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The Role of Zinc in Neonatal Indirect Hyperbilirubinemia

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ABSTRACT

Background *: Neonatal jaundice is the most common condition of newborn that require medical evaluation and treatment and it is one of the main causes of parental concern and worries. It is defined as a yellowish discoloration of skin and mucous membrane during the neonatal period.*

Aim *:This study aimed to evaluate the efficacy of Zinc sulfate on decreasing the morbidity in neonates with indirect hyperbilirubinemia.*

Pateint and method *: A case control study was performed at Salahaldeen general hospital. Seventy five patients with neonatal indirect hyperbilirubinemia were selected as a (case group) who administered zinc sulfate in a dose of 10 mg/day for two days in addition to other seventy five patients as a (control group) without zinc therapy. Estimation of indirect bilirubin level at admission, 12, 24, and 48 hrs. following admission and compared with each other.*

Results *: The results show that the mean of indirect bilirubin level, 24 and 48 hrs. after therapy, was highly significant lower in the study group when compared with the control group. The higher proportion of neonates who received zinc therapy were hospitalized for 48 hours or less, with highly significant association between receiving oral zinc therapy and the duration of hospitalization. More than three quarters of neonates who underwent blood exchange procedure, did not receive zinc therapy with a statistically significant association between blood exchange and the administration of zinc therapy.*

Conclusion*: Administration of 10 mg/day of zinc sulfate for two days was significantly reduced indirect serum bilirubin level and decreased the total needed duration for hospitalization as well as the need for blood exchange in patients having severe neonatal indirect hyperbilirubinemia.*

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INTRODUCTION

Neonatal jaundice is the most common condition of newborn that require medical evaluation and treatment. Thus, it is one of the most common causes of parental concern and worries. It is defined as a yellowish discoloration of skin and mucous membrane during the neonatal period[1].

It is caused by the inability of the immature liver of newborn to metabolize (conjugate) and then excrete bilirubin, which accumulates due to the breakdown of red blood cells which have a shorter life span than for adults. This increase in the breakdown of red blood cells and decreased newborn ability to metabolize bilirubin overwhelms the newborns capability for proper processing and excretion of bilirubin. As the newborn's liver become more matures, however, the jaundice ultimately disappears[2].

When bilirubin reaches higher levels it may cause permanent brain damage due to the toxic effect of bilirubin on brain cells.

So the maingoal of detecting and treating severe neonatal indirecthyperbilirubinemia is to prevent bilirubin encephalopathy and its chronic lifelong complications.These lifelong and chronic complications motivate the researchers for finding new and effective treatments for this condition[3].

Jaundice attributable to physiological immaturity which is usually appears between the 24-72 hours of age and between the 4th and 5th days can be considered as its peak in term neonates and in preterm at the 7th day, it disappears by 10-14 days of life.

There are non pharmacological and pharmacological modalities in treating hyperbilirubinemia. Phototherapy is widely used as a non pharmacological therapy as a treatment and prophylaxis of neonatal unconjugated hyperbilirubinemia[4]. Recent studies start working on new modalities in treating neonatal jaundice including the use of Intravenous immunoglubine, phenofibrate and more recently zinc sulfate became one of the promising medications in treating neonatal indirect hyperbilirubinemia[5].

Zinc sulfate is an inorganic compound and dietary supplement, it is important for both the growth and development of healthy body tissues[5].

Aim of Study

To evaluate the efficacy of Zinc sulfate on decreasing the morbidity in neonates with indirect hyperbilirubinemia.

Patients and Methods

This study is a case control, hospital based study, which was performed during the period extending from the 18th of February till the 15th of July 2019 in neonatal care unit at Salahaldeen General Hospital.

Seventy five patients with neonatal indirect hyperbilirubinemia were selected as a (case group) in addition to other seventy five patients as a (control group) were enrolled in this study(the total number of patients were

150).A written detailed and informed consent were taken from the parents of all the cases selected for this study. The parents of each patient were informed about the nature of the study and the ability to withdraw their patient from this study at any time they decide.

Each patient included in this study was assessed by a prepared questionnaire which includes name, age, sex, gestational age, birth weight, mode of delivery, type of feeding, serum bilirubin level, any need for exchange transfusion and the total period of hospitalization.

