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# Correlation Between Heavy Metals and Neonatal Hyperbilirubinemia Among the Patients Attended at Bangabandhu Sheikh Mujib Medical University

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## Abstract

**Background:** Neonatal hyperbilirubinemia is one of the most common clinical conditions during the early neonatal period in both term and preterm newborns. Trace elements such as magnesium (Mg), zinc (Zn), and copper (Cu) play important roles in enzymatic activity, metabolism, growth, and cellular function. Alterations in these trace elements may contribute to the development and severity of neonatal jaundice.

**Objective**: The present study aimed to evaluate the serum concentrations of magnesium, zinc, and copper among neonates with hyperbilirubinemia and to determine their correlation with serum bilirubin levels.

**Methods:** This case-control study was conducted at Bangabandhu Sheikh Mujib Medical University from January 2024 to March 2026. A total of 594 neonates were enrolled, including 319 cases with neonatal hyperbilirubinemia and 275 healthy controls. Serum bilirubin and magnesium levels were measured using an automated analyzer, while zinc and copper concentrations were determined by semi-automated colorimetric methods. Statistical analyses were performed using SPSS software, and $p<0.05$ was considered statistically significant.

**Results:** The study demonstrated statistically highly significant differences in serum Mg, Zn, and Cu levels between the case and control groups ($p<0.001$). The mean serum magnesium level was significantly higher in jaundiced neonates compared to controls (23.98 ± 2.11 mg/L vs. 19.19 ± 1.60 mg/L). Serum zinc levels were significantly lower in the case group (0.50 ± 0.03 mg/L) compared to controls (0.68 ± 0.10 mg/L). In contrast, serum copper levels were significantly elevated in jaundiced neonates (0.75 ± 0.08 mg/L) compared to healthy controls (0.43 ± 0.11 mg/L). Significant positive correlations were observed between serum bilirubin and both magnesium and copper levels, whereas serum zinc showed a significant negative correlation with bilirubin concentration.

**Conclusion:** The present study revealed significant alterations in serum magnesium, zinc, and copper levels among neonates with hyperbilirubinemia. Elevated magnesium and copper levels along with reduced zinc levels may be associated with the development and severity of neonatal jaundice. These findings suggest that trace elements may play an important role in neonatal hyperbilirubinemia and could have potential clinical significance in neonatal care.

