Original Research Article

Comparative evaluation of homocysteine levels in the serum of normal pregnant females and females with preeclampsia

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Received: 11-08-2021 / Revised: 09-09-2021 / Accepted: 16-11-2021

Abstract

Background: Levels of homocysteine are normally decreased during pregnancy. However, increased levels are associated with complications like preeclampsia and other fetal defects. Owing to the continuous association of elevated homocysteine levels in females with preeclampsia, it can be used as a potential biomarker in preeclampsia. **Aims:** The present study was carried out to compare and assess the levels of homocysteine in females with preeclampsia and normotensive females. The present study also attempted to correlate levels of homocysteine with preeclampsia severity. **Materials and Methods:** 40 multigravida and primigravida females divided into two groups controls were normal pregnant females and cases were pregnant females having preeclampsia. Following clinical examination, laboratory investigations carried out in both groups were serum homocysteine levels, hemogram, blood grouping, routine urine examination, Rh factors, Hepatitis B, and HIV, whereas, for cases, fundoscopy, liver function tests, kidney function tests, and coagulation profiles were assessed. The collected data were subjected to statistical evaluation and the results were formulated. **Results:** In controls 100% (n=20) subjects had normal values, whereas, in preeclampsia cases 20% (n=4) had normal values and 80% (n=16) had raised homocysteine levels. The mean values respectively in cases were significantly higher (19.93±11.40) compared to controls (8.0±2.28) with ($p<0.001$). Based on preeclampsia severity, in subjects with mild preeclampsia, 15% (n=3) had normal homocysteine values and 45% (n=9) had raised values, whereas in severe preeclampsia, 5% (n=1) had normal values and 55% (n=11) had raised values. **Conclusion:** The present study concludes that serum homocysteine levels are significantly higher in pregnant females with preeclampsia compared to normal pregnant females and high homocysteine levels are directly related to preeclampsia severity.

Keywords: Endothelium, Homocysteine, Preeclampsia, pregnancy

Introduction

Preeclampsia is a syndrome specifically related to pregnancy secondary to infections or hemorrhage. Preeclampsia contributes a major etiological factor in causing perinatal mortality and morbidity with a global prevalence of 5-8% in pregnant females. 15% of all the hospitalization in pregnant females is attributed to hypertension-related complications alone[1]. Female with preeclampsia during pregnancy are at high risk of other pregnancy-related complications including placenta rupture, cardiovascular effects, acute renal failure, eclampsia, cerebrovascular complications, and/or maternal death in females, and pre-term low birth weight, growth restrictions, and/or fetal death in the fetus[2].

Various theories in literature have suggested the etiology of preeclampsia to endothelial dysfunction, defective placentation, and/or inflammation. Despite the various theories, the actual etiology of preeclampsia remains largely undetermined. However, the pathophysiology of preeclampsia is largely cantered to endothelial dysfunction. The growth of various human tissues and cell depends on homocysteine which is an essential amino acid having sulfur and is synthesized by methionine demethylation[3].

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Levels of homocysteine are normally decreased during pregnancy. However, increased levels are associated with complications like preeclampsia and other fetal defects. Elevated levels of homocysteine can result from vitamin B12, folic acid, and B6 deficiency, leading to homocysteine oxidation with the production of reactive oxygen species, which further lead to endothelial dysfunction and damage, secondary to thrombomodulin and nitric oxide inactivation[4]. Homocysteine also contributes to preeclampsia pathophysiology by interfering with the fibrinolytic system. Owing to the continuous association of elevated homocysteine levels in females with preeclampsia, it can be used as a potential biomarker in preeclampsia. Also, treating elevated levels can help to reduce preeclampsia-associated adverse events[5]. Hence, the present study was carried out to compare and assess the levels of homocysteine in females with preeclampsia and normotensive females. The present study also attempted to correlate levels of homocysteine with preeclampsia severity.

Materials and methods

The present study was carried out at Department of Obstetrics and Gynaecology, GSL medical college Rajamundary, Andhra Pradesh after obtaining clearance from the concerned Ethical committee. The study subjects were recruited from the females visiting the Outpatient department, department of Obstetrics and Gynaecology, and antenatal clinics of the institution. A total of 40 females were included in the study and were divided into cases and controls with each group having a sample size of 20.

