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RESEARCH

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Effect of phototherapy on eosinophils count in neonatal hyperbilirubinemia (cross-sectional study)

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Abstract

Background Neonatal jaundice is a common disorder in neonates. Phototherapy is associated with various side effects despite being safe. This research aimed to determine the impact of phototherapy (PT) on eosinophil levels in neonates treated for unconjugated hyperbilirubinemia.

Methods In this cross-sectional prospective study, icteric neonates admitted to the Neonatal Intensive Care Unit (NICU) of Abo-Elreesh Child Hospital, Cairo University, from July 2020 to June 2021 were assessed. Laboratory data, including serum bilirubin and blood cell differentiation for eosinophils before and after phototherapy, were collected, and demographic data like age, gender, gestational age, and duration of phototherapy were taken.

Results Two hundred neonates were included in this study. Hyperbilirubinemia was most frequently related to ABO group incompatibility (44.5%). After phototherapy, total serum bilirubin and neutrophil levels were significantly decreased, and platelet levels were significantly increased. Eosinophil count and percent were significantly increased ($p < 0.001$) following phototherapy. A statistically significant positive correlation was found between eosinophil count before and after PT ($r = 0.583$, $p < 0.001$) and between eosinophil percent before and after PT ($r = 0.617$, $p < 0.001$).

Conclusion Serum eosinophils were increased after phototherapy in icteric neonates with hyperbilirubinemia.

Keywords Jaundice, Phototherapy, Eosinophils, Neonates

Background

In Cairo University Children's Hospital, severe neonatal hyperbilirubinemia accounted for 33% of total admission diagnoses to the outborn Neonatal Intensive Care Unit (NICU) in 2006, with about 10 cases of kernicterus occurring each year [1]. In 2012, Iskander et al. reported that the most common cause of indirect hyperbilirubinemia was ABO incompatibility (25.4%) [2].

Phototherapy has been used to manage neonatal jaundice since the 1970s, and it is the most commonly used therapy [3].

Studies on phototherapy and neonatal jaundice imply that allergic diseases increase after PT. The physiopathological mechanisms involved in this topic are not clear. Available information suggests that PT or hyperbilirubinemia may increase the risk of allergy atopy via many pathways [4].

Inflammatory cells, including eosinophils and lymphocytes, bind to vascular endothelial cells due to the migration of inflammatory cells in the endothelium, which is facilitated by the vascular cell adhesion molecule (VCAM1). The connection of the VCV-1 cell line (VCAM-1) with an unknown mechanism has been demonstrated to be inhibited by bilirubin [5].

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This study aims to detect how phototherapy affects the eosinophil count in newborns with hyperbilirubinemia.

Methods

Study design

This cross-sectional study was performed on neonates admitted during the first week of life to the Neonatal Intensive Care Unit (NICU) of Abo-Elreesh Child Hospital, Cairo University, from July 2020 to June 2021.

Study population

This study was conducted on 200 neonates diagnosed with unconjugated hyperbilirubinemia and treated by phototherapy. The indication of phototherapy was determined according to the phototherapy nomogram in the American Academy guidelines [6].

Clinical assessment

Neonates whose gestational age is ≥ 37 weeks were included. The following neonates: gestational age < 37 weeks; chorioamnionitis; neonatal sepsis; maternal use of tobacco; pathological weight loss; direct hyperbilirubinemia when the direct bilirubin level is more than 2 mg/dl; and family or maternal history of allergic diseases were excluded from the study.

The demographic data identified the enrolled patients' gestational age, sex, birth weight, consanguinity, mode of delivery, Apgar score, maternal blood group and RH, age of admission, and duration of therapy. Moreover, associated maternal comorbidities were screened, such as premature rupture of membrane (PROM), history of allergic diseases, pregnancy-related diseases (diabetes, preeclampsia), and antenatal drug intake. The following vital signs: heart rate, respiratory rate, and temperature were measured. Besides, blood pressure was measured using an automatic wrist blood pressure monitor.

Laboratory investigations

- Sampling

A volume of 4 ml of venous blood was obtained; 2 ml was taken in EDTA tubes for CBC and blood group evaluation, and 2 ml was taken in plain tubes for bilirubin and C-reactive protein (CRP) evaluation.

Before the treatment was started, venous blood samples were taken from all the patients, and these samples were analyzed for.

