A STUDY OF HYPERHOMOCYSTEINEMIA IN RECURRENT PREGNANCY LOSS

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Title of the Work : A Study on Hyperhomocysteinemia in Recurrent Pregnancy Loss

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The request for an approval from the Institutional Ethical Committee (IEC) was considered on the IEC meeting held on 19.11.2010 at the Modernized Seminar Hall, Stanley Medical College, Chennai-1 at 2PM

The members of the Committee, the secretary and the Chairman are pleased to approve the proposed work mentioned above, submitted by the principal investigator.

The Principal investigator and their team are directed to adhere to the guidelines given below:

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5. You should complete the work within the specified period and if any extension of time is required, you should apply for permission again and do the work.
6. You should submit the summary of the work to the ethical committee on completion of the work.

28/11/11

MEMBER SECRETARY,
IEC, SMC, CHENNAI

BONAFIDE CERTIFICATE

Certified that this dissertation is the bonafide work of **Dr.D.NAGASUTHA** on **A STUDY OF HYPERHOMO-CYSTEINEMIA IN RECURRENT PREGNANCY LOSS** during her M.D., (Obstetrics & Gynaecology) course from April 2009 to April 2012 at Govt. Raja Sir Ramasamy Mudaliar Lying-in Hospital, attached to Stanley Medical College, Chennai.

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Last but not the least, I express my thanks to all my patients who endured with me in a patient manner. I pray the Almighty to bless them with healthy babies.

CONTENTS

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 µmol/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 (GCS Smith, AM Wood, JP Pell,J Hattie)

REVIEW OF LITERATURE

DEFINITION OF RECURRENT MISCARRIAGE

Recurrent miscarriage (RM), which is also referred to as repeated pregnancy loss (RPL) and habitual abortion, is defined as three or more consecutive spontaneous miscarriages (ESHRE, 2006). The experience of repeated pregnancy loss is physically and emotionally traumatic to women who are trying to have children.

INCIDENCE

The overall frequency of RM was estimated from 1% to 3% (ESHRE, 2006, Christiansen et al., 2008). The exact prevalence of RM depends on its definition. To date there is no consensus on the definition of RM with regard to the numbers of previous miscarriages and the gestational age of RM. The American Society for Reproductive Medicine defines the numbers of previous miscarriages in RM as two or more whereas Europe Society of Reproduction and Embryology defines it as three or more (ASRM, 2008, ESHRE, 2006). In some studies only pregnancy losses in the first trimester (≤14weeks) were included whereas in other studies pregnancy losses in the second trimester (≤24weeks) were also investigated (Franssen et

al., 2007, Raffaelli et al., 2009, Tempfer et al., 2006, Garrisi et al., 2009, Jaslow et al., 2009). In some studies the gestational age of repeated miscarriages was not clarified (Zolghadri et al., 2008). Therefore, it is possible that different study populations were examined in current studies.

Proposed Etiologies for Recurrent Spontaneous Pregnancy Loss

CAUSES	INCIDENCE
Genetic factors	3.5 – 5%
Anatomic factors	12-16%
Endocrine factors	17-20%
Infectious factors	0.5-5%
Immunologic factors	20-50%
Thrombotic factors	**?**
Others	10%

GENETIC FACTORS

Chromosomal Abnormalities

Balanced translocation

Inversions and insertions

Chromosomal mosaicism

Single gene defects

Delta f 508 mutation

Cystic fibrosis

Heritable thrombophilias

X-linked disorders (result in recurrent abortion of male but not female offspring)

ANATOMIC FACTORS

Congenital

- Incomplete mullerian fusion or septum resorption
- DES exposure (T shaped uterus)
- Uterine artery anomalies
- Cervical incompetence

Acquired

- Cervical incompetence
- Synechiae
- Leiomyomas
- Adenomyosis

ENDOCRINE FACTORS

- Luteal phase insufficiency
- PCOS (including insulin resistance and Hyperandrogenism)
- Other androgen disorders
- Diabetes mellitus
- Thyroid disorders
- Prolactin disorders

INFECTIOUS FACTORS

- Bacterial vaginosis
- Chlamydial infection
- TORCH infections
- Mycoplasma & ureaplasma
- β - hemolytic streptococcus

IMMUNOLOGIC FACTORS

Cellular Mechanisms

- Suppressor cell or factor deficiency
- Alterations in major histo compatibility antigen expression.
- Alterations in cellular immune regulation.

Humoral Mechanisms

- Antiphospholipid antibodies
- Antithyroid antibodies
- Antisperm antibodies
- Antitoproblast antibodies
- Blocking antibody deficiency

THROMBOTIC FACTORS

Heritable Thrombophilias

- Single gene defects
- Protein C or S deficiency
- Prothrombin G20210A mutation
- Hyper homocysteinemia
- Factor V leiden mutation

- Antibody – mediated thrombosis
- (APAS, anti β2G1)

No. of Losses	Risk of recurrence
1	15%
2	24%
3	43%
4	54%

RECOMMENDATIONS FOR THE TESTING OF COUPLE PRESENTING WITH RECURRENT MISCARRIAGE (ESHRE)

- Basic investigations
- Obstetric and family history, age, BMI, organic solvents, alcohol, mercury, lead, caffeine, hyperthermia, smoking
- Full blood count (blood sugar level and thyroid function tests)
- Antiphospholipid antibodies (LAC and aLC)
- Parental karyotype (after 2 miscarriages)
- Pelvic ultrasound (SIS) and/or hysterosalpingogram and hysteroscopy
- Laparoscopy in case of inconclusive findings

RESEARCH INVESTIGATIONS WITHIN THE CONTEXT OF A TRIAL (ESHRE)

