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Research Article

Study to Evaluate Role of Homocysteine and Folic Acid in 1st Trimester Recurrent Pregnancy Loss

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ABSTRACT
Introduction: Recurrent pregnancy loss (RPL) is a multifactorial condition, with biochemical factors such as hyperhomocysteinemia and folate deficiency increasingly recognized as important contributors.
Aim: To evaluate the association between serum homocysteine and folic acid levels in women with RPL compared to healthy controls.
Materials and Methods: A hospital-based case–control study was conducted on 50 women with RPL and 50 age-matched controls. Serum homocysteine and folic acid levels were measured using high-performance liquid chromatography and chemiluminescent immunoassay, respectively. Hyperhomocysteinemia was defined as >15 µmol/L and folate deficiency as <4 ng/mL. Statistical analysis included *t*-test, Chi-square test, odds ratio calculation, and Pearson's correlation.
Results: Mean homocysteine levels were significantly higher in the RPL group (16.2 ± 4.5 µmol/L) compared to controls (11.8 ± 3.2 µmol/L, $p<0.0001$). Mean folic acid levels were significantly lower in cases (3.9 ± 1.1 ng/mL) than controls (6.2 ± 1.4 ng/mL, $p<0.0001$). Hyperhomocysteinemia was present in 56% of cases versus 20% of controls (OR 5.0, 95% CI 2.1–11.8), while folate deficiency was observed in 60% of cases versus 16% of controls (OR 7.5, 95% CI 3.0–18.6). A strong negative correlation between homocysteine and folic acid was noted in the RPL group ($r = -0.62$, $p<0.001$).
Conclusion: Women with RPL exhibit a significant imbalance between homocysteine and folic acid, suggesting a dual biochemical mechanism involving vascular dysfunction and impaired embryogenesis. Screening and correction of these abnormalities may improve pregnancy outcomes in high-risk populations.

Keywords: Recurrent Pregnancy Loss, Homocysteine, Folic Acid.

INTRODUCTION

Recurrent pregnancy loss (RPL) is a distressing reproductive problem, defined as two or more consecutive pregnancy losses before 20 weeks of gestation, and affects nearly 1–2% of couples worldwide[1]. It is not only a clinical challenge but also a major psychosocial burden, often leading to anxiety, depression, and marital stress[2]. The etiology of RPL is multifactorial, encompassing genetic abnormalities, uterine malformations, endocrine dysfunctions, immunological disorders, thrombophilic states, and environmental influences[3]. Despite extensive evaluation, nearly 50% of cases remain unexplained, highlighting the need to identify subtle biochemical and metabolic contributors[4]. Among the emerging risk factors, **hyperhomocysteinemia** has gained attention due to its role in vascular pathology. Elevated homocysteine levels promote endothelial dysfunction, oxidative stress, and a prothrombotic state, all of which compromise placental perfusion and fetal viability[5]. Homocysteine metabolism is critically dependent on folate and vitamin B12, which act as cofactors in the remethylation pathway[6]. Deficiency of folic acid impairs this pathway, leading to accumulation of homocysteine and subsequent vascular injury. Several studies have demonstrated that women with RPL often exhibit higher homocysteine levels and lower folate concentrations compared to healthy controls[7]. Folate deficiency itself has independent implications for pregnancy outcomes. It is essential for DNA synthesis,

methylation, and cellular division, processes that are vital during early embryogenesis[8]. Inadequate folate status has been associated with neural tube defects, fetal growth restriction, and miscarriage[9]. The interplay between folate deficiency and hyperhomocysteinemia thus represents a dual biochemical insult, potentially explaining a proportion of otherwise unexplained RPL cases. While international studies have highlighted this association, data from Indian populations remain sparse. Nutritional deficiencies, dietary patterns, and genetic polymorphisms such as MTHFR mutations may further modulate homocysteine metabolism in this context[10]. Therefore, evaluating the relationship between homocysteine and folic acid in Indian women with RPL is crucial for both clinical management and public health strategies. This study was designed to compare serum homocysteine and folic acid levels between women with RPL and healthy controls, to assess the prevalence of abnormalities, and to explore their correlation as potential predictors of adverse pregnancy outcomes.