1) Total serum bilirubin and direct serum bilirubin:

   The serum was separated by sample centrifugation, and TSB levels were measured by the spectrophotometric method (Beckman Coulter, AU 480, CA, USA). Serial serum measurements were taken daily based on clinical assessment.
2) ABO and RH blood grouping:

Knowing the ABO and RH blood groupings of the mother and neonates helps to diagnose the cause of neonatal jaundice (either ABO or RH, or both incompatibilities).

3) Reticulocytes count to exclude hemolysis.
4) Direct Coomb's test.
5) CRP was conducted to exclude sepsis and rule out the cases with positive tests.
6) CBC was performed by the hematology analyzer Sysmex XN-1000, Japan.

Manual blood films were prepared, stained, and examined microscopically to evaluate WBC, eosinophil, lymphocyte, neutrophil, monocyte, and basophil counts. Eosinophils were evaluated automatically in peripheral blood as eosinophil percent and absolute eosinophil counts. The mean value for eosinophils is reported to be 550 cells/μL in neonates, ranging between 140 and 1300 cells/μL, and if the eosinophil count is more than 1300 cells/μL, it is considered eosinophilia.

The CBC was evaluated before starting the phototherapy and after the end of the phototherapy.

The treatment was discontinued when the total serum bilirubin level fell 2 mg/dl below the phototherapy threshold for the patient's age. Subsequently, 12–24 h later, blood samples were obtained and re-analyzed for leucocytes, neutrophils, platelets, lymphocytes, and eosinophils.

Phototherapy devices

There were two types of phototherapy devices used in this study:

LED phototherapy Patients received phototherapy using an LED PT unit (Atom Phototherapy Analyzer; Atom Medical Corporation, Tokyo, Japan), a stand type with dimensions of 450(W) × 710(D) × 1900(H) mm and an irradiance level of 30–40 μW/cm^2/nm at an irradiation distance of 30 cm.

This PT type promotes early hospital discharge by reducing the treatment time by providing high irradiation intensity and an effective wavelength. Double phototherapy was used to increase the infant's exposed body surface area.

Intensive phototherapy The Novos Bilisphere 360 LED, Ankara, Turkey, was used for intensive phototherapy. It

comprises 16 blue-light LED tubes with an irradiance of > 100 μW/cm^2/nm in the 440–460 nm wavelength range. Bilisphere 360 LED gets the fastest total serum bilirubin breakdown and eliminates the need for risky blood exchange.

During this study, an LED PT unit with an irradiance level of 30–40 μW/cm^2/nm and an intensive PT unit with an irradiance of > 100 μW/cm^2/nm were used during therapy based on the guideline charts for phototherapy in hospitalized patients and the response of the patient to therapy.

Monitoring of neonates during phototherapy

During phototherapy, the babies were completely naked except for a diaper, with their eyes covered with an eye protection band. The infant's clinical status was assessed to ensure adequate hydration, proper nutrition, temperature control, and clinical improvement or deterioration of jaundice, including any sign of bilirubin encephalopathy. Nurses were advised to change the neonate's position every 2 h and to report any discomfort when handling the neonates.

Neonates were closely monitored for short-term side effects of therapy, which included loose, greenish stools, hydration status, brownish discoloration of skin, and skin rashes. The patient's vital signs were monitored every 4 h throughout phototherapy. Weight was checked daily. Feeds were calculated based on the daily kilocalorie requirement for age. Neonates were supplemented with infant formula every 3 h to maintain the total daily feed requirement. Considering the postnatal age and clinical and laboratory findings, those who did not maintain adequate oral intake were supplemented with IV fluids to maintain the total daily fluid requirement.

Statistical methods

Statistical analysis was performed using IBM SPSS® Statistics version 26 (IBM® Crop., Armonk, NY, USA). Numerical data were expressed as mean and standard deviation, or median and range, as appropriate. Qualitative data were expressed as frequency and percentage.

For quantitative data, a comparison of two consecutive measures of numerical variables was made using a paired t-test for normally distributed data, while for normally distributed quantitative data, a Wilcoxon-signed rank test (nonparametric paired *t*-test) was used.

A comparison of two consecutive measures of categorical variables was conducted using the McNemar test. The Spearman-Rho method was used to test the correlation between numerical variables. For not normally distributed quantitative data, a comparison between two groups was made using the Mann–Whitney test (nonparametric *t*-test) which is used in Table 2 hematological profile of the patients before and after the end of phototherapy, and a comparison between three groups was performed using the Kruskal–Wallis test (nonparametric ANOVA) which used in Table 3. All tests were two-tailed. A p value of < 0.05 was considered significant.

