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Can umbilical alkaline phosfatase be used as a hemolysis marker in neonates?

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ABSTRACT

Background. The use of ALP in neonatology has been intensively studied, and associations have been demonstrated between elevated alkaline phosphatase levels and hypocalcemia and hypophosphatemia in premature infants, the onset of early osteopenia and the occurrence of necrotizing enterocolitis.
Objective. The current study aimed to verify if there is any kind of statistical correlation between elevated alkaline phosphatase levels in blood from the umbilical cord and the presence of signs of hemolysis.
Material and methods. The study included 250 term newborns that met all the inclusion criteria. Alkaline phosphatase was determined in the umbilical cord serum together with hemoglobin and hematocrit.
Results. There was no statistically significant difference between the median hematocrit and hemoglobin values correlated with elevated alkaline phosphatase values for the two groups – with and without phototherapy.
Conclusion. In order to build on the correlations of alkaline phosphatase values with hemolytic markers, we need more time to conduct research and larger patient groups.

Keywords: alkaline phosphatase, hemolysis, hemoglobin, hematocrit

INTRODUCTION

Alkaline phosphatase is a hydrolase that removes phosphate groups from many molecules and is present in almost every cell in the body, including erythrocytes, bone, intestine and kidneys. During pregnancy it is secreted by the placenta (1). Studies have shown the role of the enzyme in the active transport of phosphates, the absorption of nutrients through the plasma membrane (2,3), the transfer of glucose and fatty acids through the cell membrane (4). Alkaline phosphatase has been used as a biomarker for bone metabolic disease, some cancers such as prostate cancer, and myocardial infarction (5,6). In intrauterine life it has been shown that alkaline phosphatase contributes to maintaining the health of the fetus being involved in defense against toxic substances and nutrient metabolism.

High values of the enzyme predispose the newborn to the appearance of other complications such as jaundice, bone metabolic disease, ulcer-necrotic enterocolitis.

Neonatal hemolytic disease occurs through the incompatibility between the group and the Rh of the mother with that of the newborn. In the AB0 system it occurs mainly in newborns with group A or B, from mothers with blood group 0. It has been shown that the risk of recurrence is 88% in mothers who had their first child with hemolytic disease (7). The incompatibility in the AB0 system is less severe than in the Rh system.

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Alloimmunization in the Rh system occurs when the blood of the newborn with Rh positive passes through the placenta and enters the circulation of the mother with Rh negative. The initial response of the foreign antigen in the maternal circulation is for the maternal immune system to produce Ig M-type antibodies that do not cross the placenta. This response is followed by the production of IgG-type antibodies, which then cross the placental barrier and cause fetal and neonatal complications (8).

Fetal complications are represented by anemia and fetal hydrops that appeared after week 34 of gestation, and neonatal complications are anemia and neonatal hemolytic disease that is associated with severe hyperbilirubinemia.

In Rh incompatibility, about 50% of newborns do not require treatment and 25-30% require treatment with phototherapy. It has also been shown that newborns with hemolytic disease have a higher risk of developing bilirubin encephalopathy than those with similar levels of bilirubin but who do not associate incompatibility in the Rh system (9).

Hyperbilirubinemia in neonatal hemolytic disease is severe and if left untreated will be associated with severe complications.

Incompatibility of small blood groups, although a less common cause of neonatal hyperbilirubinemia, has the potential to cause severe hyperbilirubinemia and its sequelae in newborns if left undiagnosed and untreated.

The frequency of neonatal hemolytic disease and indirect hyperbilirubinemia due to Rh sensitization has decreased with the widespread use of anti-D gamma globulin. Therefore, the contribution of incompatibility of small blood groups other than Rh (D) antigen, such as Kell, c, C, E, it gradually increased in the hemolytic disease of the newborn (10,11). The prevalence of erythrocyte antibodies, other than anti-D, which can induce hemolytic disease in the newborn is about 1 in 500 pregnancies (12). Anti-c is usually described as the next most common cause of severe hemolytic disease in the newborn after anti-D (13). More and more cases of incompatibility of small blood groups are now diagnosed due to advances in investigative methods.

Uncommon causes of neonatal hemolytic disease include antibodies directed against Kell blood group antigens (e.g., anti-K and anti-k), Kidd blood group (e.g., anti-Jka, and anti-Jkb), group blood Duffy (eg, anti-Fya), and blood group antibodies MNS and s. Until now antibodies directed against blood groups P and Lewis have not been associated with the hemolytic disease of the newborn.

