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ORIGINAL ARTICLE

Evaluation of Calcium, Magnesium, Potassium, and Sodium in Biological Samples of Pakistani Viral Hepatitis (A–E) Patients and Controls

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SUMMARY

Background: **The aim of the present study was to compare the level of calcium (Ca), magnesium (Mg), potassium (K), and sodium (Na) in biological samples (serum, blood, urine, and scalp hair) of patients suffering from different types of viral hepatitis (A, B, C, D, and E; n = 521) of both genders, ages ranged between 31–45 years. For comparative study, 255 age-matched subjects of both genders, residing in the same city, were selected as controls.**
Method: **The elements in the biological samples were analyzed by flame atomic absorption spectrophotometry prior to microwave-assisted acid digestion. The validity and accuracy of the methodology was checked by using certified reference materials (CRMs) and values obtained by conventional wet acid digestion method on the same CRMs.**
Result: **The results of this study showed that the mean value of Na was higher in blood, sera, and scalp hair samples of hepatitis patients, while Ca, Mg and K levels were found to be lower than age-matched control subjects. The urinary levels of these elements were found to be higher in the hepatitis patients than in the age-matched healthy controls ($p < 0.05$).**
Conclusions: **These results are consistent with data reported in literature, confirming that the deficiency of essential mineral elements and sodium overload can directly cause lipid peroxidation and eventually hepatic damage.**
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KEY WORDS

Calcium, Magnesium, Potassium, Sodium, Hepatitis (A–E), Gender, Atomic Absorption Spectrophotometer

INTRODUCTION

Hepatitis is an inflammation of the liver, usually caused by a virus, and is contagious. The liver becomes unable to function properly and toxins build up in the body. Symptoms include fever, nausea, headache, muscle and joint pain, dark urine, and abdominal discomfort [1]. Essential micronutrients are involved in many metabolic pathways in the liver, such as enzymatic functions, protein synthesis, oxidative damage, anti-oxidant defense, immunological competence, and interferon therapy response to virus genomes [2]. Reactive oxygen species (ROS) have also been implicated in a number of hepatic pathologies in exacerbating liver diseases [3].
The oxidant production associated with immune reactions against viral hepatitis leads to the formation of hepatocellular carcinoma. Therefore, the changes in micronutrients and their destructive effects causing oxidative stress are the factors for viral hepatitis pathogenesis [4].

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Hepatitis A virus (HAV) has no known chronic carrier state and plays no role in the production of chronic active hepatitis or cirrhosis. However, prognosis and outcome of acute hepatitis B infection are variable. As a result of subclinical infection, chronic infection occurs frequently in 10 % of infected individuals [5]. In general, it is believed that immune system-mediated processes play roles in the pathogenesis of hepatitis B virus (HBV) infection. During hepatitis delta virus (HDV) infection, many mechanisms may be involved in the development of liver lesions as described for HBV. Infection with viral hepatitis C (HCV) tends to be persistent in most of the infected individuals, apparently reflecting an inability of the immune system to mount an effective antiviral response [6]. Hepatitis E virus (HEV) infections are immunopathologic in nature; hepatitis A and E have no progression to chronic hepatitis, late hepatic sequelae, and there is no evidence for persistent infection [7].

The mechanism(s) by which cells play a role in viral hepatitis pathogenesis has been the subject of intense research in recent years. The changes in trace element levels are part of the immune defense system of organisms and are induced by hormone-like substances such as interleukin-1, tumor necrosis factor-α, and interleukin-6 [8], which are immunocytokines, liberated in a dose-dependent mode, mostly by activated macrophages, in response to several stimuli including exercise, trauma, stress, or infection [9].

Sayed et al., 2005 [10] reported that the following abnormalities, depending upon the stage and severity of hepatic diseases, were found: (a) disturbances in the concentrations of total lipids, cholesterol, phospholipids, beta lipoproteins, glycerin, glycerides, and neutral fats, (b) marked disorders of glucose tolerance as indicated by the increase in the difference between plasma and erythrocyte glucose levels in proportion to the degree of liver damage, (c) a fall in plasma and erythrocyte Mg reflecting the degree of hepatic parenchyma damage, (d) a decrease of the albumin / gamma globulin ratio in proportion to the degree of the impairment of hepatic cells. George, 2006 [11] showed that Ca, Mg, K, and Na concentrations in the serum were decreased after the induction of liver injury. Mg and K contents were significantly reduced in the hepatic tissue. The low levels of albumin and the related ascites may be one of the major causes of the imbalance of mineral metabolism in hepatic fibrosis and further aggravation of the disease. Ahn & Flamm, 2005 [12] mentioned that autoimmune hepatitis is progressive inflammatory hepatitis of unknown etiology that is responsive to immunosuppressive therapy. For the prevention and treatment of autoimmune hepatitis, good nutrition is advisable, as are the suppression of the risk factors for osteoporosis and the administration of supplements of Ca and vitamin D [13].