Results

This cross-sectional study included 248 neonates diagnosed with neonatal jaundice admitted during the first week of life in the Neonatal Intensive Care Unit (NICU) of Abo-Elreesh Child Hospital, Cairo University, from July 2020 to June 2021. Forty-eight neonates treated with exchange transfusions were excluded (Fig. 1).

One hundred seven (53.5%) of the studied newborns were males, and 93 (46.5%) were females. Most neonates (66%) were delivered by cesarean section, and 34% were delivered by vaginal delivery. The mean gestational age of newborns was 38.6 ± 0.8 weeks. The mean birth weight was 2833 ± 531 g, and the mean length was 48.1 ± 3.4 (Table 1).

These neonates had neonatal jaundice during the first week of life; 9 (4.5%) of them were during the first day, 92 (46%) on the second–fourth day, and the rest 99 (49.5%) on the fifth–seventh day. The median postnatal age at admission was 4 (1–7) days, and the median duration of phototherapy was 3 (1–9) days. The most common neonatal blood group is B + (40%), while the least common blood group is O − (1%).

Only 3% and 4% of the neonates had a history of maternal diabetes and hypertension, respectively. 5.5% of neonates had a positive antenatal drug intake. The most common maternal blood group is O + (55.5%), while the least common blood group is B − (1%).

Regarding the causes of neonatal jaundice, 89 of the admitted neonates (44.5%) were due to ABO incompatibility, and 85 neonates (42.5%) were due to physiological jaundice. The rest of the patients were due to RH incompatibility and mixed ABO and RH incompatibility.

Only 7.5% of neonates had a positive Coombs test, while the rest had a negative Coombs test.

Most neonates (94%) in this study were treated with phototherapy only, and only 6% were treated with IVIG in addition to phototherapy.

The phototherapy devices used in this study differed among the studied newborns. More than 50% of the neonates were treated by both LED and intensive PT units.

The mean duration of phototherapy among the studied neonates was 3.5 days, ranging from 1 to 9 days. The Bili sphere has the shortest duration, with a mean of one day compared to the mean of single and double LED phototherapy (1.2 and 1.3, respectively). The average hospital stay was 4.5 days.

