Effects of Vitamin E on Neonatal Hyperbilirubinemia in Preterm Newborns

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Abstract

Background: Neonatal jaundice indicates the presence of pigment in the skin and sclera. Vitamin E is an important component of the cellular antioxidant defense system. Here in the present study, we aimed to evaluate and investigate these therapeutic effects.

Materials and Methods: This is a randomized clinical trial performed on 100 premature neonates. Group 1 received 10 units of Vitamin E daily for 5 days. The other group received placebo. Amount of bilirubin was measured at the time of 0, 24, 48, 72, and 96 h after birth.

Results: Mean bilirubin in Vitamin E group was increasing until the 2nd day and then got a decreasing trend. In the control group, the increasing trend of bilirubin was going on till the 3rd day. Mean bilirubin increased significantly during the follow-up in both Vitamin E and control groups ($\chi^2$ [df] = 20.23 (1), $P < 0.001$). Although both groups showed an increasing trend in mean bilirubin, on the last day of follow-up, the average amount of increase was lower in Vitamin E group (5.06 ± 2.25 vs. 6.23 ± 3.98). Also in the 3rd and 4th days, mean bilirubin was lower in Vitamin E group.

Conclusion: This study supports the usage of oral Vitamin E therapies on reducing the bilirubin levels in neonates. We also showed that this reduced trend occurs after day 3 of life, but in the follow-ups, neonates who were treated with Vitamin E had lower bilirubin levels compared to the placebo group.

Keywords: Antioxidant, neonatal hyperbilirubinemia, total serum bilirubin, transcutaneous bilirubin, Vitamin E

INTRODUCTION

Jaundice refers to the accumulation of yellow bilirubin pigment in the skin and sclera and is one of the most common neonatal diseases.[1] Jaundice is a natural phenomenon that occurs in most infants who have had a full-term fetus and in almost all premature infants.[2] Neonatal jaundice indicates the presence of pigment in the skin and sclera. However, this phenomenon is correlated with hyperbilirubinemia.[3] This transient phenomenon is usually harmless and may play a physiological role in development, but in some conditions, the presence of bilirubin outside the peripheral circulation can be harmful, such as the accumulation of bilirubin in the brain, which could also lead to permanent neurological dysfunctions such as kernicterus.[4,5] Neonatal jaundice syndrome is caused by an imbalance between the production and elimination of bilirubin (bilirubin metabolism), which is temporarily exacerbated after birth and the embryonic stage.[6]

Based on previous studies, evolutionary and clinical features and the rate of jaundice progression in premature infants are different from term and late preterm infants.[7] These studies...
have indicated an increased bilirubin production in premature infants due to increased hemolysis.\cite{9} Bilirubin excretion in preterm infants is also reduced due to decreased ligand and decreased uridine 5'-diphospho-glucuronosyltransferase activity in hepatocytes.\cite{9} Increases in bilirubin enterohepatic cycle might also contribute to these mechanisms due to delayed feeding in premature infants.\cite{10} As a result, bilirubin levels are higher in preterm infants due to increased production and decreased excretion. Experimental data also indicated that the blood–brain barrier is more permeable to bilirubin in premature infants. The ability of brain cells to metabolize bilirubin also increases with fetal development. As a result, most treatment guidelines recommend initiating phototherapy at lower total serum bilirubin (TSB) concentrations in premature neonates.\cite{11}

Vitamin E is a fat-soluble compound that was isolated in 1936 and is also called alpha-tocopherol. This vitamin plays a significant role in the natural metabolism of all cells and acts in conjunction with many other nutrients and endogenous factors which could form a protective system against the destructive effects of reactive oxygen species.\cite{12} Vitamin E is an important component of the cellular antioxidant defense system and is also involved in other antioxidant enzyme systems, such as superoxide dismutase, glutathione peroxidase, glutathione reductase, catalase, and thioredoxin reductase.\cite{13} Studies have also shown that Vitamin E is one of the strongest fat-soluble biological antioxidants and has a protective effect against hemolysis by stabilizing the erythrocyte membrane and inhibiting the phospholipid peroxidation of the membrane.\cite{14}

