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Assessment of Some Heavy Metals and Trace Elements in a Sample of Patients with Viral Hepatitis in Baghdad

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Abstract

The liver is a vital metabolic organ that performs a variety of complex biochemical functions such as metabolism, detoxification, and excretion. Impairment of liver functions disrupts trace element metabolism, causing oxidative stress, which subsequently leads to liver inflammation and fibrosis. This study aims to evaluate zinc (Zn), copper (Cu), iron (Fe), cadmium (Cd), and lead (Pb) levels in chronic viral hepatitis (HBV or HCV) patients in Baghdad. One hundred fifty samples of blood were collected from fifty patients with hepatitis B, fifty patients with hepatitis C, and fifty individuals without viral hepatitis as controls at the Gastroenterology and Hepatology Teaching Hospital in Baghdad during the period from November 2021 to March 2022. Patients with viral hepatitis had highly significant elevated levels of lead, cadmium, and copper compared with the controls (P-value = 0.000), whereas zinc and iron levels were highly significantly lower in the patient groups than in the control group (P-value = 0.000). In conclusion, viral hepatitis patients showed varying levels of zinc, copper, iron, cadmium, and lead compared to healthy controls.

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1. Introduction

Hepatitis B virus (HBV) and hepatitis C virus (HCV) infections are the major international health issues that affect approximately 250 million people worldwide. They can infect the human liver, causing acute, transitory, and chronic illnesses [1]. Chronic infections of the HBV or HCV viruses result in a range of liver illnesses, such as chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma [2]. In the United States, the most prevalent cause of liver illness and the need for liver transplantation is HCV. Nearly 50–85% of people infected with HCV develop chronic hepatitis, with 5–30% progressing to cirrhosis. Alcohol intake can speed this progression [3].

The liver regulates the majority of trace element metabolism, and any impairment in liver function can result in a variety of metabolic disorders. The administration or depletion of these trace elements can enhance metabolic disorders and liver dysfunction [4]. Heavy metals such as arsenic (As), lead (Pb), chromium (Cr), and cadmium (Cd) are among the hepato-toxicants that accelerate the course of viral hepatitis to liver failure [5]. HBV, on the other hand, affects liver function, making Cd detoxification harder [6].

Trace elements are very small amounts of chemical elements present in natural materials. In biochemistry, the term "trace elements" refers to dietary minerals that are required in small amounts for the normal growth, development, and functioning of an organism [7]. In analytical chemistry, a trace element is defined as an element having a concentration of fewer than 100 parts per million (ppm) measured in an atomic count [8]. Heavy metal is a naturally occurring metal with an atomic number exceeding twenty and an elemental density of more than 5 g/cm3 [9]. According to Kim et al. studies, heavy metals have been categorized into two groups: essential (zinc (Zn), copper (Cu), iron (Fe), and cobalt) and non-essential (Pb, Cd, As, Cr, and mercury). At low quantities, an essential heavy metal is less harmful and serves as a coenzyme in biological processes. whereas a non-essential heavy metal is very hazardous, even at a very low concentration. It is non-biodegradable and has a severely harmful impact on living beings [10]. Trace elements have a variety of crucial roles in the human body. They are largely structural components of enzymes; some are important for enzyme processes by acting as catalysts in enzyme systems or cofactors whose activities include preventing dietary deficiency, immunological functions, gene expression regulation, antioxidant defense, and prevention of chronic disease [11].

