ORIGINAL RESEARCH

Effect of folic acid and vitamin B12 supplementation on hyperhomocysteinemia in pregnant women with recurrent pregnancy loss

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ABSTRACT
Most common adverse pregnancy outcome is spontaneous abortions affecting 10-15% pregnancies. Recurrent pregnancy loss (RPL) affects about 5% of women. High levels of homocysteine, termed hyperhomocysteinemia known to cause a number of pathologic processes in the venous and arterial vascular systems. A prospective study of 80 pregnant mothers booked at our hospital over a period of 18 months with history of unexplained RPL were included in the study and their serum homocysteine, Vitamin B12, serum Folic Acid levels were assessed. Hyperhomocysteinemia (>12 micromol/l) patients were treated with folic acid and vitamin B12 supplements irrespective of biochemical values of vitamin B12 and folic acid for 6 weeks and serum homocysteine, serum folic acid and serum vitamin B12 levels were levels were assessed again, post treatment.
In our study, post vitamin supplementation, 76.25% patients showed reduction in serum homocysteine levels but 23.75% of those who initially had hyperhomocysteinemia continued to have hyperhomocysteinemia.
At the beginning of the study, the mean serum homocysteine level was 14.43 ± 4.52 micromol/L. After 6 weeks of vitamin B12 and folic Acid supplementation, the mean value reduced to 10.42 ± 3 micromol/L with a difference of 4.01 and a statistically significant p value of < 0.001.
At the beginning of the study, the mean serum vitamin B12 level was 328.03 ± 151.91 pg/mL. After 6 weeks of vitamin B12 and folic Acid supplementation, the mean value increased to 716.65 ± 224.8pg/mL with a difference of 388.63 and a statistically significant p value of < 0.001.
At the beginning of the study, the mean serum folic acid level was 5.98 ± 2.24ng/mL. AfterweeksofvitaminB12andfolicAcidsupplementation,themeanvalue increased to 9.05 ± 1.97ng/mL with a difference of 388.63 and a statistically significant p value of< 0.001.
RPL with Hyperhomocysteinemia is associated with folic Acid and vitamin B12 deficiency. Vitamin supplementation to those with hyperhomocysteinemia, decreases homocysteine levels.
Key words: Hyperhomocysteinemia, folic acid, vitamin B12

INTRODUCTION

The most common adverse pregnancy outcome is miscarriage affecting 10 to 15% of pregnant women.[1] Spontaneous recurrent pregnancy loss can be physically and emotionally stressful for couples. The American Society of reproductive medicine has recently defined recurrent pregnancy loss as two or more consecutive pregnancy loss.[2] Studies have shown that only <5% pregnant women experience two consecutivepregnancy loss and fewer than 1% experience more than three.
The accepted number of etiologies for recurrent

pregnancy are very small at present. Most of the diagnosed etiology include uterine anomalies, endocrinal abnormalities autoimmune disorders and genetic factors. Even after evaluation, half of the causes still remain unexplained.[3]

The etiopathogenesis of spontaneous abortion involves a complex interaction of several genetic and environmental factors. The strong association between increased homocysteine levels and neural tube defects has led to the hypothesis that higher the homocysteine levels lesser are the chances of fetal viability.[4]

Homocysteine is a naturally occurring amino acid that has recently been the subject of much interest. It is a demethylated derivative involved in several key metabolic processes including the methylation and sulfuration pathways. Homocysteine is catabolized by vitamin B6 dependent enzyme cystathione b synthase into cystathionine. A significant proportion of homocysteine is regenerated into methionine-by-methionine synthase.This involves methylenetetrahydrofolate reductase and cofactor vitamin B12. The factors responsible for deficiency of folate, vitamin B12 and vitamin B6 lead toelevation of homocysteine levels. There are also several genetic polymorphisms that are associated with defects in folate and vitamin B12 dependent homocysteine metabolism.[5]

Homocysteine is a mediator of endothelial dysfunction. Hyperhomocysteinemia is one among the congenital hyper-coagulable states and it is a long known vascular disease risk factor. Elevated plasma total homocysteine is a risk factor for occlusive cardiovascular disease and that it may be cause of vascular complications associated with the uterine-placental insufficiency. Disturbance in metabolism of homocysteine has been associated with fetal neural tube defects, fetal growth retardation, fetal death in utero, thromboembolic events and condition as a result of placental vasculopathy like pre-eclampsia, abruptio-placentae and recurrent pregnancy loss.[6]

METHODOLOGY

STUDY DESIGN

Prospective observational study.

