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Biochemical Analysis of Inorganic Ions in Normal and Hepatitis C Patients

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Abstract. Trace elements are crucial for human metabolism, primarily processed in the liver. Liver diseases can alter their levels, especially during viral infections like Hepatitis C (HCV). HCV can lead to chronic liver problems. The blood samples were collected in 'gel and clot activator' tubes, allowed to clot and the serum was separated. Nitric acid was added to the serum to coagulate proteins and the samples were heated for one day at 150 °C for protein digestion. The pH was adjusted to 7 using ammonium hydroxide. Using Atomic Absorption Spectrophotometry (AAS), researchers found that HCV patients had higher levels of iron (Fe), lead (Pb), manganese (Mn), cobalt (Co) and copper (Cu) but lower levels of zinc (Zn) compared to healthy individuals.

Keywords: adsorption, trace elements, hepatitis, iron, copper, lead

Introduction

The liver is a critical organ in the human body, as it satisfies a bunch of capabilities supporting digestion, resistance, processing, detoxification and nutrient stockpiling, for instance. The grown-up liver contains both parenchymal and non-parenchymal cells (Donne *et al*., 2021). In the realm of pediatric anatomy, it commands a weight of approximately 1500 g, constituting around 2.5% of an adult's overall mass. Situated in the right upper quadrant, shielded by the thoracic cage and diaphragm, the liver boasts a smooth, dome-shaped surface that contrasts with the diaphragm's concave inferior aspect. Its nuanced positioning places it deep amid the seventh to eleventh ribs on the right side. Vascular nourishment is intricate, primarily sourced from the portal vein (75%) and the hepatic artery (25%). Hepatitis C infection (HCV) disease is surprisingly productive in laying out viral tirelessness, prompting the improvement of liver cirrhosis and hepatocellular carcinoma (HCC) (Park and Hahn, 2023)

It is a liver disease that completely destroys the liver physiology. It is marked by diarrhoea, vomiting, tiredness and jaundice, pale skin and whites of the eyes, as well as an inability to eat. The utmost prevalent source of liver disease globally is hepatitis virus infections (Cuthbert, 2001).

Hepatitis C is a deadly illness on a global scale. Both industrialized and developing nations are concerned about it. Provided that the initial unreleased assumptions of the global burden of disease (GBD) contributed to hepatitis C virus (HCV)-related chronic liver disease seem to be significant, especially in those regions of our planet where resources are scarce, public health authorities should be associated about the decline in global mortality and morbidity associated with chronic hepatitis C (Simmonds *et al*., 1994).

Hepatitis C virus (HCV), a minute enveloped RNA virus falling under the hepticvirus genus in the flaviviridae family, boasts a 9,600-nucleotide RNA genome. This genome intricately encodes a solitary polyprotein, subject to meticulous post-translational cleavage into 10 distinct polypeptides. The findings underscore HCV's pervasive impact, particularly afflicting younger demographics with heightened susceptibility to severe hepatitis C and anti-HCV seroconversion. (Pascarella and Negro, 2011)

Despite a waning incidence of acute hepatitis C the research illuminates a substantial demographic of chronically infected Americans. This population remains

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disproportionately susceptible to the grave ramifications of chronic liver disease, underscoring the persistent clinical relevance of HCV despite evolving epidemiological trends. (Shaikh *et al*., 2018)

There are at least two different types of viral particles. High-density viral particles are less infectious than low-density viral particles, which appear to be more contagious. It is uncertain what causes the differences in infectivity, however it is possible that the high-density particles have antibodies to the envelope bonded to their surface (Dustin *et al*., 2016).

The HCV genome, exhibiting the characteristic configuration of flavivirus genomes, manifests an intricate genetic structure. Encompassing a substantial open reading frame, replete with numerous nucleotides and highly conserved 5′ and 3′ terminal regions, it centres around a polyprotein boasting approximately 3300 amino acids. Within infected cells, the intricate dance of viral and host proteases comes into play, meticulously cleaving this polyprotein to engender various viral protein products.

This intricate process entails both co-translational and post-translational machinations, ultimately yielding at least 9 discernible polypeptides. Among these, El and E2 emerge as the envelope peptides, while the cohort of nonstructural proteins encompasses NS2, NS3, NS4a, NS4b, N5a and NS5b (Makris *et al*., 1990).

HCV may be classified into six phylogenetically different groups, according to nucleotide sequence evidence that has accrued since the virus's discovery. Each genotype can be further subdivided into many more closely related subtypes, each of which is indicated by a lowercase letter (e.g., 1a). Genotypes are different from one another at the nucleotide level by 30-33% and subtypes within a genotype can vary by up to 25% (Bell *et al*., 1990).

The HCV virion either circulates in the circulation as a scattered particle or is contained by host low-density lipoproteins before being taken up by the cell by a clathrin-mediated endocytosis process. It successively binds to a variety of receptor molecules before sticking to the target cell membrane. After the viral capsid in the endocytic compartment is destroyed, the virus's 9.6 kb positive polarity single stranded RNA genome is released into the cytoplasm (Everhart *et al*., 1990).