The determination of trace quantities of metals in biological samples requires the use of sensitive and selective techniques such as atomic absorption spectrometry (AAS) [14, 15]. This technique requires the solubilization of the analyte and complete or partial decomposition of the matrix using convective systems, microwave ovens, or dry ashing [16]. The main advantage of microwave assisted sample pretreatment is its requirement of a small amount of mineral acids and a reduction in the production of nitrous vapors [17- 19].

The incidence of hepatitis C viral infection is rising rapidly all over the world. In fact, over 4 million Americans are infected, and more than 40 % of those infected do not know how they contracted HCV. Approximately 75 % to 85 % of people infected with HCV develop chronic hepatitis, which can lead to cirrhosis and liver failure [20]. The incidence of viral hepatitis is also increasing rapidly in developing countries, such as Pakistan, especially due to unhygienic conditions and poverty. The present study was designed to evaluate the levels of Ca, Mg, K, and Na in biological samples (blood, serum, scalp hair, and urine) of viral hepatitis and healthy subjects of the same age group (31-45) of both genders. The patients were classified into groups based on their serological and clinical tests.

MATERIALS AND METHODS

Apparatus

A Perkin-Elmer Model A Analyst 700, Perkin Elmer (Norwalk, CT, USA) atomic absorption spectrometer equipped with deuterium background correction was used in the study. The hollow cathode lamps of Ca, Mg, K, and Na were run under the conditions suggested by the manufacturer. The instrumental conditions were described in Table 1. Integrated absorbance signals computed by the AA spectrometer were employed throughout. A Pel (PMO23, Osaka, Japan) domestic microwave oven (maximum heating power of 900 W) was used for digestion of the biological samples. Acid washed polytetrafluoroethylene (PTFE) vessels and flasks were used for preparing and storing solutions.

Reagents and Standard Solutions

Ultrapure water obtained from ELGA lab water system ELGA (Bucks, UK) was used throughout the work. Concentrated nitric acid (65 %) and hydrogen peroxide (30 %) from Merck (Darmstadt, Germany) were checked for possible trace metal contamination. Working standard solutions of calcium, magnesium, potassium, and sodium were prepared immediately prior to their use by stepwise dilution of certified standard solutions (1,000 ppm) Fluka Kamica (Buchs, Switerland), with 0.2 M HNO_3. All solutions were stored in polyethylene bottles at 4 °C. For the accuracy of methodology, certified samples (certified reference materials (CRMs)) of human hair BCR 397 (Brussels, Belgium), Clincheck control-lyophilized human urine (Recipe Chemicals + Instruments GmbH, Munich, Germany), Sero-201705 human whole blood, level-3-OK 0337 (Billingstad, Norway) and human serum ERM- DA252a

(Teddington, Middlesex, UK) were used. All glassware and plastic materials used were previously soaked for 24 hours in 2 M nitric acid, washed with distilled water, finally rinsed with Milli-Q water, dried, and stored in class 100 laminar flow hoods.

Sample Collection and Pretreatment

The study protocol was approved by the local ethics committee of higher education commission of Pakistan. This was a hospital based study. The study was carried out on a sequential sampling of 438 hepatitis patients, 22 males and 25 females of hepatitis A (HAV), 64 males and 67 females of hepatitis B (HBV), 73 males and 72 females of hepatitis C (HCV), 41 males and 31 females of hepatitis D (HDV), and 25 males and 18 females of hepatitis E (HEV), age range between 31 to 45 years, and 177 healthy subjects (82 males and 95 females) individuals as controls of same age group. They all were residents of Hyderabad City, Pakistan. All patients and contolss provided a written consent, confirming that they accepted conditions of giving biological samples (whole blood, scalp hair, and urine) and were informed about the whole experimental procedures. A questionnaire was administered to all patients and controls to collect details of their physical data, ethnic origin, dietary habits, age, and consent. Physical examinations were performed at the Basic Health Unit of Hyderabad City, Pakistan. At the start of the study weight, height, blood pressure, and biochemical data of the participants were measured and recorded. Chronic active hepatitis was diagnosed by biochemical, histological, and virological investigations (Table 2). The patient groups were serologically and clinically diagnosed and classified as viral hepatitis A, B, C, D, and E by standard clinical methods [21, 22].

