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Original Article

Paediatrics Section

Biological Reference Interval for Hematological Profile of Umbilical Cord Blood: A Study Conducted at A Tertiary Care Centre in South India

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ABSTRACT

Introduction: Umbilical cord blood (UCB) a source of hematopoietic stem cells, is also an acceptable sample to assess neonatal sepsis. Though reports are available for stem cell counts very minimal literature is available regarding hematologic parameters, which may vary on ethnicity.

Aim: To establish biological reference interval for hematological parameters of umbilical cord blood to guide neonatologists, hematopoietic stem cell transplant specialists and future analysis.

Materials and Methods: Prospective longitudinal study was done from January 2014 to April 2014 after ethics committee approval. UCB from 120 full term new borns of normal birth weight born out of uneventful pregnancy to mothers aged between 21 to 45 years with hemoglobin above 10g/dL were processed in Beckman Coulter LH780 analyzer for complete blood count and counter checked by peripheral smear. Results tabulated in Microsoft excel are analyzed using IBM SPSS statistics 16 software.

Results: Male to female ratio is 1:1.05. There is no difference in the values between males and females. When compared with few studies available, though many values are comparable a few values are not comparable.

Conclusion: This study can be a useful guide to neonatologists, hematopoietic stem cells transplant hematologists and future analysis.

Keywords: Hematology parameters, Reference range, Stem cell transplant

INTRODUCTION

Umbilical cord and placenta once regarded as precious when people used to preserve them over trees and under earth. Due to urbanization and education, people considered them as medical wastes and discarded them. Placenta was then harvested by manufacturing units for production of certain drugs. The innovation of stem cells gave umbilical cord a new insight and moreover after the first umbilical cord blood transplant in 1988 by Dr. Gluckmanto to a 5 year old boy who suffered from Fanconi anaemia. Both the donor and recipient are living models of the success of cord blood transplant [1]. Many studies are undertaken by researchers on umbilical cord and umbilical cord blood (UCB) globally. UCB is stored for years for personal and public use.

Though, many studies are available regarding stem cells count and cytometry parameters, very few studies are available regarding the hematologic parameters. Hematologic parameters reflect the health of an individual, hence UCB, the health status of new born. As the hematologic parameters vary according to age, sex and to certain geographical factors, it is essential that biological reference interval must be established before undergoing studies on various health conditions. With this background, the following study has been undertaken.

MATERIALS AND METHODS

This is a prospective longitudinal study done at the clinical pathology division of department of Pathology and Central Laboratory Services, Sri Ramachandra Medical College & Research Institute, Chennai, South India. This study was approved by the Institutional Ethics Committee, and the participants are 120 randomly selected new babies born to women at Sri Ramachandra Medical College, Hospital. Mothers gave their consent and were of 21-35 years of age having uneventful pregnancy with haemoglobin above 10g/dl and vaginal delivery of a single baby. Mothers with multiple pregnancies (more than 2), eclampsia and preeclampsia, diabetes mellitus, cardiovascular, respiratory, nervous, genitourinary diseases were excluded. The new borns were full term at birth (37-42 weeks), with normal birth weight (2.5-4kg) and normal APGAR score. They were free of congenital anomalies prenatally by ultrasound and by physical and systemic examination at birth.

As soon the umbilical cord was clamped and cut by the obstetrician, 3 ml of cord blood was collected in a pre labeled EDTA vaccutainer by milking. It was transported to lab immediately and processed within 3 hours of collection. Processing for complete blood count

	Total	Sex	
		Male	Female
Hb (g/dL)	14.9 ± 1.7	15.1 ± 1.7	14.9 ± 1.6
WBC(× 10^3/µL)	15.9 ± 5.1	15.5 ± 5.0	16.3 ± 5.2
Differential Count (%)			
Neutrophil	50.3 ± 12.2	50.8 ± 11.6	49.9 ± 12.8
Lymphocyte	35.9 ± 12.2	34.5 ± 10.6	37.2 ± 13.6
Monocyte	8.9 ± 3.1	9.5 ± 3.0	8.3 ± 3.3
Eosinophil	2.9 ± 2.0	3.4 ± 2.4	2.4 ± 1.4
Basophil	1.5 ± 1.2	2.1 ± 2.3	3.1 ± 5.5
RBC(× 10^6/µL)	4.1 ± 0.4	1.7 ± 1.3	1.3 ± 1.1
HCT (%)	44.6 ± 5.3	45.3 ± 5.4	43.8 ± 5.1
MCV (fL)	108.1 ± 4.8	107.7 ± 4.3	108.4 ± 5.3
MCH(pg)	36.0 ± 1.7	35.9 ± 1.5	36.1 ± 1.8
MCHC(g/dL)	33.3 ± 0.8	33.4 ± 0.8	33.3 ± 0.8
Platelet (10^3/µL)	215 ± 67	207 ± 69	222 ± 66
nRBCs(/100WBCs)	3.7 ± 3.5	3.8 ± 3.4	3.67 ± 3.60