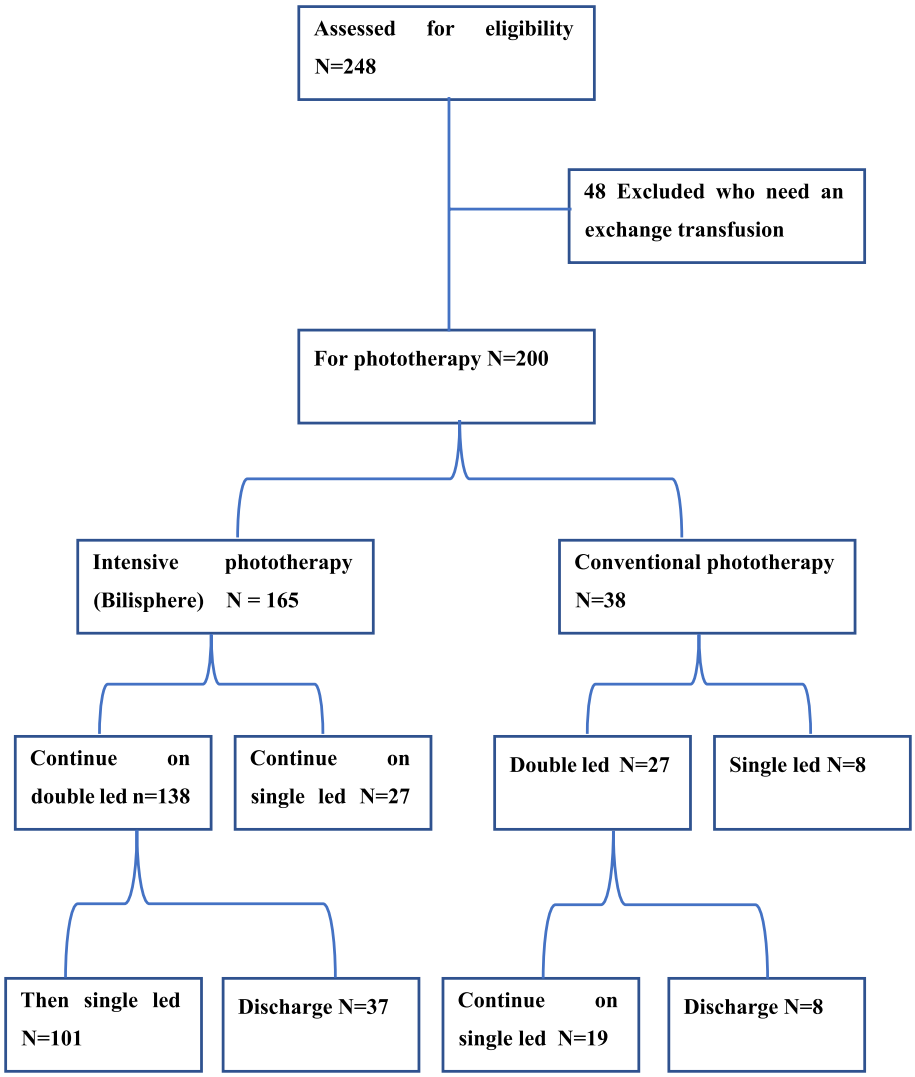

Fig. 1 Flow chart demonstrates the outcome of neonates treated with phototherapy

Table 1 Neonatal demographic data of the studied newborns

Neonatal characteristics	Mean ± SD	Median (range)
Gestational age (weeks)	38.6 ± 0.8	39 (37–41)
Postnatal age at admission (days)	4.4 ± 1.9	4 (1–7)
Weight (grams)	2833 ± 531	2793 (1686–4950)
Length (cm)	48.1 ± 3.4	48.0 (31–55)
Head circumference (cm)	33.8 ± 2	34.0 (29–48)

SD Standard deviation

Total serum bilirubin was significantly decreased from pre-PT total serum bilirubin (mg/dl) mean 19.3 (4.7) to post-PT 8.6 (2.3) following phototherapy (p value < 0.001) (Table 2).

The effect of phototherapy on the hematological profile was studied. The median platelet count increased from 313.5 ($\times 10^3$ cell/mm^3) to 340 ($\times 10^3$ cell/mm^3) with a p value of 0.007. Phototherapy is associated with significant changes in the neutrophil count. The median neutrophil count decreased from 4359.5 cells/mm^3 before the phototherapy to 3792 cells/mm^3 after the end of phototherapy, with a p value < 0.001, which was statistically significant. There was no significant effect of phototherapy regarding leucocyte count, basophil count, lymphocyte count, and monocyte count, with p values of 0.413, 0.532, 0.074, and 0.971, respectively.

The phototherapy had a highly statistically significant effect on the eosinophil counts and percent. The mean eosinophil count increased from 272 cells/mm^3 to 408.1 after the end of phototherapy, with a p value < 0.001.

Moreover, there was a significant increase in eosinophil percent before and after the end of phototherapy, from 2.4% to 3.6%, with a p value < 0.001 (Table 3) (Fig. 2).

According to the normal count of eosinophils in neonates (average 550 cells/mm^3, range from 140 to 1300 cells/mm^3), only five neonates (2.5%) following the end of phototherapy had eosinophilia, and only one neonate had eosinophilia from the start, for a total of six neonates (3%) having eosinophilia after the end of phototherapy.

There was a good positive correlation between eosinophil count and percent before and after the end of phototherapy (r = 0.583 and r = 0.617, respectively), with a p value of both < 0.001 (Fig. 3).

The mean values of eosinophils were compared before and after phototherapy based on sex and age at admission. The mean eosinophil count differed under the effect of the phototherapy regarding sex and age at admission, with no significant values (p value > 0.05).

No correlation existed between total serum bilirubin, eosinophil count, and percent before and after the phototherapy (p value > 0.05). Moreover, in our study, the highest mean eosinophil count was in neonates treated with LED and intensive PT units, although

Table 2 Hematological profile of the patients before and after the end of phototherapy

Parameter	Value before PT	Value after the end of PT	*P* value
Platelet (× 10^3/mm^3)	5.313[a] (91–802)	340[a] (95–831)	**0.007**
Leucocytes (× 10^3/mm^3)	11[a] (3.9–39.2)	8.10[a] (4.3–25.9)	0.413
Neutrophil (cell/mm^3)	5.4359[a] (358–22320)	3792[a] (950–14820)	**< 0.001**
Basophil (cell/mm^3)	0[a] (0–1800)	0[a] (0–1280)	0.532
Lymphocytes (cell/mm^3)	5.4886[a] (1773–27440)	5332[a] (1577–14245)	0.074
Monocytes (cell/mm^3)	5.913[a] (0–4074)	986[a] (0–4406)	0.971