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## Introduction

Neonatal jaundice is one of the most frequently encountered clinical conditions during the neonatal period and affects nearly 60% of term and 80% of preterm newborns during the first week of life [1]. Neonatal hyperbilirubinemia (NH), particularly unconjugated hyperbilirubinemia, remains an important cause of neonatal morbidity and hospital admission worldwide. Although bilirubin possesses antioxidant properties that may protect newborns against oxidative stress and free radical injury, excessive accumulation of bilirubin can lead to severe neurological complications including acute bilirubin encephalopathy and kernicterus, resulting in irreversible neurological damage, hearing impairment, cerebral palsy, or even death [2]. The burden of severe neonatal hyperbilirubinemia is reported to be higher in Asian countries, including Bangladesh, where neonatal jaundice remains a common clinical challenge [3]. Neonatal hyperbilirubinemia develops due to an imbalance between bilirubin production and elimination. Physiological immaturity of hepatic conjugation enzymes increased red blood cell turnover, prematurity, hemolytic disorders, infection, dehydration, and genetic predisposition are among the major contributing factors [4]. Despite significant advances in neonatal care, a considerable number of cases are still classified as idiopathic neonatal jaundice, particularly in developing countries [5]. Therefore, identification of additional biochemical and environmental factors associated with NH is important for improving prevention and management strategies. Recently, considerable attention has been directed toward the possible role of trace elements and heavy metals in neonatal diseases, including hyperbilirubinemia. Trace elements are essential micronutrients required for growth, enzymatic reactions, immune regulation, neurological development, and maintenance of cellular metabolism [6]. However, imbalance in these elements, either deficiency or excess accumulation, may contribute to oxidative stress, cellular toxicity, and metabolic disturbances. Several studies have demonstrated that alterations in serum concentrations of zinc, magnesium, copper, lead, cadmium, and other heavy metals may influence neonatal health outcomes [7]. Zinc is an essential trace element involved in protein synthesis, cellular growth, immune function, and neuronal regulation [8]. It is an important component of numerous metalloenzymes, and transcription factors required for normal metabolic activity. Previous studies have suggested that zinc may reduce serum bilirubin levels by inhibiting the enterohepatic circulation of unconjugated bilirubin through intestinal precipitation [9]. Moreover, bilirubin can chelate metal ions such as zinc, suggesting a possible interaction between bilirubin metabolism and trace element homeostasis. However, excessive intracellular zinc accumulation may exert neurotoxic effects and contribute to neuronal injury, indicating that zinc may act as a “double-edged sword” in neonatal physiology [10]. Magnesium is another important intracellular cation that plays a crucial role in cellular respiration, membrane stability, electrolyte transport, and enzymatic activity. It functions as a cofactor for more than 300 enzymatic reactions in the human body. Studies have shown a positive correlation between serum ionized magnesium levels and the severity of neonatal hyperbilirubinemia. Since ionized magnesium represents the biologically active form, disturbances in magnesium homeostasis may influence bilirubin metabolism and neurological outcomes in jaundiced neonates [9]. Copper is an essential trace element involved in oxidation-reduction reactions and acts as a component of several important metalloenzymes, including cytochrome oxidase, superoxide dismutase, and ceruloplasmin. Copper plays a vital role in hematopoiesis, connective tissue formation, antioxidant defense, and immune function. Abnormal copper metabolism has been associated with oxidative stress and hepatic dysfunction, which may contribute to the pathogenesis of neonatal hyperbilirubinemia [11]. Despite the recognized physiological importance of trace elements and heavy metals, limited studies have evaluated their relationship with neonatal hyperbilirubinemia in Bangladesh. Therefore, the present study aims to evaluate the correlation between heavy metals and neonatal hyperbilirubinemia among patients attending Bangabandhu Sheikh Mujib Medical University.

## Methods

This case-control study was conducted at the Department of Clinical Pathology and the Department of Neonatology of Bangabandhu Sheikh Mujib Medical University between January 2024 to March 2026. The study population consisted of neonates attending both outpatient and inpatient departments during the study period. A total of 594 neonatal blood samples were included in the study. Neonates of both sexes aged up to 28 days were enrolled according to the inclusion and exclusion criteria. The study participants were divided into case and control groups based on serum bilirubin status. Prior to sample collection, written informed consent was obtained from the mothers or legal guardians of all neonates. Approximately 2 mL of venous blood was collected aseptically from each participant under standard laboratory precautions. The collected blood samples were allowed to clot and subsequently centrifuged at 3000 rpm for 5 minutes to separate serum. The serum samples were then preserved at 20°C until biochemical analysis was performed. Serum bilirubin and magnesium levels were measured using an automated chemistry analyzer (Dimension RxL Max, Siemens, USA) following the manufacturer’s standard operating procedures. Serum zinc and copper concentrations were determined by semi-automated colorimetric methods using the Evolution 3000 analyzer (Italy). Internal quality control measures were maintained throughout the analytical procedures to ensure the reliability and accuracy of laboratory

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results. Collected data was processed and analyzed using Statistical Package for Social Sciences (SPSS) software version 25.0. Descriptive statistics, including mean, standard deviation, frequency, and percentage, were calculated where appropriate. Comparative and correlation analyses were performed to evaluate the association between serum heavy metal concentrations and neonatal hyperbilirubinemia. A p-value of less than 0.05 was considered statistically significant. Ethical principles were strictly maintained throughout the study period. Confidentiality, privacy, and safety of the participants were ensured at every stage of the research. The study protocol was conducted in accordance with the ethical standards of biomedical research involving human subjects.