SAMPLE SIZE:80.

INCLUSION CRITERIA

- Women willing to participate in the study.
- Pregnant women in first trimester with history of two or more consecutive pregnancy loss with hyperhomocysteinemia.

EXCLUSION CRITERIA

1. Patients with history of thromboembolic events.
2. Patients with uterine anomalies.
3. Patients with known endocrinal causes of recurrent pregnancy loss.
4. Patientsrequestingformedicalterminationofpregnancy.
5. PatientswithDiabetesmellitus.
6. Patientswithchronichypertension.
7. Patientswithhistoryofimmunologicaldiseases.
8. Patientswithchronicrenaldisease.
9. Patientswhoareconsumingdrugsthatcausesvitamin B12orfolatedeficiency.

- All pregnancies detected by a positive urinary HCG test were confirmed with ultrasound imaging.
- After obtaining institutional ethical committee's clearance all pregnant women fulfilling inclusion and exclusion criteria are recruited into the study after obtaining written informed consent.
- All the patients meeting the inclusion criteria will be selected. Data regarding maternalhistorywhichincludesmaternalage,medicalhistory,Obstetric
- history, family history, history in past pregnancy and family history, will be obtained. 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 20 weeks of gestational age.
- Thorough clinical examination and pedigree analysis done.
- Fasting EDTA Blood sample was taken after obtaining informed consent from the patient at 08:00 hours.
- Serum Homocysteine, Serum Folic Acid, Serum Vitamin B12 levels were measured after overnight fasting.
- None of the subjects of the study 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.
- Total homocysteine concentration was measured by enzymatic photometric method, after centrifugation and storing. Out of 80 Patients, all women were hyperhomocysteinemic and 38 women with decreased folic acid levels and 48 women had decreased vitamin B12 levels at the beginning of the study.

RESULTS

Table1:Comparisonofserumparameters serumhomocysteineatthe beginningofthestudy(N=80)

Parameters	Serum Homocysteine (Before)		Chi square	Fisher exact Pvalue
	12-20(N=70)	>20 (N=10)		
Serum Vitamin B12 (Before)				
<180	46 (65.71%)	2 (20%)	0.000	0.001
180-900	24(34.28%)	8 (80%)		
Serum Folic Acid (Before)				
<5	33 (47.13%)	9 (90%)	8.278	0.005
5-20	37 (52.85%)	1 (10%)		
Parameter	**Serum Folic Acid (Before)**			
	<5 (N=42)	**5-20 (N=38)**		
Serum Vitamin B12 (Before)			0.113	0.737
<180	34(18.42%)	14 (21.43%)		
180-900	8 (81.58%)	24 (78.57%)		

In the above table, correlation between each parameter is shown. 65.71% of patients with homocysteine levels more than 12 and less than 20 micromol/L have vitamin B12 deficiency and 20% of the patients with serum homocysteine levels have vitamin B12deficiency.Only24%ofpatientswithhomocysteinel evelsbetween12-20micromol/L have normal serum vitamin B12 levels with thep value being 0.001. which is significant. Hence proven from our study that there is association between vitamin B12 deficiency and hyperhomocysteinemia.

47.13% of patients with homocysteine levels more than 12 and less than 20 micromol/L have folic acid deficiency and 90% of the patients with serum homocysteine levels >20 micromol/L have folic acid deficiency. 52% of patients with homocysteine levels between 12-20micromol/L have normal serum folic acid levels with the p value being 0.005. which is significant. Hence proven from our study that there is association between folic acid deficiency and hyperhomocysteinemia.

34 patients with vitamin B12 deficiency also have folic acid deficiency.

8 patients with normal vitamin b12 values have folic acid deficiency.

14 patients with normal folic acid levels have vitamin B12 deficiency. 33 patients are with normal vitamin b12 and folic acid levels.

