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HEMATOLOGIC STUDY OF NEWBORN UMBILICAL CORD BLOOD

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Abstract Hematological parameters in newborn umbilical cord blood samples (n = 476), collected at the Hospital Provincial del Centenario, Rosario, were studied. They were divided into 3 groups: (I) full term newborns with weight according to gestational age; II) low weight and normal gestational age; (III) preterm newborns. The results were as follows: group (I) Hb: 15.5 ± 1.1 g/dl; RBC: 4.66 ± 0.33 x 10^{12}/l; PCV: 49 ± 4.3%, MCV 105.1 ± 5.3 fl; MHC: 33.2 ± 1.2 pg. Decreased Hb concentration ($p < 0.05$) and increased MCV ($p < 0.01$) were observed in preterm newborns in comparison with normal ones, and a slight PCV increase and RBC values in low weight newborns compared to the control group ($p < 0.05$). Erythrocyte morphology was normal as well as reticulocyte values in these samples. The electrophoretic pattern was (FA) with the following Hb F values 66.3 ± 6.8%, and Hb A_2 0.45 ± 0.3% in group (I), with a significant increase of Hb F in 30-35 weeks preterm newborns. Group (I) values are considered as normal hematological parameters in newborns in our country, whereas MCV < 94.7 fl is considered as a neonatal microcytosis marker, consequently an alert to investigate alpha-thalassemia. There was no influence on Hb concentration due to maternal smoking habit. The present work could be of relevance for our region since up to the present time there are no similar records.

Resumen ***Estudio hematológico en sangre de cordón umbilical de neonatos.*** Se estudió la sangre de cordón umbilical de 476 neonatos, nacidos en el Hospital Provincial del Centenario de Rosario, Argentina. Fueron divididos en tres grupos: (I) neonatos a término y peso adecuado a la edad gestacional (PAEG); (II) bajo peso y edad gestacional normal; (III) prematuros. Los resultados hematimétricos del grupo (I) fueron: Hb 15.5 ± 1.1 g/dl, GR: 4.66 ± 0.33 x 10^{12}/l, Hcto: 49 ± 4.3%, VCM 105.1 ± 5.3 fl, HCM. 33.2 ± 1.2 pg. Se encontró disminución de la concentración de Hb ($p < 0.05$) y aumento del VCM ($p < 0.01$) en los prematuros comparados con los normales y un ligero aumento del Hcto y de eritrocitos en los de bajo peso nacidos a término con respecto al grupo de referencia ($p < 0.05$). En todos los casos la morfología de los eritrocitos y la cifra de reticulocitos fue la habitual; el patrón electroforético fue FA con valores de Hb F de 66.2 ± 6.8% y de Hb A_2 de 0.45 ± 0.3% en el grupo (I), observándose un aumento significativo de Hb F en los prematuros entre 30-35 sem. Los resultados del grupo (I) se consideran parámetros normales para neonatos nacidos en nuestro medio, siendo los VCM < 94.7 fl, marcadores de microcitosis neonatal, y señal de alerta para investigar la presencia de alfa talasemias. El hábito de fumar materno no incidió sobre concentración de Hb. Finalmente consideramos que nuestro trabajo constituye un aporte importante para nuestra zona ya que hasta la fecha no existen registros similares.

Key words: newborns, umbilical cord blood, hematimetric values, electrophoretic pattern

It is widely known that newborn hematological parameters are different from those of infants or adults[1, 2], including differences according to the blood samples utilised (umbilical cord blood, venous blood, capillary blood) as well as drawing time (2, 12 or 24 hr after birth), and nutritional fetal conditions[3]. Since there are no published data in our country, the objectives of the present paper were: a) to establish reference hematological values in full term newborn umbilical cord blood, either after vaginal or cesarean delivery, with normal gestational age weight and without fetal or newborn pathology; b) to compare these results with those corresponding to preterm newborns, and low weight-normal gestational age newborns; c) to analyse the infuence of maternal smoking habit on newborn hemoglobin concentration; d) to confirm whether the electrophoretic phenotype is normal according to age, and at the same time assess Hb F and A_2; and finally e) to compare these results to those reported by other authors.

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Material and Methods

Newborn umbilical blood cord samples (n = 476), excluding twins, collected at the Hospital Provincial del Centenario, Rosario, were prospectively and consecu-tively, studied. They

were divided into 3 groups: I) full term newborns with gestational age between 37-42 weeks (wk) and normal gestational age weight (NGAW) ranging from 2.500 to 4.200 g. They were delivered either vaginally or by cesarean section without fetal or neonatal pathology and with normal Apgar score. II) Low weight newborns (range: 1.660-2.450 g) and normal gestational age. III) Preterm newborns, gestational age between 30-37 wk and weight ranging from 1.450 to 2.880 g. Maternal smoking habit was registered in every case as well as newborn ethnic origin. Blood sample was collected from umbilical cord with EDTA K_3 and processed daily; in all cases the placental cord was clamped immediately after delivery.

The following hematimetric variables: red blood cells (RBC), hematocrit (PCV), hemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) were determined in an automated counter (Cell-Dyn 1.400). The erythrocyte morphology was assessed by light microscopy in May Grünwald Giemsa stained smears. Reticulocytes (Ret) counting was manually performed in cresyl-blue stained smears. The electrophoretic pattern was determined in cellulose acetate at pH 9[4]; the Hb F level was determined by alkaline denaturation technique[5], and Hb A_2 by post-electrophoretic elution in cellulose acetate alkaline medium.