In addition to total, direct and indirect serum bilirubin level, all the patients were sent for other investigations including complete blood count, C reactive protein, stool and urine analysis to exclude any case who will not meet the criteria for the cases of this study.

The patients were divided into two groups, the (case group) who receive zinc sulfate as an oral suspension, and the control group who do not receive zinc sulfate. A detailed history was taken from each patient with full medical examination.

Estimation of serum bilirubin level for both groups at admission, 12 hrs. , 24 hrs. , and 48 hrs. following admission were measured until total serum bilirubin reaches a safe level. The procedure of serum bilirubin measurement was performed by Sulfanilic acid method (Malloy-Evelyn principle modified by Walters and al.) using specific kit (Manufactured by BIOLABO company of French origin with a reference number 80403). It was performed by collecting 100ul of un hemolysed serum, which will be added to a mixture of 1 ml of (Reagent 1 for total serum bilirubin or Reagent 2 for direct serum bilirubin) and 50 ul of Reagent 3. After waiting 5 minutes at room temperature, the absorption was read at 550 nm by spectrophotometer (Manufactured by APEL company of Japanese origin model APEL PD-303). Then, gained reading will be multiplied by 11.4 giving the level of total and direct serum bilirubin accordingly in mg/dl. Obtaining indirect bilirubin level was performed by manual method[6].

The applied phototherapy manufactured by FANEM company of Brazilian origin composed of 8 fluorescent blue tube lights from Philips company(20 w, 2 ft.),wavelength is 450 nm, each blue light tube is fully functioning (used for less than 3 months or less than 1200 hours and the ends were not blackened), the distance between the patient and the applied light was 30 cm.

Any neonate, who admitted to the hospital, was received phototherapy during the period of study. In the (case group) of the study, besides phototherapy, zinc sulfate was prescribed in a dose of 10 mg by the treating physician at a single oral daily dose for two consecutive days in the form of suspension. The administration of zinc sulfate was repeated if the neonate develops vomiting

within 10 minutes from the time of administration. Zinc sulfate which used for this study, given using a specific dropper for accurate dose administration.

Results

The total number of neonates who recruited in this study was 150. All of them were complaining from indirect hyperbilirubinemia. They were divided into two groups; experimental group (Zinc group) included 75 neonates who were administered oral zinc, and Control group included another 75 neonates without zinc supplementation.

The distribution of study groups by age and gender is shown in table (4.1).In this study, the age of neonates was ranging from 2 to 12 days with a mean of 4.86 days and standard deviation (SD) of ± 2.13 days.

Table 4.1: Distribution of study groups by Age and Gender.

Variable	Study Groups		Total (%) n= 150
	Control Group (%) n= 75	Zinc Group (%) n= 75	
Age (Days)			
< 7	60 (80.0)	58 (77.3)	118 (78.7)
≥ 7	15 (20.0)	17 (22.7)	32 (21.3)
Gender			
Male	33 (44.0)	39 (52.0)	72 (48.0)
Female	42 (56.0)	36 (48.0)	78 (52.0)

The comparison between the study groups by means of the patient's age and serum indirect bilirubin before intervention is shown in table (4.2). There were no statistically significant differences between the study groups in terms of age (P= 0.542), and indirect bilirubin before intervention (P= 0.437).

Table 4.2: Comparison between the study groups according to neonatal age and indirect bilirubin level

Variable	Zinc Group Mean±SD	Control Group Mean ± SD	P - Value
Neonatal Age (Days)	4.76 ± 2.16	4.97 ± 2.11	**0.542**
IBL	17.57 ± 2.61	17.20 ± 3.16	**0.437**

The comparison between the study groups by means of the patient's sex, is shown in table (4.3). There were no statistically significant differences between the study groups in terms of gender (P= 0.327).

Table 4.3: Comparison between the study groups according to gender

Variable	Zinc Group No.(%)	Control Group No.(%)	P - Value
Male	33 (44.0)	39 (52.0)	**0.327**
Female	42 (56.0)	36 (48.0)	

Regarding the type of feeding, more than one third of neonates in both groups were on breastfeeding (37.4% of control group, and 42.7% of zinc group), while 29.3% of study patients were on bottle feeding. As shown in figure 4.1.

