www.jmscr.igmpublication.org
Impact Factor 5.84
Index Copernicus Value: 83.27
ISSN (e)-2347-176x ISSN (p) 2455-0450
crossref DOI: https://dx.doi.org/10.18535/jmscr/v5i4.209

IGM Publication
Journal Of Medical Science And Clinical Research
An Official Publication Of IGM Publication

Prevalence of Hyperhomocysteinemia among Pre-Eclampsia Patients of A Tertiary Referral Hospital in South India

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ABSTRACT
Introduction: *Pre-eclampsia is a pregnancy-specific multisystem disorder and is a leading cause of maternal and fetal morbidity and mortality. Several studies have provided evidences that indicate the involvement of hyperhomocysteinemia in the etiology of pre-eclampsia.*
Materials and Methods: *A cross sectional descriptive study was conducted in 51 pre-eclampsia patients at ≥ 28 weeks of gestation. A fasting blood sample was collected for estimation of serum homocysteine.*
Results: *Out of 51 pre-eclampsia patients 32 had hyperhomocysteinemia. The prevalence was 62.7%*
Conclusion: *The present study found high prevalence of hyperhomocysteinemia among pre-eclampsia patients of South India. This study is relevant in our population where nutritional deficiencies are highly prevalent, and present attempt is the forerunner of further extensive studies, that hopefully come up with results that benefit future obstetrics*
Keywords: *pre-eclampsia, homocysteine, prevalence.*

INTRODUCTION

Pregnancy is a physiological process comprising of many fundamental changes in the female organisms. Although the majority of pregnant women have a normal healthy pregnancy, labour & child birth, some women experience complica-tions, which may arise due to pregnancy, or due to pre existing conditions complicated by pregnancy. Pre-eclampsia is a multisystem disorder which complicates about 3 –8% of pregnancies in Western countries and is a major source of morbidity and mortality all over the world.[1]

Even though the etiology of pre-eclampsia is not fully understood, the basic pathology include endothelial dysfunction and intense vasospasm.[2] Many mediators of pre-eclampsia produce endothelial dysfunction either by increasing oxidative damage, or by producing free radicals, or both. Many studies have proved that an elevated plasma homocysteine levels could be a

risk factor for endothelial dysfunction and vascular diseases.

Homocysteine (Hcy) is an amino acid formed of dietary methionine metabolism. Some amount of homocysteine is converted back to methionine by remethylation, which require vitamin B-12 & folic acid.[3] Rest of homocysteine is metabolized to cysteine via the transsulfuration pathway that requires vitamin B-6.[3]The cysteine is either reused for synthesis of other compounds, or is excreted as sulfate.[3]

Serum homocysteine levels fall in normal pregnancy, as early as 8 weeks and it gradually decrease with the gestational age.[4] Several mechanisms have been proposed to explain the decrease in serum homocysteine during pregnancy, including the normal increase in the glomerular filtration rate, the haemodilution, increased uptake of maternal Hcy by the foetus, increased maternal B-vitamin intake, and the hormonal effect on Hcy metabolism.[5]

The most common causes of hyperhomocysteinemia are genetic and nutritional. Nutritional deficiencies of vitamin B-6, vitamin B-12 and folic acid can increase the serum homocysteine levels.[6] Several studies have proved the crucial role of hyperhomocysteinemia in the pathogenesis of coronary artery disease, myocardial infarction, deep vein thrombosis, diabetic nephropathy etc.[7]Many prospective and retrospective studies have confirmed the association between hyperhomocysteinemia (HHcy) and pre-eclampsia.[8,9] Some of these studies have also shown that pre-eclampsia patients with elevated homocysteine levels were at increased risk of development of many adverse pregnancy outcomes like placental abruption, intra uterine growth retardation, pre term delivery, low birth weight and intra uterine death of foetus. In a famous publication "H factor solution - the best single indicator of whether you are likely to live long or die young" the author concludes the book with the statement- "if your H factor is not below 6, never attempt to become pregnant".[10]

Several mechanisms have been postulated to explain the pathomechanisms of homocysteine in pre-eclampsia. Among them the most widely accepted one is homocysteine mediated endothelial dysfunction. The mechanisms by which hyperhomocysteinemia produces endothelial dysfunction includes the reduction in bioactivity of a potent vasodilator- endothelium derived nitric oxide,[11] the increase in oxidative stress and levels of reactive oxygen species,[12] and the up regulation of components of inflammatory cascade.[13]