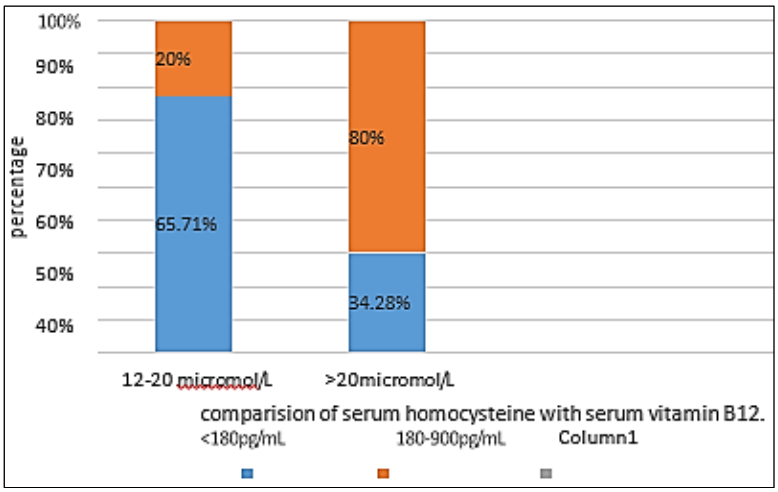

Fig 1: Staked bar chart of comparison of serum vitamin b12 (before) between serum homocysteine (before) (N=80)

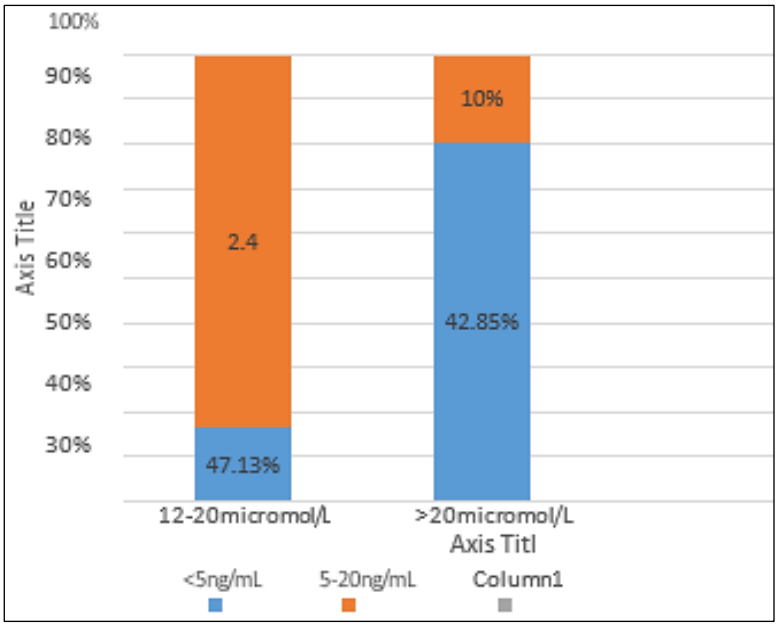

Fig 2: Staked bar chart of comparison of serum folic acid (before) between serum homocysteine (before) (N=80)

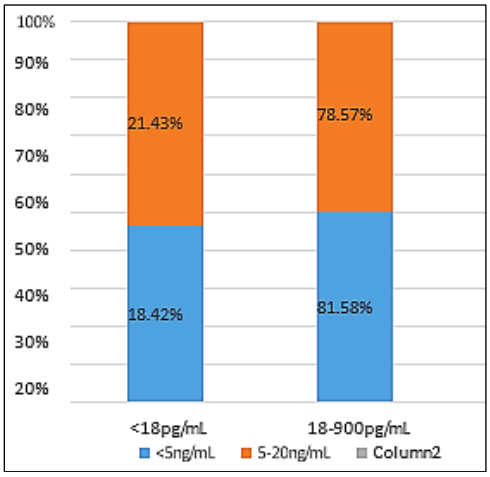

Fig 3: Staked bar chart of comparison of serum vitamin b12 (before) between serum folic acid (before) (N=80)

Table2:Comparisonofserumparameterwithinserumparameterpost supplementation (N=80)

Parameters	Serum Homocysteine (After)		Chi square	Fisher exact Pvalue
	1-12 (N=61)	12-20 (N=19)		
Serum Vitamin B12 (After)				
180-900	57 (93.44%)	8 (42.1%)	0.181	0.056
>900	4 (6.5%)	11 (57.89%)		
Serum Folic Acid (After)				
<5	0 (0%)	0 (0%)	*	*
5-20	25 (50%)	55 (50%)		
Parameter	**Serum Folic Acid (After)**			
	<5 (N=0)	**5-20 (N=80)**		
Serum Vitamin B12 (After)				
180-900	0 (0%)	65 (81.25%)	*	*
>900	0 (0%)	15 (18.75%)		

In the above table correlation of parameters is being made post vitamin B12 and Folic acid supplementation.Serum levels of homocysteine levels have drastically reduced with improvement in serum vitamin B12 and serum Folic Acid levels.