HCV, omnipresent yet subject to significant regional differentials, exhibits pronounced prevalence in Africa and Asia, while Australia, northern and western Europe, and developed north American countries record comparatively diminished rates. Economically robust nations like Germany, Canada, France, Australia and the USA portray restrained HCV seroprevalence. However, nuanced variations emerge in regions like the USA, Japan and Italy, where seroprevalence, though modest, remains perceptibly elevated.

The intricacies deepen when scrutinizing estimates in developing nations, characterized by a paucity of robust evidence compared to their developed counterparts. This complexity is starkly evident in emerging nations, representing substantial global demographics. China, hosting a fifth of the world's inhabitants, discloses an impactful seroprevalence of 32%. Conversely, a community-based study in India, harboring another fifth of the global populace, unveils a more conservative overall rate of 9%. Indonesia reports a 21% seroprevalence, albeit rooted in surveys targeting voluntary blood donors.

For a granular perspective, Pakistan emerges with a variegated seroprevalence terrain, with the majority of reported values ranging between 2 and 6 percent, (Gaetke and Chow, 2003).

An interferon-A and ribavirin treatment spanning 24 to 48 weeks is part of the therapy. Ribavirin should be taken in doses of 1200 mg daily for individuals weighing 75 Kg or more and 1000 mg daily for those who weigh less than that. To keep track of their symptoms, bilirubin levels and serum alt levels, patients should undergo regular examinations. Patients should have a comprehensive HCV RNA monitoring test after 24 weeks. For those who have genotypes 2 or 3, treatment should end after 24 weeks. If the HCV RNA test findings are positive, patients with genotype 1 should cease taking their medicines and if the results are negative at 24 weeks, therapy should continue for the entire 48 weeks (Gupta *et al*., 2019).

In addition to serving as antioxidants, the vital trace minerals zinc (Zn), selenium (Se) and copper (Cu) are also crucial for several metabolic activities in the liver. In people with chronic HCV infection, the balance of these minerals may be out of whack, leading to oxidative stress and inflammatory alterations that can exacerbate hepatic fibrosis and IR and reduce the efficacy of antiviral treatment (Sahin *et al.*, 2018). Crucial mineral called zinc is present in virtually all human cells. About 100 enzymes are activated as a result of its stimulation. Numerous foods, such as beans, nuts, several types of

seafood, red meat, oysters, whole grains and milk products, are some of the primary sources of zinc. Zinc aids in the maintenance of a strong immune system and is essential for DNA synthesis, wound healing and the senses of taste and smell. Zn inhibits HCV replication, as demonstrated by an availability of material collected over the previous few decades (Abboud and Haile, 2000).

A number of biological activities require copper and the body requires metal for several vital enzymes to function. Additionally, it contributes to the health of the neurological system, the balance of other essential metals in the body like zinc and molybdenum, as well as other bodily processes. Copper is removed by zinc from the location where it is bound, preventing the production of free radicals (Haque *et al*., 1996). Acute HCV infection induces a rise in blood copper levels, which is made worse by CHC and fibrotic liver disease. Although the exact method by which HCV promotes copper buildup in the liver is yet not known, Cu-MTs to promote the formation of hydroxyl radicals in rats, which leads to liver damage and perhaps fibrosis. Given that copper can only be eliminated through the bile, retention of biliary copper may occur as a result of HCV-mediated suppression of bile acid production. It's interesting how closely connected the liver's metabolism of copper and zinc. Zinc over supplementation can cause copper deficiency by preventing copper absorption in the gut through MT's (Gao *et al*., 2019).

Iron, the predominant trace mineral coursing through the human body, assumes a multifaceted role in indispensable physiological functions. Its involvement spans the synthesis of DNA and proteins, the orchestration of erythrocyte formation, electron transport, aerobic metabolism, cell differentiation and the regulation of gene expression. A pivotal biological role unfolds as iron facilitates the growth of functional hemoglobin and red blood cells. However, its dual nature reveals a proclivity for oxidative action, generating free radicals that pose a potential threat to tissues, notably impacting cellular membranes, particularly those of the liver (Pietrangelo, 1996). Hepatitis C treatment saves lives, forestalls transmission and is cost saving (Thompson *et al*., 2022)

Within the domain of chronic hepatitis C (CHC), iron overload emerges as a distinctive hallmark, affecting a noteworthy 10 to 42% of patients. Elevated concentrations of blood ferritin and/or transferrin manifest in up to 40% of individuals, establishing substantial correlations with hepatic fibrosis. It's imperative to discern that the elevation of serum ferritin can transpire independently of hepatic iron presence, even though it may exhibit correlation tendencies with liver iron levels. (Prie *et al*., 2016).

Materials and Methods

Materials. Nitric acid, ammonium hydroxide (commercial), ferric chloride, cupric chloride, lead carbonate, zinc sulphate and manganese chloride were used. All the standard solutions were prepared with distilled water.