## Results

A total of 594 neonates were included in the present study, comprising 319 cases and 275 controls. The mean age of the neonates was 3.08 ± 2.80 days in the case group and 3.21 ± 3.65 days in the control group. Most neonates in both groups belonged to the 1-2 days age category, accounting for 55.80% in the case group and 63.64% in the control group, followed by the 3-7 days age group. Only a small proportion of participants were observed in the 8–15 days and up to 25 days categories. No statistically significant difference was found between the two groups regarding age distribution (p=0.94). The higher frequency of neonates within the first 48 hours in the case group may be attributed to the early onset and common occurrence of neonatal jaundice during this period after birth (Table 1).

Table 2 demonstrates the sex distribution of the study participants among the case and control groups. In the case group, 187 (58.62%) neonates were male and 132 (41.38%) were female. Similarly, in the control group, 160 (58.18%) neonates were male and 115 (41.82%) were female. Male neonates constituted the majority in both groups. However, no statistically significant difference was observed between the case and control groups regarding sex distribution, indicating comparable demographic characteristics between the two study populations.

The distribution of birth weight among the neonates in both the case and control groups. The mean body weight of the respondents was 2.62 ± 0.37 kg in the case group and 2.84 ± 0.27 kg in the control group. Most neonates belonged to the 2.5-3.5 kg weight category, comprising 227 (71.16%) neonates in the case group and 271 (98.55%) neonates in the control group. A statistically significant difference was observed between the two groups regarding birth weight distribution. The findings indicate that neonatal hyperbilirubinemia was more frequently observed among low-birth-weight neonates, which is consistent with findings reported in previous studies (Table 3).

**Table 1:** Demographic variable of age among case and control groups.

| Age variables (days) | Case (n=319) | n (%) | Control (n=275) | n (%) | p value |
|---|---|---|---|---|---|
| 01-Feb | 178 | 55.8 | 175 | 63.64 | |
| 03-Jul | 115 | 36.05 | 70 | 25.45 | |
| Aug-15 | 24 | 7.52 | 27 | 9.82 | |
| Upto 25 | 2 | 0.63 | 3 | 1.09 | |
| Mean± SD | 3.08±2.80 | | 3.21±3.65 | | $^{a}0.94^{ns}$ |

Note: s= significant, ns= not significant, aP value reached from unpaired t-test

**Table 2:** Demographic variable of sex among case and control groups.

| Sex | Case (n=319) | n (%) | Control (n=275) | n (%) | p value |
|---|---|---|---|---|---|
| Male | 187 | 58.62 | 160 | 58.18 | $^{b}0.207^{ns}$ |
| Female | 132 | 41.38 | 115 | 41.82 | |

Note: s= significant, ns= not significant, bP value reached from Chi square

**Table 3:** Demographic variable of weight among case and control groups.

| Weight (kg) | Case (n=319) | n (%) | Control (n=275) | n (%) | p value |
|---|---|---|---|---|---|
| <2.5 | 90 | 28.21 | 2 | 0.73 | $^{a}0.001^{s}$ |
| 2.5-3.5 | 227 | 71.16 | 271 | 98.55 | |
| >3.5 | 2 | 0.63 | 2 | 0.73 | |
| Mean±SD | 2.62±0.37 | | 2.84±0.27 | | |

Note: s= significant; ns= not significant, aP value reached from unpaired t-test

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### Serum total bilirubin

Table 4 illustrates the comparison of serum bilirubin levels between the case and control groups. The mean serum bilirubin concentration was 171.14 mg/L in the case group, whereas it was 35.42 mg/L in the control group. A highly statistically significant difference was observed between the two groups (p<0.001). The markedly elevated serum bilirubin level in the case group indicates impaired bilirubin metabolism and reduced hepatic clearance associated with neonatal hyperbilirubinemia. These findings suggest that liver function and bilirubin conjugation processes may be compromised in jaundiced neonates compared to healthy controls.

