MAK HILL Publications Research Journal of Medical Sciences

Research Article
doi: 10.36478/makrjms.2024.6.428.431

# Effect of 6 Weeks Therapy With Vitamin B12 and Folic Acid and on Homocysteine Levels in Pregnant Women With Unexplained Recurrent Pregnancy Loss

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**Abstract**
Recurrent pregnancy loss that occurs spontaneously can impose both physical and emotional strains on couples. Identified causes often involve uterine anomalies, endocrine irregularities, autoimmune disorders, and genetic factors. Despite thorough evaluation, around half of the cases still lack a clear explanation. This study was designed to evaluate the levels of serum homocysteine, Vitamin B12 and serum Folic Acid in mothers experiencing recurrent pregnancy loss. A prospective study was conducted involving 123 pregnant mothers with a history of unexplained recurrent pregnancy loss (RPL). The study aimed to assess serum homocysteine, Vitamin B12 and serum Folic Acid levels in these mothers. Patients with hyperhomocysteinemia (>12 micromol/l) were treated with folic acid and vitamin B12 supplements for 6 weeks, regardless of their biochemical values of vitamin B12 and folic acid. Serum levels of homocysteine, folic acid and vitamin B12 were reassessed post-treatment. Results showed that after vitamin supplementation, more than 75% of patients exhibited a reduction in serum homocysteine levels, while rest continued to have hyperhomocysteinemia. Similarly, the mean serum vitamin B12 level increased significantly post-supplementation. Additionally, the mean serum folic acid level rose significantly after supplementation. Our findings indicate an association between RPL with hyperhomocysteinemia and folic acid and vitamin B12 deficiency. Vitamin supplementation effectively reduced homocysteine levels in patients with hyperhomocysteinemia.

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**Key Words**
Homocysteine, folic acid, vitamin B12, pregnancy

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**Received:** 11 April 2024
**Accepted:** 15 May 2024
**Published:** 20 May 2024

**Citation:** Dharmveer Sharma, Neelam Singh Raghuwanshi, Megha Goswami and Janki M. Kanani, 2024. Effect of 6 Weeks Therapy With Vitamin B12 and Folic Acid and on Homocysteine Levels in Pregnant Women With Unexplained Recurrent Pregnancy Loss. Res. J. Med. Sci., 18: 428-431, doi: 10.36478/makrjms.2024.6.428.431

## INTRODUCTION

The primary adverse outcome during pregnancy is miscarriage, affecting approximately 10-15% of expectant mothers. Experiencing recurrent pregnancy loss can be physically and emotionally taxing for couples. Recently, the American Society of Reproductive Medicine has delineated recurrent pregnancy loss as the occurrence of two or more consecutive pregnancy losses. Research indicates that fewer than 5% of pregnant individuals encounter two successive pregnancy losses, with fewer than 1% experiencing more than three losses[1-3].

Currently, the recognized causes of recurrent pregnancy loss are limited. Commonly identified etiologies include uterine anomalies, endocrine irregularities, autoimmune disorders and genetic factors. Despite thorough evaluation, approximately half of the underlying causes remain unexplained[4].

The etiopathogenesis of spontaneous abortion involves a multifaceted interplay of genetic and environmental factors. Notably, there is a strong correlation between elevated homocysteine levels and neural tube defects, suggesting that higher homocysteine levels are associated with reduced fetal viability. Homocysteine, an endogenous amino acid, plays a crucial role in various metabolic processes, including methylation and sulfuration pathways. It is converted into cystathionine by the vitamin B6-dependent enzyme cystathionine beta synthase. Methionine synthase, aided by methylenetetrahydrofolate reductase and vitamin B12 as a cofactor, further converts a significant portion of homocysteine back into methionine. Deficiencies in folate, vitamin B12 and vitamin B6 contribute to elevated homocysteine levels, along with genetic polymorphisms affecting folate and vitamin B12 metabolism[5,6].

