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Trace element imbalance in chronic Hepatitis B: Clinical significance of copper-to-zinc ratio as A disease activity marker

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Abstract
Background: Trace elements play crucial roles in liver health and immune function, yet their alterations in chronic hepatitis B virus (HBV) infection remain poorly characterized, particularly in resource-limited settings. The copper-to-zinc ratio has emerged as a potential biomarker for liver disease severity, but its relationship with viral replication in chronic HBV infection has not been systematically evaluated.
Objective: To characterize trace element profiles in chronic HBV patients and investigate the clinical significance of copper-to-zinc ratio as a disease activity marker correlating with viral load and liver inflammation.
Methods: A cross-sectional study was conducted among 300 chronic HBV patients in Rajasthan, India. Serum copper and zinc levels were measured using atomic absorption spectroscopy. The copper-to-zinc ratio was calculated and correlated with HBV DNA levels, liver function tests, and clinical parameters. Multivariate analysis was performed to identify independent predictors of disease activity.
Results: Zinc deficiency was present in 156 patients (52.0%), while 67 patients (22.3%) had elevated copper levels. The copper-to-zinc ratio was elevated (>1.5) in 189 patients (63.0%). The copper-to-zinc ratio showed the strongest correlation with HBV DNA levels among all measured parameters ($r = 0.467$, $p < 0.001$). Zinc levels correlated negatively with viral load ($r = -0.398$, $p < 0.001$), while copper levels showed positive correlation ($r = 0.356$, $p < 0.001$). HBeAg-positive and treatment-naïve patients had significantly worse trace element profiles. The copper-to-zinc ratio demonstrated superior diagnostic performance for identifying high viral loads (AUC = 0.751) compared to individual element measurements.
Conclusion: Trace element imbalance is highly prevalent in chronic HBV infection, with the copper-to-zinc ratio serving as a novel biomarker for disease activity. These findings suggest potential roles for nutritional assessment and intervention in comprehensive chronic HBV management.

Keywords: Hepatitis b virus, trace elements, copper, zinc, copper-to-zinc ratio, nutritional status, biomarker

Introduction

Chronic hepatitis B virus (HBV) infection affects approximately 296 million people worldwide, with the highest burden concentrated in resource-limited regions where nutritional deficiencies are common (World Health Organization, 2021) [8]. While advances in antiviral therapy have significantly improved treatment outcomes, the complex interplay between viral factors, host immune responses, and nutritional status in determining disease progression remains incompletely understood. Among the various nutritional factors that may influence chronic HBV pathogenesis, trace elements have garnered increasing attention due to their essential roles in immune function, antioxidant defense, and liver metabolism.

Trace elements are micronutrients required in minute quantities for normal physiological functioning, serving as cofactors for numerous enzymes and transcription factors involved in critical cellular processes (Osredkar & Sustar, 2011) [5]. Among these, copper (Cu) and zinc (Zn) have received particular attention in liver disease research due to their pivotal roles in hepatic metabolism and their documented alterations in various liver pathologies. However, the systematic evaluation of trace element status in chronic HBV infection, particularly in populations from resource-limited settings, remains limited. Zinc is the second most abundant trace element in the human body and serves as a cofactor for more than 300 enzymes and over 2,000 transcription factors involved in cell division, protein synthesis, DNA synthesis, immune function, and antioxidant defense (Prasad, 2014) [6]. In the liver, zinc plays crucial roles in hepatocyte regeneration, maintenance of membrane integrity, and protection against oxidative stress through its involvement in the antioxidant enzyme superoxide dismutase (Cu/Zn-SOD). The liver also plays a central role in zinc homeostasis, being involved in the regulation of zinc absorption, distribution, and storage through metallothionein binding.

Zinc deficiency has been associated with impaired immune function, increased susceptibility to infections, delayed wound healing, and enhanced oxidative stress. In the context of liver disease, zinc deficiency has been linked to impaired hepatocyte regeneration, increased liver fibrosis progression, and compromised antiviral immune responses (Stamoulis *et al*., 2007) [7]. Several studies have reported decreased serum zinc levels in patients with chronic liver diseases, including viral hepatitis, with the magnitude of reduction often correlating with disease severity.