**Table 4:** Biochemical variable of serum total bilirubin among case and control groups.

| Trace Elements | Case (n=319) Mean ±SD | Control (n=275) Mean ±SD | p value |
|---|---|---|---|
| Serum total bilirubin (mg/L) | 171.14 (±17.08) | 35.42 (±8.38) | <0.001s |

Note: s= significant; ns= not significant, p<0.001

### Serum magnesium level

The comparison of serum magnesium concentrations between the case and control groups. The mean serum magnesium level was 23.98 mg/L in the case group and 19.19 mg/L in the control group. Serum magnesium concentration was found to be significantly higher in neonates with hyperbilirubinemia compared to the healthy control group. The difference between the two groups was highly statistically significant (p<0.001). These findings suggest a possible association between elevated serum magnesium levels and neonatal hyperbilirubinemia (Table 5).

**Table 5:** Biochemical variable of serum magnesium among case and contro groups.

| Trace Elements | Case (n=319) Mean ±SD | Control (n=275) Mean ±SD | p value |
|---|---|---|---|
| Serum magnesium (mg/L) | 23.98 (±2.11) | 19.19 (±1.60) | <0.001s |

Note: s= significant; ns= not significant, p<0.001

### Serum zinc level

Table 6 shows the comparison of serum zinc levels between the case and control groups. The mean serum zinc concentration was 0.50 mg/L in the case group and 0.68 mg/L in the control group. Serum zinc levels were significantly lower in neonates with hyperbilirubinemia compared to healthy controls, and the difference was highly statistically significant (p<0.001).

**Table 6:** Biochemical variable of serum zinc among case and control groups.

| Trace Elements | Case (n=319) Mean ±SD | Control (n=275) Mean ±SD | p value |
|---|---|---|---|
| Serum Zinc (mg/L) | 0.50 (±0.03) | 0.68 (±0.10) | <0.001s |

Note: s= significant; ns= not significant, p<0.001

### Serum copper level

Table 7 presents the comparison of serum copper levels between the case and control groups. The mean serum copper concentration was 0.75 mg/L in the case group and 0.43 mg/L in the control group. Serum copper levels were significantly higher in neonates with hyperbilirubinemia compared to healthy controls, and the difference was highly statistically significant (p<0.001).

**Table 7:** Biochemical variable of serum copper among case and control groups.

| Trace Elements | Case (n=319) Mean ±SD | Control (n=275) Mean ±SD | p value |
|---|---|---|---|
| Serum Copper (mg/L) | 0.75 (±0.08) | 0.43 (±0.11) | <0.001s |

Note: s= significant; ns= not significant, p<0.001

### Correlation between serum total bilirubin level and serum magnesium level

Figure 1 demonstrates a significant positive Pearson correlation between serum total bilirubin and serum magnesium levels in both the case and control groups (r = 0.817, p<0.001). The findings indicate that increased serum bilirubin levels were associated with increased serum magnesium concentrations among the study participants.

### Correlation between serum total bilirubin level and serum zinc level

Figure 2 shows a significant negative Pearson correlation between serum total bilirubin and serum zinc levels in both the case and control groups (r = −0.773, p<0.001). The findings indicate that as serum bilirubin levels increased, serum zinc concentrations decreased among the study participants.

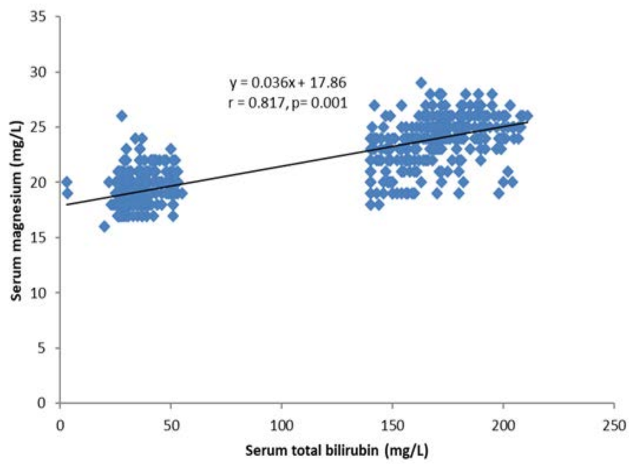


**Figure 1:** Scatter diagram of correlation between serum total bilirubin level and serum magnesium level in both groups.

