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EFFECT OF COPPER ON LIVER AND BONE METABOLISM IN MALNUTRITION*

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SUMMARY: Güler AH, Sapan N, Ediz B, Genç Z, Özkan K. (Departments of Biochemistry, Pediatrics and Medical Biology, Uludağ University Faculty of Medicine, Görükle, Bursa, Turkey) Effect of copper on liver and bone metabolism in malnutrition. Turk J Pediatr 1994; 36: 205-213.

This study was planned to investigate the effects of copper (Cu) deficiency on liver and bone metabolism in malnourished children. Serum total calcium (Ca), inorganic phosphorus (P), Ca/P, Cu/Ca, Cu/P ratios and alkaline phosphatase (ALP) activity values were analyzed. Aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma glutamyltransferase (GGT) enzyme activities and the ALT/AST (De Ritis) ratio as well as their correlations with Cu were tested to determine liver function.

The results of the study showed that Cu deficiency directly affects the organic matrix formation, and by the suppression of ALP activity, indirectly causes decalcification. In the liver, however, no direct effect of Cu deficiency was seen. Deterioration in liver function and Cu deficiency increased parallel with the severity of malnutrition. Thus we concluded that a correlation exists between Cu and the parameters that indicate liver function. *Key words: malnutrition, bone, liver, Cu-deficiency.*

Copper (Cu) is an essential trace element found in the structure of numerous enzymes[1]. In addition, Cu can affect many enzymes' activities and related metabolic processes indirectly, even if it is not a structural component. Therefore, Cu deficiency causes deterioration of many metabolic processes and results in numerous clinical symptoms. In protein-energy malnutrition (PEM), Cu deficiency is seen almost characteristically[2,3]. Thus, in this study we investigated mild and moderate malnutrition cases and evaluated in particular the effects of Cu

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deficiency on liver and bone metabolism as follows: a) determination of serum Cu, calcium (Ca) and phosphorus (P) levels, alanine amino transferase (ALT), aspartate amino transferase (AST), alkaline phoshatase (ALP) and gamma glutamyl transferase (GGT) activities, and comparison of them with normal, healthy children's values; b) investigation of the correlation between these parameters and serum Cu; c) establishment of the alterations in the Cu/Ca, Ca/P, Cu/P and ALT/AST (De Ritis) ratios in the patient groups; and d) correlations of these ratios with serum Cu levels.

Material and Methods

This study was performed on malnourished children classified into two groups according to Gomez's criteria[4]: the first group comprised cases of mild or first degree malnutrition; the second group comprised children with moderate or second degree malnutrition; and the control groups for each comprised 25 children. The children's serum Cu was measured with an atomic absorption spectrophotometry (AAS) apparatus with graphite tube[5,6]. Ca was measured with the classical complexometry, and P determination was made with Biotrol/A 02478, phosphorus UV kit. AST, ALT and GGT activities were measured with Biotrol A 03011 AST/TGO, Biotrol A 03021 ALT/TGP and Biotrol A 03026 monoreactive kits, respectively. In the determination of ALP activity, coulter's optimized kinetic method (Reagent Ref. No. 9866357) was applied. Statistical analyses of all obtained data were done on an IBM PS/2 Model 30 (UK) computer.

Results

Serum mean Ca and Cu levels were significantly ($p<0.001$) lower while P was higher ($p<0.001$) in the patient groups than in controls (Table I). Although they

Table I: Serum Ca, P, Cu Levels in the Malnutrition Groups and Comparison with the Control Group's Means

	Groups					
	Control (n=25)	1. Group (n=25)		2. Group (n=25)		
Measured Parameters	$\bar{X}\pm$SEM	$\bar{X}\pm$SEM	P	$\bar{X}\pm$SEM	P	P'
Ca (mg/dl)	10.43±0.08	9.42±0.06	<0.001	8.92±0.07	<0.001	<0.001
P (mg/dl)	3.89±0.10	4.45±0.05	<0.001	5.28±0.05	<0.001	<0.001
Cu (µg/dl)	118.35±0.97	71.84±0.70	<0.001	64.84±0.81	<0.001	<0.001

P: Statistical significance of the difference between the patient and control groups.
P': Statistical significance of the difference between the first and second groups.

were in the normal ranges, the patient groups' ALT and AST values were higher then those of controls and GGT was also increased, particulary in group 2 ($p<0.001$). ALP activities were significantly ($p<0.001$) lower in the patient groups than in controls. However, we could not establish any significant difference between the patient groups (Table II). Besides the "De Ritis" ratio, the other ratios (Cu/Ca, Ca/P and Cu/P) decreased in the first and second groups, with the lower ratios found in group 2. On the other hand, the ALT/AST ratio was higher in the second group (Table III).