[Table/Fig-1]: UCB - CBC values (Mean ± SD) in relation to sex With respect to sex, there is no statistically significant difference (p>0.05) in the parameters obtained

was done in Beckman Coulter LH780. Peripheral smears were examined to verify the results.

STATISTICAL ANALYSIS

The results were entered in Microsoft excel and analyzed with SPSS statistics 16 software. The mean and SD for various parameters were calculated.

RESULTS

Comparison of results according to sex were done. Biological reference interval for various hematologic parameters were established and compared with literature. The mean and SD values of CBC in the UCB of males and female neonates (ratio 1:1.05) are shown in [Table/Fig-1]. There was no statistically significant difference ($p>0.05$) between the results for each parameter. The results of RBC parameters of the present study with comparison of literature reports is shown in [Table/Fig-2] and WBC and platelet parameters in [Table/Fig-3].

The biological reference interval for Hb, RBC count WBC count, polymorph count and platelets counts, hematocrit, WBC count, polymorph count and platelet counts were lower in a study conducted at Greece than the present study. The Red cell distribution (RDW) was higher and Nucleated red blood cells (NRBC) count was lower in a study from Iraq when compared with ours. A study from Chandigarh, India showed a higher hemoglobin value than our result. A study from Nigeria showed low MCHC, and high RDW than ours.

DISCUSSION

The cellular components of UCB include stem cells which include mesenchymal stem cells, hematopoietic stem cells and multipotent non-hematopoietic stem cells and mature cells of hematopoietic stem cells namely RBC, WBC and platelets [2]. UCB is a source of hematopoietic stem cell transplant for various malignant and non-malignant hematologic conditions. Though the component of mesenchymal stem cells is low in number due to their high proliferation rate, cord blood can be considered as a source of mesenchymal stem cells also. Moreover, studies have indicated that cord blood reflects fetal hematopoietic and health status of new born and it can be considered an ideal source for laboratory analysis instead of new born blood, the collection of which is difficult. The disease status of new born like asphyxia, meconium staining and chorio amminonitis are reflected in UCB [3,4]. Also maternal conditions like smoking, anaemia, diabetes, preeclampsia and mode of delivery which influence the health status of new born are reflected in the UCB [5-9]. Hemogram of an individual varies according to age, sex, race and geography. The same may be true with umbilical cord hemogram also. Hence, its need of the hour to establish biological reference interval before studying the influence of maternal and new born conditions. Though it dates back in 1924 Lippman did blood count analysis on UCB, studies on this aspect were few in health and diseased condition. A study done in Chandigarh, India in 1992, did not include RBC indices. The present study is the first of its kind with analysis of all the parameters.

In the present study, no significant difference was found between the values of males and females. Adewumi et al., also found no difference when they analyzed cord blood of Nigerian neonates [10]. But a study from Greece showed statistically significant difference in RDW, WBC count, neutrophil count and platelet count and a study done in Iraq showed difference in neutrophils percentage alone [11,12]. However, both the studies showed lower neutrophil percentage in males when compared to females.

The study conducted in Greece showed lower value of Hb, RBC count, HCT, WBC count, polymorph count and platelet count when compared with the present study [11]. This may be due to dilution of cell components when blood is collected in UCB bags in their study. The RDW was higher and the NRBC were lower in a study done at Iraq when compared to the present study. However, the blood was collected from umbilical vein alone [12]. A study from Nigeria showed low MCHC and high RDW while another study showed a comparable MCHC [10,13]. Indian study from Chandigarh was comparable to the present study except for Hb and lymphocyte percentage which were found to be higher. However, the study was done manually [14]. When compared with Indian text book reference range at birth, the present study was comparable while the Hb value and RBC counts are lower than western text book reference range [15,16]. Umbilical cord blood is stored after screening which involves high cost. This can be avoided and blood can be rejected if the hematological parameters are out of range to improve outcome of UCB stem cell transplant. Also the neonate can be monitored carefully for disease state if the UCB parameters are found to be out of range.

