

# **Correlation between Neonatal Hyperbilirubinemia and Serum Magnesium and Copper Levels in Dhaka City**

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## **Authors' contributions**

*This work was carried out in collaboration among all authors. Authors AM and MEI designed the study, author MAH, conducted field works, wrote the first draft of the manuscript. Authors MSI and MNH managed the literature searches, and author SMR performed the statistical analysis. All authors read and approved the final manuscript.*

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## **ABSTRACT**

**Background:** Neonatal hyperbilirubinemia is a condition when a newborn has an excessive amount of bilirubin in the blood and is one of the most prevalent problems in neonates. Many studies reported that copper and magnesium play an important role in the pathogenesis and development of neonatal hyperbilirubinemia.

**Objectives:** The aim of this study is to find out the correlation between the level of magnesium and copper with hyperbilirubinemia.

**Methodology:** Serum bilirubin was assayed with colorimetric method by the use of diazotized sulfanilic acid reaction. A photometric automated method was used to determine the levels of magnesium and copper in the serum of neonates in both controls group (162) and cases group (220).

**Results:** In the present study a significantly higher levels of Mg was found in hyperbilirubinemia of newborn infants when compared with control groups ( $23.67 \pm 2.33$  mg/L versus  $19.74 \pm 2.18$  mg/L respectively and p value  $<0.001$  which was significant) and correlation between hyperbilirubinemia and magnesium also significant (p value  $<0.001$ ). Copper levels was significantly higher in hyperbilirubinemia of newborn infants ( $0.74 \pm 0.08$  mg/L) compared with control groups ( $0.41 \pm 0.12$  mg/L), where p value was  $<0.001$ , which was significant and correlation between hyperbilirubinemia and copper also significant (p value  $<0.001$ ).

**Conclusion:** It can be concluded that current study showed the concentrations of magnesium and copper levels were found to be significantly greater than control groups and may have a correlation with neonatal jaundice.

*Keywords: Neonatal hyperbilirubinemia; magnesium(Mg) and copper(Cu); neonatal jaundice.*

## 1. INTRODUCTION

Neonatal jaundice is a common problem that occurs in about 60% of newborns during the first week of life [1]. Severe increase of unconjugated bilirubin in blood may cause kernicterus and even death in newborn. Kernicterus is very harmful in newborn due to indirect hyperbilirubinemia but it may protect newborn from some toxic radicals in neonatal period [2]. Studies in 1990 suggest that neonatal jaundice is rare in developed countries [3]. Recent studies showed 60% of newborns exhibit signs of clinical jaundice in United States. In Asian Countries Neonatal jaundice is also recognized as a major problem. Although, large-scale prospective studies have not been reported yet from in any part of the world [4]. In Bangladesh exact data is not available. One studies showed neonatal jaundice is most common illness of hospital admission and it is about (30.71%) of total newborns [5]. The exact cause of neonatal jaundice is not known. Some factors like race, genetic polymorphisms; inherited and acquired defect, Gilbert's syndrome may responsible with neonatal jaundice [6]. So it is very difficult to find out the direct etiology.

The trace elements magnesium and copper are essential elements for growth, development, and maintenance of healthy tissues. Excess presence of toxic from elements in human may cause toxicity and their deficiency may lead to various diseases [7].

Magnesium is an essential co factor for cellular respiration, glycolysis, and trans-membrane transportation of sodium and calcium. It is also affect the enzyme activity by binding of ATP-requiring enzyme and the active site of enzyme

(pyruvate kinase, enolase), by causing conformational changes during the catalytic process (Na- K-ATPase), and by promoting aggregation of multi enzyme complexes [8]. In elderly, hypomagnesaemia is rare and mostly seen in renal failure. This may be a positive correlation between ionized magnesium level and severity of hyperbilirubinemia patients in neonate [9].

Copper is an essential micronutrient and has many important functional roles in immune system and its response. Trace element copper is involved in the function of several cuproenzymes that are essential for life. Copper deficiency affects many cuproenzymes, leading to defects in ATP production; lipid peroxidation; hormone activation; angiogenesis; and abnormalities of vasculature, skeleton, and lung [10].

Fetal liver copper levels are increased four to ten times those found in the normal adult. Copper is a very stimulating mineral to the nerves and central nervous system (CNS). Its effects on neurotransmitters can give rise to many psychological imbalances such as mood swings, depression, mental agitation, feeling overstimulated, restlessness, anxiety and insomnia. When women become pregnant, their estrogen levels rise, greatly increasing the retention of copper in the body. This metal will pass through the placenta into the unborn child. This excess presence of copper in neonates may result in toxicity [11].