The first stage of bilirubin metabolism, which is characterized by the breakdown of hemoglobin and the production of water-insoluble bilirubin, plays an important role in the process of increasing bilirubin and creating jaundice.\cite{15} Recent studies have shown that lipid autoxidation at the level of red blood cells is a possible mechanism for hemolysis and increased bilirubin production.\cite{16} On the other hand, Vitamin E deficiency is known to be a major factor in increasing peroxidase-induced hemolysis.\cite{17} From a clinical point of view, Vitamin E seems to be able to prevent physiological hemolysis and decrease bilirubin production by reducing the sensitivity of red blood cells to autoxidation processes. Different clinical experiments have indicated that decreased Vitamin E levels in neonates are correlated with increased serum bilirubin.\cite{18,19} However, very few studies have investigated the therapeutic effects of Vitamin E on jaundice among neonates. Here in the present study, regarding the importance of jaundice in premature neonates and regarding the possible therapeutic effects of Vitamin E on lowering the bilirubin levels, we aimed to evaluate and investigate these therapeutic effects.

**Materials and Methods**

This is a randomized clinical trial with a registration number IRCT code (IRCT20150423021910N4) performed on 100 premature neonates in Al-Zahra and Beheshti hospitals (affiliated to Isfahan University of Medical Sciences) in 2019–2020. This article was extracted from a research project conducted at Isfahan University of Medical Sciences, Isfahan, Iran (code: IR.MUI.REC.1396.071). Study Review Board of the university approved the written protocol and informed consent was obtained from all the parents prior to the study.

The inclusion criteria were premature neonates with 34–37 weeks of gestational age, neonates that are fed only by breastfeeding, and Apgar score more than 7 in 5 min after birth. Patients with a diagnosis of sepsis by the time of recruitment, presence of any abdominal disorders including distension, and biliary secretion did not enter the study. Our exclusion criteria were lack of regular follow-ups, diagnosis of sepsis or systemic diseases during the study, antibody-mediated hemolysis (positive direct Coombs test), enzyme deficiency-related hemolysis (such as glucose-6-phosphate dehydrogenase [G6PD] deficiency), any hemolysis due to red blood cell deformities including spherocytosis, and elliptocytosis.

A total number of 100 neonates were recruited. Informed consent was also obtained from parents. Demographic data of neonates including sex and gestational age were noted. Neonates were divided randomly into two groups (50 newborns in each group). Transcutaneous bilirubin (TCB) was measured for each neonate at the time of birth and 10 units of Vitamin E were administered for intervention group as 1 cc of Vitamin E syrup manufactured by Behsa Co. Neonates in the intervention group and control group received 1 cc (10 units) of Vitamin E and 1 cc of placebo (sterile water in the same box as Vitamin E produced by hospital pharmacy) daily for 5 days, respectively.

Amount of bilirubin was measured by an experienced nurse who was also unaware of intervention and control groups. These measurements were performed at the time of 0, 24, 48, 72, and 96 h after birth. Phototherapy was also administered based on protocols for neonates if required. We should also note that serum bilirubin, G6PD deficiency, direct Coombs, and Retic were measured in the following situations:

1. When the number obtained in the transcutaneous bilirubinometer method is higher than 75% of the Bhutani curve
2. When the number obtained in the transcutaneous bilirubinometer method is higher than 95% of the TCB nomogram curve
3. When the number obtained by transcutaneous bilirubinometer is in treatment initiation score recommendations based on the American Academy of Pediatrics tables (in this case, treatment is started and at the same time, a serum sample is sent to determine the exact amount of serum bilirubin for treatment continuation)
4. When the treatment plan is changed by adding 3 to the amount obtained in the transcutaneous bilirubinometer method
5. When the number obtained by transcutaneous bilirubinometer is 70% or higher than the recommended serum bilirubin level to begin treatment
6. In cases after discharge whenever the number obtained in the transcutaneous bilirubinometer is higher than 13.

