

Umbilical Cord Blood Haematological Parameters Reference Interval for New-borns in St. Peter Specialized Hospital Addis Ababa, Ethiopia

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ABSTRACT

Background: Several factors like altitude, age, sex, pregnancy, socioeconomic status, lifestyle and race influence haematological reference interval (RIs), which are critical to support appropriate clinical decisions and to interpret laboratory data in research. Currently there are no well-established RIs for cord blood haematological parameters of new-borns in Ethiopia.

Objective: To generate reference interval for umbilical cord blood haematological parameters of new-borns at St Peter Specialized Hospital Addis Ababa, Ethiopia.

Method: A cross-sectional study was conducted from January 1 to March 31, 2019 on healthy, term new-borns (37-42 weeks) with normal birth weight born to apparently healthy pregnant mothers who had met the eligibility criteria. From a total of 139 new-borns, 2-3ml cord blood was immediately collected from the clumped cord using EDTA tube. The samples were analysed using Sysmex KX 21 Hematology analyser. Data was entered and the 2.5th and 97.5th percentile (upper and lower reference limit) were determined using nonparametric method by SPSS version 23. The non-parametric independent Mann-Whitney U test (Wilcoxon rank-sum test) was used to compare the distribution of the parameters between genders, modes of deliveries and gestational age groups.

Result: The combined reference interval for umbilical cord blood haematological parameters of new-borns with the median and 95% reference value of cord blood parameters were as follows for WBC= 12.4 [6.55-19.35], RBC= 4.51 [3.55-5.52], HGB= 15.80 [12.41-19.65], HCT= 45.9[37.9-56.3], MCV=102.10[83.90-111.55], MCH= 35.30 [29.35-39.10], MCHC= 34.3 [32.3-37.40], PLT= 236 [146-438], LYM= 37.5% [16.6-63.0%], MXD= 7.9%[1.65-15.75%], NEU= 53.7%[30.3-78.3], RDW=15.6[12.0-19.0], PDW=11.0[9.1-15.7] and MPV= 9.4[8.1-11.8]. The current study found no significance difference between genders, except RDW (P=0.01) and gestational age group, but there was significance difference for WBC (p=0.007), RBC (p=0.018) and Absolute NEU (p=0.001).

Conclusion: Since this study is pioneer of its kind with regards to haematological reference intervals in cord blood, the values obtained from our study provide reference intervals for some haematological parameters in healthy new-borns of Addis Ababa and its surrounding special whereas. However, the results need to be confirmed by larger samples from different centres throughout the country.

Keywords: CBC; Reference interval; Umbilical cord blood; Neonates

INTRODUCTION

Reference intervals aid in the interpretation of laboratory data for appropriate result interpretation, clinical trials, and selection of participants for vaccine trials [1]. These values are affected by several factors including age, sex, race, geographical location and

dietary pattern [2]. A wealth of published studies demonstrated the age related changes in hematological parameters. The neonate exhibit profound quantitative as well as qualitative hematologic differences compared to older child and adults [3], at birth, hematological parameters of term newborns are significantly higher than those of older children and adults [4,5]. Due to the

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specific nature of RIs and biological variations it is inappropriate to use adult reference ranges for the assessment of Pedit blood [6,7]. A reference interval obtained from somewhere else and utilizing to population of interest could potentially lead to inappropriate patient management and unnecessary use of resources [8,9]. Cord blood hematological parameters reference intervals are vital in neonatal care and transplantation medicine [10,11]. Cord blood is a blood collected from long and helical cord that connects the fetus with the mother for substances exchange [12]. Establishing normal neonatal reference interval has been difficult because blood has not been drawn on healthy neonates of similar ages and other neonatal or maternal factors needed to be considered [13-16].

According to different studies maternal anemia had effect on cord blood hemoglobin and new born weight [17-20], though routine hematological values of newborns are independent to that of maternal hematological values [21, 22]. This may be due to high micronutrient intake inadequacy in pregnancy [23-25]. Factors like smoking habit [26], heavily drinking alcohol [27], medical problems like diabetic mellitus, eclampsia, hypertension [28-30] and mode of deliveries and frequency of pregnancy affect the hematological profile of neonates [31-33]. Also during the sample collection procedure though cord blood collection safe way to collect sample compared to cannula and other vascular catheters which cause thrombophlebitis, infection, and extravasation[34], the delayed cord clamping and umbilical cord milking had comparable effect on hematological parameters [35-36].