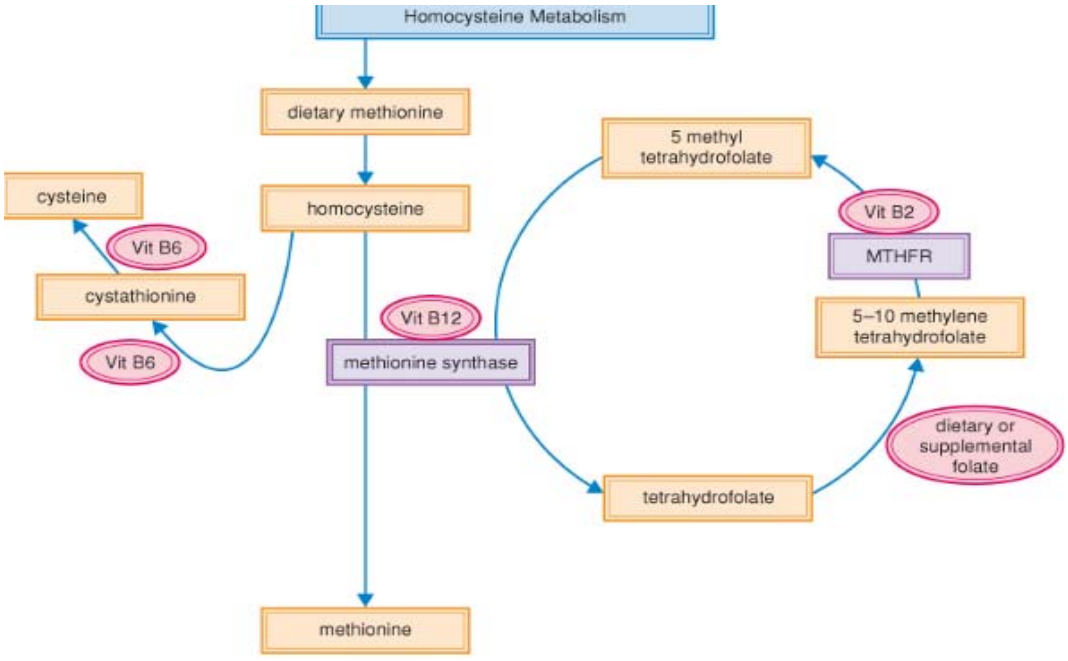
- Feto-placental karyotypes
- Testing of uterine and/or peripheral blood NK cells
- Mannan-binding lectin (MBL) level
- Luteal phase endometrial biopsy
- Homocysteine/folic acid level
- Thrombophilia screening

HOMOCYSTEINE CYCLE

Homocysteine is a sulfur-containing amino acid, rapidly oxidized in plasma to the disulfides homocystine and cysteine Circulating homocysteine is derived from dietary methionine. Homocysteine, in turn, is metabolized either into cystathione or back into methionine. The latter process involves the enzyme methionine synthase. Methionine synthase requires donation of a methyl group from 5 methyl tetrahydrofolate to produce methionine, and the enzyme methylene tetrahydrofolate reductase (MTHFR) is involved in the production of 5 methyl tetrahydrofolate from dietary folate sources The MTHFR gene is located on chromosome 1 (1p36.3), and two

common alleles, the *C677T* (thermolabile) allele and the *A1298C* allele, have been described.

The nutritional supplements folic acid, vitamins B_2, B_6, and B_{12} are all required for proper metabolism of homocysteine. Therefore, their deficiency is associated with acquired elevations in circulating homocysteine levels. Although heritable deficiencies in the enzymes required for metabolism of homocysteine have been described for the pathways leading to cystathione formation and those involved in reconversion to methionine, point mutations in MTHFR are surprisingly common, and can be associated with hyperhomocysteinemia and thrombosis.



FACTORS LOWERING HOMOCYSTEINE

- Downs syndrome
- Ethanol consumption
- Pregnancy
- Contraceptives estrogen therapy

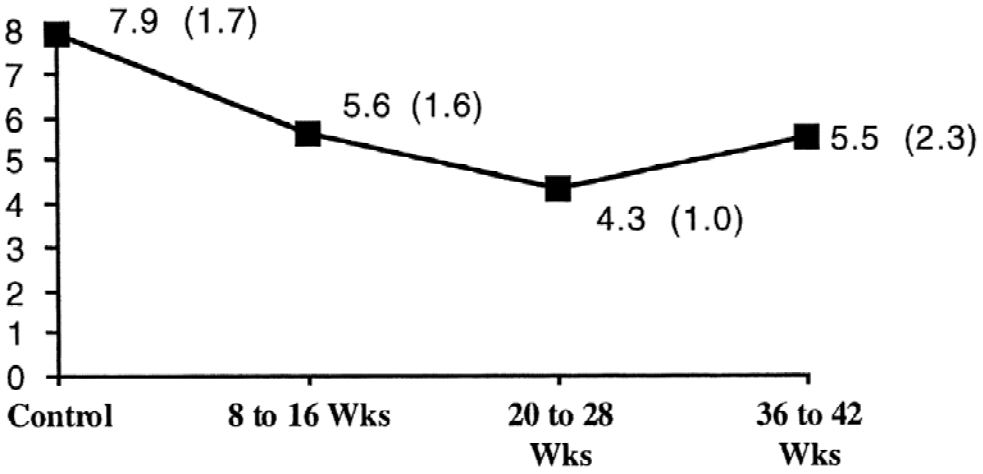
CAUSES OF ELEVATED HOMOCYSTEINE

- Deficiency of folic acid or vitamins B6/B12
- Kidney disease
- Low levels of thyroid hormones (hypothyroidism)
- Methylenetetrahydrofolate reductase (MTHFR) genetic mutations
- Psoriasis
- Systemic lupus erythematosus
- Drugs (folate antagonist, B12, B6 antagonist, niacin Ldopa, anticonvulsant)
- Cystathione synthase gene mutation
- Smoking, coffee consumption
- Unknown

HOMOCYSTEINE IN NORMAL PREGNANCY

The normal concentration of fasting homocysteine is between 5 and 15µmol/L. The mean plasma concentration in normal pregnant women is 5.4 ± 1.4 which is significantly lower than non pregnant (8.7 ± 1.7) (Bonnette et al).

	Non pregnant	1 trimester	2 trimester	3 trimester
Homocysteine (µmol/L)	4.4-10.8	3.34-11	2-26.9	3.2-21.4



HYPERHOMOCYSTENEMIA

	Values
Moderate	15-30 μmol/L
Intermediate	30-100 μmol/L
Severe	> 100 μmol/L

This table shows the values of homocysteine according to the varying degree of severity.

PATHOPHYSIOLOGY

Molecular mechanisms of homocysteine-induced cellular dysfunction include increased inflammatory cytokine expression, altered nitric oxide bioavailability, induction of oxidative stress, activation of apoptosis and defective methylation.

1. Increase endothelial cell tissue plasminogen activator binding
2. Decrease endothelial antithrombotic activity due to changes in thrombomodulin function
3. Activation of factor 7 a and 5
4. Increase in fibrinopeptide and prothrombin fragments 1&2
5. Inhibition of protein C and heparin sulfate
6. Increase in blood viscosity

HOMOCYSTEINE AND PREGNANCY COMPLICATIONS

RECURRENT MISCARRIAGE

Wouters et al. were the first to report the association between HHCh and repeated miscarriage. They noted that 14% of patients presenting with primary repeated miscarriage had HHCh, while 33% of patients with secondary repeated miscarriage (after having had normal pregnancies) had HHCh. The principal hypothesis set forth in these studies was that of premature damage to the decidual or chorionic vessels, disrupting the implantation of the conceptus.

Quere et al. reported that, in a retrospective review of 100 patients presenting with a clinical picture of repeated miscarriage, 12% had HHCh, 20% were homozygotes for the thermolabile mutation of MTHFR, and 15% had low levels of folic acid.

