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Hematological and biochemical profile of equine umbilical cord blood and comparison with peripheral blood of mare and newborn foal

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The blood profile of an animal provides a prospect to clinically investigate the presence of diverse metabolites and other constituents in the body of the animal and it plays a vital role in the assessment of physiological, nutritional and pathological status of an organism (Doyle 2006). Combined with hematology, the biochemical profile forms the data base for most diagnostic investigations. The information on hematology of adult horses (Satue *et al.* 2009, Vincze *et al.* 2010), foals up to one year of age (Rubio *et al*. 1995) and equine neonates for the first 6 h of life (Doarn *et al*. 1987) is documented. However, no information is available regarding hematological and biochemical profiles of equine cord blood.

Equine umbilical cord blood (UCB) haematology is not well defined. It is the preferred source for isolation of stem cells in equines as the collection procedure is inexpensive and non-invasive (Reed and Johnson 2008, Schuh *et al*. 2009, Iacono *et al*. 2012). Therefore, the present study was undertaken to investigate the hematological and biochemical profile of UCB and to compare it with peripheral blood of new born foal and its dam immediately after foaling. These may be used as reference values for umbilical cord blood in equines.

Freshly collected blood was used for hematological analysis and serum was used for biochemical analysis. Post-partum equine umbilical cord blood (11) was collected before spontaneous breakage of umbilical cord from an organized farm rearing thoroughbred mares. The umbilical vein of each cord was disinfected by sanitization with 70% isopropyl alcohol prior to blood collection. Umbilical cord blood was collected by venipuncture using 2.5 cm, 16-gauge needle in 15 ml centrifuge tube containing ethylene-diamine-tetra-acetate (EDTA) for hematological analysis and without EDTA for biochemical analysis. Peripheral blood of new born foal as well as dam (11) was collected from the jugular vein with EDTA and without EDTA, immediately after foaling. The collected blood was transported to laboratory on ice packs within 2 h. The serum was separated by centrifugation at 1,500 g for 15 min at room temperature and stored at –20°C till further use.

The blood for hematological parameters viz. total leucocyte count**,** total erythrocyte count, lymphocyte %, monocyte %, neutrophils %, eosionophil %, basophils %, hematocrit or packed cell volume, hemoglobin (Hb) concentration, mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RCDW), thrombocrit (PCT), mean platelet volume (MPV) and platelet distribution width (PDWC) were analyzed in duplicate using hematology analyzer.

The biochemical parameters of blood viz. glutamic pyruvic transaminase (GPT), glutamic oxaloacetic transaminase (GOT), alkaline phosphatase (ALP), total protein (TP), albumin, creatinine, urea, total bilirubin, calcium and total cholesterol were determined within 24 h of collection using fully automated chemistry analyzer. Statistical analysis of hematological and biochemical parameters of cord blood, peripheral blood of newborn foal and mare was performed using one-way ANOVA by comparing mean values using Duncan's multiple range test (DMRT) at $P<0.05$. The data was analyzed using SPSS Statistics 17.0 software.

Hematological parameters: The mean (±SE) hematological values for umbilical cord blood (11) are presented in Table 1. Total leucocyte count of umbilical cord blood ($3.63\pm0.40 \times 10^9$/L) was significantly lower ($P<0.05$) than that of mare peripheral blood ($10.16\pm0.60 \times 10^9$/L) but it did not differ significantly from that of newborn foal blood. Total erythrocyte count of umbilical cord blood ($12.53\pm1.56 \times 10^{12}$/L) was higher ($P<0.05$) than that of mare blood

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Table 1. Comparison of hematological parameters of umbilical cord blood with peripheral blood of mare and neonatal foal

Parameters	Umbilical cord blood	Peripheral blood		Normal value*
		Foal	Mare	
Total leucocyte count ($\times10^9$/L)	3.63±0.40 [a]	7.02 ± 0.41 [b]	10.16±0.60[b]	8–11
Total erythrocyte count ($\times10^{12}$/L)	12.53±1.56[a]	13.8 ± 1.03[a]	9.19±0.59[b]	10
Lymphocyte (%)	26.76±3.37 [a]	35.77 ± 6.20 [a]	21.58±2.48[b]	30–40
Neutrophil (%)	51.47±4.50[a]	64.64±6.02[a]	64.10±7.06[a]	50–60
Basophil (%)	1.22±0.27[a]	0.67±.23[a]	1.14±.20[a]	<1
Eosinophil (%)	0.59±0.11[a]	0.61±0.12[a]	0.59±0.12[a]	2–5
Monocyte (%)	6.65±0.44[a]	5.17±0.46[b]	5.22±1.61[b]	5–6
Hb (g/dl)	9.93±1.70[a]	13.34±0.48[b]	13.32±0.84[b]	11–16
HCT (%)	29.01±4.94[a]	40.51±1.66[b]	38.05±1.13[b]	34–45
MCH (pg)	12.75±0.54[a]	13.64±1.10[a]	15.87±0.90[b]	12.70–17.50
MCHC (g/dl)	32.97±0.79[a]	34.54±1.52[a]	34.92±1.76[a]	34–36
MPV (fl)	5.87±.26[a]	6.9±.37[a]	6.43±.46[a]	ND
PCT (%)	2.11±1.11[a]	2.20±.60[a]	1.15±.46[a]	ND
PDWC (%)	28.31±1.47[a]	30.81±2.01[a]	30.54±3.10[a]	ND
RCDW (%)	26.0±1.27[a]	27.03±1.45[b]	22.97±0.64[a]	ND

