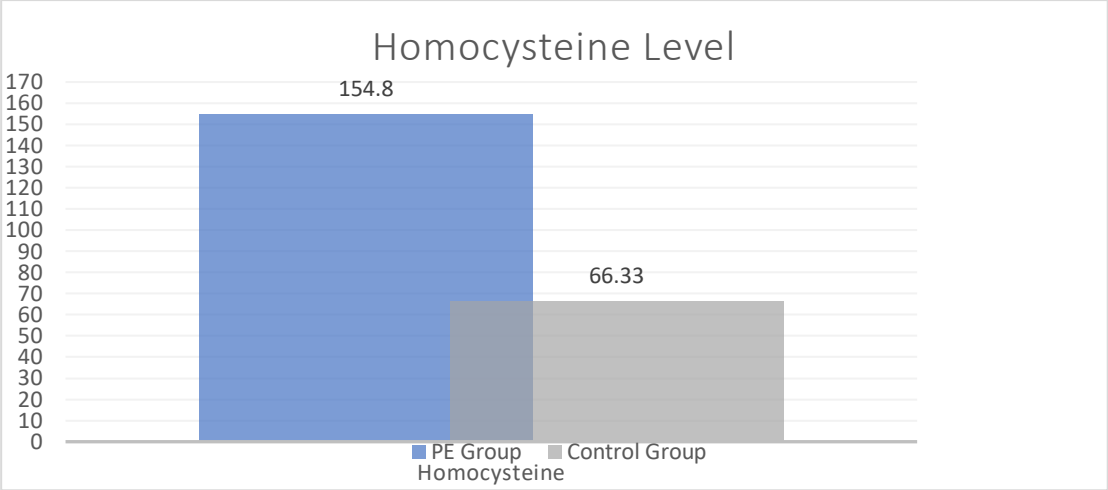

Figure 1. Distribution of study groups by mean level of homocysteine.

Receiver operating characteristic (ROC) curve analysis was constructed for homocysteine level as a predictor for PE. The cut point of the homocysteine level was 89.5 ng/ml. Hence, homocysteine level > 89.5 ng/ml is a predictor for PE, as a large significant area under the curve (AUC= 92.5%) indicating a significant association between the higher level of homocysteine and having PE. Homocysteine level was 90% sensitive, 87.5% specific, and 88.8% accurate in diagnosis of PE (Table5) and (Figure 2).

Table 5. Diagnostic accuracy of homocysteine level as a predictive marker for PE

Homocysteine Level (ng/ml)	Cut-off value	Sensitivity	Specificity	PPV	NPV	Accuracy
	89.5	**90%**	**87.5%**	**87.8%**	**89.7%**	**88.8%**

