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Serum zinc (Zn) level dynamics in blood serum of patients with acute viral hepatitis B and early recovery period

Available scientific data confirm that primary or secondary disturbances of zinc (Zn) metabolism are present in many liver diseases (3, 4, 5, 8, 10). In acute viral hepatitis B there comes to parenchymal liver damage with hepatocytes necrosis leading to the malfunction of the organ whose role in regulation of trace elements metabolism on different levels is essential in physiological conditions. Efficient immunological response against hepatitis B virus (HBV) and its antigens is crucial in pathogenesis of acute HBV infection.

The role of zinc (Zn) in regulation of biochemical processes in the body as well as its influence on the immunological system were the base for undertaking studies the aim of which was to provide answers to the following questions:

1. What are serum zinc (Zn) levels in patients during non-complicated, acute hepatitis B and early recovery period?
2. Does the serum zinc level change during acute phase of illness and early recovery period as compared with control group?

MATERIAL AND METHODS

CLINICAL MATERIAL

The studies included 39 patients, aged 18–76, hospitalised in the Department of Infectious Diseases of Medical University of Lublin because of acute hepatitis B. The diagnosis was established on the basis of anamnesis, clinical features, biochemical tests and serological tests. The presence of HBV surface antigen (HBsAg) and antibody to core

HBV antigen in M class of immunoglobulins (anty-HBcIgM) were confirmation of acute phase of infection.

The examined group consisted of patients without co-existing diseases, in whom clinical improvement and normalisation of biochemical parameters was achieved after treatment. In all the examined patients serum levels of bilirubin, total protein, activity of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase and zinc (Zn) concentration were determined. The above mentioned determinations were repeated several times during hospitalisation and once after discharging from the clinic in the early recovery period according to the following scheme: 1st examination (I) on the day of admission to the clinic, 2nd examination (II) on the 10th day of hospitalisation, 3rd examination (III) on the 20th day of hospitalisation, 4th examination (IV) on the last day of hospitalisation, after clinical improvement and normalisation of biochemical parameters, 5th examination (R) four weeks after discharge from clinic, during routine checkup.

The control group included 24 persons aged 22–69. The zinc (Zn) level and all biochemical tests of those people were determined once. All the patients and persons from the control group were informed about the purpose of examination and gave their consent to it.

METHODS

Blood used in investigations was sampled from the elbow vein in fasting state between 7 and 8 o'clock a.m. In all patients, serum levels of total bilirubin, total protein along with electrophoresis, activity of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase were determined.

Serological tests confirming HBV infection i.e. determinations of: HBsAg, antibodies to HBV surface antigen (anti-HBs), antibodies to HBV early antigen (anti-HBe), antibodies to HBV core antigen (anti-HBc) and anti-HBcIgM were made using enzyme-linked immunosorbent assay tests (ELISA), from Abbot (USA) and Organon – Technika (Holland) company.

The measurements of the zinc (Zn) serum level were made. 5 ml of sampled blood was transferred directly to demineralised test tube for centrifuge. After formation of the clot, the test tube 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°C. 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 zinc (Zn) content before each measurement. The serum for investigations was defrosted and then diluted with distilled water in proportion of 1:4 to be determined for zinc (Zn) concentration.

Determinations of the zinc (Zn) level in blood serum were made by atomic absorption spectrometry (AAS). For this purpose the AAS-3 atomic absorption spectrometer (Carl Zeiss Jena Germany) was used after previous calibration in the presence of standard sample at the wavelength of 213.9 nm.

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. The significance of differences between the zinc (Zn) 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 c-Cochran and Cox test.

RESULTS

Zinc (Zn) serum levels in the 24 persons of control group were 15.99±1.52 μmol/l, 15.78±1.25 μmol/l in men and 16.25±1.82 μmol/l in women. Serum zinc level in women was higher by 0.74 μmol/l but this difference is markedly at random (P>0.40). Lack of significant difference between men and women allowed to calculate the range of our own norm. The norm was assumed in the range of M±2SD, that is between 12.948 – 19.036 μmol/l.