Sample collection. Blood samples were collected from center for applied and molecular biology (CAMB) Lahore, Shaikh Zayad Hospital Lahore and Ghurki Trust Teaching Hospital Lahore. Blood sample (5 mL) was drawn from every patient. Blood samples were also collected from healthy donors of different areas. The syringes used for blood collection was disposed off.

Sample preparation. Blood was collected in 'Gel and Clot activator' collection tubes and allowed to clot at room temperature (25 °C). Serum was separated after one day. Separated serum was pipetted into glass vials (0.5 mL). Nitric acid (0.8 mL) was added into the serum. Proteins were allowed to coagulate and then sample was heated on digital hot plate for one day at 1500 °C for protein's digestion. The pH was adjusted to 7 by using few drops of NH_4OH.

Chemical analysis. The assessment entailed employing atomic absorption spectroscopy (AAS) to scrutinize metal concentrations, specifically Cu, Fe and Pb, in serum samples from both patients and healthy individuals. The AAS technique facilitated the determination of various trace metals, including Fe, Cu, Zn, Co, Mn and Pb, in the serum samples of both cohorts. Concentrations, measured in parts per million (ppm), were computed relative to the standard curve derived from corresponding metal salts.

The fundamental tenet of AAS revolves around the selective absorption of particular wavelengths of light by atoms and ions. At a specific wavelength, atoms absorb energy (light). AAS operates on the principle that when atoms are present at this particular wavelength, they absorb the incident light energy. In essence, AAS serves as an analytical method employed to quantitatively assess the presence of specific elements within a given sample.

Results and Discussion

Clinical studies. The clinical studies of population as shown in Table 1.

Graphical representation of absorbance of different trace minerals. The absorbance of different trace minerals as indicated in Fig. 1-6 and 8.

Hepatitis C patients and level of iron, copper and lead. The demographic results of healthy control and hepatitis C patients are showed in Table 1. These two groups data were closely relate. Majority of the patients and controls were taken from urban areas and complained about fatigue, most of the patients were reported that they don't smoke and living the unhygienic lifestyles.

Standard curves of Fe, Cu and Pb were drawn. Absorbance was analyzed by using AAS technique. Serum Fe, Cu and Pb levels in HCV patients were 1.90±0.32, 1.02±0.90 and 11.08±0.95 respectively.

Statistical analysis of selected trace minerals (Fe, Cu, and Pb) in the HCV patients and healthy controls are shown in Table 2. Serum Fe level in HCV patients and controls showed the negative correlation with no significant P value as shown in Fig. 8(a). Serum Cu level in HCV patients and controls showed the negative correlation with no significant P value as shown in Fig. 8(b). Serum Pb level in HCV patients and controls showed the negative correlation with no significant P value as shown in Fig. 8(c).

Hepatitis C patients and level of zinc, manganese and cobalt. The study encompassed a patient cohort consisting of 20 individuals with chronic HCV infection, comprising 11 females and 9 males within the age range of 20 to 65 years. A comparative control group comprised 5 healthy volunteers. Noteworthy findings revealed significantly elevated concentrations of Co and Mn in HCV patients compared to healthy controls. Conversely, the serum Zn levels in blood samples from HCV patients demonstrated a marked reduction compared to the healthy control group.

Demographic data for both healthy controls and hepatitis C patients are tabulated in Table 1, showcasing a closely intertwined relationship between the two groups.

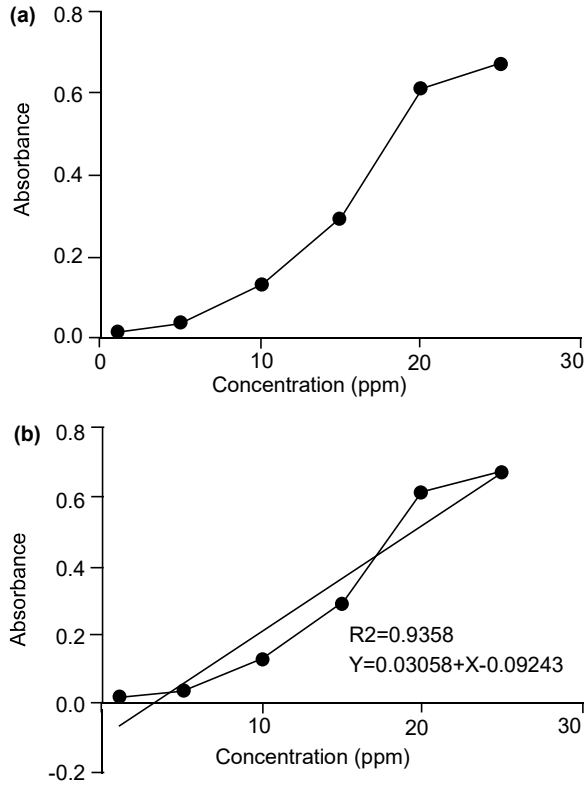

Fig. 1. Absorbance of Fe.