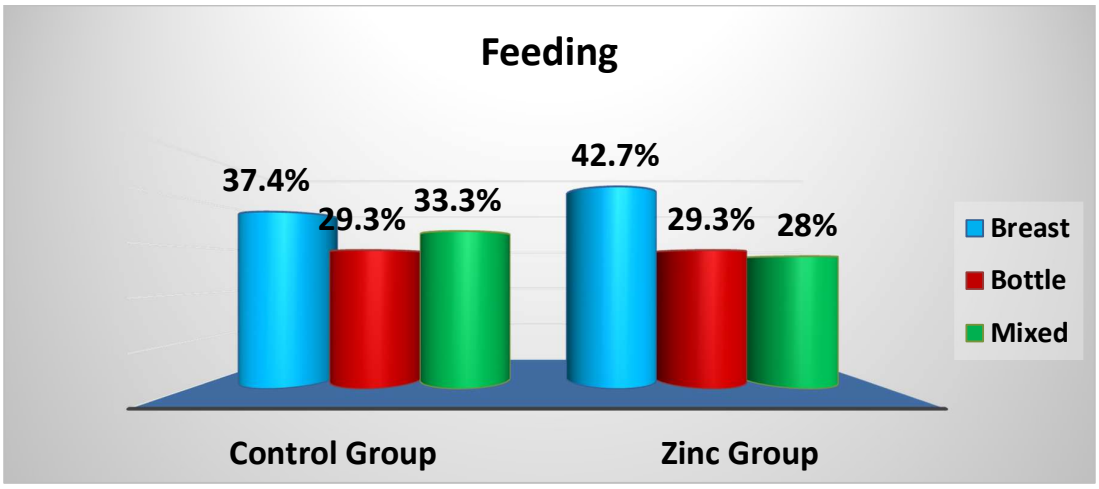

Figure 4.1: *Distribution of study groups by type of feeding*

Figure (4.2) shows the distribution of neonates who were recruited in this study according to mode of delivery. Normal vaginal delivery was the mode of delivery for the highest proportion of neonates in the two study groups (58.7% of control group and 64% of zinc group).

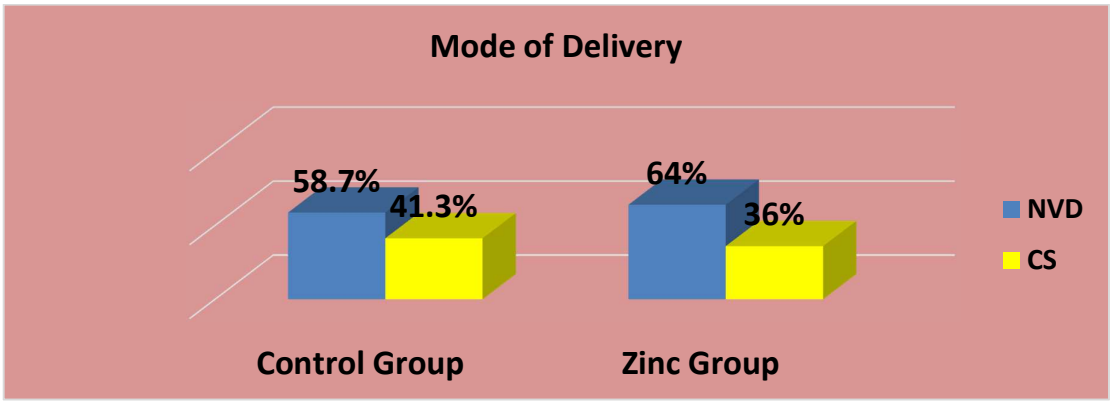

Figure 4.2: *Distribution of study groups according to mode of delivery*

As shown in figure (4.3), the highest proportion of neonates in control and zinc groups was of normal birth weight (64% of neonates in control group, and 74.7% of zinc group).

