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Investigating the relationship between serum zinc levels and red blood cell indices in anemic patients referred to the laboratories in Gorgan, Iran

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Abstract

Background: Bivalent minerals function as crucial cofactors that participate in a multitude of metabolic pathways within the organism. Specifically, zinc (Zn) assumes catalytic, structural, and regulatory roles in numerous biological processes. A severe deficiency in Zn can lead to disruptions in nucleic acid and protein synthesis, impaired cellular proliferation, increased apoptosis, and heightened lipid peroxidation of cellular membranes, a phenomenon associated with a reduced lifespan of red blood cells (RBCs). The objective of this study was to investigate the correlations between Zn status and various erythrocyte indices in a cohort of anemic patients, in comparison to a control group.

Methods: A cohort of 563 participants was enrolled in this investigation. Serum Zn concentration was quantified using a BT-3500 autoanalyzer, while hematological indices were determined via a Sysmex KX21N cell counter. Following confirmation of data normality, Spearman's rank correlation coefficient was employed to analyze the relationship between serum Zn levels and RBC indices.

Results: The mean serum Zn concentration was 102.8 ± 17.6 mg/dL. Serum Zn levels exhibited a weak correlation with RBC and hemoglobin (Hb) concentrations in healthy women, as well as a weak correlation with mean corpuscular hemoglobin concentration (MCHC) in anemic men ($p < 0.05$). Furthermore, the results indicated significantly higher serum Zn levels, RBC, Hb, hematocrite (HCT), and MCHC in men ($p < 0.01$), while mean corpuscular volume (MCV) was significantly higher in women ($p < 0.01$). Notably, in individuals with serum Zn levels < 30 mg/dL, MCHC ($p < 0.01$) and RBC ($p < 0.05$) were elevated, whereas Hb ($p < 0.05$), HCT, MCV, and MCH ($p < 0.01$) were higher than 30.

Conclusion: Considering the potential impact of varying Zn concentrations on erythrocyte indices, including Hb and MCHC, in both healthy and anemic individuals, careful regulation of its dosage is warranted.

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Introduction

Zinc (Zn) stands as a crucial micronutrient for human physiology. Functioning as a catalytic, structural, and regulatory ion, Zn participates in diverse metabolic processes (1). It is indispensable for the stabilization of cell membranes and the proper development of tissues (2), as well as for maintaining immune competence. Furthermore, Zn is a vital component of structural proteins and transcription factors, and it acts as a cofactor in various metalloenzymes, including carbonic anhydrase, alcohol dehydrogenase, carboxypeptidase, superoxide dismutase, and reverse transcriptase (3). Studies indicate a significant prevalence of Zn deficiency exceeding 20% in low- and middle-income countries (4). This micronutrient inadequacy is implicated in a range of adverse health outcomes, including impaired wound healing, osteoporosis (5,6), epithelial integrity, mental health disorders (7), delayed skeletal maturation, growth retardation, and hair fragility. It also disrupts glucose metabolism and contributes to structural and physiological abnormalities, such as the degradation of certain structural proteins, insufficient growth, and a weakened immune system (8). Zn deficiency is a prevalent issue in individuals with thalassemia major, arising from hemolysis and the resultant iron overload. This is further exacerbated by diminished intestinal absorption and increased excretion of Zn due to the use of iron-chelating agents (9). Furthermore, Zn deficiency exhibits a notable association with the development of diabetes mellitus in this patient population. The pathogenesis of this diabetes involves iron overload, which precedes a reduction in insulin sensitivity (10). Subsequently, the decline in Zn levels contributes to impaired insulin production, thereby increasing the susceptibility of these patients to diabetes (11). Within the physiological processes of iron metabolism, Zn functions as a catalytic cofactor for the enzyme α-aminolevulinate dehydratase (12,13). The activity of this enzyme is implicated in influencing the growth and proliferation of hematopoietic stem cells and megakaryocytes through the modulation of gene expression and transcriptional regulation during erythropoiesis, as well as by promoting the proliferation of immature erythroblasts (7). Consequently, the administration of Zn supplements may offer a viable approach to improving anemic conditions and alleviating the associated pathological effects (14). Prior research indicates that both excessive elevations and reductions in Zn levels are implicated in the development of anemia and impaired hemoglobin (Hb) synthesis (15). Red blood cell (RBC) indices serve as diagnostic parameters for evaluating the size, morphology, and physical attributes of RBCs. These indices include the total RBC count, the mean corpuscular volume (MCV), which reflects

