Investigating the relationship between serum zinc levels and Red blood cell indices in anemia patients referred to the laboratories of Gorgan city

Running title: Serum Zinc and RBC Indices in Anemic Patients

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Abstract:

background: Bivalent minerals have an important role as cofactors, which play vital roles in various metabolic pathways in the body. Zinc (Zn) has catalytic, structural, and regulatory roles. Severe Zn deficiency may cause the abnormal synthesis of nucleic acids and proteins, impaired cellular growth, excessive cell death, and excess lipid peroxidation of the cellular membrane, which is associated with shortening the red blood cell (RBC) lifespan. This study aimed to examine the associations between Zn status and the erythrocyte indices in anemia patients compared to the control group.

Methods: A total of 563 individuals were included in this study. The level of serum Zn was measured by a BT-3500 autoanalyzer, and blood index values were measured by using a cell counter Sysmex KX21N. After ensuring the normality of the data, Spearman's test was used to investigate the relationship between Zn and RBC indices.

Results: The average serum Zn level was 102.8±17.6 mg/dl. Serum Zn level had a weak relationship with RBC and Hb in healthy women and a weak relationship with MCHC in men with anemia(p-value<0.05). These results also determined that serum Zn level, as well as the levels of RBC, Hemoglobin (Hb), Hematocrit (HCT), and Mean Corpuscular Hemoglobin Concentration (MCHC), were significantly higher in men (Sig<0.01) but the level of MCV among women was higher (Sig<0.01). Moreover, in individuals with <30 serum Zn level, MCHC (Sig<0.01) and RBC (Sig<0.05) were higher, whereas Hb (Sig<0.05), HCT, Mean Cell Volumes (MCV), and MCH (Sig<0.01) were higher than 30.

Conclusion: Since different amounts of zinc can affect erythrocyte indices such as hemoglobin and MCHC in healthy and anemic groups, its dosage should be under control

Keywords: Zinc, Erythrocyte, Mean Cell Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC)

Introduction

Zinc (Zn) is one of the essential micronutrients for humans. Zn as a catalytic, structural, and regulatory ion plays different roles in metabolic activities(1), it is required for cell membrane stabilization, growth of normal tissues(2), immunity and is also an important part of structural proteins, transcription factors and acts as a cofactor in metalloenzymes such as (carbonic anhydrase, alcohol dehydrogenase, carboxypeptidase, superoxide dismutase, reverse transcriptase enzymes) (3). Studies have shown that the prevalence of Zn deficiency in Low- and Middle-income Countries is more than 20% (4). Zn deficiency is involved in wound healing disorders, osteoporosis(5, 6), epithelial integrity, mental health(7), delayed skeletal maturation, growth retardation, brittle hair, impaired glucose metabolism, structural and physiological disorders such as degradation of some structural proteins, insufficient growth, weakening of the immune system(8). Zn deficiency in patients with thalassemia major is also common due to hemolysis and overload caused by iron and low absorption and high excretion due to iron chelators(9) .Zn deficiency is also associated with diabetes in patients with thalassemia major. It occurs due to iron overload followed by a decrease in insulin sensitivity(10). And following the reduction of Zn, the production of insulin decreases, and the patient becomes prone to diabetes(11) .In iron metabolism, Zn acts as a catalyst for the enzyme alpha-aminolevulinic acid dehydratase(12, 13), which has a potential effect on the growth and proliferation of hematopoietic stem cells and megakaryocytes by regulating gene expression, regulating transcription during erythropoiesis, and enhancing the proliferation of immature erythroblasts(7). Zn supplements can be effective in improving anemia and reducing the effects of this disease(14). According to previous studies, excessive increases and decreases of Zn are involved in anemia and impaired hemoglobin synthesis (15). Red blood cell (RBC) indices examine their size, form, and physical characteristics of RBCs including Number of RBC, The average volume of red blood cells which indicates the size of red blood cell(MCV), The average hemoglobin of red blood cells which shows the amount of hemoglobin in each red blood cell(MCH), The average hemoglobin concentration of red blood cells, which is the amount of hemoglobin relative to cell size or the concentration of hemoglobin per red blood cell (MCHC), the red cell distribution width (RDW), These indicators are important parameters for the diagnosis and screening of anemia(16). To evaluate red blood cell size changes, the MCV index should be checked along with the RDW index. Hemoglobin can change in various pathological conditions such as infection, inflammation, bleeding, medication, etc. (RDW), which is measured in the complete count of blood cells (CBC) It is used to evaluate erythrocytes based on their size and is considered as an indicator for mortality due to pathological conditions such as heart disease, and critical diseases and bacterial infections(17). In this research, we are looking for the effect of Zn on erythrocyte indices in patients with anemia compared to the control group.