Homocysteine also acts as a mediator of endothelial dysfunction. Hyperhomocysteinemia is a recognized congenital hyper coagulable state and a well-known risk factor for vascular diseases. Elevated plasma homocysteine levels are associated with occlusive cardiovascular conditions and may contribute to vascular complications related to uteroplacental insufficiency. Disturbances in homocysteine metabolism have been linked to fetal neural tube defects, fetal growth restriction, intrauterine fetal demise, thromboembolic events and placental vasculopathy-related conditions such as pre-eclampsia, placental abruption and recurrent pregnancy loss[7].

## MATERIALS AND METHODS

The study was designed as a prospective observational study with a sample size of 123 participants. Inclusion criteria involved women who were willing to participate and pregnant women in their first trimester with a history of two or more consecutive pregnancy losses accompanied by hyperhomocysteinemia. Exclusion criteria were defined to exclude patients with a history of thromboembolic events, uterine anomalies, known endocrinal causes of recurrent pregnancy loss, those requesting medical termination of pregnancy, individuals with diabetes mellitus, chronic hypertension, immunological diseases, chronic renal disease, or those consuming drugs that could lead to deficiencies in Vitamin B12 or folate.

The procedure for the study included confirming pregnancies through a positive urinary HCG test followed by ultrasound imaging. Eligible pregnant women meeting the inclusion and exclusion criteria were enrolled after providing written informed consent. Maternal data, including age, medical history, obstetric history, family history and past pregnancy history, were recorded. Ectopic pregnancies or elective terminations were excluded and patients were categorized based on their history of pregnancies beyond 20 weeks of gestational age.

Thorough clinical examinations and pedigree analysis were conducted as part of the assessment. Fasting EDTA blood samples were collected from patients at 08:00 AM. Serum levels of Homocysteine, Folic Acid and Vitamin B12 were measured after overnight fasting. Subjects with known endocrine dysfunction, significant gastrointestinal, hepatobiliary, renal, vascular diseases, or neurological disorders such as epilepsy were excluded. Total homocysteine concentration was measured using an enzymatic photometric method after appropriate sample handling.

## RESULTS AND DISCUSSIONS

The majority of pregnant women with hyperhomocysteinemia fell between the ages of 26-29 years. About 68% of them experienced primary abortions, while about 32% had secondary abortions. Regarding vitamin deficiencies, about 60% of pregnant women with hyperhomocysteinemia had vitamin B12 deficiency, while about 50% had folic acid deficiency.

This study was undertaken as a prospective investigation. Its primary objective was to establish a correlation between hyperhomocysteinemia in cases

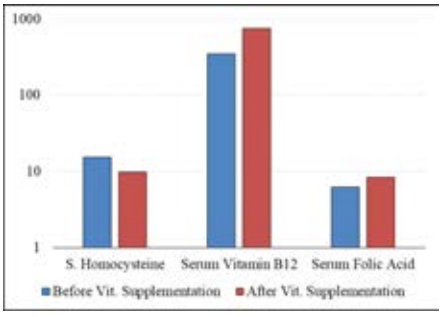


Fig. 1: Graphical depiction of effect of 6 weeks therapy with Vitamin B12 and Folic Acid

**Table 1: Correlation of biochemical parameters at the beginning of study**

| | S. Homocysteine | | | | |
|---|---|---|---|---|---|
| | 12-20 (n=108) | | >20 (n=15) | | |
| | n | percentage | n | percentage | p-value |
| S. Vit. B12 | | | | | |
| <180 | 71 | 65.74 | 3 | 20.00 | <0.05 |
| 180-900 | 37 | 34.26 | 12 | 80.00 | |
| S. Folic Acid | | | | | |
| <5 | 51 | 47.22 | 13 | 86.67 | <0.05 |
| 5-20 | 57 | 52.78 | 2 | 13.33 | |
| | S. Folic Acid | | | | |
| | <5 (n=64) | | 5-20 (n=59) | | |
| | n | percentage | n | percentage | |
| S. Vit. B12 | | | | | |
| <180 | 52 | 81.25 | 22 | 37.29 | 0.67 |
| 180-900 | 12 | 18.75 | 37 | 62.71 | |