Nomenclature			
AAS	Atomic Absorption Spectrophotometer	HCV	Hepatitis C virus
AS	Arsenic	HS	Highly Significant
Cd	Cadmium	µg/dl	Micrograms per deciliter
Cr	Cr	MT	Micrograms per deciliter
Cu	Copper	Pb	Lead
FAAS	Flame Atomic Absorption Spectrophotometer	ppm	parts per million
Fe	Iron	ROS	Reactive Oxygen Species
GFAAS	Graphite Furnace Atomic Absorption Spectrophotometer	SD	Standard Deviation
g/cm3	Gram per cubic centimeter	SPSS	Statistical Package for Social Sciences
HBV	Hepatitis B virus	Zn	Zinc

Many of the essential trace elements, such as Zn, Cu, and Fe, affect various physiological processes in the liver, including enzymatic activities, protein structure and function, oxidative-antioxidant conditions, inflammation, and immunological responses [12]. Since the liver participates in trace element metabolism, there is a strong link between the trace elements metabolism and the presence of disease and progression [13]. It is primarily the liver that is responsible for initiating the synthesis of proteins that are bound to many trace elements like Zn, Cu, Fe, and selenium to transfer or distribute them. Additionally, the liver participates in the excretion of trace metals, including Cu and magnesium, because it is a producer of bile [14]. Zn is released as free ions from food during digestion, these ions are absorbed into the jejunum and duodenum after binding to an intracellular metal binding protein called metallothionine and then passed through the cell membrane through portal circulation by Zn transporters. The portal system transport Zn absorbed into the liver and releases it into systemic circulation to transport it to other tissues [15]. A significant amount of Zn is excreted through the pancreatic, intestinal, and biliary secretions. Other routes for excretion of Zn are losses through urine and surface in squamous skin, hair, and sweat. Adjustments in the total absorption and excretion of Zn are both vital processes in Zn homeostasis regulation [16].

The absorption of Cu occurs primarily in the gut, which is bound to metallothionein, and by portal vein transported to the liver bound to albumin. 90% of Cu is integrated into ceruloplasmin, the protein that transports Cu to tissues as required. The adenosine triphosphatase enzyme participates in the connecting of Cu with ceruloplasmin in the liver, then releasing it into the blood as well as exporting excess Cu by the bile [17,18]. The absorption process of Cu is regulated by a complex homeostasis mechanism that involves both passive and active transport, which is affected by other transition metals such as Fe and Zn. High Zn consumption inhibits Cu absorption, as demonstrated by acquired hypocupremia linked to excess consumption of Zn [19]. Most of the Cu that is absorbed is excreted in bile, but a small amount is excreted in urine [20].

Dietary Fe is absorbed in different forms: inorganic, ferritin, and heme. Inorganic dietary Fe is present primarily in the oxidized form ferric (Fe^{3+}) and must be reduced to the form ferrous (Fe^{2+}) before intestinal absorption. It is thought that this reduction is mediated by ferrireductases in the apical membrane of the intestinal cell, like duodenal cytochrome B, facilitated by ascorbic acid [21]. Many proteins playing roles in Fe metabolism have been identified. Some proteins, including ferritin or transferrin, are the major carriers of Fe in the blood. Whereas peptides including Fe regulatory proteins, matriptase, and hepcidin are essential determinants of Fe regulation at various physiological levels [22].

One of the main organs that commonly accumulates Pb is the liver. Chronic Pb exposure causes Pb to build mostly in the liver. In vivo studies have demonstrated that Pb poisoning has been linked to inflammatory response changes. Chronic liver inflammation caused by chronic Pb exposure not only causes hepatic fibrosis but triggers many mechanisms that contribute to hepatic steatosis [23]. The gastrointestinal absorption of Pb may rise with reduced levels of essential elements such as Zn, Fe, and calcium, and react with proteins [24]. The route for excretion of absorbed Pb is either via renal clearance in urine or by biliary clearance through the gastrointestinal tract. Pb is excreted slowly from the body and can affect several organ systems. The major mechanism of Pb toxicity is the increased generation of ROS, which causes oxidative damage to DNA and interference with the generation of antioxidants defense systems [25,26].

