

## Reference values of cord blood transferrin, ceruloplasmin, alpha-1 antitrypsin, prealbumin, and alpha-2 macroglobulin concentrations in healthy term newborns

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**SUMMARY:** Aliyazıcıoğlu Y, Değer O, Karahan SC, Yıldırım S, Küçüködük Ş. Reference values of cord blood transferrin, ceruloplasmin, alpha-1 antitrypsin, prealbumin, and alpha-2 macroglobulin concentrations in healthy term newborns. Turk J Pediatr 2007; 49: 52-54.

Acute phase proteins are sensitive markers of tissue necrosis and inflammatory process. These markers may be especially useful in the neonatal period, in which mortality and morbidity rates are high, because fetus and baby are subjected to numerous metabolic, genetic, physiologic and environmental injuries such as neonatal asphyxia and septicemia. The purpose of the present study was to establish normal cord blood levels of some acute phase proteins in healthy term neonates. Umbilical cord blood was obtained at the time of vaginal delivery in 60 newborn infants (30 girls, 30 boys). Specific protein concentrations were measured by nephelometric assay. Transferrin, ceruloplasmin, alpha-1 antitrypsin, prealbumin, and alpha-2 macroglobulin concentrations [arithmetic mean ( $\pm$ SD)] were found to be 199.7 ( $\pm$ 34.6) mg/dl, 14.6 ( $\pm$ 4.0) mg/dl, 160.2 ( $\pm$ 23.6) mg/dl, 11.9 ( $\pm$ 2.2) mg/dl, and 284.6 ( $\pm$ 44.4) mg/dl, respectively. Prealbumin levels for girls [12.9 ( $\pm$ 2.2)] were found to be significantly higher than those of boys [10.9 ( $\pm$ 1.8)] ( $p < 0.001$ ), while there were no significant differences between the other proteins. We conclude that these results may be used as reference values for the diagnosis of pathological conditions in newborns.

**Key words:** umbilical cord blood, transferrin, ceruloplasmin, alpha-1 antitrypsin, prealbumin, alpha-2 macroglobulin, reference values.

Blood concentration of an acute phase protein (APP) increases or decreases in response to inflammation. These proteins include prealbumin (PAB, transthyretin), ceruloplasmin (CER), haptoglobin (HPT), alpha-1 antitrypsin (AAT), transferrin (TRF), and alpha-2 macroglobulin (AMG). They are synthesized by the liver, and participate together in host defenses to nonspecific and specific mechanisms including complement system, opsonization and phagocytosis, and interaction with platelets and scavengers during inflammatory processes<sup>1</sup>.

For the early diagnosis of ante-partum and intra-partum bacterial infection, the predictive value of the acute phase reactant (APR) score using C-reactive protein (CRP), AMG and HPT in cord sera has been investigated. Some

studies support the theory that an elevated APR score can aid effectively in the early diagnosis of perinatal bacterial infection in the newborn infant<sup>2</sup>. In addition, proteins such as PAB and TRF in cord blood may be used to estimate a newborn's nutritional status<sup>3</sup> and some other proteins to indicate neonatal maturity<sup>4</sup>.

The placental barrier appears impervious to CER and HPT as shown by the very low concentrations of these two proteins at birth. CRP does not cross the placenta. A protein whose concentration increases significantly in the postnatal period is said to be actively synthesized by the infant<sup>5</sup>.

Our aim was to establish reference ranges for the levels of TRF, CER, AAT, PAB, and AMG in the umbilical cord blood of newborn babies at term.

## Material and Methods

Neonates born with gestational ages between 38 to 41 weeks in Farabi Hospital, Karadeniz Technical University were included in the study. Criteria for exclusion from the study were as follows: infants with any perinatal or prenatal history of infection, malformation, respiratory distress syndrome or perinatal asphyxia (Apgar score of  $\leq 7$  at five minutes as evaluated by a pediatrician) and those mothers having gestational diabetes, pregnancy-induced hypertension, eclampsia or preeclampsia, prolonged rupture of membranes, or receiving any steroid. Sixty newborn infants (30 girls, 30 boys) meeting the inclusion criteria were enrolled in the study. Umbilical cord blood was obtained at the time of vaginal delivery in 60 healthy newborn infants (30 girls, 30 boys) admitted to Farabi Hospital, Karadeniz Technical University. All samples were collected by venipuncture from the fetal side of the placenta immediately after delivery at term. The birth weights of infants ranged between 2800 and 4200 g (mean  $\pm$  SD: 3578  $\pm$  458.7g). They were followed for at least three days after birth and there was no evidence of any pathology such as infection or hyperbilirubinemia requiring exchange transfusion. Informed consent was obtained from the parents of the children.