AIM

The aim of the present study was to verify whether there is a statistical association between the values of alkaline phosphatase collected from the umbilical cord at birth and the values of hemoglobin and hematocrit collected at the same time.

MATERIAL AND METHOD

The study was conducted in Filantropia Clinical Hospital, Bucharest, Romania in the first semester of 2019. The study included 250 full-term newborns born spontaneously or extracted by cesarean section who met the inclusion criteria.

Newborns included in the studies were selected on the basis of strict inclusion and exclusion criteria. All admitted had only inclusion criteria.

Inclusion criteria: full term, normal weight (> 2,500 grams, > 37 weeks of gestation), from investigated pregnancies, without associated pathologies.

Exclusion criteria: incompatibility in Rh or AB0 system, known hemolytic diseases, metabolic diseases, twin pregnancies.

The newborns were divided in two groups: one group that developed severe jaundice and needed phototherapy (31 newborns) and one group that didn't need phototherapy as jaundice treatment (219 newborns).

The values of hematocrit and hemoglobin were analyzed according to the values of alkaline phosphatase, as well as in relation to the need for treatment (phototherapy) for hyperbilirubinemia.

Newborns admitted in the study had a blood sample of approximately 2 ml taken from the umbilical cord from which the hemoglobin, hematocrit and alkaline phosphatase values were processed in the laboratory.

A database was compiled based on information from the neonatal observation sheets, which was analyzed using IBM SPSS 20. The significance level was considered $p < 0.05$.

RESULTS

The following table summarizes the characteristics of the patients included in the research (Table 1).

TABLE 1. Patient characteristics

	N	Minimum	Maximum	Medium +/- SD
Gestational age (weeks)	250	37	41	38.96 +/- 1.36
Weight (g)	250	2500	4050	3050.40 +/- 302.69
Alkaline phosphatase (U/l)	250	145	602	282.69 +/- 62.15

Out of the total of 250 enrolled newborns, 59.60% of the subjects (n = 149) had a spontaneous birth, while the remaining 40.40% (n = 101) were extracted by cesarean section. The radial diagram below

shows the percentage distribution of the mode of birth (Figure 1).

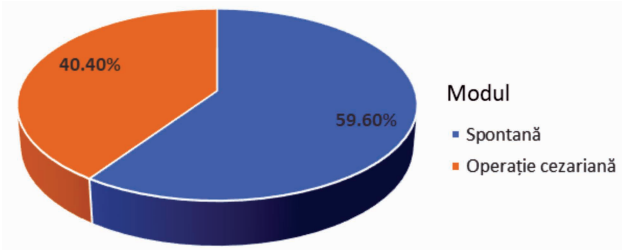

FIGURE 1. Percentage distribution according to mode of birth

The gender distribution of the subjects showed approximately equal proportions between the two sexes: 50.80% (n = 127) were female, the remaining 49.20% (n = 123) were male (Figure 2).

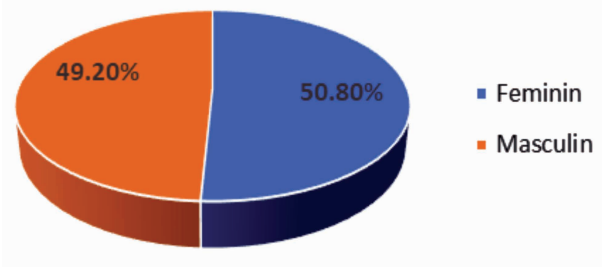

FIGURE 2. Percentage distribution by sex

Hemoglobin values ranged from 13.65 g/dl to 23.65 g/gl with a mean +/- SD of 18.34 +/- 2.26 and a median of 18.15 g/dl (Figure 3).

