EVALUATION OF BLOOD LEVELS OF COPPER, ZINC, MAGNESIUM AND CALCIUM IN FULL TERM NEONATAL UNCONJUGATED HYPERBILIRUBINEMIA

By

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ABSTRACT

Introduction: *Hyperbilirubinemia is one of the most prevalent problems in neonates. Jaundice is observed during first week of life in approximately 60% of term neonates and 80 % of preterm neonates. Phototherapy is one of the routine methods for management of hyperbilirubinemia. Many studies reported that calcium, copper, zinc and magnesium play an important role in the pathogenesis and development of neonatal hyperbilirubinemia, but the effect of phototherapy on these trace elements is unknown.*

Aim of work: *The aim of the study is to estimate the Blood Levels of Zinc, magnesium, copper and calcium and their relation to serum unconjugated bilirubin in Full term neonates with non-hemolytic unconjugated hyperbilirubinema admitted to NICU for phototherapy immediately before phototherapy and 12-24 hours after stopping phototherapy.*

Patients and methods: *The current cross sectional study was conducted at neonatal intensive care unit (NICU) of ELsayed Jalal University Hospital, Cairo, starting from December 2017 to December 2018. The study included 100 full term neonates with symptoms, signs and laboratory findings of neonatal indirect hyperbilirubinemia treated with phototherapy.*

Results of the study: *The mean serum calcium, copper and magnesium level in neonates with hyperbilirubinemia was significantly higher, while the mean serum zinc level was lower in neonates with hyperbilirubinemia; these levels were changed after phototherapy, there was statistically significant difference (decrease) in total bilirubin, calcium and magnesium. But regarding Zinc and copper there was statistically significant increase after phototherapy.*

Conclusion: *Phototherapy results in decreased serum levels of total bilirubin, calcium and Magnesium and increased serum levels of Zinc and copper after phototherapy.*

Recommendations: *Based on the findings of the current study, phototherapy can significantly decrease in the levels of calcium and magnesium and an increase in the level of zinc and copper in jaundice term infants undergoing phototherapy. Therefore, it is suggested that further studies be conducted on the effect of phototherapy on the levels of calcium, magnesium, and zinc and copper on premature and pre-term infants.*

Key words: *Hyperbilirubinemia, calcium, Magnesium, Copper, zinc, Neonates, Phototherapy.*

INTRODUCTION

Neonatal jaundice or hyperbilirubinemia is an unpreventable condition in 60%-80% of newborns worldwide , In a proportion of infants, jaundice may become sever progressing to acute bilirubin encephalopathy or kernicterus with a significant risk of neonatal mortality , Surviving infants may acquire long-term neurodevelopmental sequelae such as cerebral palsy, sensorineural hearing loss, intellectual difficulties or gross developmental delay **(Olusanya et al., 2015)**.

Phototherapy has been used since 1958 for the treatment of neonatal hyperbilirubinaemia **(Cremer et al., 1958)**. Its noninvasive nature, easy availability, low cost and occurrence of few side effects **(Tan, 1991)**. It causes unconjugated bilirubin to be mobilized from the skin by structural isomerization to a water soluble form that can be excreted in the urine. Lamps emitting light between the wavelengths of 400 - 500nanometers (peak at 460nm) are specifically used for administering Phototherapy as bilirubin absorbs this wavelength of light **(Verman et al., 2004)**.

The commonly known side effects of phototherapy are loose stool, hyperthermia, dehydration, skin burn, photo retinitis, low platelet count, increased red cell osmotic fragility, bronze baby syndrome, riboflavin deficiency, and DNA damage **(Cloherty and Martin, 2008)**.

Trace elements are essential micronutrients for growth, development, and maintenance of healthy tissues. The role of trace elements in body metabolism is of prime importance. Their deficiency causes diseases, whereas their presence in excess may result in toxicity to human life **(Hashmi et al., 2007)**.

Micronutrients and minerals levels like zinc, copper, magnesium and calcium might affect the process of bilirubin binding proteins or excretion **(Sarci et al., 2004)**.

Magnesium is a co-factor in more than 300 enzyme systems that regulate diverse biochemical reactions in the body, including protein synthesis, muscle and nerve function, blood glucose control, and blood pressure regulation **(Viering et al., 2017)**.