Aims and Objectives

Aim

To evaluate the association between serum homocysteine and folic acid levels in women with recurrent pregnancy loss.

Objectives

1. To compare mean serum homocysteine and folic acid levels between women with RPL and age-matched healthy controls.
2. To determine the prevalence of hyperhomocysteinemia and folate deficiency in the study population.
3. To analyze the correlation between homocysteine and folic acid concentrations in both groups.
4. To assess the potential role of these biochemical markers as predictors of adverse pregnancy outcomes in Indian women.

MATERIAL AND METHODS

Study Design and Setting

This was a hospital-based, case–control study conducted in the Department of obstetrics & gynaecology between duration 2022-2023. Ethical clearance was obtained from the institutional review board prior to commencement. Written informed consent was obtained from all participants.

Study Population

A. Cases (RPL group): Women with a history of ≥2 consecutive pregnancy losses before 20 weeks of gestation (n = 50).

B. Controls: Age-matched women with at least one live birth and no history of pregnancy loss (n = 50).

Inclusion Criteria

1. Women aged 20–35 years.
2. Cases: ≥2 consecutive pregnancy losses confirmed by clinical records.
3. Controls: At least one successful pregnancy outcome.

Exclusion Criteria

1. Known uterine anomalies, endocrine disorders (thyroid disease, diabetes mellitus), or autoimmune conditions.
2. Women with chronic systemic illness, renal disease, or hepatic dysfunction.
3. Current use of folate or vitamin B12 supplementation.

Procedure

Venous blood samples (5 mL) were collected under aseptic precautions after overnight fasting. Serum was separated and stored at –20°C until analysis.

- **Homocysteine Estimation:** Performed using high-performance liquid chromatography (HPLC) with fluorescence detection.
- **Folic Acid Estimation:** Measured by chemiluminescent immunoassay.

Hyperhomocysteinemia: Defined as serum homocysteine concentration >15 µmol/L, above the accepted upper limit for pregnancy reference ranges[11].

Folate Deficiency: Defined as serum folic acid concentration <4 ng/mL, consistent with established clinical thresholds for deficiency[12].

Statistical Analysis

All data were compiled and analyzed using SPSS version XX (IBM Corp., Armonk, NY, USA). Continuous variables were expressed as mean ± standard deviation (SD) and compared using Student's *t*-test. Categorical variables were analyzed using Chi-square test. Odds ratios (OR) with 95% confidence intervals (CI) were calculated for abnormal biochemical parameters. Correlation between homocysteine and folic acid was assessed using Pearson's correlation coefficient (r). A p-value <0.05 was considered statistically significant.