Copper, while essential for various physiological processes, can become hepatotoxic when present in excess. Copper serves as a cofactor for enzymes involved in energy production (cytochrome c oxidase), iron metabolism (ceruloplasmin), and connective tissue formation (lysyl

oxidase). The liver is the primary organ responsible for copper homeostasis, being the main site of ceruloplasmin synthesis and the primary route for copper excretion through bile (Araya *et al*., 2012) [1]. Disruption of this delicate balance can lead to either copper deficiency or toxicity, both of which can have serious health consequences.

In liver diseases, copper levels are often elevated due to increased synthesis of ceruloplasmin as an acute phase response to inflammation, impaired biliary excretion, or hepatocellular release following liver damage. Excessive copper can catalyze the formation of reactive oxygen species through Fenton-like reactions, leading to lipid peroxidation, mitochondrial dysfunction, and ultimately hepatocyte necrosis. This copper-mediated oxidative stress may contribute to disease progression in chronic liver conditions.

The copper-to-zinc ratio has emerged as a potentially more informative parameter than individual element measurements, as it reflects the balance between these two trace elements and may capture the combined effects of both copper excess and zinc deficiency. An elevated copper-to-zinc ratio has been associated with increased oxidative stress, enhanced inflammation, and worse clinical outcomes in various liver diseases (Zhang *et al*., 2021) [9]. However, its specific role in chronic HBV infection and its potential utility as a biomarker for disease activity have not been comprehensively evaluated.

The relationship between trace elements and viral hepatitis is complex and likely bidirectional. On one hand, trace element deficiencies may predispose to more severe viral infections and impaired viral clearance through effects on immune function and antioxidant defenses. Zinc deficiency, for instance, can impair both innate and adaptive immune responses, potentially compromising the host's ability to control viral replication. On the other hand, active viral replication and associated liver inflammation may alter trace element metabolism through increased consumption, redistribution, or impaired absorption and storage.

Previous studies investigating trace elements in chronic HBV infection have yielded variable results, often limited by small sample sizes, heterogeneous populations, or methodological differences. Chen *et al*. (2020) [2] reported significantly lower serum zinc levels in Chinese chronic HBV patients compared to healthy controls, with zinc levels inversely correlating with markers of liver inflammation. Similarly, Kumar *et al*. (2022) [4] found decreased zinc levels in Indian chronic HBV patients, with the strongest deficiency observed in those with high viral loads and elevated liver enzymes.

Regarding copper, studies have generally reported elevated levels in chronic liver diseases, including viral hepatitis. Huang *et al*. (2023) [3] found altered copper homeostasis in Taiwanese chronic HBV patients, with elevated total copper levels but reduced bioavailable copper, suggesting disturbed copper metabolism rather than simple elevation. The authors proposed that these alterations might reflect increased oxidative stress and reduced antioxidant capacity in chronic HBV patients.

Despite these emerging insights, several important knowledge gaps remain. First, the prevalence and clinical significance of trace element alterations in chronic HBV infection have not been systematically characterized in Indian populations, where the predominant HBV genotype (D) and nutritional patterns differ from those in East Asian studies. Second, the relationship between trace element status and viral replication activity has not been comprehensively evaluated, limiting understanding of whether these alterations represent causes or consequences of disease activity. Third, the potential utility of trace element measurements as biomarkers for disease monitoring or risk stratification has not been established.

The current study was designed to address these knowledge gaps by conducting a comprehensive evaluation of trace element status in a well-characterized cohort of chronic HBV patients from India. Our primary objectives were to determine the prevalence of trace element alterations, investigate their relationships with viral replication and liver inflammation, and assess the potential clinical utility of the copper-to-zinc ratio as a novel biomarker for disease activity assessment. Given the limited access to expensive molecular testing in many resource-limited settings, the identification of alternative biomarkers that could complement or partially substitute for viral load monitoring could have significant implications for clinical practice and patient outcomes.

Methods

Study Design and Setting

This cross-sectional observational study was conducted at Jhalawar Medical College and Hospital, Rajasthan, India, between January 2024 and December 2024. The institution serves as the primary referral center for the Hadoti region, providing care to a diverse patient population representative of the broader North Indian chronic HBV patient demographics. The study protocol was approved by the Institutional Ethics Committee and conducted in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines.

Study Population and Selection Criteria

Adult patients (≥18 years) with confirmed chronic HBV infection, defined as persistent hepatitis B surface antigen (HBsAg) positivity for more than six months, were consecutively recruited from the outpatient gastroenterology clinic and general medicine department. Both treatment-naïve patients and those receiving stable antiviral therapy for at least six months were included to ensure representation of the full spectrum of chronic HBV patients encountered in routine clinical practice.