Collection of Biological Samples

The blood samples of HAV and HEV hepatitis patients were taken 2 - 3 weeks after diagnosis, while samples for HBV, HCV, and HDV patients were taken after hospitalization. Venous blood samples (5 mL) were collected using seven milliliters heparinized Vacutainer ® tubes (Becton Dickinson, Rutherford, NJ, USA) between 9:30 and 11:00 A.M. About 2 mL of venous blood samples were stored at - 20 °C until elemental analysis and the remaining (3 mL) were used for separating the sera. The blood was allowed to clot at room temperature for 15 to 30 minutes. When the blood had clotted completely, it was rimmed or ringed with an applicator stick and then centrifuged for 5-10 minutes at 2,500 revolutions per minute (rpm). The supernatant fluid is then removed with a Pasteur pipette and labeled accordingly.

It was stored at - 20 °C until analysis. Morning urine samples were collected in acid-washed, decontaminated 100 mL polyethylene tubes (Kartell, Milan, Italy). Inbetween sampling sessions, the container was wrapped in a clean polyethylene bag. Urine samples were acidified with ultrapure concentrated HNO_3, (1 % v / v) and kept at - 4 °C. Prior to sub sampling for analysis, the samples were shaken vigorously for 1 minute to ensure a homogeneous suspension. The hair samples (approximately 1.0 g each) were taken from the nape of the neck. The scalp hair samples were washed and treated as reported in previous studies [23]. After washing, scalp hair samples were dried in an electrical oven at approximately 75 °C and stored in precleaned plastic bags with identity numbers.

The pathologic diagnosis of patients in our study was based on the following criteria: (1) a liver biopsy test showing the presence of steatosis (> 10 %), as well as lobular inflammation and hepatocellular degeneration, irrespective of the presence of fibrosis or Mallory bodies; (2) appropriate exclusion of other liver diseases such as drug-induced liver disease, primary biliary cirrhosis, autoimmune hepatitis, and hemochromatosis. None of the patients had consumed alcohol in their life, nor was there any history of surgery or ingestion of drugs known to produce hepatic steatosis during that time.

Microwave assisted acid digestion (MWD)

A microwave-assisted digestion procedure was carried out in order to achieve a shorter digestion time. Duplicate samples of scalp hair (200 mg) and 0.5 mL of blood, serum, and urine samples of each patient and control individual were directly placed into Teflon PFA flasks. 2 mL of a freshly prepared mixture of concentrated HNO_3- H_2O_2 (2:1, v / v) were added to each flask and kept for 10 minutes at room temperature, then the flasks were placed in a covered PTFE container. This was then heated following a one-stage digestion pro-gram at 80 % of total power (900W). Complete diges-tion of blood, serum and urine samples required 2 - 4 minutes, while 5 - 8 minutes was necessary for scalp hair samples. After cooling the digestion flasks, digest samples were filtered through Whatman 42 filter paper, transferred into a 10-mL flask, and brought to volume with ultrapure water. Blank extractions were carried through the complete procedure. Blanks, standards, and sample solutions were prepared in a similar acid matrix. The validity and efficiency of the microwave assisted digestion method was checked with certified values of CRMs of all four biological samples and with those obtained from conventional wet acid digestion method (CDM) (Table 3).

The percentage recovery of all metals in CRM samples obtained by MWD were calculated according to equation

$$\%\,\mathrm{Recovery} = \frac{[Metals\ obtained\ by\ MWD]}{[Metals\ obtained\ by\ CDM]} \times 100$$

The % recoveries varied in the range of 97.7 - 99.9 for all four analytes under study. Non significant differences were observed ($p > 0.05$) when comparing the values obtained by MDM and CDM (paired t-test) (Ta-

Table 1. Measurement conditions for flame ionization AAS 700.

Elements	Wavelength (nm)	Slit width (nm)	Lamp current (mA)	Burner height (mm)	Oxidant (Air) L/min	Fuel (Acetylene) L/min
K	766.5	0.7	10.0	7.5	17.0	2.0
Na	589.0	0.2	10.0	7.5	17.0	2.0
Ca	422.7	0.7	7.5	12.5	17.0	2.0
Mg	285.2	0.7	7.5	7.5	17.0	2.0

ble 3). Mean values for all the metals differed less than 1 - 2 % from the certified values.

Statistical analysis

All statistical analyses were performed using the computer program Excel (Microsoft Corp., Redmond, WA) and Minitab 13.2 (Minitab Inc., State College, PA). Multivariate analysis was performed for the association of metals with the risk of hepatitis. The results of the biological samples of normal and hepatitis patients are reported as mean values with standard deviation (SD) for each element (Table 4).

