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A study on role of homocysteine as a biochemical marker in various obstetrical complications

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Abstract

Low plasma homocysteine (Hyc) level during an uncomplicated pregnancy was first demonstrated by Kang *et al*. almost 20 yrs ago, & this has subsequently been confirmed by numerous investigators. Plasma homocysteine concentrations are 30-60% lower in pregnant women than in non-pregnant women & the lowest levels are observed in the second trimester. The study was a hospital based prospective study & carried out in the setting of department of Pathology & department of Obstetrics and Gynae, N.S.C.B Medical College over 100 Females. Homocysteine Tests are conducted at Path Care Labs. The cases with Pre-eclampsia have higher probabilities of having raised Hcy by 1.92% more compared with Non Pre-eclamptic cases and 0.21 at the worst being the Upper and Lower limits of 95% confidence intervals for Odds Ratio (OR=0.66). As per our study the Hcy levels were much raised in patients who were not taking Fe/Folate supplements (50.9%) as compared to those taking Fe/Folate supplement (4.3%) & this difference is highly significant indicating that Folate supplements help in keeping Hcy levels normal.

Keywords: Homocysteine, biochemical marker, obstetrical complications

Introduction

An association between elevated homocysteine levels and human disease was first suggested in 1962 by Carson and Neil, who found high homocysteine concentrations in the urine of some children with mental retardation. The elevated homocysteine levels in these patients were caused by severe enzyme defects blocking the homocysteine metabolism. This condition, homocystinuria, was later found to be associated with premature occlusive cardiovascular disease, even in childhood and about 25% of patients died as a result of cardiovascular events before the age of 30 [1].

In addition, hyperhomocysteinaemia (Hhyc) is associated with adverse pregnancy outcome, such as spontaneous early abortion, placental vasculopathy and birth defects. It is not only neural tube defects (NTDs) but also cardiac malformations and cleft lip and/or palate, which are associated with higher homocysteine levels than in controls.

Hhcy belongs among the congenital hypercoagulable states & is a long known vascular disease risk factor. The discovery that Hhcy may also be responsible for several pregnancy

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complications has only recently been made. Hyperhomocysteinemia has been suggested as a possible risk factor in women suffering from habitual abortions, eclampsia, pre-eclampsia, placental abruption, IUGR, NTD's, thromboembolic events etc.

During pregnancy there are hemostatic changes that result in a hypercoagulable state & can have thrombotic consequences. This condition can be aggravated in women who are carriers of congenital thrombophilic factors. This thrombotic tendency can manifest as thrombotic lesions in the placenta with compromise in utero-placental circulation, which are common characteristics present in obstetric complications, such as pre-eclampsia, IUGR, recurrent abortions, placental abruption [2].

Low plasma homocysteine level during an uncomplicated pregnancy was first demonstrated by Kang *et al*., almost 20 yrs ago, & this has subsequently been confirmed by numerous investigators. Plasma homocysteine concentrations are 30-60% lower in pregnant women than in non-pregnant women & the lowest levels are observed in the second trimester.

Normal human plasma contains a total concentration below 15 μmol/L of homocysteine-derived moieties, in either sulfhydryl or disulfide form. Of these, only about 2% occur as the sulfhydryl; the remaining 98% are disulfides-i.e., homocystine itself or mixed disulfides combined with either free or protein cysteine. The distinction between this total quantity, homocysteine, and homocysteine itself is almost certainly of more than trivial importance, because many of the pathophysiologic effects of homocysteine may depend on the presence of the sulfhydryl group of homocysteine [3, 4].

It is understandable that reduced methylation capacity may have profound effects on cellular growth, differentiation and function. This may be critical in many situations, not least in the ageing brain, where neurochemical processes related to methylation may be declining; in psychiatric and neurological diseases; for the rapidly growing foetus and infant; and also for carcinogenesis by reducing DNA repair.