Patients were excluded if they had: (1) co-infection with hepatitis C virus, hepatitis D virus, or HIV; (2) other liver diseases including alcoholic liver disease (defined as alcohol consumption >40g/day for men or >20g/day for women for >5 years), non-alcoholic fatty liver disease, autoimmune hepatitis, Wilson's disease, hemochromatosis, or alpha-1 antitrypsin deficiency; (3) established cirrhosis or hepatocellular carcinoma; (4) chronic kidney disease (eGFR <60 mL/min/1.73m²); (5) active malignancy; (6) current use of zinc or copper supplements within six months prior to enrollment; (7) pregnancy or lactation; (8) acute illness or hospitalization within one month; or (9) inability to provide informed consent.

Sample Size Calculation

Sample size was calculated based on the primary objective of determining correlations between trace element levels and HBV DNA. Using the standard formula for correlation studies with an expected correlation coefficient of 0.4, α =

0.05, and power = 80%, the minimum required sample size was 280 patients. To account for potential dropouts and analytical challenges, 300 patients were recruited.

Data Collection Procedures

After obtaining written informed consent, detailed clinical and demographic information was collected using structured questionnaires. This included age, gender, occupation, educational level, socioeconomic status (assessed using the modified Kuppuswamy scale), duration of known HBV infection, treatment history, and family history of liver disease. Physical examination was performed to assess for signs of chronic liver disease, and anthropometric measurements were recorded.

Laboratory Methods

Sample Collection and Processing

Blood samples were collected after a 12-hour overnight fast to minimize the influence of recent food intake on trace element levels. Venipuncture was performed using trace element-free collection tubes to prevent contamination. Samples were processed within 2 hours of collection, with serum separated by centrifugation at 3000 rpm for 10 minutes and stored at -20°C for trace element analysis and -80°C for HBV DNA quantification.

Trace Element Analysis

Serum copper and zinc concentrations were measured using flame atomic absorption spectroscopy (AAS) at the Central Research Laboratory. Sample preparation involved careful dilution with deionized water and nitric acid to achieve optimal matrix conditions while preventing interference from other serum components. Contamination prevention measures included use of metal-free containers, cleaning of all glassware with nitric acid followed by thorough rinsing with deionized water, and preparation of all reagents using high-purity chemicals.

Standard curves were prepared using certified reference standards for both copper and zinc, with concentrations spanning the expected range for human serum samples (copper: 80-155 µg/dL; zinc: 70-120 µg/dL). Quality control was maintained through analysis of certified reference materials with known copper and zinc concentrations, analysis of duplicate samples to assess precision, and regular calibration verification throughout analytical runs.

The copper-to-zinc ratio was calculated by dividing the serum copper concentration by the serum zinc concentration for each patient. Based on previous literature in liver disease, a ratio >1.5 was considered elevated, indicating potential trace element imbalance.

HBV DNA Quantification and Liver Function Tests

HBV DNA quantification was performed using the Artus HBV RG PCR Kit with real-time PCR technology, providing results in International Units per milliliter (IU/mL). The assay has a detection limit of 20 IU/mL and linear range extending to 1.7 × 10^8 IU/mL. Standard liver function tests including alanine aminotransferase (ALT), aspartate aminotransferase (AST), and serum bilirubin were performed using automated clinical chemistry platforms. HBV serological markers (HBsAg, HBeAg, anti-HBe) were analyzed using enzyme-linked immunosorbent assay (ELISA) techniques.

Statistical Analysis

Statistical analysis was performed using SPSS version 28.0. Continuous variables were assessed for normality using the Shapiro-Wilk test and visual inspection of histograms. Normally distributed variables were described using means and standard deviations, while non-normally distributed variables were described using medians and interquartile ranges. Categorical variables were described using frequencies and percentages.

Correlation analyses were performed using Pearson correlation coefficients for normally distributed variables and Spearman correlation coefficients for non-normally distributed variables. HBV DNA values were log-transformed due to their wide range and skewed distribution. Group comparisons were performed using Student's t-test or Mann-Whitney U test for continuous variables and chi-square test or Fisher's exact test for categorical variables.

Multivariate linear regression analysis was performed to identify independent predictors of HBV DNA levels, with variables showing significant univariate associations included in the model. Receiver operating characteristic (ROC) curves were constructed to evaluate the diagnostic performance of trace element parameters for identifying clinically relevant viral load thresholds. All statistical tests were two-tailed with a significance level of 0.05.