Cd is a common occupational and ecological toxicant. Cd exposure occurs mostly through two major routes: inhalation and ingestion. After absorption, Cd is transported to the liver through the bloodstream by binding to albumin. Cd stimulates metallothionein (MT) production in the liver and is bound to it. MT is a primary protein that exists at the highest level in the liver, especially following exposure to Cd. Because of the small size of MT, the compound of Cd-MT is freed back into blood circulation. This compound is readily filtered and reabsorbed by the kidney [27]**.** Heavy metal pollution, such as Cd exposure, has a large effect on the host immune system. Cd toxicity reduces immunological responses to infectious diseases, particularly in iron-deficient anemia. Cd competes with Zn for binding sites on the cell surface. Cd accumulation interferes with Zn binding to carrier molecules and reduces Zn absorption. A decrease in Zn absorption as a result of Cd pollution results in dysfunction of immune cell activities, including neutrophils and macrophages [28]. This study was conducted to evaluate the levels of Zn, Cu, Fe, Cd, and Pb in viral hepatitis patients.

2. Materials and Methods

2.1. Study design

Blood samples were collected from 100 patients with viral hepatitis (49 males and 51 females) aged 20–69 years who attended the Gastroenterology and Hepatology Teaching Hospital in Baghdad. In addition, 50 samples of blood were taken from seemingly healthy people as a control group (sex and age-matched with the patients). All participants gave their approval to be sampled. All samples in the research were taken with fundamental and ethical approvals from the Iraqi Ministry of Health. The metals were measured at the poisoning consultation center in Baghdad. A flame atomic absorption spectrometer (FAAS) was used for the determination of zinc, copper, and lead. Cadmium was estimated using a graphite furnace atomic absorption spectrometer (GFAAS), whereas iron was estimated by a CECIL CE 1011 spectrophotometer using a human manual kit.

2.2. Blood collection

From each participant, approximately 7 mL of venous blood was drawn using disposable syringes. About 3 mL of blood was dispensed into an EDTA-containing tube and stored at 4°C until lead (Pb) and cadmium (Cd) concentrations were estimated. The remaining blood was emptied into a gel tube, allowed to coagulate, and then centrifuged at 3000 rpm for 10 minutes to obtain serum. Eppendorf tubes were used to store the serum at -20 °C for two months until it was used to estimate Zn, Cu, and Fe.

2.3. Statistical analysis

The data were analyzed using the Statistical Package for Social Sciences version 26 (SPSS-26). The quantitative data were presented as mean and standard deviation (SD). The significance of the difference between the two means was evaluated using an independent sample T-test. Statistical significance was considered when the P-value was ≤ 0.05.

3. Results and Discussion

3.1. Distribution of serum zinc, copper, and iron concentrations (µg/dl) in the study groups

Table 1 illustrates that the mean zinc concentration in HBV and HCV patients (68.26±5.80 and 66.78±6.29 µg/dl, respectively) decreased significantly (P = 0.000) compared to healthy controls (87.08±5.86 µg/dl). As well, the mean iron level in HBV and HCV patients (78.94±4.88 and 77.80±4.05 µg/dl, respectively) was decreased with a significant difference (P = 0.000) from in healthy controls (87.70 4.72 µg/dl). In contrast, the mean of copper in HBV and HCV patients (161.54±7.92 and 160.76±9.25µg/dl, respectively) was highly significantly higher (P = 0.000) than in controls (115.30 ±20.71µg/dl).

Table 1 Distribution of the Zn, Cu, and Fe concentrations in the study groups.

	Mean ± SD		
	HBV	Control	HCV
Zn (µg/dl)	68.26 ±5.80	87.08 ±5.86	66.78 ±6.29
	P=.000 (HS)		P=.000 (HS)
Cu (µg/dl)	161.54 ±7.92	115.30 ±20.71	160.76 ±9.25
	P=.000 (HS)		P=.000 (HS)
Fe (µg/dl)	78.94 ±4.88	87.70 ±4.72	77.80 ±4.05
	P=.000 (HS)		P=.000 (HS)

Independent sample T-test " all data are represented as mean ±S.D.". HBV= Hepatitis B virus, HCV=Hepatitis C virus, SD= standard Deviation, HS= Highly Significant ($p < 0.01$).