PREECLAMPSIA

The oxidative stress by which Homocysteine produces endovascular cell damage have also been imputed in the pathophysiology of pre-eclampsia. First, Dekker in 1995 reported that 17.7% of women with severe pre-eclampsia had early onset had HHCh compared with 2% prevalence in the general population.

Powers et al. [52] observed in their study that HHCh is correlated with maternal blood concentration of cellular fibronectin, both in preeclamptic patients and controls.

INTRAUTRINE GROWTH RETARDATION

Burke et al. were the first to study the association between HHCh and IUGR. Leeda et al reported that 19.2% of patients with an antecedent IUGR had HHCH by a methionine challenge test performed in the post-partum period.

ABRUPTIO PLACENTAE

Goddjin-Wessel et al. [54] has reported that 31% of Patients developing an Abruptio Placentae have HHCh, compared with 9% in the control group. De Vries et al. observed that 26% of the patients who had an Abruptio Placentae had HHCh without any associated causes. Pre-eclampsia were found to have HHCh 3 months after pregnancy .

FETAL DEATH

Burke et al did however observe a 12% miscarriage rate and 10% perinatal death rate. Czeizel did not, however, observe a decrease

in the frequency of fetal death after supplementation with folic acid and multivitamins in a general population.Yet this result does not permit the extrapolation that vitamin supplementation will not reduce HHCh-associated risk.

NEURAL TUBE DEFECTS

The association between neural tube defects (NTDs) and HHCh has been reported by several authors . NTDs are not due to a direct action of HC, but indirectly to a functional abnormality of methionine synthetase. Folic acid deficiency and congenital abnormalities of MTHFR can both be responsible for NTDs and for HHCh. The association between NTDs and HHCh is thus coincidental, but these two pathologies often result from the same cause.

VENOUS THROMBOSIS

HHCh belongs among the congenital hypercoagulable states, so-called thrombophilias, with the same order of magnitude as antithrombin III, protein C, and protein S deficiencies, resistance to activated protein C (via mutation of the Leiden gene of factor V), and mutations of factor II.

Den Heijer et al. demonstrated that, in a population of 269 patients who presented with at least one venous thrombosis before the age of 70, 10% had HHCh.

In all predisposing conditions for thrombosis, it is recommended to begin prophylactic anticoagulation treatment during pregnancy and the post-partum period. Should we propose a thromboprophylaxis to pregnant patients with HHCh? The question remains without answer today.

	Early Pregnancy Loss	**Still Birth**	**Preeclampsia**	**Placental Abruptions**	**Fetal Growth Restriction**
HOMOCY-STEINE	6.3(1.4-28.4)	1(0.2-5.6)	3.5(1.2-10.1)	2.4(0.4-15.9)	N/A

(ODDS RATIO FOR HYPERHOMOCYSTEINEMIA IN PREGNANCY COMPLICATIONS)

METHODS TO MEASURE HOMOCYSTEINE

Blood samples for homocysteine assay should preferably be collected into cooled tubes if delays greater than an hour are expected prior to separation of plasma, which should be performed as soon as possible after venesection.

Methionine loading tests are not necessary to define hyperhomocysteinaemia. Pregnancy ranges for plasma homocysteine should be established for each laboratory. Homocysteine is measured by enzymatic photometric method. It can also be measured by high-pressure liquid chromatography.

MANAGEMENT

Administration of supplemental folic acid in doses between 0.2 and 15 mg/d can lower plasma homocysteine levels without apparent toxicity. On the basis of meta-analysis of 12 clinical studies, all but 1 of which was a placebo-controlled trial, it has been estimated that a 25% reduction in homocysteine concentration can be achieved with mean supplementation of 0.5 to 5.7 mg of folic acid per day; an

additional 7% lowering has been observed after the addition of vitamin B_{12} (0.02 to 1 mg/d; mean, 0.5 mg).

A recent report of the Food and Nutrition Board of the Institute of Medicine has recommended an upper limit of 1 mg/d folic acid on the basis of the possibility that higher doses may mask signs of vitamin B_{12} deficiency in some subjects. In overt cobalamin deficiency with intermediate and severe hyperhomocysteinemia, vitamin B_{12} can normalize homocysteine concentration in 70% of cases. In an open-label, uncontrolled study, vitamin B_6 at ≤250 mg/d was without effect in reducing basal homocysteine levels, but doses of 50 to 250 mg/d reduced homocysteine levels after a methionine-loading test by 25%.

Subsequently, a study that used a randomized, placebo-controlled, 2x2 factorial design demonstrated that 50 mg of vitamin B_6 per day independently reduced the post–methionine-loading increase in homocysteine levels by 22%. In a placebo-controlled study a combination of multiple agents including folic acid (0.65 mg/d), vitamin B_6 (10 mg/d), and vitamin B_{12} (0.4 mg/d) was very effective in reducing homocysteine levels in patients with moderate or intermediate hyperhomocysteinemia.

It has been reported, however, that increased vitamin intake from food sources (1 mg of folic acid, 12.2 mg of pyridoxine, and 50 μg of cyanocobalamin per day) failed to maintain normal homocyst(e)ine levels attained previously by vitamin supplementation.

Other vitamins may also influence plasma homocyst(e)ine levels. Daily food intake of 0.6 mg of riboflavin, a vitamin that can function as a cofactor for MTHFR results in modest reductions in homocysteine (0.475 μmol/L), and pharmacological doses of nicotinic acid (3000 mg/d) may cause significant elevations.

Users of multivitamin supplements in observational studies have lower homocysteine levels than nonusers, as well as higher concentrations of plasma folic acid and vitamins B_6 and $B_{12,}$ The daily intake of fortified cereals containing 499 and 650 μg of folic acid per serving and the recommended dietary amount (RDA) of other vitamins reduced homocysteine by 11% and 14%, respectively.

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.But some suggest homozygous patients with homocysteine value more than 15 mol/L expect normalization within 2 weeks of treatment with 1-4mg/day folic acid, 10-25 mg /day of vitamin B6 400-1000 microgram/day of vitamin B12.Hetrozygous will need more than 400microgram of folic acid which will be there in most of preconceptional folic acid tablets.

GENERAL MANAGEMENT

Established treatment

Tender loving care (TLC)

Health advices (diet, coffee, smoking and alcohol) (50% improvement)

TREATMENT REQUIRING MORE RCTS

- Aspirin and/or LMW heparins for women presenting with APS or (multiple) inherited thrombophilias
- Progesterone in women presenting with unexplained early and late RM

- IVIG in women presenting with unexplained secondary RM or late RM
- Folic acid in women presenting with hyperhomocysteinaemia
- Immunization with third-party donor leukocyte

TREATMENT OF NO PROVEN BENEFIT

- Immunization with paternal leukocytes or trophoblast membranes
- Multivitamins supplementation

TREATMENT ASSOCIATED WITH MORE HARM THAN BENEFIT

- Daily corticosteroids during the first half of pregnancy

AIMS OF THE STUDY

1. To analyse the incidence of recurrent pregnancy loss in the study population.

2. To estimate the relative risk of recurrent early pregnancy loss for elevated total plasma homocysteine concentrations and compare the levels with those of healthy controls.

