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Zinc content of normal human serum and its correlation with some hematic parameters

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Numerous studies have demonstrated that several diseases and stress conditions are associated with changes in the levels of zinc in the blood plasma and cellular elements. In this research the association between serum zinc concentrations and other hematic parameters of diagnostic interest has been evaluated. Quantitative determinations of zinc, total plasmatic proteins, albumin, hemoglobin and calculation of mean corpuscular volume were performed on blood samples from 58 males aged 20–61 years. Concentrations measured in our sample are comparable with reference values. Statistically significant correlation coefficients were found between age and albumin ($r = -0.562$, $P < 0.001$), serum zinc and albumin ($r = 0.328$, $P < 0.05$), serum zinc and hemoglobin ($r = 0.291$, $P < 0.05$), and total plasmatic proteins and albumin ($r = 0.463$, $P < 0.001$). These correlation coefficients were significant even after adjustment for age effect. The determination of serum zinc concentration may be useful in the assessment of clinical scenarios. Particularly, it may provide additional information for the diagnosis of specific pathologies, such as hepatic malfunctions. It could also be useful in the identification of different stages of anemia.

Keywords: albumin, hemoglobin, mean corpuscular volume, serum zinc, total plasmatic proteins

Introduction

The total amount of zinc in the human body is about 2–3 g. This element can be found in all tissues with concentrations ranging from 10 to 200 $\mu g\, g^{-1}$ of dry weight. Very high concentrations are found in the choroid of the eye and in the male sex organs (Li & Vallee 1980). Whole blood contains nearly 900 μg zinc 100 ml^{-1}: 75–88% of the total zinc is contained in the erythrocytes, 12–22% in the plasma and 3% in the leukocytes (Vallee & Gibson 1948). Almost all the zinc in the erythrocytes appears to be associated with carbonic anhydrase. The zinc content and carbonic anhydrase activity of red cells are significantly correlated in both normal and pathologic conditions. Both erythrocyte zinc concentration and carbonic anhydrase activity vary with the hematocrit level and the hemoglobin concentration (Li & Vallee 1980). Almost all the zinc in serum is protein bound: 60–80% is loosely bound to albumin (making up a metal–protein complex); 30–40% is firmly bound to an α_2-macroglobulin (forming a metalloprotein) (Parisi & Vallee 1970); 2–8% is associated with transferrin and with free amino acids (Prasad & Oberleas 1970). Albumin seems to be concerned with zinc transport; furthermore, the quantity of free albumin regulates the quantity of zinc entering the organism during zinc absorption in the small intestine (Evans *et al.* 1975). Zinc loosely bound to albumin is exchangeable for uptake by cells. Zinc firmly bound to α_2-macroglobulin is not available for the cells and its role is still unknown. The zinc fraction linked to free amino acids influences the urinary excretion of zinc (Faure *et al.* 1990).

The first human syndrome of zinc deficiency was described in Iranian and Egyptian malnourished boys (Prasad *et al.* 1961, 1963a,b). In those patients with the syndrome of iron deficiency anemia, hepatosplenomegaly, dwarfism and hypogonadism, the zinc content of plasma, erythrocytes and hair was significantly reduced as compared with normal

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subjects. Poor diet, blood loss from parasitic infestations and loss through sweating in a tropical climate were considered possible factors responsible for zinc deficiency.

From then on, numerous studies have demonstrated that several diseases and stress conditions are associated with changes in the levels of zinc in the blood plasma and cellular elements. Plasma zinc levels are depressed in patients with neoplasia, atherosclerosis, post-alcoholic cirrhosis, acute and chronic inflammations. This hypozincemia seems to be due to a flux of zinc from plasma to liver mediated by a hormone-like protein factor which is released by leukocytes. The zinc and carbonic anhydrase levels are decreased, as well as hemoglobin and erythrocyte counts, in patients with anemia (Underwood 1977).

Some causes of zinc deficiency are inadequate dietary intake, malabsorption, increased body losses and intravenous feeding. Some clinical manifestation of zinc deficiency are anorexia, impaired taste and smell, growth retardation, hypogonadism, and skin lesions (Aggett & Harries 1979).

In this research the association between serum zinc concentration and other hematic parameters of diagnostic interest have been evaluated in male blood donors. Serum zinc determination may be useful in the assessment of individual nutritional status and/or of clinical scenarios concerning particular pathologies.

Materials and methods

A random sample of males aged 20–65 years was selected from the blood donor list of the Transfusion Centre of Camposampiero Hospital (Padua). Fifty-eight subjects accepted to participate in our study.

Blood samples were collected from 12–18 h fasting subjects.

After withdrawal, blood samples were allowed to clot in a thermostatic bath at 37°C for 3 h and successively centrifuged at 1500 r.p.m. for 30 min. Serum was collected in glass tubes and stored at 4°C until analysis.