	Present	Greece [11]	Iraq [12]	India Chandigarh [14]	Nigeria [10]	Birth India ABC of CBC 2013 [16]	Birth Dacie and Lewis 2013 [15]
Sample Type	**Mixed blood**	**Mixed blood**	**Umblical vein**	**-**	**-**		
No. of samples	120	2000	220	240	130		
Male	59	987	119	-	69		
Female	61	1011	101	-	61		
Year	2014	2009	2012	1992			
Equipment	Beckman Coulter LH 780	Beckman Coulter					
ACT	Diagon D- Cell 66	Manual	Sysmex KX-21N				
Hb (g/dl)	14.9 ± 1.7	8.8 ± 2.9	13.76 ± 1.46	16.2 ± 1.5	13.29 ± 1.5	15.4-24.5	18 ± 4
RBC ($x10^3/\mu l$)	4.1 ± 0.4	2.46 ± 0.82	4 ± 0.47	-	4.07 ± 0.55	4.1 - 6.2	6 ± 1
HCT (%)	44.6 ± 5.3	25.9 ± 8.8	44.42 ± 4.74	49.4 ± 5.3	44.8 ± 5.78	42-68	60 ± 0.15
MCV (fl)	108.1 ± 4.8	105 ± 6	111.56 ± 6.09	-	110.36 ± 11.8	103-106	110 ± 10
MCH (pg)	36 ± 1.7	35.8 ± 3.1	34.41 ± 2.36	-	32.61 ± 4.13	36-38	34 ± 3
MCHC (g/dl)	33.3 ± 0.8	34.3 ± 7.3	30.93 ±1.9	-	29.76 ± 1.64	34-36	33 ± 3
RDW (%)	13.7±3.5	12.1 ± 1.6	17.01 ± 1.63	-	19.75 ± 4.26		
NRBC / 100 WBC	4.2± 3.2		0.07 ± 0.37	5.1 ± 3.6			

[Table/Fig-2]: Comparison of Present study (RBC parameters) with literature

	Present	Greece [11]	Iraq [12]	India Chandigarh [14]	Nigeria [10]	Birth India ABC of CBC 2013 [16]	Birth Dacie & Lewis 2013 [15]
Sample Type	Mixed blood	Mixed blood	Umblical vein	-	-		
WBC	15.9±5.1	7.2 ± 3.4	10.12 ± 2.8	12.1 ± 6.4	22.5 ± 7.22	4 - 40	18 ± 8
Polymorph ($x10^3$/µl)	5.7±0.9	3.4 ± 1.8		-			4 - 14
Polymorph %	50.3 ± 12.2		51 ± 11.24	52.5 ± 16			
Lymphocyte ($x10^3$/µl)	1.4±0.4	2.6 ± 1.29		-			3 - 8
Lymphocyte %	35.9 ± 12.2		39.81 ± 10.17	41.6 ± 16.2			
Monocyte ($x10^3$/µl)	0.46± 0.06	0.8 ± 1.2					0.5 - 2
Monocyte %	8.9 ± 3.1	7.85 ± 2.77			2.9	± 2.3	
Eosinophil ($x10^3$/µl)	0.2±0.05	0.29 ± 1.1					0.1 - 1
Eosinophil %	2.9 ± 2	1.22 ± 0.97		2.3 ± 1.9			
Basophil ($x10^3$/µl)	0.09± 0.14	0.1 ± 0.25		-			
Basophil %	1.5 ± 1.2	0.1 ± 0.36		-			
Platelets ($x10^3$/µl)	215 ± 67	160 ± 59	267.63 ± 60.62	199.2 ± 56.6	225.07 ± 72.21	100-300	100 - 150
MPV (Fl)	8.2± 0.9	8 ± 0.7					

[Table/Fig-3]: Comparison of present study (WBC and platelets) with literature

CONCLUSION

The biological reference interval established is a useful guide to neonatologists, UCB stem cell transplant hematologist and also for future UCB analysis in health and disease. The biological reference interval needs to be established at every region in this era of stem cell research.

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