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Research Article

Association of Serum Ferritin and Vitamin D Levels with Severity of Iron Deficiency Anaemia and Response to Oral Ferrous Sulfate Therapy in Pregnant Women

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ABSTRACT

Background: Iron deficiency anaemia is the most prevalent nutritional problem in pregnancy and can have negative effects on maternal health and pregnancy outcomes. Serum ferritin, a marker of iron stores is highly used and iron deficiency is now seen to be linked with a disturbed erythropoiesis and iron metabolism.The other factor is serum ferritin, which is a marker of iron stores, and vitamin D deficiency has been linked with altered iron metabolism and disturbed erythropoiesis. The understanding of the relationship between these biomarkers and response to oral iron therapy may help to enhance the management of anaemia during pregnancy.

Objective: To determine the association of serum ferritin and vitamin D levels with the severity of iron deficiency anaemia and response to oral ferrous sulfate therapy in pregnant women.

Methodology: This is a prospective, observational study at Bacha Khan Medical College, Mardan, from January 2025 to June 2025. In total 75 iron deficiency anaemic pregnant women were enrolled. Hb, haematological indices, serum ferritin and serum 25-hydroxyvitamin D levels were determined. Anaemia was classified into mild, moderate and severe. Oral ferrous sulfate therapy was administered to all participants and a re-evaluation was made after the treatment period. Increase in Haemoglobin of 1.0 g/dL or more was regarded as satisfactory response. $P<0.05$ was considered statistically significant.

Results: The mean age of participants was 27.81 ± 4.63 years, and the mean baseline haemoglobin level was 9.21 ± 1.12 g/dL. Mean serum ferritin and vitamin D levels were 12.84 ± 6.71 ng/mL and 19.76 ± 8.39 ng/mL, respectively. Serum ferritin decreased significantly from 17.62 ± 6.14 ng/mL in mild anaemia to 5.46 ± 2.73 ng/mL in severe anaemia, while vitamin D decreased from 24.83 ± 8.11 ng/mL to 12.98 ± 5.44 ng/mL (both $p<0.001$). After oral ferrous sulfate therapy, mean haemoglobin increased to 10.87 ± 1.09 g/dL ($p<0.001$). Overall, 78.7% of women achieved a satisfactory treatment response. Responders had significantly higher baseline ferritin and vitamin D levels than non-responders.

Conclusion: Lower serum ferritin and vitamin D concentrations were significantly associated with greater severity of iron deficiency anaemia in pregnancy. Higher baseline levels of both biomarkers were also associated with a better haematological response to oral ferrous sulfate therapy. Assessment of vitamin D status alongside conventional iron parameters may provide additional information for the clinical evaluation and management of pregnant women with iron deficiency anaemia.

Keywords: Iron Deficiency Anaemia, Pregnancy, Serum Ferritin, Vitamin D, Ferrous Sulfate, Haemoglobin, Treatment Response.

INTRODUCTION

Iron deficiency anaemia (IDA) is a significant nutritional and haematological condition that is experienced by pregnant women worldwide, and is a significant cause of maternal morbidity. In pregnancy, iron needs increase gradually as a result of expansion of maternal blood volume, development of the placenta and fetal iron needs. If the dietary intake and maternal iron stores are not adequate to meet these needs, iron stores become depleted and can lead to decreased haemoglobin production and iron deficient anaemia. Iron requirements are significantly elevated during the second and third trimesters and pregnant women are especially susceptible to deficiency (1-3).

The clinical significance of Iron Deficiency Anaemia (IDA) in pregnancy is that it can lead to maternal fatigue, decrease in physical capacity, impaired immunity and susceptibility to infection. More serious and complicated cases can lead to less tolerance of blood loss when giving birth and may make it more likely that someone will need more medical help. Anaemia in pregnancy has also been found to have a negative impact on fetal and neonatal outcomes, such as low birth weight, pre-labour delivery and impaired fetal growth. Therefore, early detection and good correction of ID is essential in regular antenatal care (4-6).

The concentration of haemoglobin is generally used to assess anaemia status but it is not a direct indicator of maternal iron stores status. A biochemical indicator of stored iron, serum ferritin is thought to decrease prior to significant decreases in haemoglobin. Low ferritin levels thus offer vital clues to the degree of iron deficiency. The evaluation of serum ferritin along with the routine haematological parameters may be helpful to differentiate iron deficiency from other anaemias, and may also give an idea about the reserve of available iron needed for a satisfactory response to oral iron supplementation (7, 8).

