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EVALUATION OF SERUM VITAMIN B12, FOLATE, AND HEMATOLOGICAL PARAMETERS IN WOMEN WITH RECURRENT PREGNANCY LOSS

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ABSTRACT

Background: Recurrent pregnancy loss is a painful reproductive disorder, which impacts maternal physical and psychological health. Nutritional and biochemical factors like low levels of vitamin B12 and folic acid, and other related hematologic dysfunctions can be one of the causes of recurrent miscarriage by disrupting DNA production, erythropoiesis, and placental development.

Objective: To evaluate serum vitamin B12, folate, and hematological parameters in women with recurrent pregnancy loss.

Methods: The cross-sectional study was an analytical study that was designed and conducted at Pak International Medical College, Peshawar between January 2023 and June 2023. There were 72 women having recurrent pregnancy loss. Structured proforma was used to record demographic and obstetric information. Blood samples were taken to be estimated of serum vitamin B12, serum folate and hematological parameters such as hemoglobin, red blood cell count, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, red cell distribution width, white blood cell count and platelet count. The data analysis was done using SPSS version 26 and a p-value was taken as 0.05.

Results: The mean age of participants was 29.8 ± 5.1 years. Vitamin B12 deficiency was found in 37.5% of women, while folate deficiency was observed in 26.4%; 19.4% had combined deficiency. Anemia was present in 63.9% of participants, with mild anemia being most common. Women with a

higher number of pregnancy losses had significantly lower mean vitamin B12, folate, and hemoglobin levels ($p<0.05$). First-trimester loss was the most frequent presentation.
Conclusion: Vitamin B12 deficiency, folate deficiency, and hematological abnormalities were common among women with recurrent pregnancy loss. Lower micronutrient levels were significantly associated with increasing number of pregnancy losses. Screening for these deficiencies may help in the evaluation and management of recurrent pregnancy loss.

Keywords: Recurrent pregnancy loss, Vitamin B12, Folate, Anemia, Hematological parameters, Miscarriage

INTRODUCTION

Recurrent pregnancy loss (RPL) is a significant issue in reproductive health, which is typically described as having two or more consecutive pregnancy losses prior to fetus viability. It is a multifactorial disorder that has various etiologies such as genetic abnormalities, endocrine disorders, uterine abnormality, autoimmune disorders, thrombophilia, infections and environment or nutritional factors. Although there has been progress in reproductive medicine, a significant percentage of cases are still unaccounted hence the need to investigate the possible risk factors that may be altered in order to lead to recurrent miscarriage [1-3].

The micronutritional status has become a growing interest in the evaluation of women with poor pregnancy outcomes. The vitamin B12 and folate are also one of the most important nutrients due to their key role in DNA production, cell division, methylation processes, and RBC maturation. Other uses of these vitamins include homocysteine metabolism and lack of them may result in hyperhomocysteinemia, endothelial impairment, and poor placental circulation. These interference can disrupt implantation, trophoblastic invasion, and early fetus development, thus raising the risk of pregnancy loss [4-6].

Deficiency of folate is not a new concept as it is known to lead to embryogenesis defects and poor obstetric outcomes. Similarly, vitamin B12 deficiency can also influence the maternal hematology as well as fetal development due to disrupted erythropoiesis and disturbed metabolism. This may lead to decreased levels of these nutrients in the body of reproductive- age women due to poor food consumption, malabsorption, multiple pregnancies, and insufficient vitamin supplementation. It is possible that in low-resource environments, where case of anemia and nutritional deficiencies are prevalent, the role of vitamin B12 and folate deficiency in repeated instances of pregnancy loss is especially pronounced [7-9].

Hematological parameters also can also be used to give a helpful insight into the nutritional and physiological condition of women with recurrent miscarriage. Hemoglobin level, red blood cell count, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, and red cell distribution width can indicate anemia, macrocytosis and mixed nutritional deficiencies. These malformations have the potential to damage oxygen supply and tissue circulation, which might have an impact on placental activity and pregnancy support. Hence, the analysis of both biochemical and hematological indicators can be used to gain a better insight into maternal factors related to pregnancy loss recurrence [10].