Magnitude and significance of variations of serum zinc levels in patients between individual examinations are shown in Table 1. Comparing the results of examination II with those of examination I, the serum zinc level in patients changed from –19.7 μmol/l (decrease) to +5.5 μmol/l (increase). The medium decrease is 1.99 μmol/l and is statistically

Table 1. Magnitude and significance of variation of zinc (Zn) level between individual examinations

Comparable examinations	n	μmol/l				Significance of variation	
		from	to	mean	SDd	t	p
II z I	36	-19.7	+5.5	-1.99	4.46	2.674	<0.02
III z II	29	-12.8	+8.6	+0.57	3.95	0.781	>0.40
IV z III	20	-9.7	+4.9	+0.06	4.08	0.060	>0.90
IV z R	20	-9.2	+6.1	-1.31	3.95	1.476	>0.10
I z R	39	-9.2	+18.7	+0.46	6.08	0.476	>0.60
B z A	39	-10.0	+6.4	-1.69	3.32	3.178	<0.01

B = II+III+IV, A = I+R, p – level of statistical importance

Table 2. Zinc (Zn) serum level in patients with acute hepatitis B compared with control group

Group examination	n	μmol/l			V%	Compared to control group		
		M	SD	SE		difference of means	c	P
Control	24	15.99	1.52	0.31	9.5	-	-	-
I	39	16.77	4.77	0.76	28.4	+0.78	0.948	>0.30
II	36	14.64	3.15	0.53	21.5	-1.35	2.217	<0.04
III	32	15.00	3.60	0.64	24.0	-0.99	1.401	>0.10
IV	20	14.80	2.18	0.49	14.8	-1.19	2.060	0.06
R	39	16.31	3.46	0.55	21.2	+0.32	0.501	>0.60
A (I+R)	39	16.55	2.80	0.45	16.9	+0.56	1.015	>0.30
B (II+III+IV)	39	14.86	2.52	0.40	17.0	-1.13	2.229	<0.04

M – arithmetical mean, SD – standard deviation, V% – variation coefficient, c – value calculated with c – Cochran and Cox test, P – level of statistical importance

significant ($P<0.02$). Changes between examination II and III, as well as those between examination III and IV were highly at random ($P>0.40$; $P>0.90$). Differences between examination I and R were highly at random as well ($P>0.60$).

Mean zinc (Zn) serum concentration of the first (I) and the last (R) examination is marked with the letter A, however, mean of the other three examinations (II, III, IV) is marked with the letter B in Table 1. Comparing mean zinc (Zn) serum concentrations from period B (examination II, III, IV) to mean from period A (examination I and R) the difference ranges between –10.0 μmol/l and +6.4 μmol/l. In the comparison of period B with period A the average decrease of the serum zinc level is 1.69 μmol/l and that difference is highly significant ($P<0.01$). Comparing the serum zinc level observed in control group amounting to mean 15.99 μmol/l with level of this element in patients in period A (Table 2, Fig. 1), the mean increase comes to merely 0.56 μmol/l and this difference is markedly at random ($P>0.30$), however, in period B the mean decrease comes to 1.13 μmol/l and this difference is highly significant ($P<0.04$). Percentage of patients with the serum zinc level differing from the norm is given in Table 3. During the first examination the increased serum zinc level was found in 25.6% of patients while the decreased level was found in 15.4% of patients. During the second examination the increased level was observed in 8.3% of patients and decreased level in 30.6% of patients. The third determination revealed the increased serum zinc level in 12.5% of patients and decreased level in 30.4% of patients. None of them showed the increased serum zinc level during the fourth examination and 1 out of 4 (25%) patients showed the serum zinc