Studies on children with severe inborn errors resulting in defective methyl group synthesis, support the theory that deficient methylation is one of the leading causes of demyelination. The synthesis of glutathione is dependent on the trans-sulfuration of homocysteine. Glutathione is an important endogenous antioxidant. It protects many cellular components against oxidative damage and other types of injury. Glutathione also maintains α-tocopherol in its reduced form, either by a direct reaction or by a pathway involving ascorbate. Glutathione may also have protective vascular effects, possibly by interaction with nitric oxide. Finally, certain forms of homocysteine itself are proposed to have oxidative effects and to react with proteins leading to protein damage [4].

Methodology

Place of study

The study was a hospital based prospective study & carried out in the setting of department of Pathology & department of Obstetrics and Gynae, N.S.C.B. Medical College over 100 females. Homocysteine Tests are conducted at Path care labs.

Inclusion criteria

- All pregnant females developing hypertension & Oedema.
- All pregnant females with previous history of miscarriages/recurrent abortions.
- All pregnant females having history of NTD in previous pregnancy.
- All pregnant females complaining of bleeding p/v, in second & third trimester.
- All postpartum females giving birth to Low Birth Weight babies.
- Pregnant females showing no fetal movements on examination.

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Exclusion criteria

- Pregnant females coming for routine ANC visits, no complications.
- Females with previously done MTP because of some ailment/or medically indicated.

For testing homocysteine levels

Collect an overnight fasting blood sample either plain or in EDTA/Lithium Heparinized, taking all aseptic precautions, by venipuncture. Separate serum or plasma to perform test. Haemolysed samples should not be used.

Equipment used: ADVIA-Centaur.

Method used: Chemiluminescence.

Materials required

HYC Calibrator, Ready pack primary reagent pack containing Advia Centaur HYC Lite reagent & Solid phase, Advia Centaur iPTH master curve card, HYC Diluent.

Principle

The Advia Centaur Homocysteine assay is a competitive immunoassay using direct chemiluminometric technology. The different forms of Homocysteine in the patient sample are reduced to free HCY by the Reducing Reagent. Free HCY is then converted to S-adenosylhomocysteine (SAH) by the enzyme reagent. Converted SAH from the patient sample competes with SAH covalently coupled to paramagnetic particles in the Solid Phase for a limited amount of acridinium ester-labelled anti-SAH in the Lite Reagent.

Results

Table 1: Clinically diagnosed complication v/s Serum Hcy cross tabulation

Clinical diagnosis	Serum Homocysteine		Total	Significance
	Normal	Abnormal		
Abruptio Placentae Abru	15 (88.2%)	2 (11.8%)	17 (100.0%)	p>0.05
Pre-eclampsia/eclampsia	23 (76.6%)	7 (23.3%)	30 (100.0%)	p>0.05
IUFD	13 (92.9%)	1 (7.1%)	14 (100.0%)	χ^2=3.78 p>0.05
IUGR	10 (66.7%)	5 (33.3%)	15 (100.0%)	p>0.05
NTD	0 (0.0%)	1 (100.0%)	1 (100.0%)	p>0.05
Recurrent Abortion	10 (43.5%)	13 (56.5%)	23 (100.0%)	*p*<0.001
Total	71 (71.0%)	29 (29.0%)	100 (100.0%)	-

Above table shows that out of 17 cases of Placental Abruption, 11.8% cases had elevated Hcy levels & rest 88.2% were normal. Out of 30 cases included in Pre-eclampsia/eclampsia, 23.3% had raised Hcy levels & 76.6% had normal levels. In 14 cases of IUFD, 7.1% had raised Hcy & 92.9% were normal. In 15 cases of IUGR, 33.3% had elevated Hcy levels & 66.7% were normal. Out of 23 cases of Recurrent Abortions, 56.5% had raised Hcy levels & 43.5% were normal. There was only 1 case of NTD & that had raised Hcy level, making 100%.

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Table 2: H/o of CVD v/s S. Hcy cross tabulation

H/o CVD	Serum Homocysteine		Total
	Normal	Abnormal	
Yes	1 100.0%)	0 (0.0%)	1 (100.0%)
No	70 (70.7%)	29 (29.3%)	99 (100.0%)
Total	71 (71.0%)	29 (29.0%)	100 (100.0%)

p>0.05

Above table shows that only 1 case had history of cardiovascular disease but had normal Hcy levels, whereas 29.3% with no history of cardiovascular disease had elevated Hcy levels & 70.7% had normal level.