RESULTS

The present hospital based study on patients suffering from hepatitis was carried out to determine the concentrations of Ca, Mg, K, and Na in biological samples (scalp hair, blood, serum, and urine). It was found that hepatitis was associated with a pronounced imbalance of the analytes under study (Table 4). The analyzed biological samples were categorized according to the hepatitis patients, controls, and gender. The hepatitis patients were further divided into five subgroups according to A, B, C, D, and E genotypes.

The mean values of Ca in scalp hair samples of male hepatitis patients of all genotypes were found in the range of 1110 - 2270 μg / g, which were significantly lower than control subjects of the same age group, 2450 - 3990 μg / g ($p < 0.001$) The same trend was observed in females. In sera samples, the mean concentration of Ca in control subjects of the 31 - 45 age group was found to be higher (44.9 - 62.7 mg / L) than those obtained from sera samples of hepatitis patients of all genotypes, male (27.4 - 43.3 mg / L) and female (26.2 - 39.7 mg / L). The range of Ca concentrations in blood samples of controls of both genders (47.8 - 63.9 mg / L) was significantly higher compared to the range of Ca concentrations observed in blood samples of all genotypes, male and female, hepatitis patients (26.3 - 42.8 mg / L) ($p < 0.01$). The excretion of Ca was found to be higher in all the genotypes of hepatitis patients of both genders.

The lower levels of Mg in scalp hair of male hepatitis patients of all genotypes were found in the range of 192 - 610 μg / g versus controls at 709 - 746 μg / g. The level of Mg is significantly lower in HBV patients (217 - 304 μg / g) and HCV patients (142 - 257 μg / g) ($p < 0.001$) compared to control values. The same trend was observed in females. Lower Mg was observed in blood and sera of all hepatitis patients compared to the healthy male and female groups ($p < 0.001$). Significantly lower concentrations of Mg in sera of HBV (24.3 - 31.6 mg / L) and HCV (18.5 - 27.8 mg / L) patients versus controls (45.8 - 63.7 mg / L) ($p < 0.001$) were observed. The excretion of Mg in urine was found to be significantly higher in all hepatitis patients (92.3 - 134 mg / L) than in controls (35.4 - 81.3 mg / L) ($p < 0.01$). The mean values of K in scalp hair samples of male hepatitis patients of all genotypes were found in the range of 27.4 - 37.2 μg / g. These were significantly lower than control subjects of the same age group (38.2 - 60.6 μg / g) ($p < 0.001$). The same trend was observed in females. In sera samples, the mean concentration of K in control subjects of the 31 - 45 age group was found to be higher (1762 - 2350 mg / L) than those obtained in sera samples of hepatitis patients of all genotypes, male (664 - 1652 mg / L) and female (632 - 1540 mg/L) patients. The range of K concentrations in blood samples of controls of both genders (1620 - 2650 mg / L) was significantly higher compared to the range of K concentrations observed in blood samples of all genotypes, male and female hepatitis patients (1030 - 1810 mg / L) ($p < 0.01$). The excretion of K was found to be higher in all the genotypes of hepatitis patients of both genders. The mean values with standard deviation for Na in controls and hepatitis patients are shown in Table 4, which indicates that the level of Na was altered in the biological samples (scalp hair, blood, urine, and serum) of hepatitis patients. The elevated levels of Na in scalp hair of male hepatitis patients of all genotypes were found in the range of 320 - 335 μg / g versus controls at 313 - 320 μg / g. The same trend was observed in females. Elevated Na was observed in blood and sera of all hepatitis patients compared to the healthy male and female groups ($p < 0.01$ - 0.02). The excretion of Na was found to be significantly higher in all hepatitis patients (3270 - 3910 mg / L) than controls (2950- 3540 mg / L) in urine ($p < 0.02$).

Table 2. Clinical and biochemical characteristics of controls and hepatitis patients.