If the above indications occur, the complementary tests will be sent and treatments of the patient will continue according to the protocols of the American Academy of Pediatrics. In the current study, information regarding age; weight; TCB at 0, 24, 48, 72, and 96 h after birth; need for phototherapy; duration of phototherapy; neonatal blood pressure; complications; and results of other tests were collected.

**Statistical analysis**

Differences between the control and intervention group in in term infants’ characteristics and serum bilirubin levels at baseline were tested using Student’s *t*-tests and Chi-squared tests for continuous and categorical data, respectively. Mean bilirubin changes in the Vitamin E group were compared with control group using the generalized estimating equation (GEE) modification of linear regression to account for intra-individual correlation over time. The models were adjusted for baseline serum bilirubin. The GEE approach for mixed-model regression using the available data was applied to address missing data. The intervention effect on the outcome was examined based on the assigned treatment at randomization, regardless of adherence or study retention (i.e., intention-to-treat). Statistical analyses were performed using SPSS version 22.0 (SPSS Statistics, Armonk, NY, USA, IBM Corp., 2013).

**RESULTS**

In the current study, 100 infants were assessed. Two groups were similar regarding gender distribution (*P* = 0.545). The mean age was 35.81 ± 0.99 days and not significantly different in the intervention and control group (*P* = 0.245). The mean weight was 2600.55 ± 344.68 and not significantly different between groups (*P* = 0.903). The baseline mean of serum bilirubin was not different between Vitamin E and control group (4.64 ± 2.08 vs. 4.52 ± 2.30, *P* = 0.785) [Table 1].

| Variable          | Vitamin E (n=50) | Control (n=50) | Total (n=100) | *P*   |
|-------------------|-----------------|---------------|--------------|-------|
| Gender            |                 |               |              |       |
| Girl              | 23 (46.0)       | 20 (40.0)     | 43 (43.0)    | 0.545 |
| Boy               | 27 (54.0)       | 30 (60.0)     | 57 (57.0)    |       |
| Age (day)         | 35.93±0.99      | 35.70±1.05    | 35.81±0.99   | 0.245 |
| Baseline bilirubin| 4.64±2.08       | 4.52±2.30     | 4.58±2.19    | 0.785 |
| Weight (g)        | 2604.80±287.97  | 2596.30±396.32| 2600.55±344.68| 0.903 |

*a* (%), *b* Mean±SD. *P* values resulted from Pearson’s Chi-square test or independent samples *t*-test. SD: Standard deviation

Regarding the significant interaction effect of trial groups and days of follow-up, there was a significant difference in the pattern of change in bilirubin level between two groups ($\chi^2$ [df] = 3.81 (1), *P* = 0.050). Although both groups showed an increasing trend in mean bilirubin, on the last day of follow-up, the average amount of increase was lower in Vitamin E group (5.06 ± 2.25 vs. 6.23 ± 3.98). Also in the 3rd and 4th days, mean bilirubin was lower in the Vitamin E group [Table 2].

According to the Fisher exact test, the need for TSB measurement was 18% and 26% in Vitamin E group and control group, respectively (*P* = 0.065). Also according to the Likelihood ratio test, the need for phototherapy was 8% and 22% in the Vitamin E group and control group, respectively (*P* = 0.046) [Table 4].

Figure 1 visualizes mean bilirubin at baseline and the days after intervention in both groups. Mean bilirubin in Vitamin E group is increasing until the 2nd day and then gets a decreasing trend. In the control group, the increasing trend of bilirubin is going on till the 3rd day. For a better comparison between groups, Table 2 represents the mean of bilirubin level as well as the mean difference between intervention and control group at each study point. Changes from baseline in the days of follow-up are provided, too.