Phototherapy decrease serum Mg level as it decreases serum bilirubin. We suggest that increase in serum Mg may be due to extracellular movement of Mg, resulting from generalized cellular injury including neurons and erythrocytes with possibility of a neuroprotective role or a compensatory mechanism of increased serum Mg levels to reduce bilirubin toxicity **(El Frargy et al., 2016)**.

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 **(Schulpis et al., 2004)**.

Zinc (Zn) is essential component of many metalloenzymes involved in virtually all aspects of metabolism. Zinc is an integral component of nearly 300 enzymes **(Mansi et al., 2009)**.

Serum zinc level increase after phototherapy by reducing the bilirubin level so that an additional zinc supplementation can lead to zinc toxicity **(Mosayebi et al., 2016)**.

Calcium is crucial for many biochemical processes including blood coagulation, neuromuscular excitability, cell membrane integrity, and many of the cellular enzymatic activities **(JAIN et al., 2010)**.

Phototherapy inhibits pineal secretion of melatonin which blocks the effect of cortisol on bone calcium, so cortisol increases bone uptake of calcium and induces hypocalcaemia **(Hunter and Abrams, 2004)**.

The aim of the study is to estimate the blood levels of zinc, magnesium, copper and calcium and their relation to serum unconjugated bilirubin in full term neonates with non-hemolytic unconjugated hyperbilirubinema admitted to NICU for phototherapy immediately before phototherapy and 12-24 hours after stopping phototherapy.

PATIENT AND METHODS

We conducted this study at neonatal intensive care unit

(NICU) of ELssayed Jalal University Hospital, Cairo, starting from December 2017 to December 2018. The study included 100 full term neonates with symptoms, signs and laboratory findings of neonatal indirect hyperbilirubinemia treated with phototherapy. Apart from jaundice, their physical examination was completely normal.

Ethical Considerations:

1. Approval of ethical committee, Faculty of Medicine, Al-Azhar University.
2. Written consents from the parents of the patients.
3. The patients have the right to withdraw from the study at any time.
4. All the obtained data are confidential and the patients have the right to keep them.
5. The authors declare that there is no any financial conflict regarding the research and publication.
6. No conflict of interest regarding the study and publication.

Inclusion criteria:

1. Full term neonates (gestational age: >37 weeks), age weighing >2500 g with non-hemolytic unconjugated hyperbilirubinemia need management with phototherapy.
2. Unconjugated bilirubin/total bilirubin ≥ 80%.

Exclusion Criteria:

Preterm and low birth weight newborns. Direct bilirubin > 20% - Exchange transfusion cases. Neonates with cephalohematoma, congenital malformation, respiratory distress, hypoxic ischemic encephalopathy, inborn errors of metabolism, infant of diabetic mother, sepsis. Hemolytic hyperbilirubinemia. All parents or guardians of neonates gave a written informed consent to participate in this study. The study was approved by the Ethics Committee of Faculty of Medicine, AL-Azhar University.

Methods:

All neonates enrolled in the study will be subjected to Full history taking laying stress on:

1. A family history of jaundice, anemia, liver disease, sibling with jaundice or anemia.
2. Perinatal history including (maternal illness, mode of delivery, gestational age, birth weight, gender, Apgar score, history of cyanosis or convulsions).

3. Clinical examination (with special emphasis on vital signs, anthropometric measures, presence of Cephalhematoma and neurological examination).
4. Laboratory investigations including:
   a. Complete blood count.
   b. Serum total and direct bilirubin level.
   c. Blood Levels of Zinc, magnesium, copper and calcium.

Blood samples for determination of Zinc, magnesium, copper, calcium and serum bilirubin levels were obtained from infants during venipuncture. Zinc, magnesium, copper and calcium concentrations were determined using atomic absorption spectrophotometry.

5. Phototherapy.

Phototherapy equipment containing four blue light fluorescent lamps with wave length of 410-470 nm, was placed at a distance of 30-40 cm. We used the AAP guidelines for phototherapy.

Statistical analysis:

Data were statistically described in terms of mean ± standard deviation (±SD), median and range, or frequencies (number of cases) and percentages when appropriate. Comparison of numerical variables between the study groups was done using Student t test for independent samples and Paired t test for paired samples. For comparing categorical data, Chi square (x2) test was performed. Exact test was used instead when the expected frequency is less than 5. p values less than 0.05 was considered statistically significant. All statistical calculations were done using computer programs SPSS (Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA) version 20 for Microsoft Windows.