Table II: Serum Mean ALT, AST, GGT and ALP Activities in the Patient Groups and Comparison with the Control Group's Means

	Groups					
	Control (n=25)	1. Group (n=25)		2. Group (n=25)		
Measured Parameters	$\bar{X}\pm$SEM	$\bar{X}\pm$SEM	P	$\bar{X}\pm$SEM	P	P'
ALT (U/L)	17.40±0.97	29.30±0.24	<0.001	38.44±0.46	<0.001	<0.001
AST (U/L)	22.12±0.92	31.72±0.34	<0.001	39.36±0.36	<0.001	<0.001
GGT (U/L)	12.28±0.56	13.66±0.25	<0.05	20.48±0.56	<0.001	<0.001
ALP (U/L)	476.24±2.75	385.92±11.31	<0.001	380.16±2.41	<0.001	>0.05

Table III: The Mean Ratios Established in the Patient and Control Groups and Their Comparisons with Each Other

	Groups					
	Control (n=25)	1. Group (n=25)		2. Group (n=25)		
Ratios	$\bar{X}\pm$SEM	$\bar{X}\pm$SEM	P	$\bar{X}\pm$SEM	P	P'
Cu/Ca	11.36±0.12	7.64±0.09	<0.001	7.28±0.11	<0.001	<0.001
Ca/p	2.72±0.07	2.13±0.03	<0.001	1.69±0.03	<0.001	<0.001
Cu/p	30.82±0.72	16.22±0.29	<0.001	12.28±0.13	<0.001	<0.001
De Ritis (ALT/AST)	0.79±0.04	093.92±0.01	<0.01	0.98±0.02	<0.001	<0.05

We could not establish any correlation between Cu and Ca in any of the groups; however, the correlation between P and Cu was negative, and this inverse relation increased gradually in the first and second groups, respectively. The relation between Cu and ALP became negative in the first group and was lost in

the second group, while the correlations of ALT and GGT with Cu increased inversely only in the second group (r= −0.24 and −0.40 respectively). The relation between AST and Cu was found to be negative as well, but only in the first group (Table IV).

Table IV: Linear Regression Equations and the Correlation Coefficients (r) Established Between Cu, Ca, P and the Enzymes

Parameters Compared with Cu	Groups					
	Control		1. Group		2. Group	
	Linear Regression Equations	P	Linear Regression Equations	P	Linear Regression Equations	P
Ca	y=110.14+0.78x r=0.07	N.S.	y=60.63+1.191x r=0.10	N.S.	y=71.431−0.739x r=−0.07	N.S.
P	y=106.941+2.931x r=0.30	N.S.	y=96.88−5.629x r=−0.41	<0.05	y=19.201−8.644x r=−0.53	<0.01
ALT	y=117.937+0.024x r=0.02	N.S.	y=77.351−0.188x r=−0.07	N.S.	y=81.014−0.421x r=−0.24	N.S.
AST	y=117.162+0.054x r=0.05	N.S.	y=98.191−0.831x r=−0.40	<0.05	y=47.914+0.43x r=0.19	N.S.
GGT	y=116.096+0.183x r=0.11	N.S.	y=65.245+0.483x r=0.17	N.S.	y=75.076−0.05x r=−0.40	<0.05
ALP	y=80.431+0.080x r=0.23	N.S.	y=78.344−0.017x r=−0.27	N.S.	y=71.981−0.019x r=−0.06	N.S.

P : Statistical significance of the correlation coefficients.
N.S. : Non-significant.

Correlations between Cu and Cu/Ca ratios were significantly ($p<0.001$) higher in the patient groups and somewhat increased compared to controls. Again, the negative correlation between Cu and Ca/P ratios was slightly increased in the first and second groups. There was no correlation between Cu and Cu/P in the control group, but this relation increased significantly in the patient groups. Cu and "De Ritis" were inversely related, while a direct relation existed between GGT and ALT/AST ($p<0.05$) in these groups (r=0.4 and 0.5 respectively) (Table V).