the mean size of individual RBCs, and mean corpuscular hemoglobin (MCH), which quantifies the mean amount of Hb within each RBC. The mean corpuscular hemoglobin concentration (MCHC), representing the mean Hb concentration within RBCs relative to their volume, and the red blood cell distribution width (RDW) are significant hematological parameters employed in the diagnosis and screening of anemia (16). Assessment of RBC size variation necessitates the evaluation of the MCV in conjunction with the RDW index. Hb levels are susceptible to alterations in various pathological states, including infection, inflammation, hemorrhage, pharmacological interventions, etc. (RDW), which is measured in the complete blood count (CBC). In addition, it serves as an evaluation metric of erythrocytes based on their dimensions and is recognized as a prognostic indicator for mortality arising from pathological states, including cardiovascular disease, critical illnesses, and bacterial infections (17). The present research aims to determine the impact of Zn on erythrocyte indices in anemic patients, in comparison to a control cohort.

Methods

In this study, a cohort of 563 participants who presented at Kavosh Medical Laboratory in Gorgan, Iran, and requested CBC and Zn assays were enrolled. Following the fulfillment of requisite criteria, a 5 mL venous blood sample was collected from each individual. Subsequently, hemolysis-free specimens were aliquoted into separate CBC-specific tubes and gel-containing tubes for hematological and biochemical analyses, respectively. The biological samples underwent centrifugation using a Universal centrifuge (Model BH-1200, manufactured in the Netherlands). Subsequently, Zn concentrations were quantified employing a BT-3500 autoanalyzer (Biotecnica Instruments, Italy). Hematological parameters, including RBC, Hb, HCT, MCV, MCH, MCHC, white blood cell (WBC) 1 count, and platelet count (PLT), were determined utilizing a Sysmex KX21N cell counter (Japan).

Data were collected, coded, and subsequently entered into a computer database. Statistical analysis was performed using SPSS version 16.00. Initial analysis involved assessing the normality of the dataset via the Shapiro-Wilk test. Statistical significance was defined as $P > 0.05$. Participants were further categorized into healthy and anemic groups based on their Hb, RBC count, and HCT levels. To determine the relationship between serum Zn levels and hematological markers, a correlation analysis was conducted utilizing the Spearman rank correlation coefficient. The study protocol received ethical approval from the Ethics Committee of Golestan University of Medical Science (IR.GOUMS.REC.1397.25).

Results

This study examined 563 cases, and the demographic data revealed that 35.8% (n = 201) of the participants were male, while 64.2% (n = 362) were female (Figure 1A). Subsequently, the male and female subjects were each stratified into two distinct groups: A healthy group and an anemic group. This classification was based on Hb, HCT, and RBC levels (Figure 1B).

The Shapiro-Wilk test results indicated a normal distribution of the data. Consequently, a one-way analysis of variance (ANOVA) was employed for data analysis. Given a mean participant age of 30 years, the sample was stratified into two groups: Age < 30 and age > 30. Descriptive statistics, including the mean and standard deviation, alongside the outcomes of the one-way ANOVA for these two age cohorts, are presented in Table 1.

The independent samples t-test results demonstrated statistically significant differences ($p < 0.05$) between male and female groups for Zn levels and erythrocyte indices, with the exception of MCH, as detailed in Table 1.

The independent samples t-test revealed a statistically significant difference ($p < 0.05$) in both Zn levels and erythrocyte indices between the two age groups examined (age < 30 and age > 30), as detailed in Table 1.

Pearson's correlation analysis revealed a statistically non-significant association between serum Zn concentrations and erythrocyte indices within the male cohort ($p > 0.05$). Conversely, in the female cohort, a statistically significant correlation was observed between serum Zn levels and MCHC ($p < 0.05$, Table 2). Based on the correlation analysis in healthy women, serum Zn levels exhibited a significant association with RBC, Hb, and MCHC ($p < 0.05$). In anemic men, serum Zn demonstrated a significant correlation with MCHC and PLT ($p < 0.05$). Conversely, in healthy men and anemic women, no significant correlations were found between serum Zn and any erythrocyte indices ($p > 0.05$, Table 2).

