ADDIS ABABA UNIVERSITY

COLLEGE OF HEALTH SCIENCES

DEPARTMENT OF MEDICAL LABORATORY SCIENCES

Establishing Umbilical Cord Blood Hematological Parameters Reference Interval for Newborns in St. Peter Specialized Hospital Addis Ababa, Ethiopia from January 1 to March 31, 2019

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A Research Thesis to be Submitted to the Department of Medical Laboratory Sciences, College of Health Sciences, Addis Ababa University, in Partial Fulfillment of Master of Science Degree in Clinical Laboratory Sciences (Hematology and Immunohematology).

July, 2019

Addis Ababa, Ethiopia

Addis Ababa University

School of Graduate Studies

This is to certify that the thesis prepared by **Ammanuel Angelo**, entitled:

Establishing Reference Interval for Umbilical Cord Blood Hematological Parameters of Newborns in St. Peter Specialized Hospital Addis Ababa, Ethiopia from January 1 to March 31 2019 and submitted in partial fulfillment of the requirements for Master of Science degree in Clinical Laboratory Sciences (Hematology and Immunohematology) complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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Acknowledgement

It is impossible to express with few sentences my gratitude for so many people for helping me to do this research thesis and cooperate finally come to an end.

Firstly, my heart felt gratitude goes to my Advisor Dr. Aster Tsegaye (PhD) Associate Professor of Immunology, Addis Ababa University. She has been always thoughtful and helpful from the very first days of the course and encouraging us to do on topics related to problems we face throughout our profession. One of them is lack of our own reference range for hematological value for Ethiopia a country with varied altitudinal variation and other factors like age, sex and socioeconomic conditions. It is an honor to work with you again and to be taught. Then to my appreciation also goes to my collaborator Girma Derbie (MD, ObGyn) to direct me well with respect to his area of specialty by giving some important suggestion and comments.

Secondly, I would like to thank Addis Ababa University, College of Health Sciences, Department of Medical Laboratory Sciences to have the chance to join you again for the Master's program and it is a privilege to learn here and to be taught by such friendly instructors.

Finally, my great respect and thanks goes to study participants who had sought their time for the interview with full voluntary and support for the study. My innermost thanks also to Midwifery professionals, OR (operation room) nurses and Medical Laboratory Technologists who had took the full responsibility for interviewing participants, collecting cord blood and analyzing sample respectively besides having the usual heavy workload from the beginning to end.

My special and from bottom of my heart I would like to thank Mr. Abebe and his wife Mrs. Helen (Importer and Wholesaler) for their support by providing with EDTA Test Tube and Wright's Stain. I would like to finalize my acknowledgment by noticing and appreciating St. Peter Specialized Hospital research directorate and to all staffs and my friends who were by my side during these periods.

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List of Abbreviations

Ba	Basophil
CBC/FBC	Complete Blood Count/Full Blood Count
CI	Confidence Interval
CLSI	Clinical Laboratory Standards Institute
ECS	Emergency/Elective Cesarean Section
Eos	Eosinophil
GVHD	Graft-versus-host disease
Gyn	Gynecology
Hct	Hematocrit
Hgb	Hemoglobin
HLA	Human leukocyte antigen
HSC	Hematopoietic Stem Cell
HSCT	Hematopoietic stem cell transplantation
Lym	Lymphocyte Count
MCH	Mean Corpuscular Hemoglobin
MCHC	Mean corpuscular hemoglobin concentration
MCV	Mean corpuscular volume
MNCH	Maternal Neonatal and Child Health
Mon	Monocyte
MPV	Mean platelet volume
Neu	Neutrophil count
NICU	Neonatal Intensive Care Unit
Obs	Obstetrics
OR	Operation Room
PI	Principal Investigator
Plt	Platelet
RBC	Red blood cell
RDW	Red cell distribution width
SOP	Standard Operating Procedure
SD	Standard Deviation
SVD	Spontaneous Vaginal Delivery
UCB	Umbilical cord blood
UCBB	Umbilical Cord Blood Bank
WBC	White blood cell count
WHO	World Health Organization

Abstract

Background: Several factors like altitude, age, sex, pregnancy, socioeconomic status, life style and race influence hematological 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 hematological parameters of newborns in Ethiopia.