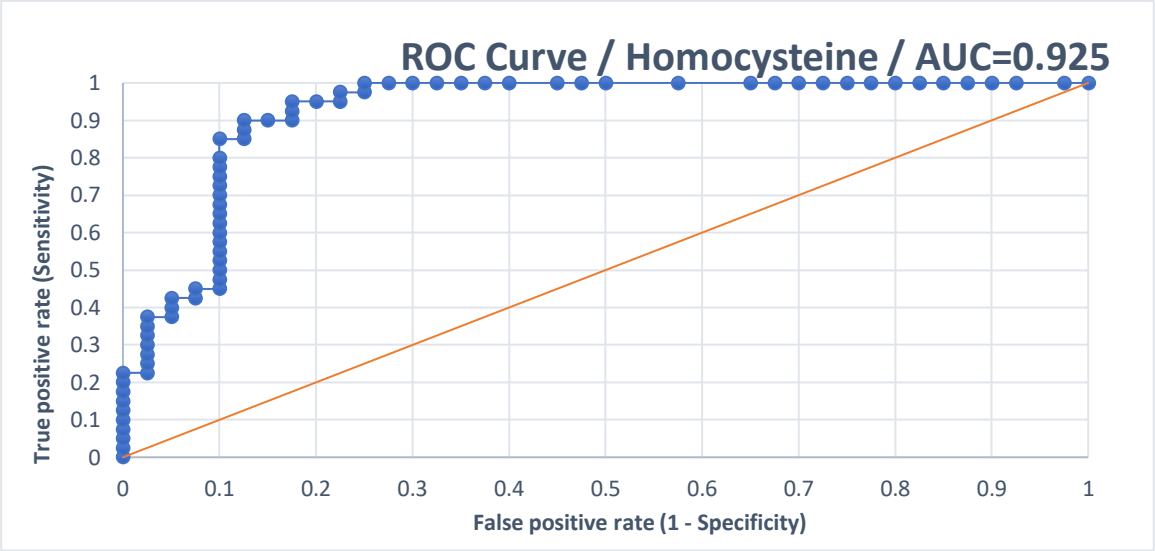

Figure 2. ROC curve for homocysteine in predicting of PE

It was clear that there was a statistically significant difference in homocysteine levels between patients of PE group. Pregnant women with severe PE had significantly higher mean of serum homocysteine compared to those with mild PE (192.3 ng/ml versus 102.8 ng/ml, P= 0.001) (Table 6).

Table 6. Comparison in mean level of homocysteine between PE group

Homocysteine (ng/ml)	PE Severity		P – Value
	Mild Mean ± SD	Severe Mean ± SD	
	102.8 ± 24.32	192.3 ± 85.1	0.001

DISCUSSION

Pre eclampsia (PE), a pregnancy specific syndrome, is a major cause of maternal and perinatal morbidity and mortality of all pregnancies. Despite a fairly high incidence,the underlying etiology of pre eclampsia is still incomplete[11] . Although the pathogenesis is still under investigation, impaired placental function seems to play a pivotal role in the etiology of this syndrome, it is hypothesized that pathophysiology of PE is characterized by a cascade of impaired early trophoblast invasion, decreased placental perfusion, placental ischemia,

oxidative stress, and consequentially impaired placental factors (imbalance in angiogenic and prothrombotic factors) which are playing a key role in inducing systemic maternal endothelial dysfunction[12]. Clinical evidence had reported a hyperhomocysteinaemia features in hypertensive disorders of pregnancy [13].

It is a risk factor for endothelial dysfunction and vascular disease like atherosclerosis. In normal pregnancy there is conversion of spiral arteries from its highly tortuous thick-walled vessels to flaccid sinusoidal conduits of low resistance, however, the muscular coats of spiral arteries are retained in pre eclampsia [14]. In PE there is elevated homocysteine injuries and abnormal vascular endothelium [9]. The main hypothesis of this study is that maternal homocysteine level may differ between pre eclampsia women and healthy pregnant women and also between sever and mild pre eclampsia patients.

The current study showed that the mean and standard deviation (SD) of age was 30.22 ± 6.02 years, and of of BMI of PE group was 23.73 ± 3.40 kg/m2, 60% of cases had normal weight; there were no significant differences between the two groups in terms of age (P= 0.467) and BMI (P= 0.093). An agreement reported by Al-Sakarneh and other co-authors in their study in 2021, in which no significant differences was observed between cases and controls in terms of age and BMI [14] another agreement was found in consistent with result of Dundar et al that found no statistical difference in BMI between studied groups[15], and in disagreement with the results observed in AlSheeha et, al. study in 2016, in which mean and standard deviation (SD) of age was 30.6±6.0 years, the mean and SD of BMI was 31.0 ± 6.3Kg/m2[16].

Concerning parity, 55% of cases were nulliparous. The GA was ≥ 37 weeks in 60% and 70% of the pregnant women in PE group and control group, respectively.

In this study, there were no significant differences between the two groups in terms of parity (P= 0.787), and GA (P= 0.867).This agrees with the findings of Ayala et al, 2001 in which no significant relation between parity and elevation of BP was observed, The larger, although not statistically significant, differences as a function of parity in our study were found for women ≥36 years of age; surprisingly, for both SBP and DBP measured at all 3 trimesters of pregnancy in this age group, multiparous women tend to have a slightly higher BP than do nulliparous women, in opposition to previous reports based on casual measurements ; a slightly higher BP can also be observed for all multiparous women compared with nulliparous women, independent of maternal age for data sampled by ABPM in the second trimester of pregnancy, although differences are not statistically significant[17].

However, it differs from the result of Košir Pogačnik, et al,2020 that showed multipara had a significant lower incidence of pre eclampsia[18].

Our study revealed a significant difference in levels of SBP and DBP. The means of SBP and DBP were significantly higher in PE group than control group (P= 0.001). The results in Al-Sakarneh,et al study in 2021, agreed to the current one, as SBP and DBP was significantly

higher in women with PE than in controls (154.16 ± 2.57 and 114.16±1.44 mmHg vs 95.16±1.05 and 75.33±0.89 mmHg, respectively, P < 0.001) [14] . In the same accordance, an agreement reported in a study conducted by Özdemir and colleagues in 2018, in which patients in the PE group, had a systolic and diastolic blood pressure significantly higher than that observed in patients of control group(P<0.001)[19].