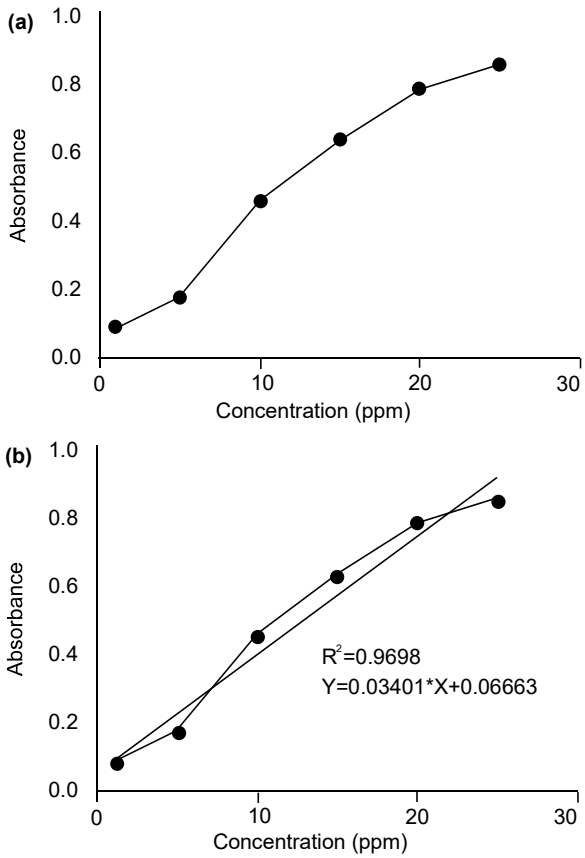

Fig. 2. Absorbance of Cu.

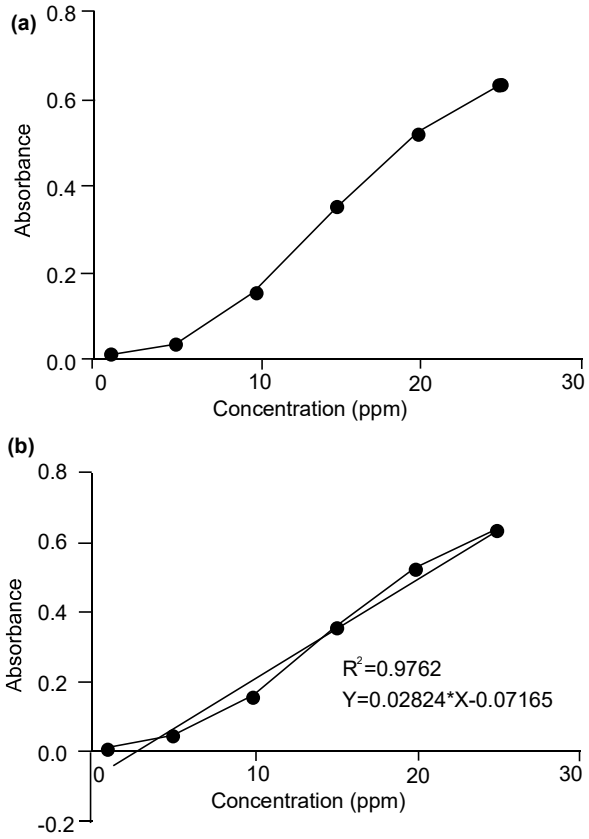

Fig. 3. Absorbance of Pb.

Predominantly sourced from urban locales, a substantial number of participants across both cohorts reported fatigue as a prevailing concern. Notably, a majority refrained from smoking, yet exhibited lifestyles marked by unhygienic practices.

Employing the AAS technique, standard curves for Zn, Mn and Co were meticulously delineated and absorbance data as shown in Fig. 1 to 6 were analyzed. Serum levels of Zn, Mn and Co in HCV patients were quantified as 0.5±0.48, 0.19±0.11 and 0.35±0.03, respectively.

Statistical scrutiny of selected trace minerals (Zn, Mn, and Co) in HCV patients and healthy controls is presented in Table 2. Interestingly, serum Zn levels in HCV patients and controls exhibited a negative correlation without attaining statistical significance, suggesting intricate interplays in the context of HCV infection, as shown in Fig. 7.

Statistical analysis of selected trace minerals (Zn, Mn and Co) in the HCV patients and healthy controls are shown in Fig. 10. Serum Zn level in HCV patients and controls showed the negative correlation and do not show any significant P value. Serum Zn level in HCV patients and controls showed the negative correlation and do not show any significant P value. Serum Zn level in HCV patients and controls showed the negative correlation and do not show any significant P value.