Objective: To generate reference interval for umbilical cord blood hematological parameters of newborns 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 newborns (37-42 weeks) with normal birth weight born to apparently healthy pregnant mothers who had met the eligibility criteria. From a total of 139 newborns, 2-3ml cord blood was immediately collected from the clumped cord using EDTA tube. The samples were analyzed using Sysmex KX 21 hematology analyzer. Data was entered and the 2.5^{th} and 97.5^{th} percentile (upper and lower reference limit) were determined using non parametric 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 hematological parameters of newborns with the median and 95% reference values 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: The values obtained from our study provide reference intervals for some hematological parameters in healthy newborns of Addis Ababa and its surrounding special weredas. However, the results need to be confirmed by larger samples from different centers throughout the country.

Key words: CBC, Reference interval, Umbilical Cord Blood, Neonates

1. INTRODUCTION

1.1 Background

Complete blood count (CBC) also known as full blood count (FBC) is one of the most common laboratory tests ordered to assess anemia, polycythemia, infection and thrombocytopenia in new born, and it offers a great deal of information about the hematopoietic system in general within short period of time (1-3).

Hematopoiesis is shortly described as the developmental process of all the three blood cells, i.e. red blood cells (RBC), white blood cells (WBC) and Platelet (PLT) from a pool of undifferentiated cells, hematopoietic stem cells (HSC), which give rise to all of the bone marrow cells by the processes of proliferation and differentiation (4). The development takes in 3 stages in embryonic yolk sack (1^{st}-2^{nd} months or 2^{+4} weeks of gestation), liver (3^{rd}-4^{th} months of gestation) and bone marrow from 5^{th} months of gestation on become the primary and major site of hematopoiesis, therefore, both mature and progenitor cells found in term cord blood(5).

Umbilical cord is long and helical cord that allows the fetus get nutrients, oxygen or other substances critical for growth and development within the womb. It achieves final form by the 12^{th} week of gestation and connects one end to the abdomen area of the fetus and the other end to placenta, which enables the mother and fetus exchange contents from both circulations (6). It is surrounded by an extracellular matrix, specialized connective tissue known as Wharton's jelly which is important to keep the cord moist and appear bluish white (7).

After delivery, the cord is clamped into two places and the clamps extensively cleaned with povidone-iodine solution and alcohol (8-10) and cut to separate the infant from the placenta. During this procedure, cord blood could be collected for hematological (CBC, Blood Grouping, Cross matching etc.) and biochemical (Bilirubin, Ferritin etc.) studies and for therapeutic purpose (cord transplants) (3, 8) due to its enrichment with stem cells(11).

In order to make clinical decision for the observed or measured laboratory results, it is necessary to have reference data that is derived from well-defined population of healthy subjects and this reference data is termed as reference range/value/interval(12). The borderline abnormalities should be in detail interpreted in context of history and physical examination(13). This is because, a value outside the

range have higher statistical probability of having a specific disease or at least the observed result is not normal for healthy individual (14).

Reference interval can be described as the lower and upper reference limits assumed to take the values 2.5^{th} and 97.5^{th} percentile respectively enclosing the central value (95%)(15). Reference ranges may also be established for a population with diseases or conditions by taking diseased individuals among those having disease or conditions (12). It is understood that established reference range is specific for subgroup of the population whose measurements are greatly affected by several factors including age, sex, ethnicity, race, altitude (16)

Since 1969 when the concept of reference range was raised by Grasbeck and Saris to describe analyte concentration variation of known groups, the clinical laboratories test result is reported and interpreted based on it and nearly 80% of physician's clinical decisions are based on the information generated by the laboratories(17, 18).

1.2 Statement of the Problem

Reference intervals aid in the interpretation of laboratory data for appropriate clinical decisions. Cord blood hematological parameters reference intervals are vital in neonatal care. The etiology of neonatal anemia can be classified into i) hemorrhage (ii) hemolysis (iii) failure of red cell production. In 50% of pregnancies some fetal cells are passed in maternal circulation sometimes during gestation or during birth process (19). Sepsis is the commonest cause of neonatal mortality; it is responsible for about 30-50% of the total neonatal deaths in developing countries that may be caused by bacterial, viral, fungal and parasitic infections which can be indicated by thrombocytopenia as one of the early but non-specific indicator of neonatal sepsis more than WBC (20).

