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Gynaecology

A STUDY OF HOMOCYSTEINE LEVEL AS A RISK FACTOR FOR RECURRENT EARLY PREGNANCY LOSS

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ABSTRACT This study is a hospital based prospective study in a teaching hospital.

AIMS AND OBJECTIVES

1. To estimate the relative risk of recurrent early pregnancy loss for different total plasma homocysteine concentrations.
2. To study the incidence of recurrent pregnancy loss in the study population.
3. To estimate the relative risk of recurrent early pregnancy loss for different total plasma homocysteine concentrations and compare the levels with those of healthy controls.
4. To study coexisting anomalies,if any are present.

MATERIAL AND METHODS: 60 Patients attending genetic op and antenatal clinic with recurrent miscarriage (>2 pregnancy losses) were enrolled in the study as cases. The control group consisted of women who had delivered at least one live born infant and no spontaneous abortions and comparable for age, geographical area, and social class. 30 antenatal women from antenatal op taken as control.All pregnancies to be confirmed by a positive urinary hCG test or ultrasound imaging.Fasting Blood sample taken after obtaining consent from the patient.All patients started on routine folic acids.For those with elevated homocysteine B6 and B12 added.Pedigree analysis of the patients was done in detail and results analysed.

CONCLUSION: Our study has an incidence of recurrent pregnancy loss of 1% in our study population. The study of our 60 RPL cases showed 6 patients with hyperhomocysteinemia which has a 10% rate of occurrence. Our odds ratio with 3.22 indicates that hyperhomocysteinemia has an increased rate of occurrence among the people with RPL with correlates well with other studies. Therapeutic normalization of homocysteine in supplementation with folic acid B6,B12 in 4 out of 7 indicates the management options that can be tried. Our study indicates that there is no correlation of age with hyperhomocysteinemia.Primary aborters have a higher incidence of hyperhomocysteinemia when compared with secondary aborters. As suggested by our case report, therapeutic normalization of hyperhomocysteinemia might lead to metabolic restoration, which may favor a successful pregnancy outcome.

KEYWORDS : Recurrent Pregnancy Loss, Homocysteinemia

INTRODUCTION

Recurrent pregnancy loss is defined as the number of consecutive miscarriages which is more than 3 occurred within the 20th week of gestation and it is a very miserable condition for the patient.

Recurrent pregnancy loss (Recurrent Miscarriage) affects 0.5-1% of couples. The pathophysiology of Recurrent miscarriage is complex. The suggested causes include anatomical, genetic and molecular abnormalities, endocrine disorders, thrombophilias and antiphospholipid syndrome. In approximately 50% of the cases neither of the above can be identified. (Szekeres-Bartho J et al.,2008).

The pathogenesis of human spontaneous abortion involves a complex interaction of several genetic and environmental factors. The firm association between increased homocysteine concentration and neural tube defects (NTD) has led to the hypothesis that high concentrations of homocysteine might be embryotoxic and lead to decreased fetal viability.

There are several genetic polymorphisms that are associated with defects in folate- and vitamin B12-dependent homocysteine metabolism. (Henrik ZetterBerg et al.,2002) Homocysteine results from the transmethylation of methionine. Its metabolism depends primarily on three enzymes and several vitamin cofactors. Genetic abnormality in these enzymes or deficiency of these vitamins lead to hyperhomocysteinemia (HHCh). HHCh is usually biologically defined by a fasting value >15 micromol/l. HHCh belongs among the congenital hypercoagulable states and is a long-known vascular disease risk factor. The discovery that HHCh may also be responsible for several pregnancy complications has only recently been made. Studies in this area are still scarce and report on limited numbers of patients.

It nevertheless appears clear that HHCh is associated with the syndromes of repeated miscarriage, pre-eclampsia, placenta abruptio, thromboembolic events, neural tube defects, and perhaps with fetal death-in-utero and intra-uterine growth retardation.

The prevention of thromboembolic events during pregnancy by anticoagulant treatment is also desirable in these patients.(Aubard Y et al.,2000) Folates belong to the vitamin B group and are involved in a large number of biochemical processes, particularly in the metabolism of homocysteine. Dietary or genetically determined folate deficiency leads to mild hyperhomocysteinemia, which has been associated with various pathologies.

Any research in this field would prove immense help to patients with recurrent miscarriage as well help them in preventing future complications not only of pregnancy but also in preventing coronary artery disease, dementia osteoporosis, lack of concentration and underachievement for which they are more prone. It is shown by studies that women with recurrent miscarriage were more likely to have family history of cardiovascular disease .