Subjects born at 37 weeks had hemoglobin values between 13.65 g/dl and 23.6 g/dl with a mean +/- SD of 18.10 +/- 2.27 g/dl and a median of 17.85 g/dl. Subjects born at 38 weeks had hemoglobin values between 14.15 g/dl and 22.45 g/dl with a mean +/- SD of 18.40 +/- 2.19 g/dl and a median of 18.05 g/dl. Subjects born at 39 weeks had hemoglobin values between 14.15 g/l and 22.65 g/dl with a mean +/- SD of 18.09 +/- 2.18 g/dl and a median of 17.95 g/dl. Subjects born at 40 weeks had hemoglobin values between 14.15 g/dl and 23.15 g/dl with a mean +/- SD of 18.36 +/- 2.49 g/dl and a median of 18.47 g/dl. Subjects born at 41 weeks had hemoglobin values between 14.70 g/dl and 23.05 g/dl with a mean +/- SD of 18.70 +/- 2.24 g/dl and a median of 18.55 g/dl. Depending on the distribution of hemoglobin values, the One-Way ANOVA test was chosen with subsequent post-hoc tests to make comparisons between groups and they showed that there were no statistically significant differences between the mean hemoglobin values according to gestational age (Figure 4).

Given the fact that there is a normal distribution of values, the ANOVA statistical test was chosen. It indicated that there was no linear relationship between hemoglobin and alkaline phosphatase levels (Figure 5).

In subjects who did not require phototherapy, hemoglobin values were between 13.65 g/dl and 23.65 g/dl with a mean +/- SD of 18.26 +/- 2.22 g/dl and a median of 18.15 g/dl. In those for whom phototherapy was necessary, hemoglobin values were between 14.15 g/dl and 23.05 g/dl with a mean +/- SD of 18.85 +/- 2.55 g/dl and a median of 18.45 g/dl. The distributions of hemoglobin values were non-parametric. A Mann-Whitney test showed that there was no statistically significant difference between the hemoglobin medians depending on the treatment (U = 2,930.00, p = 0.22) (Figure 6).