Table 3 represents the effect of Vitamin E on bilirubin level during follow-up in a GEE modification of linear regression. The results were adjusted for baseline bilirubin values. Mean bilirubin increased significantly during the follow-up in both Vitamin E and control groups ($\chi^2$ [df] = 20.23 (1), *P* < 0.001). Overall, the effect of Vitamin E was not significantly different compared to controls ($\chi^2$ [df] = 3.21 (1), *P* = 0.073) [Table 3].

![Figure 1](image_url)
Table 2: Bilirubin level changes during the follow-up

| Days   | Vitamin E | Control | SMD (95% CI) | Absolut mean difference (95% CI) |
|--------|-----------|---------|--------------|----------------------------------|
|        | n         | Bilirubin (mg) | Change from baseline | n | Bilirubin (mg) | Change from baseline |          |
| 0 (baseline) | 50 | 4.64±2.08a |                   | 50 | 4.52±2.30 |                   | 0.06 (−0.34−0.45) |
| 1      | 50 | 8.31±1.78 | 3.67±2.60       | 50 | 8.32±2.42 | 3.80±1.92       | −0.01 (−0.40−0.39) |
| 2      | 50 | 10.47±1.80 | 5.82±2.44       | 49 | 9.57±2.52 | 5.04±2.89       | 0.41 (0.01−0.81) |
| 3      | 47 | 10.35±1.47 | 5.74±2.46       | 47 | 10.67±3.27 | 6.30±3.67       | −0.13 (−0.53−0.28) |
| 4      | 39 | 9.61±1.38  | 5.06±2.25       | 37 | 10.55±3.37 | 6.23±3.98       | −0.37 (−0.83−0.08) |

Entries are mean±SD. Absolut mean difference=absolut changes from baseline between Vitamin E and control groups. SMD: Standardized mean difference, CI: Confidence interval, SD: Standard deviation

Table 3: Results of generalized estimating equation modification of linear regression measuring bilirubin change across intervention study time points

| Variable                  | β (SE) | 95% CI | Wald χ² (df) | P     |
|---------------------------|--------|--------|-------------|-------|
| Group                     |        |        |             |       |
| Vitamin E                 | 0.96 (0.54) | −0.019−2.01 | 3.21 (1) | 0.073 |
| Control                   | 0.99 (0.20) | 0.51−1.31 | 20.23 (1) | <0.001|
| Day                       | 0.00 (0.08) | −0.09−0.41 | 9.70 (1) | 0.002 |
| Day × Group               | −0.46 (0.23) | −0.91−0.002 | 3.81 (1) | 0.050 |
| Baseline bilirubin        | 0.00 (0.08) | 0.00−0.19 |              |       |

β: Coefficient regression, Df: Degree of freedom, CI: Confidence interval, SE: Standard error

Table 4: Result of Fisher’s exact test and likelihood ratio test for total serum bilirubin measurement and phototherapy

| Variable                  | Case | Control | Total | P     |
|---------------------------|------|---------|-------|-------|
| TSB check                 |      |         |       |       |
| No need for serum bilirubin check | 41 (82.0) | 37 (74.0) | 78 (78.0) | 0.065a |
| Serum bilirubin check at h 24 | 1 (2.0) | 1 (2.0) | 2 (2.0) |       |
| Serum bilirubin check at h 48 | 6 (12.0) | 2 (4.0) | 8 (8.0) |       |
| Serum bilirubin check at h 72 | 2 (4.0) | 7 (14.0) | 9 (9.0) |       |
| Serum bilirubin check at h 96 | 0     | 3 (6.0) | 3 (3.0) |       |
| Phototherapy              |      |         |       |       |
| No                        | 46 (92.0) | 39 (45.9) | 85 (85.0) | 0.046a |
| Yes                       | 4 (8.0) | 11 (22.0) | 15 (15.0) |       |

aMean±SD, b n (%), c Fisher’s exact test, d Likelihood ratio test. TSB: Total serum bilirubin, SD: Standard deviation

Discussion

In the present study, 100 infants were assessed and we showed that the mean bilirubin levels increased in Vitamin E group until the 2nd day and then showed a decreasing trend afterward. In the other group, the trend of bilirubin increased until the 3rd day. Furthermore, we showed that the mean bilirubin levels increased significantly during the follow-up in both Vitamin E and control groups and we also noted that the effect of Vitamin E was not significantly different compared to controls. On the other hand, both groups showed an increasing trend in mean bilirubin, but on the last day of follow-up, the average amount of increase is lower in Vitamin E group, and in the 3rd and 4th days mean bilirubin was lower in Vitamin E group.

