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Department of Infectious Diseases, Medical University of Lublin

HANNA FOTA-MARKOWSKA, ROMANA ROLLA-SZCZEPAŃSKA, ROMA MODRZEWSKA, JOANNA LIS-TØNDER, IRENA BOROWICZ

# *Copper/Zinc ratio in measles patients*

Primary measles virus infection activates an immunological reaction and induces an effective and long-term antiviral immune response (6). Experimental research and clinical observation have confirmed the participation of copper (Cu) and zinc (Zn) in stimulating humoral and cellular immunity and granulocyte phagocytosis (3,7). Mutual interactions between Cu and Zn motivated us to undertake the research whit was to provide answers to the following questions: 1.What is the serum Cu and Zn level in the acute stage of the disease? 2. Do the Cu and Zn levels change in the acute stage of disease as compared with convalescence? 3. What is the serum Cu and Zn level in the acute stage of the disease and convalescence as compared to the control group? 4. Is the Cu/Zn ratio useful for clinical evaluation of the disease?

## MATERIAL AND METHODS

The study was conducted on 26 measles patients (8 men and 18 women) aged 18–34, hospitalized at the Infectious Diseases Department of Medical University in Lublin. Nine of these patients had been vaccinated against measles according to the vaccination calendar (i.e. 16–18 months after birth). The average hospitalization period was 14 (±2) days, from the appearance of eruption. The presence of the disease was established on the basis of anamnesis, clinical symptoms, laboratory analysis and virus diagnosis. The patients examined by the authors did not show any symptoms of co-existing diseases. Clinical improvement was achieved after the treatment. In all the examined patients, the serum Cu, Zn levels and Cu/Zn ratios were determined according to the following scheme: twice during hospitalization and, additionally, once more after discharging them from the clinic: First examination (I) – on the first day of hospitalization; Second examination (II) – on the last day of hospitalization after attaining clinical improvement; Third control examination (Cv) – after 3 weeks.The control group included 24 persons aged 15–19 chosen from Lublin school students. The serum Cu, Zn levels and Cu/Zn ratios in those persons were determined once. All patients and persons from the control group were informed about the purpose of the examination.

Blood used in investigations was sampled from the elbow vein before breakfast between 7 and 8 o'clock a.m. Measurements of Cu, Zn level were made in the serum. Five ml of the sampled blood was transferred directly to a demineralised centrifuge test-tube. After the

formation of a clot, the content was separated at 2000 r.p.m. One ml of serum was pipetted to scintillation Plastomed containers and frozen at –20°C. Samples thus protected were stored until the measurements were taken, but no longer than for 2 months. Scintillation containers, centrifuge test-tubes and automatic pipette tips were soaked before use for 24 h in 10 % hydrochloric acid in a vessel placed under exhaust. Demineralized laboratory vessels were flushed several times with distilled water and then dried. Before each measurement was taken, the reagents were tested with respect to Cu, Zn content. The serum for the investigations was defrosted and then diluted with distilled water in 1:40 proportion. Determination of the level of these elements in blood serum was made by atomic absorption spectrometry (AAS) at the Central Instrumental Laboratory of Lublin Agricultural University. For this purpose, a Carl Zeiss Jena AAS-3 atomic absorption spectrometer was used after previous calibration in the presence of a standard sample at a wavelength of 324.8 nm – Cu, 213.9 nm – Zn. The levels of Cu and Zn are expressed in μmole/l. The numerical data obtained in the study were subjected to a statistical analysis. The significance of differences between Cu, Zn levels in the patient and control groups were verified with the t-Student test for independent variables. In the case of significant differences between the variances, at different sample sizes in the compared groups, verification was performed with the Cochran-Cox test. The significance of differences between frequencies of occurrence of decreased and increased values between successive tests being compared was verified with the $\chi^2$ test. The probability P value was read, respectively, from the t-Student and $\chi^2$ distribution tables. The risk of committing a Type I Error was set at an $\alpha$=0.05. Individual Cu, Zn levels found on comparing the results for the examinations (I with II, I with Cv, II with Cv) were obtained by studying correlation. The range of our own norm (shown in the plots) is determined on the basis of Cu, Zn levels in the control group.