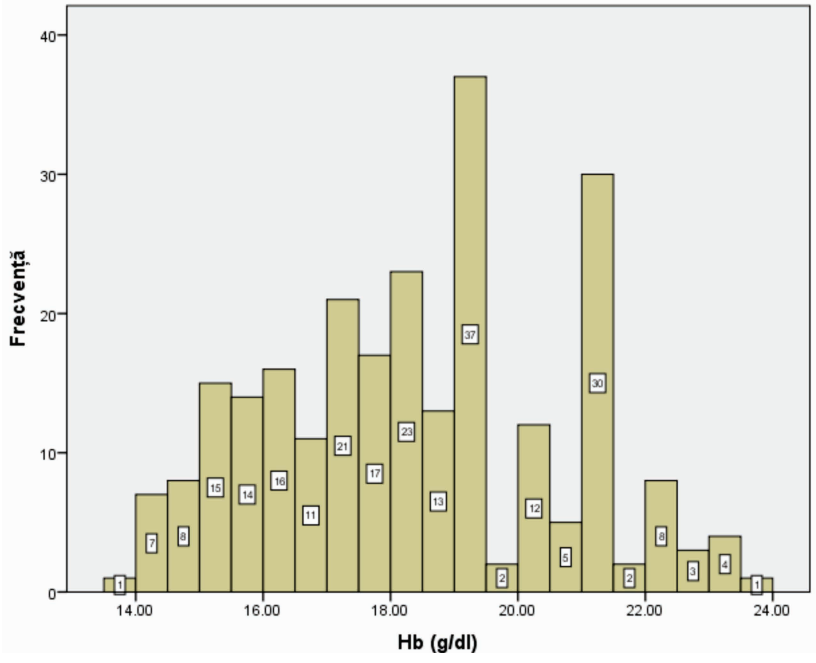

FIGURE 3. Histogram of hemoglobin values

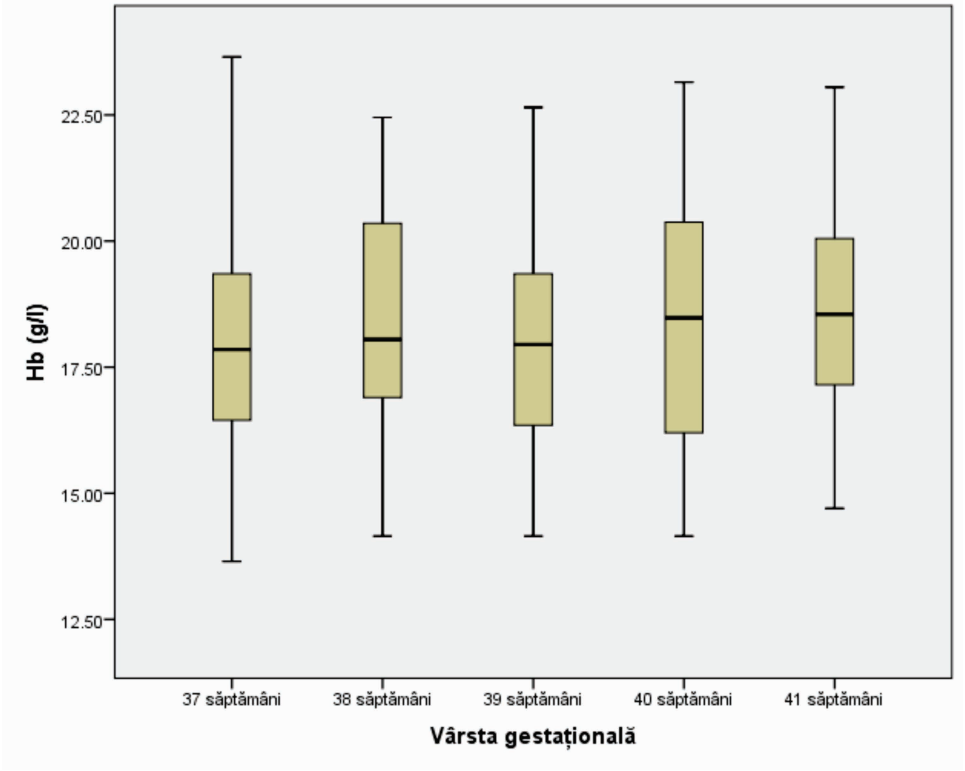

FIGURE 4. Box-plot graph for hemoglobin values by gestational age

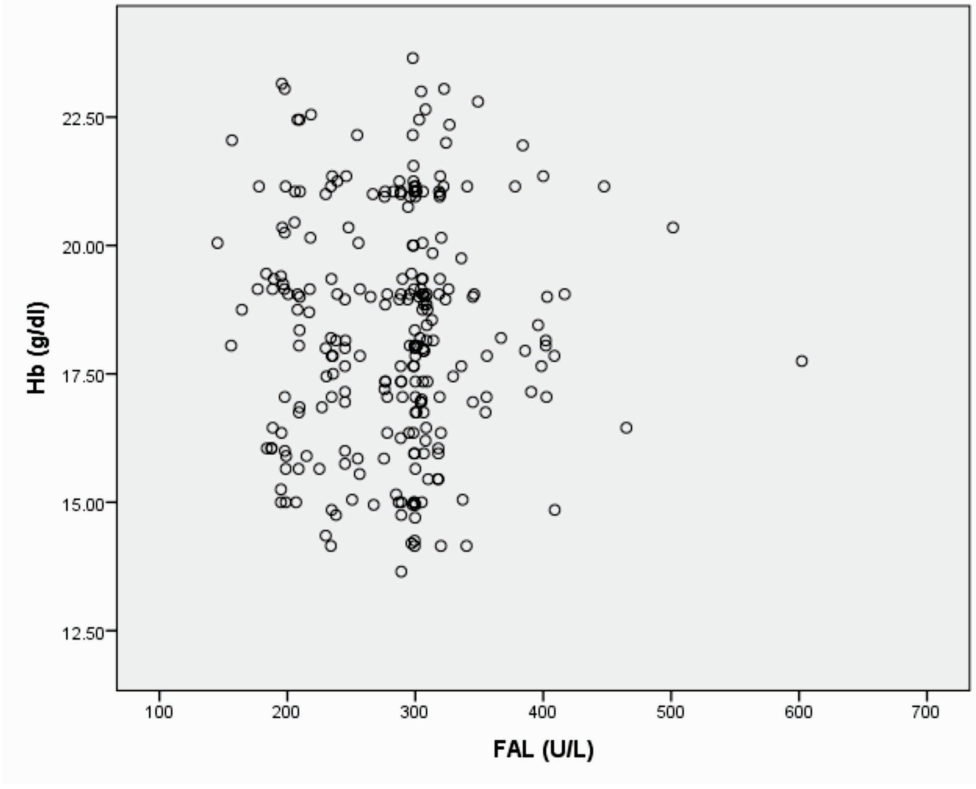

FIGURE 5. Relationship between alkaline phosphatase and hemoglobin values

The hematocrit had values between 41.90% and 67.30% with an average +/- SD of 55.37 +/- 6.09% and a median of 56.3%. The step of the histogram was 1.25%, the interval in which the highest frequency was recorded (n = 28) being between 56.25% and 57.50% (Figure 7).