Parameter	Normal	Hepatitis A	Hepatitis B	Hepatitis C	Hepatitis D	Hepatitis E	Range
Male							
BMI	26.2 ± 1.1	27.3 ± 2.5	26.9 ± 1.9	26.5 ± 1.5	27.1 ± 2.3	25.9 ± 1.4	24.4 - 29.9
Hb (13.2-17.3 g/dL)	16.2 ± 1.1	11.1 ± 1.2	13.3 ± 1.1	14.2 ± 1.31	13.3 ± 0.54	13.9 ± 1.45	10.1 - 17.3
Htc (39-49 %)	45.3 ± 2.4	43.4 ± 2.6	32.9 ± 3.92	28.9 ± 4.51	34.9 ± 2.6	42.4 ± 6.8	23.4 - 48.2
Urine Creatinine (27.0–260 mg/dL)	112 ± 23.5	189 ± 23.5	246 ± 13.4	255 ± 27.6	233 ± 8.37	213 ± 12.5	79.2 - 282
ALT (0-40 U/L)	26.5 ± 1.45	158.9 ± 7.9	302.5 ± 14.5	343.2 ± 21.1	283.7 ± 13.6	173.2 ± 18.8	150 - 389
AST (0-37 U/L)	14.9 ± 2.9	147.6 ± 3.9	276.7 ± 14.8	305.4 ± 23.6	242.6 ± 12.5	163.2 ± 8.74	142 - 336
ALP (37-147 U/L)	72.6 ± 4.6	172.5 ± 11.2	213.4 ± 12.8	358.2 ± 25.9	195.6 ± 7.8	178.9 ± 15.6	156 - 392
GGT (0-57 U/L)	29.6 ± 4.9	68.2 ± 6.7	95.2 ± 7.1	139.2 ± 10.2	83.6 ± 9.4	78.7 ± 4.3	61.5 - 152
SIBC (150-560 ng/dL)	241.5 ± 6.6	308 ± 15.6	536 ± 27.8	628 ± 23.7	483 ± 13.5	376 ± 10.9	282 - 658
TS (20-55 %)	25.3 ± 1.65	29.2 ± 4.9	47.9 ± 7.8	59.2 ± 6.1	43.6 ± 2.4	33.9 ± 3.9	24.8 - 66.2
Ferritin (28-80 ng/mL)	52.8 ± 2.9	194.6 ± 10.9	348.7 ± 19.8	354.4 ± 29.4	294.5 ± 16.8	239.7 ± 29.8	163 - 439
Total cholesterol (123-200 mg/dL)	138.9 ± 9.9	182.8 ± 13.7	263.2 ± 32.4	285.6 ± 22.9	247.9 ± 15.7	231.8 ± 10.3	169 - 317
Triglyceride (60-165 mg/dL)	92.3 ± 7.5	147.5 ± 12.7	214.5 ± 19.8	325.6 ± 28.5	198.4 ± 16.8	159 ± 19.2	132 - 358
AAT (1.4-3.2 g/dL)	2.24 ± 0.46	1.34 ± 0.35	0.74 ± 0.18	0.48 ± 0.08	0.95 ± 0.07	1.25 ± 0.27	0.30 - 1.71
Female							
BMI	25.3 ± 1.6	26.4 ± 2.1	26.2 ± 1.7	24.9 ± 2.9	25.7 ± 2.3	23.5 ± 3.8	20.3 - 28.7
Hb (13.2-17.3 g/dL)	15.5 ± 1.5	11.2 ± 1.9	13.5 ± 0.85	14.7 ± 1.5	12.8 ± 0.68	13.8 ± 2.5	10.4 - 17.2
Htc (39-49 %)	44.6 ± 3.1	42.2 ± 3.5	33.9 ± 4.5	30.9 ± 6.02	36.8 ± 3.1	41.6 ± 7.2	24.5 - 49.5
Urine Creatinine (27.0–260 mg/dL)	106 ± 14.9	182 ± 18.3	249 ± 9.82	256 ± 20.8	239 ± 12.3	219 ± 10.2	82.6 - 277
ALT (0-40 U/L)	26.2 ± 2.6	156.8 ± 5.8	299.8 ± 12.5	345.8 ± 43.5	285.6 ± 15.3	176.9 ± 11.3	150 - 421

BMI: body mass index; Hb: hemoglobin; Htc: hematocrit; ALT: alanine aminotransferase; AST: aspartate aminotransferase; ALP: alkaline phosphatase; GGT: gamma glutamyl transferase; SIBC: serum iron binding capacity; TS: transferrin saturation; AAT: alpha-1 antitrypsin.

Table 3. Determination of Ca, Mg, Na, and K in certified samples by CDM and MWD (N = 10).