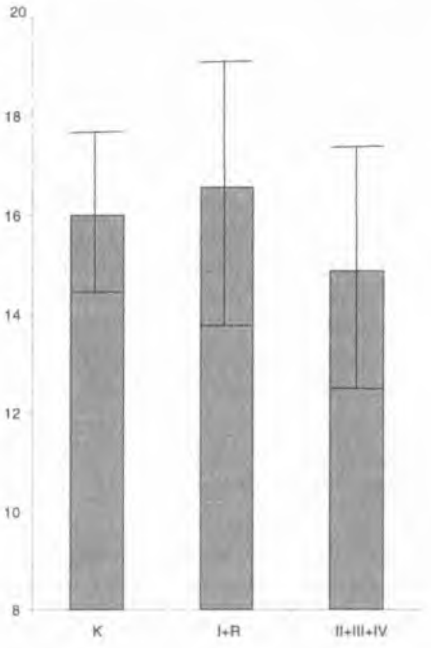

Fig. 1. Zinc (Zn) serum level in control group (K) and in patients

Table 3. Number (f) and percentage (%) of patients with the serum zinc level below (<ON), above (>ON) and within (=ON) our norm (ON) range

Examination	f	Zn		
		<ON	=ON	>ON
I	f	6	23	10
	%	15.4	59.0	25.6
II	f	11	22	3
	%	30.6	61.1	8.3
III	f	11	17	4
	%	34.4	53.1	12.5
IV	f	5	15	0
	%	25.0	75.0	0
R	f	6	24	9
	%	15.4	61.5	23.1

Our norm (ON) of the zinc (Zn) serum level ranges: Zn – from 12.95 to 19.04 μmol/l

level below the norm at that time. During early recovery period (R) the increased serum zinc level was observed in 23.1% of patients and decreased level in 14.4 % of cases. Thus, in examination I and R (period A) the increased values were observed slightly more frequently, however, in examinations II, III and IV (period B) the determined values were more often found to be decreased than increased. To recapitulate, the significantly decreased serum zinc level was observed in examination II, III and IV, while the differences stated in the serum zinc level in examination I and early recovery determinations compared with control group results were markedly at random.

DISCUSSION

Numerous reports confirm that in many liver diseases, depending on the cause and degree of damage, there comes to the disturbances of zinc (Zn) metabolism (3, 4, 5, 8, 10). For clinical use, the evaluation of the serum zinc level is considered as one of important criteria revealing the disturbances of its metabolism. Nowadays, the atomic absorption spectrometry (AAS) is the most valuable analytic method of trace elements level determination (11). Zinc (Zn) serum levels noted in this work in control group ranged between 12.948 – 19.036 μmol/l and are consistent with SI Unite Conversion Guide (6). In this research no significant difference in the serum zinc level between men and women was observed.

Among all sex hormones, only progesterone is the one that affects with the decreasing serum zinc level while estrogens activity is disputable. Anterior pituitary hormones exert indirect influence on mechanisms keeping serum zinc concentration stable and the growth hormone (GH) is the one of special importance amongst them. However, there are divergent reports on the serum zinc level depending on the age (7). We do not analyse this parameter in the presented study due to homogenous age structure of control group. Analysis of serum zinc level dynamics shows significant decrease during hospitalisation and correlates with the acute phase of HBV infection. Although the serum zinc level constitutes only 1% of the whole body zinc content, its decreased serum level reveals disturbances of this trace element metabolism on different stages, not determining their nature. From the practical point of view it is important whether the observed hypozincemia accompanies real zinc deficiency symptoms, and thus whether it can influence the course and outcome of acute hepatitis B. Decrease of the serum zinc level in patients observed in our research did not fall below 9 μmol/l considered to be the border value describing clinical manifestation (9). Presently avaliable data, proving the presence of a different zinc level in all tissues do not rule out liver as a temporary store of this metal in the body. However, it is thought that biological function of the liver is limited to accumulation of zinc excess rather than storing it for later use, especially as zinc mobilisation from muscles or bones is difficult and takes place only in states of increased catabolism (2). Real zinc deficiency often goes along liver pathology, which is shown by

decreased level of this trace element in hepatocytes and leucocytes (3, 4, 5). Decrease of serum zinc level below values seen in healthy men in the course of acute hepatitis B, observed in our study may be a sign of secondary deficiency due to a low-level-trace-element diet and coexisting dyspeptic symptoms during illness. In this depiction it is possible to explain similar values obtained on the first day of hospitalisation and in early recovery period with probable influence of diet on serum zinc level and so related to individual diet. Perhaps decreasing zinc level during escalation of illness does not prove the element deficiency, but is caused by translocation from the serum to the liver, similarly to its accumulation in hard-healing wounds and burns. However, studies revealing the decrease in zinc hepatocytes concentration deny that (3, 4, 5). Thus, other causes of hypozincemia observed in acute hepatitis B in our patients cannot be excluded.