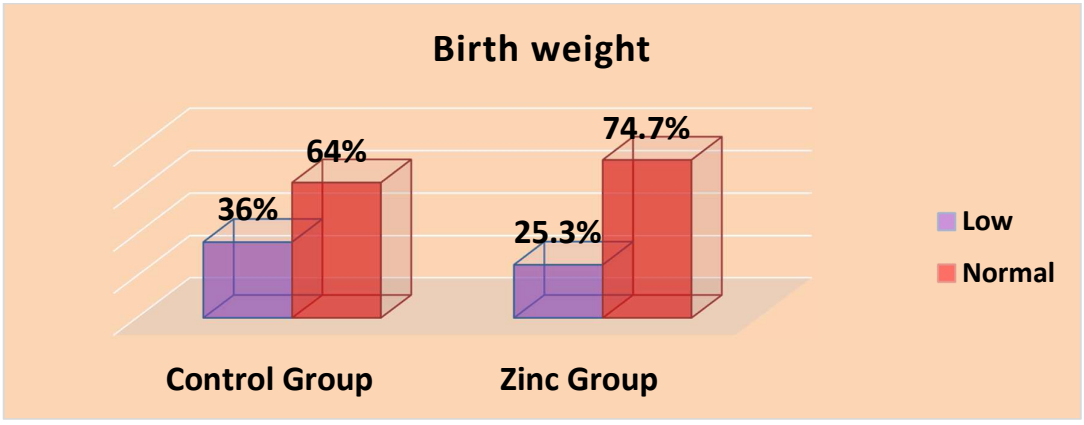

Figure 4.3:*Distribution of study groups by birth weight*

The comparison between the study groups by means of birth weight are shown in table (4.4). There were no statistically significant differences between the study groups in terms of birth weight (P= 0.157).

Table 4.4: Comparison between the study groups according to Birth weight

Variable	Zinc Group No.(%)	Control Group No.(%)	P - Value
Low Birth Weight	27 (36.0)	19 (25.3)	**0.157**
Normal Birth Weight	48 (64.0)	56 (74.7)	

Concerning gestational age, more than three quarters of patients in the two groups were delivered with full term pregnancies(77.3% of control group and 81.3% of zinc group).

The comparison between the study groups by means of the patient's Gestational age is shown in table (4.5). There were no statistically significant differences between the study groups in terms of gestational age (P= 0.545).

Table 4.5: Comparison between the study groups according to Gestational Age(Weeks)

Variable	Zinc Group No.(%)	Control Group No.(%)	P - Value
Preterm	17 (22.7)	14 (18.7)	**0.545**
Full-term	58 (77.3)	61 (81.3)	

The distribution of study groups by duration of staying in hospital is shown in figure (4.4). The majority of neonates (94.7%) who received zinc therapy, and less than two thirds of those without zinc therapy (62.7%) were hospitalized for ≤ 48 hrs.

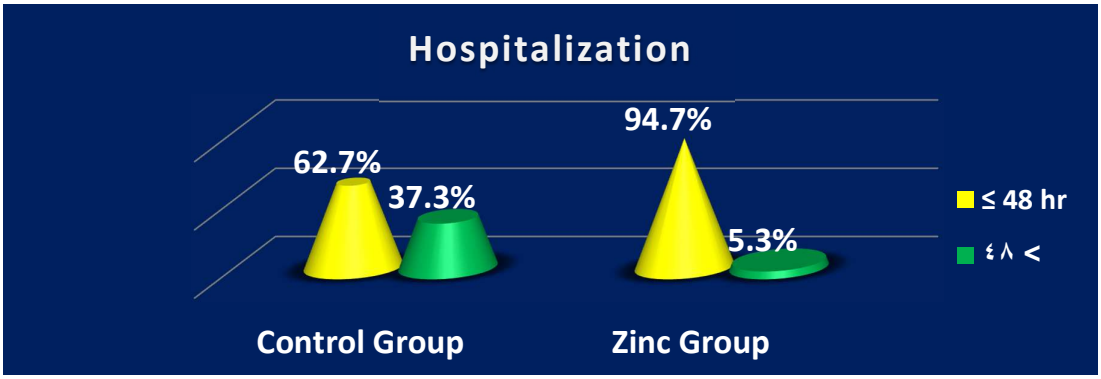

Figure 4.4: *Distribution of study patients according to the duration of staying in hospital*

It is found that, the higher proportion of neonates who received zinc therapy (60.2%) were hospitalized for 48 hours or less, with highly significant association (P=0.001) between receiving oral zinc therapy and the duration of hospitalization. This association is shown in table (4.6).

