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Role of Vitamin A, Iron, Zinc and Copper in Relation to Anemia Severity in Pregnancy

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Abstract
Background: *Anemia is a common health problem during pregnancy, and nutritional deficiencies during pregnancy are associated with adverse maternal and fetal outcomes, with various factors influencing its occurrence which significantly impacting maternal and fetal health.*
Objective: *This study aims to investigate the complete blood count and nutritional status, including iron, zinc, copper, and vitamin A in anemic and non-anemic pregnant women.*
Materials and Methods: *The study involved 200 participants, out of which 121 anemic pregnant and 79 non-anemic pregnant women. The severity of anemia among the participants was classified according to WHO guidelines. The iron, zinc, and copper were estimated using ICPMS and Vitamin A was estimated using LCMS/MS.*
Results: *The Hb and TLC were significantly reduced in anemic pregnant women than non-anemic ($p<0.05$). The iron (53.35±30.85 vs. 63.40±36.04; $p=0.036$), zinc (898.95±755.78 vs. 578.23±507.44; $p=0.006$), copper (397.92±205.34 vs. 1056.56±833.86; $p=0.002$), and vitamin A (506.24±223.42 vs. 662.52±220.15 pg/mL; $p=0.0001$) were significantly reduced in anemic women as compare to non-anemic women. Among the anemic severity, the severe anemic women had lower levels of vitamin A, iron, zinc, and copper than moderate and mild ($p<0.05$. The Hb was also positively correlated with iron ($r=0.38$; $p=0.001$), zinc ($r=0.32$; $p<0.0001$), and copper ($r=0.27$; $p<0.0001$), and vitamin A ($r=0.29$; $p=0.019$).*
Conclusion: *Anemia severity was correlated with reduced levels of iron, zinc, copper, and vitamin A. These findings provide valuable insights into the nutritional status of pregnant women and also highlight that during pregnancy, nutritional deficiency needs to addressed by targeted interventions.*
Keywords: *Pregnancy, Anemia, ICPMS, LCMS/MS, Vitamin A, Copper, Zinc*

INTRODUCTION

Anemia, a health concern of global proportions, is notably prevalent during pregnancy. This condition, influenced by geographical location, socioeconomic status, and dietary habits, can lead to unfavorable outcomes for both the mother and the baby. Nutritional deficiencies affect all age groups, but pregnant women and children are more vulnerable [1-3]. The worldwide prevalence of anemia in pregnant women is 38% [4], and a significant burden of anemia was reported in Asia (60%) and Africa (52%), respectively [5]. Anemia during pregnancy is more prevalent in developing countries (43%- 56%) compared with developing countries (9%- 18%). Pregnancy increases metabolic demands, requiring adequate nutrients for placental, fetal, and maternal tissue development. However, nutritional needs often go unmet in low-resource settings, where access to diverse, nutrient-rich foods is limited [6]. Poor maternal nutrition during pregnancy is associated with serious outcomes, such as premature birth, intrauterine growth restriction, and stillbirth [7]. The risk of these outcomes increases with multiple micronutrient deficiencies prevalent in food-insecure communities.

Globally, anemia affects about 38% of pregnant women, with iron deficiency as the leading cause. In South Asia, anemia rates are particularly alarming, with profound implications for maternal health [10] While it is suggested that iron deficiency in pregnant women could result in their babies having deficient iron status, more evidence is needed to confirm this. Some research indicates that the fetus obtains iron independently from the mother, potentially leading to a state of deficiency [9,10].

Vitamins A, iron, zinc, and copper are essential for various physiological processes crucial for both maternal and fetal health. Vitamin A is critical for fetal immune development, while thiamine and folate are essential for red blood cell production. Similarly, iron and zinc are vital for oxygen transport and fetal growth.

Vitamin A supports immune function and fetal organ development. Deficiencies during pregnancy can increase the risk of maternal morbidity, including night blindness [11]. Iron, a key component of hemoglobin, is essential for oxygen transport, and its deficiency leads to iron-deficiency anemia, the most common form of anemia in pregnancy. The demand for iron increases during pregnancy, making pregnant women particularly susceptible to iron deficiency [12]. Zinc and copper are vital for various physiological processes, including immune function, DNA synthesis, and fetal development. These minerals play synergistic roles in iron metabolism and the synthesis of hemoglobin, and their deficiencies can further compromise red blood cell production and overall maternal health [13, 14].