Table 1. Clinical characteristics of the study of population

	HCV positive (n=50)	Controls (n=25)
Gender (M:F)	M: 23 F: 27	M: 11 F: 14
Age (years)	45±20	35±10
Fatigue (Yes/No)	39/11	16/9
Fever (Yes/No)	11/39	7/18
Vomiting (Yes/No)	14/36	5/20
Smoking (Yes/No)	15/35	12/13
Loss of appetite (Yes/No)	37/13	3/22
Cleanliness (Yes/No)	17/33	24/1
Filtered water (Yes/No)	42/8	19/6
Ortho treatment (Yes/No)	21/29	14/11

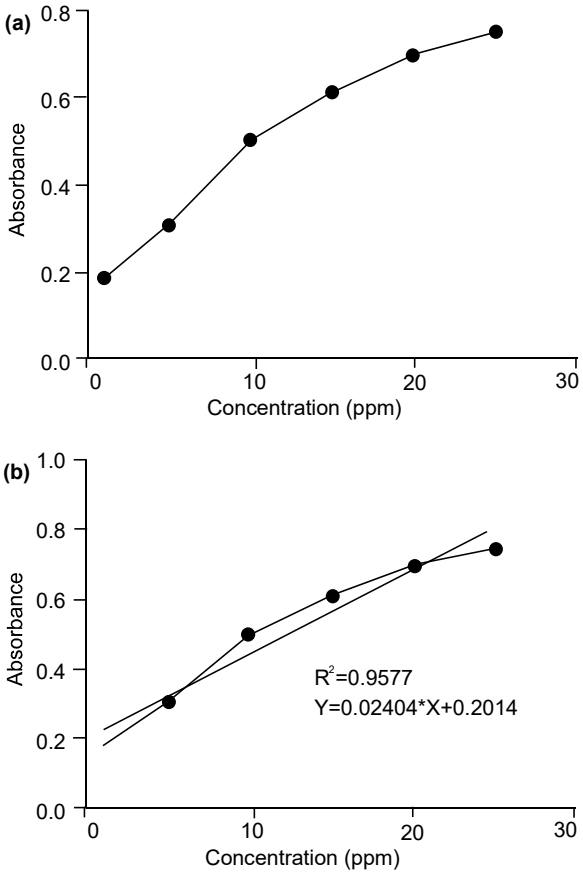

Fig. 4. Absorbance of Zn.

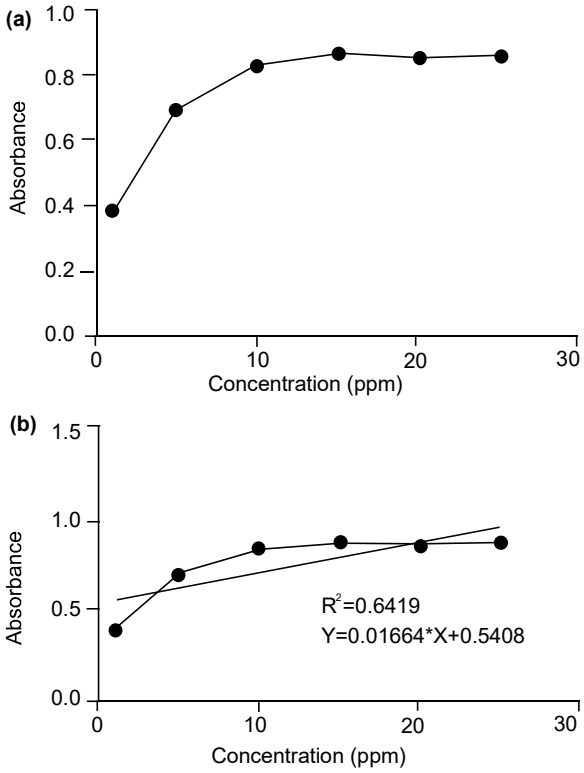

Fig. 5. Absorbance of Mn.

Hepatitis C virus (HCV) is one of the several viruses that can cause hepatitis. Hepatitis C is a haptotropic RNA virus that cause progressive liver damage. Trace minerals plays an important role in liver disease. At the moment, five distinct hepatitis viruses account for more than 95% of viral-induced hepatitis cases worldwide (Afridi *et al*., 2009). HCV infection appears to have a significantly different natural history than HBV infection. As viremia and infectivity decline in the latter, sero-conversion from e antigen to anti-e. The antibody typically takes place (Ohto *et al*., 1994). Chronic hepatitis brought on by HCV infection frequently increases the risk of liver cirrhosis and HCC. When lacking, out of balance, or toxic, the necessary micronutrients (Zn, Cu, Fe and Se) may aggravate liver disease. According to clinical research, patients with chronic liver disease caused by the hepatitis C virus (HCV) exhibit decreased trace element metabolism at various stages of liver damage (Mohammed *et al.*, 2012). In our study we correlate the serum mineral levels (Fe, Cu, Zn, Co, Pb and Mn) in HCV patients and healthy controls as shown in Fig. 9 and 10. By demographic study we checked that majority of HCV patients complained about fatigue, fever and vomiting on other hand some of controls also complained about the same symptoms. 80% of HCV patients were drawn from urban areas that don't have proper hygienic conditions in their areas. 10% HCV patients were never used filtered water, which may cause several heavy metals induction into their bodies and may contaminate their blood too. Hepatitis C infection (HCV) is a huge supporter of viral-prompted hepatitis, causing moderate liver harm. This haptotropic RNA infection has a particular normal history contrasted with hepatitis B infection (HBV), while HBV disease frequently includes seroconversion from e antigen to

