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Clinical studies

Liver tissue trace element levels in HepB patients and the relationship of these elements with histological injury in the liver and with clinical parameters

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ABSTRACT

Aim: In this study, the relationship of liver tissue trace element concentrations with hepatitis B disease and the effects of several environmental factors were analysed.
Method: The liver tissue concentrations of Al, Fe, Cd, Mn, Cr, Cu, Pb, Ni, Zn, Ag, and Co were evaluated in 92 patients with hepatitis B using the Inductively Coupled Plasma − Mass Spectrometry (ICP/MS) method in the analyses. The patients were divided into the following two groups: low-high Ishak histologic activity index (HAI) (0–6: Low Histologic Activity, 7–18: High Histologic Activity) and low-high fibrosis (FS) (Fibrosis 1,1,2 and Fibrosis 3,4,5,6). The metal levels were compared between the groups.
Results: The Cd concentration was found to be statistically higher in the group with low HAI scores ($p = 0.019$). The hepatic Cu concentration was found to be higher in women than in men ($p = 0.046$). The hepatic Fe concentration was found to be higher in the group with increased FS compared to the group with decreased FS ($p = 0.033$). Cd was found to be higher in patients who worked in positions involving exposure to heavy metals and in individuals with an ALT level above 40 IU/L ($p = 0.008$). Several correlations have been found between the hepatic tissue metal levels in our study. In a linear regression analysis, Fe and Zn were found to be correlated with the fibrosis scores ($p = < 0.001$ and $p = 0.029$), and Cu was correlated with HAI ($p = 0.023$). In the linear regression model, Ni ($p = 0.018$) and Cr ($p = 0.011$) were correlated with gender. There was a correlation between the hepatic Fe level and the location where hepatitis B patients were living (village/city) ($p = 0.001$), frequency of fish consumption ($p = 0.045$) and smoking ($p = 0.018$) according to the linear regression analysis. Using a logistic regression analysis, Cr ($p = 0.029$), Ni ($p = 0.031$) and Pb ($p = 0.027$) were found to be correlated with smoking habit, and Zn ($p = 0.010$), Ag ($p = 0.026$), Cd ($p = 0.007$) and Al ($p = 0.005$) were correlated with fish consumption.
Conclusion: The liver tissue trace element levels are correlated with disease activity and histologic damage in patients with HepB disease. Additionally, smoking, the environment in which the patient works and the amount of fish consumption affect the accumulation of trace elements in the liver.

1. Introduction

Hepatitis B (HepB) infection is a significant health problem worldwide, and serologic tests in 30% of the population show positive HepB (hepatitis B virus) [1]. Approximately 4,3 million individuals are estimated to be infected with HepB in the East Mediterranean region [2]. Most (60–85%) patients with chronic HepB are asymptomatic and a significant portion of the patients is not diagnosed until cirrhosis develops [3,4]. HepB is transmitted between individuals by the prenatal route, percutaneous route, sexual contact and open wound contact. Using intravenous agents and haemodialysis increases the risk of contagion [5,6].

It has been reported that the serum heavy metal levels are highly sensitive in hepatic diseases [7]. It is thought that HepB virus activity is

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affected by the levels of trace elements. Although it is known that the plasma trace element levels vary in most viral infections, the effect of this variability on the creation of tissue injury is not known. Trace elements play a significant role in hepatic diseases including mainly hepatocyte degeneration [8].

In recent years, Inductively Coupled Plasma − Mass Spectrometry (ICP/MS) has emerged as a method for measuring more sensitively trace elements. ICP/MS is an analytical technique in which molecular bounds are broken and atoms are ionized by transferring argon to a plasma with a high temperature. This method is widely used in qualitative analyses and in determining isotope rates because of its ability to analyse multiple elements simultaneously [9–12].

In this study, we aimed to assess the liver tissue trace element concentrations using the ICP-MS method and evaluate the relationship of these concentrations with HepB activity and pathologic injury level. In our study, the effects of different factors, including nutritional habits, social environment and occupational states, on the liver tissue trace element levels were also examined.

2. Material and methods

Ninety-two HepB patients (17–71 years of age) who presented to our outpatient clinic consecutively and who were found to have an HBV DNA value above 2000 IU/ml were included in this study. A liver biopsy was performed before treating these patients to evaluate the histologic activity of the disease according to the ISHAK score. (Fibrosis is scored between 0 and 6; the histologic activity index is scored between 1 and 18) [13]. The patients were divided into two groups: those with a low histologic activity index (HAI) score (0–6) and those with a high histologic activity index score (7–18). The trace element levels were compared between these groups. Additionally, patients with a fibrosis score of 0, 1, or 2 were in the low fibrosis score group, and those with a fibrosis score of 3, 4, 5, or 6 were in the high fibrosis score group. The patients' hepatic tissue samples were stored at −80 °C. Local ethics committee approval was obtained for this study.