Vitamin D deficiency is another common nutritional problem encountered during pregnancy. Vitamin D is known primarily for its effect on calcium and bone metabolism but current interest is being focused on the potential role in haematopoiesis and iron regulation. Vitamin D can regulate the proliferation of erythroid cells, inflammation and hepcidin-regulatory pathways. When activated, hepcidin plays an important role in regulating the availability of iron in the body by blocking iron absorption in the intestine and iron release from iron stores. Therefore, low vitamin D status could potentially coexist and/or contribute to iron metabolism abnormalities, but this requires further elucidation in pregnant women (9, 10).

Oral ferrous sulfate remains one of the most commonly used treatments for iron deficiency anaemia during pregnancy because it is inexpensive, widely available, and effective in many patients. However, the response to therapy varies considerably between individuals. Poor adherence, gastrointestinal intolerance, inadequate absorption, severity of iron depletion, dietary factors, inflammation, and associated micronutrient deficiencies may all influence improvement in haemoglobin levels. Determining whether baseline ferritin and vitamin D concentrations are associated with treatment response may therefore help identify women who are less likely to respond adequately to routine oral iron therapy. The present study was conducted to assess the association of serum ferritin and vitamin D levels with the severity of iron deficiency anaemia and to determine their relationship with the haematological response to oral ferrous sulfate therapy among pregnant women**.**

METHODOLOGY

This prospective observational study was conducted at Bacha Khan Medical College, Mardan, from January 2025 to June 2025. A total of 75 pregnant women diagnosed with iron deficiency anaemia were enrolled after obtaining informed consent. Participants were recruited from the antenatal outpatient and obstetric services using a non-probability consecutive sampling technique. The study was designed to assess the association of serum ferritin and vitamin D levels with the severity of iron deficiency anaemia and to evaluate their relationship with haematological response to oral ferrous sulfate therapy.

Women who were pregnant in the 2nd or 3rd trimester with known iron deficiency anaemia were included. Iron deficiency anaemia (IDA) was diagnosed by both low haemoglobin concentrations and low serum ferritin levels. Based on haemoglobin concentration anaemia was graded as mild, moderate and severe. Women who had been previously diagnosed with haemoglobinopathies, chronic kidney or liver diseases, active infections or inflammatory conditions, recent blood transfusion, malabsorption syndromes,

multiple pregnancy or current parenteral iron therapy were excluded. Women taking medications or supplements that could significantly affect iron or vitamin D levels (apart from regular ante-natal supplements) were also excluded (when applicable).

Demographic and obstetric data such as age, gestational age, gravidity, parity, residence and related clinical history were collected on a structured data collection form after enrolment. Haemoglobin, haematocrit, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, serum ferritin and serum 25-hydroxyvitamin D were measured from baseline venous blood samples collected under aseptic conditions. Serum ferritin was used as an indicator of body iron stores, and serum 25-hydroxyvitamin D as an indicator of vitamin D status. The vitamin D levels were classified as deficient, insufficient, or sufficient based on the laboratory reference ranges predetermined by the study laboratory.

All the participants were given ferrous sulfate treatment orally as per the standard protocol for routine antenatal treatment of the institution. Participants were educated about regular medication usage and given advice on possible gastrointestinal side effects and how to adhere to them. Haematological indices, serum ferritin and haemoglobin were re-evaluated after the intended duration of treatment. Treatment response was mainly assessed as the difference in haemoglobin concentration between baseline and follow-up. An increase of 1.0 g/dL in haemoglobin was regarded as a good haematological response and the patients were then divided into responders and non-responders. Other indices of red cell function were also measured as secondary endpoints of treatment efficacy, such as changes in serum ferritin.

Data was entered and analysed in SPSS (version 20) software from IBM. The continuous measurements were presented as mean ± standard deviation (SD) and categorical measurements were expressed as frequencies and percentages. Baseline and follow-up lab parameters were compared with paired-samples t-test for parameters with normal distribution. The differences between anaemia severity groups in serum ferritin and vitamin D levels were compared by one-way analysis of variance and relationships between categorical variables were determined by chi-square test or Fisher's exact test as appropriate. Relationships between serum ferritin, vitamin D, haemoglobin concentration and the change in haemoglobin after treatment were evaluated using Pearson correlation analysis. A p-value less than 0.05 was deemed as being statistically significant. Ethical approval was granted by the relevant institutional ethics committee prior to the commencement of the study and participant information was always kept confidential throughout the research process.