Table 2. Concentration of trace metals

	HCV positive (n=50)	Controls (n=25)
Trace Metals		
Fe (µg/mL)	1.90±0.32	1.19±0.56
Cu (µg/mL)	1.02±0.90	0.84±0.81
Zn (µg/mL)	0.51±0.48	0.82±0.09
Mn (µg/mL)	0.19±0.11	0.16±0.15
Co (µg/mL)	0.35±0.03	0.16±0.02
Pb (µg/mL)	11.08±0.95	6.69±0.93

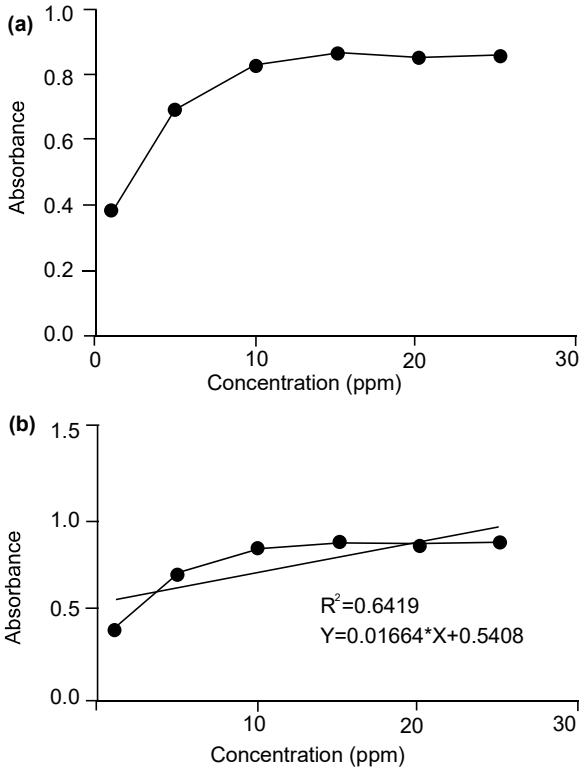

Fig. 6. Absorbance of Co.