Borderline micronutrient deficiencies, especially in early pregnancy, can exacerbate anemia later in gestation, suggesting that early nutritional status may predict later risks. This study examines the prevalence of vitamin A, iron, zinc, and copper among anemic and non-anemic pregnant women and their association with anemia severity.

MATERIALS AND METHODS

Study Population

The cross-sectional study was carried out at maternity centre, Gonda Medical centre, Gonda and the experimental work was done in the Department of Biochemistry, Dr. Ram Manohar lohia institute of Medical Sciences, Lucknow. Total 200 pregnant women was recruited in this study. Based on the hemoglobin status women were divided into two groups: anaemic pregnant women (n=121) and non-anaemic pregnant women (n=79). The classification of anemia followed the WHO guidelines, categorizing the women into Mild anemia (Hb: 9-11g/dl), Moderate anemia (Hb: 7-9g/dl), and Severe anemia (Hb: <7g/dl) [15].

Ethic

The study was approved by the Institutional Ethical Committee (IEC) of NIMS University, Rajasthan, Jaipur (Ref. NIMSUR/URC/2024/400). It adhered to ethical guidelines as per the Helsinki Declaration. Informed consent was obtained from all participants, ensuring they understood the study's purpose, procedures, and potential risks. Participants were assured of their right to withdraw at any time without consequence, and their confidentiality was strictly maintained throughout the study.

Sample collection and processing

Sample was collected during the first visit to the maternity centre at Gonda Medical centre Blood sample (5mL) was collected by veinpuncture: 2 mL in EDTA vial and 3 mL in a plain vial for serum. Serum sample was collected after centrifugation at 3500 rpm for 15 minutes. EDTA sample was used to analysed the CBC while, serum sample was used to analyse the essential minerals.

Estimation of complete blood count (CBC)

The complete blood count (CBC) was measured using a fully automated 5-part differential hematology analyzer, which provided detailed counts of red blood cells, white blood cells (with differentiation into neutrophils, lymphocytes, monocytes, eosinophils, and basophils), haemoglobin concentration, platelet count.

Estimation of Iron, zinc, and copper:

The essential metals were estimated using the protocol of Singh et al. (2025) [16] on an Inductively Coupled Plasma Mass Spectrometry (ICP-MS) analyzer.

Preparation of diluent: A diluent was prepared using a mixture of 0.5% HNO3, 0.05% triton-X, 2% methanol, and internal standard (scandium, germanium, and yttrium).

Standards and sample dilution and calculation: Standards (stock 1000 µg/dL) were diluted using the ready-to-use diluent to prepare a stock solution of 1000 µg/L. Subsequently, serial dilution was prepared for calibration, i.e., 2, 5, 10, 25, 50, 100, and 500 ppb, respectively. A 100 µL serum sample was mixed with 100 µL internal standards (scandium, germanium, and yttrium) and made up to 5 mL using the diluent (Fig. 1). The following formula was used to calculate the elements: Elements (µg/L) = (Value in ppb – blank value) × 50 (dilution factor).