Discussion

One of the characteristic findings of PEM is Cu deficiency[2,3]. In malnourished children, bone defects, depigmentation, connective tissue defects and neurological disorders can be seen as a result of Cu deficiency[7,8]. After observing the

Table V: Linear Regression Equations and the Correlation Coefficients Between Cu and the Ratios (Cu/Ca, Ca/P, Cu/P and ALT/AST) in the groups

	Groups					
	Control		1. Group		2. Group	
Parameters Compared with Cu	Linear Regression Equations	P	Linear Regression Equations	P	Linear Regression Equations	P
Cu/Ca	y=54.26+5.64x r=0.71	<0.001	y=20.40+6.73x r=0.82	<0.001	y=20.56+6.08x r=0.84	<0.001
Cu/P	y=130.41−4.43x r=−0.32	N.S.	y=55.25+7.80x r=0.36	N.S.	y=86.87−13.00x r=−0.40	<0.05
Cu/P	y=118.34+34.0x r=0.00	N.S.	y=41.08+1.89x r=0.80	N.S.	y=14.06+4.13x r=0.70	<0.001
ALT/AST	y=118.49−0.18x r=−0.01	N.S.	y=56.61+16.44x r=0.29	N.S.	y=78.91−14.37x r=−0.28	N.S.
GGT ALT/AST	y=11.27+1.26x r=0.1	N.S.	y=6.49+7.74x r=0.40	<0.05	y=4.82+15.99x r=0.5	<0.05

occurrence of spontaneous fractures in domestic animals consuming forage low in Cu, investigators are exploring the effects of Cu on bone metabolism.

Bone defects have been observed experimentally in rabbits, pigs, chickens and dogs. In these experiments, total ash as well as calcium and phosphorus remain within normal limits in bones, despite Cu deficiency. In conclusion, the biochemical defect of Cu deficiency is believed to reside only in the organic matrix[1]. In an another experiment, Cu-deficient chick bone was found to contain a higher-than-normal proportion of soluble collagen (immature collagen)[9,10]. This shows that the reactions with lysyl oxidase (a cuproenzyme) and the formation of allysin (an aldehyde form of lysin) are lesser than normal. The implication is that collagen cross-linking in bones is impaired by Cu deficiency. Failure of collagen maturation in the organic matrix of bone can account for the greater fragility of bone and associated abnormalities seen in PEM cases.

The second effect of Cu deficiency on bones is related to zinc (Zn) and ALP. Zn is an essential trace element for carbonic anhydrase and ALP enzyme activities[11]. These enzymes play important roles in both organic matrix formation and calcification processes (especially ALP in the latter)[12,13]. Most of the body Zn is found in the bones. In Cu deficiency, to maintain the equilibrium between Zn and Cu, Zn enters into the plasma in larger than normal quantities and in time, total

body Zn may be decreased. However, as long as the plasma Zn concentration is high, Cu absorption by the intestine is depressed. In the intestine, Zn and Cu are absorbed after binding to metallothionein (MT), and they compete for this binding process[14]. The relation between Zn and Cu is very similar to the relations between Ca-P and Na-K. High dietary Zn intake can depress Cu absorption and may result in Cu deficiency. Although adequate Zn is found in the initial phases of malnutrition, Zn deficiency may also supervene gradually as a result of inadequate diet and other causes of malnutrition (diarrhea, etc.). Secondary to Zn deficiency, ALP activity decreases and thus calcification slows down and may be depressed completely.

In PEM there is another factor which causes decalcification, independent of Cu deficiency. In mild and moderate malnutrition cases, ALP activity is suppressed secondary to the increase in cortisol levels[15,16]. In this condition, the organism's first aim is to maintain the plasma Ca concentration in the normal range. Therefore, as a result of mobilization of Ca from the bones (i.e., decalcification), osteoporosis develops. In this period, formation of bone and osteoblastic activity are also suppressed, and growth retardation occurs. As malnutrition progresses, growth hormone (GH) compensates by increasing parallel to the severity of the disease, which in turn can activate the bone ALP isoenzyme and increase the Ca and P absorption by the intestines.