The study included a total of 40 multigravidas and primigravida in the age range of 18 years to 36 years with a mean age of 23.7 years and a gestational age range of 26 weeks to 40 weeks. The exclusion criteria

for the study were females with systemic diseases such as diabetes mellitus, hypertension, thrombophilia, systemic lupus erythematosus, and/or anemia (severe). Also, the females with multiple gestations and abruptio placentae were excluded from the study. Females taking medications like xanthopterin, carbamazepine, tamoxifen, phenytoin, 6-azauridine, and antifolic acids were also excluded from the study.

After the final inclusion of 40 subjects, they were divided into two groups: controls (n=20) and cases (n=20) where controls were normal pregnant females and cases were pregnant females having preeclampsia. Preeclampsia was defined by raised blood pressure with the systolic blood pressure of more than 140mm hg and diastolic blood pressure of higher than 90mm Hg and proteinuria with more than 300 mg protein excretion in 24-hour urine. After explaining the study design and obtaining informed consent, demographic and obstetrics history was recorded for both cases and controls followed by clinical examination.

Following clinical examination, the samples were collected for laboratory investigation. The laboratory investigations carried out in both groups were serum homocysteine levels, hemogram, blood grouping, routine urine examination, Rh factors, Hepatitis B, and HIV, whereas, for cases, fundoscopy, liver function tests, kidney function tests, and coagulation profiles were assessed. Following homocysteine levels assessment, the value of >15 µmol/Liter was considered as Hyperhomocysteinemia.

The collected data were subjected to the statistical evaluation using SPSS software version 21.0, 2012, Armonk, NY, and t-test. The results were formulated keeping the level of significance at $p<0.05$.

Results

The study included a total of 40 multigravidas and primigravida in the age range of 18 years to 36 years with a mean age of 23.7 years and a gestational age range of 26 weeks to 40 weeks. The demographic and obstetrics characteristics of the study subjects are summarized in Table 1.

Table 1: Demographic and Obstetric characteristics of the study subjects

Characteristics	Controls		Cases		p-value
	%	n	%	N	
Age Range(years)					
Less than 20	5	1	20	4	
20-30	75	15	65	13	
30-36	20	4	15	3	
Mean±S.D	26.73±4.28		25.06±4.78		0.2517
Gestational Age (weeks)	%	n	%	N	
28-32	5	1	25	5	
33-36	10	2	35	7	
37-40	85	17	40	8	
40-42	0	0	0	0	
Mean±S.D	37.87±1.99		35.14±3.49		<0.001
Systolic Blood Pressure (mm Hg)	%	n	%	N	
Less than 140	100	20	0	0	
141-159	0	0	55	11	
160 or more	0	0	45	9	
Mean±S.D	117.58±8.47		151.98±12.62		<0.001
Diastolic Blood Pressure (mm Hg)	%	n	%	N	
Less than 90	100	20	0%	0	
91-109	0	0	70%	14	
110 or more	0	0	30%	6	
Mean±S.D	75.53±5.95		97.98±8.85		<0.001
Gravida Status	%	n	%	N	
Primi Gravida	35	7	65%	13	
Multi Gravida	65	13	35%	7	
BMI (kg/m^2)	26.81±4.01		27.96±3.97		0.367
Eclampsia Severity	**Percentage (%)**		**Number (n)**		
Mild	55		11		
Severe	45		9		

The maximum study subjects were within the age of 20-30 years with 75% (n=15) in controls and 65% (n=13) females among cases with respective means of 26.73±4.28 and 25.06±4.78 and p-value of 0.2517 showing that age differences in two groups were statistically non-significant. For gestational age, the means for controls and cases were 37.87±1.99 and 35.14±3.49 respectively which was statistically significant ($p<0.001$) showing females with preeclampsia had significantly less age compared to normal females. Significantly higher systolic and diastolic blood pressure was seen in cases compared to controls ($p<0.001$). The respective mean systolic blood pressure in controls was 117.58±12.62 and 151.98±12.62 and diastolic blood pressure respectively was 75.53±5.95 and 97.98±8.85mm Hg. Mild eclampsia was seen in 55% (n=11) study subjects and severe eclampsia in 45% (n=9) subjects. The non-significant difference in BMI was seen in the two groups (p=0.367).