In spite of the fact that reference range can play an important role in guiding the interpretation of laboratory results in assessing the hematological changes, there is no published reference interval for hematological parameters in cord blood of Ethiopians.

METHODS

A cross-sectional study was conducted from January 1 to March 31, 2019 in Addis Ababa, Ethiopia at an elevation of about 2440 m (about 8000 ft) above sea level, among 139 newborns delivered in St. Peter Specialized Hospital using convenient non-probability techniques. Priori selection method was implemented to include eligible mothers aged 18 to 45 years with no medical conditions (infectious (e.g. Hepatitis B, HIV, Syphilis), chronic (e.g. Insulin-dependent diabetes mellitus), obstetric (e.g. less than six months from abortion, preeclampsia) and psychological problems) and social habits (e.g. smoking, heavily alcohol drinking) by using diagnostic tests (lab tests, ultrasound) and history from the card. Also mothers who had Hgb \geq 11.0g/dL and inter-pregnancy interval of more than or exactly 18 months as per WHO recommendation included (51, 52). Whereas, posteriori selection method was used to include eligible newborns who were term (37- 42 weeks) and had 5th minute Apgar score of \geq 7 and birth weight of term newborn within 2.5-4 Kg (4). Babies with respiratory distress, meconium stain, gross congenital anomalies, umbilical cord with true knot, and babies delivered by instrumental deliveries were excluded.

Data Collection Procedure

All the professionals who were participated in data collection were oriented about the aim of the study, in selecting study participants, data confidentiality, safety and precautions to follow in collecting, transporting, analyzing and storing cord blood samples. Predesigned questionnaire was used to collect demographic information and a brief medical history from consented mother. The umbilical cord

clamped (in $<$ 1min after birth); 2-3 ml of blood were collected by Midwives or operation room (OR) nurse by EDTA tube and well mixed (10x) sample was transported to the laboratory for analysis.

Screening Tests

The laboratory screening tests for pregnant mother was done only for those tests were not done, missed or suspicious about the result during the antenatal care (ANC) follow up. The routine serological screening tests were Hepatitis B surface antigen test, HIV and syphilis antibody test. The ultrasonography test was also used to rule out fetal gross congenital anomalies.

Hematological Analysis

Complete blood count (CBC) tests namely WBC count, Diff count (NEU, LYM and MXD), RBC, HGB, HCT, MCV, MCH, MCHC, RDW, PLT, MPV and PDW were analyzed using Sysmex KX-21N, a speedy and 18 parameters performing automated hematology analyzer by the three detector blocks (WBC (DC detection method), HGB (Non-cyanide HGB method) RBC and PLT (DC Detection method)). Peripheral blood smears were prepared using wright's stain for cord blood samples for investigation of red blood cell morphology, white blood cell and platelets abnormalities.

Statistical Analysis

All the data from the questionnaires and laboratory results were coded and checked for completeness. Then data were entered and analysed using SPSS-version 23 statistical software for windows. 2.5th and 97.5th percentiles for each haematological parameter with 90% CI reference Intervals for 139 new-borns of both genders were determined according to Clinical Laboratory Standard Institute (CLSI) guideline (2) and descriptive statistic. The non-parametric Independent Mann-Whitney U test was used to compare the distribution of the parameters between genders, delivery modes, and gestational age groups. Additionally, descriptive statistics (minimum, maximum, mean, SD, median) were also determined.

Ethical Considerations

The study was conducted after getting ethical clearance and support letter from the Department Research and Ethics Committee (DREC) of the Department of Medical Laboratory Sciences, College of Health Sciences, Addis Ababa University and St. Peter Specialized Hospital research department. The mother of neonates was informed about the aim of the study, participation and confidentiality of the information.

RESULTS

A total of 139 healthy full-term newborns consisting of 67[47.9%] males and 72[51.8%] females (Table 1) were enrolled in this cross-sectional study conducted in St. Peter Specialized Hospital from January 1 to March 31, 2019. About 15.8% of the mothers were from outside Addis, 84.9% were literate, and 47.5% (66/139) were having first time delivery.

On the other hand, for all hematological parameters there were no statistically significant difference within gestational age groups [37-39.1 versus 39.2-42 months] ($p>$ 0.05) (Table 2). Independent Mann-Whitney U (Wilcoxon rank-sum) test between delivery modes shows significant difference ($p<$ 0.05) of which newborns delivered through C/S had lowered value for WBC=11.1[6.6-19.4], RBC=4.39[3.55- 5.52], and absolute NEU =6.0[2.7-12.8] compared to newborns delivered through SVD with value for WBC=12.9[6.6-19.4], RBC=4.55[3.55-5.52], and absolute NEU

Table 1. Demographic and Medical Information.

