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The Frequency Of Vitamin B_{12} Derangements In Females Presenting For Workup Of Iron Deficiency Anemia

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Abstract

Objective: This study aims to determine the frequency of Vit B_{12} deficiency in women presenting with IDA at child-bearing age.

Materials and methods: Venous blood samples of the non-pregnant female population (N=133) from Fauji Foundation Hospital, Rawalpindi, were collected and analyzed for complete blood count (CBC), Vit $B_{12,}$ and serum Ferritin. The population was further subdivided into two groups. Group A, included females with low serum Ferritin and low MCV, group B was restricted to normal serum Ferritin and low MCV, whereas the control group had females with normal serum Ferritin and MCV levels.

Results: Demographic data suggested that only height was slightly lower in IDA patients other than reduced serum Ferritin levels. Group A also showed reduced levels of Vit B_{12} as compared to Group B and the control group. CBC analysis indicated a marked reduction in all hematological parameters except platelet count in group A.

Conclusion: Our findings suggest that IDA and Vit B_{12} deficiency may coexist in young females. Monitoring Vit B_{12} along with iron deficiency parameters is essential to treat anemia and prevent gynecological complications. The paucity of information on their coexistence in females of childbearing age requires further investigation.

Keywords: Vitamin B_{12}, Iron deficiency anaemia, Microcytosis, Serum Ferritin.

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1. Introduction

Iron plays a vital role in several biochemical processes such as the electron transport chain, synthesis of RNA and DNA, and storage and transport of oxygen[1]. Physiological conditions, namely pregnancy, and menstruation, and several disease conditions like malnutrition, GI cancer, malaria, chronic kidney disease, and hemophilia lead to the development of IDA[1,2]. Symptoms of IDA are portrayed as glossitis, lassitude, anorexia, weakness, pallor, dyspnea, and palpitation[3]. Moreover, IDA in pregnancy may lead to feto-maternal morbidity and mortality due to chronic insufficiency of the placenta, postpartum hemorrhage, heart failure, and impairment of normal physiology[4]. Reportedly, during the postpartum period depletion of iron stores may lead to emotional instability, postpartum depression, reduced period of lactation, and production of milk[5]. World Health Organization report claims that more than two billion population suffer from IDA globally[6]; among these 30% population belongs to women of childbearing age[7]. Levels of serum Ferritin are the predictor of IDA. The Serum Ferritin threshold for diagnosis of IDA is claimed to be 15 ng/mL[8]. Furthermore, CBC is a diagnostic tool for IDA as it indicates microcytosis, hypochromic cells with a reduction in mean cell volume (MCV), mean cell hemoglobin (MCH), and mean cell hemoglobin concentration (MCHC)[9]. Hematological parameters like low MCV from CBC in conjunction with low ferritin levels are clear evidence of IDA[10]. A study indicates that approximately 26.8% Pakistani population has low stores of serum iron and among these 50% population belongs to childbearing age women[10]. WHO recommends a 60mg/day dose of supplemental iron[11]. It had been claimed that ferric carboxymaltose is effective in IDA management as it enhances the body's stores of iron, increases Hb% levels, and depicts better tolerance[12].

Vit B_{12} deficiency occurs due to pernicious anemia, gastrectomy, inflammatory bowel disease, metformin, nitrous oxide gastritis, lactation, and a vegetarian diet[13]. Lack of Vit B_{12} leads to anemia, thrombocytopenia, leukopenia, increased risk of stroke and myocardial infarction (MI), and neurological and psychiatric defects such as paresthesia, dementia, and neuropathic pain[14]. The

true prevalence of Vit B_{12} deficiency is still unknown in the general population[14] whereas, more than 40% population of reproductive age and pregnant women deal with this condition[15]. Due to its high prevalence in childbearing-age women, it is necessary to diagnose and treat the deficiency of vitamin B_{12}. The daily requirement of Vit B_{12} is 2.4µg[16]. Vit B_{12} deficiency anemia can be treated by oral administration of methylcobalamin at a therapeutic dose ranging from 1.5mg-6mg per day[17].

The current study aims to assess the frequency of Vit B_{12} derangements in females presenting for workup of IDA, between the ages of 18 to 25 years referred to the laboratory of Fauji Foundation Hospital, Rawalpindi.

2. Materials & Methods

The current study is cross-sectional and descriptive in nature. The study duration was six months. Population size (N=100) of young females aged range from 18 to 25 years were selected from the laboratory of Fauji Foundation Hospital, Rawalpindi.

The inclusion criteria were:

- Young unmarried non-pregnant females with an age range from 18-25 years
- Low levels of Hb, cut off value of Hb for anemia in non-pregnant females is 12 g/dL, whereas the reference value is 12.1 to 15.1 g/dL in females
- Low levels of MCV, cut off value of MCV is 100fL whereas the reference range is 80-100fL.

The exclusion criteria were:

- Deranged liver and renal functions
- Infection and chronic systemic ailments.

