Original Article

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Correlation of Serum Zinc Level with Hyperbilirubinemia in Neonates- A Case-control Study

Biochemistry Section

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ABSTRACT

Introduction: Neonatal jaundice is the common cause of hospitalisation in the first month of life. Factors that affect the severity of neonatal jaundice includes maternal, pre-natal, and neonatal factors as well as environmental factors. Zinc (Zn) is one of the factors that affect the severity of neonatal jaundice as it prevents the lipid depolarization of the cell membranes. Hypozincemia may alter the erythrocyte membrane resulting in deficient synthesis of enzymes which play a key role in bilirubin metabolism.

Aim: To correlate the serum Zinc level with bilirubin level in neonates with hyperbilirubinemia and also to compare with healthy controls.

Materials and Methods: This case-control study was done in Clinical Biochemistry Laboratory of Father Muller Medical College and Hospital, Mangalore, Karnataka, India, from April 2018 to June 2018. Total 60 subjects were included in the study, which was divided into two groups: Neonates with clinically diagnosed hyperbilirubinemia (Group I) and normal healthy neonates as controls (Group II). Serum levels of zinc, total bilirubin, conjugated and unconjugated bilirubin were compared among groups. Correlation among the biochemical parameters was analysed by Karl Pearson's Correlation Analysis.

Results: In present study, 30 subjects were males and 30 females with mean age of 4.90±1.06 days among Group I and 2.50±1.61 days among Group II. This study observed significantly decreased mean levels of serum Zinc (171.24±19.82 µg/dL) in Group I patients when compared to mean levels of serum Zinc (189.23±17.90 µg/dL) in Group II controls. There was statistically significant positive correlation among serum levels of Zinc and total bilirubin in Group I patients ($p<0.05$) when compared to Group II.

Conclusion: There was statistically significant positive correlation was found among serum levels of Zinc and total bilirubin in cases when compared to healthy controls. Current results showed, zinc may have a protective effect in the incidence of neonatal jaundice.

Keywords: Jaundice, Lipid depolarization, Newborn, Trace element

INTRODUCTION

Neonatal jaundice is the most common cause of hospitalisation in the first month of life and most of the causes of severe jaundice is due to prolonged hyperbilirubinemia. Hyperbilirubinemia is defined as Total bilirubin level higher than 10 mg/dL over two and three weeks of life in term and preterm neonates, respectively [1]. In the first week of life around 60% of term and 80% of preterm babies develop jaundice, which is associated with a wide variety of biochemical disturbances [2].

Physiological jaundice of the newborn is a result of deficiency in the enzyme glucuronyl transferase, one of the last liver functions to be activated in prenatal life since bilirubin processing is handled by the mother of the fetus. In premature births, infants may be born without glucuronyl transferase, the enzyme responsible for bilirubin conjugation. This deficiency results, increase in unconjugated bilirubin, which can be life threatening [3].

Trace elements are necessary for normal body functions. Zinc (Zn) is an essential trace element with various biological effects and it's regulation in human body have been associated with disease like jaundice. It plays three well-known physiological roles-catalytic, structural, and regulatory, especially in an enormous number of enzymes and "Zn-finger" proteins. Decreased serum levels of zinc may result in deficient synthesis of some enzymes such as glucuronyl transferase that play a role in the bilirubin metabolism and may cause structural defects in the erythrocyte membranes by causing lipid depolarization of the cell membranes resulting in hemolysis [4].

Similar observations were found in previous studies that Mineral like Zn might affect the process of bilirubin binding proteins or excretion [5,6]. Some studies evaluated, role of zinc in decreasing the bilirubin levels by the inhibition of the normal enterohepatic circulation of unconjugated bilirubin [7,8]. But, there is paucity of Indian studies; hence present study was conducted to estimate and correlate the serum zinc levels in neonates with clinically diagnosed hyperbilirubinemia, in comparison to normal healthy neonates.

MATERIALS AND METHODS

This case-control study was conducted in Clinical Biochemistry Laboratory of Father Muller Medical College and Hospital Mangalore, Karnataka, India, over a period of three months, between April 2018 to June 2018. Study was performed after getting approval from the Institutional Ethics Committee (IEC No: FMMCIEC/CCM/175/2018). All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the Helsinki Declaration of 1975 that was revised in 2013 and Informed consent was obtained from all individual participants included in the study.

Inclusion criteria: Full term neonates (gestational age ≥38 weeks) with clinically diagnosed hyperbilirubinemia (Total bilirubin >12 mg/dL), admitted in NICU were included as cases. Age and sex-matched, full term newborns without idiopathic hyperbilirubinemia (Total Bilirubin: <8 mg/dL) were included as controls.

Exclusion criteria: Neonates with sepsis, any congenital abnormalities, Rh incompatibility, glucose-6-phospate dehydrogenase deficiency were also excluded from the study.

Sample size: Sample size calculated was 90 and calculated by using the sample size calculator for the power of >80% and alpha value <0.05) [5].