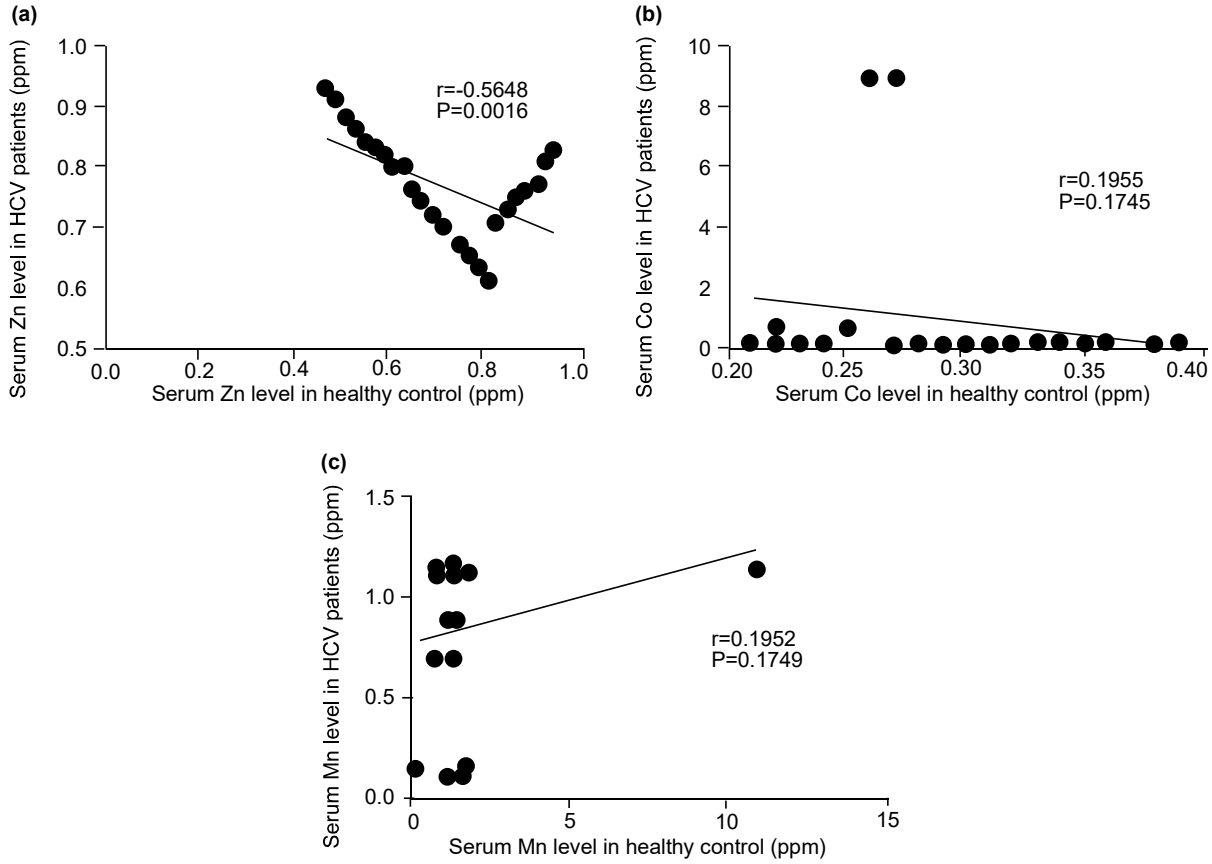

Fig. 7. Correlation analysis of different minerals with blood serum between healthy control and HCV patients. **(a)** Zn analysis with serum having r value 0.5648, P value 0.0016; **(b)** Co analysis with serum having r value -0.1955, P value 0.1745; **(c)** Mn analysis with serum having r value 0.1952, P value 0.1749.

hostile to e neutralizer as viremia and infectivity decline, HCV contamination presents interesting difficulties. Persistent hepatitis coming about because of HCV disease essentially expands the gamble of liver cirrhosis and hepatocellular carcinoma (HCC). In the domain of liver illnesses, the job of minor elements becomes vital. Minor elements, including zinc (Zn), copper (Cu), iron (Fe), and selenium (Se), assume crucial parts in keeping up with liver wellbeing. Be that as it may, when these micronutrients are missing, imbalanced, or present in harmful sums, they can compound liver illness. Clinical exploration demonstrates that patients with constant liver infection brought about by HCV display changed minor component digestion at different phases of liver harm (Mohammed *et al*., 2012). Our review expects to relate serum mineral levels, explicitly iron (Fe), copper (Cu), zinc (Zn), cobalt (Co), lead (Pb) and manganese (Mn), in HCV patients and solid controls. By leading a segment study, we saw that a larger part of HCV patients revealed side effects like exhaustion, fever and regurgitating. Curiously, a few controls likewise grumbled of comparable side effects, proposing that these protests probably won't be selective to HCV patients. Besides, 80% of HCV patients were drawn from metropolitan regions with sub-par sterile circumstances. This raises worries about the expected commitment of natural elements to the predominance and seriousness of HCV diseases. Absence of appropriate cleanliness in metropolitan regions can work with the spread of irresistible specialists, including HCV. Prominently, 10% of HCV patients detailed never utilizing sifted water. This is a basic perception as the shortfall of water filtration might open people to different impurities, including weighty metals. The ingestion of unfiltered water can prompt the presentation of weighty metals into the body, possibly adding to the pollution of blood. Weighty metals are known to negatively affect wellbeing and their presence in the circulatory system