A reference interval obtained from somewhere else and utilizing to population of interest could potentially lead to wrong classification of individuals (healthy or ill), inappropriate patient management and unnecessary use of resources (21, 22). This is due to the reason that there is biological variation within or between individuals for nearly all laboratory measurements (23). Quantitative and qualitative differences are present as a reflection of the developmental changes during fetal hematopoiesis and, so, correlate with gestational age. At birth, the hemoglobin, mean corpuscular volume, and WBC counts of term newborns are significantly higher than those of older children and adults; in preterm neonates the differences are even more pronounced (24).

On the other side, it is difficult to establish reference intervals for each physiological change, but some indirect techniques used in some situations like that of neonates (25). Establishing normal neonatal ranges has been difficult because blood has not been drawn on healthy neonates of similar ages and other factors needed to be considered include sample site, timing of the sample, gestational age, the neonate's degree of health and maternal factors like being anemic, pregnancy related conditions, mode of delivery, cord clumping, pregnancy interval, life style and socioeconomic status can affect the CBC results (1, 26).

For example, a study from Malawi on healthy individuals investigated whether there are any changes of hematological values in age and sex; they found that the total WBC count decreases with the advancement of age and on the other hand, with the same age female neonates had significantly higher MCV compared to that of males (27).

According to the studies from Nigeria, India and Turkey maternal anemia had effect on cord blood hemoglobin and new born weight(28-32), though routine hematological values of newborns are

independent to that of maternal hematological values (33). This may be due to high micronutrient intake inadequacy in pregnancy especially in those women who had the previous pregnancy complicated with pre-eclampsia (34). Such conditions leads to maternal and neonatal deaths in developing countries, associated with 10–15% of direct maternal deaths and nearly a quarter of stillbirths and newborn deaths(35).

Maternal depression during pregnancy could also have association with neonatal anemia(36). As the study from Sao Paulo, Brazil indicated that pregnant mother with smoking habit could give birth to new born with decreased birth weight depending on the number of packs per day compared to non-smoking mother(37), in turn low birth weight lead to early neonatal death or other health complication in later life(38). Similarly, mothers who are heavily drinking alcohol could give birth to low birth weight, with a range of lifelong effect like brain disorders baby and also be at high risk of miscarriage and still birth (39).

Other factors like, eclampsia that may be caused by young age, null parity, poor antenatal care and pre-existing medical problems like diabetic mellitus which worsen the condition of pregnant woman with hypertension(40) could lead to morbidity and mortality of both the mother and neonate (41). All these factors affect the hematological profile of neonates. Also during the sample collection procedure though safe way to collect sample compared to cannula and other vascular catheters which cause thrombophlebitis, infection, and extravasation(42), the delayed cord clamping and umbilical cord milking had comparable effect on hematological status at 6 weeks of life, in term neonates (43).

Generally, reference range can play an important role in guiding the interpretation of laboratory results of newborn if it is determined considering the above mentioned different influencing factors. Despite these, there is no published reference interval for hematological parameters in cord blood of Ethiopians, a gap which this study tries to address.

1.3 Significance of the Study

The data generated from this study will aid the clinical decision by providing suitable reference interval in assessing the hematological changes in the newborns associated with anemia, thrombocytopenia and infection that result in the morbidity and mortality of neonates. This study could be used as baseline information for researchers. Finally, introducing cord blood analysis in neonatal care also will be another target of this study.

2: LITERATURE REVIEW

2. Literature Review

A study aimed at obtaining large scale population based reference range for healthy neonates with samples obtained from public umbilical cord blood bank (UCBB) done for Taiwanese neonates from September 2001 to November 2005 showed significance differences between the male and female neonates ($p<0.001$) and routes of delivery. The female neonates had higher MCV, PLT and WBC, but lower RBC, HGB, HCT and MCHC as compared to the male neonates. They got result closer to the Korean study with lower HGB (9.7-12.7vs10.4-13.2gm/dL) and uncorrected WBC (6.9-12.7x10^3/uLvs7.7-14.1x10^3/uL), but higher PLT (172-262 vs 169-247 x10^3/uL). They collected a total of 5602 UCB and ran with Sysmex XE2100 automated analyzer and the WBC diff count was performed manually by experienced technician. To conclude, they obtained the reference range for the targeted group and also tried to indicate the influence of gender and mode of delivery on reference values (44).