Results

Results regarding maternal smoking habit (Table 1) showed no influence on cord blood Hb concentration in the three neonatal groups considered. Hematimetric values of the three groups under study are presented in Table 2. Decreased Hb concentration ($p < 0.05$) and increased MCV ($p < 0.01$) were observed in preterm newborns in comparison with normal ones. The erythrocyte morphology showed: slight anisocytosis and abundant macrocytes, slight poikilocytosis and polychromatophilia. In many cases there were crenate erythrocytes, some spherocytes and presence of polychromatophilia and orthochromatic erythroblasts, with values attaining 6 ± 3/100 leukocytes, which is considered normal in newborns, and 9 ± 3 in preterm newborns.

The electrophoretic pattern was normal in all the groups (FA). In Table 3 are presented the Hb F mean values in the groups. Within the preterm newborn group it was observed that between 30-34 wk values are significantly higher than those between 35-37 wk ($p < 0.001$). In the same table normal Hb A_2 mean value can be observed.

Discussion

Normal hematimetric values in full term newborn umbilical cord blood are similar to those reported by Tchernia in France[2], and slightly smaller than those from other authors[6, 7]. Variables remarkably affecting hemoglobin concentration in umbilical cord blood, such as umbilical cord clamping and materno-fetal transfusion[8, 9] have been controlled during the study. The ethnic origin of the population under study was as follows: 83% Spanish with a high percentage of "mestizos", 14% Italian and 3% Arabs, German and French. In all cases mothers lived in the city outskirts with a low socioeconomical status, coming from the Northeastern region of our country due to economical reasons.

In low weight neonates there is a slight increase in hematocrit and red blood cells values compared with the control group ($p < 0.05$), similar to that observed by Humber et al.[10].

In preterm neonates, Hb is significantly lower ($p < 0.05$) with a remarkable MCV increase ($p < 0.01$) in comparison with full term newborns, but without differences either in RBC or in PCV. It must be pointed out that preterm newborns were not under 30 weeks of age where differences are more remarkable[2, 11].

TABLE 1.– Hb concentration in neonatal umbilical cords from smoking and non smoking mothers

Group	Non smoking mothers		Smoking mothers	
	n (%)	[Hb] g/dl	n (%)	[Hg] g/dl
Group I n: 438	391 (89.3)	15.5 ± 1.1[a]	47 (10.7)	15.5 ± 1.1
Group II n: 12	10 (83.4)	15.0 ± 1.6	2 (16.6)	15.2
Group III n: 26	24 (92.3)	14.7 ± 1.6	2 (7.7)	14.9
Total n: 476	425 (89.3)		51 (10.7)	

[a] *Data are presented as mean ± standard deviation*

TABLA 2.– *Hematimetric values in umbilical cord blood*

	n	RBC ($x10^{12}$/l)	PCV (%)	Hb (g/dl)	MCV (fl)	MHC (pg)	Ret. (%)
Group I	438	4.66 ± 0.33 [a]	49.0 ± 4.3	15.5 ± 1.1	105.1 ± 5.30	33.3 ± 1.2	6.5 ± 2.3
Group II	12	5.03 ± 0.51	54.3 ± 6.3	15.0 ± 1.6	107.1 ± 5.8	29.8 ± 1.9	6.9 ± 3.7
Group III	26	4.40 ± 0.51	48.0 ± 6.1	14.7 ± 1.9	109.1 ± 2.90	32.9 ± 2.1	8.7 ± 3.7

[a] *Data are presented as mean ± standard deviation*

TABLE 3.– *Hb F and Hb A_2 in umbilical cord blood*

Group	n	Hb F (%)	Hb A_2 (%)
Group I	438	66.2[a] ± 6.8	0.45 ± 0.3
Group II	12	66.0 ± 10.8	-
Group III-a (30-35 wk)	11	75.9 ± 4.3	-
Group III-b (35-37 wk)	15	65.1 ± 5.3	-

[a] *Data are presented as mean ± standard deviation*

The MCV is a very interesting parameter since neonatal microcytosis has been proposed as an alarm criterion to diagnose alpha thalassemia[12]; however, it is necessary to apply molecular biology techniques to attain a reliable diagnosis. In the present study, the MCV in full term newborns with weight according to gestational age was 105.1 ± 5.3 fl, consequently, we considered MCV values less than 94.7 as a microcytosis marker.

There was no increase in the umbilical cord blood concentration hemoglobin in smoking mothers compared with non smoking ones. It is already known that smoking mothers have a greater content of carboxyhemoglobin, which produces a greater fetal hypoxia and secondary erythrocytosis[13, 14]. It must be pointed out that in all cases the smoking habit corresponded to less than 10 cigarettes per day; however, there was an increased percentage of low weight infants. The Hb F levels in umbilical cord blood samples were within normal range, with no significant differences either among groups or in comparison with other authors' data[15, 16]. Performing a subanalysis within the premature group, it could be observed that the presence of Hb F in newborns aged 30-34 weeks, is significantly higher than in newborns aged 35-37 weeks ($p < 0.001$), a fact that has been reported by other authors.

Finally, we think that the present work could be relevant regarding neonatal normal parameters in our region since up to the present time there are no such records.

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