Many studies have established that patients with elevated homocysteine values benefitted from therapies that lowered homocysteine levels, in the form of folic acid, vitamin B-6 and B-12. A Meta analysis proved that administration of 0.5 to 5mg of folic acid daily can reduce serum homocysteine levels by about 25%. The same study has shown that if 0.5mg of vitamin B-12 daily was also included in the regimen, it produced an additional 7% decrease in homocysteine levels.[14] Being amenable to therapy makes the estimation of serum homocysteine a relevant investigation in pregnant women.

An elevated homocysteine level among the general population of India was reported in many studies, which was mainly attributed to the nutritional deficiency of B vitamins.[15] The present study was intended to find out prevalence of hyperhomocysteinemia among pre-eclampsia patients $\geq$ 28 weeks of gestation, who got admitted to a tertiary care hospital of South India.

Normal pregnancy is associated with lower Hcy levels compared to non-pregnant state. Hyperhomocysteinemia is a controversial term, and the cut-off value differs with the population under study. An elevated Hcy level is an indirect measure of B vitamins deficiency. Therefore, identifying the cut-off value of HHcy should be achieved only in the light of B-vitamin status. In the current study, the cut-off value was taken as 8.2 μ mol/L, which represented the 95th percentile of Homocysteine distribution in normotensive pregnant women who have had adequate status of

folate and vitamin B12, based on a study conducted in Syrian population.[16]This particular reference was selected, since this study population was found to have a higher prevalence of vitamin deficiencies, as in our native population.

MATERIALS AND METHODS

A cross sectional descriptive study was conducted in the Department of Obstetrics and Gynaecology at a tertiary referral hospital of South India. The study was conducted after obtaining clearance from the Research committee and Ethical committee. The study group included 51 pre-eclampsia patients ≥ 28 weeks of gestation, who got admitted to this hospital. The total period of study was one year.

The pre-eclampsia patients were diagnosed using the following criteria:

1. Development of hypertension after 20 weeks of gestation in a pregnant woman who was normotensive before pregnancy.
2. Systolic blood pressure ≥140 mm of Hg
3. Diastolic blood pressure ≥ 90 mm of Hg [Both blood pressures recorded on two separate occasions, atleast 6 hours apart.]
4. Proteinuria described as ≥ 300 mg / 24 hour or ≥ 1+ dipstick urinary protein in random urine samples.

The exclusion criteria included patients with known vascular disease, renal disease, diabetes mellitus and those with any other medical disorders like thyroid dysfunction. Patients on antifolate drugs like antiepileptics, methotrexate etc, were also excluded from the study.

Baseline characteristics of the study group were recorded. Body mass index (BMI) was calculated from height and weight of each patient. 5ml of venous blood was collected after an overnight fast using standardized techniques. Blood collected was transferred to bottles containing Ethylene Diamine Tetra Acetic acid (EDTA) as anticoagulant for total homocysteine estimation. Specimens were centrifuged at 3000 rpm for 10 minutes to obtain plasma. Plasma was immediately separated and stored at -20°c for estimation of total homocysteine using Globe Diagnostics Homocysteine Enzymatic test kit.

Statistical Analysis

Data Analysis was done using SPSS version 16.0 statistical software.

Quantitative variables were expressed as mean+/- SD. Qualitative variables are expressed as proportions or percentages. Chi square test was applied to find any association between selected base line data and hyperhomocystinemia.

A ‘p value’ less than or equal to 0.05 was taken as statistically significant.