The population was further subdivided into two groups. Group A (IDA and microcytosis) n=60, included females with low serum Ferritin and low MCV. Group B (normal iron stores and microcytosis) n=40, consisted of females with normal serum Ferritin and low MCV while the control group (normocytic picture) n=33, females exhibited normal levels of serum Ferritin and MCV. All experimental procedure was conducted after taking approval from Institutional Ethical Committee (IRC) and verbal informed consent from participants was obtained.

A 2.5 mL blood was collected in serum tubes and the same blood volume was collected in K3 ethylenediaminetetraacetic acid (EDTA-tubes) from the study population to perform CBC with the aid of Sysmex X-1000 automated hematology analyzer[18]. Serum tubes were then centrifuged at 3500 rpm for 3 minutes and serum was collected. Serum Ferritin and Vit B_{12} levels were then analyzed by Advia Centaur XP Immunoassay System as per the manufacturer's guidelines.

The results obtained from anthropometric measurements, ferritin, and Vit B12 levels, and hematological and biochemical parameters were expressed as mean ± standard deviation (S.D) and statistically analyzed by applying analysis of variance (ANOVA). Pearson coefficient (r) analysis was also used. The figures and scattered plots were analyzed by using GraphPad Prism 6. software. A value of $p<0.001$ was considered statistically significant.

3. Results

Anthropometric measurements of the study subjects

The age, height, weight, and body mass index (BMI) of the study subjects were quantified by the noninvasive method. The results of the current study do not show any statistically significant difference in age, weight, and BMI of groups A, B, and the control group whereas group A depicts shorter height as compared to group B and the control group with ***p<0.001 as shown in Table 1.

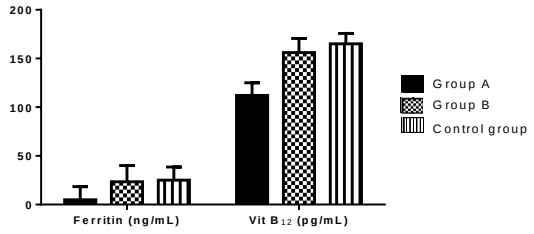

Figure-1 Comparison of Ferritin and Vit B_{12} levels in different groups.

Ferritin and Vit B_{12} levels of different groups

Ferritin and Vit B_{12} levels of different groups are shown in Figure 1. A marked reduction in Ferritin and Vit B_{12} is shown in group A. However, group B signifies normal iron stores and Vit B_{12} levels as compared to group A. When Ferritin and Vit B_{12} of group A were compared with the control group, the p-value was <0.001. A comparison of group B with the control group showed similar findings. Significant comparison of group A with group B also showed p value <0.001 as shown in Table. 2

Table-1 Anthropometric measurements of the study subjects

Parameters (Mean ± SD)	**Group A n=60**	**Group B n=40**	**Control group n=33**	**p-value**
Age	20 ± 1.5	20.4 ± 1.2	19.3 ± 1.3	p>0.05
Weight	52 ± 1.3	52.5 ± 0.2	51.5 ± 0.1	p>0.05
Height	154 ± 1.3	164.5 ± 1.5	162 ± 1.7	***p<0.001
BMI	22.4 ± 1.4	21.7 ± 1.5	22 ± 0.4	p>0.05

Table-2 Biochemical parameters of the study groups.

Parameters (Mean ± SD)	**Group A**	**Group B**	**Control group**	**p-value**
Serum Ferritin (ng/mL)	4.98 ± 13.4	23.45 ± 16.70	25.11 ± 13.50	***p<0.001
Vit B_{12} (pg/mL)	112 ± 13.10	156 ± 14.50	165 ± 10.70	###p<0.001

Hematological parameters of the study subjects.

Hematological analysis of study groups revealed that group A had lower levels of mean corpuscular hemoglobin concentration (MCHC), mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), hematocrit (Hct%), and hemoglobin (Hb) as compared to group B and control group with p values <0.001 as shown in Table 3. Similarly, RBC and white blood cell count also exhibited a marked reduction in group A compared to group B and the control group. Platelet count did not show any significant change (Table 3).

Table-3 Hematological indices of the study subjects.

Parameters (Mean ± SD)	**Group A**	**Group B**	**Control group**	**p-value**
WBC (x 10^9 L^{-1})	5.80 ± 2.30	7.50 ± 1.10	7.87 ± 0.80	***p<0.001
RBC (x 10^9 L^{-1})	4.23 ± 2.10	4.55 ± 1.10	4.67 ± 0.50	***p <0.001
Hb (g/dL)	7.80 ± 1.20	11.03 ± 1.50	12.30 ± 0.5	***p <0.001
Hct%	28.60 ± 2.5	34.50 ± 1.50	35.50 ± 0.4	***p <0.001
MCV (fL)	72.60 ± 1.50	74.89 ± 1.20	80.30 ± 1.30	***p <0.001
MCH (pg)	21.20 ± 1.40	25.20 ± 1.40	27.80 ± 1.30	***p <0.001
MCHC (g/dL)	28.60 ± 2.20	33.20 ± 2.00	34.50 ± 1.80	***p <0.001
PLT (x 10^9 L^{-1})	1.55 ± 0.50	1.56 ± 0.50	1.54 ± 0.50	p>0.05

Correlation between Serum Ferritin and Vit B_{12}

Pearson correlation coefficient (r) analysis between serum Ferritin and Vit B_{12} indicates a positive correlation as shown in Figure 2.