There have been also some studies, evaluating the effects of different agents including Vitamin E on bilirubin levels. Two randomized trials were performed evaluating the effects of Vitamin E on bilirubin levels in neonates. In a study by Fischer et al. in 1987, they showed that supplement therapy with Vitamin E has no major effects on bilirubin levels during the first 3 days of life, especially in premature neonates, but afterward, they observed a significant decline in bilirubin levels.[20] Smith et al. also showed that the bilirubin production was not significantly different on day 3 in both neonates receiving Vitamin E supplements and placebo, but a significant decline was reported in bilirubin production in neonates who received Vitamin E by day 7 of age.[21] Another recent study was performed by El Mashad et al. on 150 full-term neonates and concluded that the levels of bilirubin decreased following Vitamin E treatments compared to placebo. They also showed that there was no correlation between age and body weight in bilirubin decline in all groups. We should also note that they measured TCB levels in all cases. They also suggested that the addition of oral Vitamin E 25 mg/kg/day to phototherapy in the treatment of indirect hyperbilirubinemia is better than oral Vitamin E 50 mg/kg/day or phototherapy alone.[22] These data are in line with the findings of our study. We also found an increasing trend of bilirubin until the 3rd day, but afterward, the levels of bilirubin decreased significantly. We also showed that the decreased trend in bilirubin levels was greater in the neonates who were treated with Vitamin E.

Another review study was conducted by Westergren and Kalikstad in 2010 on the effectiveness of Vitamin E supplement therapy in children. They showed that treatments with Vitamin E are useful in childhood and are associated with decreased bilirubin levels in neonates, but they also suggested that comparative studies should be carried out to determine the proper dosage for treatments.[23] Another study was performed by Kwak et al. in 2012 on 17348 subjects. They showed that serum bilirubin levels are inversely associated...
with the prevalence of nonalcoholic fatty liver disease. Usage of supplements, especially antioxidants against fatty liver disease and hyperbilirubinemia, has been supported in different lines of evidence. Abdul-Razzak et al. evaluated 196 live birth neonates. They showed that hyperbilirubinemia in neonates with G6PD deficiency is almost greater than other healthy neonates and they also showed that the levels of Vitamin C and E were decreased in this group. We believe that Vitamin E treatments are useful and beneficial in lowering the bilirubin levels, but these effects occur after day 3 of life.

Some previous studies have also supported the use of oral supplements and antioxidants for neonatal hyperbilirubinemia, but very few studies compared the results of Vitamin E therapies. In the current study, we showed that usage of Vitamin E is beneficial and associated with decreased bilirubin levels. This was in line with most of the previous studies, but we also believe that more studies on larger populations and also neonates with special medical conditions should be performed.

**Conclusion**

This study supports the usage of oral Vitamin E therapies on reducing the bilirubin levels in neonates. We also showed that this reduced trend occurs after day 3 of life, but in the follow-ups, neonates who were treated with Vitamin E had lower bilirubin levels compared to the placebo group. These results were in line with the finding of most previous studies; however, we believe that more studies on larger populations should be performed.

**Limitations**

One of the limitations of this study was related to the sample size which was small to be generalizable to the whole community. Another limitation was the lack of measuring Vitamin E level before starting the program because some neonates might have appropriate levels of Vitamin E and it was more advisable to prescribe Vitamin E in neonates with lower levels of Vitamin E. Further studies are needed to investigate the factors that potentially affect the results.

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**Conflicts of interest**

There are no conflicts of interest.

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