

Department of Infectious Diseases, Medical University of Lublin

HANNA FOTA-MARKOWSKA, ROMA MODRZEWSKA,  
IRENA BOROWICZ, SŁAWOMIR KICIAK

*Evaluation of serum copper (Cu) level dynamics  
in measles patients*

In the view of several clinical and experimental studies, cuprum (Cu) as a trace element is indispensable for anatomical and functional integrity of organism. It is active in all the basic physiological processes on the organ, tissue, cellular and molecular level. Experimental research has established the participation of copper in the proper course of immunological reactions and cellular and humoral types. Primary measles virus infection is characterised immunologically by immune activation and the induction of an effective and long-lived antiviral immune response. (4)

The aim of undertaking research was to provide the answers to the following questions: 1. What is the serum Cu in the acute stage of disease? 2. Does the Cu change in the acute stage of disease as compared with convalescence? 3. What is the serum Cu in the acute stage of disease and convalescence as compared with control group?

MATERIAL AND METHODS

CLINICAL MATERIAL

The studies included 26 measles patients, namely 8 men and 18 women aged 18-34, hospitalised in the Infectious Diseases Department of Medical University from 1990 to 1991 (9 of these patients were vaccinated against measles according to vaccination calendar (i.e. 16-18 months after birth). The average hospitalisation period was 14 (+2) days, from the appearance of eruption. The diagnosis was established on the basis of anamnesis, clinical symptoms, laboratory analysis and viral examinations. The group examined consisted of patients not showing symptoms of co-existing diseases. Clinical improvement was achieved after the treatment. In all the examined patients serum Cu level was determined twice during hospitalisation and additionally one time after discharging from clinic according to the following scheme: First examination – during the first day of hospitalisation, second examination – during the last day of hospitalisation after attainment of clinical improvement, Cv examination – after 3 weeks, during periodic control.

The control group included 24 persons aged 15-19 selected among the schoolboys and schoolgirls of Lublin schools. The serum Cu level of those persons was determined once. All patients and persons from the control group have been informed about the purpose of examination.

## METHODS

Blood used in investigations was sampled from elbow vein in fasting state between 7 and 8 o'clock in the morning. The measurements of serum Cu level were made.

5ml of sampled blood was transferred directly to demineralised test tube for centrifuge. After the formation of the clot the centrifuge content was centrifuged at rotation speed equal to 2000 rot./min. 1 ml of serum was pipetted to scintillation containers (Plastomed) and frozen at  $-20^{\circ}\text{C}$ . Thus protected samples were stored until the beginning of measurements, but no longer than 2 months. Scintillation containers, test-tubes for centrifuge and automatic pipette tips were soaked before use for 24 h in 10% hydrochloric acid in a vessel placed under digestorium. Demineralised laboratory vessels were flushed several times with distilled water and then dried. The reagents were tested in the respect of Cu content before each measurement. The serum for investigations was defrosted and then diluted with distilled water in proportion of 1:40. Determinations of copper level in blood serum were made by atomic absorption spectrometry (AAS) in Central Apparatus Laboratory of Lublin Agricultural University. For this purpose the AAS-3 atomic absorption spectrometer (Carl Zeiss Jena, Germany) has been used after previous calibration in the presence of standard sample at the wavelength of 324.8 nm. The level of Cu is expressed in mmole/l.

## STATISTICAL METHOD

The obtained numerical data were subjected to statistical analysis. On the basis of the results obtained from  $n$  persons the following statistical characteristics were determined:  $M$  – arithmetic mean,  $SD$  – standard deviation,  $SE$  – average error of arithmetic mean,  $V\%$  – coefficient of variability.