RESULTS

The current study carried out on 100 Full term neonates. They admitted in the neonatal intensive care unit (NICU) of Elsayed Galal University Hospital.

Table (1): Demographic data of the studied group

Variable	The case group (100) Range (mean ± SD)
Age, (hours)	48-96(71.7±16.9)
Gestational age, (weeks)	37-39(37.8±0.7)
Gender, (Male/ Female)	56(56%) 44(44%)
MOD, (NVD/ CS)	32(32%) 68(68%)
Weight , (Kg.)	2.5-3.5(3.04±0.2)

Table 1 shows demographic data of the studied neonates: Atotal of 100 cases were followed-up consisted of 56 (56%) males and 44 (44%) females. 68 (68%) in¬fants were born by cesarean section and (32%) by vaginal delivery. Apgar scores were normal (8 - 9) at birth in all cases. The mean gestational age was 38 weeks and the mean birth weight 3100 grams.

Table (2): Bilirubin and minerals levels before and after phototherapy in the studied group

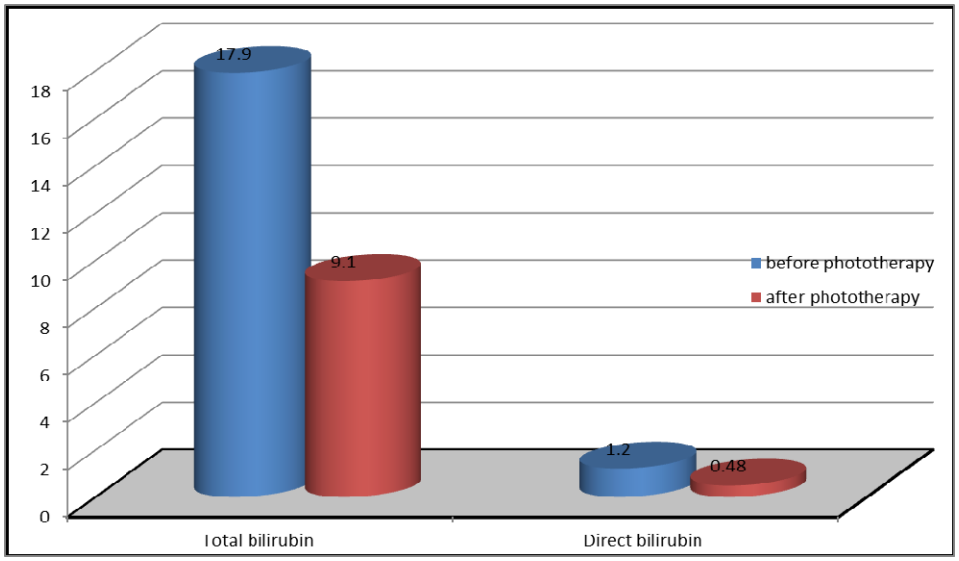
Variable	Before phototherapy mean ± SD (Range)	After phototherapy mean ± SD (Range)	paired t-test	p-value
Total bilirubin (mg/dl)	17.9±1.5 (15.9-20.1)	9.1±0.8 (7-10.9)	53	**0.001****
Direct bilirubin (mg/dl)	1.2±0.04 (0.2-1.6)	0.48±0.16 (0.2-0.7)	12	**0.001****
Calcium (mg/dl)	9.8±2.89 (7.9-11.4)	8.1±1.7 (7.3-10.9)	13	**0.001****
Magnesium (mg/dl)	2.7±0.6 (1.8-3.9)	1.9±0.7 (1.2-2.8)	3.4	**0.002****
Copper (ug/dl)	109.8±11.8 (98.2-130)	145.3±19.8 (98.5-175.5)	3.5	**0.001****
Zinc (ug/dl)	60.9±13.5 (33.5-86.3)	65.2±14.7 (31.5-96.2)	2.6	**0.003***

** Statistically highly significant difference ($P \leq 0.001$)

Table 2 shows the relation between bilirubin and minerals levels before and after phototherapy in the studied group: Before phototherapy the mean serum calcium, copper and magnesium levels in neonates with hyperbilirubinemia were significantly higher, while the mean serum zinc level were lower in neonates with hyperbilirubinemia; these levels were changed after phototherapy.