Methods

In this study, 563 individuals referred to Kavosh Medical Laboratory in Gorgan who requested CBC and Zn tests participated. Initially, 5 cc venous blood samples were taken from the individual after meeting the necessary conditions. In the next step, free hemolysis samples were placed in CBC and gelled tubes separately For Biochemical and Hematological tests. The samples were centrifuged with the Universal centrifuge (type BH-1200/ Netherlands), and Zn levels were assessed by a BT-3500 autoanalyzer (Biotecnica Instruments/Italy). Hematological parameters, including RBC, hemoglobin, hematocrit, MCV, MCH, MCHC, WBC, and platelets, were determined by the cell counter Sysmex KX21N/ Japan.

Data were collected, coded, and entered into the computer, then SPSS 16.00 statistical software was used for analysis. initially, the normality of the data was measured by the Shapiro-Wilk test, and Data were considered statistically significant at a P value > 0.05. Further, people were divided into healthy and anemic groups according to Hb, RBC, and hematocrit levels. Correlation analysis was performed using the Spearman correlation method to identify the relationship between the serum Zn levels and hematological markers. The protocol of the study was ethically approved by the Ethics Committee of the Golestan University of Medical Science (IR.GOUMS.REC.1397.25).

Result

IIn this study, 563 cases were examined, and according to the demographic information, 35.8 % (201 cases) of the study participants were male and 64.2% (362 cases) were female (Figure 1, A). Then, we divided each of the male and female subjects into two groups, healthy and anemic, based on their hemoglobin, hematocrit, and RBC levels (Figure 1, B).

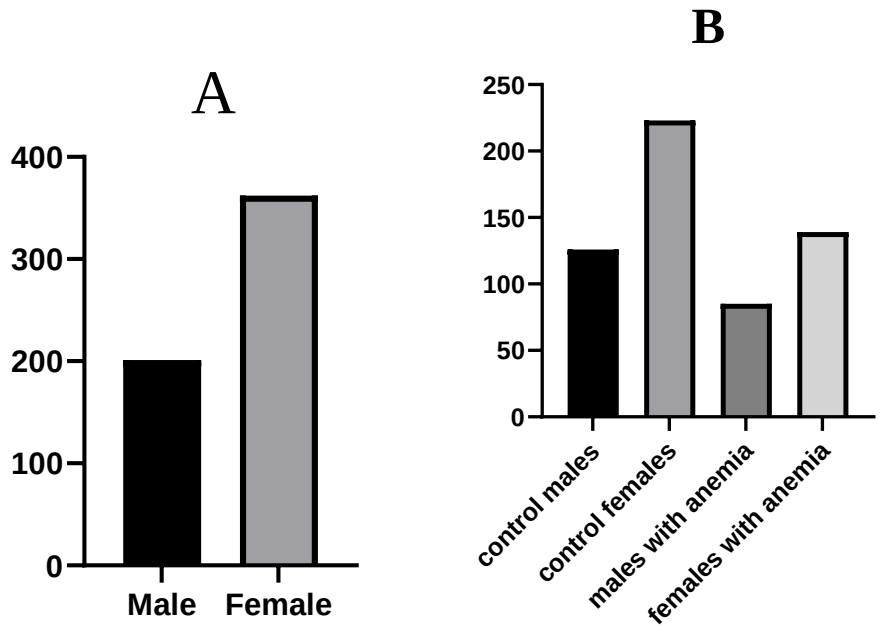

Figure 1. Frequency of males and females in the control and anemia groups in the study

The results of the Shapiro-Wilk test show that the data distribution is normal. Therefore, a one-way analysis of variance test was used to analyze the data. Considering that the average age of the participants was 30 years, the participants were divided into two groups (age<30 and age>30). The mean, standard deviation, and one-way analysis of variance test results in two age groups(age >30 and age<30) are shown in Table 1.

The results of the independent t-test showed that Zn level and erythrocyte indices, except MCH, are significant between male and female groups (p-value<0.05) and are shown in Table 1.

The results of the independent t-test showed that Zn level and erythrocyte indices are significant between the two age groups, less than 30 years old and more than 30 years old (p-value<0.05), and are shown in Table 1.