Inter-measurements variations were described by variability range (from – to), and variability mean and  $SDd$  – by variability standard deviation. The significance of differences between Cu level in patients and in control group was verified by means of Students t-test for the resolved variables. In the case of significant differences in variances, at different numbers of compared groups, such verification was performed by means of Cochran-Cox test. In individual tables the types (t, c) and the values of test functions are listed in Column T. The significance of differences between decrease and increase frequencies between comparative tests was verified by  $c^2$  test. The probability  $P$  was read from the tables containing t-Student distribution functions or  $c^2$  functions respectively. It has been assumed that the risk of conclusion error is equal to 5%. The individual Cu level found during comparative studies (I with II), (I with R), (II with R) presented in the figures are obtained by means of correlation diagrams. The range of our norm (marked in the diagrams) is determined on the basis of Cu level in the control group. Mean values together with the values of standard deviations determined for the control group and the same values obtained in the three tests performed in patients were illustrated in histograms.

## RESULTS

Copper (Cu) levels in the control group as well as in three examinations made in patient group are listed in Table 1, whereas the variations in Cu level observed between the three examinations are listed in Table 2 and illustrated in Figure 1. Cu level in the control group was  $17.40 \pm 3.09$  mmole/l ( $M \pm SD$ ) and in patient group  $23.84 \pm 4.03$  mmole/l,  $23.00 \pm 4.43$  mmole/l and  $19.79 \pm 4.19$  mmole/l in the first and second examination and in convalescence respectively (Table 1, Fig. 1). Comparing the

serum Cu level in the examined group to the analogous level in the control group it has been found that in the first examination this level was higher by 6.44 mmole/l and this difference is highly significant ( $P<0.001$ ), in the second examination it was higher by 5.60 mmole/l ( $P<0.001$ ) and in convalescence only by 2.39 mmole/l but even this last difference is highly significant ( $P<0.05$ ). Assuming that our norm of Cu level ranges from 11.23 to 23.58 mmole/l in the first examination the elevated Cu level has been observed in 14 among 26 examined patients (i.e. in 53.8%), in the second examination in 12 among 26 patients (46.2%) and in 3 among 18 (16.7%) patients in convalescence. In the period between the first and second examination the decrease in Cu level has been observed in 12 patients (46.2%), the increase also in 12 patients (46.2%), and the level has remained unchanged in 2 patients. In this period the Cu level changed from  $-8.2$  mmole/l (decrease) to  $+10.1$  mmole/l (increase), therefore it has been reduced only by 0.845 mmole/l and this difference is markedly at random ( $P>0.30$ ). Comparing the Cu level observed in convalescence to the one observed in the first examination the increase has been found in 4 and the decrease in 14 among 18 patients (22.2 and 77.8% respectively). The decrease in Cu level was observed more frequently than the increase ( $P<0.02$ ). The variations in Cu level ranged from  $-13.2$  to  $+9.8$  mmole/l, therefore the average decrease was 3.95 mmole/l and was highly significant ( $P<0.01$ ) – Tab.2. Comparing the Cu level in convalescence to the observed one in the second examination the increase in this level has been found in 3 patients (16.7%), the decrease in 14 patients (77.8%) and unchanged level in 1 patient. The decrease in Cu level was observed more frequently than the increase ( $P<0.01$ ). The differences ranged from  $-8.8$  mmole to  $+4.4$  mmole/l, was on an average 2.993 and was highly significant (Tab.2). Thus the results of this analysis indicate that the significant increase in serum Cu level occurs in measles patients. During the disease period (between the first and second examination) the Cu level does not undergo significant variations, whereas in convalescence period it decreases in highly significant manner, nevertheless it remains significantly higher than the one observed in the control group. No statistically significant differences in serum Cu ratio in the acute stage of disease were found. In convalescence serum Cu ratio is statistically significantly lower in comparison to the acute stage of disease. Statistically significantly higher Cu ratio both in the acute state and in convalescence as compared with the control was ascertained.