Results

Study Population Characteristics

A total of 300 patients with chronic HBV infection were included in the final analysis. The mean age was 42.3 ± 12.7 years, with 178 (59.3%) males and 122 (40.7%) females. HBeAg was positive in 134 patients (44.7%), while 186 patients (62.0%) were treatment-naïve. The mean duration of known HBV infection was 8.4 ± 6.2 years. Complete demographic and clinical characteristics are presented in Table 1.

Table 1: Demographic and Clinical Characteristics by Trace Element Status

Characteristic	Overall (n=300)	Normal Cu/Zn Ratio (n=111)	Elevated Cu/Zn Ratio (n=189)	p-value
Age (years)	42.3 ± 12.7	40.8 ± 11.9	43.2 ± 13.1	0.128
Male gender, n (%)	178 (59.3)	58 (52.3)	120 (63.5)	0.073
HBeAg positive, n (%)	134 (44.7)	36 (32.4)	98 (51.9)	0.002
Treatment-naïve, n (%)	186 (62.0)	56 (50.5)	130 (68.8)	0.003
Duration of infection (years)	8.4 ± 6.2	7.8 ± 5.9	8.8 ± 6.4	0.187
ALT elevation, n (%)	198 (66.0)	54 (48.6)	144 (76.2)	<0.001
AST elevation, n (%)	174 (58.0)	46 (41.4)	128 (67.7)	<0.001
Log10 HBV DNA (IU/mL)	4.23 ± 1.87	3.41 ± 1.64	4.72 ± 1.82	<0.001

Cu/Zn = Copper-to-Zinc; ALT = Alanine Aminotransferase; AST = Aspartate Aminotransferase; HBV = Hepatitis B Virus; HBeAg = Hepatitis B e Antigen

Trace Element Levels and Distribution

Serum zinc levels ranged from 45.2 to 98.7 μg/dL, with a mean of 71.8 ± 15.3 μg/dL. Using the reference range of 70-120 μg/dL, 156 patients (52.0%) had zinc deficiency. The distribution showed 89 patients (29.7%) with severe zinc deficiency (<60 μg/dL), 67 patients (22.3%) with moderate deficiency (60-70 μg/dL), and 144 patients (48.0%) with normal or high levels.

Serum copper levels ranged from 78.4 to 165.3 μg/dL, with a mean of 118.7 ± 24.6 μg/dL. Using the reference range of 80-155 μg/dL, 67 patients (22.3%) had elevated copper levels, while 23 patients (7.7%) had levels below normal. The copper-to-zinc ratio ranged from 0.89 to 3.47, with a mean of 1.72 ± 0.48. A total of 189 patients (63.0%) had an elevated ratio >1.5.

Table 2: Trace Element Levels by Patient Characteristics

Parameter	Overall	HBeAg+	HBeAg-	p-value	Treatment-naïve	Treated	p-value
Zinc (μg/dL)	71.8 ± 15.3	68.7 ± 14.2	74.3 ± 15.8	0.002	69.8 ± 15.1	75.1 ± 15.2	0.007
Copper (μg/dL)	118.7 ± 24.6	124.5 ± 26.8	114.2 ± 21.7	<0.001	122.4 ± 25.9	112.6 ± 21.3	0.002
Cu/Zn Ratio	1.72 ± 0.48	1.88 ± 0.54	1.59 ± 0.38	<0.001	1.82 ± 0.52	1.55 ± 0.35	<0.001
Zinc Deficiency, n (%)	156 (52.0)	78 (58.2)	78 (47.0)	0.081	104 (55.9)	52 (45.6)	0.111
Elevated Copper, n (%)	67 (22.3)	38 (28.4)	29 (17.5)	0.037	49 (26.3)	18 (15.8)	0.047
Elevated Cu/Zn, n (%)	189 (63.0)	98 (73.1)	91 (54.8)	0.002	130 (69.9)	59 (51.8)	0.003

Cu/Zn = Copper-to-Zinc; HBeAg = Hepatitis B e Antigen

Correlation Between Trace Elements and HBV DNA

The copper-to-zinc ratio demonstrated the strongest correlation with log10 HBV DNA levels among all measured parameters ($r = 0.467$, $p < 0.001$). Individual trace elements also showed significant correlations: zinc levels correlated negatively with HBV DNA ($r = -0.398$, $p < 0.001$), while copper levels showed a positive correlation ($r = 0.356$, $p < 0.001$).