3. To assess response after supplement with folicacid B6 and B12 for those with elevated homocysteine and sample repeated after 2 months.

STUDY DETAIL

This study was conducted over a period of 30 months at department of Obstetrics and Gynaecology, Govt.R.S.R.M. Lying in Hospital attached to STANLEY MEDICAL COLLEGE, Royapuram, Chennai.

STUDY PERIOD

The period of study was from OCTOBER 2009 – OCTOBER 2011

STUDY DESIGN

Prospective case-control study

Patient selection criteria

INCLUSION CRITERIA

Recurrent pregnancy loss with at least two consecutive spontaneous early pregnancy losses within 16 weeks of menstrual age.

EXCLUSION CRITERIA

- Serum creatinine greater than 90 μmol/L ,
- Serum alanine aminotransferase greater than 30 IU/L ,
- Medication that might interfere with homocysteine metabolism (like folate antagonist, B12, B6 antagonist, niacin Ldopa, anticonvulsant)
- Ectopic pregnancy
- Molar pregnancy

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.

- Detailed history with pedigree analysis done
- 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 done detail

MATERIAL AND METHODS

Between 2009 and 2011, 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 atleast 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 hyperhomocysteinemic if the measured levels exceeded 95 percentile level in healthy controls or lab control values. All patients were started on oral folicacid 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 are 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.

RESULTS AND ANALYSIS

The median total fasting homocysteine concentration in the study group was 8.59µmol/L and in the control group was 6.43 µmol/L. The distribution of homocysteine concentrations in the study group is given in Table 1.

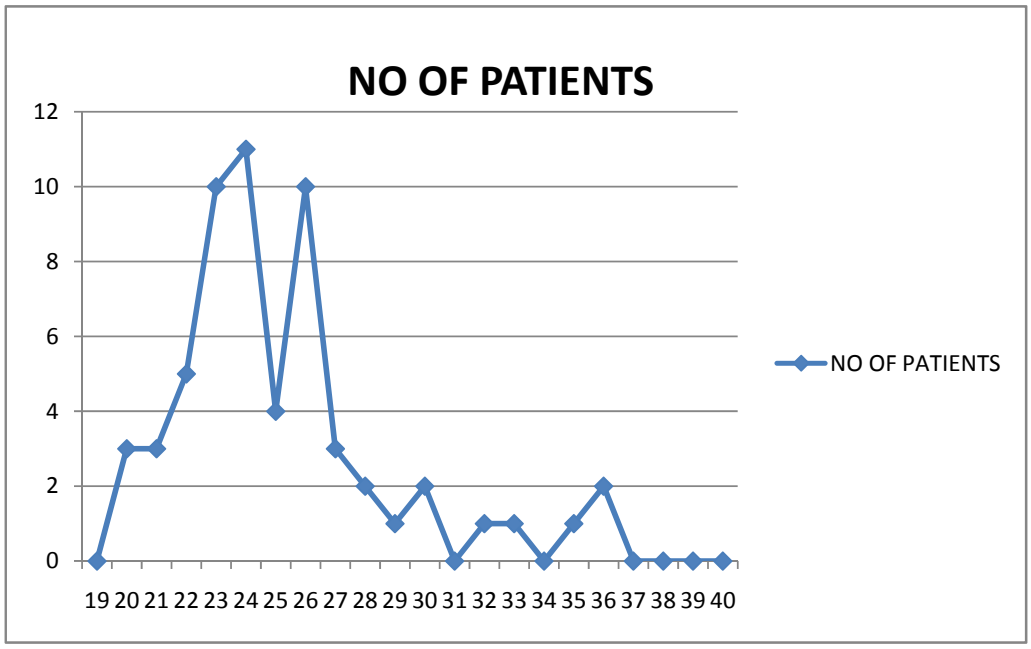
TABLE 1

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).

In the study group the effect of parity on homocysteine concentration is statistically significant.

THE AGE DISTRIBUTION BETWEEN CASES IS AS DEPICTED BELOW.



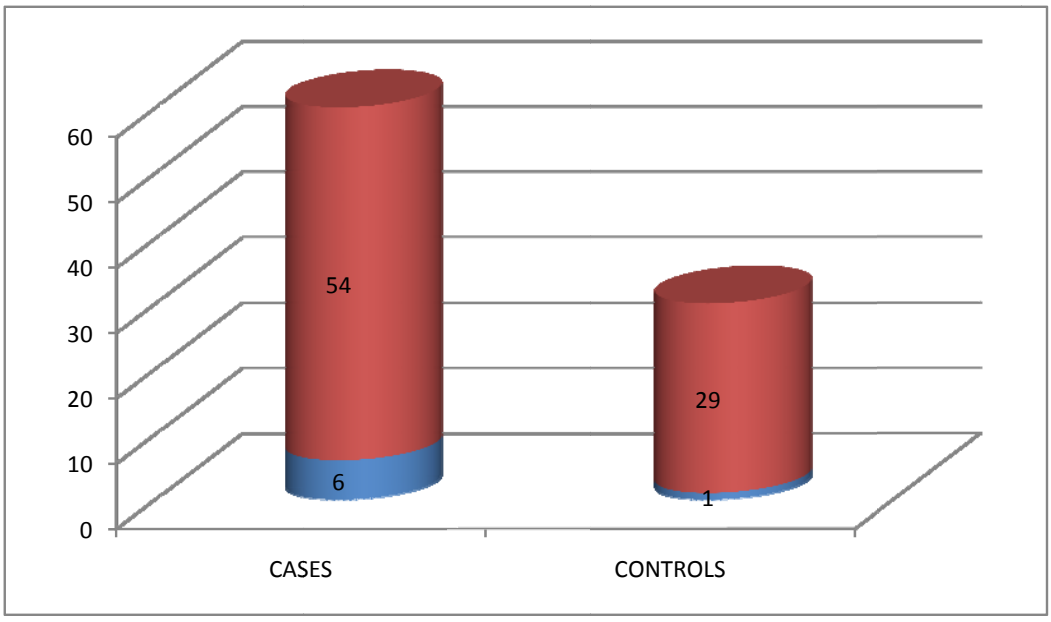
No correlation was found between age and homocysteine levels between the two groups.