RESULTS

The study was conducted in 51 pre-eclampsia patients. Tables 1 & 2 shows the baseline data of the study group

Table-1 Base line characteristics of cases (Categorical values)

		Frequency	Percent
Age	<20	7	13.7
	21-30	37	72.55
	>30	7	13.7
Gestational age	28-34	22	43.1
	34-37	24	47.1
	>37	5	9.8
BMI	<25	27	52.9
	25-30	20	39.2
	>30	4	7.8
Prenatal folic acid intake	Yes	2	3.9
1st trimester folic acid intake	Yes	50	98.0

Table-2 Base line characteristics of cases (Quantitative variables)

Descriptive Statistics	N	Minimum	Maximum	Mean	Sd
AGE [years]	51	19	38	25.4	4.5
Gestational age [weeks]	51	28	38.3	33.8	2.8
BMI [Kg/m²]	51	20.1	32.3	24.7	2.6
Hcy[µmol/L]	51	2.8	20.1	9.37	5.0

The proportion of pre-eclampsia patients who tested positive for hyperhomocysteinemia was found out. Among the 51 pre-eclampsia patients, 32 patients [62.7%] had hyperhomocysteinemia. It is shown in Figure 1.

Fig.1 Pi diagram showing the prevalence of hyperhomocysteinemia in pre-eclampsia patients.

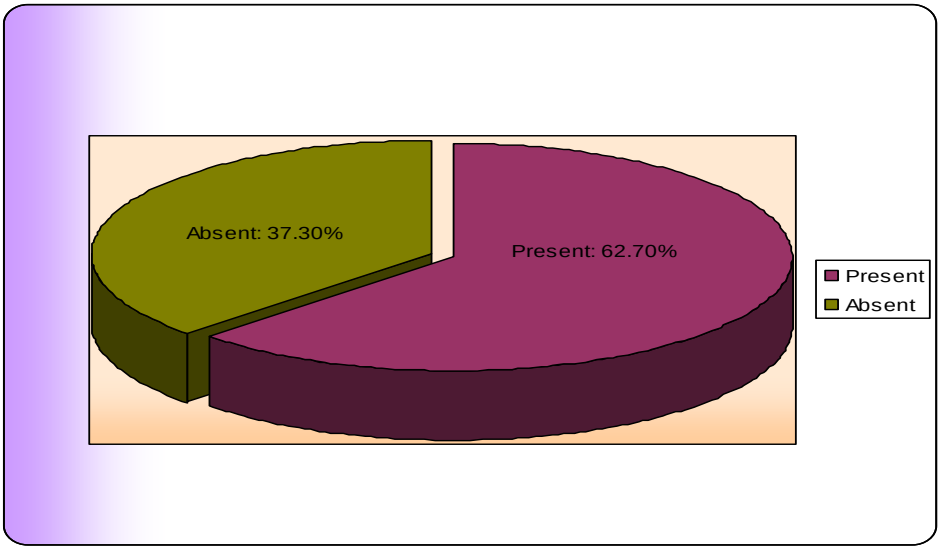

Table-3 shows the distribution of hyperhomocysteinemia in different age groups. In our study the age of the patients was within the range of 19 to 38 years. Maximum number of cases was present within the age group of 21 to 30 years and maximum proportion of hyperhomocysteinemia was also observed in the same group. This observation was statistically significant. [p = 0.042]

Table-3 Distribution of hyperhomocysteinemia in different age groups

Age	Hyperhomocysteinemia				Total	
	Present		Absent			
	N	%	N	%	N	%
<20	2	28.6	5	71.4	7	100.0
21-30	27	73.0	10	27.0	37	100.0
>30	3	42.9	4	57.1	7	100.0
Total	32	62.7	19	37.3	51	100.0

χ^2 =6.337df =2 p=0.042

Among the cases who were in the gestational age between 34 and 37 weeks, 58.3% had hyperhomocysteinemia. The same was present in 63.6% and 80.0% of cases who belonged to the gestational age group of 28 – 34, and >37 weeks respectively. The chi-square test showed no significant association. [Table-4]

Table-4 Distribution of hyperhomocysteinemia in different gestational age groups

Gestational age	Hyperhomocysteinemia				Total	
	Present		Absent			
	N	%	N	%	N	%
28-34	14	63.6	8	36.4	22	100.0
34-37	14	58.3	10	41.7	24	100.0
>37	4	80.0	1	20.0	5	100.0
Total	32	62.7	19	37.3	51	100.0

χ^2 =0.844 df =2 p=0.656

Among the two patients who took folic acid in the pre natal period, none of them had hyperhomocysteinemia. Although there was no statistically significant association, the p value was found to be 0.061. [Table- 5]

Table-5 Distribution of hyperhomocysteinemia with respect to history of prenatal folic acid intake

Prenatal folic acid intake	Hyperhomocysteinemia				Total	
	Present		Absent			
	N	%	N	%	N	%
Yes	0	0	2	100.0	2	100.0
No	32	65.3	17	34.7	49	100.0
Total	32	62.7	19	37.3	51	100.0

χ^2 =3.056 df =1 p=0.061

Table-6 shows that among the 51 patients, 50 took folic acid in the first trimester. Among this, 31 patients (62.0%) had hyperhomocysteinemia. There was no significant association between first trimester folic acid intake and hyperhomocysteinemia on chi square analysis.