These correlations remained significant across different patient subgroups, though with varying strength. In HBeAg-positive patients, the copper-to-zinc ratio correlation was $r = 0.523$ ($p < 0.001$), compared to $r = 0.387$ ($p < 0.001$) in HBeAg-negative patients. Treatment-naïve patients showed stronger correlations ($r = 0.512$, $p < 0.001$) compared to treated patients ($r = 0.298$, $p = 0.002$).

Table 3: Correlation Between Trace Elements and Clinical Parameters

Parameter	Zinc	Copper	Cu/Zn Ratio	ALT	AST	Log10 HBV DNA
Zinc	1.000	-0.234**	-0.768***	-0.412***	-0.367***	-0.398***
Copper	-0.234**	1.000	0.723***	0.378***	0.334***	0.356***
Cu/Zn Ratio	-0.768***	0.723***	1.000	0.489***	0.442***	0.467***
ALT	-0.412***	0.378***	0.489***	1.000	0.823***	0.421***
AST	-0.367***	0.334***	0.442***	0.823***	1.000	0.387***
Log10 HBV DNA	-0.398***	0.356***	0.467***	0.421***	0.387***	1.000

*Correlation coefficients shown; **p < 0.01, **p < 0.001 Cu/Zn = Copper-to-Zinc; ALT = Alanine Aminotransferase; AST = Aspartate Aminotransferase; HBV = Hepatitis B Virus

Trace Elements by HBV DNA Categories

When patients were stratified by HBV DNA levels, clear trends emerged in trace element status. Patients with higher viral loads had progressively lower zinc levels and higher copper levels, resulting in higher copper-to-zinc ratios. This relationship is detailed in Table 4.

Table 4: Trace Element Levels by HBV DNA Categories

HBV DNA Category	Number	Zinc (μg/dL)	Copper (μg/dL)	Cu/Zn Ratio	Zinc Deficiency n (%)	Elevated Cu/Zn n (%)
Undetectable/Very Low (<2000 IU/mL)	89	79.4 ± 17.2	106.8 ± 18.4	1.39 ± 0.28	28 (31.5)	23 (25.8)
Low (2000-20,000 IU/mL)	67	74.8 ± 14.6	115.2 ± 21.9	1.59 ± 0.35	31 (46.3)	34 (50.7)
Moderate (20,000-2×10^6 IU/mL)	78	68.9 ± 13.7	123.4 ± 24.8	1.86 ± 0.46	47 (60.3)	58 (74.4)
High (>2×10^6 IU/mL)	66	64.2 ± 11.9	131.7 ± 27.3	2.13 ± 0.52	50 (75.8)	60 (90.9)
p-value for trend		<0.001	<0.001	<0.001	<0.001	<0.001

Cu/Zn = Copper-to-Zinc; HBV = Hepatitis B Virus

Diagnostic Performance of Trace Element Parameters

ROC curve analysis was performed to evaluate the diagnostic performance of trace element parameters for identifying patients with clinically significant viral replication. For detecting HBV DNA >2000 IU/mL, the copper-to-zinc ratio achieved an AUC of 0.751 (95% CI: 0.701-0.801), superior to individual zinc (AUC = 0.698) or copper (AUC = 0.623) measurements.

For higher viral load thresholds, the copper-to-zinc ratio maintained superior performance. For HBV DNA >20,000 IU/mL, the AUC was 0.743 (95% CI: 0.691-0.795), while for HBV DNA >200,000 IU/mL, the AUC was 0.732 (95% CI: 0.677-0.787).

Table 5: Diagnostic Performance of Copper-to-Zinc Ratio for HBV DNA Thresholds

HBV DNA Threshold	AUC (95% CI)	Optimal Cut-off	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
>2000 IU/mL	0.751 (0.701-0.801)	1.65	73.9	71.2	82.1	60.7

>20,000 IU/mL	0.743 (0.691-0.795)	1.78	71.4	73.8	68.2	76.7
>200,000 IU/mL	0.732 (0.677-0.787)	1.95	68.9	75.1	59.3	81.8

AUC = Area Under the Curve; CI = Confidence Interval; PPV = Positive Predictive Value; NPV = Negative Predictive Value; HBV = Hepatitis B Virus

Multivariate Analysis

Multivariate linear regression analysis was performed to identify independent predictors of HBV DNA levels. In the final model, the copper-to-zinc ratio remained the strongest independent predictor ($\beta = 0.298$, $p < 0.001$), followed by ALT ($\beta = 0.187$, $p = 0.006$) and HBeAg status ($\beta = 0.156$, $p = 0.018$). This model explained 31.4% of the variance in HBV DNA levels ($R^2 = 0.314$, $p < 0.001$).

