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# Effect of Intensive Phototherapy and Exchange Transfusion on Copper, Zinc and Magnesium Serum Levels in Neonates with Indirect Hyperbilirubinemia

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## *Abstract*

### *Background*

Many studies reported that copper, zinc and magnesium play important roles in the pathogenesis and development of neonatal hyperbilirubinemia. Exchange transfusion and intensive phototherapy are known two modalities of therapy for severe neonatal hyper bilirubinemia, but the effect of them on those trace elements is unknown.

### *Materials and Methods*

Copper, Zinc and Magnesium serum levels were measured before and after treatment with intensive phototherapy and exchange transfusion in full term neonates with indirect hyperbilirubinemia admitted to neonatal intensive care unit (NICU) of Minia and Sohag University hospitals, Egypt, during 2014-2016 and comparison with normal healthy neonates was done.

### *Results*

There were significant higher copper and magnesium and lower zinc serum levels in neonates with indirect hyperbilirubinemia than controls before and after intensive phototherapy. These levels were significantly changed after exchange transfusion to be comparable with controls. Significant positive correlations between the total bilirubin levels and hemoglobin, copper, and magnesium serum levels and significant negative correlations with serum zinc levels were present. There were no significant correlations between maternal and neonatal copper, zinc or magnesium serum levels.

### *Conclusion*

Neonates with indirect hyperbilirubinemia had significant higher copper and magnesium and lower zinc serum levels than healthy neonates which were not related to their maternal serum levels. Intensive phototherapy had no effect on their levels while exchange transfusion changed these levels to be comparable with that of normal healthy neonates.

***Key Words:*** Copper, Hyperbilirubinemia, Magnesium, Neonates, Phototherapy, Zinc.

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## 1- INTRODUCTION

Neonatal hyperbilirubinemia is a frequently encountered problem in 60%–80% of newborns worldwide (1, 2). In a proportion of infants, jaundice may become severe with a significant risk of neonatal morbidity and mortality (3, 4). Surviving infants may acquire long-term neurodevelopmental sequelae especially on central nervous system (5, 6).

Micronutrients and minerals levels like zinc, copper and magnesium might affect the process of bilirubin binding proteins or excretion (7, 8). Copper is an active component of several enzyme systems, including cytochrome oxidase and superoxide dismutase and is essential for the prevention of anemia and leucopenia (9, 10). In neonates with jaundice, the high serum copper may be of intracellular (erythrocyte) origin due to the slight hemolysis which occurs in neonates (11).
Zinc (Zn) is an essential trace element with various biological effects depending on its catalytic and structural role in an enormous number of enzymes and "Zn-finger" proteins. Decreased serum levels of zinc may result in deficient synthesis of some enzymes that play a role in the bilirubin metabolism, and may also cause structural defects in the erythrocyte membranes resulting in hemolysis (12, 13).

Magnesium is needed for more than 300 biochemical reactions in the body and it maintains normal muscle and nerve functions, keeps heart rhythm steady, supports a healthy immune system and keeps bones strong (14). The serum levels of magnesium become higher in serum depending on the existence of mild hemolysis in neonates, the fact that magnesium is high in erythrocytes, especially reticulocytes than in serum concentrations, so its serum levels increase after hemolysis (15). Exchange transfusion and intensive phototherapy are known two modalities of therapy for severe neonatal hyper bilirubinemia but the effect of them on the previous minerals serum levels is unknown (16-20). The aim of this study was to investigate the effect of exchange transfusion versus intensive phototherapy on copper, zinc and magnesium serum levels in full term neonates with severe indirect hyperbilirubinemia.

## 2- MATERIALS AND METHODS

### 2-1. Methods

This was a prospective case control study included 60 full term neonates with severe indirect hyperbilirubinemia (36 males and 24 females), during the first week of life admitted to neonatal intensive care unit (NICU) of Minia and Sohag University hospitals, Egypt during the period from January 2014 to April 2016. They were divided into 2 subgroups: **group I** included 30 (50%) neonates exposed to intensive phototherapy and **group II** included 30 (50%) neonates exposed to exchange transfusion according to World Health Organization (WHO) and American Academy of Pediatrics guidelines (21, 22) after written and verbal consents of caregivers as a routine in our NICU.