Information		Frequency	Percent	
Age of Mother (Years)	18-31	115	82.7	
	31-45	24	17.3	
Residence	Addis Ababa	117	84.2	
	Outside Addis Ababa	22	15.8	
Educational level	Illiterate	21	15.1	
	Literate	118	84.9	
Employment Status	Employed	62	44.6	
	Unemployed	77	55.4	
Marital Status	Married	133	95.7	
	Other	6	4.3	
Pregnancy History	None	66	47.5	
	Yes	73	52.5	
Pregnancy Interval (n=73)*	1-1year & 6months	6	8.2	
	>=2years	67	91.8	
Delivery mode	Previously	SVD	56	76.7
		CS	17	23.3
	Currently	SVD	93	66.9
		CS	46	33.1
Sex of Baby	Male	67	48.2	
	Female	72	51.8	
Weight of Baby	2-3Kg	74	53.2	
	>3Kg	65	46.8	
Gestational age	37-39.1 weeks	60	43.2	
	39.2-42 weeks	79	56.8	

Table 2 Shows Independent (2 Groups) Mann-Whitney U Test in 95% Confidence Interval with Significance Level 0.05.

Parameters	P- value by sex	P-value by mode of delivery	P-value by gestational age
WBC(x10 ⁹ /L)	0.787	0.007	0.366
RBC(x10 ¹² /L)	0.075	0.018	0.852
HGB (g/dL)	0.374	0.11	0.904
HCT (%)	0.861	0.193	0.707
MCV (fL)	0.171	0.117	0.99
MCH (Pg)	0.139	0.094	0.832
MCHC (g/L)	0.386	0.519	0.54
RDW-CV(%)	0.01	0.318	0.197
PLT (x10 ⁹ /L)	0.369	0.291	0.855
LYM%	0.274	0.348	0.325
MXD%	0.807	0.666	0.915
NEU%	0.227	0.262	0.315
LYM#	0.188	0.42	0.781
MXD#	0.657	0.157	0.765
NEU#	0.684	0.001	0.264
PDW-CV(%)	0.199	0.296	0.188
MPV (fL)	0.913	0.858	0.503

=7.6[2.7-12.8] (p<0.05). Sex specific 2.5th and 97.5th percentile for complete blood count parameters from umbilical cord blood is summarized in Table 3. Statistically significant differences by sex were not detected for any of the parameters except RDW-CV in which females showed lower median value (p <0.05) than males. The combined median and 95% reference value of cord blood parameters as shown in Table 3 were for WBC = 12.4 x10⁹/L [6.6-

19.4], RBC = 4.51 x10¹²/L [3.55-5.52], HGB = 15.8g/dL [12.4-19.7], HCT = 45.9% [37.9-56.3], MCV= 102.1fL [83.9-111.6], MCH = 35.3pg [29.4-39.1], MCHC = 34.3% [32.3-37.4], PLT = 236 x10⁹/L[146-438], LYM = 37.5% [16.6-63.0%], MXD = 7.9% [1.7-15.8%], and NEU = 53.7% [30.3-78]. The study also tried to compare the established cord blood RI with that provided by Sysmex for 0- 24 hours old newborns and other previous studies.

Table 3: Percentile Reference Intervals for umbilical cord hematological parameters by Sex of Newborns from January 1 to March 31, 2019 G.C in St. Peter Specialized Hospital, Addis Ababa, Ethiopia.