MATERIALS AND METHODS

This study was conducted over a period of 20 months at department of obstetrics and gynaecology, Madurai Medical College, Madurai. Between 2017 and 2019, 75 women who suffered early pregnancy losses, attending our genetic clinic and antenatal OP were evaluated clinically and 60 women were enrolled for this study. Recurrent Pregnancy Loss was defined as three or more consecutive miscarriages within 16 weeks gestation with confirmation by biochemical pregnancy test [β-human chorionic gonadotrophin (HCG 100 IU/l] and/or sonography. Ectopic pregnancies or elective terminations of gestations were excluded.

They were categorized as primary and secondary aborters, based on whether they had at least one pregnancy beyond 16 weeks of gestational age. A control group of 30 women with normal menstrual history and an obstetric history of uncomplicated pregnancies alone was registered. All pregnancies confirmed by a positive urinary hCG test or ultrasound imaging. Detailed history with pedigree analysis done. Of the 60 women, 42 experienced 2 abortions, 14 experienced 3 abortions, 4 experienced more than 3 abortions.

The patients and controls did not take any vitamin B supplementation or oral contraceptives 6 months before performing homocysteine test. Fasting EDTA Blood sample was taken after obtaining informed consent from the patient at 0800 hours. Homocysteine levels were

measured after overnight fasting.Women were excluded if they had elevated serum creatinine or SGOT.None of the subjects of either the study or the control group had a known endocrine dysfunction or suffered from gastrointestinal, hepatobiliary, renal or vascular disease. Patients with neurological disorders such as epilepsy were also excluded. Before admittance,informed consent was obtained from all subjects.

In four women other investigations revealed abnormalities like thyroid dysfunction, gestational diabetes, bicornuate uterus etc. Total homocysteine concentration was measured by enzymatic photometric method, after centrifugation and storing. Patients were considered hyperhomocysteinemia if the measured levels exceeded 95 percentile level in healthy controls or lab control values. All patients were started on oral folic acid supplementation. For those with elevated homocysteine, vitamin B6 and B12 were added.

Fasting homocysteine measurement was repeated after 2 months of management. Data were analysed using conventional statistical tools and appropriate software (LaMorte statistical tool for MS excel) where needed. Results were given as Mean, Median, Standard Deviation (SD).Odds ratio,95% confidence interval was also calculated. Consequently statistical significance was determined using F-test, Pearson Chi square test, Fischer linear test. A p- value <0.05 was considered to indicate a statistically significant difference.

OBSERVATION AND RESULTS

The median total fasting homocysteine concentration in the study group was 8.59µmol/L and in the control group was 6.43 µmol/L.

TABLE 1: Distribution of homocysteine concentrations in study group

GROUP	NUMBER	MEAN	SD	RANGE	2 Tailed P - Value
Study group	60	8.59	4.22	3.1-22	0.1161
Primary aborters	42	8.79	4.51	3.1-22	0.052
Secondary aborters	18	8.13	3.53	3.8-20.2	0.015
Control group	30	6.43	3.06	2.5-19.32	

(There was no significant difference between the mean values of homocysteine concentration between the two groups).

No correlation was found between age and homocysteine levels between the two groups.In the study group the effect of parity on homocysteine concentration is statistically significant.

Fig 1: Age distribution between cases

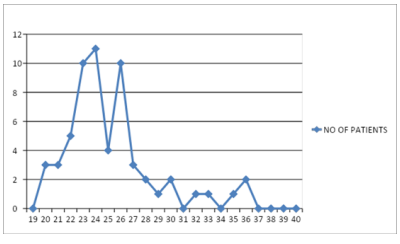

TABLE 2 : HYPERHOMOCYSTEINEMIA IN CASES AND CONTROL A COMPARISION

GROUP	PERCENTAGE WITH HYPERHOMOCYSTEINEMIA
CASES	10%
CONTROL	3.33%

A level of homocysteine greater than 15 µmol/L was considered positive. Six patients in the study group had elevated homocy steine,while only one patient in the control group had elevated homocysteine.Subgroup analysis showed, 5 were positive in the primary aborters and only one case was positive in the secondary aborters group. On comparison of odds ratio and 95% CI between primary and secondary aborters, hyperhomocysteinemia is more likely to occur in the primary aborters group.(TABLE 3)This correlated well with other studies.Further analysis of the positive patients in the form of serum folate,methionine loading ,genetic screening were done in selected cases. All seven patients were treated with vitamins B6 and B12 apart from routine folic acid supplementation. Fasting homocysteine measurement was repeated after 2 months of management.