Table 3: Presence of Seizures v/s S. Hcy cross tabulation

Seizures	Serum Homocysteine		Total
	Normal	Abnormal	
Yes	9 (81.8%)	2 (18.2%)	11 (100.0%)
No	62 (69.7%)	27 (30.3%)	89 (100.0%)
Total	71 (71.0%)	29 (29.0%)	100 (100.0%)

In above table, 18.2% of cases, having seizures, had raised Hcy levels, rest 81.8% had normal levels. Of cases having no seizures, 30.3% had raised Hcy levels & 69.7% had normal levels.

Table 4: Fe/Folate intake v/s S. Hcy cross tabulation

Fe/Folate intake	Serum Homocysteine		Total
	Normal	Abnormal	
Yes	45 (95.7%)	2 (4.3%)	47 (100.0%)
No	26 (49.1%)	27 (50.9%)	53 (100.0%)
Total	71 (71.0%)	29 (29.0%)	100 (100.0%)

χ^2= 26.37; p<0.0001

As per above table, out of cases taking Fe/Folate supplement, only 4.3% were having raised Hcy levels & 95.7% were having normal levels. On the contrary, out of cases not taking Folate supplement, 50.9% had elevated levels of Hcy & 49.1% were having normal levels.

Table 5: Presence of P/v bleeding v/s S. Hcy cross tabulation

P/v Bleeding	Serum Homocysteine		Total
	Normal	Abnormal	
Yes	24 (61.5%)	15 (38.5%)	39 (100.0%)
No	47 (77.0%)	14 (23.0%)	61 (100.0%)
Total	71 (71.0%)	29 (29.0%)	100 (100.0%)

As per the table, out of 39 cases who had vaginal bleeding, 38.5% had raised Hcy levels & rest 61.5% were normal. Out of 61 cases who had no vaginal bleeding, 23% had raised Hcy levels & rest 77% were normal.

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Table 6: Fundal ht. correspondence with gestational age v/s S. Hcy cross tabulation

Fundal ht. with gestational age		Serum Homocysteine		Total
		Normal	Abnormal	
Yes		41 (66.1%)	21 (33.9%)	62 (100.0%)
No	30 78.9%	8 21.1%		38 100.0%
Total	71 71.0%	29 29.0%		100 100.0%

As per the table, out of 62 cases whose fundal height was corresponding to the gestational age, 33.9% had raised Hcy levels & rest 66.1% were normal. Out of 38 cases whose fundal height & gestational age was not corresponding, 21.1% had raised Hcy levels & rest 78.9% were normal.

Table 7: Presence of Fetal cardiac activity v/s S. Hcy cross tabulation

Fetal cardiac activity	Serum Homocysteine		Total
	Normal	Abnormal	
Yes	58 (68.3%)	27 (31.7%)	85 (100.0%)
No	13 (86.7%)	2 (13.3%)	15 (100.0%)
Total	71 (71.0%)	29 (29.0%)	100 (100.0%)

Above table shows that out of 85 cases showing fetal cardiac activity, 27 (31.7%) had raised Hcy levels & rest 58 (68.3%) had normal levels. Out of 15 cases showing no fetal cardiac activity, only 2 (13.3%) had elevated Hcy levels & rest 13 (86.7%) were normal.

Table 8: Presence of Fetal movement's v/s S. Hcy cross tabulation

Fetal movements	Serum Homocysteine		Total
	Normal	Abnormal	
Yes	58 (68.3%)	27 (31.7%)	85 (100.0%)
No	13 (86.7%)	2 (13.3%)	15 (100.0%)
Total	71 (71.0%)	29 (29.0%)	100 (100.0%)

Above table shows that out of 85 cases showing fetal movements, 27 (31.7%) had raised Hcy levels & rest 58 (68.3%) had normal levels. Out of 15 cases showing no fetal movements, only 2 (13.3%) had elevated Hcy levels & rest 13 (86.7%) were normal.