Quantitative analysis of zinc was performed with an atomic absorption spectrophotometer (Model 2380; Perkin-Elmer, Norwalk, CT). For this analysis, serum was diluted 1:5 with bidistilled water (Butrimovitz & Purdy 1977). Instrumental calibration was obtained with standard solutions, prepared by dilution of 1000 p.p.m. Normadose (Prolabo, Paris, France) solution, with concentrations in the range 0.5–4.0 p.p.m. Standard solutions were prepared in 5% glycerol (Prolabo) to overcome the differences of viscosity and superficial tension between diluted serum samples and calibration standards (Peaston 1973, Butrimovitz & Purdy 1977, Smith & Butrimovitz 1979, Delves 1987).

For the blood samples we determined the concentration of total plasmatic proteins (biuret method), of albumin (brominecresol red method) and of hemoglobin (cyanmethemoglobin method) using commercial kits (CliniCals, Carlo Erba, Milan, Italy) (Henry *et al.* 1974).

Mean corpuscular volume (MCV) was calculated for each sample with the following formula: MCV = hematocrit (%) × 10/red cell count (10^6 μl^{-1}) (Davidsohn & Henry 1974).

Statistical evaluation of data was performed by means and standard deviations, coefficient of variation, coefficients of skewness and kurtosis, correlation coefficient and partial correlation coefficient, and multiple linear regression (Sokal & Rohlf 1981).

Results

Mean, standard deviation, range and coefficient of variation of age, serum zinc, total protein, albumin, MCV and hemoglobin for the sample ($n = 58$) are reported in Table 1.

The coefficient of variation (%) shows that the sample is more variable for serum zinc concentrations while it is less variable for MCV. Estimation of skewness and kurtosis in the sample distribution detects no departures from normality for any hematic parameters (Table 2).

Next we evaluated the association between age and hematic parameters. The correlation matrix is reported in Table 3. Statistically significant correlation coefficients are those between age and albumin ($r = -0.562$, $P < 0.001$), serum zinc and albumin ($r = 0.328$, $P < 0.05$), serum zinc and hemoglobin

Table 1. Mean, standard deviation, range and coefficient of variation (%) of hematic parameters for the sample ($n = 58$)

	$\bar{Y} \pm s$	Range	Coefficient of variation (%)
Age (years)	37.5 ± 9.8	22–61	26.14
Serum zinc ($\mu g\ ml^{-1}$)	1.25 ± 0.28	0.68–2.13	22.12
Total protein (g $100\ ml^{-1}$)	7.33 ± 0.36	6.46–8.30	4.88
Albumin (g $100\ ml^{-1}$)	5.06 ± 0.23	4.60–5.60	4.51
MCV (μm^3)	88.8 ± 3.5	81–97	3.98
Hemoglobin (g $100\ ml^{-1}$)	15.6 ± 0.8	13.7–17.6	5.17

Table 2. Coefficient of skewness and kurtosis of the sample distribution for hematic parameters considered

Hematic parameters	Coefficient of skewness	Coefficient of kurtosis
Serum zinc	0.613	3.925
Total protein	0.056	3.101
Albumin	0.199	2.348
MCV	−0.192	2.590
Hemoglobin	0.085	3.000

($r = 0.291$, $P < 0.05$), and total plasmatic proteins and albumin ($r = 0.463$, $P < 0.001$).

Correlation coefficients adjusted for age effect were calculated and they are reported in Table 4. Correlation coefficients between serum zinc and albumin ($r = 0.263$, $P < 0.05$), serum zinc and hemoglobin ($r = 0.268$, $P < 0.05$), and total plasmatic protein and albumin ($r = 0.452$, $P < 0.001$) are still significant.

Table 5 shows the results of multiple linear regression which revealed that, together, albumin and hemoglobin levels account for 18.25% of the variance in serum zinc concentrations. The coefficient of multiple correlation is also significant ($R = 0.427$, $P < 0.01$). When age and total protein levels were introduced as independent variables into the multiple regression, the coefficient of multiple determination was not as appreciably affected ($R^2 = 18.42\%$, $P < 0.05$) as the coefficient of multiple correlation ($R = 0.429$, $P < 0.05$). Partial regression coefficients with age and total protein concentration were not significant (respectively, $\beta \pm SE = 0.008 \pm 0.004$ and -0.034 ± 0.109).

Discussion

Normal ranges for hematic parameters are reported in the literature (Colloca 1989). Our values are comparable with reference values. Particularly, with regard to hemoglobin concentration, a level below the limit set for anemia (below 13 g 100 ml^{-1}) was not found in any case.

In a previous study on 107 males aged 20–59 years, no age influence was found on serum zinc concentrations (Folin *et al.* 1991). This result is confirmed by literature data. In a study on controls and on patients with atherosclerosis obliterans, serum zinc levels were demonstrated to be significantly lowered in both controls and patients over 61 years than in those below this age (Uza & Vlaicu 1989).