Table 4.6: Association of study groups with the duration of hospitalization

Variable	Study Groups		Total (%) n= 150	P- Value
	Zinc Group (%) n= 75	Control Group (%) n= 75		
≤ 48	71 (60.2)	47 (39.8)	118 (78.7)	**0.001**
> 48	4 (12.5)	28 (87.5)	32 (21.3)	

Out of the 75 neonates in zinc group, blood exchange was done just for3 patients (4%). While this procedure was done for 11 patients out of 75 (14.7%)in the control group as shown in figure (4.5).

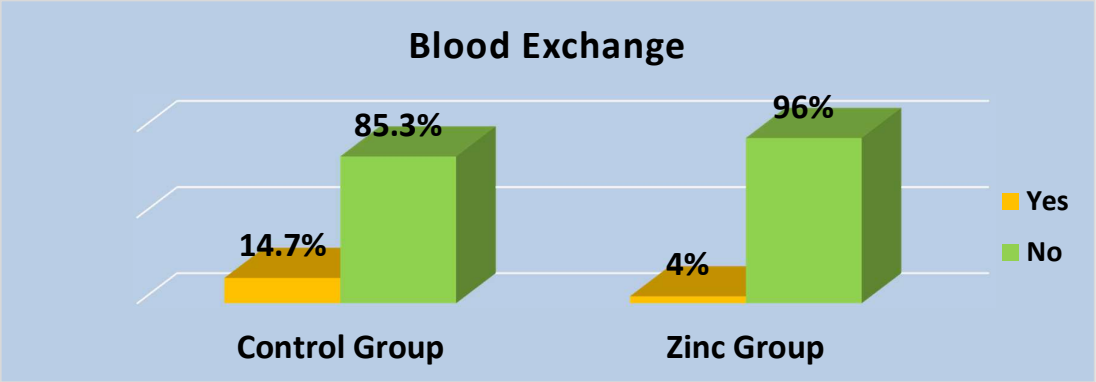

Figure 4.5: *Frequency of blood exchange among study patients*

In this study, more than three quarters (78.5%) of neonates who underwent blood exchange procedure, did not receive zinc therapy with a statistically significant association (P=0.025) between blood exchange and the administration of zinc therapy. This association is shown in table (4.7).

Table 4.7: Association of study groups according to blood exchange

Variable	Study Groups		Total (%) n= 150	P- Value
	Zinc Group (%) n= 75	Control Group (%) n= 75		
Yes	3 (21.4)	11 (78.6)	14 (9.3)	**0.025**
No	72 (52.9)	64 (47.1)	136 (90.7)	

The comparison between study groups by the mean of indirect bilirubin level is shown in table (4.8). After 24 and 48 hrs. of therapy, mean of indirect bilirubin level was highly significant lower in the neonates who were administered oral zinc compared to that in those without zinc therapy (11.20 mg/dl versus 13.65 mg/dl, P= 0.001; and 8.18 mg/dl versus 11.60 mg/dl, P=0.001, respectively). Also, the mean of indirect bilirubin was lower in zinc group than that in control group, after 12hrs.but this difference in mean was not statistically significant(P= 0.150).

Table 4.8: Comparison between the study groups by means of IBL after 12, 24, and 48 hrs. of therapy

Variable	Zinc Group Mean±SD	Control Group Mean ± SD	P - Value
IBL After 12 hrs.	14.71 ± 2.72	15.40 ± 3.07	**0.150**
IBL After 24 hrs.	11.20 ± 2.27	13.65 ± 3.18	**0.001**
IBL After 48 hrs.	8.18 ± 1.60	11.60 ± 2.34	**0.001**

Discussion

Medication treatment has dependably been a strategy in most of medical conditions including neonatal indirect hyperbilirubinemia. A lot of therapeutic approaches had been proposed to treat high neonatal indirect bilirubin level away from the standard methods of treatment including phototherapy and exchange transfusion. Phototherapy has certain complication when applied on patient with neonatal jaundice[7], while blood exchange has a high risk of complications and mortality[8].