The cord blood samples were centrifuged immediately, and the serum was then kept at  $-70^{\circ}\text{C}$  until analyzed. Specific protein concentrations were measured by nephelometric assay, which is based on the detection of the light scattering by the antigen-antibody complex in the medium (Beckman, Array 360, USA). Their values were assigned with reference to the reference values of the International Federation of Clinical Chemistry (IFCC). Within-run coefficient of variations (CVs) of all the parameters was less than 4%.

## Statistical Analyses

For statistical analysis, 5<sup>th</sup> and 95<sup>th</sup> percentiles and the arithmetic means including standard deviations (SD) were calculated for all specific proteins according to sex. Values for all proteins showed a normal distribution by Kolmogorov-Smirnov test. Difference between sexes was found to be significant by Student's t test.

## Results

Table I shows the arithmetic mean ( $\pm$ SD) and 5<sup>th</sup> and 95<sup>th</sup> percentiles of cord blood TRF, CER, AAT, PAB, and AMG levels for 30 girls and 30 boys (60 newborns in total). PAB levels in girls were found to be significantly higher than

**Table I.** Mean Concentration [X( $\pm$ SD)] and Reference Intervals (5<sup>th</sup>–95<sup>th</sup> Percentiles) of Cord Blood Specific Proteins

| Parameter mg/dl           |              | Girls n:30         | Boys n:30          | Total n:60         |
|---------------------------|--------------|--------------------|--------------------|--------------------|
| Transferrin               | X( $\pm$ SD) | 195.7( $\pm$ 37.7) | 203.6( $\pm$ 31.8) | 199.7( $\pm$ 34.6) |
|                           | 5%           | 147.2              | 129.8              | 139.0              |
|                           | 95%          | 268.6              | 289.0              | 262.0              |
| Ceruloplasmin             | X( $\pm$ SD) | 15.1( $\pm$ 4.2)   | 14.0( $\pm$ 3.7)   | 14.6( $\pm$ 4.0)   |
|                           | 5%           | 8.6                | 7.9                | 8.1                |
|                           | 95%          | 24.2               | 20.9               | 22.2               |
| $\alpha$ -1 Antitrypsin   | X( $\pm$ SD) | 158.9( $\pm$ 25.3) | 161.6( $\pm$ 22.1) | 160.2( $\pm$ 23.6) |
|                           | 5%           | 103.8              | 124.0              | 124.0              |
|                           | 95%          | 202.6              | 205.8              | 203.8              |
| Prealbumin                | X( $\pm$ SD) | 12.9( $\pm$ 2.2)*  | 10.9( $\pm$ 1.8)   | 11.9( $\pm$ 2.2)   |
|                           | 5%           | 10.2               | 7.9                | 8.1                |
|                           | 95%          | 17.6               | 13.8               | 17.1               |
| $\alpha$ -2 Macroglobulin | X( $\pm$ SD) | 291.1( $\pm$ 39.4) | 278.0( $\pm$ 48.7) | 284.6( $\pm$ 44.4) |
|                           | 5%           | 201.1              | 173.2              | 193.0              |
|                           | 95%          | 357.2              | 342.1              | 341.0              |

\*Significantly different from boys at  $p < 0.001$ .

in boys ( $p < 0.001$ ). No significant difference in TRF, CER, AAT, and AMG concentrations was observed between sexes.

## Discussion

Acute phase proteins show an increase in plasma concentration in the case of acute inflammatory reactions, which is related to the activity of the inflammatory response. In organ dysfunctions without inflammation, an increase in APPs does not occur. The determination of APPs is therefore an important criterion for the differentiation between inflammatory and functional organ involvement<sup>1</sup>.

Acute phase proteins in infancy are of special interest because changes in their levels are helpful for diagnosing and following up the course of systemic infections<sup>6-8</sup> and for assessing intrauterine and postnatal status<sup>3,9</sup>.

Various biochemical markers have been studied to determine systemic fetal inflammation or umbilical cord inflammation<sup>10,11</sup>. Especially cord blood PAB concentration related to intrauterine growth was studied for nutritional status. This protein could potentially be used as a marker to document adverse nutritional effects on the fetus resulting from perinatal factors causing placental dysfunction<sup>12,13</sup>.