## RESULTS

### COPPER (CU)

The Cu level in the control group was 17.40±3.09 μmole/l (M.±SD), and in the patient group, 23.84±4.03 μmole/l, 23.00±4.43 μmole/l and 19.79 ± 4.19 μmole/l in the first and second examinations and in convalescence, respectively. Comparing the Cu level in blood serum in the examined group to an analogous level in the control group revealed that in the first examination this level was higher by 6.44 μmole/l and this difference is highly significant (P<0.001), in the second examination it was higher by 5.60 μmole/l (P<0.001) and in convalescence, it was higher only by 2.39 μmole/l and even the latter difference is highly significant (P<0.05). Assuming that our norm of the Cu level ranges from 11.23 to 23.58 μmole/l, in the first examination an elevated Cu level was observed in 14 among 26 examined patients (i.e. in 53.8%); in the second examination, it was found in 12 among 26 patients (46.2%) and in 3 among 18 (16.7%), in convalescence. In the period between first and second examination, we observed a decrease of the Cu level in 12 patients (46.2 %), an increase in 12 patients (46.2%), and in 2 patients this level remained unchanged. In this period, the Cu level changed from –8.2 μmole/l (decrease) to +10.1 μmole/l (increase), therefore it reduced only by 0.845 μmole/l and this difference is markedly accidental (P>0.30). Comparing the Cu level observed in convalescence to the one observed in the first examination, we found an increase in 4 and a decrease in 14 among 18 patients (22.2 and 77.8%, respectively). A decrease of Cu level was observed significantly more frequently than an increase (P<0.02). The variability in the Cu level ranged from –13.2 to + 9.8 μmole/l; therefore an average decrease was 3.95 μmole/l and was highly significant (P<0.01). Comparing the Cu level in convalescence to the one observed in the second examination, it was found that this level increased in 3 patients (16.7%), decreased in 14 patients (77.8%) and remained unchanged in 1 patient. A decrease of Cu level was observed significantly more frequently than an increase (P<0.01). The differences ranged from –8.8

μmole/l to +4.4 μmole/l, with an average of 2.993, and it was highly significant. Thus, the results of our analysis indicate that in measles patients a significant increase of Cu level in blood serum occurs. During the period of the disease (between first and second examinations), the Cu level does not vary significantly, whereas in the convalescence period, it decreases in a highly significant manner, while remaining significantly higher than the one observed in the control group. No statistically significant differences in the serum Cu level in the acute stage of the disease were found. In convalescence, the serum Cu level is significantly lower in comparison to the acute stage of the disease. A significantly higher Cu levels both in the acute stage and in convalescence, as compared with the control were found.

### ZINC (ZN)

The Zn level in the control group was 15.99±1.52 μmole/l (M±SD) in the patient group, 13.10±2.92 and 17.31±3.99 μmole/l in the first and second examinations, respectively, and in convalescence, 14.49±3.43 μmole/l. A comparison of the Zn level in blood serum in the experimental and control groups revealed that in the first examinations it was lower by 2.89 μmole/l and this difference is highly significant ($P<0.001$), in the second examination, it was higher by 1.32 μmole/l and this difference is accidental ($P>0.10$), and in convalescence, it was lower by 1.50 μmole/l, and this difference is also accidental ($P>0.10$). Assuming that our norm of Zn level ranges between 12.95μmole/l and 19.04μmole/l, it was observed in the first examination that the values of Zn level reduced in 61.5 % of the patients, whereas they were normal in 38.5% of the patients. In the second examination either reduced or elevated values were observed (in 11.5% and 30.8 % of the patients, respectively). In convalescence we observed reduced Zn values in 38.9% of the patients, normal in 55.6 %,and elevated in 5.6%. In the period between first and second examinations, a decrease was observed only in 1 patient, whereas in 25 (96.2%) patients Zn level increased. Zn level changed from −1.85 μmole/l (decrease) to +12.22 μmole/l (increase). This level increased by an average of 4.21 μmole/l and this increase is highly significant ($P<0.001$). Comparing the Zn level observed in the convalescence group to the one observed in the first examination revealed a decrease in 8 patients (44.4%) and an increase in 9 patients (50%). The differences observed between the frequencies of increase and decrease were clearly accidental ($P>0.80$). The values ranged between −7.95μmole/l and + 9.18 μmole/l. Thus, an average increase by 0.93 μmole/l occurred which, however, is clearly accidental ($P>0.30$). Comparing the Zn level in convalescence to the one in the second examination, we found an increase in 4 patients (22.2%) and a decrease in 14 patients (77.8%). The decrease of Zn level was observed significantly more frequently than the increase ($P<0.02$). The values ranged from −11.0 μmole/l to +6.1 μmole/l, hence a decrease by -2.91 μmole/l was found, which in this case is statistically significant ($P<0.02$). Thus, the results of examinations indicate that in measles patients the Zn level is highly significantly reduced in the acute period of the disease. The level observed in the second examination and in convalescence does not differ from the one observed in the control group. Significant lowering of the serum Zn level in measles patients in the eruptive period of the disease was ascertained, as compared to the serum Zn level in the period of the regression of the clinical symptoms. Reduction of Zn level in this period as compared with the control group is statistically significant. Normalization of serum Zn level in convalescence, three weeks after the disappearance of the clinical symptoms was ascertained.