[a] The value is expressed as the median and range. Mann–Whitney test

Table 3 The effect of phototherapy on eosinophils counts and percents

Parameter	Mean (SD)	Median	Range	*P* value
Eosinophil percent before PT	2.4 (2)	2	0–10	< 0.001
Eosinophil count before PT (cell/mm^3)	272.6 (258.8)	222	0–1800	
Eosinophil percent after the end of PT	3.6 (2.4)	3	0–12	
Eosinophil count after the end of PT (cell/mm^3)	408.1 (324.1)	324.5	0–1960	

Kruskal–Wallis test

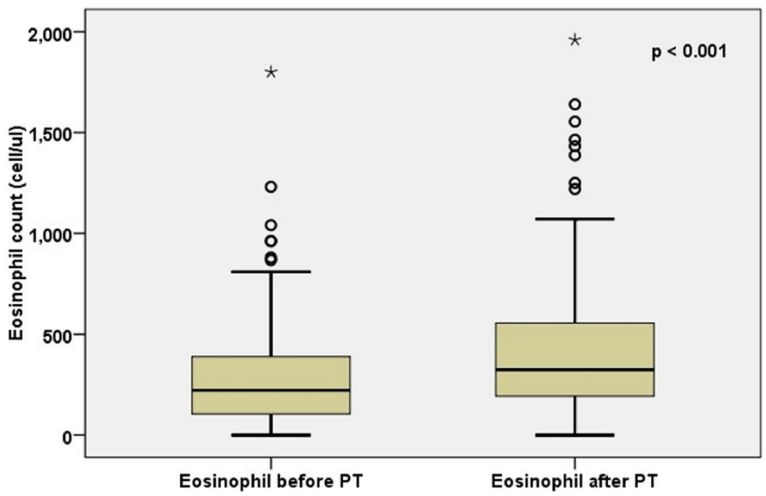

Fig. 2 The eosinophil count before and after the phototherapy

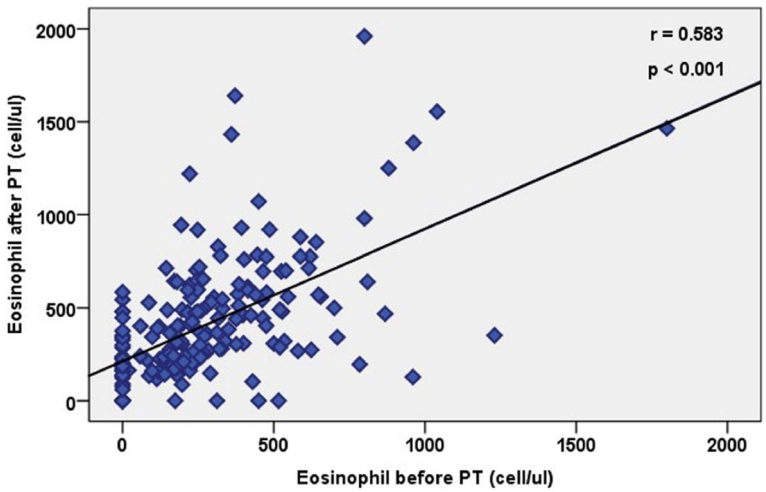

Fig. 3 Correlation between eosinophil percent before and after phototherapy

this had no significant value. There was no correlation between the duration of the phototherapy and eosinophil count, or percent, before and after the phototherapy (p value > 0.05). Consequently, the increased eosinophil count after phototherapy was not affected by the duration of phototherapy.

Discussion

Phototherapy is universally recognized as the first option for treating neonatal jaundice. However, several studies have suggested that phototherapy may have many short- and long-term adverse reactions associated with pediatric diseases, including hemolysis, allergic diseases, DNA damage, or cancer [7].

Fifty to 60% of term neonates develop neonatal jaundice, and 10% to 20% are treated with phototherapy to prevent bilirubin encephalopathy and kernicterus. A possible association between neonatal jaundice and phototherapy and increasing risks of allergic diseases has been reported [8].

This study aimed to observe the changes in eosinophil count after therapeutic phototherapy for neonatal hyperbilirubinemia.