5. Discussion

Depletion of iron stores leads to a severe health condition, IDA targets people of all ages and groups including children, elderly, and childbearing-age women, and substantially affects the quality of life[19]. Literature survey reveals that by restoring serum iron stores the patient's quality of life can be improved[20]. A myriad of constant fatigue, shortness of breath (SOB), cold feet and hands, irritability, impaired memory function, and tachycardia, can be due to IDA[21]. Vit B_{12} deficiency may occur in pregnancy, lactation, metformin use, nitrous oxide, pancreatic insufficiency, hemolytic anemia, birth control pills, less intake of

meat and dairy products, and vegetarians of all age groups irrespective of gender[22].

In the current study, 100 non-pregnant females participated. Among these 60 participants had IDA signifying 60% of subjects suffered from IDA. A similar study was conducted in Quetta showing 25% of reproductive-age women were suffering from IDA[23]. IDA may occur even if biochemical analysis shows normal levels of serum iron stores in various inflammatory disorders for example rheumatoid arthritis, hyperthyroidism and adult-onset Still's disease[24]. Our study showed no significant difference between weight and BMI of all three groups but patients with low serum Ferritin stores had shorter height as compared to study groups who showed normal levels of serum Ferritin stores. Similar results were shown by Sidrah et al, 2014 in female patients of the Gaza Strip of Palestine[18].

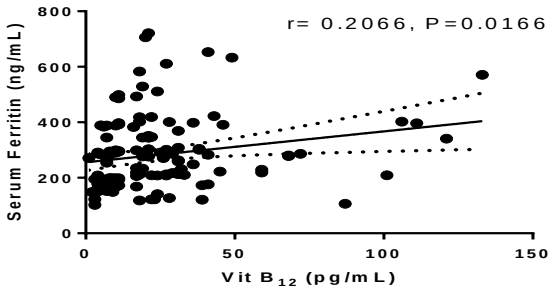

Figure-2 Pearson correlation coefficient analysis of serum Ferritin and Vit B_{12}

CBC analysis is a diagnostic tool for the detection of IDA. CBC test includes Hb, MCV, MCH, MCHC, Hct% and RBC, WBC and platelet count. Our study reveals that the IDA group shows a prominent reduction in levels of Hb, MCV, MCH, MCHC, and Hct% as compared to group B and the control group. Reduced levels of MCV, MCH and MCHC were also seen in IDA caused by gastrointestinal surgery[25]. Our data suggest that in IDA there is a significant reduction in hematocrit levels and no change was observed in platelet count which is in line with the findings of Chai et al. 2022[26]. Lower levels of white blood cell count and red blood cell count are observed in our study for the IDA group and similar results are found in the population of pregnant females living at low altitudes[27].

We found a positive correlation between serum stores of iron and Vit B_{12} in the population of reproductive-age women. Pearson correlation coefficient (r) was found to be 0.2066 with P=0.0166 which is statistically significant. Our study revealed that despite the typical manifestation of IDA, that is microcytosis seen in group B, Vit B_{12} deficiency was also present. Our findings are in line with the literature survey which concludes that if certain anemias occur concurrently, the clinical presentation of one of the anemia would be masked[28]. Similarly, research conducted in India on the IDA population of reproductive-age women with Vit B_{12} deficiency anemia showed marked microcytic cells[29,30]. Vit B_{12} deficiency anemia can be masked by concomitant IDA, showing the absence of macrocytosis in the blood smear.

5. Conclusion

A significant proportion of childbearing-age women with IDA were found to be Vit B_{12} deficient in our study. Our study shows that clinicians need to be made aware of the overlap of these two anemias in reproductive-age women. Secondly, it highlights Vit B_{12} monitoring as a primary parameter in this age group to reduce poor feto-maternal outcome rates. A diagnostic model of Vit B_{12} measurement in IDA with low serum Ferritin and microcytosis needs to be further investigated at a larger scale in women of childbearing age.

CONFLICTS OF INTEREST- None

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Contributions:
N.A, S.S- Conception of study
N.A, M.K- Experimentation/Study Conduction
N.A, M.K, F.T.Z, J.A- Analysis/Interpretation/Discussion
N.A, J.A- Manuscript Writing
M.K, S.K, S.S- Critical Review
S.K, S.S- Facilitation and Material analysis

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