Progress of the disease depends on the dynamics between virus replication and immune humoral and cellular response. Efficient activation of CD4 expressing lymphocytes intensifies induction of cytotoxic CD8 lymphocytes. Subpopulation of Th1 frees gamma interferon (IFN), which activates macrophages and interleukin (IL) 2, which additionally intensifies cellular response. It was proved that zinc plays important role in creating proper immune reactions. It activates T and NK lymphocytes, takes part in production of gamma INF, Il 1, IL 2 and tumour necrosing factor (TNF) (1). The presented data do not explain all stages of zinc metabolism disturbances in acute hepatitis B. Perhaps the observed changes in serum zinc level are only passive reflection of disease process, but their influence on the course of illness cannot be excluded.

CONCLUSIONS

The significantly decreased serum zinc level was observed in examination II, III and IV, while the differences stated in the serum zinc level in the first (I) and early recovery determination compared with control group results were markedly at random.

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SUMMARY

The aim of the present study was an analysis of serum zinc level dynamics in patients with acute hepatitis B and early recovery period compared with control group. The investigation included 39 patients aged 18–76 hospitalised in the Department of Infectious Diseases of the Medical University of Lublin, because of acute hepatitis B. Determinations of zinc (Zn) level in blood serum were made four times during hospitalisation and once, four weeks after discharging from the clinic in the early recovery period using atomic absorption spectrometry (AAS). The control group included 24 persons aged 22–69. Zinc (Zn) levels of those people were determined once. The obtained numerical data were subjected to statistical analysis. Lack of significant differences between men and women allowed to calculate the range of our norm, which was assumed at the level of M±2SD, that is between 12.948 – 19.036 μmol/l. The significantly decreased serum zinc level was observed during hospitalisation while the differences stated in the serum level of this element in initial and early recovery determination compared with control group results are markedly at random.

Dynamika stężenia cynku (Zn) w surowicy krwi chorych w ostrym okresie wirusowego zapalenia wątroby typu B oraz we wczesnej rekonwalescencji

Celem niniejszej pracy była analiza dynamiki stężenia cynku (Zn) w surowicy krwi chorych w ostrym okresie wirusowego zapalenia wątroby typu B (wzw B) oraz we wczesnej rekonwalescencji – w porównaniu z kontrolą. Badania dotyczyły 39 chorych w wieku od 18 do 76 lat, hospitalizowanych z powodu ostrego wzw B w Klinice Chorób Zakaźnych AM w Lublinie. W okresie hospitalizacji czterokrotnie oznaczano metodą absorpcyjnej spektrometrii atomowej (AAS) stężenie cynku (Zn) w surowicy krwi oraz jednorazowo we wczesnej rekonwalescencji po czterech tygodniach od wypisania z Kliniki. Grupa kontrolna obejmowała 24 osoby w wieku od 22 do 69 lat, u których stężenie cynku (Zn) oznaczono jednorazowo. Uzyskane dane liczbowe poddano analizie statystycznej. Nie stwierdzono istotnych różnic w stężeniu (Zn) w surowicy krwi w zależności od płci w grupie kontrolnej, co pozwoliło na wyznaczenie normy własnej. Przyjęto za nią stężenia w zakresie M ± 2SD, tj. 12,948 do 19,036 μmol/l. Stwierdzono istotne obniżenie stężenia cynku (Zn) w surowicy krwi w okresie hospitalizacji, natomiast w badaniu wstępnym i we wczesnej rekonwalescencji obserwowane różnice w stężeniu tego pierwiastka w porównaniu z kontrolą były wybitnie losowe.