Table 3: Comparison of mean in serum parameters different follow-up periods(N= 80)

Follow-up periods	(Mean± STD)	MeanDifference	P-value
Serum Homocysteine			
Before 6 weeks	14.43 ± 4.52	4.01	<0.001
After 6 weeks	10.42 ± 3		
Serum Vitamin B12			
Before 6 weeks	328.03 ± 151.91	388.63	<0.001
After 6 weeks	716.65 ± 224.8		
Serum Folic Acid			
Before 6 weeks	5.98 ± 2.24	-3.08	<0.001
After 6 weeks	9.05 ± 1.97		

The above table depicts the mean values of all the three parameters before and after vitamin supplementation.

At the beginning of the study, the mean serum homocysteine level was 14.43 ± 4.52 micromol/L. After 6 weeks of vitamin B12 and folic Acid supplementation, the mean value reduced to 10.42 ± 3 micromol/L with a difference of 4.01 and a statistically significant p value of < 0.001.

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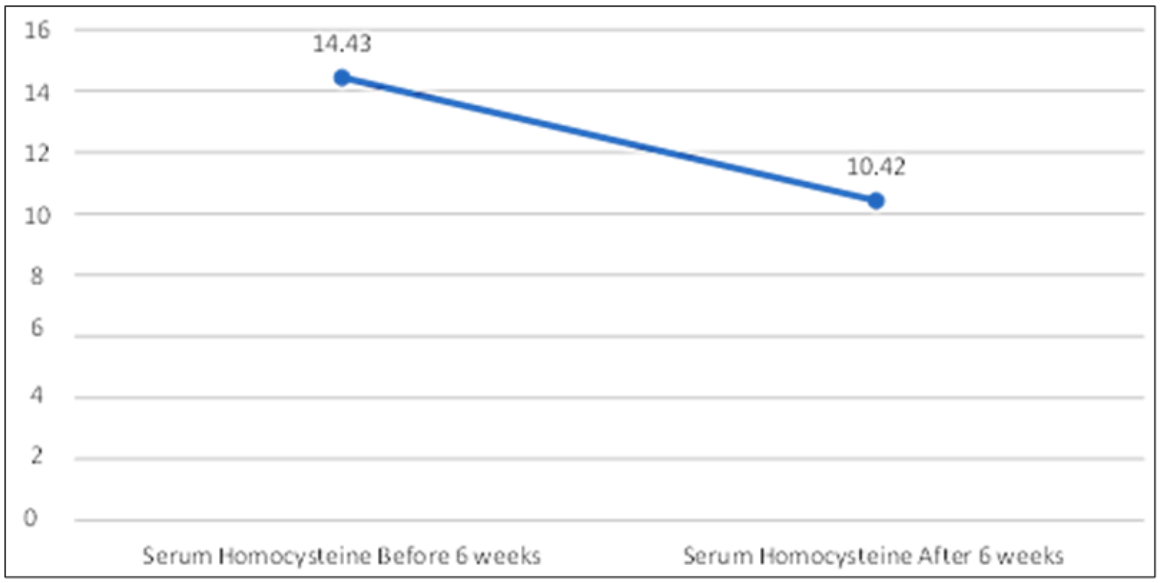

Fig 4: Line chart for comparison of mean in serum homocysteine different follow-up periods (N= 80)