Treatment Status and Trace Element Profiles

Patients receiving antiviral therapy demonstrated significantly better trace element profiles compared to treatment-naïve patients. Treated patients had higher mean zinc levels (75.1 ± 15.2 vs. 69.8 ± 15.1 μg/dL, $p = 0.007$) and lower copper levels (112.6 ± 21.3 vs. 122.4 ± 25.9 μg/dL, $p = 0.002$), resulting in a lower copper-to-zinc ratio (1.55 ± 0.35 vs. 1.82 ± 0.52, $p < 0.001$).

Discussion

This study represents the most comprehensive evaluation of trace element status in chronic HBV infection conducted in an Indian population and provides several novel insights with important clinical implications. Our findings demonstrate that trace element imbalance is highly prevalent in chronic HBV patients, with over half experiencing zinc deficiency and nearly two-thirds having an elevated copper-to-zinc ratio. Most importantly, we have established that the copper-to-zinc ratio serves as a strong biomarker for disease activity, showing the strongest correlation with HBV DNA levels among all measured parameters.

The high prevalence of zinc deficiency (52.0%) observed in our study population is consistent with previous reports in chronic liver disease but represents a more detailed characterization of this problem in chronic HBV infection specifically. This finding is clinically significant because zinc deficiency has been associated with impaired immune function, increased oxidative stress, and enhanced liver fibrosis progression, all of which could contribute to worse outcomes in chronic HBV infection (Prasad, 2014) [6]. The inverse correlation between zinc levels and HBV DNA ($r = -0.398$, $p < 0.001$) suggests that zinc status may influence viral replication or that viral activity affects zinc metabolism.

The mechanistic basis for the relationship between zinc deficiency and viral replication likely involves multiple pathways. Zinc is essential for the function of numerous immune cells and enzymes involved in antiviral responses. Deficiency could impair natural killer cell activity, T cell proliferation and function, and the production of antiviral cytokines, potentially compromising viral clearance. Additionally, zinc deficiency reduces the activity of antioxidant enzymes, leading to increased oxidative stress that could favor viral replication or impair hepatocyte function (Stamoulis *et al*., 2007) [7].

The pattern of serum copper elevation observed in 22.3% of our patients, while the majority maintained normal copper levels, reflects the complex nature of copper metabolism in liver disease. The positive correlation between copper levels and HBV DNA ($r = 0.356$, $p < 0.001$) likely reflects increased synthesis of ceruloplasmin as an acute phase response to liver inflammation, although impaired biliary excretion due to hepatocellular dysfunction may also contribute. The tendency toward higher copper levels in patients with more active disease supports the acute phase response mechanism and suggests that copper levels may serve as a marker of disease activity.

The copper-to-zinc ratio emerged as the most informative parameter in our study, with the strongest correlation with HBV DNA ($r = 0.467$, $p < 0.001$) among all measured variables. This parameter appears to capture the combined effects of both zinc deficiency and copper elevation, making it a more comprehensive indicator of trace element imbalance than either element alone. The ratio may also reflect the balance between pro-oxidant (copper) and antioxidant (zinc) activities, with higher ratios indicating greater oxidative stress that could facilitate viral replication or impair viral clearance.

The superior diagnostic performance of the copper-to-zinc ratio (AUC = 0.751 for detecting HBV DNA >2000 IU/mL) compared to individual element measurements validates its potential utility as a clinical biomarker. The optimal cut-off value of 1.65 provides a practical threshold that could be implemented in clinical practice, offering 73.9% sensitivity and 71.2% specificity for identifying significant viral replication.

The relationship between HBeAg status and trace element levels represents a novel finding that has not been extensively reported in previous literature. HBeAg-positive patients had significantly lower zinc levels and higher copper levels compared to HBeAg-negative patients, resulting in higher copper-to-zinc ratios. This observation suggests that the degree of viral replication and immune activity may directly influence trace element metabolism, providing biological plausibility for the correlations observed between trace elements and viral load.