The parameters with different superscripts in a same row differ significantly (P< 0.05). Hb, hemoglobulin; HCT, hematocrit; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MPV,mean platelet volume; PCT, thrmbocrit; RCDW, red cell distribution width; platelet distribution width (PDWC); pg, picogram; fl, femto litre; ND, not defined. * Normal values of adult (Reece and Swenson 2005).

($9.19\pm0.59 \times 10^{12}$/L). Lymphocyte concentration was higher in umbilical cord blood (26.76±3.37%) than in the mare blood but lower than that of newborn foal peripheral blood (35.77 ± 6.20%). Monocytes were 6.65±0.44% in umbilical cord blood which was significantly higher (P<0.05) than that of newborn foal and mare blood (Table 1). Hemoglobin concentration and HCT% were found lowest in UCB. No significant difference (P<0.05) was observed in the rest of hematological parameters among umbilical cord blood, foal and mare peripheral blood.

Biochemical parameters: Ten serum biochemical parameters including 3 enzymes viz. GPT, GOT ALP were analyzed (Table 2). GPT concentration was the lowest in UCB followed by foal and mare serum. Statistically significant difference (P<0.05) was observed in GOT concentration of cord blood from that of mare. ALP activity in umbilical cord blood was significantly different (P<0.05) from that of mare and foal blood. The calcium concentration in umbilical cord blood was lowest (Table 2). Concentration of total protein (TP) was the lowest in umbilical cord blood compared to newborn foal and mare. Similarly albumin content of umbilical cord blood was significantly different (P<0.05) from that of foal and mare blood (Table 2). Creatinine content of umbilical cord blood was significantly higher than that of foal and mare blood. Urea and total bilirubin content of umbilical cord blood was lowest among three (Table 2). Triglyceride content of umbilical cord blood was the highest but cholesterol value was lower than that of foal and mare.

Total leucocyte count of equine umbilical cord blood was lower (P<0.05) than that of mare peripheral blood. This may be due to presence of more immature granulocytic and agranulocytic cells. Total erythrocyte (RBC) count was significantly higher in umbilical cord blood compared to newborn foal and its mare. A higher RBC count has earlier been reported in foal during early hours of birth as compared to that of later life (Chavatte *et al.* 1991). It was reported that the RBC counts declines within 2 h of birth and WBC count rises to normal levels (Chavatte *et al.* 1991). Lymphocyte and monocyte counts in umbilical cord blood were higher than that of mare blood. It might be due to the fact that precise hematologic changes occurring in the horse during parturition (Doarn *et al.* 1987). The lowest hemoglobin concentration and hematocrit (%) of umbilical cord blood as reported in human beings (Katsares *et al.* 2009), was also observed in equine UCB in the present study. The consistent lower hemoglobin and hematocrit may be due to physiological hemolysis (Doarn *et al.* 1987). The higher values of these parameters of newborn foal could be a physiologic condition that is necessary to get the neonate through the low blood oxygen condition at birth (Doarn *et al.* 1987). Red cell distribution width (%) of umbilical cord blood was close to mare but different from that of newborn foal. No significant difference (P<0.05) was found in rest of hematological parameters among umbilical cord blood, foal and mare peripheral blood at the time of foaling. Similar findings have also been reported in human being (Katsares *et al*. 2009).

GPT activity was the lowest in umbilical cord blood than foal and mare. But the reason for the higher GOT activity in the umbilical cord blood is not known. Alkaline phosphatase

Table 2. Comparison of biochemical parameters of serum separated from umbilical cord blood with peripheral blood of mare and neonatal foal

Parameters	Umbilical cord blood	Peripheral blood		Normal value*
		Foal	Mare	
GPT/ALT (IU/L)	13.3±1.70[a]	15.15±0.94[b]	26.43±1.13[c]	14±11(3–25)
GOT/AST (IU/L)	332.3±15.90[a]	306.4±4.80[a]	282.1±8.49[b]	296±70
ALP (IU/L)	209.0±3.27[a]	253.6±2.24[b]	151.3±0.95[c]	184±57
Calcium (mg/dl)	8.1±0.09[a]	9.96±0.15[b]	9.1±0.12[c]	11.2–13.6
TP (g/dl)	2.75±0.25[a]	5.65±0.13[b]	6.53±0.09[c]	4.60–7.90
Albumin (g/dl)	1.81±0.004[a]	3.03±0.09[b]	3.28±0.03[c]	2.50–4.20
Creatinine (mg/dl)	2.00±0.02[a]	1.28±0.01[b]	1.76±0.02[c]	0.60–1.90
Urea (mg/dl)	17.43±0.18[a]	42.46±0.35[b]	28.51±0.20[c]	28–78
Total billirubin (mg/dl)	0.31±0.03[a]	0.76±0.02[b]	1.79±0.04[c]	0.10–2
Triglyceride (mg/dl)	25±4.50[a]	14±2.30[b]	8±0.50[c]	4–44
Cholesterol (mg/dl)	72.17±1.0[a]	75.17±0.70[b]	92.67±0.66[c]	75–150