Table 1. Cu level in blood serum in control group (Ct) and in measles patients in examinations I, II and Cv

Group	n	Cu level ( $\mu\text{mole/l}$ )				Comparison to control group									
			SD	SE	V %	number (f) and percentage (%)									
						mean differ.	T	p	reduced		norm		elevated		
									f	%	f	%	f	%	
Ct	24	17.40	3.09	0.63	17.7	(our norm: 11.23-23.58)									
P I exam.	26	23.84	4.03	0.79	16.9	+6.44	t	6.307	<0.001	0	0	12	46.2	14	53.8
P II exam.	26	23.00	4.43	0.87	19.3	+5.60	c	5.213	<0.001	0	0	14	53.8	12	46.2
P Cv	18	19.79	4.19	0.99	21.2	+2.39	t	2.123	<0.05	1	5.6	14	77.8	3	16.7

Symbols used in first left column: control group, P – patents, Cv – convalescence.

Table 2. Magnitude and direction of variation of Cu level between individual examinations

Comparable examinations		Magnitude of variations ( $\mu\text{mole/l}$ )			Significance of variations		Direction of variations					
		from - to	mean	SDt	t	P	number (f) and percentage (%)					
							decrease		increase		$\chi^2$	P
f	%	f	%									
II and I	26	-8.2 +10.1	-0.845	4.888	0.882	>0.30	12	46.2	12	46.2	0	>0.99
Cv and I	18	-13.2 +9.8	-3.950	5.619	2.982	<0.01	14	77.8	4	22.2	5.56	<0.02
Cv and II	18	-8.8 +4.4	-2.993	3.630	3.498	<0.01	14	77.8	3	16.7	7.12	<0.01

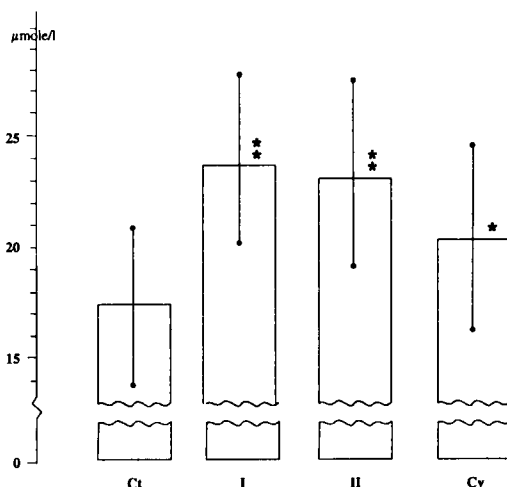


Fig. 1. Cu level ( $M \pm SD$ ) in control group (Ct) and in patients in examinations I, II, and convalescence (Cv); significant differences in comparison to control group are denoted as follows:  $P < 0.01$ ,  $P < 0.05$

## DISCUSSION

It is difficult to fix correlation between the changes of Cu content in biological material and to qualify the state of excess and deficiency in organism. Analytic methods are applied in order to examine the dynamics of serum Cu ratio in patients with different period of pathology development. The method of absorbent atomic spectrometry (AAS) is most valuable owing to/because of its high sensitivity and specificity.

Our research showed that serum Cu level in control group amounted to 11.22 - 23.58  $\text{mmol/l}$  and is consistent with "Si Conversion Guide" (7). Serum Cu level is conditioned by the sequence of homeostatic mechanisms dependent, among others, on diet, age and it can vary in the course of

twenty-four hours (5, 8, 10).

Hormonal regulation of Cu level and, especially, stimulating influence of oestrogen can be explained by higher, if statistically insignificant, value in women group (8).

In the research we observed significant increase in serum Cu ratio during the acute stage of disease and convalescence in comparison with control group. The highest figures were observed during the first test, namely during the eruptive period of the disease. Slightly lower but still significantly lower figures were observed in the second test after clinical symptoms regression. During convalescence serum Cu concentration was statistically significantly higher in comparison with control group although serum Cu level lowered.

Cu metabolism may be disturbed at absorption stage and circulation in blood in pathology caused by morbillivirus infection. Ionised Cu released from food or Cu in amino acid complex is bound by legends and stored in enterocytes where it passes to blood (10). Morbillivirus infection in prodromal stage may cause non-specific dyspeptic symptoms and lack of appetite. It is possible that this mechanism leads to disturbances in Cu absorption and is compensated by activating supplies. However, hypercupremia observed during convalescence suggests that some other mechanisms are involved.