Neonates of group I received intensive phototherapy through (Bilisphere 360 phototherapy system) which consisted of a chamber containing 16 blue TL 20W/52 fluorescent tubes arranged cylindrically. The baby was laid on a gauze hammock suspended along the center of the chamber which was illuminated on all sides and serum copper, magnesium and zinc levels were measured before and 6-8 hours after intensive phototherapy. Eyes and genitalia were covered during the period of exposure to phototherapy (23).

Exchange transfusions were done for group II using double volume irradiated and washed blood of blood group (o negative blood), and all recommended precautions of WHO guidelines were applied during the process of transfusion

and serum levels of cupper, magnesium and zinc were measured before and 6-8 hours after exchange transfusion (24, 25). Thirty apparently healthy full term neonates (17 males and 13 females) with age and gender matched were selected from neonatal follow up clinic as controls (**group III**).

Inclusion criteria for patients: full term neonates (37-42 weeks) with good general condition, good Moro and sucking reflexes during the first 72 hours of life with total bilirubin levels matched with intensive phototherapy (group I), or exchange transfusion (group II) according to WHO and American Academy of Pediatrics (AAP) guidelines.

Exclusion criteria: preterm or post-term infants, full term infants beyond the first week of life, neonates with history of birth trauma, Hypoxic-ischemic encephalopathy (HIE), major congenital malformations, sepsis and those with cholestasis (direct bilirubin > 20%). Also, neonates who were fed with formula or milk fortifier, enteral supplement, and parenteral nutrition with trace elements, infants of diabetic, preeclamptic or hypertensive mothers were excluded from the study. Neonates who were exposed to intensive phototherapy and then to exchange transfusion due to failure of phototherapy in lowering their bilirubin levels were excluded from the study. All cases were subjected to perinatal history (including maternal illness, mode of delivery, gestational age, weight, gender, Apgar score, history of cyanosis or convulsions), and clinical examination (with special emphasis on vital signs, anthropometric measures, presence of Cephalhematoma and neurological examination).

Four ml of venous blood were obtained from each neonate under sterile venipuncture and divided as follow: one ml on Ethylenediaminetetraacetic acid (EDTA) containing tube for performing complete blood count and reticulocyte count (by automated cell counter sysmex KX 21N, Japan). Three ml were putted on a plain plastic tube and kept in the incubator for 20 minutes and centrifuged at 3000 revolutions per minute RPM for 10 minutes to obtain serum which was then processed for bilirubin (by automated chemical analyzer Konelab 20I, Finland), and C-Reactive Protein (CRP) test (by qualitative and semi quantitative determination latex agglutination, Spinreact-Spain), while the remaining serum stored at -20 $c^o$ in Eppendorf cups until processing serum copper, zinc and magnesium serum levels using atomic absorption spectrophotometer (AAS) (by direct colorimetric method using Qumica Clinica Applicada SA/Spain) (26-28).

### 2-2. Statistical analyses

Statistical analyses were performed using the SPSS statistics version 16.0. Differences in the mean of continuous variables were analyzed using parametric test (independent sample t-test). Bivariate correlation and scatter plot curves were used to assess the significance of correlation between the numerical variables. For all tests, the values $P<0.05$ were regarded statistically significant.

## 3- RESULTS

There were significant higher serum levels of copper and magnesium and lower serum zinc levels in patients (groups I and II) than controls (group III). Hemoglobin levels, total bilirubin and direct bilirubin levels were significantly higher in patients than controls while there were no significant differences between both groups as regards age, gestational age, weight, gender, maternal age, mode of delivery, white blood cells (WBCs), reticulocyte count, or maternal copper, zinc and magnesium serum levels (**Tables 1, 2**).

Copper, zinc and magnesium serum levels were not significantly changed in group Ia

(patients after intensive phototherapy) (168.5±16.1, 53.4±3.7 and 3.7±0.8 versus 113.6±15.2, 87.4±14.6 and 2.2±0.2, respectively, P = 0.001, 0.001 and 0.01, respectively); while copper, zinc and magnesium serum levels were significantly changed after exchange transfusion in group IIa (patients after exchange transfusion) and were comparable with normal healthy neonates of group III (123.1±17.4, 70.2±4.5 and 2.7±0.7 versus 113.6±15.2, 87.4±14.6 and 2.2±0.2, respectively, (P>0.05). Total and direct bilirubin levels were significantly decreased after intensive phototherapy, but still significantly higher than controls (**Tables 3, 4**).