RESULTS

A total of 75 pregnant women with iron deficiency anaemia were studied. The participants' mean age was 27.81 ± 4.63 years and the mean gestational age was 25.67 ± 6.18 weeks. Most women were aged 25–34 years (57.3%), and 61.3% were multigravida. Moderate anaemia was found in 45.3%, mild anaemia in 38.7% and severe anaemia in 16.0% of participants.

Table 1. Demographic and Obstetric Characteristics of Study Participants (N = 75)

Variable	**n (%) / Mean ± SD**
Age, years	27.81 ± 4.63
Age group	
<25 years	22 (29.3)
25–34 years	43 (57.3)
≥35 years	10 (13.3)
Gestational age, weeks	25.67 ± 6.18
Trimester	
Second trimester	42 (56.0)
Third trimester	33 (44.0)
Gravidity	
Primigravida	29 (38.7)
Multigravida	46 (61.3)
Parity	
Nulliparous	27 (36.0)

1–2 previous births	34 (45.3)
≥3 previous births	14 (18.7)
Severity of anaemia	
Mild	29 (38.7)
Moderate	34 (45.3)
Severe	12 (16.0)

The mean haemoglobin level in the baseline laboratory evaluation was 9.21 ± 1.12 g/dL and the mean serum ferritin level was 12.84 ± 6.71 ng/mL. Mean serum 25-hydroxyvitamin D was 19.76 ± 8.39 ng/mL. Forty-two women were vitamin D deficient, 21 women were vitamin D deficient and 12 women were vitamin D sufficient.

Table 2. Baseline Haematological, Ferritin and Vitamin D Parameters

Laboratory parameter	**Mean ± SD**
Haemoglobin, g/dL	9.21 ± 1.12
Haematocrit, %	29.18 ± 3.74
MCV, fL	72.64 ± 6.82
MCH, pg	23.49 ± 3.12
MCHC, g/dL	30.81 ± 2.47
Serum ferritin, ng/mL	12.84 ± 6.71
Serum 25(OH) vitamin D, ng/mL	19.76 ± 8.39
Vitamin D status	n (%)
Deficient (<20 ng/mL)	42 (56.0)
Insufficient (20–29.9 ng/mL)	21 (28.0)
Sufficient (≥30 ng/mL)	12 (16.0)

As anaemia got worse, there was a gradual decrease in ferritin and Vitamin D levels in the serum. The mean level of ferritin (ng/mL) was 17.62 ± 6.14 in women with mild anaemia, 11.37 ± 4.89 in women with moderate anaemia and 5.46 ± 2.73 in women with severe anaemia. This difference was significant (P<0.001). The vitamin D levels were also similar, as the mean levels were 24.83 ± 8.11 ng/mL in mild anaemia, 17.82 ± 6.72 ng/mL in moderate anaemia and 12.98 ± 5.44 ng/mL in severe anaemia (p < 0.001).

Table 3. Serum Ferritin and Vitamin D Levels According To Severity of Iron Deficiency Anaemia

Anaemia severity	**n**	**Ferritin, ng/mL Mean ± SD**	**Vitamin D, ng/mL Mean ± SD**
Mild	29	17.62 ± 6.14	24.83 ± 8.11
Moderate	34	11.37 ± 4.89	17.82 ± 6.72
Severe	12	5.46 ± 2.73	12.98 ± 5.44
p-value		<0.001	<0.001

One-way ANOVA was used for comparison among the three groups.

After ferrous sulphate (FeSO4) treatment, haematological and iron-storage parameters showed a marked improvement. Mean haemoglobin increased from 9.21 ± 1.12 g/dL at baseline to 10.87 ± 1.09 g/dL at follow-up, corresponding to a mean increase of 1.66 ± 0.72 g/dL (p < 0.001). Serum ferritin increased from 12.84 ± 6.71 ng/mL to 22.63 ± 9.17 ng/mL, with a mean increase of 9.79 ± 6.13 ng/mL (p < 0.001). Other improvements were noted in haematocrit, MCV and MCH.