Table 9: Pre-eclampsia v/s S. Hcy

Case	Serum Homocysteine		Total
	Normal	Abnormal	
Pre-eclamptic cases	23	7	30
Non pre-eclampic cases	48	22	70
Total	71	29	100

OR=0.66; 95% CI- exact Upper 95% Confidence Limit =1.92 and exact Lower 95% Confidence Limit =0.21.

This indicates that at the best, the cases with Pre-eclampsia have higher probabilities of having raised Hcy by 1.92% more compared with Non Pre-eclamptic cases and 0.21 at the worst being the Upper and Lower limits of 95% confidence intervals for Odds Ratio (OR=0.66).

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Table 10: Evaluation of Hcy test

Homocysteine	Cases	Control
Raised	29	0
Normal	71	10
Total	100	10

- Sensitivity among cases is 29 & among controls is 100.
- Specificity is 100, with positive predictive value of 100 & Negative predictive value of 12.3.

Discussion

In present study the majority (30%) of pregnant females were having Pre-eclampsia/ eclampsia as a complication. This shows that Pre-eclampsia is the most common complication occurring during pregnancy.Next bulk of cases were having Recurrent Abortions (23%) as complication [5].

However, association of Pre-eclampsia with high Hcy level could not be established in this study, but it has been found that the cases with Pre-eclampsia have higher probabilities of having raised Hcy by 1.92% more compared with Non Pre-eclamptic cases.

In our study majority of patients belong to lower socio-economic strata of society, as total of 70% were in the annual income group <20,000/- & 20,000-40,000/-. This indicates that these complications are more prevalent in lower socio-economic groups, however, it showed no significant association with elevated levels of Hcy.

In this study we have seen that still maximum (53%) pregnant females are not aware of taking Fe/Folate supplement. This again is responsible for the complications occurring because of Iron & Folic acid deficiency (NTD, etc) superimposed over physiological plasma volume expansion during pregnancy which itself causes deficiency of Fe/Folate as well as increased demand.

In this study patients belonging to age <20 yrs, none have raised Hcy level. Also 50% patients belonging to age group 40-49 yrs show raised Hcy level. This may indicate a need for more routine screening in the elderly population.

In this study serum Hcy was found to be raised in 56.6% cases of Recurrent Abortions with P value <0.001 which is highly significant. This shows that raised Hcy is definitely associated with Recurrent Abortions. Similar observations were reported by K.S.D. Kumar, V. Govindaiah, S.E. Naushad, R.R. Devi and A. Jyothy *et al*. in a study. Nelen, Willianne L.D. M. MD; Blom, Henk J. Ph.D. *et al*, in a case-control study also concluded similar findings.

In this study significant association was found between raised Hcy level & adverse pregnancy outcome. Patients having miscarriage as previous pregnancy outcome, 56% showed elevated Hcy levels which was highly significant with $p<0.0001$. Similarly patients with history of still birth, 66.7% showed elevated Hcy levels which was also highly significant with $p<0.0001$. On the contrary, patients having normal previous births, majority (92.1%) showing normal Hcy levels & this was also highly significant with $p<0.0001$.

As per our study the Hcy levels were much raised in patients who were not taking Fe/Folate supplements (50.9%) as compared to those taking Fe/Folate supplement (4.3%) & this difference is highly significant indicating that Folate supplements help in keeping Hcy levels normal.

In our study patients having per vaginal bleeding had no significant association with raised Hcy levels with p>0.05, as raised Hcy levels were seen in 38.5% cases having p/v bleeding & 23% cases having no bleeding.

Variation of fundal height according to gestational age also had no significant association with elevated Hcy with p>0.05, as abnormal Hcy levels were seen in 33.9% cases of fundal

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height corresponding to gestational age & in 21.1% cases of fundal height not corresponding to gestational age [6, 7].

Conclusion

- Adverse pregnancy outcomes like miscarriages & still births are significantly associated with raised Homocysteine during pregnancy. Also cases with previous live birth history, showed normal Homocysteine & this finding is also statistically significant.
- Testing Homocysteine can be used along with other tests to screen pregnant females against developing complications. Although as an individual test it has low sensitivity but when combined with other tests (clinical examination, blood tests, USG, uterine artery colour Doppler etc.) can very well predict the forthcoming complication.

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