In developing nations like Pakistan, women are affected by nutritional issues such as poor nutrition, little health education and lack of prenatal care. In spite of the significant emotional and social burden of recurrent pregnancy loss, local evidence on the correlation between serum vitamin B12, folate, and hematological variables and RPL is still scarce. The detection of these abnormalities that can be corrected potentially may enhance clinical evaluation and assist in informing preventive measures in women who have them. The objective of this study was to evaluate serum vitamin B12, folate, and hematological parameters in women with recurrent pregnancy loss.

METHODOLOGY

This was an analytical cross-sectional study carried out in the Pak International Medical College, Peshawar by a duration of six months between January 2023 and June 2023. It involved 72 women

with recurrent pregnancy loss (RPL) who were referred to the gynecology and obstetrics unit where the study took place within the study time. The operational definition of recurrent pregnancy loss was any two or more consecutive pregnancy losses before fetal viability. The study included women of reproductive age whose history of frequent miscarriage was confirmed. The participants who have known uterine abnormalities, chromosomal abnormalities, antiphospholipid syndrome, thyroid disease, diabetes mellitus, chronic liver or kidney disease, active infection, malignancy, recent hormonal therapy, or malignant vitamin B12/folate supplement were excluded to eliminate confounding factors.

A structured proforma was used to gather pertinent data after institutional approval and informed consent were obtained of all the participants. Data on age, residence, socioeconomic status, gravidity, parity, the number of prior pregnancy losses, and gestational age at pregnancy loss were taken. Biochemical and hematological analysis of venous blood samples were done under aseptic conditions.

The measurement of serum vitamin B12 and serum folate was done through the standard laboratory procedures available in the institution whereas the complete blood count parameters such as the hemoglobin, red blood cell count, hematocrit, red cell mean corpuscular volume, red cell mean corpuscular hemoglobin, red cell mean corpuscular hemoglobin concentration, red cell distribution width, white blood cell count and platelet count were done through automated hematology analyzer. Based on the normal laboratory reference ranges, anemia and micronutrient deficiencies were classified.

All collected data were entered and analyzed using SPSS version 26. Quantitative variables such as age, vitamin B12, folate, and hematological indices were presented as mean ± standard deviation, while qualitative variables were expressed as frequency and percentage. Stratification was performed for relevant obstetric categories, particularly the number of pregnancy losses. Associations between micronutrient levels and recurrent pregnancy loss were assessed using appropriate statistical tests, including one-way ANOVA or independent sample t-test for comparison of means, while categorical variables were compared using the chi-square test**. A** p-value of ≤0.05 was considered statistically significant.

RESULTS

A total of 72 women with recurrent pregnancy loss (RPL) were included in the study. The mean age of the participants was 29.8 ± 5.1 years. Most women were between 26–30 years of age. The majority belonged to urban areas and were housewives. Most participants had experienced 2–3 previous pregnancy losses, while first-trimester loss was the most common presentation. Biochemical analysis showed that a considerable proportion of women had low serum vitamin B12 and folate levels, and hematological assessment revealed a high frequency of anemia and altered red cell indices.

Table 1: Socio-demographic and obstetric characteristics of women with recurrent pregnancy loss (n=72)

Variable	Frequency (%) / Mean ± SD
Age (years)	**29.8 ± 5.1**
20–25 years	18 (25.0%)
26–30 years	28 (38.9%)
31–35 years	17 (23.6%)
>35 years	9 (12.5%)
Residence	
Urban	41 (56.9%)
Rural	31 (43.1%)
Occupation	
Housewife	58 (80.6%)
Employed	14 (19.4%)