In the present study, serum Ca and P levels were found to be in the normal ranges. However, Ca levels were lower and P higher as compared to controls' parallel to the severity of the disease. We can say that these changes were an attempt to maintain the equilibrium between Ca and P in the blood. ALP activities were also in the normal ranges in the first and second groups, but they were lower than controls. This finding may be accepted as a suppressive effect of cortisol on ALP activity in addition to probable Zn deficiency. In the first group, the negative correlation found between Cu and ALP acvitiy may also support the latter finding. In the second group, we have found this relation to be lacking. Therefore we believe that GH has entered into the cycle.

ALT, AST and GGT activities are specific indicators of liver function. In mild cases, as a response to an organism's stress, cortisol increases in plasma. In stressful conditions, an organism primarily needs energy and cortisol to supply energy and tries to activate gluconeogenesis. If there is inadequate glucose in the diet, the tissues and primarily the muscle (proteins) degrade into amino acids. These amino acids then lose their amino groups by transamination reactions and convert to glucose via gluconeogenesis[17]. Thus, cortisol-activating transaminases stimulate glucose formation.

GGT (gamma glutamyl-peptide: amino acid gamma glutamyl transferase) is a peptidase[18] that can also be used as an indicator of malnutrition. AST and ALT

activities were found to be increased compared to controls in every stage of malnutrition, although the causes of the elevations differ. For example, in mild malnutrion cases, the increase of cortisol causes AST and ALT to increase, while in the severe cases, AST and ALT increases may be seen secondary to the development of fatty liver. In other words, GGT increases in proportion to the degree of fatty degeneration of the liver. The correlation between GGT and the De Ritis ratio supports the latter idea as well. In various conditions affecting the liver, ALT is characteristically as high as or higher than AST, and the ALT/AST (De Ritis) ratio, which is normally less than 1.0, becomes greater than unity. In our study, De Ritis ratios were also found to be less than 1.0 in all groups. They were higher in the patient groups than in controls, and highest in the second group. This finding may support the idea that the De Ritis ratio can show the severity of liver pathology (Table III). In our work, the gradual increase in ALT and AST values in the patient groups and the fact that GGT was significantly higher in the second group than in the controls is consistent with the above results. The inverse relation between Cu and GGT in the second group again shows the severity of PEM (Table IV).

Cu is a trace element transported 95 percent by ceruloplasmin and about 5 percent by albumin in the plasma. Ceruloplasmin, besides its many other functions, is also an acute phase reactant. Especially in acute infections, trauma and in any type of acute disease, ceruloplasmin levels increase in serum. Secondary to the leukocyte endogenous mediator (LEM)'s acute phase action, initially serum ceruloplasmin and therefore the serum Cu level rises[19]. The finding of Cu and ceruloplasmin levels generally low in chronic and degenerative diseases supports these observatious[20-22]. In malnutrition the phenomenon is rather complex, and can vary with the severity of disease. In the early periods of malnutrition, the first aim is to maintain the organism's integrity. Generally PEM is a chronically progressing disease. Therefore, even in the beginning of PEM, neither ceruloplasmin nor Cu is increased in serum as an acute response. Ceruloplasmin synthesis continues as long as there is adequate substrate (protein and amino acids) and the liver functions are normal in the organism. As the disease progresses, because of protein inadequacy and breaking down of liver function[23], ceruloplasmin synthesis gradually decreases. As a result, Cu cannot be transported to the places where it is needed in the body, and serum Cu decreases. In malnutrition, Cu absorption can be lessened as a result of MT synthesis suppression secondary to protein inadequacy, in addition to local intestinal disorders such as diarrhea. The third reason which can cause hypocupremia in PEM is the inadequacy of dietary Cu. In such cases, Cu deficiency is not a cause but a result of PEM, and the severity of Cu deficiency is proportionate to the severity of PEM.

We conclude that Cu deficiency directly affects the organic matrix formation in bones and, by the suppression of ALP activity, indirectly causes decalcification. In the liver, on the other hand no direct effect of Cu deficiency was seen. However, the breaking down of liver functions and the amount of the Cu deficiency increases parallel with the severity of malnutrition. Therefore we believe that some correlations exist between Cu and the parameters that indicate liver function.

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