Observation and Result

Table 1. Baseline Characteristics of Study Population

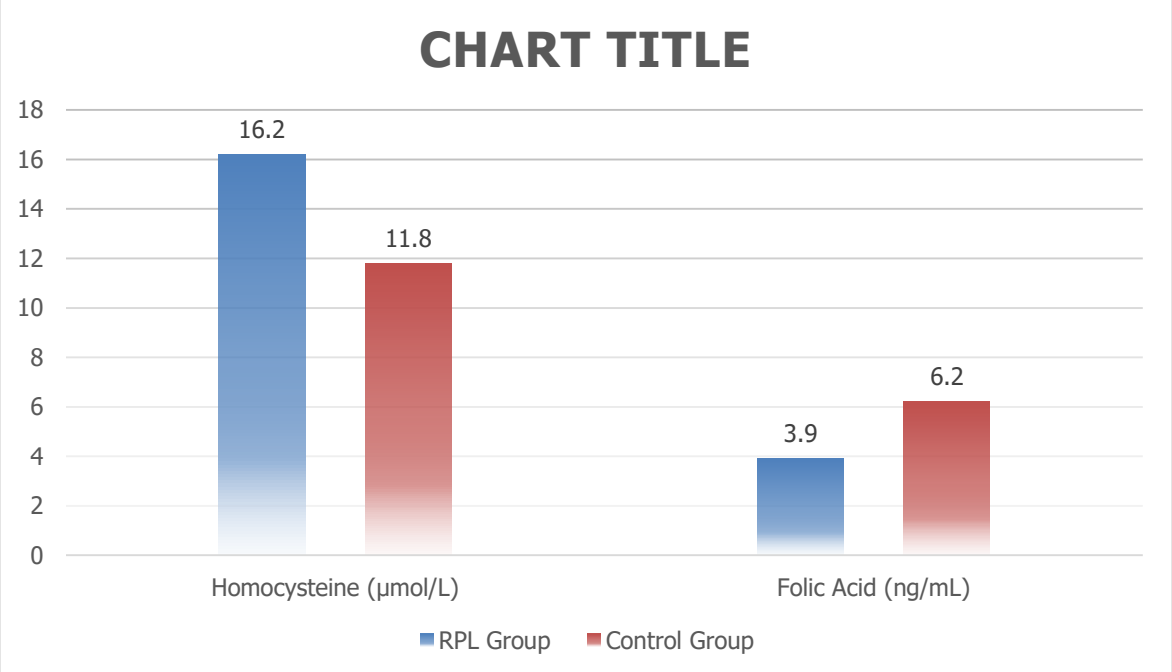
Sr No	Variable	RPL Group (n=50)	Control Group (n=50)	p-value
1	**Mean Age (years)**	29.8 ± 4.2	28.6 ± 3.9	**0.14 (NS)**
2	**Mean BMI (kg/m²)**	24.7 ± 3.1	23.9 ± 2.8	**0.17 (NS)**
3	**Nulliparous (%)**	30 (60 %)	27 (54 %)	**0.68 (NS)**
4	**Family history of RPL**	10 (20 %)	3 (6 %)	**0.04 (S)**

The baseline comparison shows that the **mean age** and **BMI** of women in the recurrent pregnancy loss (RPL) group and controls were similar, with no statistically significant difference. This indicates that age and BMI are not confounding factors in this study. The proportion of **nulliparous women** was also comparable between groups. However, a **family history of RPL** was significantly higher in the case group (20% vs. 6%, p=0.04), suggesting a possible genetic or hereditary predisposition to recurrent pregnancy loss.

Table 2. Mean Homocysteine and Folic Acid Levels

Sr No	Parameter	RPL Group (n=50)	Control Group (n=50)	p-value
1	**Homocysteine (µmol/L)**	16.2 ± 4.5	11.8 ± 3.2	**<0.0001 (S)**
2	**Folic Acid (ng/mL)**	3.9 ± 1.1	6.2 ± 1.4	**<0.0001 (S)**

Women in the RPL group had **markedly elevated homocysteine levels** (16.2 µmol/L vs. 11.8 µmol/L in controls, p<0.0001). Elevated homocysteine is known to impair vascular function and may contribute to placental insufficiency. At the same time, **folic acid levels were significantly lower** in the RPL group (3.9 ng/mL vs. 6.2 ng/mL, p<0.0001). Since folate is essential for homocysteine metabolism, deficiency may explain the higher homocysteine levels observed. Together, these findings highlight a biochemical imbalance strongly associated with pregnancy loss.



Graph 1. Mean Homocysteine and Folic Acid Levels

Table 3. Distribution of Abnormal Levels

Sr No	Parameter	RPL Group (n=50)	Control Group (n=50)	Odds Ratio (95% CI)	p-value
1	**Hyperhomocysteinemia >15**	28 (56%)	10 (20%)	5.0 (2.1–11.8)	**0.0003 (S)**
2	**Low Folic Acid <4 ng/mL**	30 (60%)	8 (16%)	7.5 (3.0–18.6)	**<0.0001 (S)**

The distribution analysis shows that **hyperhomocysteinemia (>15 µmol/L)** was present in 56% of RPL cases compared to only 20% of controls. The odds ratio of 5.0 indicates that women with high homocysteine are five times more likely to experience RPL. Similarly, **low folic acid (<4 ng/mL)** was found in 60% of cases versus 16% of controls, with an odds ratio of 7.5. This means folate deficiency is an even stronger risk factor, increasing the likelihood of RPL more than sevenfold. Both associations were highly significant ($p<0.0001$), reinforcing the role of these biochemical markers in pregnancy outcomes.