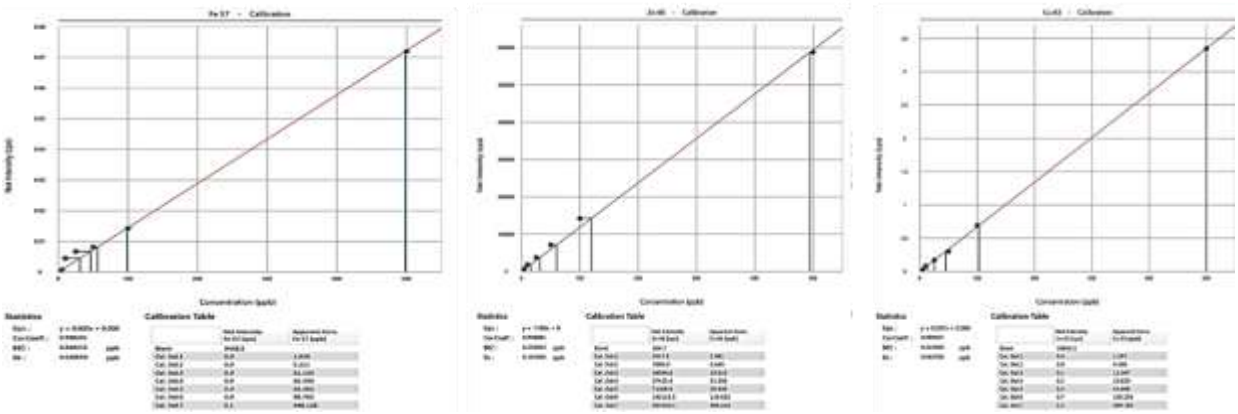

Fig. 1: Calibration curve of Iron, zinc, and copper on ICPMS

Instrument setup: The diluent (a mixture of 5% HNO3, 0.05% triton-X and 2% methanol) was used as a carrier and rinse solution, excluding the internal standards. The sample introduction rate was 300 µL/min, the spray chamber temperature was 2°C, and the mass spectrometry analysis was conducted in collision mode (collision-induced dissociation). Helium was used as a collision gas due to its inertness and low atomic mass, which allows for efficient collision-induced dissociation without interfering with the analyte ions.

Estimation of Vitamin A

Vitramin A was estimated using Liquid chromatography tandem mass spectrometry (LCMS/MS).

Sample preparation

A 500µL serum sample was used to measure vitamins A using LC-MS/MS. First, Protein precipitation was done using a 0.125 mL of 0.1 M trichloroacetic acid concentration. The supernatant was separated, and fat-soluble vitamins were extracted using 1 mL hexane and centrifugated, then evaporated using a nitrogen evaporator. The residue was reconstituted in 100 µL of mobile phase. The residue was reconstituted in 100 µL of mobile phase, consisting of water (phase 1) and methanol (phase 2), modified with 0.5 mM ammonium formate. The mixture was sonicated for 30 seconds to ensure complete dissolution. The final solution was transferred to an autosampler vial for injection into the LC-MS/MS system.

Instrumental Conditions

The analysis used an AB Sciex UPLC system with a triple quadrupole 5500+ MS/MS (Sciex, Framingham, MA, USA). The ionization interface was operated in positive ion mode with the following settings: curtain gas at 50 PSI, collision-activated dissociation gas at 10 psi, a temperature of 450°C, nebulizer gas at 50 psi, and turbo ion spray voltage at 4500 V. Chromatographic separation was performed using a C18 2.5-µm analytical column (Kinetex, 2.1 × 50 mm) maintained at 40°C. The residue was reconstituted in 100 µL of mobile phase, consisting of water (phase 1) and methanol (phase 2), modified with 0.5 mM ammonium formate. The mixture was sonicated for 30 seconds to ensure complete dissolution. Serial dilutions were made to establish a calibration curve with levels ranging from 0.0, 0.5, 1, 2, 5, and 10 ng/L (Fig.1 & 2). Quality control was maintained by analysis of standard reference materials (National Institute of Standards and Technology, NIST, Gaithersburg, MD, USA).

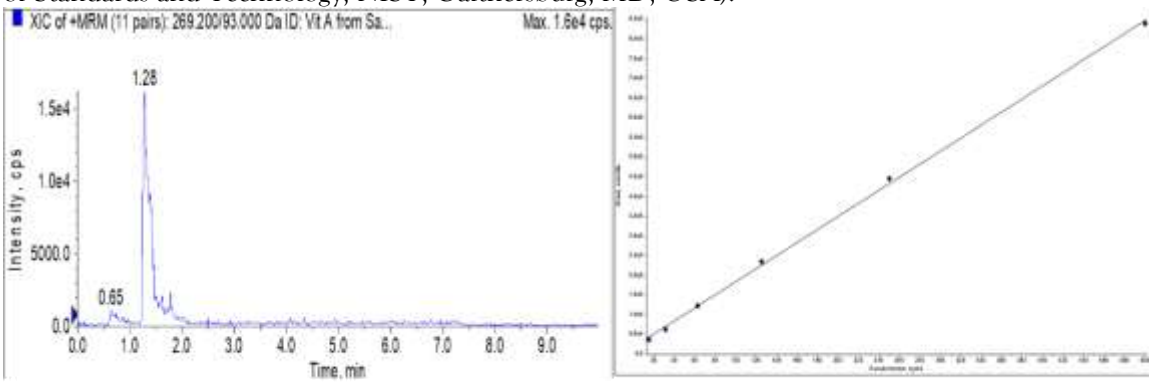

Fig. 2: Chromatogram and Calibration curve of Vitamin A on LCMS/MS

Data analysis

Variables	Anaemic (Hb<11) N=121 (Mean±SD)	Non-anaemic (Hb>11) N=79 (Mean±SD)	p-value
Copper (µg/L)	898.95±755.78	1056.56±833.86	0.002*
Zinc (µg/L)	397.92±205.34	578.23±507.44	0.006*
Iron (µg/dL)	53.35±30.85	63.40±36.04	0.036*
Vitamin A (pg/mL)	506.24±223.42	662.52±220.15	0.0001*