Most of our neonates were admitted at the age of the fifth-seventh day, with a mean age on admission of 4.4 ± 1.9 days; this agrees with the study conducted by [9].

Our study had a higher frequency of ABO incompatibility (44.5%) compared with Rh incompatibility (6%). This agrees with Erdeve et al. (2018) due to anti-D immunoglobulin administration.

Our study's average length of hospital stay was 4 days, which is close to the study of Meslhy et al. (2020), in which the average length was 3.8 days. They mentioned that bilisphere caused a marked reduction in the serum bilirubin level in a short period and decreased the duration of hospital stay.

In our study, an increased eosinophil count was observed more on the first day of life than the rest of the first week but was of no significant value. Sangsari et al. (2020) mentioned in their study that eosinophilia is related to the baby's age. This suggests that at an age of less than a week, the influence of exposure to phototherapy is higher than that of older ones, which can be due to the lack of maturity of the body's early immune system at the beginning of life.

A significant increase in the eosinophil count and percent was observed before and after PT. Eosinophil levels were increased after PT for 48–72 h [10].

High bilirubin levels may decrease eosinophil levels by suppressing the vascular cell adhesion molecule (VCAM-1). The vascular cell adhesion molecule cellular connection molecule plays a crucial role in the migration of inflammatory cells in the endothelium, which causes the binding of inflammatory cells, like eosinophils and lymphocytes, to vascular endothelial cells. Bilirubin has been shown to inhibit the VCV-1 cell line (VCAM-1) connection with an unknown mechanism [5].

A study by Kuniyoshi et al. (2021) showed a relationship between phototherapy, neonatal jaundice, and childhood allergic diseases. They explained that, first, unconjugated bilirubin may suppress interleukin (IL)-2 production, which induces the T helper type 2 (Th-2)/T helper type 1 (Th-1) switch disorder. Second, phototherapy for neonatal jaundice could alter the levels of cytokines, such as tumor necrosis factor-alpha, IL-1-beta, IL-8, and IL-6, and directly result in DNA damage in lymphocytes. These changes may result in the Th-2/Th-1 switch disorder. The Th-2/Th-1 switch disorder can influence Th-2

predominance, which may, in turn, contribute to the subsequent development of allergic diseases.

Thus, any treatment that aims to decrease total serum bilirubin levels may be expected to increase the levels of eosinophils. They concluded that bilirubin is protective against allergic disease and that phototherapy may trigger allergic disease. These studies showed that jaundice and phototherapy increase the risk of asthma later in life [11].

Sangsari et al. (2020) stated in their study that there was a significant negative correlation between patients' mean total bilirubin concentration before phototherapy and mean eosinophilia. As in this study, there was no significant correlation between total serum bilirubin and mean eosinophil count before and after phototherapy. On the other hand, a study by El-Sheikh et al. (2022) and Can and Hamilcıkan (2019) found a statistically significant positive correlation between bilirubin and eosinophil levels before PT.

In our study, following phototherapy, the patients who received more sources of LED PT had higher levels of eosinophils, although not significantly. This aligns with the study by Beken et al. (2014). The fact that LED PT emits a larger amount of irradiance at a longer wavelength might account for this situation. As a limitation of our study, the effects of LED and conventional PT on eosinophil levels were not compared. Indeed, conventional PT is not used in our unit anymore [12].

Conclusion

In conclusion, serum eosinophils increased after phototherapy in term neonates with hyperbilirubinemia. As eosinophils are considered inflammatory markers in the asthma pathway, icteric neonates treated with phototherapy are prone to developing asthma in childhood .

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Authors' contributions
DS collected the data. ST and SA analyzed and interpreted the data. DM, ND, and OA supervised and revised the work and SA contributed to writing the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials
The data sets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
Research ethics committee, Faculty of Medicine, Cairo University, Registration no.: (approval code: MD-208-2020). This study was approved by the Ethical Research Committee of the Faculty of Medicine at Cairo University in Egypt. The ethics committee reference number is not available. Verbal consent was taken from the legal guardians of all patients accepting to participate in our research work. Written approval was taken from the ER for asking patients by questionnaire. Also, the study was approved by the ethics committee of Cairo University Children's Hospital. The study has been performed in accordance with the ethical standards laid down in the Helsinki Declaration of 1975 and its late amendments. All methods were carried out in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards.

Consent for publication
All patients included in this research gave written informed consent to publish the data contained within this study. All authors read and approved the final manuscript.

Competing interests
The authors declare that they have no competing interests.

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