It is also reported that high plasma levels of ionized Mg in neonatal is related to nonhemolytic hyperbilirubinemia in newborns.

Neonatal jaundice is the most common cause of hospitalization in the first month of life. Factors that affect the severity of neonatal jaundice include: maternal, prenatal and neonatal factors as well as environmental factors.[12]

To date, very few studies have been conducted in Bangladesh on this issue. Therefore, this study was undertaken to assess the presence of heavy metal specially, copper and magnesium in blood of hyperbilirubinemic neonates and correlate it with serum bilirubin level in neonatal jaundice.

## 2. MATERIALS AND METHODS

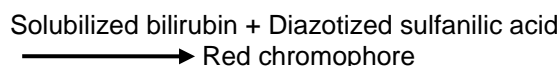
This was a correlation study carried out on 382 neonates admitted to Department of Clinical Pathology (Laboratory Medicine) and Neonatology at Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka. This study was carried out in the period January 2016 to December 2017. Infants with severe congenital malformation, sepsis, or birth asphyxia were excluded from this study. Neonates were divided into case and control groups. Every infant has undergone two blood sampling.

### 2.1 Blood Collection and Serum Separation

Two milliliter (2ml) blood was collected from neonate under complete aseptic conditions and was dispensed into a plain tube left to clot at room temperature (25°C) for 30 minutes, centrifuged in 3000 rpm for 5 minutes, then the serum was separated and stored in -20°C for further test.

### 2.2 Estimation of Serum Bilirubin

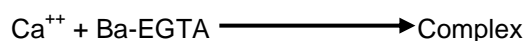
The level of bilirubin was assessed by photometric automated methods (Dimension RxL Max, USA) at 540 nm using a bichromatic (540, 700 nm) endpoint technique [13] which can be represent as the following scheme.



### 2.3 Estimation of Serum Magnesium

The level of magnesium in serum sample was assessed by photometric methods (Dimension RxL Max, USA) [13]. Briefly, methyl thymol blue (MTB) (0.0528 g/L) is mixed with the serum sample to form a blue complex with magnesium.

Calcium interference is minimized by forming a complex between calcium and Ba-EGTA (chelating agent). The amount of MG-MTB complex formed is proportional to the magnesium concentration and is measured using a bichromatic (600 and 510 nm) endpoint technique.



The concentration of magnesium in the sample was measured by the following equation-

$$\text{Conc. of Mg} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times \text{concentration of standard}$$

### 2.4 Estimation of Serum Copper

Copper was assessed by colorimetric method (Semi-automated Biochemistry analyzer, Evolution 3000, Italy) using ready for use kit (Italy). The serum sample was mixed well with all the reagents and incubated at room temperature for 10 min. In acidic medium copper reacts with Di-Bar-PAESA to form a colored complex [14]. The increase of absorbance of this complex can be measured and the formation of the complex is proportional to the concentration of total copper in the sample. The absorbance of the standard and sample against blank was measured at 580nm.



Concentration of copper in plasma sample was calculated by the following formula-

$$\text{Copper in } \mu\text{g/dl} = \frac{\text{Abs of test sample}}{\text{Abs of std. sample}} \times 200$$

### 2.5 Statistical Analysis

Statistical analysis was done by using the SPSS (statistical package for the social sciences) statistical software to obtain the mean, standard deviation and frequencies. The following statistical tests were used:

- Mean and standard deviation (SD) to describe quantitative data.
- Student t test was used to compare between two groups.

- c. Chi-square test was used to compare between two groups.
- d. Pearson correlation was used to correlate two quantitative variables. All tests, a probability (*p*) of <0.05 was considered significant.

group and (19.74 mg/L) in control group. It is also showed concentration of magnesium higher in case group than control group. Serum copper result was higher in cases (0.74 mg/L) than control group (0.41 mg/L). It is highly significant (*p*<0.001) when for all serum level in case group compared with control group.

### 3. RESULTS

#### 3.1 Total Serum Bilirubin, Mg and Cu Level

Total serum bilirubin, Magnesium (Mg) and copper (Cu) level were determined of all neonates and compared to the control group. Table1 showed that the total serum bilirubin was higher in case group (169.43 mg/L) than control group (39.38 mg/L). The level of serum magnesium was (23.67 mg/L) in case