Parameter	Sex	N	Median	Min	Max	2.5	97.5	P-value
WBC (x10 ⁹ /L)	M	67	12.6	6.5	19.6	6.6	19.3	0.787
	F	72	12.4	5.8	23.5	6.1	23.2	
	Combined	139	12.4	5.8	23.5	6.6	19.4	
RBC (x10 ¹² /L)	M	67	4.59	3.54	5.7	3.59	5.59	0.075
	F	72	4.44	3.1	5.7	3.37	5.3	
	Combined	139	4.51	3.1	5.7	3.55	5.52	
HGB (g/dL)	M	67	15.9	12.3	20	12.4	19.8	0.374
	F	72	15.7	10.8	20.6	11.1	18.8	
	Combined	139	15.8	10.8	20.6	12.4	19.7	
HCT (%)	M	67	46	36	60.2	37.9	57.8	0.861
	F	72	45.45	36.4	61.8	37.3	56.3	
	Combined	139	45.9	36	61.8	37.9	56.3	
MCV (fL)	M	67	102	81.9	115.5	82.1	112.8	0.171
	F	72	102.3	85.4	123.3	88.5	113.5	
	Combined	139	102.1	81.9	123.3	83.9	111.6	
MCH (Pg)	M	67	35.1	28.6	39.7	28.8	39.4	0.139
	F	72	35.3	27.8	41.1	31.6	38.7	
	Combined	139	35.3	27.8	41.1	29.4	39.1	
MCHC (g/L)	M	67	34.6	32.6	37.5	32.8	37.2	0.386
	F	72	34.3	30	37.9	31	37.7	
	Combined	139	34.3	30.2	37.9	32.3	37.4	
PLT (x10 ⁹ /L)	M	67	230	145	469	146.7	466.2	0.369
	F	72	241.5	141	433	145.95	418.2	
	Combined	139	236	141	469	146	438	
%LYM	M	67	36.6	4.8	71.2	10.8	64.6	0.274
	F	72	37.8	14.7	76	19.4	66.4	
	Combined	139	37.5	4.8	76	16.6	63	
%MXD	M	67	8.3	1.5	17.8	1.7	15.8	0.807
	F	72	7.85	0	17.3	0	16.6	
	Combined	139	7.9	0	17.8	1.7	15.8	
%NEU	M	67	54.7	22.5	88.4	26.9	84.1	0.227
	F	72	53.4	18.6	82.3	29.7	75.4	
	Combined	139	53.7	18.6	88.4	30.3	78.4	
#LYM	M	67	4.4	0.8	7.7	1.9	7.7	0.188
	F	72	4.6	1.7	10.6	1.9	10.4	
	Combined	139	4.5	0.8	10.6	1.9	8.3	
#MXD	M	67	1	0	2.4	0.1	2.3	0.657
	F	72	1	0	2.5	0	2.4	
	Combined	139	1	0	2.5	0.1	2.4	
#NEU	M	67	6.8	1.9	15.7	2	15.4	0.684
	F	72	6.3	2.5	11.2	2.8	10.8	
	Combined	139	6.6	1.9	15.7	2.7	12.9	
RDW-CV (%)	M	67	16	12.9	18.3	13	18.3	0.01
	F	72	15	12	19	12	19	
	Combined	139	15.6	12	19	12	19	
PDW-CV (%)	M	67	11.2	9.1	16.5	9.3	16.2	0.199
	F	72	10.8	8.6	15.8	8.6	14.8	
	Combined	139	11	8.6	16.5	9.1	15.7	
MPV(fL)	M	67	9.4	7	11.3	7.4	11.2	0.913
	F	72	9.35	8	12.5	8.1	12.3	
	Combined	139	9.4	7	12.5	8.1	11.8	

Table 4 Mean±SD or Median Comparison with Previous Studies and other given reference values.

Parameter	Our Study		Sysmex KX-21	From Book	Sudan	Nigeria	S.Arabia	Pakistan
	Median	Mean±SD	(0-24hrs newborns)	(58)	(47)	(46)	(43)	(41)
WBC	12.6	12.4±3.38	9.0-30.0	5.0-23.0	12.3±4.17	13.1±5.20	16.1	13.7±4.00
RBC	4.5	4.51±4.49	4.1-6.7	3.13-4.85	4.34±0.60	4.05±0.55	5.1	-
HGB	15.8	15.8±1.64	15.0-24.0	11.3-17.6	14.4±1.55	13.9±1.50	17.7	15.4±1.90
HCT	45.9	46.1±4.62	44-70	46-75	44.1±5.14	44.8±5.78	53.2	-
MCV	102.1	101.2±5.97	102-115	99-115	105.5±5.14	110.4±11.88	106	103.4±4.60
MCH	35.3	35.1±1.97	33.0-39.0	-	33.5±1.99	32.6±4.13	35.5	33.8±1.60
MCHC	34.3	34.5±1.17	32.0-36.0	-	33.1±1.19	29.8±1.64	33.2	-
RDW-CV	15.6	15.4±1.60	11.8-15.6	-	19.8±4.26	-	-	18.5±18
PLT	236	245.5±69.78	140-385	180-428	261± 83.16	225.1±72.21	234	285±62
LYM%	37.5	38.2±10.96	-	-	-	-	-	-
MXD%	7.9	8.0±3.44	-	-	-	-	-	-
NEU%	53.7	53.9±10.84	-	-	-	-	-	-
#NEU	6.6	6.7±2.42	6.0-26.0	1.7-19.0	-	-	7.7±3.00	-
#LYM	4.5	4.7±1.93	2.3-10.8	1.0-11.0	-	-	5.1±1.80	-
#MXD	1	1.1±0.88	0.1-3.6	0.2-5.7	-	-	-	-
PDW	11	11.6±2.22	-	-	-	-	-	-
MPV	9.4	9.5±0.90	-	-	-	-	-	-