TABLE 3 : COMPARISON BETWEEN PRIMARY AND SECON DARY ABORTERS

PARAMETER	PRIMARY ABORTERS	SECONDARY ABORTERS	CONTROL
MEDIAN	7.9	7.55	5.65
SD	4.51	3.53	3.06
ODDS RATIO	3.91	1.76	3.22
95%CI	0.434 to 35.417	0.1 to 29.073	0.37 -28.07

(Primary aborters are more likely to have HHCh than Secondary aborters on comparison of Odds ratio and 95%CI)

TABLE 4 : COMPARISON OF HOMOCYSTEINE LEVELS IN VARIOUS STUDIES

	Our Study	Nelen Et Al	Maristell D'va Et Al	Steegers Et Al
Mediancontrol	5.65	12.6	7.85	6.7
Sdcontrol	3.06	-	3.31	2.4
Median Cases	7.69	13.1	19.2	6.9
Sd Cases	4.22	-	6.14	2.3

The odds ratio is the ratio of the odds of an event occurring in one group to the odds of it occurring in another group. These groups might be men and women, an experimental group and a control group, or any other dichotomous classification. An odds ratio greater than 1 indicates that the condition or event is more likely to occur in the first group(usually the control group). And an odds ratio less than 1 indicates that the condition or event is less likely to occur in the first group. Our study has an odds ratio of 3.22.(TABLE 5).Nelen et al and Quere et al derived an odds ratio of 3.6 and 2.6 respectively. Steegers et al derived an odds ratio of 5.6.Steegers et al included both fasting and methionine loaded homocysteine levels in their study, resulting in a higher odds ratio in contrast with our study and those of Nelen et al and Quere et al.

TABLE 5 : COMPARISON OF ODDS RATIO BETWEEN VAR IOUS STUDIES

Parameter	Our Study	Nelen Et Al	Quere Et Al	Steegers Etal
Odds Ratio	3.22	3.6	2.6	5.6
CI	0.37 -28.07	1.3-10.0	0.9-7.7	0.5-57.9

Four among them showed reduction in homocysteine values. Two patients progressed to term pregnancy. One patient went in for preeclampsia and IUD. One went for missed abortion.(TABLE 6) Two patients are still under follow up. Among the control group, only one patient had hyper homocysteinemia. She went for a miscarriage at 14 weeks of gestation.

TABLE 6 : PREGNANCY OUTCOME AMONG CASES

TERM	IUD	MISSED ABORTION	FOLLOW UP
2	1	1	2

Fig 2 : Pregnancy among cases

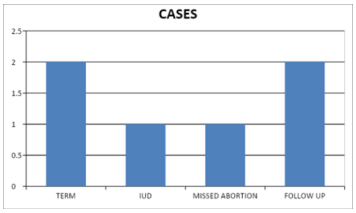

Table 7 : ABORTION AMONG CASES AND CONTROL

CASES	CONTROL
15	4

Among the four controls who went in for abortions,only one had hyperhomocysteinemia.the abortions in the other three controls may be attributed to chromosomal abnormalities,embryonic anamolies or increased maternal age.

DISCUSSION

Multiple mechanisms are said to play a role in the aetiology of

spontaneous and recurrent abortions. Not only immunological and genetic disorders but also endocrine and psychological factors as well as infections or endometriosis may be responsible for embryo loss (Bulletti *et al.*, 1996).Several studies reveal the pathogenetic role for inherited thrombophilia in RPL .These studies delineate the pathogenetic role of various factors like protein C, protein S and antithrombin and the role of inherited gene polymorphism of factor V Leiden and A20210G of prothrombin, and also for acquired thrombophilia, in particular antiphospholipid syndrome. Of these factors moderate hyperhomocysteinemia has also been found to be a risk factor for recurrent early pregnancy loss (RPL

A recent meta-analysis confirmed an increased risk of hyperhomo cysteinemia for RPL, defined as two or more spontaneous abortions before 16 weeks of menstrual age (Nelen et al., 2000). First, there is no homogeneous definition of RPL (at least two or three consecutive spontaneous abortions), as well as of hyperhomocysteinemia (fasting or afterload concentrations).Second, the possible impact of the fetal MTHFR genotype on the risk of RPL has not been investigated in most of the studies. Recently, Zetterberg et al. (2002) emphasized the importance of this parameter, as they observed an OR of 14.2(95% confidence interval: 1.78–113) in spontaneously aborted embryos presenting with one or more MTHFR 677T and 1298C alleles when compared with the wild-type (677CC and 1298AA) genotype.