Socioeconomic status	
Low	30 (41.7%)
Middle	32 (44.4%)
High	10 (13.9%)
Body Mass Index (kg/m²)	**25.1 ± 3.7**
Gravidity	**3.8 ± 1.2**
Parity	**1.2 ± 0.9**
Number of previous pregnancy losses	
2 losses	34 (47.2%)
3 losses	23 (31.9%)
≥4 losses	15 (20.8%)
Gestational age at loss	
First trimester	49 (68.1%)
Second trimester	23 (31.9%)

As indicated in Table 1, majority of women with recurrent pregnancy loss were within the reproductive age of 26 30 years. Many of them had two prior pregnancy losses with first-trimester miscarriage being more prevalent than second-trimester losses. These results demonstrate that repeated pregnancy loss in this group was mainly observed in women who were young to middle aged with early pregnancy losses.

Table 2: Serum vitamin B12 and folate levels among study participants (n=72)

Biochemical parameter	**Mean ± SD**
Serum Vitamin B12 (pg/mL)	248.6 ± 86.4
Serum Folate (ng/mL)	5.9 ± 2.1
Deficiency status	**Frequency (%)**
Vitamin B12 status	
Deficient (<200 pg/mL)	27 (37.5%)
Borderline (200–300 pg/mL)	24 (33.3%)
Normal (>300 pg/mL)	21 (29.2%)
Folate status	
Deficient (<4 ng/mL)	19 (26.4%)
Normal (≥4 ng/mL)	53 (73.6%)
Combined deficiency (B12 + folate)	14 (19.4%)

In Table 2, it is shown that more than one-third of the women had vitamin B12 deficiency and approximately one-quarter had folate deficiency. A significant percentage also expressed deficiency of both micronutrients combined, which is a likely nutritional factor to recurrent pregnancy loss. The average level of serum vitamin B12 and folate was relatively low in comparison to the desired optimum levels of reproductive health.

Table 3: Hematological parameters of women with recurrent pregnancy loss (n=72)

Hematological parameter	**Mean ± SD**
Hemoglobin (g/dL)	10.4 ± 1.5
Red blood cell count (×10⁶/µL)	3.86 ± 0.52
Hematocrit (%)	32.8 ± 4.3
MCV (fL)	91.6 ± 10.8
MCH (pg)	29.1 ± 3.8
MCHC (g/dL)	31.6 ± 2.4
RDW (%)	15.8 ± 2.1

WBC ($\times 10^3/\mu L$)	7.4 ± 1.9
Platelet count ($\times 10^3/\mu L$)	271.3 ± 64.7

Table 3 indicates that the average hemoglobin level was low, which means that anemia was prevalent among women who experience a frequent pregnancy loss. Mean MCV was also within the normal and slightly high range with RDW being higher, which indicates mixed nutritional deficiency patterns, particularly that is of vitamin B12 and folate. The level of the white blood cell and platelet counts were mostly in the normal range, which means that the primary issues were connected with the red cell line.

Table 4: Frequency of anemia and red cell morphological patterns in women with recurrent pregnancy loss (n=72)

Variable	Frequency (%)
Anemia status	
Anemic	46 (63.9%)
Non-anemic	26 (36.1%)
Severity of anemia	
Mild	24 (33.3%)
Moderate	18 (25.0%)
Severe	4 (5.6%)
Red cell morphology by MCV	
Microcytic	14 (19.4%)
Normocytic	35 (48.6%)
Macrocytic	23 (31.9%)
Raised RDW	39 (54.2%)

Table 4 also shows that almost two-thirds of the respondents were anemic with mild anemia being the most common. Even though the most frequent type of morphology was normocytic, a significant percentage of women had macrocytosis, which confirms the presence of vitamin B12 and folate deficiency in women. Increased RDW in more than half of the women is another indication of anisocytosis and blood nutritional abnormality.