Table 4. Correlation between Homocysteine & Folic Acid

Sr No	Group	Number of cases n	Correlation Coefficient (r)	p-value	Interpretation
1	**RPL Group**	50	-0.62	<0.001 (S)	**Strong negative correlation**
2	**Control Group**	50	-0.28	0.05 (S)	**Weak negative correlation**

Correlation analysis revealed a **strong negative correlation** between homocysteine and folic acid in the RPL group ($r = -0.62$, $p<0.001$). This suggests that as folic acid levels decrease, homocysteine levels rise substantially, which may directly contribute to pregnancy loss. In the control group, the correlation was weaker ($r = -0.28$, $p=0.05$), indicating that while the relationship exists in healthy pregnancies, it is much less pronounced. This difference underscores the pathological significance of folate-homocysteine imbalance in women with RPL.

DISCUSSION

The present study demonstrated that women with recurrent pregnancy loss (RPL) had significantly higher mean serum homocysteine levels and lower folic acid concentrations compared to age-matched healthy controls. Hyperhomocysteinemia was observed in 56% of cases versus 20% of controls, while folate deficiency was present in 60% of cases compared to 16% of controls. These findings suggest that both elevated homocysteine and reduced folate are strongly associated with adverse pregnancy outcomes. Similar results have been reported in earlier studies. Nelen et al. found that hyperhomocysteinemia was significantly associated with recurrent early pregnancy loss, supporting its role as an independent risk factor[13]. Vollset et al. also demonstrated that elevated homocysteine levels were linked to pregnancy complications, including miscarriage and fetal growth restriction[14]. In an Indian cohort, Sharma et al. reported that folate and vitamin B12 supplementation reduced homocysteine levels and improved pregnancy outcomes, highlighting the therapeutic potential of correcting these deficiencies[15]. Kanani et al. further confirmed that targeted micronutrient therapy normalized homocysteine levels in women with unexplained RPL[16]. The strong negative correlation between homocysteine and folic acid observed in our study ($r = –0.62$, $p<0.001$) underscores the biochemical interplay between these two parameters. Folate acts as a cofactor in the remethylation of homocysteine to methionine, and deficiency disrupts this pathway, leading to accumulation of homocysteine[17]. Elevated homocysteine contributes to endothelial dysfunction, oxidative stress, and a prothrombotic state, which compromise placental perfusion and fetal viability[18]. In addition, folate deficiency impairs DNA synthesis and methylation, resulting in defective embryogenesis and increased risk of miscarriage[19]. Genetic polymorphisms such as MTHFR mutations may further exacerbate this

imbalance, particularly in populations with low dietary folate intake[20]. Taken together, these mechanisms explain the dual insult observed in RPL: vascular compromise due to hyperhomocysteinemia and impaired embryogenesis due to folate deficiency. The consistency of our findings with international and Indian studies reinforces the importance of screening for these biochemical markers in women with recurrent pregnancy loss. Early identification and correction of folate deficiency and hyperhomocysteinemia may represent a simple, cost-effective strategy to improve pregnancy outcomes.

CONCLUSION

Women with recurrent pregnancy loss exhibited significantly higher homocysteine levels and lower folate concentrations compared to healthy controls. Hyperhomocysteinemia and folate deficiency were strongly associated with RPL, with a clear negative correlation between the two parameters. These findings suggest that folate–homocysteine imbalance plays a pivotal role in the pathogenesis of RPL and highlight the importance of biochemical screening and targeted nutritional interventions to improve pregnancy outcomes.

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