The association between RPL and vitamin B12 has been illustrated by two case reports concerning a 38 year-old woman with four episodes of early spontaneous abortion vitamin B12 deficiency and bone marrow megaloblastosis (Candito et al., 2003), and a 36year-old patient with documented familial and personal history of Addison–Biermer disease, who had experienced 12 episodes of spontaneous abortion in the absence of any other known causes of RPL (Gueant et al., 2004).

Despite several pathophysiological hypotheses including impaired cell proliferation, increased oxidative stress, apoptosis,reduced extra-embryonic Vascular development and hypomethylation(Zetterberg, 2004; Latacha and Rosenquist, 2005), it is not clear whether hyperhomocysteinemia is causally related to RPL or whether it is only a marker of the increased risk of RPL. Actually, lowering homocysteine concentration by B-vitamin supplementation ,that has been shown to have a positive effect in several case reports and in our series also, with spontaneous pregnancies occurring after a few months of treatment in patients who had previously experienced between 4 and 12 early spontaneous abortions (Quere et al., 1998, 2001; Candito et al.,2003; Gueant et al., 2004).

There is evidence in the literature that some miscarriages result from elevated levels of homocysteine factors present in the sera. Increasing evidences are available for the relationship between hyperhomo cysteinemia and MTHFR C677T gene polymorphism and unexplained recurrent pregnancy loss. Several reports, in fact, described an association between early RPL and HHCY and/or MTHFR C677T gene polymorphism [2,3,26,27]. A different point of view on the association between hyperhomocysteinemia and RPL has been reported only by Makino et al. [28].

In the present study we evaluated homocysteinemia in women showing RPL. In our study out of 60 cases with RPL 7 cases had hyperhomocysteinemia which is not statistically significant.

Out of the seven positives,6 were primary aborters and 1 was secondary aborter. The one in the control group with hyperhom ocysteinemia also went in for a miscarriage. The number of primary aborters and secondary aborters with hyperhomocysteinemia ,and the odds ratio for the subgroups are compared with other studies in the following tables.

With regards to subgroup analysis, our study showing that disturbed homocysteine metabolism may have a greater effect in primary aborters than in secondary aborters, suggests that, in primary aborters the disturbances are permanent. That could be because of an intrinsic metabolic disorder, rather than environment. This contrasted with previous reports that showed increased incidence of hyperhomoc ysteinemia in parous women compared with women who had primary recurrent early pregnancy loss. Since our study was limited to fasting homocysteine levels measurement alone, further detailed investig ations of both the groups may enlighten the reasons for this discrepancy.

A possible cause of hyperhomocysteinemia in these 7 patients will be diminished remethylation induced by decreased concentration of active folate,B12,or enzymes involved in remethylation.On analyzing after folate and B vitamin complex supplementation ,four showed reduced values. This results correlate with other studies as discussed below. Meta-analysis of individual data on 1114 people in 12 randomized controlled trials assessed the effects of folic acid-based supplements on blood homocysteine concentrations. Multivariate regression analysis was used to determine the effects on homocysteine concentration of different doses of folic acid and of the addition of vitamin B12 or B6. The results showed that the proportional and absolute reductions in blood homocysteine produced by folic acid supplements were greater at higher pre-treatment blood homocysteine concentrations ($p<0.001$) and at lower pre-treatment blood folate concentrations ($p<0.001$). After standardization to pre-treatment blood concentrations of homocysteine of 12 micromol/L and of folate of 12 nmol/L (approximate average concentrations for Western populations), dietary folic acid reduced blood homocysteine concentrations by 25 percent (95% confidence interval 23%-28%; $p<0.001$), with similar effects in the range of 0.5-5 mg folic acid daily. Vitamin B12 (mean 0.5 mg daily) produced an additional 7 percent (3%-10%) reduction in blood homocysteine. Vitamin B6 (mean 16.5 mg daily) did not have a significant additional effect. In conclusion, typically in Western populations, daily supplementation with both 0.5-5 mg folic acid and about 0.5 mg vitamin B12 would be expected to reduce blood homocysteine concentrations by about a quarter to a third (for example, from about 12 micromol/L to 8-9 micromol/L.There are studies regarding the beneficial effect of folic acid supplementation in women with preeclampsia/eclampsia and hyperhomocysteinemia in lowering homocysteine levels. The prospective cohort study by Linda Dodds et al has confirmed strong association between increased homocysteine levels in early pregnancy and pregnancy loss and the development of preeclampsia, But it could not establish whether supplemental folic acid will reduce the risk of pregnancy loss or preeclampsia, as a consequence of a reduction in total homocysteine levels. Large intervention trials as well as prospective studies measuring homocysteine and folate status before and during pregnancy are needed to establish the role of these and related factors as predictors or etiologic factors of adverse pregnancy outcomes.
Regardless of whether you have an *MTHFR* mutation in both genes or not, the treatment for elevated homocysteine is the same—dietary intervention and supplementation with folic acid and vitamins B_6 and B_{12}. The amount of each of these supplements should be adjusted on the basis of the degree of homocysteine elevation, not your genetic status. If you have mutations in both *MTHFR* genes but have normal homocysteine levels, you do not need to be on folic acid or vitamin B_6or B_{12} therapy.