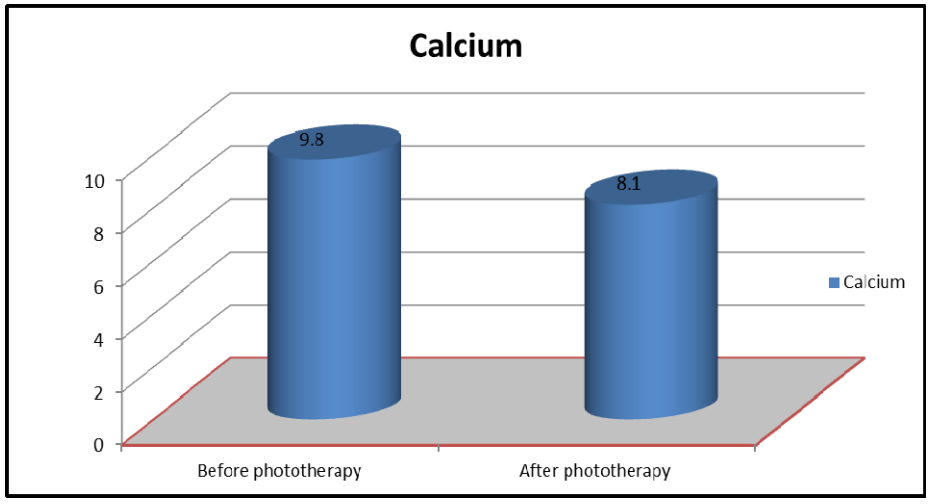
There were statistically significant difference (decrease) in total bilirubin, direct bilirubin, calcium and magnesium. But regarding Zinc and copper there were statistically significant increase after phototherapy.

Fig (1): Bar chart for comparing bilirubin before and after phototherapy



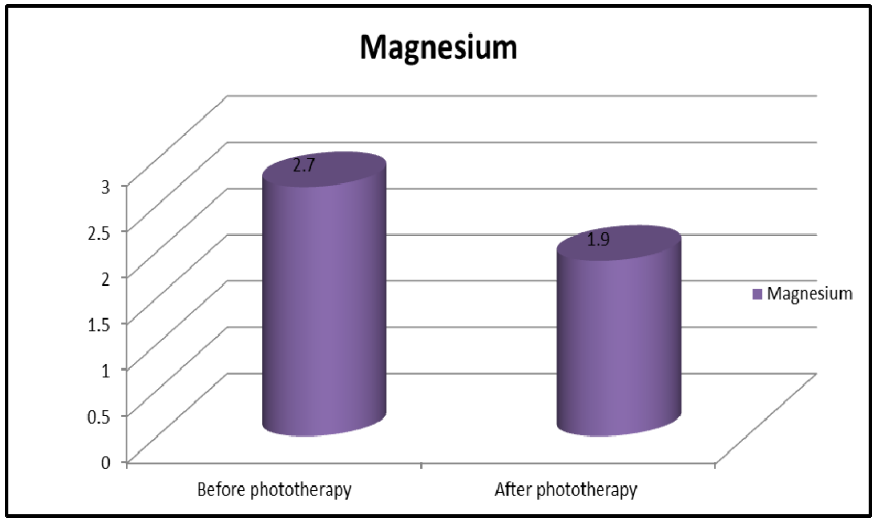
There is statistically significant decrease in total and direct bilirubin after phototherapy.

Figure (2): Bar chart for comparing Calcium before and after phototherapy



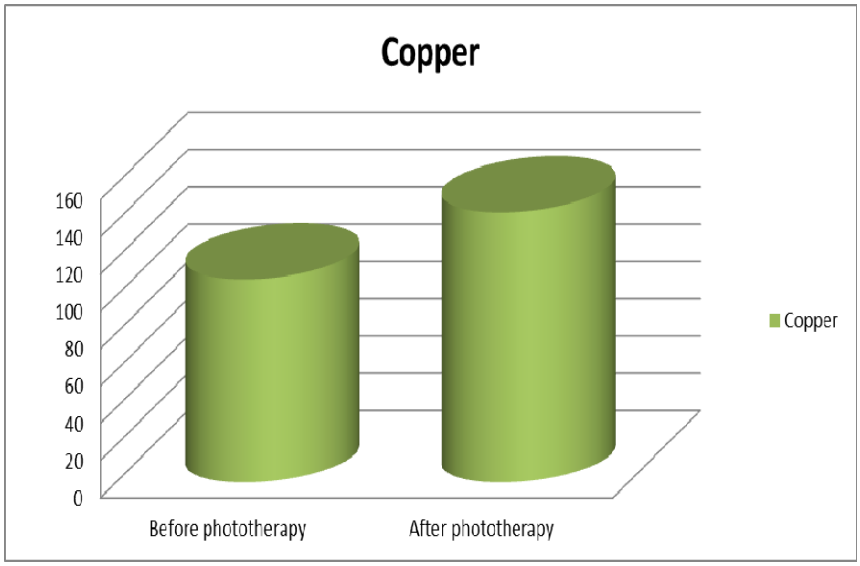
There is statistically significant decrease in serum calcium level after phototherapy.