Serum metal levels are extremely sensitive in the diagnosis of liver disease. Each trace element's concentration differs with different forms of liver illness because these elements can be directly harmful to the liver or may be reduced due to impaired liver function [29]. Essential elements like Zn and Cu are a portion of the active sites of several enzymes and are also involved in immunological responses and signaling pathways [30]. Even little changes in the levels of these elements may lead to a change in antioxidant activity. Thus, changes in trace element homeostasis influence the progression of liver disease, particularly in individuals with persistent infections of HBV and HCV [31,32].

Zinc is an important trace element that acts as a cofactor for several enzymes that participate in metabolism and the growth of cells [33]. It plays a key role in immune response, DNA synthesis, wound healing, synthesis of protein, and cell division. It's important for proper growth and development throughout pregnancy, childhood, and puberty, and it is needed for a proper sense of smell and taste [34]. Zinc, as a whole, has a crucial role in preserving human health, particularly in terms of anti-inflammation and anti-oxidative stress [35]. Copper is necessary for the proper functioning of several enzymes in the human body, including cytochrome c oxidase, Cu/Zn superoxide dismutase, ceruloplasmin, and amine oxidases [36,37]. Copper has anti-oxidant and pro-oxidant properties. It neutralizes free radicals and minimizes or prevents damage. When copper acts as a pro-oxidant, it promotes free radical damage and disease development [17]. The metabolism of copper and zinc in the liver is strongly linked. Over-supplementation of zinc can result in a deficiency of copper via metallothionein-mediated suppression for the absorption of copper in the intestines [38].

In the current study, the levels of zinc were highly significantly lower whereas the levels of copper were highly significantly higher in the patient groups than in the control group. These findings back up a prior study conducted in Turkey, which found that chronic infection of viral hepatitis affects Zn and Cu homeostasis, resulting in an elevation in serum copper levels and a reduction in zinc levels [39].

Zinc deficiency has been noticed in many types of hepatic disease, including viral hepatitis. Some mechanisms for zinc deficiencies include reduced dietary intake, increased urinary excretion, activation of some zinc transporters, and stimulation of hepatic metallothionien [40]. During inflammation and cell damage, liver cells use more zinc to manufacture proteins, nucleic acids, and zinc-related enzymes. As liver damage progresses, Zn intake and absorption diminish because of loss of appetite, poor bowel and gut function, and high portal venous pressure. Additionally, low serum albumin content leads to less association with zinc, and blood zinc is easily lost via urine and sweating due to its diffusion characteristic [41].

Alterations in serum copper concentrations have been found in several liver illnesses, including liver cell degeneration, viral hepatitis, and hepatocellular carcinoma [31]. Since Cu is excreted only in the bile, suppression of bile acid secretion by HCV may lead to biliary copper retention [38]. According to Kalkan et al., the levels of copper were significantly higher in people with viral hepatitis, indicating that Cu levels may be changed by certain cytokines [42]. The elevated level of copper might be attributed to increasing absorption from the intestine, decreased liver excretion, and tissue breakdown, which results in the release of copper stored in the body [13].

Iron is an important mineral that is required for growth and development, appropriate cellular function, hormone, and connective tissue synthesis, and, most significantly, acts as a constituent of hemoglobin, which transports oxygen to bodily tissues [43]. Chronic liver disease of any etiology is linked to hematological abnormalities. Anemia is a common occurrence among them, appearing in approximately 75% of individuals with advanced liver disease [44]. The iron concentration in the current study was within the normal range in all participants (patients and controls), and this result was consistent with another study done in Iraq [45]. However, the mean Fe in the patient groups was lower than in the control group. Iron homeostasis is mostly regulated by hepcidin, which is accountable for the transport of iron from the intracellular region into systemic circulation [46]. When the liver is injured, the expression of hepcidin is reduced. As a result, the iron liberation from cells into plasma is stopped [44]. Furthermore, interleukin-6 upregulates hepcidin in acute and chronic inflammation, resulting in hypoferremia. Hence, decreased serum iron levels are linked to chronic liver disease [47].