There was no linear relationship between hematocrit values and alkaline phosphatase values determined from umbilical cord blood (Figure 8).

In subjects who did not require phototherapy, hematocrit values were between 41.9% and 67.30% with a mean +/- SD of 55.33 +/- 6.16% and a median

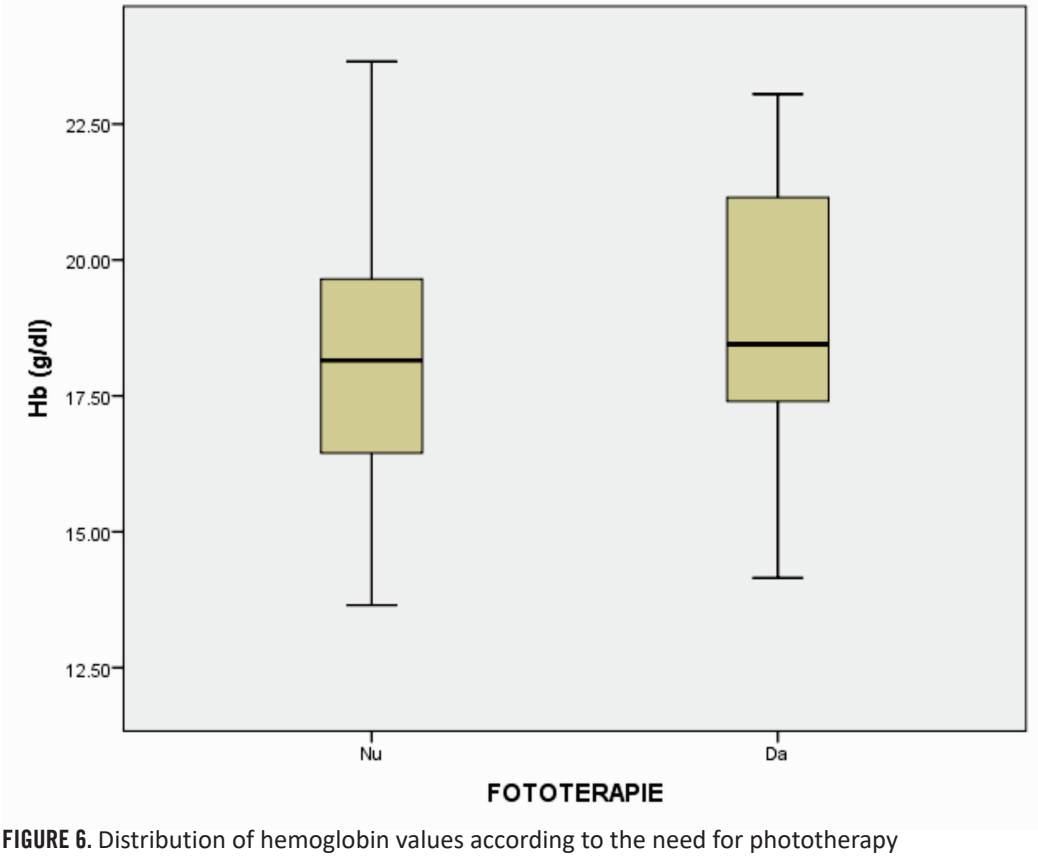

FIGURE 6. Distribution of hemoglobin values according to the need for phototherapy