Table1. Significant differences in the studied variables

Variable	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 (×10^6/µL)	4.81±0.55	4.45±0.415	*0.000	4.59±0.47	0.49±4.50	*0.033
WBC (×10^3/µL)	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

The results of Pearson's correlation test indicate that there was no significant relationship between the Zn serum level and erythrocyte indices in the men's group(p-value>0.05), while these changes in the women's group had a significant relationship between the MCHC value and the Zn serum level (p-value<0.05, Table2). Based on the results of the correlation test in healthy women, serum Zn has a significant correlation with RBC, Hb, and MCHC(p-value<0.05). In men with anemia, serum Zn has a significant correlation with MCHC and platelets(p-value<0.05). Also, in healthy men and women with anemia, serum Zn has no significant correlation with any erythrocyte index (p-value>0.05, Table 2).

Table2. Correlation between erythrocyte indices and serum Zn level in gender and age group

Variable	Control				Anemia			
Zn (mg/dl)	Male		Female		Male		Female	
	r	p-value	r	p-value	r	p-value	r	p-value
WBC (×10^3/µL)	0.162	0.125	0.133	0.083	0.114	0.408	-0.093	0.397
RBC (×10^6/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 (µm^3)	-0.105	0.363	-0.132	0.085	-0.106	0.441	-0.058	0.585
PLT (×10^3/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

Discussion

Zn is present in the structure of many enzymes and metalloproteases. Zn plays a role in heme synthesis due to its role in alpha-aminolevulinic acid dehydratase enzyme activity(13). Zn is additionally found within the structure of the GFI-1B Zn finger protein, which acts as a major controller in erythroid cell development by balancing quality expression specific to the erythroid arrangement, performs transcriptional regulation amid erythropoiesis, bolsters multiplication of immature erythroblasts, and provides normal erythropoiesis by taking a potential part within the serial improvement of hematopoietic stem cells and megakaryocytes(18). The role of Zn deficiency in iron metabolism abnormalities has been known for a long time(19). Zn is known to evacuate overabundance of copper by inducing enterocyte metallothionein, which specially binds copper, prevents its absorption, and enhances its excretion و For this reason, Zn supplements are used to treat Wilson's disease(20, 21) and the anemia was severer in patients receiving high dose Zn treatment (22, 23).

The average level of erythrocyte RBC, WBC, Hb, HCT, and MCHC in women was lower than in men, and in women, Zn level with MCHC index was statistically significant and in healthy women, serum Zn has a significant correlation with RBC, Hb and MCHC. In a study conducted by Pilch during 1976-1980 under the supervision of FAO, mean serum Zn levels were higher in men than in women(24) While in our study, the mean serum level of Zn was higher in women. But it should be noted that the normal range of plasma Zn concentration is always wider than the normal range of serum Zn concentration .Many researchers also stated in their studies that the average concentration of Zn in boys is significantly lower than that in girls(25, 26). which was consistent with our results. Plasma or serum evaluation is the most widely used test and is a very simple technique that is readily available in many laboratories, and a substandard decrease in serum Zn does not necessarily reflect a deficiency, as normal serum Zn levels in acrodermatitis. Enteropathica have observed and lower amounts were also observed without deficiency(27). On the other hand, hair and plasma Zn tests are useful in detecting population groups at risk of mild Zn deficiency(28) and are a valid and useful indicator of replaceable Zn reserves in the body(29). Various factors are involved in Zn deficiency, especially in the elderly, such as reduced intestinal absorption, malnutrition, diuretics, and drugs used in diabetes(30). The results of our study showed that, there is not significant correlation between Zn concentration and erythrocyte indices in healthy men and women with anemia. In other words, it was found that there is no linear or non-linear relationship between erythrocyte parameters and body surface area. This study shown that significant correlation between Zn and Hb , RBC and MCHC in control women. We also found a significant correlation of Zn with MCHC and platelets in men with anemia. In their study in 2018, Abbaspour et al investigated the relationship between serum Zn level and hematological indicators and parathyroid hormone concentration in patients undergoing hemodialysis. They stated that no significant relationship was found between Zn concentration and hematological indicators. The results indicate that with increasing age, the serum level of Zn and the number of red blood cells decrease, followed by a decrease in MCHC content. Therefore, the effectiveness of Zn supplements to control anemia is discussed and studies with a larger sample size are recommended.

Conclusion

Anemia is one of the most important diseases in the world, which reduces the quality of life of patients. On the other hand, Zn is one of the micronutrients found in food and pharmaceutical supplements. This study showed that Zn has a positive correlation on erythrocyte indices such as Hb, RBC and MCHC in healthy women, and on the other hand, it has a positive correlation with indices such as MCHC in men with anemia. Therefore, it is suggested to reduce the negative effects of anemia in patients by taking the exact dose of Zn.

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Conflict of interest

The authors declare that they have no conflict of interest.

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NA.

Ethical statement

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

Author contributions

All authors contributed equally.

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