TABLE 2

HYPERHOMOCYSTEINEMIA IN CASES AND CONTROL A COMPARISION

GROUP	PERCENTAGE WITH HYPERHOMOCYSTEINEMIA
Cases	10%
Control	3.33%

(Number of persons with abnormal levels of homocysteine in the two groups)



A level of homocysteine greater than 15 µmol/L was considered positive. Six patients in the study group had elevated

homocysteine, 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.

TABLE 3

COMPARISON BETWEEN PRIMARY AND SECONDARY 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)

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 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

(Homocystiene levels in various studies compared with our study)

TABLE 5

COMPARISON OF ODDS RATIO BETWEEN VARIOUS 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

(Odds ratio of our study correlates with Nelen et al)

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.

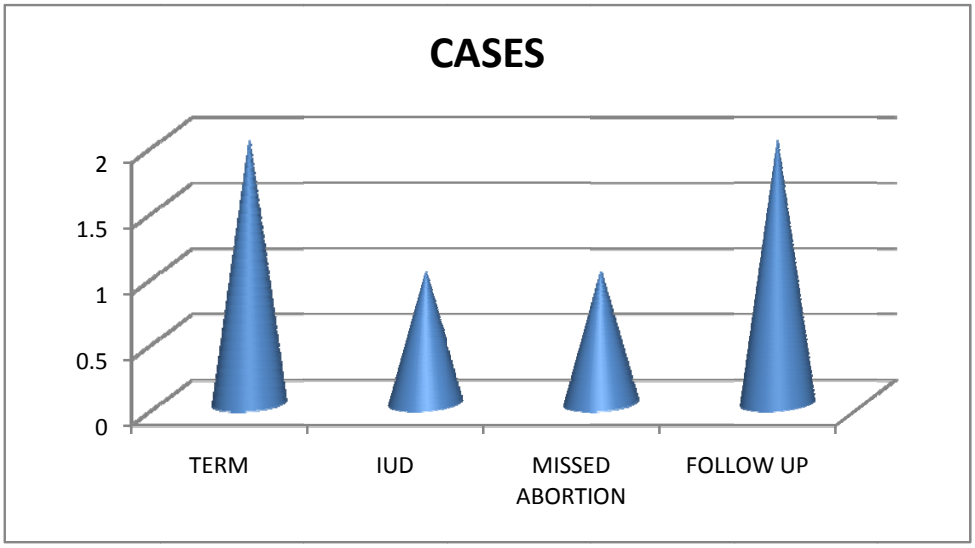
TABLE 6

PREGNANCY OUTCOME AMONG CASES

TERM	IUD	MISSED ABORTION	FOLLOW UP
2	1	1	2

(Two patients among the study group completed the term pregnancy)

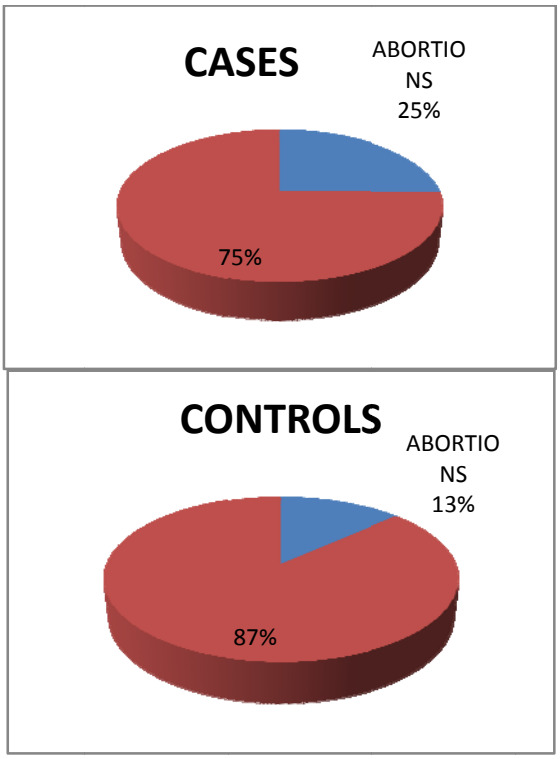
Four among them showed reduction in homocysteine values. Two patients progressed to term pregnancy. One patient went in for preeclampsia and IUD. One went for missed abortion.(TABLE 6) Two patients are still under follow up. Among the control group, only one patient had hyper homocysteinemia. She went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



PATIENTS WHO WENT IN FOR 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) (Steegers-Theunissen et al., 1992a; Wouters et al., 1993; Coumans et al.,1999; Del Bianco et al., 2004) and even for first early pregnancy loss (Gris et al., 2003).

A recent meta-analysis confirmed an increased risk of hyperhomocysteinemia for RPL, defined as two or more spontaneous abortions before 16 weeks of menstrualage (Nelen et al., 2000). As to

the possible impact of MTHFRpolymorphism, 677 TT homozygosity has been shown to increase the risk in some studies, whereas in others, no such effect could be detected; these studies have been reviewed elsewhere (Zetterberg,2004). In a recent meta-analysis by Rey et al.(2003), this polymorphism was not found to increase the risk for RPL.There are several reasons to explain the inconsistency of these results.

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.

Thus the risk could even be higher when both the mother and her fetus are homozygous,As an example, the interference of a

transcobalamin (TC) polymorphism,TC C776G, which influences homocysteine metabolism has been investigated (Zetterberg et al., 2003). TC is a vitamin B12-binding protein and plays a role in the transport and bioavailability of this vitamin. Patients with a heterozygous or homozygous TC 776 mutation have lower serum TC concentration and a tendency toward a higher homocysteine concentration(Namour et al., 2001).

Genotype analysis in fetal tissuesfrom 76 spontaneous abortions, most of them occurring earlier than week 12, showed that embryos presenting with a combined MTHFR 677TT and TC 776CG or TC 776GG genotype had an increased risk for RPL when compared with embryos that had only one of these mutated genotypes (Zetterberg et al., 2003).Fourth, as suggested by the preceding observation, etiologies other than folate deficiency or MTHFR polymorphism may lead to hyperhomocysteinemia and subsequent pregnancy loss.

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 ascular 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 byB-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

hyperhomocysteinemia and MTHFR C677T gene polymorphism and unexplained recurrent pregnancy loss. Several reports, in fact,described an association between early RPL and HHCh 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.