The present study also assessed homocysteine levels and it was seen that in controls 100% (n=20) subjects had normal values, whereas, in preeclampsia cases, 20% (n=4) had normal values and 80% (n=16) had raised homocysteine levels. The mean values respectively in cases were significantly higher (19.93±11.40) compared to controls (8.0±2.28) with ($p<0.001$). Based on preeclampsia severity, in subjects with mild preeclampsia, 15% (n=3) had normal homocysteine values and 45% (n=9) had raised values, whereas in severe preeclampsia, 5% (n=1) had normal values and 55% (n=11) had raised values. Concerning systolic B.P, in subjects with <140mm Hg, 141-159mm Hg, and >160mm Hg, the mean homocysteine values respectively were 8.53±3.16, 16.73±8.97, and 23.93±13.41 ($p<0.001$). For diastolic B.P, in subjects with <90mm Hg, 91-109mm Hg, and >110mm Hg, the mean homocysteine values respectively were

8.19±2.28, 17.63±9.17, and 25.29±14.47 (p<0.001) as shown in Table 2.

Table 2: Homocysteine levels based on various parameters in the study females

Homocysteine levels (µmol/L)	Controls		Cases		p-value
	%	N	%	N	
Serum Values					**<0.001**
Normal	100	20	20	4	
Increased	0	0	80	16	
Mean±S.D	8.0±2.28		19.93±11.40		<0.001
Based on preeclampsia severity	**Cases**		**Mean±S.D**		
	Normal % (n)	**Raised % (n)**			
Mild Preeclampsia	15(n=3)	45(n=9)	5.76±15.55 (11)		<0.001
Severe Preeclampsia	5 (n=1)	55 (n=11)	3.97±24.85 (9)		
Controls	80 (n=16)	0	8.19±2.28		
Based on Blood Pressure (B.P)	Less than 140	141-159	160 or more		<0.001
systolic B.P	8.53±3.16	16.73±8.97	23.93±13.41		
	Less than 90	91-109	110 or more		<0.001
diastolic B.P	8.19±2.28	17.63±9.17	25.29±14.47		

The present study also assessed delivery and fetal outcomes. Concerning delivery modes, FTVD, PTVD, and LSCS among controls was seen in 45% (n=9), 15% (n=3), and 40% (n=8) subjects, whereas, in cases these values respectively were 15% (n=3), 30% (n=6), and 55% (n=11) females. Pre-term deliveries in controls and cases were done in 10% (n=2) and 45% (n=9) study females. For low birth weight, it was seen that the difference between cases and controls were statistically non-significant (0.0048) with respective mean values of 2140±798.94 and 2779.93±523.38. On assessing the perinatal outcomes, it was seen that stillborns, normal, IUGR, and neonatal ICU requirements were respectively seen among controls in 0% (n=0), 80% (n=16), 5% (n=1), and 15% (n=3) females, these values respectively in cases were 5% (n=1), 35% (n=7), 10% (n=2), and 50% (n=10) newborns as described in Table 3.

Table 3: Fetal Outcomes in Study subjects

Delivery and Fetal Outcomes	Controls		Cases		p-value
	%	n	%	N	
Delivery modes					
Full-term Vaginal Delivery (FTVD)	45	9	15	3	
Pre-term Vaginal Delivery (PTVD)	15	3	30	6	
Lower segment Caesarean section (LSCS)	40	8	55	11	
Term					
Term	90	18	55	11	<0.001
Preterm	10	2	45	9	
Birth weights					
Less than 1000 gms	0	0	10	2	
1001-2000 gms	15	3	15	3	
2001-3000 gms	55	11	45	9	
More than 3000 gms	30	6	30	6	
Mean±S.D	**2779.93±523.38**		**2140±798.94**		**0.0048**
Perinatal Outcomes	**Controls**		**Cases**		
	%	**n**	**%**	**N**	
Stillborn	0	0	5	1	
Normal	80	16	35	7	
Intrauterine Growth Retardation (IUGR)	5	1	10	2	
Neonatal ICU	15	3	50	10	