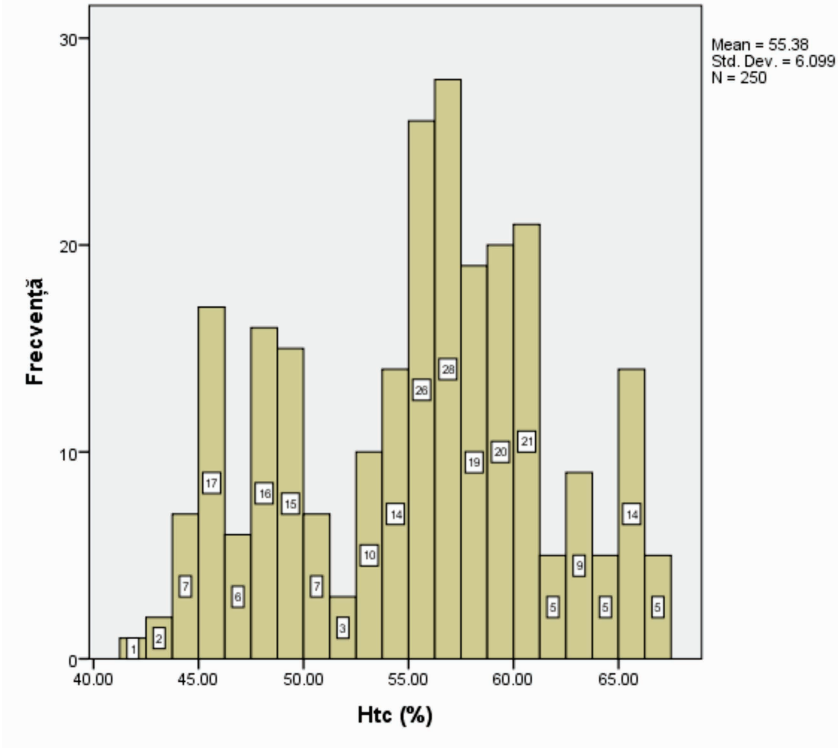

FIGURE 7. Histogram of hematocrit values

of 56.20%. In those who needed phototherapy, hematocrit values were between 44.30 and 65% with a mean +/- SD of 55.67 +/- 5.69% and a median of 56.87%. The distribution of hematocrit values was non-parametric. A Mann-Whitney test showed that there was no statistically significant difference between the median hematocrit values (U = 3,260.00, p = 0.72) (Figure 9).