There were significant higher serum levels of copper and magnesium and significant lower serum zinc levels in neonates of group Ia (after phototherapy) than neonates of group IIa (after exchange transfusion) (**Table.5**).

Positive significant correlations between total bilirubin and hemoglobin, copper and magnesium serum levels while significant negative correlations with serum zinc levels were present in patients (**Figures. 1-3**). There were no significant correlations between maternal and neonatal copper, zinc and magnesium serum levels (not shown in results) or total bilirubin levels (**Table.6**).

As regards the main causes of severe hyperbilirubinemia in our patients: there were 43 out of 60 cases (71.6%) were secondary to Hemolytic disease of the newborn (ABO) and/or Rh incompatibility, 7 cases (11.6%) with G6PD deficiency, 4 cases (6.6%) with cephalhaematoma, one case (1.6%) was pyruvate kinase deficiency, one case (1.6%) was Criggler-Najjar syndrome and four cases (6.6%) were idiopathic.

**Table-1**: Demographic and laboratory data of studied groups

| Items | Patients | | Controls | P-value |
|---|---|---|---|---|
| | Group I (No. = 30) Range (Mean± SD) | Group II, (No. = 30) Range (Mean± SD) | Group III, (No. = 30) Range (Mean± SD) | |
| Age, (days) | 1-7 (4.3±1.6) | 1-7 (4.5±1.9) | 1-7 (4.9±1.3) | 0.82 |
| Gestational age, (weeks) | 37-42(38.2±1.34) | 37-42 (39.9±1.54) | 37-42 (38.0±1.04) | 0.76 |
| Maternal age, (years) | 18-37 (22.5±4.15) | 18-39 (24.3±3.85) | 18-41(23.2±6.15) | 0.42 |
| Weight, (Kg.) | 2.8-4.0 (3.55±0.15) | 2.8-4.0 (3.00±0.15) | 2.5-4.200 (3.16±0.25) | 0.91 |
| Gender, (Male/ Female) | 17(56.7%)/13(43.3%) | 19(63.3%)/11(36.7%) | 17 (56.7%)/13(43.3%) | 0.35 |
| MOD, (NVD/ CS) | 17(56.7%)/13(43.3%) | 10(33.3%)/20(66.7%) | 19 (63.3%)/ 11(36.7%) | 0.23 |
| Hb, (gm/dl) | 11.8-19.3 (14.1±3.7) | 11.4-18.9(17.22±2.7) | 12.4-14.6 (13.7±1.3) | 0.01 |
| Reticulocyte count, ($x10^3$) | 0.1-5.9 (3.24±0.6) | 0.3-6.3 (4.16±0.8) | 0.2-4.2 (1.2±0.3) | 0.07 |
| WBCs, ($x10^3$) | 3.5-10.5 (9.2±2) | 3.8-11.8 (7.67±2) | 4.2-12.2 (7.8±1.8) | 0.83 |
| Platelets ($x10^3$) | 130-432(196.3±86.2) | 110-431(187.3±79.2) | 155-480 (335±67.9) | 0.12 |

| | | | | |
|---|---|---|---|---|
| Total bilirub, (mg/dl) | 16.8-29.2 (24.7±2.6) | 19.3-31.2 (30.7±3.7) | 0.2-2.4 (1.8±0.9) | 0.001 |
| Direct bilirub, (mg/dl) | 0.8-2.2 (1.11±0.4) | 0.9-2.6 (2.1±0.3) | 0.0-0.9 (0.4±0.1) | 0.006 |
| Serum Cu (ug/dl) | 100-185.7(161.9±11.6) | 98.5-175.6(164.6±12.3) | 88.6-122.4(113.6±15.2) | 0.001 |
| Serum Zn, (ug/dl) | 33.7-81.3 (50.1±4.6) | 35.5-86.3 (51.9±7.1) | 36.8-102.5 (87.4±14.6) | 0.001 |
| Serum Mg, (mg/dl) | 1.9- 3.8 (3.1±0.5) | 1.8-4.3 (3.4±0.3) | 1.8-2.9 (2.2±0.2) | 0.01 |
| Maternal Serum Cu, (ug/dl) | 175.4-281 (218 ± 61) | 179.8-288.5(236±54) | 182.4-290.4 (236 ± 36) | 0.71 |
| Maternal Serum Zn, (ug/dl) | 68.7-72.5 (70± 11) | 70.7-77.9 (75 ± 10) | 57.6-74.5 (68 ± 10) | 0.68 |
| Maternal Serum Mg, (mg/dl) | 1.4 - 2.1 (1.5 ±0.8) | 1.6 - 2.3 (1.6 ±0.7) | 1.2- 2.0 (1.3 ±0.7) | 0.81 |