Total number of 60 subjects were included in the study, which was categorised into two groups; Group I (cases): Thirty (n=30) subjects with hyperbilirubinemia, Group II (controls): Thirty (n=30) healthy controls.

Blood samples (3 mL) were collected with aseptic precautions as per requirement. Following Biochemical parameters were analysed using standard spectrophotometric methods in Cobas 6000 autoanalyzer.

1. Zinc: photometric method using Nitro-PAPS reagent method in Cobas 6000 autoanalyzer [9]. Normal Reference range-Serum Zinc: 60-120 µg/dL [10].
2. Total bilirubin, Direct bilirubin: Photometric method by diazo method in Cobas 6000 autoanalyzer. Normal Reference range: Total bilirubin: <8 mg/dL, Conjugated Bilirubin: <0.5 mg/dL, Unconjugated Bilirubin: Total bilirubin-Conjugated bilirubin (By calculation) (Normal Reference range for Unconjugated Bilirubin: <7.5 mg/dL) [10].

STATISTICAL ANALYSIS

All statistical analysis were conducted by using Statistical Package for the Social Sciences (SPSS V.17.0). Continuous variables were analysed by measures such as sample mean, standard deviation, and statistical significance was tested by paired 't' test. Correlation among the biochemical parameters was analysed by Karl Pearson's Correlation Analysis.

RESULTS

In present study, out of total 60 subjects, 30 were males and 30 females with mean age of 4.90±1.06 days among Group I and 2.50±1.61 days among Group II. The serum levels of Total bilirubin, conjugated and unconjugated bilirubin were significantly higher in Group I (p value <0.01) when compared to Group II. Serum zinc level was significantly higher among Group II (189.23±17.90 µg/dL) when compared to Group I (171.24±19.82 µg/dL) (p value <0.01) [Table/Fig-1].

		Group I (Neonates with hyperbilirubinemia; n=30)	Group II (Healthy neonates; n=30)	p-value
Age (Days)		4.90±1.06	2.50±1.61	0.072
Gender distribution	Males	16	16	0.098#
	Females	14	14	
Gestation (Weeks)		38.07±0.81*	38.43±0.77	<0.001
Birth weight (g)		3126.27±340.10* (2999.27-3253.26)	2765.23±383.23 (2622.13-2908.34)	<0.001
Total bilirubin (mg/dL)		15.72±3.88* (14.27-17.17)	6.99±3.78 (5.57-8.40)	<0.001
Unconjugated bilirubin (mg/dL)		15.12±3.82* (13.69-16.54)	6.55±3.74 (5.16-7.95)	<0.001
Conjugated bilirubin (mg/dL)		0.60±0.32* (0.49-0.72)	0.43±.25 (0.34-0.52)	<0.001
Serum zinc (µg/dL)		171.24±19.82* (163.84-178.64)	189.23±17.90 (182.55-195.91)	<0.001

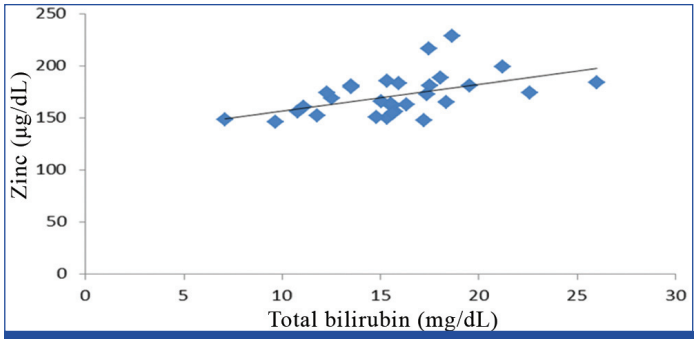
[Table/Fig-1]: Demographic details and Biochemical parameters (Total bilirubin, unconjugated bilirubin, Conjugated bilirubin and serum Zinc levels) in both the groups: Group I and Group II.
*Significance of difference p<0.001; NS=Non-significant (p>0.05) Paired 't' test; #chi-square

A statistically significant positive correlation of serum Zn level with Total bilirubin (r value 0.506, p-value 0.004), and Unconjugated bilirubin (r-value 0.501, p-value 0.004) was found in Group I. In Group II, a significant positive correlation of serum zinc level was found with age only (r value 0.525, p-value 0.003) [Table/Fig-2].

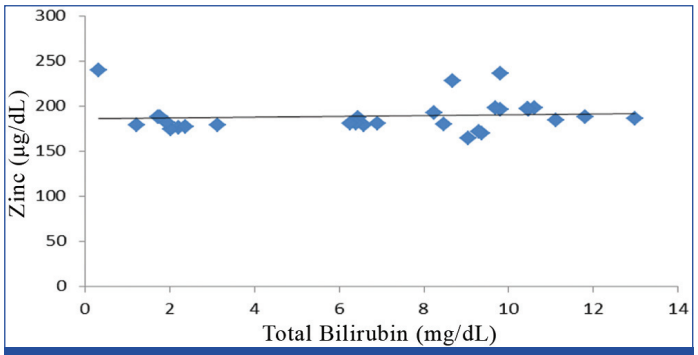
[Table/Fig-3] shows statistically significant positive correlation of serum Zn level with total bilirubin in the cases. [Table/Fig-4] shows that there was a positive correlation among serum levels of Zn and Total bilirubin in Group II patients but was not found statistically significant.