A 4 months (January to April 2014) longitudinal study from South India by Suman FR *et al* published in 2015 revealed no significance difference in the reference range of neonates between the two sexes. The study was done on 120 full term newborns with normal birth weight born to mother with hemoglobin >10g/dL, ages between 21 and 45 years and fulfilling other exclusion criteria. They found comparable results by running samples on Diagon D-Cell 66 automated analyzer with that of a study from Nigeria by Adewumi *et al (45)*. However, they found a result of Hgb (13.2-16.6g/dL), RDW (10.2-17%), WBC (10.8-21.0x10^3/uL), Platelet (146-284x10^3/uL), and absolute neutrophil count (ANC) (4.8-6.6x10^3/uL) with significant difference to that of Greece's findings (46) with Hgb (5.9-11.7g/dL), RDW (10.5-13.7%), WBC (3.8-10.6x10^3/uL), Platelet (101-219x10^3/uL), and ANC (1.6-5.2x10^3/uL. The difference in hemoglobin measurement is so visible and the possible reason may be the dilution effect during collection. They came into the conclusion that the established reference range could be helpful for Neonatologists and Transplant Specialist for important clinical decision (47).

The study undertaken to establish reference range for Nepal neonates also showed there were no significant differences in the value of HGB (15.16 ± 1.95 and 15.31 ± 1.96g/dL), RBC (4.28 ± 0.64 and 4.30 ± 0.62x10^{12}/L), WBC (14.68 ± 4.29 and 14.99 ±4.59 x10^9/L) and PLT (229.38 ± 59.56 and 224.6 ± 62.98 x10^9/L) for male and female, respectively. The study enrolled 210 full term healthy neonates from Jan 2014 to Feb 2015 and analyzed on automated hematology analyzer. The result for HGB (13.28-17.2), RBC (3.67-4.93 x10^{12}/L), WBC (10.49-19.37 x10^9/L) and PLT (165.60-288.16 x10^9/L)

was comparable to the findings of Buenos Aires (48), Pakistan (Karachi and Sindhi) (49, 50), Iraq (51), and Malawi (52). The RBC value (4.30±0.63 $x10^{12}$/L) in this study was slightly lowered from the Malawian (52)neonates with the value of RBC (4.52±0.57 $x10^{12}$/L). However, the study showed higher HGB value compared to findings from Greece (8.8±2.9g/dL) (46), Nigeria (13.29±1.5g/dL) (45) and Taiwan (11.2±1.5g/dL) (44). They finalized the study by successfully establishing the required reference range; however, they indicated it was not sufficient and recommended to confirm this result with large population samples (53).

For the establishment of reference range for Greece neonate from 2000 umbilical cord blood (UCB) collected on collecting bag and analyzed by multi-parameter Beckman coulter hematology analyzer in the years between Jun 2008 and Jan 2009. Like the previous study, in this study there were no significant variations of the results between male and female neonates. But some differences were noted in the value of WBC (7.1±3.4 for male and 7.4±3.4 for female $x10^9$/L), RDW (12.3±1.7 for male and 12.0±1.6 for female) and PLT (157±58 for male and 164±60 $x10^9$/L for female). They used the manufacturer reference values of Beckman Coulter and European reference values for comparison of the results. Even though they could not find other studies of evidence they proposed the use these reference values for hematological analysis, hematopoietic stem cell therapy and future analysis (46).

A different cross-sectional study from Iraq determined the reference range for neonates by collecting UCB in EDTA tube and analyzed with Diagon D-Cell 60 hematology analyzer and had done manual differential white cell counts. In their study they included 220 healthy term neonates born to mother with ages between 15-45 years and with no any pregnancy related problems from Feb 2011 to Jan 2012. They obtained results for HGB 13.88±1.34g/dL, WBC 10.12±2.8$x10^9$/L, and PLT 267.63±60.62$x10^9$/L. Similarly they did not find significant difference between values of genders, except significant neutrophil value differences. Comparing to the previous studies they had lower values for HGB, RBC and WBC. For example in their study the HGB value was 13.88±1.34g/dL, but the Pakistan study showed a value of 14.99±1.47g/dL. They concluded that even though most results fall within the range, further study should be done on large number samples from throughout the country (51).