CU/ZN RATIO

In the control group, the Cu/Zn ratio was 1.102±0.231(M±SD), our own norm ranging from 0.64 to 1.56. In the examined group of patients, this ratio was 1.875±0.382 in the first examination, 1.363±0.273 in the second examination and 1.393±0.247 in convalescence. In comparison to the control group, this ratio was on the average higher by 0.773 in the first examination, by 0.261 in the second one and by 0.291 in convalescence. In each of the three examinations, significantly ($P<0.001$) higher values of the ratio were found than those in the control group (Tab.1). In no case were reduced values found. On the contrary, the elevated values (i.e. above 1.56) were observed in 76.9% of the patients in the first examination, in 23.1% in the second examination and in 27.8% in the convalescence group (Tab.1). In the period between the first and the second examinations, a decrease in 84.6% was found, while an increase was seen only in 7.7% of the patients. Therefore this ratio was reduced by an average of 0.513 and this decrease is highly significant ($P<0.001$). Similarly, comparing the values of this ratio obtained in the first examination to the one obtained in the convalescence period, it was found that, during the convalescence period a decrease by 0.398 occurred, and this decrease is highly significant ($P<0.001$). In the period between the first examination and the convalescence period, a decrease in the ratio of the values occurred significantly more frequently than an increase (77.8% in comparison to 22.2%, $P<0.02$). On the contrary, the differences in the ratio values observed in the period between the second examination and the convalescence period seemed definitely accidental ($P>0.50$), and in connection with this, the results obtained permit to draw the conclusion that in the acute period of the disease a significant and highly significant increase in Cu/Zn ratio takes place. In spite of the significant decrease observed as early as in the second examination, this ratio remains highly significantly increased in comparison to the values obtained for the control group. In the convalescence period, the ratio value remains at the level observed in the second examination.

Table 1. Cu/Zn ratio in control and experimental group

| Group | n | Cu/Zn ratio | | | | Comparison to control group | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | number (f) and percentage (%) | | | | | |
| | | M | SD | SE | V % | mean differ. | | T | p | reduced | | in norm | | elevated | |
| | | | | | | | | | | f | % | f | % | f | % |
| Ct | 24 | 1.102 | 0.231 | 0.047 | 21.0 | (our norm:) 0.64 – 1.56 | | | | | | | | | |
| P I exam. | 26 | 1.875 | 0.382 | 0.075 | 20.4 | +0.773 | c | 8.766 | <0.001 | 0 | 0 | 6 | 23.1 | 20 | 76.9 |
| P II exam. | 26 | 1.363 | 0.273 | 0.053 | 20.0 | +0.261 | t | 3.633 | <0.001 | 0 | 0 | 20 | 76.9 | 6 | 23.1 |
| P Cv | 18 | 1.393 | 0.247 | 0.058 | 17.7 | +0.291 | t | 3.929 | <0.001 | 0 | 0 | 13 | 72.2 | 5 | 27.8 |