3.2. Distribution of lead and cadmium concentrations (µg/dl) in the study groups

Table 2 shows a highly significant (P = 0.000) increase in the mean lead levels in the HBV and HCV patient groups (26.00±3.57 and 26.10±2.96 µg/dl, respectively) when compared to the control group (16.34±2.60 µg/dl, respectively). The mean cadmium level was highly significantly (P = 0.000) higher in the hepatitis B and hepatitis C patient groups (0.33±0.06 and 0.34± 0.06 µg/dl) than in the healthy control group (0.19± 0.03 µg /dl).

Table 2 Distribution of the Pb and Cd concentrations in the study groups

	Mean ±SD		
	HBV	Control	HCV
Pb (µg/dl)	26.00 ±3.57	16.34 ±2.60	26.10 ±2.96
	P=.000 (HS)		P=.000 (HS)
Cd (µg/dl)	0.33 ±0.06	0.19 ±0.03	0.34 ±0.06
	P=.000 (HS)		P=.000 (HS)

Independent sample T-test "all data are represented as mean ±S.D.". HBV= Hepatitis B virus, HCV=Hepatitis C virus, SD= standard Deviation, HS= Highly Significant ($p < 0.01$).

Heavy metal exposure in humans can lead to impairment of innate and humoral immune responses, making people more susceptible to infections and the development of autoimmune diseases [48]. Inhibition of the immune response may increase the host's susceptibility to chronic infection. Heavy metals may have an impact on the intensity of infection in addition to their immunotoxic effects [49]. Lead and cadmium are common, non-biodegradable contaminants that pose a significant risk to human health. They are naturally present in the environment, but industrial activity has dramatically raised their levels. Because of their presence in the air, food, and tobacco leaves, the primary modes of lead and cadmium exposure are inhalation and ingestion [50]. These heavy metals are both hepatotoxic and have been demonstrated to increase viral activity. The immunological impacts of cadmium and lead poisoning may be linked to an increased predisposition to chronic infection [48]. Lead is hazardous to humans and animals and may harm a variety of tissues. For example, continuous exposure to Pb has been associated with liver damage, osteoporosis, neurological problems, and many cancers [51, 52]. It could stimulate the production of ROS, reducing antioxidant defenses, and substitute monovalent and divalent cations in the proteins of cells. As a result, Pb causes oxidative stress in cells and affects enzyme activity, ion transport, metabolism, and signaling pathways [53]. Cadmium generates ROS indirectly and promotes the oxidation of lipids, proteins, and DNA. The indirect production of free radicals results from the ability of cadmium to substitute for copper and iron in intracellular and membrane proteins, which in turn releases and increases unbound iron and copper, which participates in oxidative stress [26]. Cd may cause acute and chronic inflammation of the liver, heart, kidney, lung, and reproductive system, potentially resulting in tissue injury and systemic inflammation [28]. On the other hand, the HBV infection probably impairs the liver's normal functioning, preventing it from producing the threshold antioxidant levels and other biomolecules required for cadmium detoxification [54].

Many studies showed that essential metal deficiency like zinc, iron, or calcium can result in increased Pb, Cd absorption, and toxicity in both humans and animals [55-57]. Pb and Cd have chemical and physical characteristics that are similar to Zn, and they compete for the enzymatic and absorptive protein binding sites of metal [58]. Table 2 shows that the mean lead and cadmium in viral hepatitis patients were higher than in controls. The probable reason that explains the increase in the levels of lead and cadmium in patients might be the decrease in zinc levels. Zinc deficiency leads to increased heavy metal accumulation and its toxicity in various organs. The adequacy of essential elements influences the gastrointestinal absorption of heavy metals and sensitivities to their effects. Since lead and cadmium compete with zinc in the intestine for the same transport system as well as a binding site in metallothionein protein, zinc deficiency leads to the use of lead and cadmium instead of zinc by the body [59,60].

4. Conclusion

Viral hepatitis infection alters the homeostasis of trace elements, which subsequently leads to the progression of the disease.

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