TABLE 1

STUDY	CASES WITH HYPERHOMO-CYSTENEMIA	CONTROLS WITH HYPERHOMO-CYSTENEMIA	P-VALUE	CORRELATION
Our study	6/60	1/30	0.26	No Statistical Significance
Quere et al	12/100	5/100	0.07	No Statistical Significance
Nelen et al	19/123	5/104	0.09	No Statistical Significance
Wouters et al	22/180	3/46	0.27	No Statistical Significance

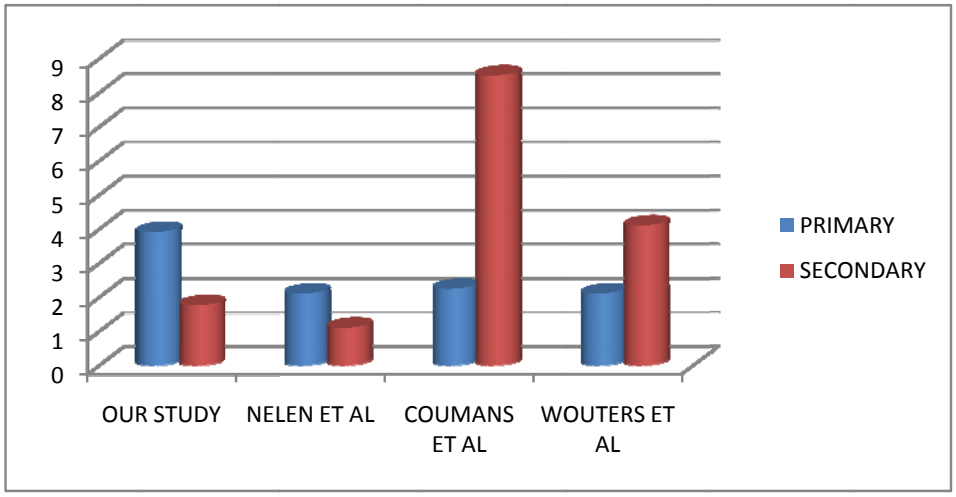
(The women with RPL show non significant higher mean homocysteine concentrations than controls, correlating with other studies).

TABLE 2

COMPARISON OF ODDS RATIO BETWEEN PRIMARY AND SECONDARY ABORTERS

GROUP	PRIMARY	SECONDARY
Our study	3.91	1.76
Nelen et al	2.1	1.1
Coumans et al	2.24	8.5
Wouters et al	2.1	4.1

(Coumans et al and Wouters et al show greater effect of hyperhomocystenemia in secondary aborters)



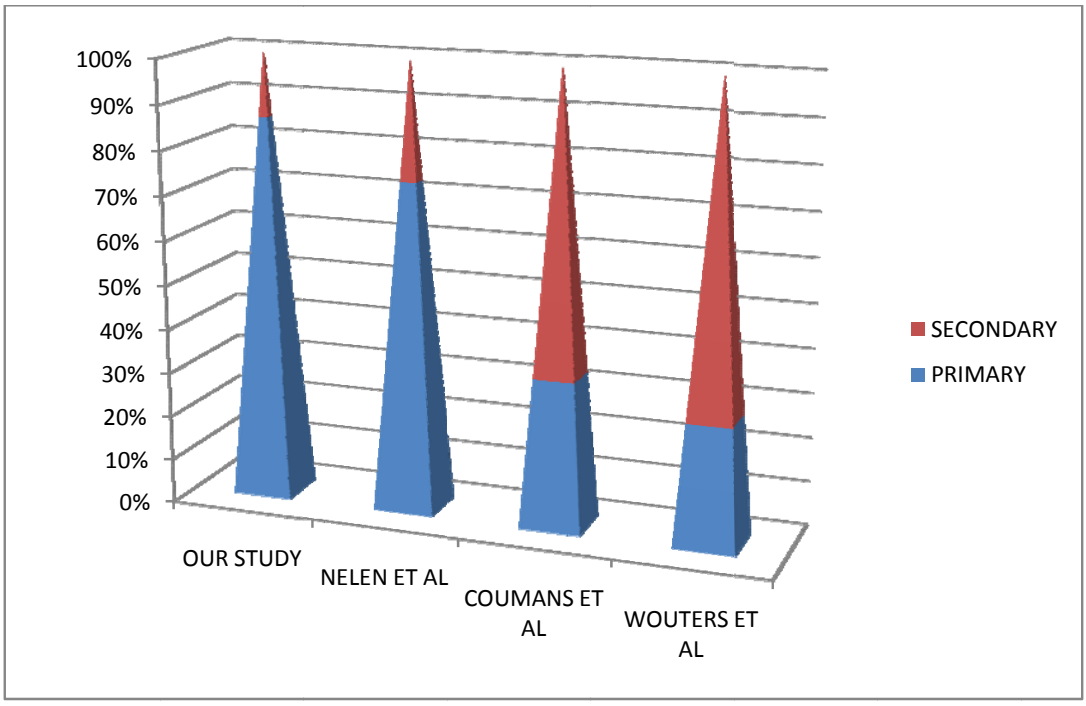
Out of the seven positives,6 were primary aborters and 1 was secondary aborter.The one in the control group with hyperhomocysteinemia also went in for a miscarriage. The number of primary aborters and secondary aborters with hyperhomocysteinemia and the odds ratio for the subgroups are compared with other studies in the tables. (TABLE 2, 3)

TABLE 3

NUMBER OF PRIMARY AND SECONDARY ABORTERS HAVING HYPERHOMOCYSTENEMIA

GROUP	PRIMARY	SECONDARY
OUR STUDY	6	1
NELEN ET AL	14	5
COUMANS ET AL	2	4
WOUTERS ET AL	6	16

(Our study and Nelen et al show that primary aborters are greatly affected by disturbed homocysteine metabolism)



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 hyperhomocysteinemia 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 investigations of both the groups like 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 randomised 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 standardisation 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.(*Homocysteine Lowering Trialists' Collaboration).*T *Am J Clin Nutr* 2005;82:806 –12. F4.There are studies regarding the beneficial effect of folic acid supplemenataion in women with preeclampsia/eclampsia and hyperhomocystenemia in lowering homocysteine levels(Seema

Bibi Qureshi et al,Leeda et al)F2. 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.(F3). Large intervention trials as well as prospective studies measuring tHcy 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.a 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 hyperhomocysteinemia patients to normal. Such information could only be obtained by a multicenter trial.

Many unknowns remain regarding the impact of hyperhomocysteinemia 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 hyperhomocysteinemia. Therapeutic trials are also necessary to study the impact of vitamin supplementation and the best manner to administer it.

SUMMARY

1. Our study has an incidence of recurrent pregnancy loss of 1% in RSRM lying hospital.
2. The study of our 60 RPL cases showed 6 patients with hyperhomocysteinemia which has a 10% rate of occurrence.
3. 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.
4. Therapeutic normalization of homocysteine in supplementation with folic acid B6,B12 in 4 out of 7 indicates the management options that can be tried.
5. Our study indicates that there is no correlation of age with hyperhomocysteinemia.
6. Primary aborters have a higher incidence of hyperhomocysteinemia when compared with secondary aborters.

CONCLUSION

Recent studies on recurrent early pregnancy loss and hyperhomocysteinemia 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 homocysteinebut 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.