Concerning the other hematic parameters, an inverse correlation has been found between age and albumin concentrations. Serum albumin levels decrease with age (Natelson & Natelson 1980). This could be related to changes in physiological function that occur between the ages of 30 and 80 (e.g. protein synthesis has a reduction of 48%) (Kritchevsky 1990).

Serum proteins are prevalently albumin and some

Table 3. Correlation matrix for the sample

	Age	Serum zinc	Total protein	Albumin	MCV
Serum zinc	−0.205	—			
Total protein	−0.168	0.117	—		
Albumin	−0.562***	0.328*	0.463***	—	
MCV	0.219	−0.017	0.017	−0.200	—
Hemoglobin	−0.157	0.291*	0.013	0.056	0.037

*$P < 0.05$.
***$P < 0.001$.

Table 4. Correlation coefficients adjusted for age effect

	Serum zinc	Total protein	Albumin	MCV
Total protein	0.086	—		
Albumin	0.263*	0.452***	—	
MCV	0.029	0.056	−0.095	—
Hemoglobin	0.268*	−0.014	−0.039	0.074

*$P < 0.05$.
***$P < 0.001$.

Table 5. Multiple linear regression

Dependent variable	Independent variable	$\beta \pm$ SE	$R^2 \times 100$
Serum zinc	constant	-2.142 ± 0.970*	18.25**
	albumin	0.381 ± 0.149*	
	hemoglobin	0.094 ± 0.042*	
	$R = 0.427$**		
Serum zinc	constant	-2.154 ± 1.297	18.42*
	age	0.0008 ± 0.004	
	total protein	-0.034 ± 0.109	
	albumin	0.424 ± 0.205*	
	hemoglobin	0.095 ± 0.043*	
	$R = 0.429$*		

R^2, coefficient of multiple regression; R, coefficient of multiple correlation.
*$P < 0.05$.
**$P < 0.01$.

α and β globulins which perform carrier functions and act in water balance. This explains the positive correlation that has been found between total protein and albumin concentrations, even after adjustment for any age effect. The level of total proteins depends on their quantity and on water quantity in blood. Quantitative determination of total serum proteins has little clinical interest. Evaluation of single component concentrations (such as albumin) and their reciprocal relationship may provide more interesting clinical information, concerning hepatic functionality, considering their metabolism (Burlina & Bonessa 1981).

We found a positive significant correlation between serum zinc and albumin concentrations; this correlation is still significant after adjustment for any age effect. The significant positive correlation between serum zinc and albumin concentrations was also found by other authors (Vir & Love 1979) in a study on males and females aged over 65 years. This correlation might explain the lowered serum zinc levels they measured, since albumin levels are reduced with increasing age. The significant positive correlation between levels of albumin and serum zinc could explain the fact that some pathologic conditions, considered as causes of the lowering of albumin levels (hepatocellular insufficiency, malnutrition, burns), are also characterized by reduced serum zinc levels.

Therefore, the determination of serum zinc concentration may provide additional information compared with that of usual hematic proteic parameters. This may be important for the diagnosis of hepatic malfunctions and of proteinuria. It may also be useful to control patients subjected to dialysis and to plasmapheresis.

Hemoglobinometry is the main examination for the diagnosis of anemia. Normal levels of hemoglobin vary with individual age and consolidate on 16 g 100 ml^{-1} in adult men and on 14 g 100 ml^{-1} in adult women. For optimum morphological definition of anemia, determination of MCV is particularly important: there is a microcytic anemia when MCV $< 83\ \mu m^3$, a normocytic anemia when $83 <$ MCV $< 95\ \mu m^3$ and a macrocytic anemia when MCV $> 95\ \mu m^3$. Thus, several phases of anemia may be distinguished: a mild form of anemia when hemoglobin is 13 g per 100 ml and MCV is lowered and a severe form when hemoglobin is 5 g per 100 ml and MCV is very low. Our data, however, did not reveal any correlation between hemoglobin concentrations and MCV. On this subject the correlation we found between serum zinc and hemoglobin concentrations seems to be more interesting. The same correlation was found only in females by other authors (Vir & Love 1979). In erythrocytes, besides being a cofactor of carbonic anydrase, zinc is bound to the cell membrane, hemoglobin and other proteins. Between 2 and 3% of zinc in human erythrocytes is exchangeable with that bound to albumin (Van Wouwe *et al.* 1990). A $[Cl^-/HCO_3^-]$ exchange mechanism has been described that transports free zinc into the human erythrocyte as the $[Zn(HCO_3)_2Cl^-]$ complex (Kalfakakou & Simons 1986, Torrubia & Garay 1989). Zn^{2+} is known to increase the O_2 affinity on human hemoglobin by binding directly to hemoglobin (Gilman & Brewer 1978).

As stated above, it has been demonstrated that, in boys with microcytic hypochromic anemia, plasma zinc levels were significantly low compared with control subjects (Prasad *et al.* 1963a,b). It seems to

us that serum zinc determination would also be useful in the identification of different stages of anemia.

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