Discussion

The study results showed that maximum study subjects were within the age of 20-30 years with 75% (n=15) in controls and 65% (n=13) females among cases with respective means of 26.73±4.28 and 25.06±4.78 and p-value of 0.2517 showing that age differences in two groups were statistically non-significant. For gestational age, the means for controls and cases were 37.87±1.99 and 35.14±3.49 respectively which was statistically significant (p<0.001) showing females with preeclampsia had significantly less age compared to normal females. Significantly higher systolic and diastolic blood pressure was seen in cases compared to controls (p<0.001). The respective mean systolic blood pressure in controls was 117.58±12.62 and 151.98±12.62 and diastolic blood pressure respectively was 75.53±5.95 and 97.98±8.85mm Hg. Mild eclampsia was seen in 55% (n=11) study subjects and severe eclampsia in 45% (n=9) subjects. The non-significant difference in BMI was seen in the two groups (p=0.367). These findings were similar to the study by Khosrowbeygi A et al[6] in 2011 where similar results concerning age, systolic, and diastolic blood pressure were seen, and Georgios M et al[7] in 2007 concerning BMI and gestational age were seen.

In the present study, concerning delivery modes, FTVD, PTVD, and LSCS among controls was seen in 45% (n=9), 15% (n=3), and 40% (n=8) subjects, whereas, in cases these values respectively were 15% (n=3), 30% (n=6), and 55% (n=11) females. Pre-term deliveries in controls and cases were done in 10% (n=2) and 45% (n=9) study females. For low birth weight, it was seen that the difference between cases and controls were statistically non-significant (0.0048) with respective mean values of 2140±798.94 and 2779.93±523.38. On assessing the perinatal outcomes, it was seen that stillborns, normal, IUGR, and neonatal ICU requirements were respectively seen among controls in 0% (n=0), 80% (n=16), 5% (n=1), and 15% (n=3) females, these values respectively in cases were 5% (n=1), 35% (n=7), 10% (n=2), and 50% (n=10) newborns. These findings were similar to

Roberts JM et al[8] in 2005 and Laskowska M et al[9] in 2011 where authors reported similar findings as present study.

For homocysteine levels, it was seen that in controls 100% (n=20) subjects had normal values, whereas, in preeclampsia cases, 20% (n=4) had normal values and 80% (n=16) had raised homocysteine levels. The mean values respectively in cases were significantly higher (19.93±11.40) compared to controls (8.0±2.28) with ($p<0.001$). Based on preeclampsia severity, in subjects with mild preeclampsia, 15% (n=3) had normal homocysteine values and 45% (n=9) had raised values, whereas in severe preeclampsia, 5% (n=1) had normal values and 55% (n=11) had raised values. Concerning systolic B.P, in subjects with <140mm Hg, 141-159mm Hg, and >160mm Hg, the mean homocysteine values respectively were 8.53±3.16, 16.73±8.97, and 23.93±13.41 ($p<0.001$). For diastolic B.P, in subjects with <90mm Hg, 91-109mm Hg, and >110mm Hg, the mean homocysteine values respectively were 8.19±2.28, 17.63±9.17, and 25.29±14.47 ($p<0.001$). These findings correlated with the studies of Romerio SC et al[10] in 2004 and Mignini LE et al[11] in 2005 where homocysteine corresponded to the present study.

Conclusion

Within its limitations, the present study concludes that serum homocysteine levels are significantly higher in pregnant females with preeclampsia compared to normal pregnant females and high homocysteine levels are directly related to preeclampsia severity. These high homocysteine levels in females with preeclampsia can be attributed to endothelial injury caused by high homocysteine levels leading to preeclampsia. However, the present study had few limitations including geographical area bias, smaller sample size, and single-institution nature. Hence, more longitudinal studies with a larger sample size will help in reaching a definitive conclusion.

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Conflict of Interest: Nil Source of support: Nil