A study aiming at establishing reference values for Korean neonates was done on 2129 samples from donated UCB from August 2007 to December 2007. The samples were analyzed using Sysmex XE2100 and reference values estimated using parametric method (mean±SD) and non-parametric method (2.5-

97.5 percentile). They came with result of HGB (9.0-14.4g/dL), WBC (5.6-13.5x10^3/uL), WBC-DIFF (Neu-40.8-72.4%, Lym-17.2-46.7%, Mon-4.9-12.8%, Eos-0.7-7.0% and Bas-0.0-1.6%), PLT (130-287x10^3/uL) and NRBC (0.0-13.1/100WBC). They established this reference values as they are significant for CBC result interpretations and to assess whether the donated UCB is suitable for transplantation (54).

In Pakistan, Karachi a cross-sectional study aimed on establishing reference values for hematological parameters in full term healthy newborns in order to be used in clinical decision making by physicians. A total of 404 samples were collected from Jul 2006 to Apr 2008. Out of 404 newborns, 271 and 133 were delivered by Normal Vaginal Delivery (NVD) and Elective Cesarean Section (ECS), respectively with mean weight of 3.03±0.385Kg. The samples were arrived to the laboratory within 3-6 hours and analyzed by Beckman Coulter hematology analyzer and manual diff count was performed and the findings were as follows: HGB (14.99±1.47g/dL), WBC (13.61±4.23x10^3/uL) and PLT (256.25±76.54x10^3/uL). The HGB was lower than from Indian (14.9±1.7g/dL) reports (47, 49). Another relatively recent descriptive study was conducted on 316 full term normal neonates from rural Sindh, Pakistan analyzed by Sysmex KX-21 and found nearly the same HGB (15.4±1.9g/dL) and WBC (13.7±4.0x10^9 /L), while a few difference to PLT (285±62x10^9/L) result (50).

A research from Nigeria by Adewumi A *et al* revealed there were no significant differences in CBC values regarding gender. The obtained result for male and female were HGB (13.27±1.60g/dL M and 13.32±1.61g/dL F), WBC (13.16±5.43x10^9/L M and 13.07±4.95x10^9/L F) and PLT (223.64±64.21x10^9/L M and 226.69±80.83x10^9/L F). They got relatively closer findings with that of Iraq (51) and Pakistan (49). For example the RBC in their study was 4.07±0.55x10^{12}/L, while for Iraq and Pakistan was 4.0±0.47x10^{12}/L and 4.29±0.44x10^{12}/L respectively (45).

A study performed from September 2007 to March 2008 in Iran to determine hematological reference values for neonates done on 447 full term healthy neonates. The samples were analyzed by Sysmex automated analyzer and manual diff count was performed for white cells. The results obtained were HGB (15.9g/dL (13.0-18.8)), WBC (11.62x103/uL(5.16-18.2)), DIFF (Neu-48%, Lym-42%, Mon-7%, Eos-3% and Bas-0%), and PLT (257x103/uL(131-383)). This study values compared with Brugnara C et al had some differences of values like HGB (15.9g/dL vs 15.3g/dL (12.7-17.9)), WBC (11.62x103/uL vs 18x103/uL(9-30)) and PLT (257x103/uL vs 288x103/uL(182-394)) (55).

A cross-sectional, descriptive and analytic study aimed on identifying expected normal hematological values as a baseline to facilitate early detection of disorders occurred after birth. The study was conducted from July 2011 to June 2013 on 500 full term healthy Sudanese neonates with 2.5 Kg weight born to normal pregnant women. They found results for HGB (14.35±1.55g/dL), MCV (105.5±5.14fL), WBC (12.3±4.17x10^9/L), and PLT (261±83.16x10^9/L). In their conclusion they tried to indicate that HGB and RBC indices values of UCB fall within the normal values but slightly lowered due to ethnological and lifestyles differences compared to previous studies (56).

A cross-sectional study was conducted on 2,163 healthy newborns from January 1 to April 30, 2013, in Jeddah-Saudi Arabia and established the reference range of hematological parameters from cord blood and found no statistical difference between males and females, except total WBC (male= 16.2 ±5.3×10^9/L and female= 17.2±12 ×10^9/L (p= 0.017)) and monocytes (male= 1.2± 0.2 × 10^9/L and female=1.3 ± 0.4 × 10^9/L (p=0.037)) (57).