SD: Standard deviation; WBCs: white blood cells; Hb: hemoglobin; MOD: mode of delivery; NVD: normal vaginal delivery; CS: caesarean section.

**Table-2**: Demographic and laboratory data for patients sub-groups

| Items | Patients | | P-value |
|---|---|---|---|
| | Group I, (No. = 30) Range (Mean± SD) | Group II, (No. = 30) Range (Mean± SD) | |
| Age, (days) | 1-7 (4.3±1.6) | 1-7 (4.5±1.9) | 0.71 |
| Gestational age, (weeks) | 37-42(38.2±1.34) | 37-42 (39.9±1.54) | 0.62 |
| Maternal age, (years) | 18-37 (22.5±4.15) | 18-39 (24.3±3.85) | 0.81 |
| Weight , (Kg.) | 2.8-4.0 (3.55±0.15) | 2.8-4.0 (3.00±0.15) | 0.88 |
| Hb, (gm/dl) | 11.8-19.3 (14.1±3.7) | 11.4-18.9 (17.22±2.7) | 0.07 |
| Reticulocyte count, ($x10^3$) | 0.1-5.9 (3.24±0.6) | 0.3-6.3 (4.16±0.8) | 0.16 |
| WBCs, ($x10^3$) | 3.5-10.5 (9.2±2) | 3.8-11.8 (7.67±2) | 0.42 |
| Platelets, ($x10^3$) | 130-432 (196.3±86.2) | 110-431 (187.3±79.2) | 0.89 |
| Total bilirubin, (mg/dl) | 16.8-29.2 (24.7±2.6) | 19.3-31.2 (30.7±3.7) | 0.05 |
| Direct bilirubin, (mg/dl) | 0.8-2.2 (1.11±0.4) | 0.9-2.6 (2.1±0.3) | 0.23 |
| Serum Cu, (ug/dl) | 100-185.7 (161.9±11.6) | 98.5-175.6 (164.6±12.3) | 0.76 |
| Serum Zn, (ug/dl) | 33.7-81.3 (50.1±4.6) | 35.5-86.3 (51.9±7.1) | 0.82 |
| Serum Mg, (mg/dl) | 1.9- 3.8 (3.1±0.5) | 1.8-4.3 (3.4±0.3) | 0.86 |

SD: Standard deviation; Hb: hemoglobin; WBCs: white blood cells.

**Table-3**: Comparison between patients (after intensive phototherapy) and controls as regarding laboratory data

| Items | Group Ia, (after photo) Range (Mean± SD) | Group III, (Controls) Range (Mean± SD) | P-value |
|---|---|---|---|
| Hb, (gm/dl) | 11.7-15.2 (14.8±1.7) | 12.4-14.6 (13.7±1.3) | 0.07 |
| Reticulocyte count, ($x10^3$) | 1.8-3.9 (3.1±0.6) | 0.2-4.2 (1.2±0.3) | 0.06 |
| WBCs, ($x10^3$) | 4.0-12.1 (8.1±1.8) | 4.2-12.2 (7.8±1.8) | 0.8 |
| Platelets, ($x10^3$) | 170-460 (292±77.6) | 155-480 (335±67.9) | 0.1 |
| Total bilirubin, (mg/dl) | 9.6-15.1 (14.5±1.7) | 0.2-2.4 (1.8±0.9) | 0.001 |
| Direct bilirubin, (mg/dl) | 0.3-2.8 (1.9±0.8) | 0.0-0.9 (0.4±0.1) | 0.001 |
| Serum Cu, (µg/dl) | 102-181.4 (168.5±16.1) | 88.6-122.4 (113.6±15.2) | 0.001 |
| Serum Zn, (µg/dl) | 30.4-96.4 (53.4±3.7) | 36.8-102.5 (87.4±14.6) | 0.001 |
| Serum Mg, (mg/dl) | 2.1-3.9 (3.7±0.8) | 1.8-2.9 (2.2±0.20 | 0.01 |

SD: Standard deviation; Hb: hemoglobin; WBCs: white blood cells.