Ct - control group, P - patients, Cv - convalescence

## DISCUSSION

In pathology caused by Morbilivirus infection, the Cu metabolism may be disturbed at the absorption and blood transportation stages. The ionised Cu released from food or Cu in the amino acid complex is bound by ligands and stored in enterocytes, from where it passes to blood (10). A measles virus infection in the 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 for by activating the supplies. However, hypercupremia observed during conva-lescence suggests that some other mechanisms are involved. Cu occurs in blood in a labile form bound with amino acids and in stabile form with coeruloplasmin. Since Morbilivirus belongs to hepatotropic viruses, hypercupremia observed in our patients may be related to this mechanism; especially that hypoalbuminemia, which usually accompanies lever pathology, was not observed in the course of measles. Thus, it cannot be ruled out that hypercupremia observed in our patient may have been caused mainly by the increase of coeruloplasmin which binds physiologically more than 90% of circulating Cu (10). It would be helpful to estimate coeruloplasmin level in our patients, especially that it was observed that its activity is higher in numerous virus infections (1,4). Immune reactions caused by measles virus antigens may also be of high importance (5,6,8). Therefore, it seems that the mechanisms leading to an increase of serum Cu value in our patients and the biological importance of this phenomenon are very complex. In our research we observed that the serum Zn level dynamics indicates a significant lowering in the eruptive period in comparison with the control group. Normalization of the serum Zn level was observed during regression of clinical symptoms and convalescence. The fact that the serum Zn level decreases although its content in plasma is less than 1% of the whole in organism means that Zn metabolism is disturbed at various stages, without determining their nature. Clinical symptoms of Zn deficiency appear when serum Zn level falls below 9 umol/l and remains at this level for a longer time (7). The decrease of the serum Zn level that we observed in the acute stage of measles never exceeded this value, even though the value was smaller than in healthy population. Therefore, lack of clinical symptoms of Zn deficiency in our patients is the result of this phenomenon. Furthermore, hypozinkemia does not last long; normalization of Zn level was observed during the regression of clinical symptoms. Hypozinkemia that we observed in our patients at the early stage of measles may be related to diet deficiency caused by lack of appetite. In addition, the data provided by various sources indicate that hypozinkemia may be the result of Zn translocation from serum to infected tissues because Zn is important in nucleic acids transformations and protein biosynthesis. Available literature also emphasises the importance of stress that accompanies infectious diseases. It is claimed that stress influences the serum Zn level. From this point of view, it may be stated that hypozinkemia is caused by activating adrenal cortex hormones (9) Immunological reactions released by Morbillivirus are important in mechanisms influencing the decrease of serum Zn value in measles (2). It was proved that considerable liability of the serum Zn level in measles is only a passive image of a disease process, whereas hypozinkemia can condition its course in the initial period. Measles belongs to serious viral diseases because of complications caused in the central nervous system and respiratory system that are difficult to predict. The analysis of Cu/Zn ratio in our patients indicates its significant increase in the acute stage and decrease following the regression of clinical symptoms. It should also be emphasised that no complications in the patients were observed. It seems that comparative research would be interesting. Ratio Cu/Zn values reflect the disturbances in homeostasis of these trace elements, whereas its clinical use seems to be tantamount to the determination of serum Cu and serum Zn levels.

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SUMMARY

The aim of this study was to evaluate the serum copper (Cu), zinc (Zn) levels and Cu/Zn ratio in measles patients in comparison to the control group. The study was conducted on 26 patients. The serum Cu and Zn levels were determined three times using atomic absorbtion spectrometry. The control group included 24 healthy persons. The serum Cu and Zn levels in those persons were determined once. Conclusion: in the acute period of the disease, a significant and highly significant increase in Cu/Zn ratio takes place.

Wskaźnik Cu/Zn u chorych na odrę

Celem pracy była ocena stężenia miedzi (Cu) i cynku (Zn) w surowicy krwi oraz wskaźnika Cu/Zn u chorych na odrę. Badaniami objęto 40 osób, w tym 26 chorych. Oznaczenia wykonano metodą spektrometrii atomowo-absorbcyjnej. Stwierdzono statystycznie znamienny wzrost wskaźnika Cu/Zn w ostrym okresie choroby w porównaniu z okresem rekonwalescencji.