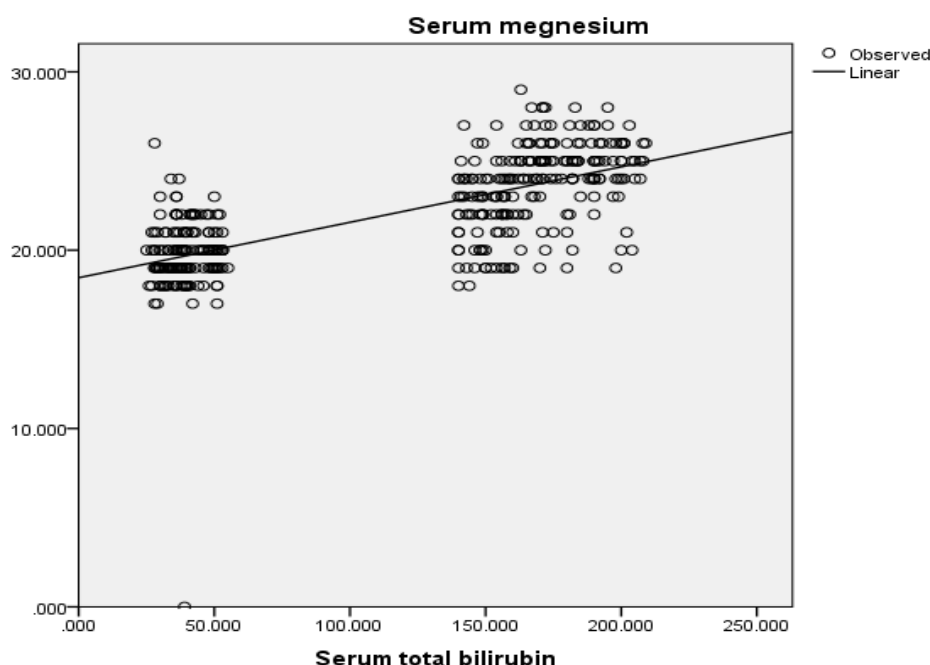
#### 3.2 Correlation between Serum Total Bilirubin with Serum Magnesium and Serum Copper Level

Figs. 1 and 2 showed that the positive correlation between serum total bilirubin with serum magnesium and copper 68% and 82% indicates that when serum bilirubin was increased then serum magnesium and copper also be increased.

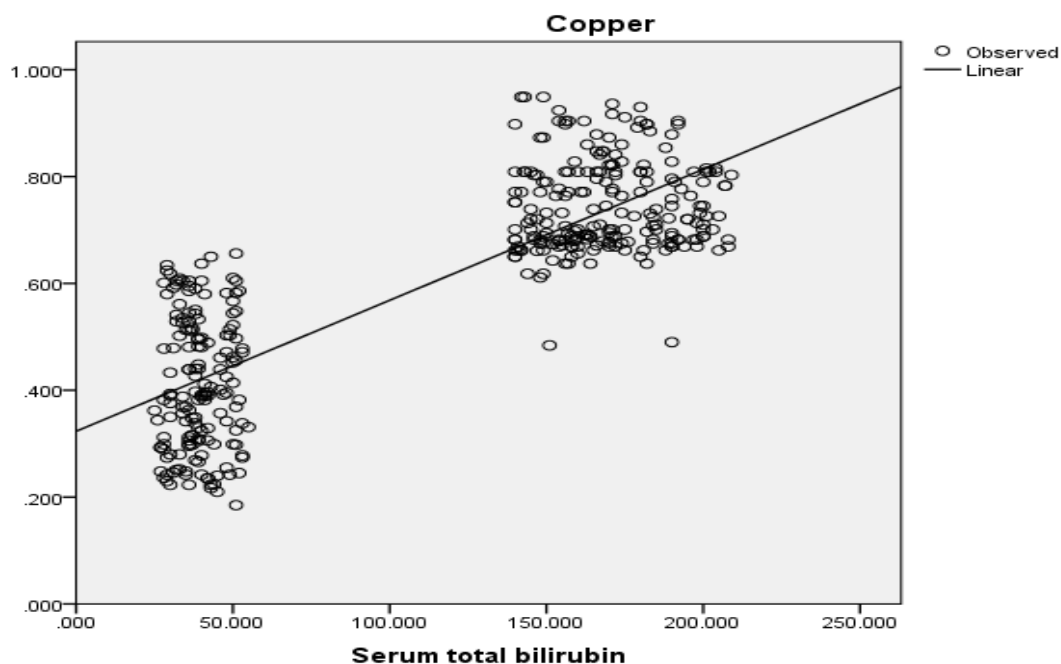
**Table 1. Mean difference Serum total bilirubin, serum magnesium, serum copper between cases and controls**

Elements	Case Mean ±SD	Control Mean ±SD	p value
Serum total bilirubin (mg/L)	169.43 (±19.36)	39.38 (±7.65)	<0.001 <sup>s</sup>
Serum magnesium (mg/L)	23.67 (±2.33)	19.74 (±2.18)	<0.001 <sup>s</sup>
Serum Copper (mg/L)	0.74 (±0.08)	0.41 (±0.12)	<0.001 <sup>s</sup>