Although folate deficiency is one of the factors that may lead to alterations in DNA synthesis and chromosome structure in rapidly dividing cells (e.g. heritable folate-sensitive fragile site in human autosomal chromosome 1p21.3; Baker and Sutherland, 1991),and the serum concentration is a sensitive indicator of the folate available for replicating cells (Neiger *et al.*, 1993), Abir *et al.* found the mean serum concentration of folic acid to be similar in so called 'high risk sera' from women with at least two abortions and in control sera from women after a normal pregnancy or during a normal second trimester of gestation.Several studies on the relationship of the vitamin B complex, particularly of folate, to spontaneous abortion have been published, but available data with regard to recurrent miscarriage are rare.

The question of whether to add anticoagulant treatment to vitamin supplementation is a reasonable one. Prescribing anticoagulant therapy is recommended in patients with congenital hypercoagulable state when this can be identified.

This prophylaxis consists of treatment by heparin during pregnancy and delivery, and during the post-partum However, in contrast to the congenital hypercoagulable states, in hyperhomocysteinemia we have treatment options capable of normalizing homocysteine levels available to us, in the great majority of cases.

One may thus ponder whether it is useful to give anticoagulant treatment throughout pregnancy in a patient whose homocysteine levels have been normalized with vitamin supplementation. It will not be possible to answer this question until it is known whether vitamin supplementation reduces the thrombotic risk in hyperhomo cysteinemia patients to normal. Such information could only be obtai

ned by a multicenter trial.

Many unknowns remain regarding the impact of hyperhomo cysteinemia on pregnancy and the optimal manner in which to manage this condition during pregnancy. Large-scale case–control studies are needed to clearly define the disease states linked to hyperhomoc ysteinemia. Therapeutic trials are also necessary to study the impact of vitamin supplementation and the best manner to administer it.

CONCLUSION

Our study has an incidence of recurrent pregnancy loss of 1% in our institute. The study of our 60 RPL cases showed 6 patients with hyperhomocysteinemia which has a 10% rate of occurrence. Our odds ratio with 3.22 indicates that hyperhomocysteinemia has an increased rate of occurrence among the people with RPL with correlates well with other studies. Therapeutic normalization of homocysteine in supplementation with folic acid B6,B12 in 4 out of 7 indicates the management options that can be tried. Our study indicates that there is no correlation of age with hyperhomocysteinemia. Primary aborters have a higher incidence of hyperhomocysteinemia when compared with secondary aborters.

Recent studies on recurrent early pregnancy loss and hyperhomoc ysteinemia suggested a positive association between the two.In the present study elevated fasting homocysteine concentrations were associated with higher odds ratio in women with 2 or more early pregnancy losses.The level showed no significant difference in ages. In primary aborters disturbed homocysteine metabolism seemed to have a greater effect than secondary aborters in our study indicating that intrinsic metabolic disorder rather than environment as a cause of homocysteinemia.

In animal studies folic acid supplementation seemed to improve survival of fetuses during early gestations and increases the number of living fetuses. In our study also folic acid supplementation along with B12 and B6 has an effect on lowering the homocysteine but the still more studies are needed to conclude the effect of folic acid B6,B12 on pregnancy outcome. Still more intervention trials as well as prospective studies measuring folate and tHcy status before and during pregnancy are needed to establish the role of folic acid B6 and B12 either as predictors or etiologic factors for recurrent pregnancy losses.

We therefore believe that women with hyperhomocysteinemia should be identified earlier. The folic acid-vitamin B6,B12 combination, a nonteratogenic treatment, should be tried. As suggested by our case report, therapeutic normalization of hyperhomocysteinemia might lead to metabolic restoration, which may favor a successful pregnancy outcome.

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