PROFORMA

NAME

AGE

IP/OP NO

ADDRESS

TELEPHONE NO

MENSTURAL H/O

- MENARCHY
- CYCLES REGULAR/IRREGULAR
- DURATION
- LMP

MARITAL H/O

- AGE AT MARRIAGE
- CONSAGNUITY

OBSTETRIC H/O

- YEARS AFTER MARRIAGE
- NATURAL/ASSISTED
- ANTENATAL PERIOD
- 1^{ST} TRIMESTER

- 2^{ND} TRIMESTER
- 3^{RD} TRIMESTER
- PEDIGREE
- PERI CONCEPTIONAL EXPOSURE
- ILLNESS(FEVER,JAUNDICE)
- MEDICATION
- EXPOSURE

FAMILY H/O

- ABORTION,INFERTILITY,BIRTH
- DEFECTS,MENTAL RETARDATION

RESIDENCE H/O

- (FACTORY,TANNERY,POLLUTED
- WATER,RADIATION,BURNING)
- OCCUPATION H/O

GENERAL EXAMINATION

- WIFE HUSBAND
- VITALS
- OBSTETRIC EXAMINATION

INVESTIGATIONS

- Hb
- BLOOD UREA
- BLOOD SUGAR
- SERUM CREATINE
- HIV/VDRL
- HbSAg
- CERVICAL SWAB
- HOMOCYSTEINE
- USG
- LFT

ABBREVIATION

RPL	-	Recurrent Pregnancy Loss
HHCH	-	Hyperhomocysteinemia
NTD	-	Neutral tube Defect
RM	-	Recurrent Miscarriage
PCOS	-	Polycystic Ovarian Syndrome
A PAS	-	Antiphospholipid antibody syndrome
MTHFR	-	Methylene tetrahydro folate Reductase
LAC	-	Lupus Anticoagulant
SIS	-	Saline Infusion Sonography
TLC	-	Tender Loving Care
HCG	-	Human Chronic Gonadotrophin
SD	-	Standard Deviation
IUD	-	Intra Utrine Death
TC	-	Transcobalamin

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MASTER CHART

SL. NO	NAME	AGE	REG NO.	OBSTETRIC CODE	GESTATIONAL AGE	LMP	EDD	USG	ASSOCIATED COMPLICATIONS	HOMOCYSTEINE VALUES
1	Mrs vasantha	26	15431	G4A3	11 weeks 2 days	8/6/11	15/3/12			8.6
2	Mrs subanthini	21	12341	G4A3	10 weeks 3 days	12/3/11	19/12/12			3.1
3	Mrs muniyamal	28	12309	G4A3	12 weeks 2 days	16/4/11	23/1/12			4
4	Mrs vasanthi	23	13891	G3A2	10 weeks 3 days	2/6/11	9/3/12			6.1
5	Mrs selvi	24	15432	G5A4	11 weeks 4 days	12/2/11	19/11/11			7.1
6	Mrs baby	20	61/10	G3A2	12 weeks 3 days	21/8/10	28/5/11	MISSED ABORTION		5.8
7	Mrs nithya	26	14365	G4A3	10 weeks 2 days	25/8/10	1/6/11			4
8	Mrs ramani	25	1752	G3A2	11 weeks 4 days	15/2/11	22/11/11	BICORNUATE UTERUS		6.9
9	Mrs janaki	24	1583	G3A2	12 weeks 2 days	16/6/11	23/3/12			6
10	Mrs karolin	26	1764	G3A2	10 weeks 5 days	18/4/11	25/1/12		HYPOTHYROID	8
11	Mrs kameshwari	26	1930	G5A4	13 weeks 5 days	15/6/11	22/3/12	MISSED ABORTION		6.8
12	Mrs tasleema	23	1783	G3A2	12 weeks 2 days	6/8/10	13/5/11			7.5
13	Mrs Naomi	24	104/10	G5A4	11 weeks 4 days	6/12/10	13/9/11		GDM	6.3
14	Mrs poongodi	36	96/10	G3A2	10 weeks 2 days	26/6/10	2/4/11			8.4
15	Mrs revathy	24	77/10	G3A2	13 weeks 3 days	5/5/10	12/2/11			3.4
16	Mrs jeyanthi	23	100/10	G3A2	12 weeks 2 days	2/11/10	9/8/11			22
17	Mrs karpagavalli	24	14501	G3A2	10 weeks 2 days	5/4/11	12/1/12			20
18	Mrs ramya	20	10/10	G4A3	11 weeks 3 days	7/7/10	14/4/11			19.32
19	Mrs jegadha	32	1583	G5P1L1A3	13 weeks 1 day	3/6/10	10/3/11	IUD		20.2
20	Mrs vani	23	1892	G3A2	12 weeks 6 days	4/3/11	11/12/11			4.6
21	Mrs chellamal	40	14761	G3A2	10 weeks 3 days	18/4/11	25/1/12	MISSED ABORTION	HYPOTHYROID	8.5
22	Mrs allirani	35	29/10	G6P2A3	11 weeks 2 days	4/2/10	11/11/11			4.5
23	Mrs maheshwari	28	1820	G4P1A2L1	12 weeks 5 days	16/8/11	23/5/12			6.7
24	Mrs thangamaheshwari	26	25/11	G3A2	13 weeks 3 days	15/12/10	22/9/11			4.3
25	Mrs muthulakshmi	27	26/11	G4P1A2	14 weeks 1 day	12/11/11	19/1/12	MISSED ABORTION		8.3
26	Mrs rameshwari	24	17832	G3A2	10 weeks 4 days	18/2/11	27/11/11			7.5
27	Mrs latha	23	1920	G3A2	12 weeks 2 days	12/3/11	19/12/11		HYPOTHYROID	8.3
28	Mrs Rekha Malaiappan	22	3482	G3A2	10 weeks 1 days	14/4/11	21/1/12			7.5
29	Mrs kameshwari	26	4192	G4A3	13 weeks 3 days	25/4/11	1/1/12			5.4