**Table-4**: Comparison between patients (after exchange) and controls as regarding laboratory data

| Items | Group IIa, (after exchange) Range (Mean± SD) | Group III, (Controls) Range (Mean± SD) | P-value |
|---|---|---|---|
| Hb (gm/dl) | 11.4-14.8 (13.1±2.1) | 12.4-14.6 (13.7±1.3) | 0.11 |
| Reticulocyte count, ($x10^3$) | 1.2-3.6 (1.9±0.4) | 0.2-4.2 (1.2±0.3) | 0.07 |
| WBCs ($x10^3$) | 4.3-11.4 (8.5±2.4) | 4.2-12.2 (7.8±1.8) | 0.81 |
| Platelets ($x10^3$) | 130-380 (210±80.7) | 155-480 (335±67.9) | 0.68 |
| Total bilirubin(mg/dl) | 10.3-14.2 (13.7±3.8) | 0.2-2.4 (1.8±0.9) | 0.001 |
| Direct bilirubin(mg/dl) | 0.4-2.6(1.23±0.16) | 0.0-0.9 (0.4±0.1) | 0.04 |
| Serum Cu (µg/dl) | 98.2-133.4 (123.1±17.4) | 88.6-122.4 (113.6±15.2) | 0.17 |
| Serum Zn (µg/dl) | 31.5-92.6 (70.2±4.5) | 36.8-102.5 (87.4±14.6) | 0.08 |
| Serum Mg (mg/dl) | 1.3-2.8 (2.7±0.7) | 1.8-2.9 (2.2±0.2) | 0.42 |

SD: Standard deviation; Hb: hemoglobin; WBCs: white blood cells.

**Table-5**: Comparison between patients after intensive phototherapy (group Ia) and patients after exchange transfusion (group IIa) as regarding laboratory data

| Items | Group Ia, (after photo) Range (Mean± SD) | Group IIa, (after exchange) Range (Mean± SD) | P-value |
|---|---|---|---|
| Hb, (gm/dl) | 11.7-15.2 (14.8±1.7) | 11.4-14.8 (13.1±2.1) | 0.13 |
| Reticulocyte count, ($x10^3$) | 1.8-3.9 (3.1±0.6) | 1.2-3.6 (1.9±0.4) | 0.13 |
| WBCs, ($x10^3$) | 4.0-12.1(8.1±1.8) | 4.3-11.4 (8.5±2.4) | 0.69 |
| Platelets, ($x10^3$) | 170-460 (292±77.6) | 130-380 (210±80.7) | 0.78 |
| Total bilirubin, (mg/dl) | 9.6-15.1 (14.5±1.7) | 10.3-14.2 (13.7±3.8) | 0.12 |
| Direct bilirubin(mg/dl) | 0.3-2.8 (1.9±0.8) | 0.4-2.6 (1.23±0.16) | 0.23 |
| Serum Cu, (µg/dl) | 102-181.4 (168.5±16.1) | 98.2-133.4 (123.1±17.4) | 0.001 |
| Serum Zn, (µg/dl) | 30.4-96.4 (53.4±3.7) | 31.5-92.6 (70.2±4.5) | 0.001 |
| Serum Mg, (mg/dl) | 2.1-3.9 (3.7±0.8) | 1.3-2.8 (2.7±0.7) | 0.01 |

SD: Standard deviation; Hb: hemoglobin; WBCs: white blood cells.