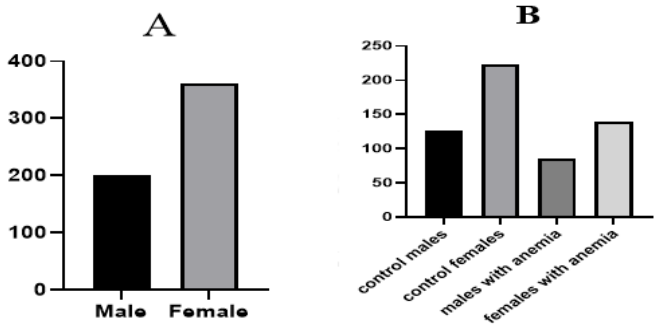

Figure 1. Frequency of males and females in the control and anemic groups in the study

Table 1. Significant differences in the studied variables

Variable (Unit)	Gender			Age		
	Male	Female	P-Value	< 30	> 30	P-Value
Zn (mg/dL)	106.10±16.57	101.59±17.89	0.007*	105.48±20.94	100.24±13.29	0.000*
RBC ($\times 10^6/\mu L$)	4.81±0.55	4.45±0.415	0.000*	4.59±0.47	0.49±4.50	0.033*
WBC ($\times 10^3/\mu L$)	7.73±2.15	7.26±2.02	0.018*	7.78±2.38	7.01±1.63	0.000*
Hb (g/dL)	13.60±1.51	12.67±1.05	0.000*	12.78±1.18	13.05±1.33	0.012*
HCT (%)	39.54±4.59	37.40±2.99	0.000*	37.33±3.5	38.6±3.63	0.000*
MCH (pg)	28.41±2.86	28.66±2.97	0.359	28.03±2.8	29.13±2.97	0.000*
MCHC (%)	34.42±1.07	32.52±7.99	0.000*	34.26±1.06	33.79±0.97	0.000*
MCV (μm^3)	33.87±0.99	84.52±8.11	0.009*	81.7±7.55	86.16±8.06	0.000*

Zn: Zinc; RBC: Red Blood Cell; WBC: White Blood Cell; Hb: hemoglobin; HCT: Hematocrit; MCH: Mean Corpuscular Hemoglobin; MCHC: Mean Corpuscular Hemoglobin Concentration; MCV: Mean Cell Volume.

Table 2. The correlation between erythrocyte indices and serum Zn level in gender and age group

Variable (Unit)	Control				Anemia			
Zn (mg/dL)	Male		Female		Male		Female	
	r	P-value	r	P-value	r	P-value	r	P-value
WBC ($\times10^3$/µL)	0.162	0.125	0.133	0.083	0.114	0.408	- 0.093	0.397
RBC ($\times10^6$/µL)	0.17	0.106	0.167	0.029*	0.155	0.258	0.068	0.521
HB (g/dL)	0.158	0.135	0.255	0.001*	0.09	0.512	- 0.009	0.992
HCT (%)	0.055	0.604	0.134	0.081	0.121	0.38	0.014	0.892
MCH (pg)	- 0.097	0.363	- 0.088	0.256	- 0.155	0.258	- 0.046	0.666
MCHC (%)	0.094	0.375	0.199	0.009*	- 0.31	0.021*	0.026	0.810
MCV (µm³)	- 0.105	0.363	- 0.132	0.085	- 0.106	0.441	- 0.058	0.585
PLT ($\times10^3$/µL)	- 0.029	0.789	0.073	0.347	- 0.289	0.032*	0.177	0.093
Iron (mg/dL)	0.083	0.489	0.093	0.317	0.269	0.054	0.054	0.66
Ferritin (mg/dL)	0.103	0.378	0.052	0.528	0.01	0.998	- 0.005	0.969

Zn: Zinc; WBC: White Blood Cell; RBC: Red Blood Cell; Hb: hemoglobin; HCT: Hematocrit; MCH: Mean Corpuscular Hemoglobin; MCHC: Mean Corpuscular Hemoglobin Concentration; MCV: Mean Cell Volume; PLT: Platelet Count.