Elements	Conventional digestion method CDM	Microwave digestion method MWD	T value^	% recovery[$]	Certified values
Certified sample of whole blood (mg/L)					
Ca	14.60 ± 1.1 (7.53)	14.58 ± 1.3 (8.92)	0.898	99.9	14.7 ± 0.3
Mg	16.88 ± 1.3 (7.70)	16.86 ± 1.4 (8.30)	0.978	99.9	17.0 ± 0.3
Na	1202.7 ± 33.8 (2.81)	1193.1 ± 19.8 (1.66)	0.310	99.2	1210 ± 40
K	1011 ± 48.7 (4.82)	1009 ± 36.7 (3.64)	0.936	99.8	1014 ± 30
Certified sample of serum (mg/ L)					
Ca	57.6 ± 0.83 (1.44)	56.3 ± 0.65 (1.15)	0.00376	97.7	58 ± 1.9
Mg	8.06 ± 0.095 (1.18)	8.01 ± 0.104 (1.30)	0.0003	99.4	8.1 ± 0.36
Na	2340 ± 150 (6.41)	2310 ± 165 (7.14)	0. 321	98.7	2400 ± 120
K	66.6 ± 1.03 (1.54)	66.2 ± 1.09 (1.65)	0. 628	99.4	67 ± 1.90
Certified sample of urine (mg / L)					
Ca	104.5 ± 7.9 (7.56)	104.3 ± 6.8 (6.52)	0.938	99.8	105 ± 5
Mg	62.68 ± 5.6 (8.93)	62.56 ± 4.5 (7.19)	0.937	99.8	63 ± 3
Na	2609.5 ± 59.8 (2.29)	2604.3 ± 73.5 (2.82)	0.947	99.8	2620 ± 140
K	1495.5 ± 28.9 (1.93)	1491.0 ± 39.8 (2.67)	0.876	99.7	1500
Certified sample of human hair (µg/ g)					
Ca	1550.6 ± 55.3 (3.56))	1546.0 ± 45.6 (2.95)	0.889	99.7	1560.0 ± 40[‡]
Mg	199.0 ± 13.5 (6.79)	198.2 ± 12.3 (6.2)	0.838	99.6	200 ± 5[‡]
Na	1.07 ± 0.08 (7.48)	1.06 ± 0.06 (5.66)	0.855	99.1	1.08 ± 0.007
K	8.55 ± 0.5 (5.85)	8.53 ± 0.7 (8.21)	0.941	99.8	8.6 ± 0.03

Key: ^Paired t-test between CDM and MWD DF = 9,
T (critical) at 95 % CI = 2.262, p = 0.05
‡ informative value
# indicative value
Values in ( ) are RSD
[$] % recovery was calculated according to : [MDM] X 100
[CDM]

Table 4. Calcium, magnesium, potassium, and sodium concentrations in biological samples (scalp hair, serum, blood, and urine samples) of controls and in all genotypes of male and female viral hepatitis patients.