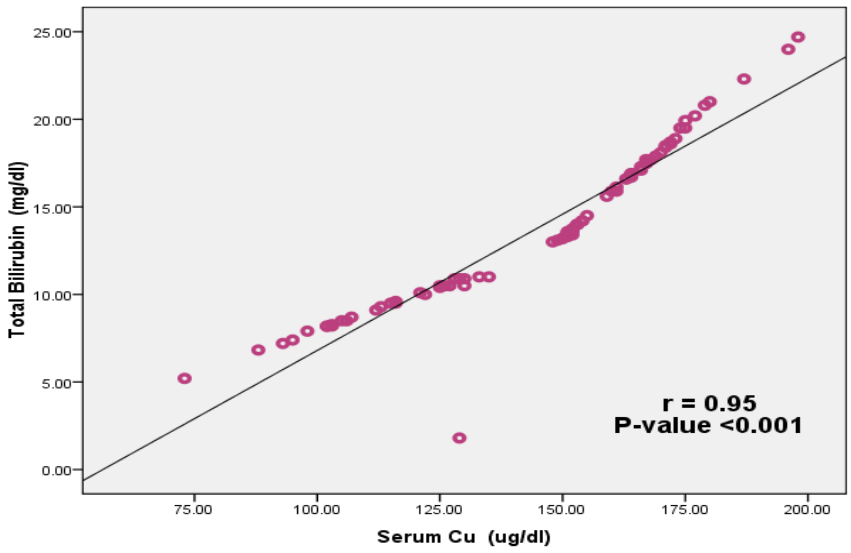


**Fig. 1**: Scatter plot showing the correlation between total bilirubin and serum copper in patients

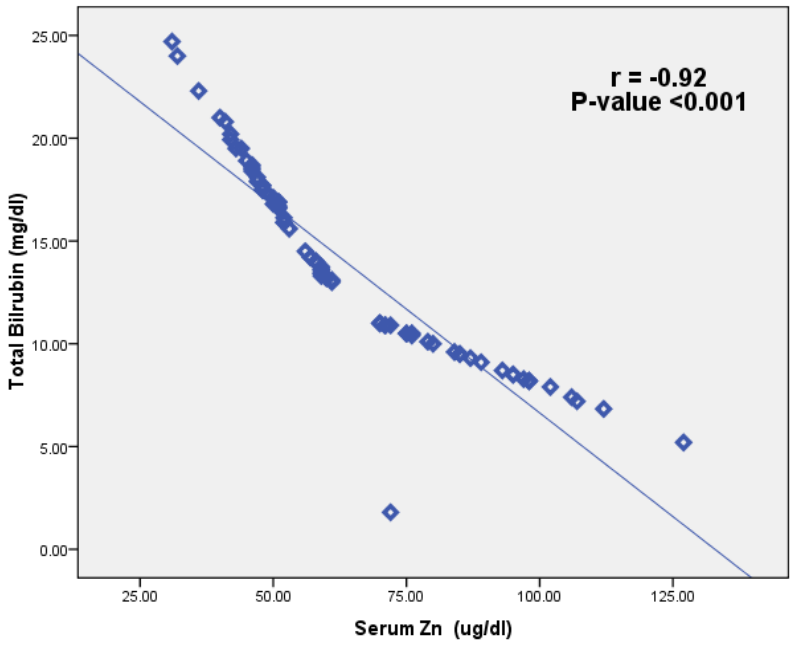


**Fig. 2**: Scatter plot showing the correlation between total bilirubin and serum zinc in patients

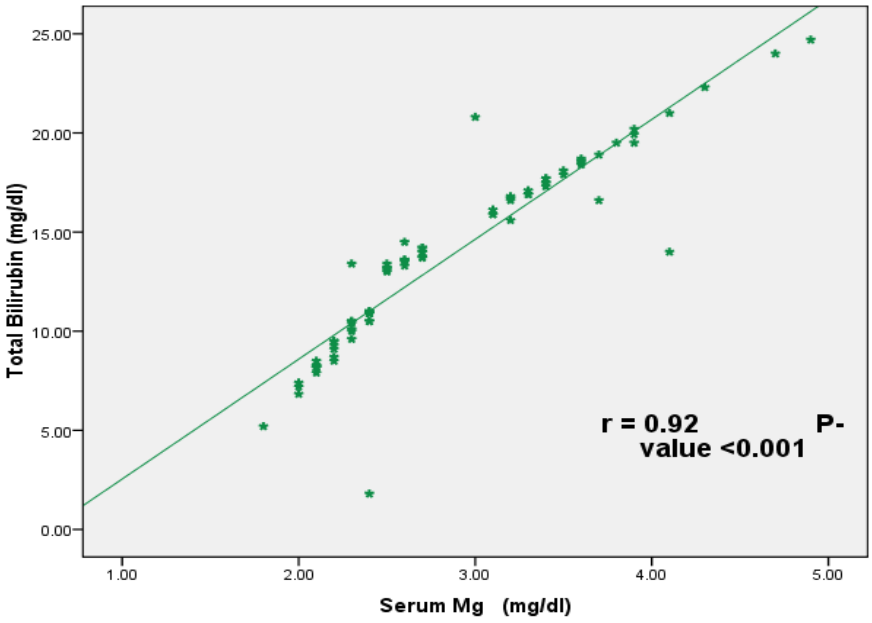


**Fig. 3**: Scatter plot showing the correlation between total bilirubin and serum magnesium in patients

**Table-6**: Correlation between total bilirubin and other laboratory data in patients

| Items | Total bilirubin | |
|---|---|---|
| | r | P-value |
| Hb, (gm/dl) | 0.39 | 0.01 |
| Reticulocyte count, ($x10^3$) | 0.15 | 0.06 |
| WBCs, ($x10^3$) | 0.16 | 0.1 |
| Platelets, ($x10^3$) | -0.08 | 0.4 |
| Serum Cu, (µg/dl) | 0.95 | 0.001 |
| Serum Zn, (µg/dl ) | -0.92 | 0.001 |
| Serum Magnesium, (mg/dl) | 0.92 | 0.001 |
| Maternal Serum Cu, (µg/dl) | 0.17 | 0.51 |
| Maternal Serum Zn, (µg/dl ) | -0.19 | 0.33 |
| Maternal Serum Mg, (mg/dl) | 0.23 | 0.41 |

SD: Standard deviation; Hb: hemoglobin; WBCs: white blood cells.

## 4- DISCUSSION

This study revealed that the mean serum copper and magnesium levels in neonates with hyperbilirubinemia were significantly higher, while the mean serum zinc levels were lower in neonates with hyperbilirubinemia than normal healthy neonates which were not related to their maternal serum levels. These levels were changed after exchange transfusion but not after intensive phototherapy. In agreement with this study, Schulpis et al., 2004 (10) reported that the serum copper increasedtwofold in neonates with moderate hemolytic jaundice and almost threefold in neonates with severe hemolytic jaundice. Also, Hassan, 2011 (11) reported that in neonates with jaundice, the higher serum copper may be due to intracellular (erythrocyte) origin due to the slight hemolysis. Copper overload may result from hemolysis, such as in cases with glucose-6-phosphate dehydrogenase deficiency, ABO and Rh incompatibility and other causes of hemolytic anemias in which cases Non-Ceruloplasmin copper is greatly increased in plasma.