The liver biopsy samples were thawed on the same day, and the liver tissue heavy metal concentrations were analysed by an experienced biologist. In this analysis, the atomic absorption spectrophotometric methods were applied. Copper, iron, aluminium, chromium, lead, mangan, silver, zinc, cobalt and cadmium concentrations in the liver tissue were evaluated. Trace element measurements are based on milligrams per kilogram (mg/kg). Considering this objective, the tissue samples that were used in the metal analysis were maintained at 105 °C for 72 h, constant weighing was obtained and the dry weights were determined. A mixture of two millilitres of nitric acid (HNO3, 65%, Ö.A.: 1.40, Merck) and 1 ml of perchloric acid (HClO4, 60%, Ö.A.: 1.53, Merck) was added to the tissue samples, which had been transferred into the experimental tubes [Muramoto, 1983], and were burned at 120 °C for 8 h. The tissue samples were transferred into polyethylene tubes after the burning procedure was completed, and the total volume was prepared for analysis by adding deionised water to reach 10 ml. The heavy metal content of the tissue samples was determined using Inductively Coupled Plasma Mass Spectrometry (ICP-MS) [Okamoto, 1997]. IAEA-407 was used as reference material. Detailed results on the amounts of trace elements obtained from the reference material and LOD- LOQ values cited in Table 1.

Additionally, the patients' baseline liver function tests [AST (U/L), ALP (U/L), ALT(U/L), GGT (U/L), and total bilirubin (mg/dl)] were measured. In several patients, the blood lead (mg/L) (n: 35) and zinc (mg/L) (n:36) values were analysed.

At the time of the diagnosis, the factors that might have affected the deposits of heavy metals in the liver, including the habitat (village/city), frequency of fish consumption, occupation and smoking,were evaluated using a questionnaire. This questionnaire asked about the following aspects: 1) the habitat (village/city; living in cities was considered risky); 2) frequency of fish consumption (once a week and more frequently/once a month/less than once a week/none; consuming fish once a week was considered risky); 3) working environments carrying risks (working in different branches of industry, including 1-Petrochemistry, 2-Mine, 3-Closed conservatory, 4-Metal and 5-Textile, and individuals who had close contact with the products made in branches of a mentioned industry) and working environments that did not carry any risk (housewives or patients who were not exposed to metal, petrochemistry, textile chemicals and pesticides; and 4) smoking (smoker/non-smoker; smoking was considered risky).

Table 1
Validation parameters of the analytical method.

Trace elements mg/kg	LOD (ng g-1)	LOQ (ng g-1)	R^2	Certificated Values Concentration (mg kg^{-})	Certificated Values 95% Confidience Interval (mg kg^{-})
Aluminium	1.74	4.18	0.9998	13.8	12.4–15.2
Chromium	1.13	3.78	0.9999	0.73	0.67–0.79
Mangan	0.21	0.75	0.9999	3.52	3.44–3.60
Iron	1.49	5.01	0.9998	146	143–149
Cobalt	0.24	0.69	0.9999	0.10	0.09–0.11
Nikel	0.64	2.23	1	0.60	0.55–0.65
Copper	0.59	2.04	0.9999	3.28	3.20–3.36
Zinc	2.51	8.29	0.9999	67.1	66.3–67.9
Silver	0.13	0.43	0.9999	0.037	0.033–0.041
Cadmium	0.47	1.51	0.9999	0.189	0.185–0.193
Lead	0.36	1.18	0.9999	0.12	0.10–0.14

IAEA-407 was used as reference material.
IAEA (International Atomic Energy Agency), LOD:Limit of detection,LOQ:Limit of quantification.

2.1. Statistical analysis

In the homogeneity evaluation, the variables with skewness-kurtosis values ranging between +1,5 and −1,5 were homogeneous in our study. The dual independent variables, which showed a normal distribution as a result of homogeneity tests, were analysed using an independent sample T test. In groups that did not show homogeneity, comparisons were performed using the Mann-Whitney *U* test. The parameters that did not show a normal distribution were analysed using Spearman correlation test, and the parameters that showed a normal distribution were analysed using Pearson correlation analysis.

Each heavy metal level that was measured to evaluate the parameters, which were related to the heavy metal levels to the greatest extent, was considered a dependent variable. The independent variables included the habitat, smoking status, frequency of fish consumption, hepatic fibrosis score and histologic activity score. The control variables included age and gender. Analyses were performed based on these variables by constituting linear regression models. Considering smoking, fish consumption, habitat and occupation to be separate categorical variables, the relevant liver tissue heavy metal levels were evaluated using a logistic regression analysis. In these tests, $p < 0.05$ was considered statistically significant.

3. Results

Fifty-two (61,5%) of the 92 HepB patients included in the study were male, and 36 (38,5%) were female. The mean age in the patient group was 38,5 ± 13.4 years (17–71 years). The liver tissue trace element levels of the patients are shown in Table 2. When the trace element levels were compared between genders, it was found that only the hepatic Cu levels were higher in women than in men ($p = 0.046$). No difference was found in the levels of the other trace elements ($p > 0.05$). Among the liver function tests, only GGT levels were found to be significantly higher in men ($p = 0.027$). Comparisons of the liver