*s*= significant



**Fig. 1. Positive significant Pearson correlation (r=0.688, p value <0.001) between serum total bilirubin level and serum magnesium level in both groups**



**Fig. 2. Positive significant Pearson Correlation ( $r=0.829$ ,  $p$  value  $<0.001$ ) between serum total bilirubin level and serum copper level in both groups**

#### 4. DISCUSSION

In early neonatal period hyperbilirubinemia is a very common problem in term and preterm babies [15]. In newborns, it occurs in both the physiological and pathological processes [16]. In healthy term newborn, physiological jaundice follows a typical pattern where total serum bilirubin peaks at 50 to 60 mg/L on third to fourth day of life and then slowly decreases during first week of life [17]. The results of current study demonstrated that the concentration of total bilirubin is 169.43 mg/L and 39.38 mg/L in case and control group respectively which is highly significant ( $p<0.001$ ) when case group compared with control group. It may be increased turnover of erythrocyte and lower activity of the enzyme uridine diphosphoglucuronate (UDP) glucuronyl transferase as a result decreased clearance by slow intestinal motility and increase small amounts of bilirubin reuptake by the enterohepatic circulation [18].

Magnesium is the second most important cation in serum. It protects central nervous system against the neurotoxic effects of bilirubin [15]. The current study showed the serum magnesium level is higher in hyperbilirubinemia group or case group than control group and the value is 23.67 mg/L in case group and 19.74 mg/L is

control group. It is also significant ( $p<0.001$ ) when compared with control group. This may be due to early destruction of red blood cell and haemolysis of fetal red blood cells in newborn hyperbilirubinemia extracellular movement of intracellular Mg because of cellular injury by high bilirubin that may cause neuronal and generalized cellular injury [19]. The serum levels of magnesium become high, depending on the existence of mild hemolysis in newborn in case group. Magnesium is a cofactor in multiple enzymatic reactions, including those involving energy metabolism as well as DNA and protein synthesis, and it participates in the regulation of ion channels [20].

Copper is an integral component of many metalloenzyme including ceruloplasmin, cytochrome oxidase, superoxide dismutase, dopamine- $\beta$ -hydroxylase, ascorbate oxidase, and tyrosinase. Copper synthesis in liver by ceruloplasmin a major serum copper-transporting protein but its metabolic role is unclear. After birth neonatal hepatic ceruloplasmin synthesis occurs and is associated with gradually increase of plasma concentrations [21]. The case group showed serum copper level is 0.74 mg/L in case and 0.41 mg/L in control group. The level of copper is also high in case group than control group and statistically significant ( $p<0.001$ ) when compared

with control group. Copper is a strong antioxidant and helps glucose oxidation, destroys free radicals and prevents cellular damage as a result in the body its release energy, iron absorb, oxidase to body tissue, balance thyroid hormone and synthesis of adrenaline [22]. In newborn with jaundice, the high serum copper may be of intracellular (erythrocyte) origin [23].

Past study showed that the elevated level of serum copper is documented in liver disease like cirrhosis, obstructive jaundice and cholestasis. In intentional or accidental ingestion and hemolysis, such as glucose-6-phosphate dehydrogenase deficiency hemolysis, results increase nonceruloplasmin copper in plasma due to copper overload. Copper also plays an essential role in mitochondrial electron transport and activate the two enzymae intracellular copper zinc superoxide dismutase and cytochrome oxidase that work as antioxidant [24].

The correlation between serum billirubin and serum magnesium also showed 68% which indicates positive correlation with increased billirubin and increased magnesium. Another result also showed that the increased serum level of copper has a positive correlation with increased billirubin and that is 80%.

## 5. CONCLUSION

Our study showed that the concentrations of magnesium (Mg) and copper (Cu) levels were found to be significantly greater than control groups. On the other hand serum magnesium and copper levels were increased in neonatal hyperbilirubinemia. There was significant positive correlation between serum magnesium level and neonatal hyperbilirubinemia. Significant positive correlation between serum copper level and hyperbilirubinemia was also found in neonates. Therefore, magnesium, and copper may have relationship with development of jaundice in neonates.

## CONSENT

Consent was taken from the neonate Patients mother and legal Guardian.

## ETHICAL APPROVAL

This study was approved by the institutional ethical committees of Institute of Biological Sciences (IBSc), University of Rajshahi,

Bangladesh (Approval Memo no-82/320/IAMEBBC/IBSc, 20 August, 2017).

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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