The favorable impact of antiviral treatment on trace element profiles represents an important finding with potential clinical implications. Treated patients showed significantly better trace element status compared to treatment-naïve patients, suggesting that effective viral suppression may help normalize trace element metabolism. This could occur through reduction of liver inflammation, improvement in hepatic synthetic function, or decreased viral-induced metabolic disturbances. This finding also raises the possibility that trace element monitoring could serve as an additional marker of treatment response.

The clinical implications of these findings extend beyond the immediate correlations observed. The high prevalence of zinc deficiency indicates a significant but previously underrecognized problem that could affect patient outcomes through its impacts on immune function and liver health. Systematic assessment of zinc status and consideration of supplementation for deficient patients could potentially improve viral control and liver outcomes, though this hypothesis requires validation through intervention studies.

The copper-to-zinc ratio's strong predictive value for viral activity has practical applications for clinical monitoring, particularly in resource-limited settings where HBV DNA

testing may not be readily available. While not sufficiently accurate to completely replace molecular testing, the ratio could serve as a screening tool to identify patients most likely to have significant viral replication and therefore most likely to benefit from HBV DNA testing and potential treatment.

From a pathophysiological perspective, our findings provide insights into the complex interactions between viral replication, immune responses, and micronutrient metabolism in chronic HBV infection. The progressive worsening of trace element profiles with increasing viral loads suggests that these alterations are closely linked to disease activity rather than being simply coincidental findings. This relationship could reflect either causal mechanisms where trace element imbalances contribute to viral persistence or consequential effects where viral activity drives metabolic disturbances.

The stronger correlations observed in treatment-naïve patients compared to those receiving antiviral therapy likely reflect the suppression of viral replication by treatment, which would naturally attenuate the relationships between viral load and biochemical parameters. However, the persistence of significant correlations even in treated patients suggests that these relationships reflect fundamental aspects of viral pathogenesis that are not completely eliminated by antiviral therapy.

Several limitations of our study should be acknowledged. The cross-sectional design prevents assessment of the temporal relationship between trace element levels and viral load changes over time. Longitudinal studies would be valuable for determining whether trace element alterations precede viral rebound or follow changes in viral activity. The exclusion of patients with cirrhosis, while necessary to isolate the effects of viral replication, limits applicability to the full spectrum of chronic HBV patients.

The study did not include comprehensive dietary assessment, which could provide important context for interpreting trace element alterations. Dietary factors, nutritional status, and supplement use could influence trace element levels independently of liver disease. Future studies should consider including detailed nutritional assessment to better understand the mechanisms underlying trace element alterations.

The focus on copper and zinc, while well-justified by previous literature, may have missed other trace elements that could be relevant to chronic HBV infection. Elements such as selenium, iron, and manganese have also been implicated in liver health and immune function, and broader trace element panels could provide more comprehensive assessment.

Conclusion

This study demonstrates that trace element imbalance is highly prevalent in chronic HBV infection, with zinc deficiency affecting over half of patients and elevated copper-to-zinc ratios present in nearly two-thirds. The copper-to-zinc ratio emerges as a novel biomarker for disease activity, showing the strongest correlation with viral load among all measured parameters and superior diagnostic performance compared to individual element measurements.

These findings have important implications for comprehensive chronic HBV management, suggesting that nutritional assessment and intervention should be considered as integral components of patient care rather than peripheral concerns. The potential utility of the copper-to-zinc ratio as a biomarker for disease activity could be particularly valuable in resource-limited settings where expensive molecular testing is not readily available.

The favorable impact of antiviral treatment on trace element profiles suggests that effective viral suppression may help normalize metabolic disturbances, potentially providing additional benefits beyond viral suppression alone. The high prevalence of zinc deficiency indicates a need for systematic nutritional evaluation and consideration of supplementation in chronic HBV patients.

Future research should include intervention studies examining the effects of zinc supplementation on viral control and liver outcomes, longitudinal studies assessing the predictive value of trace element measurements for disease progression, and technology development efforts aimed at creating accessible testing platforms for trace element assessment in resource-limited settings.

The integration of trace element assessment into chronic HBV care protocols could contribute to more comprehensive patient management and potentially improve outcomes through addressing previously unrecognized nutritional deficiencies. The copper-to-zinc ratio's potential as a cost-effective biomarker for disease activity represents a valuable addition to the limited arsenal of affordable monitoring tools available in resource-limited settings, where such innovations are most needed.

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