Data were presented as mean and standard deviation for continuous variables and as number and percentage for categorical variables. The Student's t-test and Chi-square test were used to assess statistical differences between anaemic and non-anaemic pregnant women. Pearson correlation analysis was performed to examine the relationships between variables. Data were analysed using SPSS software version 24 (IBM Corp., Chicago, USA), and graphs were prepared using GraphPad Prism software. A p-value of <0.05 was considered statistically significant.

RESULT

Variables	Anaemic (Hb<11) N=121 (Mean±SD)	Non-anaemic (Hb>11) N=79 (Mean±SD)	p-value
Age (Years)	26.49±3.99	26.88±3.76	0.490
Hb(g/dL)	9.81±0.90	11.82±0.53	<0.0001*
FHR (bpm)	139.41±6.68	140.14±6.58	0.448
POG (weeks)	29.27±3.76	29.93±4.19	0.240
TLC (cells/mm^3)	11.68±10.21	16.78±7.60	0.002*
N (%)	68.38±6.27	68.50±11.12	0.922
L (%)	26.21±6.47	26.52±6.79	0.745
E (%)	1.97±0.56	2.14±0.95	0.113
M (%)	3.49±1.03	3.48±0.90	0.943
PC (cells/mm^3)	319782.61±475431.75	209227.27±154230.85	0.047*

Demographical and CBC status among anemic and non-anemic women

No significant differences were found in age, gestation week, and FHR. The hemoglobin level was significantly reduced in the anemic pregnant women than the non-anemic pregnant women (p<0.0001). The TLC count was higher in non-anaemic pregnant women than the pregnant women (p=0.002). There were no significant differences were found in differential leucocyte counts (p>0.05) (Table 1).

Table 1: Demographical and clinical variables of study population

Abbreviations: FHR: Fetal heart rate, DLC: Differential leucocyte counts, PC: Platelet counts. The student t-test was used to calculate the p-value. *p-value <0.05 was considered as statistically significant.

Status of nutritional differences among anemic and non-anemic women

Table 2 evident the status of vitamin A, zinc, copper, iron levels among anemic and non-anemic pregnant women. The vitamin A, zinc, iron, and copper were significantly reduced in anemic women as compare to non-anemic women (p<0.01).

Table 2: Comparison of iron, zinc, copper, and vitamin A among anaemic and non-anaemic pregnant women

The student t-test was used to calculate the p-value. *p-value <0.05 was considered as statistically significant.

Status of nutritional markers Levels and Severity of Anemia in Women

Zinc levels decreased from mild anemia (523.87 ± 198.98 vs. 143.38 ± 21.56 µg/L, p=0.01) to severe anemic and from moderate to severe anemia (323.41 ± 65.53 vs. 143.38 ± 21.56 µg/L, p<0.0001). Similarly, Iron levels exhibited the most dramatic decline in mild anemia (81.08 ± 6.81 vs. 48.38 ± 6.98 µg/dL, p<0.0001) compared to moderate anemia and decrease in severe anemia (27.9 ± 4.11 µg/dL) compared to mild anemia (p < 0.0001). Copper levels showed a significant decrease in severe anemia (234.01 ± 23.38 µg/L, p < 0.0001) in compare to mild anemia. Vitamin A levels were decreased

progressively with severity of anemia; women with severe anemia had lower levels (344.68 ± 63.98 pg/mL, p<0.0001), than the moderate anemia (1034.78 ± 293.83 pg/mL) and mild anemia (1270.32 ± 67.15 pg/mL) (Fig. 3).