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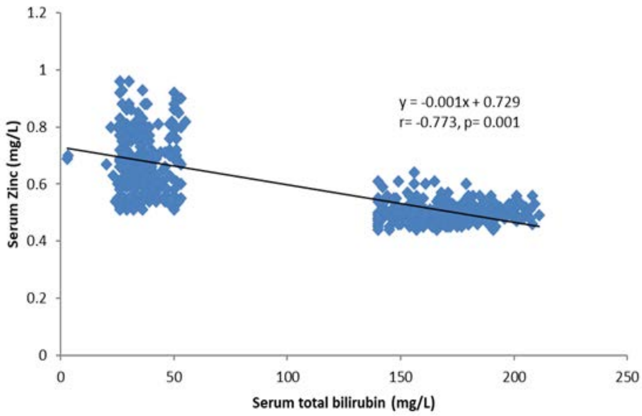


**Figure 2:** Scatter diagram of correlation between serum total bilirubin level and serum zinc level in both groups.

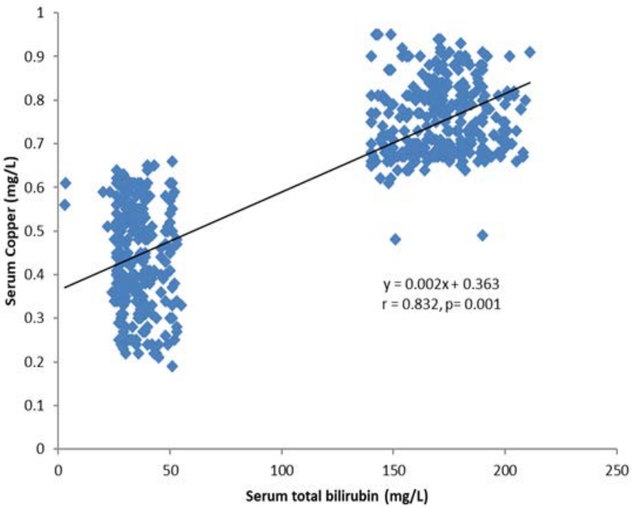


**Figure 3:** Scatter diagram of correlation between serum total bilirubin level and serum copper level in both groups.

### Correlation between serum total bilirubin level and serum copper level

Figure 3 demonstrates a significant positive Pearson correlation between serum total bilirubin and serum copper levels in both the case and control groups ($r = 0.832$, $p<0.001$). The findings indicate that increased serum bilirubin levels were associated with increased serum copper concentrations among the study participants.