Afify et al., 2012 (29) reported that the plasma ionized magnesium levels were significantly higher in jaundiced neonates compared to controls and this may be due to mild hemolysis not detectable by the ordinary investigations. Also, the increased magnesium levels may be due to the extracellular movement of intracellular magnesium secondary to cellular injury caused by high bilirubin levels that may cause neuronal and generalized cellular injury. They reported that magnesium levels were significantly higher in jaundiced infants than controls and he postulated that the serum levels of magnesium become higher in serum depending on the existence of mild hemolysis in neonates, the fact that magnesium is high in erythrocytes, especially reticulocytes than in serum concentrations, so its serum levels increase after hemolysis.

Different from our results are Tuncer et al., 1972 (30), who reported lower serum total magnesium concentrations in both umbilical cord and maternal blood of neonates with hyperbilirubinemia, when compared with normal neonates and they

postulated that hypomagnesaemia may result from intracellular shift of magnesium ions, this difference may be due to different methodology and the source (maternal and cord blood) of blood samples. In contrary to our results, Pintov et al., 1992 (31) in their study about the predictive value of zinc, magnesium and copper levels of cord blood on the development of benign hyperbilirubinemia, they concluded that the cord serum concentrations of copper are not useful in predicting which neonates will develop hyperbilirubinemia. This difference may be explained in the light of that copper and magnesium levels were increased after hemolysis (and not before), so the cord blood is not a good indicator or predictive of expected hemolysis which will occur in the future.