Correlations of Zinc (Zn)	Group I (neonates with hyperbilirubinemia)		Group II (neonates without hyperbilirubinemia)	
	Correlation coefficient (r)	p-value and significance	Correlation coefficient (r)	p-value and significance
Age	0.271	0.147; NS	0.525	0.003; S
Gestation	-0.130	0.494; NS	-0.232	0.218; NS
Birth weight	-0.30	0.874; NS	-0.22	0.910; NS
Total bilirubin	0.506	0.004; S	0.95	0.617; NS
Unconjugated bilirubin	0.501	0.004; S	0.113	0.551; NS
Conjugated bilirubin	0.141	0.458; NS	-0.255	0.174; NS

[Table/Fig-2]: Correlation of Age, Gestation, Birth weight, Total Bilirubin, Unconjugated Bilirubin, Conjugated Bilirubin with Zinc in Neonates with hyperbilirubinemia (Group I) and Neonates without hyperbilirubinemia (Group II).
Note: Statistically significant positive correlation among serum levels of Zinc and total bilirubin in Group I patients when compared to Group II by Karl Pearson's Correlation Analysis
S: Significant (p<0.05); NS: Non-significant



[Table/Fig-3]: Correlation between Total bilirubin and Zinc in Group I.



[Table/Fig-4]: Correlation between Total bilirubin and Zinc in Group II.

DISCUSSION

As Zn is an essential trace element crucial for the function of more than 300 enzymes and it is important for cellular processes like cell division and apoptosis. Hence, the concentration of zinc in the human body is tightly regulated and disturbances of zinc homeostasis have been associated with several diseases including diabetes mellitus and jaundice [10]. In the human body, most Zn is found in the muscle and bone and smaller concentrations are found in some organs including the liver, gastrointestinal tract, and kidney. Low levels of zinc may lead to decreased synthesis of glucuronyl transferase that play a vital role in bilirubin metabolism and may cause hemolysis [4]. Mineral like Zn might affect the process of bilirubin binding proteins or excretion [6].

There was a significant alteration in serum levels of Zn in neonates with hyperbilirubinemia when compared to controls. This study revealed that the mean serum zinc levels were significantly lower in neonates with severe hyperbilirubinemia than controls, which is consistent with the previous study by Boskabadi H et al., [1] showed significant decrease in mean serum levels of zinc in neonates with jaundice when compared to healthy neonates, as it leads to deficient synthesis of enzymes (glucuronyl transferase) which is needed for bilirubin conjugation [4].

The observed relation between zinc and bilirubin levels investigated previously as in-vitro study [11], showing that zinc salts can

precipitate Unconjugated bilirubin at physiological pH because the chemical structure of bilirubin has the potential to chelate with metal ions, such as Zn [11]. In-vivo study, showed that Zn salts can causes a decrease in serum Unconjugated bilirubin but an increase in the fecal bilirubin excretion by inhibiting the entero-hepatic circulation of Unconjugated bilirubin [12]. Recently some studies have proposed using Zn salts for lowering bilirubin levels in neonates with jaundice or preventing the incidence of neonatal jaundice. The results of these studies should be considered when evaluating the effect of Zn therapy in hyperbilirubinemic neonates [11-13].

A statistically significant positive correlation of serum Zn levels with Total bilirubin and Unconjugated bilirubin was found in Group I patients, which was in consistent with previous study [1], as zinc is an inhibitor of heme oxygenase enzyme in both in-vitro and in-vivo, which plays a key role in bilirubin metabolism and also zinc acts as a co-factor for important enzymes involved in the proper functioning of the antioxidant defense system [1]. According to the results of one study, serum zinc levels were significantly lower in Low birth weight neonates with hyperbilirubinemia than in normal controls [14], but in present study there was non-significant negative correlation between serum zinc levels and birth weight in both the groups.

Limitation(s)

The limitation of the study was small sample size. Also hyperbilirubinemia is more common in pre-term neonates, so similiar study with one more group (pre-term neonate) and large sample size can be conducted in future.

CONCLUSION(S)

The current study showed statistically significant positive correlation of serum Zn levels with Total bilirubin and Unconjugated bilirubin in neonates with hyperbilirubinemia when compared to healthy newborns. The decreased serum Zn level in neonates with hyperbilirubinemia suggests increased susceptibility to jaundice and hemolysis. The findings also suggest involvement of oxidative stress, antioxidant activity and trace elements (Zn) in the pathogenesis of hyperbilirubinemia. In conclusion, zinc have oxidant-antioxidant effect in the incidence of neonatal jaundice. However, more evaluations with larger sample size are needed for better decision making.

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