	Male				Female			
Calcium								
Specimens	**Scalp hair (µg/g)**	**Serum (mg/L)**	**Blood (mg/L)**	**Urine (mg/L)**	**Scalp hair (µg/g)**	**Serum (mg/L)**	**Blood (mg/L)**	**Urine (mg/L)**
Controls	3120 ± 831	53.2 ± 8.24	57.3 ± 6.7	54.5 ± 11.2	2950 ± 920	52.4 ± 5.62	55.6 ± 8.8	63.9 ± 13.7
Hepatitis A	2270 ± 588	43.3 ± 5.37	42.8 ± 5.2	75.9 ± 8.3	2300 ± 639	41.5 ± 3.58	39.7 ± 6.6	74.5 ± 9.2
Hepatitis B	1140 ± 274	34.7 ± 6.82	27.4 ± 9.9	109 ± 22.2	1670 ± 57.4	33.9 ± 2.82	29.8 ± 7.8	97.8 ± 28.4
Hepatitis C	1110 ± 48.8	27.4 ± 5.67	28.0 ± 6.4	113 ± 24.4	1230 ± 69.9	26.2 ± 1.45	26.3 ± 6.5	113 ± 24.2
Hepatitis D	2000 ± 168	35.6 ± 2.87	36.4 ± 4.1	87.2 ± 15.7	2060 ± 116	33.9 ± 3.84	38.5 ± 4.9	78.3 ± 15.7
Hepatitis E	2190 ± 486	40.2 ± 5.74	39.7 ± 4.8	79.3 ± 13.8	1980 ± 150	38.4 ± 2.84	37.8 ± 5.5	76.6 ± 18.3
Magnesium								
Controls	728 ± 17.7	56.6 ± 7.95	83.8 ± 16.4	56.7 ± 18.6	789 ± 9.5	54.9 ± 8.31	87.7 ± 13.9	64.8 ± 16.5
Hepatitis A	610 ± 39.9	40.3 ± 5.64	60.3 ± 6.7	106 ± 18.2	626 ± 34.6	39.6 ± 7.92	62.6 ± 5.4	109 ± 18.2
Hepatitis B	262 ± 40.2	28.5 ± 3.41	31.9 ± 2.9	113 ± 12.5	286 ± 45.3	27.5 ± 3.98	34.8 ± 4.1	118 ± 12.7
Hepatitis C	192 ± 59.4	24.1 ± 1.52	25.2 ± 2.6	134 ± 12.4	195 ± 72.2	22.5 ± 5.28	28.2 ± 3.5	123 ± 15.8
Hepatitis D	480 ± 45.5	30.7 ± 4.92	40.4 ± 7.7	97.2 ± 17.9	504 ± 45.3	29.8 ± 2.38	42.5 ± 5.9	98.3 ± 17.6
Hepatitis E	512 ± 48.4	38.6 ± 3.26	53.5 ± 8.9	92.3 ± 19.8	538 ± 47.9	36.9 ± 5.98	57.8 ± 9.2	96.6 ± 15.3
Potassium								
Controls	49.9 ± 11.5	1960 ± 364	2170 ± 490	1260 ± 106	46.7 ± 10.2	1843 ± 350	2100 ± 513	1050 ± 97.3
Hepatitis A	37.2 ± 9.6	1652 ± 214	1810 ± 212	1270 ± 227	35.9 ± 12.6	1540 ± 168	1690 ± 149	1150 ± 55.4
Hepatitis B	32.0 ± 7.6	830 ± 172	1270 ± 133	1600 ± 220	31.5 ± 6.3	824 ± 137	1250 ± 98.4	1390 ± 68.9
Hepatitis C	27.4 ± 4.8	664 ± 95.2	1030 ± 109	1760 ± 139	25.9 ± 5.2	632 ± 160	1050 ± 77.9	1700 ± 45.3
Hepatitis D	35.2 ± 5.7	960 ± 145	1560 ± 181	1490 ± 73.8	32.7 ± 6.9	931 ± 128	1410 ± 92.7	1340 ± 94.2
Hepatitis E	36.4 ± 7.6	1527 ± 137	1760 ± 79.9	1340 ± 81.5	37.5 ± 9.8	1470 ± 260	1600 ± 164	1250 ± 103
Sodium								
Controls	317 ± 3.5	2560 ± 239	1060 ± 106	3490 ± 105	293 ± 4.2	2539 ± 450	1050 ± 97.3	3060 ± 90.2
Hepatitis A	322 ± 39.6	3027 ± 176	1370 ± 137	3540 ± 105	306 ± 18.6	3104 ± 138	1140 ± 211	3270 ± 33.5
Hepatitis B	335 ± 20.6	3456 ± 332	1240 ± 340	3740 ± 82.6	324 ± 21.3	3428 ± 280	1270 ± 226	3700 ± 44.4
Hepatitis C	356 ± 15.8	3678 ± 259	1540 ± 267	3820 ± 73.3	326 ± 21.2	3750 ± 460	1380 ± 305	3910 ± 52.9
Hepatitis D	321 ± 31.7	3237 ± 178	1430 ± 210	3630 ± 63.7	319 ± 29.9	3280 ± 430	1170 ±1 29	3390 ± 51.5
Hepatitis E	320 ± 23.6	3180 ± 418	1340 ± 330	3520 ± 58.2	309 ± 35.8	3258 ± 541	1120 ± 128	3360 ± 97.7

DISCUSSION

Currently, five different identifiable hepatitis viruses are responsible for more than 95 % of the cases of viral-induced hepatitis in the world. Treatment options have expanded, but no cure is available for some of them.

Of the five identified viruses, three are associated with chronic disease (HBV, HCV, and HDV). Hepatitis viruses A and E are enterically transmitted and do not cause chronic disease [24].

In our study, high levels of cholesterol, triglycerides, ferritin, and creatinine in urine were found in all groups of hepatitis patients whereas significant decreases in hemoglobin and hematocrit were observed. The liver is the primary target organ for glucagon action where it promotes glycogenolysis, gluconeogenesis, and ketogenesis [25]. The availability of serum blood chemistry tests for screening both symptomatic and asymptomatic patients has resulted in a marked increase in the number of abnormal liver chemistry tests that must be interpreted by physicians. Usually the first step in the evaluation of a patient with elevated liver enzymes is to repeat the test to confirm the result. There is a hypothesis that enzymes conventionally associated with liver dysfunction, AST, ALT, and γ-glutamyltransferase (GGT), may predict hepatitis. In our study, significantly increased levels of AST, ALT, GGT, ALP, SIBC, and TS were found, whereas AAT was decreased in all groups of hepatitis patients. A recent study showed that one-third of the hospitalized patients with liver cirrhosis are infected with HBV or HCV, with raised ALT / AST and ALP being more common with superadded viral infection [26].