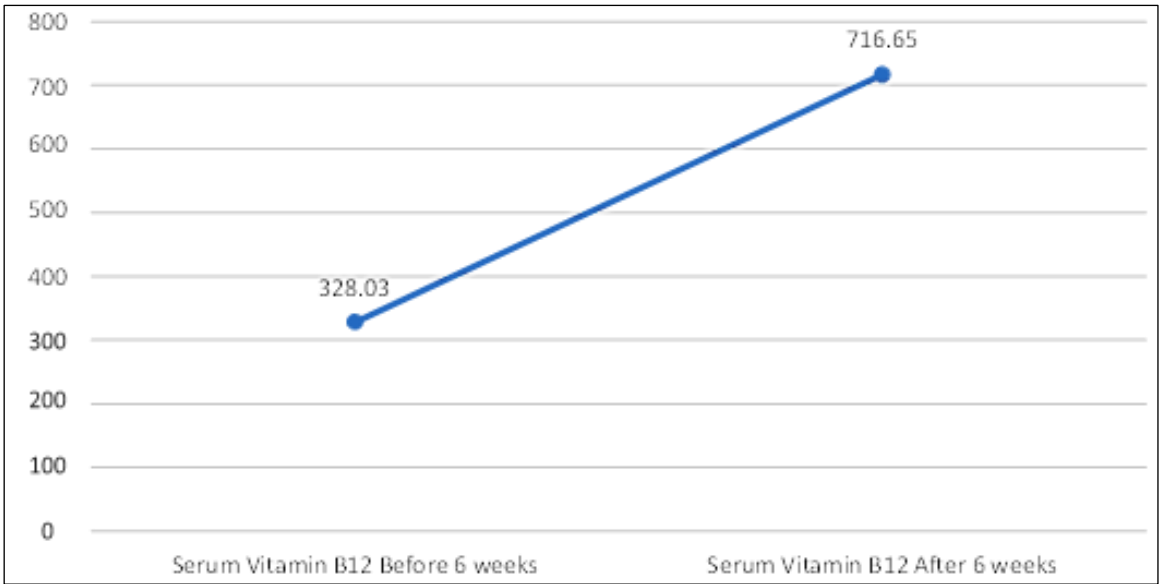

Fig 5: Line chart for comparison of mean in serum vitamin B12 different follow-up periods (N= 80)

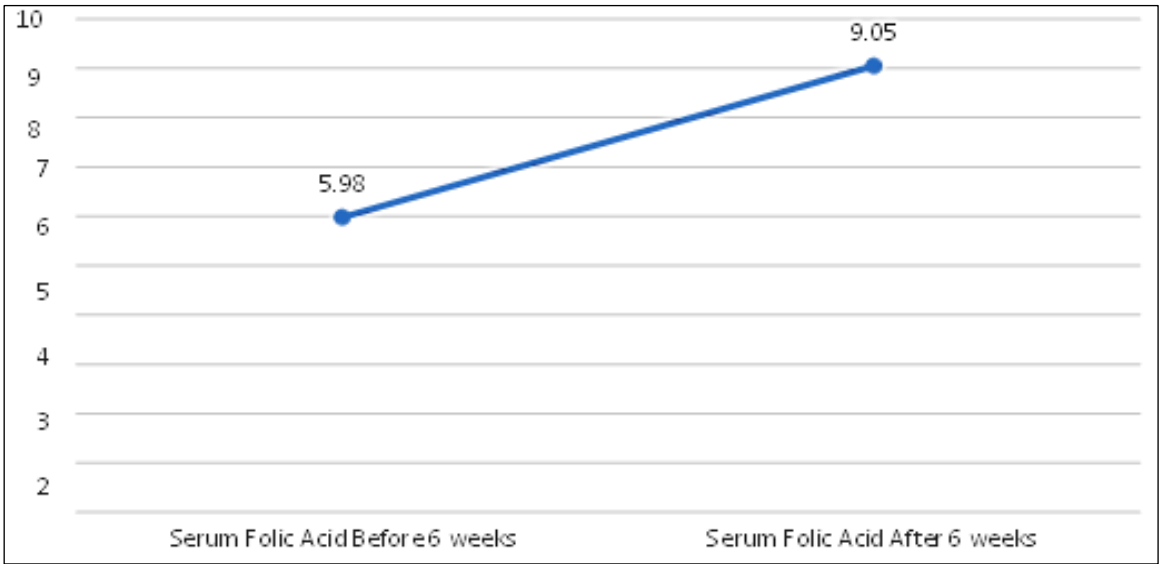

Fig 6: Line chart for comparison of mean in serum folic acid different follow-up periods (N= 80)

Table 4: Pregnancy outcome

Outcome	Frequency	Percentage
Recurrence ofabortion	5	6.25%
PTVD	8	12%
FTVD	34	42.5%
PRETERM LSCS	2	2.5%
FULLTERMLSCS	30	37.5%
Maternal death	0	

All pregnant women in our study were followed up to assess the pregnancy outcome. Recurrence of abortion was found in only 6.25% of the patients.
74(92.5%) of them had live birth.
Preterm vaginal delivery was the outcome in 12% of the patients, 42% of them delivered vaginally at full term, 40% of them underwent LSCS in view of various indications. 1 patient had antepartum stillbirth.

DISCUSSION

This study was conducted as a prospective study over a period of 18 months. The main aim of the study was to correlate hyperhomocysteinemia in recurrent pregnancy loss and the effect of folic acid and Vit

B12 supplementation on levels of homocysteine and to assess the pregnancy out come.