We established reference ranges for some of the specific proteins advocated for an indication of nutritional status (PAB and TRF) and infection status (CER, AAT, and AMG).

A limited number of studies were found regarding reference ranges for the above-mentioned specific proteins in cord blood. Pittard et al.<sup>12</sup> reported that the mean cord blood PAB concentration was  $10.7 \pm 3.7$  mg/dl and Sasanow et al.<sup>13</sup> reported it as  $12.0 \pm 3.9$  mg/dl. Our mean PAB level was close to the above results ( $11.9 \pm 2.2$  mg/dl). In addition, we found that PAB levels were higher in girls than in boys. It has been shown that the levels of gonadotropins in umbilical cord vein were significantly higher in males than in females<sup>14</sup>. The different levels of PAB in males and females might be speculated to result from hormonal changes in both sexes. Cord blood CER reference interval was reported to be 5-33 mg/dl by Thomas<sup>15</sup>. Except for that CER reference interval, cord blood specific proteins levels were lower than those of newborn serum levels, especially for upper reference limits<sup>16</sup>.

In conclusion, our results of acute phase proteins of cord blood may be used as reference values for both sexes. The low level of prealbumin and high levels of the other acute phase proteins should alert us to give more attention to the monitorization of newborns.

## REFERENCES

1. Thomas L. Inflammatory reaction. In: Thomas L (ed). Clinical Laboratory Diagnostics. Frankfurt: TH-Books; 1998: 689-697.
2. Sakaida H, Imanishi H, Kitamura T. Predictive value of acute phase reactants (APR)-score for perinatal infections. Nippon Sanka Fujinca Gakkai Zasshi 1983; 35: 929-933.
3. Raubenstine DA, Ballantine TV, Greecher CP, Webb SL. Neonatal serum protein levels as indicators of nutritional status: normal values and correlation with anthropometric data. J Pediatr Gastroenterol Nutr 1990; 10: 53-61.
4. Warner A, Jang R. Biochemical measurement in cord blood as an indicator of neonatal maturity. Ann Clin Lab Sci 1990; 20: 398-408.
5. Gitlin D, Kumate J, Urrusti J, Morales C. The selectivity of the human placenta in the transfer of plasma proteins from mother to fetus. J Clin Invest 1964; 43: 1938-1946.
6. Sann L, Bienvenu F, Bienvenu J, Bourgeois J, Bethenod M. Evolution of serum prealbumin, C-reactive protein and orosomucoid in neonates with bacterial infection. J Pediatr 1984; 105: 977-981.
7. Philip AG. The protective effect of acute phase reactants in neonatal sepsis. Acta Paediatr Scand 1979; 68: 481-483.
8. Speer CH, Bruns A, Gahr M. Sequential determination of CRP,  $\alpha_1$ -antitrypsin and haptoglobin in neonatal septicemia. Acta Paediatr Scand 1983; 72: 679-683.
9. Georgieff MK, Sasanow SR, Mammel MC, Ophoven J, Pereira GR. Cord prealbumin values in newborn infants: effect of prenatal steroids, pulmonary maturity, and size for dates. J Pediatr 1986; 108: 972-976.
10. Kim CJ, Yoon BH, Romero R, et al. Umbilical arteritis and phlebitis mark different stages of the fetal inflammatory response. Am J Obstet Gynecol 2001; 185: 496-500.
11. Volante E, Moretti S, Pisani F, Bevilacqua G. Early diagnosis of bacterial infection in the neonate. J Matern Fetal Neonatal Med 2004; 16: 13-16.
12. Pittard WB 3<sup>rd</sup>, Anderson DM, Gregory D, Rothstein FC. Cord blood prealbumin concentrations in neonates of 22 to 44 weeks gestation. J Pediatr 1985; 107: 959-961.
13. Sasanow SR, Spitzer AR, Pereira GR, Heaf L, Watkins JB. Effect of gestational age upon prealbumin and retinol binding protein in preterm and term infants. J Pediatr Gastroenterol Nutr 1986; 5: 111-115.
14. Herruzo AJ, Mozas J, Alarcon JL, et al. Sex differences in serum hormone levels in umbilical vein blood. Int J Gynaecol Obstet 1993; 41: 37-41.
15. Thomas L. Ceruloplasmin. In: Thomas L (ed). Clinical Laboratory Diagnostics. Frankfurt: TH-Books; 1998: 660-662.
16. Soldin SJ, Brugnara C, Hicks JM. Pediatric Reference Ranges. Washington, DC: AACC Press, 1999.