Cu occurs in blood in labile form bound with albumin and in stabile form with coeruloplasmin. Liver is the only place where coeruloplasmin is synthesised and released. Since morbillivirus belongs to hepatotropic viruses, hypercupremia that was observed in our patients may be related to this mechanism; especially that hypoalbuminemia, which usually accompanies liver pathology, has not been observed in the course of measles. Thus, it cannot be excluded that hypercupremia observed in our patient may be caused mainly by the increase of coeruloplasmin which binds physiologically more than 90% of circulating copper. (15). It would be helpful to estimate coeruloplasmin level in our patients, especially that it has been observed that its activity is higher in numerous virus infections (1, 3).

Immune reactions caused by morbillivirus antigens may also be of high importance. Experiments and clinical observations prove that viral infection activates cytokinin cascade. The activation of macrophages, neutrophilic granulocytes leads to releasing the so-called inflammatory cytokinins and stimulating protein synthesis, including coeruloplasmin. (4, 6, 9)

Cu is active in generating IFN $\gamma$ , Ia – 1, Ia – 2, TNF. (1, 2) The increase of coeruloplasmin and copper level serum and increased synthesis of metallothionein constitute one of the elements of the acute stage.

Therefore, it seems that mechanisms leading to the increase of serum Cu level in our patients and biological importance of this phenomenon are very complex. Mutual correlation between aetiological agent and immunity as well as interaction between elements may be important in the processes.

It is possible that hypercupremia we observed in our patients during the acute stage and convalescence is only the reflection of measles although it cannot be excluded that hypercupremia also influences the course of the disease.

## CONCLUSIONS

1. No statistically significant differences in serum Cu ratio in the acute stage of disease were found. In convalescence serum Cu ratio is statistically significantly lower in comparison to the acute stage of disease.

2. Statistically significantly higher Cu ratio both in the acute state and in convalescence as compared with the control was ascertained.

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## SUMMARY

The aim of the present study was to estimate the cuprum(Cu) level in blood serum in patients ill from measles in the acute period of the disease and three weeks after regression of clinical symptoms in comparison with the control group.

The investigations included 26 patients aged 14-34, hospitalised due to measles in the Department of Infectious Diseases of the Medical University of Lublin. The analysed group included patients without coexisting diseases, in which clinical improvement after the carried out treatment was obtained. All the patients had Cu concentration in the blood serum twice determined during hospitalisation period and once during the period of convalescence.

The control group included 24 healthy persons aged 15-19, who had Cu concentration in blood serum only once determined. The obtained in numbers data were subject to a statistical analysis.

Both in the acute period of measles and during convalescence a statistically significantly higher Cu level in blood serum in comparison to the control group was observed.

#### Ocena dynamiki stężenia miedzi (Cu) w surowicy krwi u chorych na odrę

Celem niniejszej pracy była ocena poziomu miedzi (Cu) w surowicy krwi u chorych na odrę w ostrym okresie choroby i po trzech tygodniach od ustąpienia objawów klinicznych w porównaniu z grupą kontrolną.

Badania dotyczyły 26 chorych na odrę w wieku 14 – 34 lat, hospitalizowanych z powodu odrę w

Klinice Chorób Zakaźnych AM w Lublinie. Analizowaną grupę stanowili pacjenci bez chorób współistniejących, u których uzyskano poprawę kliniczną po przeprowadzonym leczeniu. U wszystkich badanych oznaczono stężenie Cu w surowicy krwi dwukrotnie w okresie hospitalizacji oraz jednorazowo w okresie rekonwalescencji.

Grupa kontrolna obejmowała 24 osoby zdrowe w wieku od 15 do 19 lat, u których stężenie Cu w surowicy krwi oznaczono jednorazowo. Uzyskane dane liczbowe poddano analizie statystycznej.

Zarówno w ostrym okresie odry, jak i w rekonwalescencji stwierdzono statystycznie istotnie wyższy poziom miedzi (Cu) w surowicy krwi w porównaniu z kontrolą.