Figure (3): Bar chart for comparing Magnesium before and after phototherapy



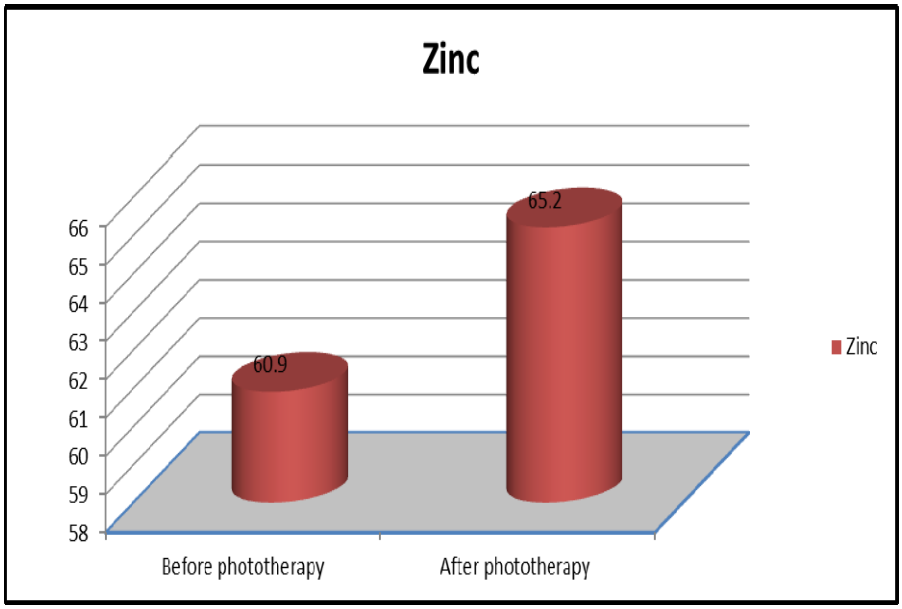
There is statistically significant decrease in serum magnesium level after phototherapy.

Fig (4): Bar chart for comparing Copper before and after phototherapy



There is statistically significant increase in serum copper level after phototherapy.

Fig (5): Bar chart for comparing Zinc before and after phototherapy



There is statistically significant increase in serum zinc level after phototherapy

DISCUSSION

Hyperbilirubinemia or jaundice is a common problem and it is often considered benign in infants. Jaundice might be observed at the birth or any time during infancy. Therefore, if hyperbilirubinemia, which is non-conjugated, is not treated early, bilirubin crosses the blood-brain barrier and exerts its neurotoxic effects. Its therapy includes medicine therapy, blood exchange, and phototherapy **(Martin et al., 2015)**.

Phototherapy has a significant role in the treatment of hyperbilirubinemia in neonates **(Barrington et al., 2014)**. However, this method may result in the development of some complications **(Basiglio et al., 2010)**.

This study revealed that the mean serum calcium, copper and magnesium levels in neonates with hyperbilirubinemia were significantly higher, while the mean serum zinc level were lower in neonates with hyperbilirubinemia; these levels were changed after phototherapy.

There were statistically significant difference (decrease) in total bilirubin, direct bilirubin, calcium and magnesium. But regarding Zinc and copper there were statistically significant increase after phototherapy.

This reduction in mean serum calcium value was found to be statistically significant based on t-test (p value -<0.001). This was in correlation with studies done by **Bahbah et al., 2015** (8.58±0.76) and **Singh et al., 2017** (8.42±1.19).