This study demonstrated that the supplementation of zinc in a single daily dose of 10 mg/day for two days can significantly decrease TSB level. In addition, a significant difference in the mean of TSB levels at 24 and 48 hrs. of treatment for both groups, proving that zinc can play a certain role in decreasing neonatal indirect hyperbilirubinemia. In other words, the action of zinc in hyperbilirubinemia depends on its ability to reach the distal intestine where it is once again absorbed into the blood, and therefore, reduces the enterohepatic circulation[9].

These results agree with Mendez-Sanchez et al 2002 which was the first study applied on 15 patients having Gilbert syndrome compared to 5 normal volunteers. This study showed that short and long termadministrationof 40 mg of ZnSo4 orally decreased serum indirect bilirubin levels significantly in patients with Gilbert´s syndrome. Most likely due tothe inhibitionof the normal enterohepatic cycling of indirect bilirubin[10].

Also this study agrees with Hashemian S et al 2017, which was a double blind, placebo

controlled trial, applied on 70 neonates with high unconjugated serum bilirubin level, revealed thatadministering of a single dose of 10 mg/day of oral zinc sulfate reduced the mean TSB level and total needed duration of phototherapy. Zinc, as a micronutrient, can be administered as an effective and safe medication in neonatal indirect jaundice besides phototherapy[11].Another study carried out by Agrawal K et al 2018, which was a randomized double-blind placebo-controlled trial applied on 100 neonates born at more than35 weeks of gestation, showing similar results[12].

Another study carried out by MohammadZadeh A. et al 2016which was carried out on very low birth weight neonates showing significant reduction of indirect serum bilirubin in the first 24 hrs of starting treatment with oral zinc[13].All is due to the action of zinc by reaching the distal part of intestine and binding to bilirubin resulting in significant reduction in enterohepatic circulation and enhancing excretion.

Rana N et al 2011 found that the administration of zinccanreduce the duration of phototherapy needed for the treatment of neonates having indirect hyperbilirubinemia; but it showed no any benefit to decrease the incidence ofhyperbilirubinemia and the need of phototherapy in at-risk neonates in the first seven days of life[12]. Patton et al 2011 applied on term neonates with umbilical cord bilirubin ≥2 mg/dl , they used zinc sulphate salt in a low dose of 5 mg/day. The sample size selected for this study was small which was 30 neonates in each group. This study did not find any effect of zincsulphate on incidence and the mean total duration of hyperbilirubinemia[14].The discrepancy may be due to dose of zinc used in this study which was 5 mg.

Aprevious study by Maamori et al 2014 which tried to use zinc as a protective medication in preventing neonatal high bilirubin level, but showed that oral administration of zinc sulfate does not affect hyperbilirubinemiaanddoes not delaythe development of jaundice [15]. However, admission rate and phototherapy duration were lower in the zinc group when compared with the placebo group. In the above mentioned study, the usedtranscutaneous bilirubinmeasurment in infants above 35 weeks of age, while in the present study,serum indirect bilirubin level was analyzed; the discrepancy between the findings may be due to this difference. In addition, in the above mentioned study, zinc was used for prophylaxis, whereas in the present study, the role of zinc as a therapeutic effect was analyzed.However, the ineffectiveness of zinc in diminishing bilirubin level in this study might be attributed to various factors such as the delayed release of zinc sulfate to reach the active area, and the need for other zinc preparations or requirement for higher doses. The role of zinc in neonatal indirect hyperbilirubinemia relies upon its ability in reaching the terminal ileum, where it

precipitates indirect bilirubin to prevent enterohepatic circulation. In general, zinc seemed to be a safe agent.

CONCLUSIONS

A highly significant reduction of serum indirect bilirubin level after 24 and 48 hrs. follows the administration of Zinc sulfate for 2 days in patients with neonatal indirect hyperbilirubinemia.(Mean of indirect bilirubin level was highly significant lower in the neonates who were administered oral zinc compared to that in those without zinc therapy (11.2 mg/dl versus 13.65 mg/dl, and 8.18 mg/dl versus 11.60 mg/dl respectively)

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