30	Mrs valliammal	23	3418	G3A2	10 weeks 4 days	12/6/11	19/3/12	MISSED ABORTION		6.8
31	Mrs Halima	24	2891	G4A3	14 weeks 2 days	6/5/11	13/2/12			6.4
32	Mrs kalaiselvi	24	14526	G4P1L1A2	12 weeks 1 day	17/7/11	24/4/12			5.6
33	Mrs saritha	26	16/10	G3A2	14 weeks 3 days	20/5/10	27/2/11		GDM	8.6
34	Mrs lakshmi	23	5643	G3A2	13 weeks 5 days	15/6/11	22/3/12			19.32
35	Mrs deivanayagi	36	81/10	G4P1L1A2	12 weeks 2 days	25/2/10	2/12/10			7.5
36	Mrs selvi	26	5632	G5P2L2A2	10 weeks 3 days	15/7/11	22/4/12			8.6
37	Mrs aruna	23	7645	G4P1A2	13 weeks 4 days	16/5/11	23/2/12			5.8
38	Mrs mathiyalagi	24	3421	G3A2	12 weeks 3 days	11/1/11	18/10/11			7.8
39	Mrs surya	27	9821	G4P1L1A2	10 weeks 1 day	22/5/11	29/2/12			3.89
40	Mrs suseela	25	4501	G4P1L1A2	13 weeks 4 days	12/8/11	19/5/12		GDM	8.6
41	Mrs nithya	24	5621	G3A2	14 weeks 2 days	14/8/11	21/5/12			10.4
42	Mrs shamim nisha	24	2091	G4P1L1A2	12 weeks 3 days	5/3/11	12/12/11	BELIGHTED OVUM		11.4
43	Mrs tamil malar	23	3210	G7P4L2A2	13 weeks 2 days	14/3/11	21/12/11			6.7
44	Mrs aparna	22	2150	G4P1L1A2	10 weeks 1 day	16/3/11	23/12/11		HYPOTHYROID	7.4
45	Mrs radhika	25	2317	G3A2	13 weeks	6/8/11	13/5/12	MISSED ABORTION		18.32
46	Mrs anitha	23	27/10	G4A3	12 weeks	20/9/10	27/6/11			8.6
47	Mrs vatsala	26	33/10	G4P1L1A2	10 weeks 3 days	7/10/10	14/7/11			9.8
48	Mrs nagavalli	22	53/10	G3A2	12 weeks 2 days	5/1/10	12/10/10			8.62
49	Mrs ramya	21	10/10	G3A2	13 weeks	7/7/10	14/4/11			10.75
50	Mrs nishanthi	25	2310	G5P1L1A3	10 weeks 4 days	5/5/10	12/2/11			8.67
51	Mrs deviragavan	38	9/10	G4P1L1A2	13 weeks 2 days	13/7/10	20/4/11			7.1
52	Mrs sulekha sudhakar	22	63/10	G3A2	12 weeks 5 days	7/7/10	14/4/11	BELIGHTED OVUM		8.6
53	Mrs subulakshmi	23	64/10	G5P1L1A3	14 weeks 3 days	9/1/10	16/10/10		OVERT DIABETIC	6.5
54	Mrs bhavani	29	29/10	G3A2	10 weeks	1/2/10	8/11/10			8.4
55	Mrs Baby Tthulasidas	21	83/10	G3A2	13 weeks 3 days	19/6/10	26/4/11			8.6
56	Mrs shobana	20	84/10	G3A2	12 weeks 2 days	23/8/10	30/5/11			11.5
57	Mrs anjalai	23	92/10	G3A2	14 weeks 1 days	3/8/11	10/5/12			9.2
58	Mrs shyamala	22	103/10	G4A3	10 weeks 3 days	12/10/10	19/7/11			6.8
59	Mrs sheela	27	15621	G4P1A2	10 weeks	2/5/11	9/2/12	MISSED ABORTION		8.7
60	Mrs gomathi	26	11983	G7P3A4	12 weeks 5 days	3/6/11	10/3/12			7.6

VALUES FOR CONTROL

S.NO.	NAME	AGE	REG NO	OBSTETRIC CODE	LMP	EDD	GESTATIONAL AGE	USG	ASSOCIATED COMPLICATIONS	HOMOCYSTEINE VALUE
1	Devi	25		G3P2L2	12/5/11	19/2/12	14 weeks 2 days			4.5
2	Selvi	23	25527	G2P1L1	23/5/11	30/2/12	14 weeks 1 day			3.6
3	Renu	22	25907	G2P2L2	2/7/11	9/4/12	13 weeks 3 days			4.6
4	Vinodhini	24	25333	G2P2L2	26/6/11	2/4/12	14 weeks 2 days			5.4
5	Mallika	23	25284	G2P2L2	24/7/11	31/4/12	14 weeks 3 days	MISSED ABORTION		6.7
6	Lakshmi	24	24832	G3P2L2	23/8/11	30/5/12	15 weeks 2 days			8.6
7	Usha	25	22179	G3P2L2	17/8/11	24/5/12	12 weeks 3 days			8.4
8	Kalaivani	22	20377	G2P2L2	19/7/11	26/4/12	14 weeks 3 days			6.8
9	Banupriya	23	23971	G2P2L2	26/7/11	2/5/12	15 weeks 2 days			7.8
10	Dhinakari	24	23964	G2P2L2	31/8/11	7/6/12	15 weeks 4 days			3.5
11	Selvi	23	24688	G2P2L2	26/7/11	2/5/12	13 weeks 2 days			2.5
12	Nirmala	22	24690	G2P2L2	18/6/11	25/3/12	14 weeks 5 days			4.6
13	Umamaheshwari	25	13525	G2P1L1	10/7/11	17/4/12	12 weeks	MISSED ABORTION		19.32
14	Revathy	21	24586	G2P2L2	3/7/11	10/4/12	13 weeks 3 days			7.8
15	Anjali	22	24357	G2P2L2	16/8/11	25/5/12	14 weeks 5 days			8.7
16	Jayashree	30	23451	G3P2L2	12/6/11	19/3/12	13 weeks			6.7
17	Thulasi	29	24350	G4P3L3	19/4/11	26/1/12	13 weeks 3 days		INEVITABLE ABORTION	4.6
18	Annalakshmi	23	23170	G2P2L2	4/8/11	11/5/12	14 weeks			5.7
19	Devi	21	23199	G2P2L2	13/5/11	20/2/12	12 weeks			9.4
20	Deepa	22	22515	G2P2L2	17/6/11	26/3/12	12 weeks 2 days			5.4
21	Amul	23	22511	G2P2L2	29/8/11	6/6/12	14 weeks	MISSED ABORTION		6.7
22	Saritha	24	27617	G2P2L2	15/7/11	22/4/12	13 weeks 3 days			3.6
23	Sumathy	23	26511	G2P2L2	30/6/11	7/4/12	15 weeks 4 days			8.3
24	Shoba	24	13979	G2P2L2	14/5/11	21/2/12	13 weeks			5.3
25	Aseena	23	20989	G2P2L2	27/6/11	4/4/12	12 weeks			5.6
26	Sangeetha	20	21343	G2P2L2	24/8/11	31/5/12	12 weeks 4 days			7.8
27	Amutha	23	21347	G2P2L2	19/5/11	26/2/12	14 weeks 1 day	MISSED ABORTION		3.5
28	Mala	22	22901	G2P2L2	31/7/11	7/4/12	13 weeks			4.5
29	Vijayalakshmi	21	23413	G2P2L2	14/6/11	21/3/12	12 weeks			5.4
30	Meenakshi	23		G2P2L2	25/6/11	2/4/12	14weeks 3 days			7.5