(Romagnoli et al., 1979) was the first to suggest the association of hypocalcaemia in newborn following phototherapy. **(Hakinson, 1987)** and **(Hunter, 2004)** hypothesized that phototherapy inhibits secretion of melatonin from pineal gland which blocks the effect of cortisol on bone calcium. So, cortisol increases bone uptake of serum calcium and induces hypocalcemia. **(Kim, 2001)** suggested that decreased secretion of parathyroid hormone is the cause of hyopcalcemia in photoherapy.

In the present study we detected hypocalcaemia after 48 hours phototherapy in 18 % of our neonates.

In previous studies, the incidence of hypocalcaemia after 48 hours of phototherapy was 14.4% **(Karamifar et al., 2002)**, 15% **(Ehsanipour et al., 2008)**, 26.3% **(Kargar, Marzieh, et al.,**

2014), 20.23% **(Nishant Prabhakar et al., 2016)**, 26% **(Mohammed Hamed Bahabah et al., 2015)**, 7% **(Paymaneh et al., 2013)**, 7.5% **(Fatemeh Haji, 2014)**, 16.9% **(Ezzeldin et al., 2015)**, 22.2% **(Maha A. Nouh et al.,2013)** , 25.7% **(Abd-Elmagid et al.,2017)**, 35% **(Goyal S et al.,2018)**.

None of the hypocalcaemia neonates were clinically symptomatic. This was in correlation with studies done by **Tehrani et al., 2014** and **Reddy et al., 2015**.Different from our study **Goyal S et al., 2018** symptomatic hypocalcaemia was observed in 2.86% of neonates, **Bahbah et al., 2015** observed jitteriness in 14% and convulsions represented 10% of hypocalcaemia cases.

Magnesium (Mg++) ion is one of the most important antagonistic regulators of the NMDA receptor/ion channel complex (Ascher and Nowak 1987) it protects the CNS against hypoxia and exerts its neuroprotective effects by blocking excitotoxic and NMDA receptor–mediated neuronal injury mechanisms **(Marret et al., 1995)**.

In the present study, there are statistically highly significant differences in serum bilirubin level as well as magnesium level before starting phototherapy and after 48 hours of phototherapy in full term neonates.

In agreement with our study, **Imani et al., 2012** reported that serum bilirubin and magnesium levels were measured before and after phototherapy, both of which showed a significant decrease. Also, **Khosravi et al., 2011** reported that phototherapy can decrease the total magnesium.

Ravichander, B. and Kundu, S., 2018, suggest that there is statistically highly significant difference in serum bilirubin level as well as magnesium level before starting phototherapy and after 48 hours of phototherapy in term and late preterm neonates.

In this study, the mean serum Cu level in neonates with hyperbilirubinemia was significantly higher.

In agreement with this study, **Schulpis et al., 2004** reported that the serum Cu increased 2-fold in neonates with moderate hemolytic jaundice and almost threefold in neonates with severe hemolytic jaundice. Also, **Hassan, 2017** reported that in neonates with jaundice, the higher serum Cu may be due to intracellular (erythrocyte) origin due to the slight hemolysis.

Different from our results are **Pintov et al., 1992** 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.

In the present study, there are statistically highly significant differences in serum bilirubin level as well as zinc level before starting phototherapy and after 48 hours of phototherapy in full term neonates.

Al-Hajjiah et al., 2018 reported that serum concentration of zinc in neonates with jaundice was significantly lower than that in healthy control.

Mosayebi et al., 2016, reported that phototherapy increases the serum zinc levels in neonates with hyperbilirubinemia.

Boskabadi et al., 2015 the level of serum zinc in the neonates with hyperbilirubinemia jaundice was lower than that of the ones without jaundice. It seems that zinc has a protective effect. However, more studies are needed for better decision making.

El-Farrash et al., 2018, reported that more detailed analysis of the effect of phototherapy on serum trace elements, we observed significant increase in serum Cu, Zn and Fe levels post-phototherapy.

Hassan EJ.2017, reported that the concentration of Cu, Zn, Mg, and Mn were measured using atomic absorption spectrophotometer (AAS). It has been found that zinc level is significantly lower in newborn jaundice patients compared with normal subjects at ($p<0.05$), also the level of Cu Mg, and Mn were significantly higher in newborn jaundice compared with control group.