The parameters with different superscripts in a same row differ significantly ($p<0.05$). GPT, glutamic pyruvic transaminase; GOT, glutamic oxaloacetic transaminase; ALP, alkaline phosphatase, IU, international unit; TP, total protein. * Normal values of adult horse (Kaneko *et al.* 2008).

activity of umbilical cord blood was significantly lower ($P<0.05$) than that of mare and foal .The high value of serum ALP level in newborn foal might be due to osteogenesis in immature animals, which is probably a secondary phenomenon and related to an increased liver cell metabolism immediately after birth (Thorén-Tolling 1988). Calcium concentration of umbilical cord blood was lower ($P<0.05$) than foal blood. The significantly higher calcium value was observed in newborn foal, which may be due to the fact that young and growing animals have more serum calcium concentration as it helps in bone formation along with ALP activity (Thorén-Tolling 1988). Concentration of total protein (TP) and albumin was found to be the lowest in umbilical cord blood compared to newborn foal and mare. It might be possibly due to the limited activity in liver of foal during gestational period. Triglyceride content of umbilical cord blood was found to be highest but cholesterol value was lower than that of foal and mare. Higher triglyceride value of umbilical cord blood may be due to limited activity of liver function of foal which increases immediately after foaling so that a lower triglyceride value was found in newborn foal (Kaneko *et al*. 2008). Urea and total bilirubin content was the lowest in the umbilical cord blood. Urea was found to be significantly higher in newborn foal. High blood urea levels in foal blood indicate the more activity of liver (Kaneko *et al*. 2008) immediately after foaling. Total bilirubin content was highest in mares followed by newborn foal and umbilical cord blood. Lowest bilirubin content of UCB may be due to absence of hemolysis of red blood cells, highest liver uptake of bilirubin, or more albumin binding (Kaneko *et al*. 2008).

The hematological and biochemical profiles of equine umbilical cord blood differed significantly from those of newborn foal and mare blood. The reasons for such differences are fully not known. There might be some unknown biological mechanisms occurring within umbilical cord during gestation, making the difference in their constituents. Mare and foal blood originate from hematopoietic centre in their respective bone marrows. The exact origin of the equine umbilical cord blood is not known, but it is presumed that during embryogenesis, hematopoiesis occurs in umbilical cord itself making the components of umbilical cord blood. This is substantiated by the fact that no mixing of umbilical cord blood takes place during gestation with either mare or foal blood, due to epitheliochorial type of placentation in equines (MacDonald and Fowden 1997). Further studies are needed to know the mechanism of variation between umbilical cord blood and foal or mare blood and to know the origin of UCB in equines.

This study for the first time reports the hematological and biochemical parameters of equine umbilical cord blood and their comparison with those of peripheral blood of newborn foal and their mare, immediately after foaling. Based on 15 hematological and 10 biochemical parameters, it appears that UCB is more intimate to foal blood than to mare blood. However, cellular content and biochemical profile of umbilical cord blood differs significantly from either newborn foal or mare blood.

SUMMARY

The haematological and biochemical profile of equine umbilical cord blood (UCB) is not well known presently and very little information is available about peripheral blood of equine neonates immediately after foaling. This information has relevance as UCB is a rich source of progenitor stem cells and neonatal hematology may be useful in early disease diagnosis and management in equines. In the present study, hematological and biochemical values for umbilical cord blood (11) collected at natural full term were compared with

peripheral blood of neonatal foal and its dam. Comparative hematological analysis indicated that total leucocyte count in UCB (3.63±0.40 × 10^9/L) was significantly lower than that of mare, whereas total erythrocyte count (12.53±1.56 × 10^{12}/L), lymphocyte (26.76±3.37%) and monocyte (6.65±0.44%) in UCB were significantly higher than those of mare blood. Biochemical analysis revealed that glutamic pyruvic transaminase (GPT) concentration (13.3±1.70 IU/L) was lower in UCB, whereas glutamic oxaloacetic transaminase (GOT) concentration (332.3±15.90 IU/L) and alkaline phosphatase concentration (209.0±3.27 IU/L) were higher in UCB than mare blood. Calcium, total protein and albumin concentration was lowest in UCB in comparison to mare and neonatal foal blood. These findings indicated that UCB hematological and biochemical profile is significantly different from mare and foal blood. These differences in UCB could be due to independent hematopoiesis of UCB, probably occurring in the umbilical cord itself in equines as no mixing of blood occurs in equine UCB, foal and mare during pregnancy.

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