Discussion

Zn is a structural component in numerous enzymes and metalloproteases. It participates in heme synthesis by contributing to the enzymatic activity of alpha-aminolevulinic acid dehydratase (13). Furthermore, Zn is integral to the structure of the growth factor independence 1B (GFI-1B) Zn finger protein. GFI-1B acts as a crucial regulator in erythroid cell development by modulating gene expression specific to the erythroid lineage. It plays a role in transcriptional regulation during erythropoiesis, promotes the proliferation of immature erythroblasts, and supports normal erythropoiesis potentially through its involvement in the sequential development of hematopoietic stem cells and megakaryocytes (18). The long-established role of Zn deficiency in disrupting iron metabolism is well-documented (19). Zn is understood to facilitate the removal of excess copper by stimulating the production of enterocyte metallothionein. This protein exhibits a specific affinity for copper, thereby inhibiting its intestinal absorption and promoting its excretion. Consequently, Zn supplementation is employed in the therapeutic management of Wilson's disease (20,21). However, clinical observations have indicated a more pronounced severity of anemia in patients undergoing treatment with high-dose Zn regimens (22,23).

The mean concentrations of RBC, WBC, Hb, HCT, and MCHC were observed to be lower in women compared to men. Furthermore, a statistically significant correlation was found in women between Zn levels and the MCHC index. In healthy women, serum Zn demonstrated a significant positive correlation with RBC, Hb, and MCHC. Consistent with these findings, a study conducted by Pilch under the supervision of the Food and Agriculture Organization (FAO) between 1976 and 1980 reported higher mean serum Zn levels in men than in women (24). While our study indicated a higher mean serum Zn level in females, it is important to acknowledge that the typical reference interval for plasma Zn concentration is generally broader than that for serum Zn concentration. Furthermore, a number of studies have reported that the mean Zn concentration is significantly lower in male subjects than in female subjects (25,26), a finding that aligns with the results obtained in our current study. Plasma or serum analysis represents the most frequently employed diagnostic assay for Zn status due to its straightforward methodology and widespread availability in clinical laboratories. However, it is crucial to note that a marginal reduction in serum Zn concentration does not invariably indicate a true Zn deficiency. This is evidenced by observations of normal serum Zn levels in individuals with acrodermatitis enteropathica, and conversely, the presence of lower serum Zn levels in the absence of a demonstrable deficiency (27). On the other hand, hair and plasma Zn assays serve as valuable tools for identifying population subgroups at elevated risk of marginal Zn deficiency (28) and represent a reliable and practical measure of the body's readily available Zn reserves (29). Several factors contribute to Zn deficiency, particularly among older adults, including diminished intestinal absorption, inadequate nutrition, the use of diuretics, and medications prescribed for diabetes (30). The findings of this study demonstrated a statistically non-significant correlation between Zn concentration and erythrocyte indices in a cohort of healthy men and women presenting with anemia. Furthermore, the investigation revealed the absence of any linear or non-linear relationship between erythrocyte parameters and body surface area. This study demonstrated that Zn had a significant correlation with Hb, RBC, and MCHC in women within the control group. Additionally, Zn was found to have a significant correlation with both MCHC and PLT in anemic men. In a study, Toida T et al. (2020) investigated the correlation of serum Zn levels with hematological parameters and parathyroid hormone concentration in patients undergoing hemodialysis and reported the absence of a statistically significant correlation between Zn concentration and hematological parameters. Their findings suggest an inverse relationship between advancing age and serum Zn levels, as well as RBC count, which is subsequently followed by a reduction in MCHC (31). Consequently, the efficacy of Zn supplementation in managing anemia is deliberated, and future research employing larger sample sizes is advised.

Conclusion

Anemia constitutes a significant global health concern, demonstrably diminishing the life quality of patients. Concurrently, Zn is recognized as an essential micronutrient obtainable through dietary intake and pharmaceutical supplementation. This research revealed a positive correlation between Zn levels and key erythrocytic indices, including Hb, RBC, and MCHC, in a cohort of healthy women. Furthermore, a positive association was observed between Zn and MCHC in male subjects diagnosed with anemia. Consequently, it is proposed that the judicious administration of Zn may serve to mitigate the adverse effects of anemia in patients.

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Ethical statement

The protocol of this study was ethically approved by the Ethics Committee of Golestan University of medical science (IR.GOUMS.REC.1397.259).

Conflicts of interest

The authors declare that they have no conflict of interest.

Author contributions

All authors contributed equally in the present research.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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