Zinc prevents the lipid depolarization of the cell membranes and hypozincemia may modulate the erythrocyte membrane resulting in deficient synthesis of assorted enzymes that play a role in the bilirubin metabolism. Also hypozincemia may cause structural defects in the erythrocyte membranes resulting in hemolysis (12, 13). It may result in deficient synthesis of assorted enzymes that play a role in the bilirubin metabolism, especially the Y and Z proteins and as a result could lead to indirect hyperbilirubinemia (9, 13). This study revealed that the mean serum zinc levels were significantly lower in neonates with severe hyperbilirubinemia than controls and similar results reported by other studies (7, 11).

Early studies by Tuncer et al., 1982 (9) hypothesized that low levels of zinc are encountered in neonates with hyperbilirubinemia and their mothers and this may be due to insufficient and unbalanced nutrition in their country which might be among the causes of idiopathic hyperbilirubinemia in the neonates. This is not the case in our study where maternal serum zinc levels were normal, and not correlated with the serum bilirubin levels and there were no statistical differences in maternal serum zinc levels between mothers of neonates with hyperbilirubinemia and control groups. In this study, the serum levels of copper, zinc and magnesium were changed after exchange transfusions, but not after intensive phototherapy. This may be explained by the following mechanisms: first; through the dilution effect of transfused normal plasma and/or blood on the increased levels of copper and magnesium and second; through the effect of citrate or EDTA present in the transfused blood used in the process of exchange transfusion causing hypocalcaemia, hyperkalemia and pH changes (32, 33), and lastly exchange transfusion can affect the total capacity of oxidative stress for neonates through the changes of the trace elements, micronutrients like selenium, zinc and magnesium serum levels (34).

Newborns are subject to increased oxidative stress and in cases where there is a depressed antioxidant system, which is significantly shown in cord blood and on the $4^{th}$ day of life in babies with high bilirubinemia compared to less jaundiced babies (35). Neurotoxicity of severe indirect hyperbilirubinemia is reported in many studies and this effect may be due to the effect of bilirubin itself or secondary to changes of micronutrients and trace elements serum levels like copper, zinc and magnesium (36-39); as Magdy and co-workers, 2011 (40) reported that in a group of Egyptian children with Attention deficit hyperactivity disorder (ADHD) the serum copper, zinc and magnesium levels were changed according to the type of ADHD reflecting the neuronal toxicity resulting from changes in these micronutrients. In contrary to our results, Mosayebi et al, 2016 (41), reported that phototherapy increases the serum zinc levels in neonates with hyperbilirubinemia.

The difference between our results and Mosayebi may be due to small sample size as well as the mean of bilirubin levels and methodology used in treatment of those neonates. As regards the main causes of severe hyperbilirubinemia in our patients; the ABO and/or Rh incompatibility were the main cause of severe jaundice during the first week of life followed by Glucose-6-phosphate dehydrogenase deficiency (G6PD deficiency), then by cephalohematoma, and these results were in agreement with others (2-4).

Few side effects for phototherapy use in management of neonates with hyperbilirubinemia like chromosomal effects, DNA damage and oxidant effects were reported (42-45). We recommend more studies for evaluation of the effect of copper, zinc and magnesium serum levels changes on the long run in neonates with severe hyperbilirubinemia exposed to intensive phototherapy and exchange transfusion.

### 4-1. Limitations of the study

The small sample size and the long term effect of these trace elements changes on different neurological functions were two limitations of this work.

## 5- CONCLUSION

In this study, neonates with indirect hyperbilirubinemia had significant higher copper and magnesium and lower zinc serum levels than healthy neonates which were not related to their maternal serum levels. Intensive phototherapy had no effect on their levels while exchange transfusion changed these levels to be comparable with that of normal healthy neonates; so more studies are needed for evaluation of the effect of those minerals on neonates exposed to intensive phototherapy and exchange transfusion on the long run.

## 6- CONFLICT OF INTEREST

There are no any financial or non-financial competing interests to declare in relation to this manuscript

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