Table 5: Association of vitamin B12 and folate deficiency with number of pregnancy losses (n=72)

Number of pregnancy losses	n	Mean Vitamin B12 (pg/mL) Mean ± SD	Mean Folate (ng/mL) Mean ± SD	Mean Hemoglobin (g/dL) Mean ± SD
2 losses	34	276.4 ± 79.3	6.5 ± 2.0	10.9 ± 1.3
3 losses	23	241.8 ± 81.7	5.7 ± 1.8	10.3 ± 1.4
≥4 losses	15	198.2 ± 73.6	4.8 ± 1.7	9.6 ± 1.5
p-value		**0.008**	**0.021**	**0.014**

Table 5 will indicate that the level of serum vitamin B12, folate and hemoglobin will significantly decrease with the number of pregnancy losses. Women who lost four and above had the lowest vitamin B12, folate, and hemoglobin mean. It indicates that nutritional deficiency and hematological impairment might be linked to a higher degree of recurrent pregnancies loss.

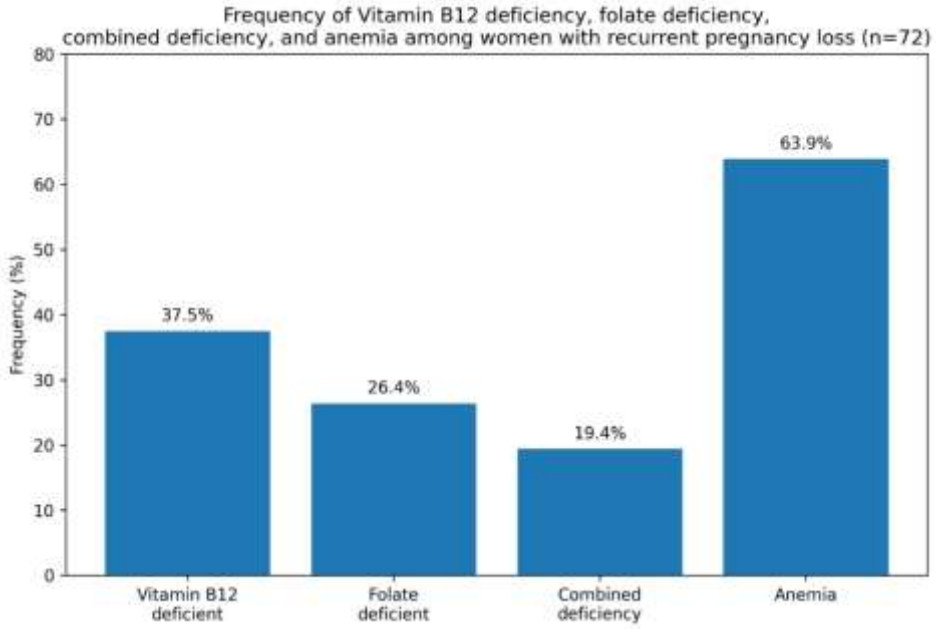


Figure 1: Frequency of vitamin B12 deficiency, folate deficiency, combined deficiency, and anemia among women with recurrent pregnancy loss (n=72).

DISCUSSION

This study evaluated serum vitamin B12, folate, and hematological parameters in women with recurrent pregnancy loss at Pak International Medical College, Peshawar. The results indicated that unusual results and absence of micronutrients and hematological abnormalities had been prevalent in this category of women. A significant percentage of the respondents were deficient in serum vitamin B12, folate, anaemia, increased RDW and macrocytic or normocytic red blood cell distribution. Moreover, women who experienced more pregnancies in which the losses occurred had very low means of vitamin B12, folate and hemoglobin, indicating a potential relation between nutritional deficiency and the extent of recurrent pregnancy loss [11-13].

A high rate of vitamin B12 deficiency was one of the key findings of this study. Vitamin B12 is significant in DNA replication, maturation of red blood cells and metabolism of the homocysteine. The lack can negatively affect the implantation and early fetal development by interfering with cell division and placenta development. The presence of low vitamin B12 in women with recurrent pregnancy loss can be a factor in impaired trophoblastic activity, endothelial dysfunction, and altered methylation events, which predisposes to recurrent miscarriage. The biological plausibility of this association is supported by the relatively low mean serum vitamin B12 level that was noted during the present study [14-17].