**Table 2: Correlation of biochemical parameters after vitamin supplementation**

| | S. Homocysteine | | | | |
|---|---|---|---|---|---|
| | 12-20 (n=94) | | >20 (n=29) | | |
| | n | percentage | n | percentage | p-value |
| S. Vit. B12 | | | | | |
| 180-900 | 88 | 93.62 | 12 | 41.38 | 0.06 |
| >900 | 6 | 6.38 | 17 | 58.62 | |
| S. Folic Acid | | | | | |
| <5 | 0 | 0.00 | 0 | 0.00 | - |
| 5-20 | 94 | 100.00 | 29 | 100.00 | |
| | S. Folic Acid | | | | |
| | <5 (n=0) | | 5-20 (n=123) | | |
| | n | percentage | n | percentage | |
| S. Vit. B12 | | | | | |
| 180-900 | 0 | 0.00 | 100 | 81.30 | - |
| >900 | 0 | 0.00 | 23 | 18.70 | |

**Table 3: Effect of 6 weeks therapy with Vitamin B12 and Folic Acid**

| Parameter | Before Vit. Supplementation | After Vit. Supplementation | p-value |
|---|---|---|---|
| S. Homocysteine | 15.15 ± 4.75 | 9.90 ± 3.15 | <0.05 |
| Serum Vitamin B12 | 344.44 ± 159.51 | 752.48 ± 236.04 | <0.05 |
| Serum Folic Acid | 6.28 ± 2.35 | 8.51 ± 1.87 | <0.05 |

of recurrent pregnancy loss (RPL) and the impact of folic acid and Vitamin B12 supplementation on homocysteine levels, alongside evaluating pregnancy outcomes. The study involved 123 antenatal cases with a history of RPL and hyperhomocysteinemia (defined as two or more consecutive pregnancy losses) where no identifiable etiology for RPL could be determined (e.g., diabetes mellitus, hypothyroidism, or negative APLA screening in cases of three or more RPL).

During the first antenatal visit, serum levels of homocysteine, folic acid and vitamin B12 were assessed. Patients with hyperhomocysteinemia (>12 micromol/l) were prescribed Tab. Folic acid 5mg once daily until delivery, along with inj. Vitamin B12 1mg I.M every other day for a week, followed by once-weekly injections for six weeks. After six weeks of vitamin supplementation, homocysteine levels were re-evaluated to gauge the decrease post-treatment.

The majority of pregnant women with hyperhomocysteinemia fell between the ages of 26-29 years. About 68% of them experienced primary abortions, while about 32% had secondary abortions. Regarding vitamin deficiencies, about 60% of pregnant women with hyperhomocysteinemia had vitamin B12 deficiency, while about 50% had folic acid deficiency. A previous found significantly higher levels of homocysteine in RPL patients compared to control subjects[8]. Similarly, another study reported statistically significant elevations in fasting total plasma homocysteine levels among RPL patients compared to the control group[9].

In the present study, post-supplementation, more than 75% of patients showed reduced serum homocysteine levels, with a significant decrease observed post-treatment. This aligns with findings from previous study, where supplementation with folic acid, vitamins B6 and B12 led to a notable decrease in homocysteine concentrations and improved pregnancy outcomes[10]. Another study supported the efficacy of high-dose folic acid and vitamin B12 supplementation in lowering total plasma homocysteine concentrations, particularly in hemodialysis patients[11].

Furthermore, a double-blind randomized controlled trial involving survivors of myocardial ischemia demonstrated a 28% reduction in homocysteine values with folic acid and vitamin B12 supplementation. These collective findings underscore the potential benefits of vitamin supplementation in managing hyperhomocysteinemia-related complications and improving pregnancy outcomes.

## CONCLUSION

The combination treatment of folic acid and Vitamin B6/B12 should be administered, as indicated

by our study, suggesting that therapeutic normalization of hyperhomocysteinemia might contribute to metabolic restoration, potentially leading to a more favorable pregnancy outcome. Our study also observed that folic acid supplementation, when combined with Vitamin B12, has an effect on reducing homocysteine levels. However, further studies are necessary to conclusively determine the impact of folic acid and Vitamin B12 on pregnancy outcomes. Additional intervention trials and prospective studies measuring folate and serum total homocysteine status before and during pregnancy are needed to establish the role of folic acid, Vitamin B6 and Vitamin B12 as either predictors or etiological factors for recurrent pregnancy losses. We, therefore, advocate for the early identification of women with hyperhomocysteinemia.

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