The findings of the present study clearly demonstrate that the concentration of essential elements, Ca, K, and Mg were found to be lower in the biological samples (scalp hair and blood) of hepatitis patients, while Na levels were found to be higher compared to controls, as shown in Table 4.

Similarity between the symptomatology of hypomagnesaemia and hepatic precoma suggests that hypomagnesaemia may play an important role in the aetiopathogenesis of hepatic coma [27]. It may lead to a new approach in the management of hepatic coma. In infective hepatitis serum Mg was raised suggesting the release of magnesium from the necrosed liver cells. Similar observations were made in three cases of liver abscess. Serum Mg levels thus may serve as an indicator of liver cell necrosis [28]. There was no significant variation in serum Mg levels in cases of amoebic hepatitis (without frank abscess), obstructive jaundice, and malignancy of liver [29, 30]. In addition, magnesium isoglycyrrhizinate on rat hepatic stellate cells (HSC) significantly inhibits the proliferation of, and compliance with the dose relationship and it also has anti-cell-oxidation [31, 32].

Advanced scarring of the liver [cirrhosis] may lead to an abnormal accumulation of fluid in the abdomen referred to as ascites. Patients with hepatitis C who have ascites must be on Na [salt] restricted diets [33]. Every gram of Na consumed results in the accumulation of 200 mL of fluid. The lower the salt content in the diet, the better this excessive fluid accumulation is controlled. While often difficult, Na intake should be restricted to 1000 mg each day and, preferably, to 500 mg per day. One must become a careful shopper, carefully reading all food labels. It is often surprising to discover which foods are high in Na [34]. Meats, especially red meats, are high in Na content, and adherence to a vegetarian diet may often become necessary. Patients with chronic hepatitis C without ascites, are advised not to overindulge in salt intake, although their restrictions need not be as severe [35, 36].

Calcium is essential for healthy teeth and bones, for normal muscle contraction, and for blood clotting. Almost all of the calcium in the body resides in the bones. Without an adequate amount of calcium the bones become soft and brittle [37].

Osteoporosis is characterized by a reduced bone mass and the resulting increased risk for bone fractures. Since people with chronic liver disease are at increased risk for the development of osteoporosis, it is important to consume foods rich in calcium and / or to supplement their diets with calcium [38]. Other factors, such as cigarette smoking, lack of exercise, excessive alcohol consumption, and abnormal hormone levels also play roles in the development of bone loss. Alcohol has been shown to be directly toxic to bone cells and may impair calcium absorption [39]. Thus, it is especially important for patients with alcoholic liver disease to take calcium supplementation. In fact, it is a good idea for all people with chronic liver disease to take a calcium and vitamin D supplement [39].

Good sources of calcium include dairy products, leafy dark green vegetables (except spinach), tofu, canned sardines with bones, or salmon with bones. Excessive calcium consumption may interfere with the absorption of iron, in addition to causing many medical problems such as kidney stones, constipation, and fatigue [40]. Furthermore, as with all supplements, regardless of how much is consumed, the body will only utilize the amount needed. If calcium supplementation is taken, it should be limited to no more than 1000-2000 milligrams / day, and should be taken with a vitamin D supplement (which is usually included in the calcium tablet) [40].

Insulin regulates glucose and K metabolism by acting differently upon peripheral tissues (e.g., skeletal muscle) and the splanchnic bed, including the liver [41, 42]. Liver disease is accompanied by "insulin resistance" of glucose metabolism, whereby glucose intolerance occurs despite increased plasma insulin concentration. However, it is unknown whether insulin resistance extends to K metabolism [43]. Further, it is uncertain whether the hyperglycemia and alterations of plasma K concentration observed during liver transplantation result from changes in circulating insulin concentration, altered sensitivity to insulin, or both, as the diseased liver is removed and replaced with a graft organ [44].

CONCLUSION

These results demonstrate the low level of essential elements (Mg, K, and Ca) in all types of hepatitis. These changes may be of pathophysiological significance in acute hepatitis, in particular in patients with pre-existing elemental deficiencies. Changes in the content of microelements depended on the severity of the disease. HAV and HEV patients showed less disturbance in metabolism of metals under study. The deficiency of Ca, Mg, and K in fibrotic livers caused by hepatitis (A–E) may contribute to hepatic injury. The real mechanism is not known at present.

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Declaration of Interest:
There is no conflict of interest.

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