Table 4. Changes in Haematological Parameters Following Oral Ferrous Sulfate Therapy

Parameter	**Baseline Mean ± SD**	**Follow-up Mean ± SD**	**Mean change**	**p-value**
Haemoglobin, g/dL	9.21 ± 1.12	10.87 ± 1.09	+1.66 ± 0.72	<0.001
Haematocrit, %	29.18 ± 3.74	33.46 ± 3.91	+4.28 ± 2.47	<0.001
MCV, fL	72.64 ± 6.82	78.39 ± 6.27	+5.75 ± 4.18	<0.001
MCH, pg	23.49 ± 3.12	26.17 ± 3.04	+2.68 ± 1.83	<0.001

Serum ferritin, ng/mL	12.84 ± 6.71	22.63 ± 9.17	+9.79 ± 6.13	<0.001

Paired-samples t-test was used to compare baseline and follow-up values.

Those who had an increase in haemoglobin of at least 1.0 g/dL were defined as responders for analysis of treatment response. In general, there was a satisfactory response from 59 women (78.7%) and non-respondents were 16 women (21.3%). Baseline serum ferritin and vitamin D levels were significantly related to treatment response. The outcome of the therapy was 91.7% of women with sufficient vitamin D levels, 90.5% of women with vitamin D insufficiency, and 69.0% of women with vitamin D deficiency (p = 0.048). The serum ferritin level was significantly higher at baseline in women who responded to treatment compared to non-responders (14.16 ± 6.45 ng/mL vs 7.97 ± 4.91 ng/mL; p < 0.001). Baseline vitamin D levels were also higher among responders (21.23 ± 8.31 ng/mL) than non-responders (14.34 ± 6.22 ng/mL; p = 0.003).

Table 5. Association of Baseline Ferritin and Vitamin D with Response to Oral Ferrous Sulfate Therapy

Variable	Responders (n = 59)	Non-responders (n = 16)	p-value
Baseline haemoglobin, g/dL	9.34 ± 1.06	8.72 ± 1.20	0.047
Serum ferritin, ng/mL	14.16 ± 6.45	7.97 ± 4.91	<0.001
Vitamin D, ng/mL	21.23 ± 8.31	14.34 ± 6.22	0.003
Mean rise in Hb, g/dL	1.88 ± 0.54	0.84 ± 0.19	<0.001
Mean rise in ferritin, ng/mL	11.08 ± 5.72	5.04 ± 4.83	<0.001

A positive correlation was observed between baseline serum ferritin and haemoglobin concentration (r = 0.56, p < 0.001) and was statistically significant. Baseline haemoglobin was also significantly and directly correlated with serum vitamin D (r = 0.41, p < 0.001). Also, there was a significant and positive correlation between baseline ferritin (r = 0.44, p < 0.001) and Vitamin D (r = 0.35, p = 0.002) with magnitude of haemoglobin improvement after 6 weeks of oral ferrous sulfate. Overall, the results showed that women with lower serum ferritin and vitamin D levels were more likely to have severe iron deficiency anaemia and females with relatively high baseline ferritin and vitamin D levels had a better haematological response to oral ferrous sulfate treatment.

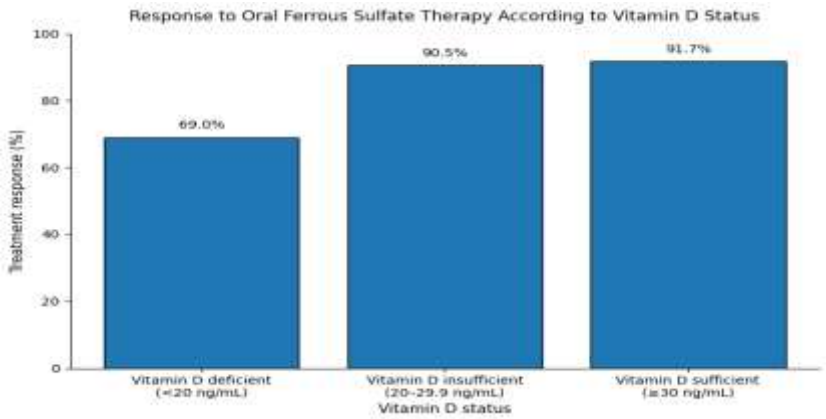

Figure 1. Treatment Response to Oral Ferrous Sulfate Therapy According To Baseline Vitamin D Status among Pregnant Women with Iron Deficiency Anaemia (N = 75)

Treatment response was 69.0% in vitamin D-deficient women, 90.5% in those with vitamin D insufficiency, and 91.7% in women with sufficient vitamin D levels.