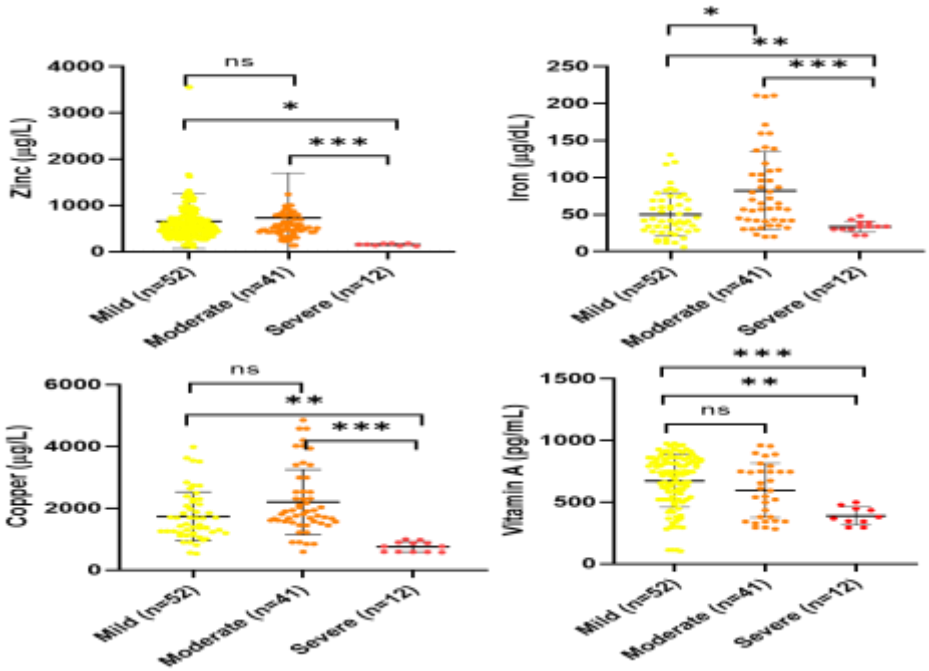

Fig. 3: Status of iron, zinc, copper, and vitamin A based on anaemic severity. **0.001, *<0.0001, ns: Not significant. p<0.05 was considered as statistically significant.**

Correlation of hemoglobin with essential metals and vitamins

Hb was positively correlated with iron (r=0.38; p=0.001), zinc (r=0.32; p<0.0001), and copper (r=0.27; p<0.0001), and vitamin A (r=0.29; p=0.019) (Fig. 4).

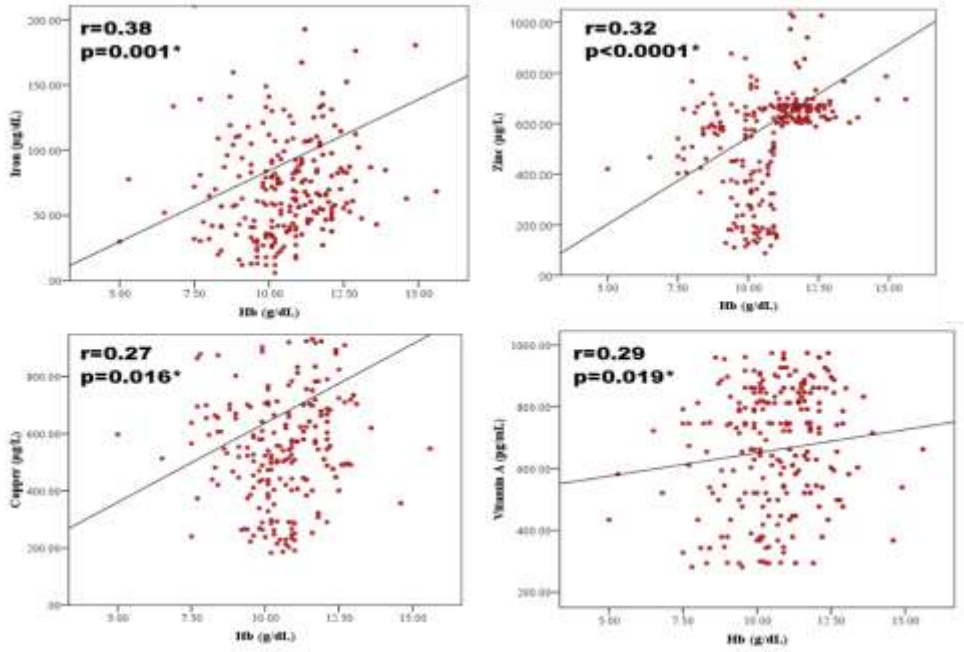

Fig 4.: Correlation of hemoglobin with iron, zinc, copper, and vitamin A. *p<0.05 was considered as statistically significant.