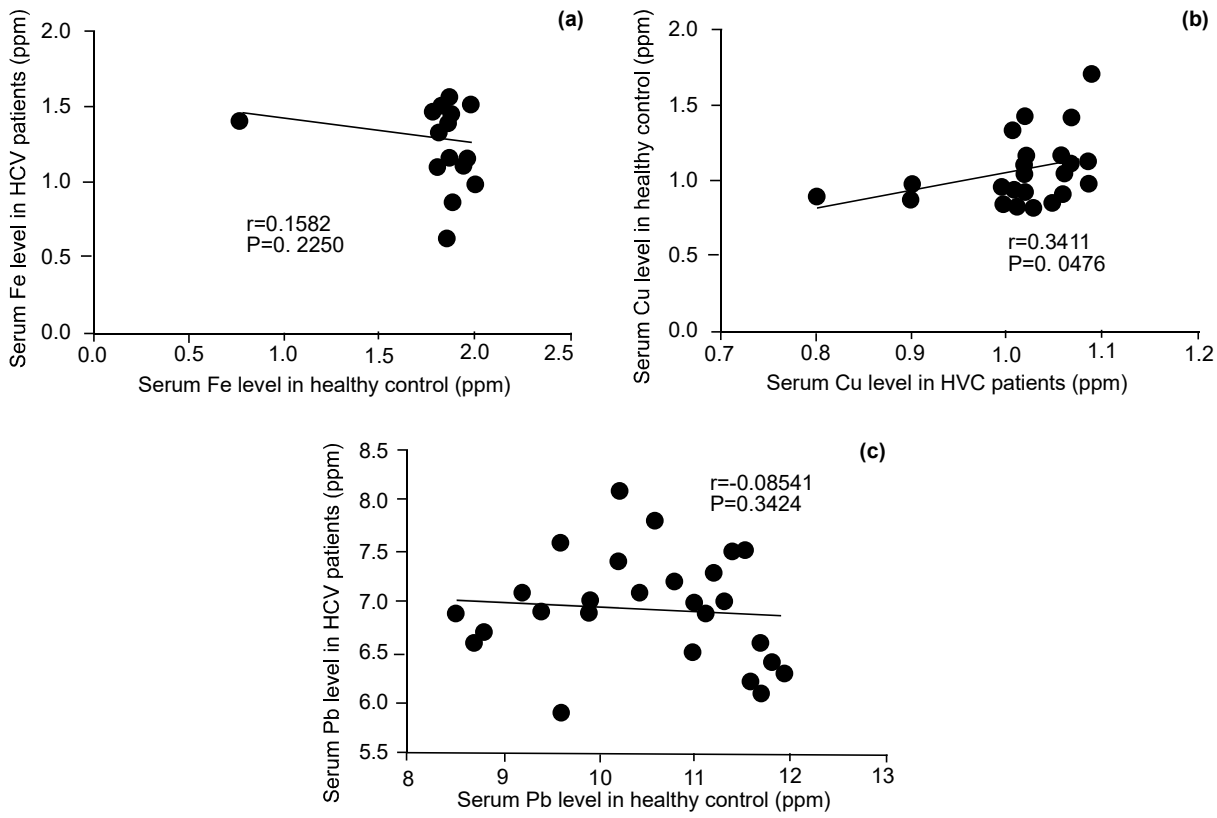

Fig. 8. Correlation analysis of different minerals with blood serum between healthy control and HCV patients. **(a)** Fe analysis with serum having r value -0.1582, P value 0.2250; **(b)** Cu analysis with serum having r value 0.3411, P value 0.0476; **(c)** Pb analysis with serum having r value 0.08541, P value 0.3424.

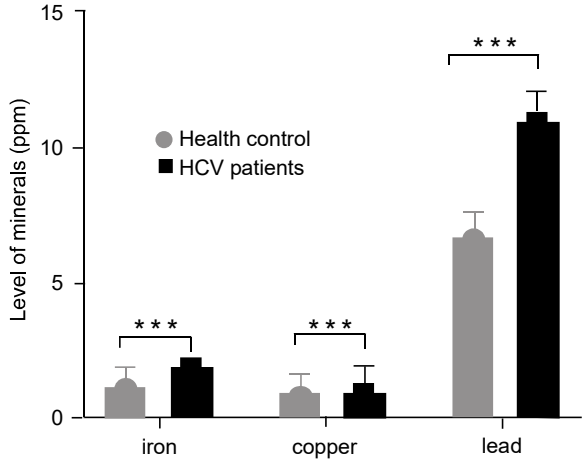

Fig. 9. Statistical analysis of selected trace minerals (Fe, Cu and Pb) in the HCV patients and healthy controls.

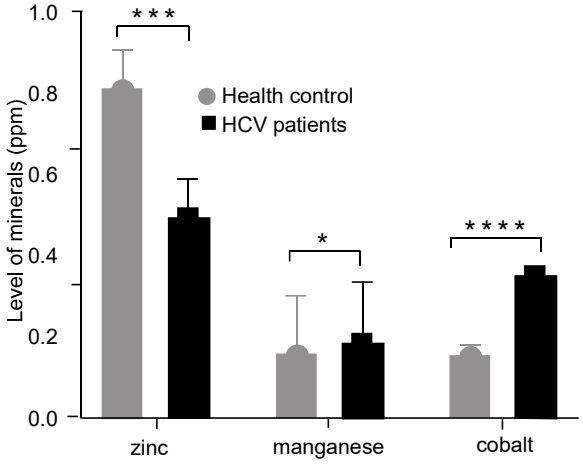

Fig. 10. Statistical analysis of selected trace minerals (Zn, Mn and Co) in the HCV patients and healthy controls.

might fuel the movement of liver illness in HCV patients. The relationship between's the segment information and serum mineral levels highlights the complex idea of HCV diseases. Factors like ecological circumstances,

water quality and way of life decisions can essentially influence the course and seriousness of the sickness. As we dive further into figuring out the transaction between minor elements, natural variables and HCV disease, our discoveries might give significant bits of knowledge to creating exhaustive systems for the avoidance and the board of hepatitis C.

Conclusion

This study delves into the pivotal role of trace minerals in the human body, scrutinizing the disparities in their content between HCV patients and their healthy counterparts. Distinct levels of trace minerals emerge as crucial differentiators, holding the potential for both detrimental and advantageous outcomes. Perturbations in these concentrations serve as potential indicators of liver dysfunction, adding a layer of significance to their evaluation.

The investigation meticulously examined the serum metal concentrations, measured in parts per million (ppm), in both healthy controls and HCV patients. Strikingly, the findings unveil elevated levels of Fe, Cu, Co, Mn and Pb in HCV patients compared to their healthy counterparts. This conspicuous elevation is attributed to the impact of HCV virus infection, substantiating its influence on the perturbation of these mineral concentrations. Notably, compared to the healthy control group, adding another dimension to the intricate interplay between HCV infection and trace mineral dynamics. Its worth noting that 80% of the HCV patients were from urban areas with poor hygienic conditions. That could definitely contribute to the spread of the virus. And the fact that 10% of the HCV patients never used filtered water is concerning. It could potentially lead to heavy metal contamination in their bodies and even their blood.

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Conflict of Interest: The authors declare that they have no conflict of interest.

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