DISCUSSION

The present study evaluated the relationship of serum ferritin and vitamin D levels with the severity of iron deficiency anaemia and the response to oral ferrous sulfate therapy among pregnant women. The findings demonstrated that both serum ferritin and vitamin D concentrations declined with increasing severity of anaemia. Women with severe anaemia had markedly lower mean ferritin and vitamin D levels compared with

those with mild or moderate anaemia. In addition, both biomarkers showed significant positive correlations with baseline haemoglobin concentration. These observations suggest that depletion of iron stores and reduced vitamin D status may coexist in pregnancy and may contribute to a more pronounced haematological deficit (11-13).

Ferritin in serum was found to have a particularly strong relationship with anaemia severity which was biologically expected as serum ferritin is an indicator of body iron stores. Progressive decrease in iron stores during pregnancy due to increased iron demands to enlarge red cell mass, growth of the placenta, and fetal growth. The present study showed a significant reduction in mean serum ferritin levels, from 17.62 ng/mL (mild anaemia) to 5.46 ng/mL (severe anaemia), reflecting the progressive deterioration of iron stores with the severity of the disease. The strong positive correlation between Ferritin and Haemoglobin also confirms the usefulness of Ferritin as another important biochemical marker for measuring the extent of Iron deficiency in Pregnant women (14-16).

Another significant finding was the relationship between the anaemia severity and vitamin D status. Woman with mild anaemia had the highest vitamin D level, whereas severe anaemia had the lowest vitamin D level. The mechanism of vitamin D's effect on erythropoiesis may be mediated by its anti-inflammatory actions, through the regulation of iron metabolism, and by its effects on erythroid precursor activity. Vitamin D deficiency can activate inflammatory pathways, and may indirectly decrease the hepcidin activity, which decreases the absorption of iron in the intestine and decreases the availability of iron in the erythropoiesis. Inadequate vitamin D status is not only associated with iron deficiency, but may also accompany iron depletion, and may be associated with increased haematological impairments during pregnancy, as suggested by the results of the current study, even though iron deficiency is the primary mechanism underlying iron deficiency anaemia (16-19).

Oral ferrous sulfate therapy was seen to have resulted in a significant improvement. The mean values of haemoglobin at baseline and follow-up were 9.21 g/dL and 10.87 g/dL, respectively, with a significant increase observed for the serum ferritin. Overall, the 78.7% of the participants had satisfactory treatment response. baseline, the serum ferritin and Vitamin D levels were significantly higher in women who responded to iron versus those who were non-responders. The treatment response also improved as vitamin D levels increased, from 69.0% in vitamin D deficient women to more than 90% in women with either insufficient or sufficient vitamin D levels. The results of this study indicate that oral iron treatment effects vary according to baseline nutrition status and that nutrition status might be used as a therapeutic target. Other factors that may be associated with a relatively slower response include adherence, diet, gastrointestinal absorption, gestational age, and women with significant depletion of iron stores or Vitamin D deficiency (20).

The study has certain limitations, which should be taken into account in the interpretation of the results. It was carried out in one institution and involved a small number of women (N = 75), which may reduce the generalizability of the findings. Dietary intake, sunlight exposure, inflammatory markers, concentration of hepcidin and detailed adherence to oral iron therapy have not been fully evaluated and these can affect ferritin, vitamin D and response to oral iron therapy. Furthermore, the observational study design does not allow for causal linking of the vitamin D deficiency and decrease in iron therapy response. Larger prospective multicentre studies are needed to assess the inflammatory markers, dietary habits and compliance to oral iron treatment during pregnancy and the effect of correcting VDD on the haematological response to oral iron treatment.

CONCLUSION

Lower serum ferritin and vitamin D levels were significantly associated with greater severity of iron deficiency anaemia among pregnant women. Oral ferrous sulfate therapy produced a significant improvement in haemoglobin concentration, red-cell indices, and serum ferritin levels; however, women with higher baseline ferritin and vitamin D concentrations demonstrated a better treatment response. These findings indicate that assessment of both iron stores and vitamin D status may provide useful additional information when evaluating pregnant women with iron deficiency anaemia and monitoring their response to oral iron therapy. Larger prospective studies are required to determine whether targeted correction of vitamin D

deficiency can further improve treatment outcomes.

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