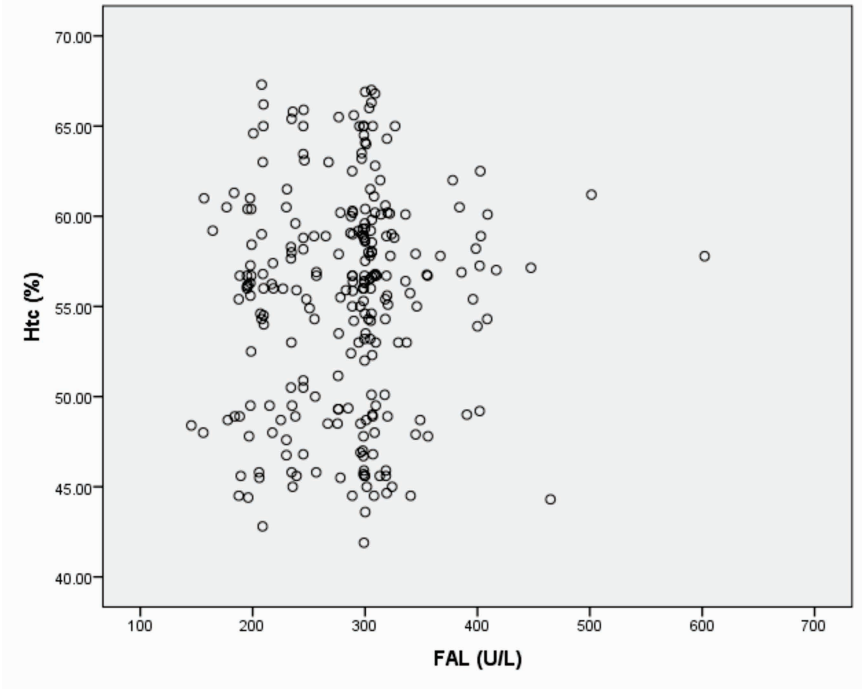

FIGURE 8. Relationship between alkaline phosphatase and hematocrit values

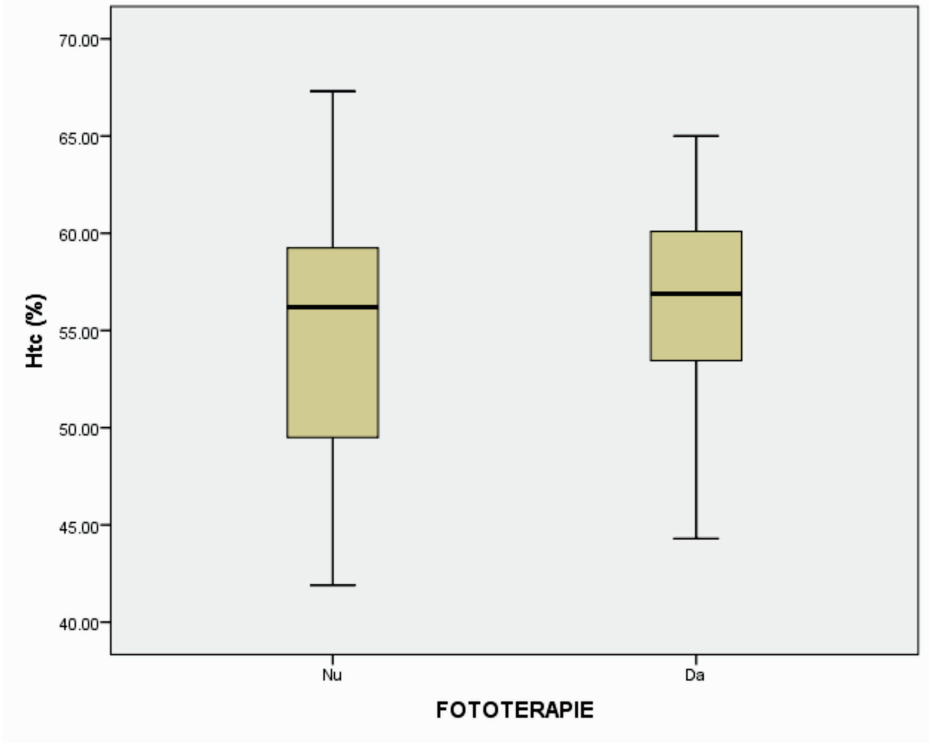

FIGURE 9. Distribution of hematocrit values according to the need for phototherapy

DISCUSSION

The most well-known late complication of hemolytic disease due to isoimmunization is the development of anemia in the first months of life. This risk may be close to 80% in cases where intrauterine transfusion has been required to treat the affected fetus (14). Thus, was born the idea of this study to associate the occurrence of neonatal anemia with alkaline phosphatase values to be used as a predictive factor.

FAL levels in umbilical cord blood in the first day of life can be used to determine the course of jaundice (15,16). Alkaline phosphatase is found in all cells, including red blood cells, and is secreted into the plasma by destroying these cells. Thus, our research aimed to associate the values of bilirubin with those of alkaline phosphatase as a marker for the occurrence of hemolysis.

In the literature, Sarici and colleagues found high levels of serum lactate dehydrogenase and α-fetoprotein (AFP) in patients with significant hemolysis (17,18). Tan and colleagues also found that the level of AFP in umbilical cord blood can also be used to predict hyperbilirubinemia (19).

Yılmaz et al. found high levels of FAL in a patient with Rh incompatibility and hAemophagus syndrome (20). El-Mauhoub and colleagues also found high levels of FAL in patients with Rh hemolytic disease, although aspartate aminotransferase and alanine aminotransferase levels were shown to be normal (21).

In our previous studies, alkaline phosphatase levels were significantly higher in patients with hyperbilirubinemia who required treatment regardless of etiology, and we previously demonstrated that it can be used as a predictive marker for the onset and progression of jaundice (22,23).

Unlike the study conducted by Nalbantoglu and collaborators (24), in our research no correlations could be made between hemoglobin and hematocrit and alkaline phosphatase values. He showed an association between low hematocrit values and high alkaline phosphatase values.

In our study, no adverse reactions in phototherapy or significant complications of jaundice were reported. Most forms have evolved favorably with the remission of the yellow coloration of the sclera and skin without the appearance of neurological sequelae or the association of other complications.

CONCLUSIONS

Our study aimed at associating alkaline phosphatase values with hemoglobin and hematocrit values. Although following the application of Mann-Whitney and One-Way ANOVA statistical tests, there was no statistically significant difference between the median hematocrit and hemoglobin values correlated with elevated alkaline phosphatase values for the two groups - with and without phototherapy. We cannot clearly conclude that they cannot associate. A slight increase in hematocrit and hemoglobin values was observed in the group that required phototherapy.

In order to build on the correlations of alkaline phosphatase values with hemolytic markers, we need more time to conduct research and larger patient groups.

Conflict of interest: none declared
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