## Discussion

In the current study, serum magnesium levels were significantly higher in neonates with hyperbilirubinemia compared to the control group (23.98 ± 2.11 mg/L vs. 19.19 ± 1.60 mg/L; $p<0.001$). Furthermore, a strong positive correlation was observed between serum bilirubin and magnesium levels ($r=0.817$, $p<0.001$), indicating that serum magnesium concentration increased proportionately with increasing bilirubin levels. These findings suggest that magnesium may play an important role in the pathophysiology of neonatal jaundice. Elevated serum magnesium levels in jaundiced neonates may be associated with increased erythrocyte turnover, mild hemolysis, or intracellular electrolyte imbalance during hyperbilirubinemia. Since magnesium is predominantly an intracellular cation and acts as a cofactor for numerous enzymatic reactions, alterations in magnesium homeostasis may reflect metabolic disturbances occurring in jaundiced neonates. Similar observations have been reported in previous studies where increased magnesium concentrations were associated with the severity of neonatal hyperbilirubinemia. The present findings therefore support the possibility that elevated serum magnesium may be linked to bilirubin metabolism and disease progression in neonatal jaundice [12]. The present study also demonstrated significantly lower serum zinc levels among neonates with hyperbilirubinemia compared to healthy controls (0.50 ± 0.03 mg/L vs. 0.68 ± 0.10 mg/L; $p<0.001$). In addition, a significant negative correlation was observed between serum bilirubin and zinc levels ($r=-0.773$, $p<0.001$), indicating that serum zinc concentration decreased as bilirubin levels increased. Zinc is an essential trace element involved in numerous biological functions, including enzymatic activity, antioxidant defense, immune regulation, and cellular growth. It also plays an important role in bilirubin metabolism by reducing enterohepatic circulation of unconjugated bilirubin [13]. Reduced zinc levels in jaundiced neonates may impair antioxidant protection and membrane stability, thereby contributing to increased oxidative stress and bilirubin accumulation. The findings of the present study are consistent with earlier reports suggesting that zinc deficiency may increase susceptibility to neonatal jaundice. Therefore, lower serum zinc concentrations observed among hyperbilirubinemia neonates may indicate a protective role of zinc against the development and severity of neonatal jaundice [14]. Regarding serum copper levels, the present study found significantly higher concentrations in the case group compared to the control group (0.75 mg/L vs. 0.43 mg/L; $p<0.001$). Moreover, serum copper showed a strong positive correlation with serum bilirubin levels ($r=0.832$, $p<0.001$), suggesting that copper concentration increased with increasing bilirubin levels. Copper is an essential component of several metalloenzymes involved in oxidation-reduction reactions and antioxidant defense mechanisms. However, excessive copper accumulation may contribute to oxidative stress, hepatocellular dysfunction, and impaired bilirubin metabolism. Elevated serum copper levels observed in jaundiced neonates may reflect altered hepatic handling, increased oxidative activity, or inflammatory responses associated with neonatal hyperbilirubinemia [15]. Similar findings from previous studies have also demonstrated increased copper concentrations in neonates with jaundice, supporting the possible involvement of copper in the pathogenesis of hyperbilirubinemia [16]. The findings of

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the present study indicate that alterations in trace element concentrations are significantly associated with neonatal hyperbilirubinemia. Increased serum magnesium and copper levels along with decreased zinc levels may contribute to the development or progression of neonatal jaundice through mechanisms involving oxidative stress, impaired hepatic metabolism, and altered enzymatic activity [17]. These observations highlight the potential clinical importance of monitoring trace element status in jaundiced neonates. Further large-scale multicenter studies are recommended to better understand the exact mechanisms linking trace elements with neonatal hyperbilirubinemia and to evaluate their potential role in diagnosis, prognosis, and therapeutic management.

## Conclusion

The present study demonstrated significant alterations in serum magnesium, zinc, and copper levels among neonates with hyperbilirubinemia compared to healthy controls. Serum magnesium and copper levels were significantly increased, whereas serum zinc levels were significantly decreased in jaundiced neonates. Significant correlations between serum bilirubin and these trace elements suggest that Mg, Zn, and Cu may play an important role in the development and severity of neonatal hyperbilirubinemia. Further studies are recommended to clarify their clinical significance in neonatal jaundice.

## Recommendations

Further large-scale multicentre studies are recommended to evaluate the relationship between serum magnesium, zinc, and copper levels and neonatal hyperbilirubinemia in different geographical and socioeconomic regions of Bangladesh, including rural, urban, and slum populations. Future research should also investigate the influence of maternal nutrition, environmental exposure, genetic factors, and dietary supplementation on trace element imbalance among jaundiced neonates and their mothers. In addition, improving maternal nutritional status during pregnancy, particularly in low-income populations, may help identify and reduce the risk factors associated with zinc deficiency and elevated magnesium and copper levels in neonatal jaundice.

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