Table-6 Distribution of hyperhomocysteinemia with respect to history of folic acid intake in the first trimester.

1st trimester folic acid intake	Hyperhomocysteinemia				Total	
	Present		Absent			
	N	%	N	%	N	%
Yes	31	62.0	19	38.0	50	100.0
No	1	100.0	0	.0	1	100.0
Total	32	62.7	19	37.3	51	100.0

χ^2 =0.606 df =1 p=0.436

A‘t’ test was done to find out the correlation between BMI of the patients and hyperhomocysteinemia. The mean BMI of the cases who had hyperhomocysteinemia was 25.79 ± 2.53 Kg/m² and that of the patients without hyperhomocysteinemia was 22.82 ± 1.57 Kg/m² .There was a positive correlation between the two [r = 0.559, p = 0.001].It is shown in Table-7

Table-7 Mean BMI of patients with and without hyperhomocysteinemia

Hyperhomocysteinemia	N	Mean BMI	Sd	T	p
Present	32	25.79	2.53	4.614	0.001
Absent	19	22.82	1.57		

DISCUSSION

In our study 62.7% of pre-eclampsia patients had hyperhomocysteinemia. Similar studies have been conducted in many parts of the world. Many studies revealed a higher value for serum homocysteine among Indain population. The SHARE study[17] and the UK study[18] found that the levels of homocysteine in the South Asians / Indians were higher than those found in other ethnic groups. Several explanations have been put forward to account for this observation that include the reduced intake of vitamin-B12 and the habit of prolonged cooking of vegetables. The latter one may deteriorate up to 90% of their folate content. [19]

The high prevalence rate of hyperhomo-cysteinemia (62.7%) noted among the pre-eclampsia patients in the present study could be due to the widespread dietary deficiency of vitamin B12 and folic acid and / or due to genetic causes like genetic mutations of enzymes influencing homocysteine metabolism.

Many studies have shown a high prevalence of vitamin B deficiency in Indian general population.[19,20] But there are only few studies done in our pregnant population, showing the homocysteine status. Our study recommends screening of all pregnant women for an elevated homocysteine level, in all the three trimesters, especially in those women with an increased risk for developing pre-eclampsia. Since the estimation of serum homocysteine level is an expensive test, the affordability can pose problems. So prophylactic administration of folic acid through-out the period of pregnancy and if possible in the pre-conceptional period also, can be considered. Addition of other B vitamins especially B-6 and B-12 along with folic acid can be well thought-out, since these are cost effective and safe measures to reduce serum homocysteine levels.

Anyway, further studies are required to find out the reference value for homocysteine in normotensive pregnants of our native population. For this, the maternal folate and other B vitamin status of our population need to be explored, since they are the major determinants of serum homocysteine levels. Also, more studies on MTHFR Gene Polymorphism and its distribution are needed so as to elucidate the reasons for the high prevalence of Hyperhomocysteinemia among the pregnant population of South India.

CONCLUSIONS

The present study found a high prevalence of hyperhomocysteinemia among the pre-eclampsia patients. There was a statistically significant association between hyperhomocystinemia and the age of the patients.

A positive correlation between BMI and hyperhomocysteinemia was also found.

Further extensive studies may be required to elucidate the reasons for this observation. Also, interventional studies are required to find out the need of modifying the current nutritional supplementation given to pregnant women, in terms of duration and dosage, and also to decide upon, whether to include B 12 and B 6 vitamins along with folic acid, in the prevention and / or treatment of hyperhomocysteinemia.

ACKNOWLEDGEMENTS

The authors are grateful to all those staff members in the Departments of Physiology, Obstetrics, Community Medicine and Biochemistry, who helped us at various stages of our work.