Accordingly, an agreement observed in Vyakaranam et al study in 2015, in which both SBP and DBP was significantly higher in pregnant women with PE in comparison to those with normal pregnancy (P<0.05) [20] .

In this study, pregnant women with PE had significantly higher mean of serum homocysteine compared with controls (P= 0.001). Moreover, ROC curve was constructed for homocysteine level as a predictor for PE, in which cut point was 8.95 μmol/l. So, homocysteine level > 8.95 μmol/l is a predictor for PE. Homocysteine level was 90% sensitive, 87.5% specific, and 88.8% accurate in diagnosis of PE.

As compared to results of Al-Sakarneh et al study in 2021, an agreement found. They found that Serum levels of homocysteine was significantly higher in women with PE compared with controls (16.35±0.43 vs 7.25±0.21, respectively; *P*<0.001) [14]. In the same accordance, Oluwole and colleagues in a study done in 2020, found of the 167 participants, 10.8% had PE, the final analysis included 41 (24.6%) patients had a homocysteine concentration above the reference range (>15μmol/L) and 126 (75.4%) with a normal homocysteine concentration and the rate of PE was three times higher among women with high homocysteine level than those with normal homocysteine level (P= 0.01) [21].

On the other hand, results from studies investigating the relation between serum homocysteine and PE are contradictory. A case control study conducted by Shilpa et al. (2017) demonstrated significantly lower serum Hcy level in PE patients (8.90±4.33 μmol/L) compared with normal non-pregnant women (13.31±5.81 μmol/L). However, mean serum Hcy levels of the PE patients (8.90±4.33 μmol/L) were not significantly higher than those of normal pregnant women (7.52±2.25 μmol/L). The authors indicated that this insignificant minor difference in serum Hcy levels may be due to PE patients receiving folic acid supplementation during their first trimester of gestation. (P<0.05). However, mean homocysteine levels of the PE patients were not significantly higher than those of normal pregnant women (P > 0.05) [22].

Out of 40 women with PE enrolled in this study, 42.5% were with mild PE while the remaining 57.5% were with severe PE. Concerning homocysteine level in severe cases of PE in this study, pregnant women with severe PE had significantly higher mean of serum homocysteine compared to those with mild PE (P= 0.001). In consistent to other studies, a similarity was reported with a study done by Al-Sakarneh et al study in 2021, in which a strong association between the severity of PE and levels of serum homocysteine. An increase in serum homocysteine of 1 μmol/L was associated with a 3.5 mmHg increase in SBP and 1.8 mmHg

increase in DBP (P<0.05)[14]and this is also consistent with Shahbazian et al study in 2016, that involved 51 PE pregnant women, in which they reported a significantly higher serum homocysteine levels in pregnant women with severe PE compared with women with non-severe disease (P<0.05)[23]. Similarly, in Maru et al study in 2016, the association between homocysteine levels and severity of PE and complications of PE and eclampsia was confirmed as There was a direct correlation with a statistical significance in the study, of the 32 patients, 30 patients of eclampsia had an elevated level of homocysteine which is as high as 93.75 %. Of the 87 patients with elevated homocysteine levels, 81 patients had hypertension of varied severity. Si (P<0.05) [24]. A different finding observed while the current results compared to that obtained in Oluwole et al study in 2020, the final analysis of this study included 167 women; 41 (24.6%) patients had a homocysteine concentration above the reference range (>15μmol/L) and 126 (75.4%) with a normal homocysteine concentration. in which severe pre eclampsia was recorded in 7 (38.9%) of mothers with PE, moreover, no significant association was found between high homocysteine level and severity of PE (P= 0.29) [21]. Finally, our study had some limitation as the small sized sample and homocysteine level was measured at 2nd half of pregnancy when sign and symptoms of pre eclampsia have already been started.

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Nil.

Conflicts of interest:
There are no conflicts of interest**.**

CONCLUSION

1. Maternal serum homocysteine biomarker represents a non-invasive, and excellent predictor of pre-eclampsia at a cut off >8.95 μmol/l.

2. Maternal serum homocysteine is a biomarker that is also helpful to determine the severity of pre-eclampsia.

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