Some other literatures tried to describe the effect of associated factors involved in establishing reference range for specific subgroup of population. In the study on 476 samples from Buenos Aires, Argentina in 1999, Noguera NI *et al* aimed on investigating the cord blood hematological profiles of newborns born to different groups pregnant women with different habits (alcoholic, smokers) and mode of delivery; neonates' birth weight and gestational age also included on the study. The study was done on three groups; 1st group: normal birth weight and full term gestational age, 2nd group: low birth weight and normal gestation age, and 3rd group: pre term newborn. In their study they found a slight rise on the HCT (54.3±6.3%) and RBC (5.03±0.51) in the 2nd group, but a significant decrease on the HGB (14.7±1.9) and increase on the MCV (109.1±2.90) 3rd group comparing to the 1st group HCT (49.0±4.3%), RBC (4.66±0.33x10^6/uL) HGB (15.5±1.1g/dL) and MCV (105.1±5.3fL), respectively. In the RBC morphology they had seen large number of macrocytes, polychromatophilia and slight anisocytosis and poiklocytosis. They also tried to look the effect of smoking habit and they found no effect on the three of the groups(48).

The study by Chang YH *et al* 2011 from Taiwan compared the normal reference values of RBC (3.22±0.44 x10^6/uL), HGB (11.2±1.5g/dL), NEU (9.0-66%), MON (1.0-17.0%), NRBC (3.4±5.5), HCT (36.9±4.6%) and PLT (217±45x10^3/uL), and showed the newborns delivered by normal vaginal delivery (NVD) had significant increase in RBC (3.14±0.41x10^6/uL), HGB (10.9±1.4g/dL), WBC (9.5±2.7x10^3/uL) and HCT (36.9±4.6%)($p<0.001$). They also showed elective cesarean section (ECS)

could significantly decrease NEU (8-61%), MON (1.0-15.9%), PLT (202±41x10^3/uL) and NRBC (3.1±6.5). On the other hand ECS had no significant effect on MCV, MCH and MCHC (44).

A comparative study by El Gendy FM *et al* 2014 conducted in 72 newborns to assess the hematological parameters born in NVD (31) and ECS (41) modes of delivery. They found significantly higher RBC (4.6±0.57 vs 4.03±0.55 x10^6/uL), HGB (15.6±2.16 vs 14.1±1.70g/dL), HCT (44.92±3.66 vs 41.07±5.31%), WBC (16.1±1.93 vs 14.62±1.95x10^3/uL), PLT (292.38±67.19 vs 248.95±43.08 x10^3/uL), NEU (53.74±8.39 vs 48.58±6.72%), EOS (2.22±2.50 vs 1.29±1.19%) and BAS (1.48±2.01 vs 0.31±0.19%) in NVD compared to ECS, respectively. Similarly to the previous study mentioned above, there were no significant differences on the MCV, MCH and MCHC (58). Conversely, a study from Sudan by Younis MS *et al* 2017 had come into a conclusion that mode of delivery had no significant effect on CBC parameters like HGB (14.6±1.6 vs 14.7±1.56g/dL), HCT (45.2±4.9 vs 45.8±5%), MCH (34.1±2.2 vs 33.4±2.5 pg), MCHC (32.3±1.3 vs 32.2±1.3%), EOS (2.6±0.6 vs 2.5±0.7%) and BAS (0.60±0.5 vs 0.57±0.5%) (59).

A cross-sectional study aimed on finding any correlation exist between maternal and UCB CBC results. The study was conducted on 114 mothers who delivered through normal vaginal delivery and on their healthy neonates. They found moderately positive Pearson correlation ($p<0.001$ & $R=0.496$) between maternal and neonatal HGB, MCH and MCHC; but there were no statistically significant correlation with WBC, RBC and PLT. This was because decreased HGB occur due to severe anemia even though there was no significant correlation existed. So the fetal hematological values are not the reflective of maternal hemogram (60).

A prospective study for a period of two years conducted on 300 cord bloods to see the effect of feto-maternal factors on cord blood hematological parameters showed neonates were born with fairly constant hematological parameters in the presence of a number of factors. The study also included control group consists of normal vaginal delivery, full term, and >2.5Kg birth weight for comparison. As the CBC evaluated for 106 (36%) ECS delivery, pre term and early parity showed higher MCH and MCHC values. MCH and MCHC values were not affected by gestational age and birth weight. In the context of gender, females had significantly lower HGB, RBC and PCV. WBC counts were raised in preterm, NVD and early parity. There were significantly higher count for NRBCs and Retics count in preterm and ECS. The effects of mode of delivery were seen in all of the parameters except PCV, MCV and PLT (61).