CONCLUSION

From this study we concluded that:

1. Phototherapy results in decreased serum levels of total bilirubin, calcium and Magnesium after phototherapy.

2. Phototherapy results in increased serum levels of Zinc and copper after phototherapy.

RECOMMENDATIONS

1. Based on the findings of the current study, phototherapy can significantly decrease in the levels of calcium and magnesium and an increase in the level of zinc and copper in jaundice term infants undergoing phototherapy. Therefore, it is suggested that further studies be conducted on the effect of phototherapy on the levels of calcium, magnesium, and zinc and copper on premature and pre-term infants.

2. The values of using these minerals as prophylactic treatment of neonatal hyprebilirubinemia deserve further studies.

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دراسة مستوي الزنك والنحاس والمغنيسيوم والكالسيوم في حالات الاطفال حديثي الولادة مكتملي النمو المصابين بارتفاع نسبة البليروبين الغير مباشر في الدم

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هيئة الطاقة الذرية-مصر

اليرقان الوليدي هو اصفرار لون الجلد والأغشية المخاطية للأطفال حديثي الولادة وذلك لزيادة نسبة الصفراء في الدم ويحدث اليرقان فيما يقارب 50% من المواليد كاملي النمو وحوالي 80%من المواليد ناقصي النمو.

اليرقان الوليدي أحد أسباب القلق للآباء و أيضا الأطباء حيث أنه يتسبب ببعض المضاعفات للأطفال حديثي الولادة و يزيد من مدة إقامتهم بالمستشفى مع زيادة في الكلفة المادية وإمكانية إعادة إدخال الطفل حديث الولادة المستشفى مرة أخري.

العلاج الضوئى له دور هام فى العلاج من اليرقان فى الأطفال حديثى الولادة . ومع ذلك فإن هذهالطريقة قد تؤدى إلى حدوث بعض المضاعفات.

الهدف من هذه الدراسة هو دراسة مستوي الزنك والنحاس والمغنيسيوم والكالسيوم في الدم في حالات الاطفال

حديثي الولادة مكتملي النمو المصابين بارتفاع نسبة البليروبين الغير مباشر في الدم.

هذه الدراسة تم إجراؤها علي عدد 100 مولود بمستشفي السيد جلال الجامعي. و تم الحصول علي موافقة من آباء الأطفال المشاركين في هذه الدراسة.

وشملت الدراسة 100طفلا حديثى الولادة مكتمل النمو تعرضوا للعلاج (56 ذكور , 44 إناث). 68 مولود ولادة قيصرية ويمثلون نسبة 68% , بينما 32 مولود ولادة طبيعية ويمثلون نسبة 32% وتتراوح أوزانهم ما بين 2500- 3500 جم.

متوسط البليروبين الكلي قبل العلاج الضوئي (1.5±17.9) متوسط البليروبين المباشرقبل العلاج الضوئي (0.04±1.2) متوسط عنصر الكالسيوم قبل العلاج الضوئي (2.89±9.8) متوسط عنصر المغنيسيوم قبل العلاج الضوئي (0.6±2.7) متوسط عنصر النحاس قبل العلاج الضوئي(11.8±109.8) متوسط عنصر الزنك قبل العلاج الضوئي (13.5±60.9).

متوسط البليروبين الكلي بعد العلاج الضوئي(0.8±9.1) متوسط البليروبين المباشربعد العلاج الضوئي (0.16±0.48). متوسط عنصر الكالسيوم بعد العلاج الضوئي(1.7±8.1). متوسط عنصر المغنيسيوم بعد العلاج الضوئي(0.7±1.9). متوسط عنصر النحاس بعد العلاج

الضوئي (145.3±19.8) متوسط عنصر الزنك بعد العلاج الضوئي(65.2±14.7).

وقد أسفرت نتائج هذه الدراسة عن ظهور ارتفاع فى مستوى الكالسيوم والمغنسيوم والنحاس واظهرت انخفاض في مستوي الزنك في الدم في الاطفال حديثي الولادة مكتملي النمو والمصابين بارتفاع نسبة البليروبين الغير مقترن وان هذه النتايج قد تغيرت بعد العلاج الضوئي.

واسفرت هذه الدراسه عن وجود انخفاض ملحوظ في مستوي البليروبين الكي واللبليروبين المباشر ومستوي عنصري الكالسيوم والمغنيسيوم بعد العلاج الضوئي واسفرت ايضا عن وجود ارتفاع ملحوظ في مستوي عنصري النحاس والزنك بعد العلاج الضوئي.