*Units: WBC ($\times 10^9/L$), RBC ($\times 10^{12}/L$), HGB (g/dL), PLT ($\times 10^9/L$), MCV (fL), MCH (Pg), MCHC (g/L), RDW-CV (%).

Most studies present their findings as Mean±SD and hence the comparison was made accordingly as described in Table-4 (All tables are found at the end of this article)

DISCUSSION

Comparison of results according to sex, delivery modes and gestational age group were done. There were no statistically significant gender difference ($p>0.05$) for all haematological parameters except RDW, which was a consistent finding with that of Greece, in addition to WBC, NEU and PLT [37]. Other previous studies from Korea, South India, Nepal, Saudi Arabia, Iran, Iraq, Nigeria and Sudan on the other hand, conclude there were no statistically significant differences by gender at this early life [38-47].

In the current study all the measured values were located within interval compared to reference value given for Sysmex KX-21 haematology analyser new-borns (0-24hrs) reference interval. Even though, the current study has lowered and narrowed reference interval for WBC, RBC, HGB, HCT and absolute differential counts (#NEU, #LYM, #MXD) parameters. On the other hand, there were no significant difference of MCH and MCHC reference interval of our findings to that of them.

In the current study, there were higher RBC, HGB and HCT values compared to Taiwan, Greece, Korea, South India, Pakistan, Nepal, Iraq, Nigeria and Sudan [33, 37-42, 45-47]. Our findings for both MCH and MCHC were also higher than those from Taiwan, South India, Nepal, Iraq, Nigeria and Sudan [33, 39, 42, 45-47], these might be due to our study was performed on high altitude and mode of deliveries which was majorly spontaneous vaginal delivery; also supported by previous studies by Quiser DH et al [16], Younis et al, El Gendy et al and YH Chang et al [31-33]. Birth is stressful event accompanied with hormone regulated inflammatory action which increase cell mobilization [47-55]. MCV value in this

study was surprisingly lower from all the studies included in this literature [33, 37-47]. RDW values in our finding was lower than Saudi Arabia [43], Iran [44] and Nigeria [46]; this might be due to nutritional variation [50] but higher from that of Greece [37].

In the current study, we found higher total WBC compared to studies from Taiwan, Greece, Korea, Nepal and Iraq [33, 37, 38, 42, 45]. This might be due to spontaneous vaginal delivery was the major mode of delivery in our country which affect the fetal hemogram [51-53]. However, the reference values from South India[39], Saudi Arabia[43], Pakistan[40, 41], and Nigeria[46] are slightly higher than our findings. On the differential part, we found higher neutrophil value compared to Taiwan, Korea and Pakistan [33, 38, 40, 41]. However, we found lower lymphocyte and mixed parts when we come across with most of the studies included in this literature may be due to nutrition and ethnic variation [48-50].

In the present study, the PLT value was higher than Taiwan, Korea, Nepal and Nigeria [33,38, 42, 46]; however, lower than South India, Pakistan, Saudi Arabia, Iran, Iraq and Sudan [39, 40, 41, 43-45]. On the other hand, the platelet indices (MPV=8.1-11.8 and PDW=9.1-15.7) values in the current study were lower than findings from Iran (MPV=8.5-11.6 and PDW=9.4-16.4) but higher than Greece (MPV=6.0- 10.0)[37,44]. There might be possibly due to sample collection, processing and analysis may greatly affect reference interval establishment as described by different textbooks and literatures [14,33,35,56-58].

CONCLUSION

Since this study is pioneer of its kind with regards to haematological reference intervals in cord blood, the values obtained from our study could provide reference intervals for some haematological parameters in healthy new-borns (0-24rs) of Addis Ababa and its surrounding special whereas. However, the results need to be confirmed by larger samples from different centres throughout the country.

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