Generally, the literatures above broadly describe reference intervals are influenced by several factors. As far as to my literature search goes, there are no published studies that have assessed hematological reference interval in a controlled set up in Ethiopia for neonate population from umbilical cord blood, despite some attempts were made in other areas(62, 63) Therefore, this study is the pioneer of its kind with regards to hematological reference intervals in cord blood. In addition, the findings from this study were compared to other similar studies from Africa and some parts of world who had established reference range in the same controlled set up.

3: OBJECTIVES

3.1 General Objective

The aim of this study was to establish umbilical cord blood hematological parameters reference interval for newborns delivered in St. Peter Specialized Hospital Addis Ababa, Ethiopia from January 1 to March 31, 2019.

3.2 Specific Objectives

- To determine reference interval of cord blood hematological parameters (WBC, LYM, MXD, NEU, RBC, HGB, HCT, MCV, MCH, MCHC, RDW, PLT, MPV and PDW)
- To compare umbilical cord blood CBC results distribution of newborns by sex, deliveries modes (NVD vs ECS) and gestational week.

4: MATERIALS & METHODS

4.1 Study Area

The study was conducted in Addis Ababa a city situated in central Ethiopia at an elevation of about 2440 m (about 8000 ft) above sea level, among newborns delivered in St. Peter Specialized Hospital. The hospital which was established in June 1953 and is currently located in Gulele Sub city, Shiromeda is administered under the Federal Democratic Republic of Ethiopia Ministry of Health (FMoH). It has more than 900 medical and none medical staff. The hospital currently has 245 functional beds. According to the 4th quarter of 2010 EC report of the hospital HMIS a total of 28,788 clients visited the hospital for various services; more than 23,999 patients had received services in its outpatients department and about 2985 were seen in Maternal, Neonate and Child (MNCH). While, the remaining 1807 patients had received inpatient services and from this 270 and 142 were admitted to Gynecology/Obstetrics (GYN/OBS) and Neonatal Intensive Care Unit (NICU), respectively.

Source: St. Peter Specialized Hospital Health Management and Information System (HMIS) Department 4th Quarter Report June 2010 (E.C)

4.2 Study Design and Period

A cross-sectional study was conducted in St. Peter Specialized Hospital in Addis Ababa from January 1 to March 31, 2019.

4.3 Population

4.3.1 Source Population

All newborns delivered in St. Peter Specialized Hospital, Addis Ababa city were the source population.

4.3.2 Study Population

Term apparently healthy newborns delivered from volunteer apparently healthy mothers and fulfill the eligibility criteria in St. Peter Specialized Hospital, Addis Ababa city during the study period were the study population.

4.4 Inclusion and Exclusion Criteria for the Pregnant Mother and Newborns

Priori selection method will be implemented to include eligible or exclude mother with medical conditions (infectious, chronic, obstetric and psychological) and social habits (smoking, alcohol) by

using diagnostic tests (lab tests, ultrasound) and history from the card, whereas posteriori selection method was used to include eligible or exclude newborns with certain conditions like low birth weight (LBW), preterm.

4.4.1.1 Inclusion for Pregnant Mother

- 18-45 years of age
- >=11.0g/dL HGB (WHO Recommendation…2011)
- Inter-pregnancy Interval >=1 year and 6months (WHO Recommendation…2005)

4.4.1.2 Inclusion for Newborn

- Newborns full term (37-42 weeks)
- 5^{th} minute APGAR score >=7 (appearance, muscle tone, pulse and respiratory rate)
- Normal Birth weight =2.5-4 Kg

4.4.2.1 Exclusion for Pregnant Mother

- Smoking, alcoholic habit, khat chewing and drug abuses during pregnancy
- Pregnant mother with medical (Hepatitis B, HIV, Syphilis and Insulin-dependent diabetes mellitus), obstetric (<6 months of abortion, preeclampsia) and psychological conditions
- Lack of willingness to sign the consent form

4.4.2.2 Exclusion for Newborn

- Babies with respiratory distress, meconium stain, fever and gross congenital anomalies
- Abnormal umbilical cord appearance, length and true knot
- Babies delivered by instrumental deliveries

4.5 Study Variables

4.5.1 Dependent

All hematological parameters to be measured are WBC, RBC, HGB, WBC Diff count (NEU, LYM and MXD), HCT, RBC indices (MCV, MCH, MCHC, RDW), PLT, PLT indices (MPV, PDW).