A significant proportion of the participants also had the deficiency of folate. Folate is critical in the synthesis of nucleic acid, high cellular division and normal embryonic development. Poor folate status can result in poor placentation and elevated oxidative stress in the early stages of pregnancy. Folate and vitamin B12 are metabolically related, and joint deficiency can further promote the impaired erythropoiesis and reproductive failure. Combined deficiency in vitamin B12 and folate was identified in a significant proportion of women in this study and this suggests that nutritional deficiency could be an unacknowledged cause of recurrent pregnancy loss in low-resource environments [18].

This relationship is also supported by the hematological results of the present study. Over fifty percent of the women were anemic of which mild to moderate anemia was the predominant type. The prevalence of RDW upraised and the occurrence of macrocytosis in such a large percentage of the participants is of particular significance since hepatoblastic alterations due to vitamin B12 and folate deficiency are traditionally related to these factors. Even though microcytosis was present in some women, which suggests that mixed nutritional anemia might be observed, the general hematological image is that the deficiencies of hematinic factors are prevalent in women with

recurrent miscarriage. These malformations might interfere with the maternal oxygen delivery and tissue perfusion and impact the pregnancy retention in an adverse way .[19]

The other significant outcome was a major reduction in the level of vitamin B12, folate and hemoglobin as the number of pregnancy losses increased. Women who had four or more previous losses were the worst biochemical and hematological. This trend shows that depletion of micronutrients can either increase with repeated pregnancies and miscarriages or can be one of the underlying factors and constant in exposing women to repeated pregnancy failure. This result has clinical implications as it implies that nutrition screening can be particularly significant when dealing with women who have had multiple miscarriages [20, 21].

The predominance of first-trimester losses in this study is also notable. Early pregnancy loss is often linked with chromosomal, endocrine, immunologic, and nutritional factors. The high proportion of first-trimester miscarriages in women with low vitamin B12 and folate levels may indicate that these micronutrients are particularly important during implantation and early embryogenesis. Since these stages require rapid DNA synthesis and effective placental vascular development, any nutritional disruption may have a greater impact early in gestation.

It has significant consequences to the health of the population. Women of reproductive age in most of the developing areas are exposed to improper diet, unidentified anemia and reduced access to preconception services. There are biological, emotional, and social implications of recurrent pregnancy loss not only to women and families but also to pain. Avoidable nutritional deficiencies including low folate and vitamin B12 can be identified and provide a low-cost and effective approach to enhance the reproductive outcome. Repeated miscarriage should also be subject to routine laboratory examination of hematinic status in women, which would be useful in the environment where nutritional issues are widespread [21].

There are some limitations to this study. The sample was comparatively small and data collection was done in only one center, which could be a limiting factor to generalization. It has not included any control group of women who had successful pregnancies, thus direct comparison was not possible. No evaluations on other relevant factors related to recurrent pregnancy loss including serum homocysteine, antiphospholipid antibodies, genetic factors and detailed dietary assessment were carried out. In spite of these weaknesses, the research is useful in offering local evidence on the potential role of vitamin B12, folate, and hematological disturbances in recurrent pregnancy loss.

CONCLUSION

Women with recurrent pregnancy loss in this study had a high frequency of vitamin B12 deficiency, folate deficiency, anemia, and abnormal hematological indices. Lower serum vitamin B12, folate, and hemoglobin levels were significantly associated with an increasing number of previous pregnancy losses. These findings suggest that micronutrient deficiency and related hematological abnormalities may play an important role in the pathophysiology of recurrent pregnancy loss. Screening for vitamin B12, folate, and anemia should be considered in the evaluation of women with recurrent miscarriage so that timely nutritional correction and supportive management can be provided.

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