REFERENCES

1. Duley L. The global impact of pre-eclampsia and eclampsia. Semin Perinatol. 2009;33:130–137
2. Roberts JM, Taylor RN, Musci TJ, Rodgers GM, Hubel CA, Mclaughlin MK (1989) Preeclampsia: an endothelial cell disorder. Am J Obstet Gynecol 161: 1200-1204
3. Selhub J, Miller JW,:The pathogenesis of homocysteinemia: Interruption of the coordinate regulation by S-adenosyl methionine of the remethylation and

transsufuration of homocysteine . Am J Clin Nutr 55:131-138,1992.

4. Bonnette RE, Caudill MA, Boddie AM, Hutson AD, Kauwell GP, Bailey LB (1998) Plasma homocyst(e)ine concentr-ations in pregnant and nonpregnant women with controlled folate intake. Obstet gynecol 92: 167-170
5. Walker MC, Smith GN, Perkins SL, Keely EJ, Garner PR. (1999) Changes in homocysteine levels during normal pregnancy. Am J Obstet Gynecol 180: 660-664
6. De Bree A, Verschuren WM, Blom HJ, Kromhout D (2001) Lifestyle factors and plasma homocysteine concentrations in a general population sample. Am J Epidemiol 154: 150-154
7. Herrmann W (2001) The importance of hyperhomocysteinemia as a risk factor for diseases: an overview. Clin Chem Lab Med 39: 666-674
8. Singh Urmila,Gupta HP,Singh RK et al.Homocysteine: Association with preeclampsia and normotensive pregnancy.J Obstet Gynecol India Vol.59,No . 3:May/June 2009 pg 235-238
9. Rajkovic A, Catalano PM, Malinow MR (1997) Elevated homocyst(e)ine levels with preeclampsia. Obstet Gynecol 90: 168-171
10. The H Factor Solution: Homocysteine, the Best Single Indicator of Whether You Are Likely to Live Long or Die Young Authors: James Braly and Patrick Holford 2003 edition
11. Stuhlinger MC, Tsao PS, Her JH, Kimoto M, Balint RF, Cooke JP (2001)Homocysteine impairs the nitric oxide synthase pathway: role of asymmetric dimethylarginine. Circulation 104: 2569–2575
12. Yamamoto M, Hara H, Adachi T (2000) Effects of homocysteine on the binding of extracellular-superoxide dismutase to the endothelial cell surface. FEBS Lett 486: 159–162
13. Wang G, Siow YL, O K (2000) Homocysteine stimulates nuclear factor κB activity and monocyte chemoattractant protein-1 expression in vascular smooth-muscle cells: a possible role for protein kinase C. Biochem J 352: 817–826
14. Homocysteine Lowering Trialists' Collaboration (1998) Lowering blood homocysteine with folic acid based supplements: meta-analysis of randomi-sed trials. British Medical Journal 316, 894-898.
15. Chambers JC, Ireland H, Thompson E, et al. MTHFR C677T mutation and CHD risk in UK Indian Asians. Arterioscler Thromb Vas Biol 2000; 20; 2448-52.
16. Isber S (2006) The role of poor nutritional status and hyperhomocysteinemia in complicated pregnancy in Syria.Dissertation for awarding the Degree of Theoritical Medicine .University of Sarland; Faculty of Medicine, Clinical chemistry and Laboratory Medicine / Central laboratory, Homburg.
17. Anand SS, Yusuf S, Vuksan V, et al. Difference in risk factors, atherosclerosis and cardiovascular disease between ethnic groups in Canada: the study of health assessment and risk ethnic groups (SHARE). Indian Heart J 2000; 52:535-43.
18. Chambers JC, Obeid, Refsum H, et al. Plasma homocysteine concentrations and risk of coronary heart disease in UK Indian Asian and European men. Lancet 2000; 355:523-7.
19. Abraham R, Brocon MC, North WR, Mc Fadyen IR. Diets of Asian pregnant women in Harrow: iron and vitamins. Hum Nutr Appl Nutr 1987; 41:164-73.
20. Mathews JH, Wood JK. Megaloblastic anaemia in vegetarian Asians. Clin Lab Haematol 1984; 6:1-7.