80 booked antenatal cases with history of recurrent pregnancy loss with hyperhomocysteinemia (two or more consecutive pregnancy loss) and in whom any etiology for RPL (diabetes mellitus, hypothyroidism, and in case of 3 or more recurrent pregnancy loss-APLA screening could not be found were considered in the study.

Serum homocysteine, serum folic acid and serum vitamin B12 levels were assessed in the first antenatal visit and those who had hyperhomocysteinemia (>12micromol/l) were given Tab. Folic acid 5mg once daily until delivery and inj. Vitamin B12 1mgI.M every 2nd day for a week and once a week for 6 weeks and homocysteine levelswere repeated after 6 weeks after Vitamin supplementation to assess decrease in homocysteine levels post treatment.

Maximum number of all pregnant women with hyperhomocysteinemia were age between 26-29 years. 31/80 patients were26-29 years old, 22/80 patients were 21-24 years old, 16 patients were 30-34 years old, 6 patients were 24-25 years old.

68% of them were primary aborters. 32% of them were secondary aborters.

70% pregnant women had previous 2 abortions, 22% had previous 3 RPL, 5% hadprevious 4 RPL and 2% had previous 6 RPL.

60%of the pregnant women with hyperhomocysteinemia had vitaminB12 deficiency. 50% of them had folic acid deficiency.

In a study done by WaelAlhalaki and his associates, homocysteine was higher amongpatients with RPL 36/100 (36%) as compared to control subjects 17/100 (17%), the difference was statistically significant ($p<0.003$).

In a study conducted by Maristella *et al*.[7] statistically significant levels of fasting total plasma homocysteine levels were found in those with RPL and unexplained sterility as compared to the control group. The median fasting total plasma homocysteine levels was 19.2 micromol/l in RPL group as compared to 7.85 micromol/l in that of study group.

Acase-controlstudybyGovindaiahandassociatesfoundthatmaternalhyperhomocysteinemia increases RPL[8].

In our study, post vitamin supplementation, 76.25% patients showed reduction in serumhomocysteinelevelsbut23.75%ofthosewhoinitiallyhad hyperhomocysteinemiacontinuedtohavehyperhomocysteinemia.(Pre-treatment±SD in hyperhomocysteinemia patients) =14.46±2.53. Post treatment (Mean ± S D =10.42 ± 2.33). The decrease in homocysteine levels post vitamin supplementation is by 31.22% and this decrease was found to be strongly significant (<0.01).

In a study conducted by Danielius Serapinas *et al*., [9]the impact of folic acid, vitamins B6 and B12 supplementation for the lowering of total homocysteine concentrations and pregnancy outcome was evaluated. 16 patients who had had 3 or more miscarriages and MTHFR mutations were used in the study. They received methyl folate (5mg/day), vitamin B6 (50mg/day) and vitamin B12 (1mg/week). Supplementation induced a decrease in homocysteine from 19.4±5.3μmol/L to 6.9±2.2μmol/L after folate supplementation ($p<0.05$) which is statistically significant. During one year 7 women became pregnant and delivered. In conclusion, supraphysiologic methyl folate, vitamins B6 and B12 supplementationin woman withhyperhomocysteinemiahas a beneficial effect on pregnancy outcome.

In a study conducted by NassimAzadibaksh*et al*.,[10] they evaluated the efficacy of supplementation with high dose folic acid and vitamin B12 in lowering total plasma homocysteine concentrations in hemodialysis patients.36 HD(hemodialysis) patients were included in the study and supplemented with high dose folic acid and vitamin B12. After supplementation, plasma total homocysteine increased by 1.35% in group I and decreased by 6.99%, 14.54% and 30.09% in groups II, III and IV respectively, whichwasonlysignificantingroupIV(P=0.014).concludingthatoral supplementation with 15 mg/day folic acid together with 1 mg/day of vitamin B 12 is effective in reducing total homocysteine levels in HD patients.

In a double blind randomized controlled trial of 12,064 survivors of myocardial ischemia, providing folic acid plus vitamin B12 reduced homocysteine values by 28%(3.8micromol/l).

Conclusion:

In our study also folic acid supplementation along with B12 has an effect on lowering the homocysteine but the still more studies are needed to conclude the effect of folic acid,B12 on pregnancy outcome. Still more intervention trials as well as prospective studies measuring folate and serum total homocysteinestatus before and during pregnancy are needed to establish the role of folic acid B6 and B12 eitheras 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 treatment, should be given. 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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