DISCUSSION

This study highlights the complex association between anemia, vitamin, A, iron, zinc, and copper among pregnant women in low-resource settings. Women with anemia showed significantly lower iron, zinc, copper, and vitamins A. There was no significant difference found in age, FHR, and POG. The iron, zinc, copper, and vitamin A were significantly reduced in anemic and non-anemic pregnant women.

Anemic women had significantly lower hemoglobin (Hb) levels (<10 g/dL) compared to non-anemic women (12 g/dL), aligning with global data that identifies iron deficiency as the leading cause of anemia in pregnancy [17].

A correlation was observed between Hb and iron levels, with anemic women demonstrating significantly lower iron levels. Iron is a critical component of hemoglobin, and its deficiency leads to impaired erythropoiesis, resulting in microcytic, hypochromic red blood cells that cannot effectively carry oxygen to tissues [18].

In addition to iron, our study found that deficiencies in other micronutrients, including vitamin A was significantly associated with anemia. The vitamin A status in anemic women was lower than in non-anemic women, supporting its role in iron mobilization and erythropoiesis. Vitamin A is essential for the efficient utilization of iron for hemoglobin synthesis, and its deficiency impairs these processes, contributing to anemia [19,20].

Iron deficiency, a key driver of anemia, emerged as the most significant factor, with anemic women exhibiting drastically reduced iron levels. This finding reinforces the need for targeted iron supplementation in at-risk populations [21,22].

Zinc deficiency was also associated with anemia, vital in heme synthesis and red blood cell maturation [23,24]. Its deficiency was observed in anemic women. Copper is essential for iron metabolism, aiding in the conversion of iron to its ferric form for hemoglobin synthesis [25]. Thus, iron, zinc and copper, often overlooked, are crucial for maintaining normal erythropoiesis and preventing anemia.

Among severity of anemia, iron, zinc, copper, and vitamin A were significantly lower in severe anemic pregnancy than the moderate and mild. In addressing anemia, the interplay among iron, vitamin A, zinc, and copper is crucial: iron is the core component of hemoglobin and its absorption from the small intestine relies on transporters like DMT1; once absorbed, iron must be oxidized to Fe^{3+} by copper-dependent enzymes, hephaestin in enterocytes and ceruloplasmin in the bloodstream, to bind transferrin and reach the bone marrow for erythropoiesis [26-28]. Vitamin A enhances iron mobilization from storage sites and improves hemoglobin synthesis, thereby synergistically reducing anemia when combined with iron supplementation [29,30]. Zinc affects this balance in multiple ways: deficiency may contribute to anemia, while excessive zinc intake can impair copper absorption by inducing metallothionein in enterocytes, leading to copper deficiency and, consequently, compromised iron transport and utilization. Together, these nutrients form a tightly linked network, adequate vitamin A and copper support iron's availability and use, but imbalance, especially due to excess zinc, can disrupt the system and precipitate or worsen anemia [31,32].

Thus, anemia in pregnancy is a multifactorial condition driven by interactions between iron, zinc, copper, and vitamins A. The absorption and bioavailability of iron are influenced by other micronutrients, which enhances iron absorption, and vitamin A, which facilitates iron mobilization [33]. Moreover, deficiencies of zinc and copper can disrupt iron metabolism, contributing to anemia [34,35]. The complex interactions among these micronutrients highlight the importance of a balanced intake to prevent and manage anemia effectively. The study has some limitation like this study's focus on a specific low-middle population in India may limit the generalizability of the findings to other populations. Additionally, the lack of data on dietary intake and genetics makes it difficult to account for the multifactorial nature of anemia in pregnancy.

CONCLUSION

This study underscores the importance of targeted interventions, particularly for iron, in managing anemia among pregnant women. The interrelationship between vitamins A and iron, zinc, copper is critical for preventing and managing anemia during pregnancy. These nutrients support red blood cell production, immune function, and fetal development. Deficiencies in these nutrients can disrupt hematopoiesis, leading to anemia and significant maternal and fetal health risks in low-resource settings.

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