4.5.2 Independent

Gestational weeks, Age of mother, Sex of newborn, Socio-economic status, modes of delivery (SVD, ECS) and medical history.

4.6 Measurement and Data Collection

4.6.1 Sample Size Calculation

The Clinical Laboratory Standard Institute (CLSI) recommends that studies to establish reference intervals should have a minimum of 120 individuals by non-parametric means in each grouping variable. In the case of difficult-to-obtain subclass reference values for certain populations, such as newborn whatever number of values is obtained, the data should still be analyzed by the nonparametric method and reported by percentiles appropriate to the number of values obtained. (12). Therefore, in this reference interval study, 139 neonates were availed.

Participant mothers were approached through the department head, then their assigned respective midwives interviewed them. Finally, the mothers who were willing to sign both the consent and assent form were proceeded to cord blood collection after the baby is delivered alive.

4.6.2 Sampling Method

Non probability convenience sampling method was used.

4.6.3 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. From those consented participants, demographic information and a brief medical history were collected using predesigned questionnaire by assigned Midwives. The umbilical cord were clamped (in <1min after birth); 2-3 ml of blood were collected by Midwives or operation room (OR) nurse and transferred into an EDTA tube and well mixed (10x) by inverting with anticoagulant. Then the blood sample was transported to the laboratory for hematologic analysis. The samples were analyzed using Sysmex kx-21 automated hematology analyzer and peripheral blood smear by experienced Laboratory Technologist and for every CBC results smear review was performed alongside counting NRBC for WBC correction. The whole data collection process will be supervised by the assigned professional and principal investigator.

4.6.4 Screening Tests

The laboratory screening tests for pregnant mother was done only if the tests were not done, missed or suspicious about the result during the antenatal care (ANC) follow up. Currently used serological screening test routinely for pregnant women includes:

- Hepatitis B surface antigen test one step strip for Hepatitis B virus
- HIV antibody test with three steps (algorithm)
- Syphilis antibody test one step strip
- Ultrasonography tests to rule out fetal gross congenital anomalies

Currently, there are no laboratory tests available for maternal and newborn genetic screening in public hospitals.

4.6.5 Hematological Analysis

Complete Blood Count (CBC)

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 automated hematology analyzer. The KX-21N performs speedy analysis of 18 parameters including a 3-part WBC differential, plus histograms for RBC, PLT and WBC in blood. The KX-21 employs three detector blocks and two kinds of reagents for blood analysis. The WBC count is measured by the WBC detector block using the DC detection method. The RBC count and platelets are taken by the RBC detector block, also using the DC detection method. The HGB detector block measures the hemoglobin concentration using the non-cyanide hemoglobin method.

In brief, a well-mixed cord blood sample is aspirated and measured to predetermined volume, diluted at a specific ratio, and fed into each transducer. The transducer chamber has 2 mini holes called aperture. Blood cells suspended in the diluted sample pass through an aperture causing a change in the direct current resistance between electrodes. The size of the blood cell is detected as electric pulses. The number of blood cells is calculated by counting the pulses.

Peripheral Blood Morphology

Peripheral blood smears were prepared from all cord blood samples for investigation of red blood cell morphology, white blood cell abnormality and platelets abnormalities. In addition, nucleated red cells (NRBCs) were counted by the formula:

$$\text{Corrected WBC} = \frac{\text{Uncorrected WBC X 100}}{100+\text{NRBC}}$$

In brief, a thin smear was prepared, air dried and stained by wright stain. Wright's stain is a polychromatic stain consisting of a mixture of eosin and methylene blue. When applied to blood cells, the dyes produce multiple colors based on the ionic charge of the stain and the various components of the cell. The eosin ions are negatively charged and stain basic cell components giving them an orange to pink color. The methylene blue ions are positively charged and stain the acid cell components in varying shades of blue. The neutral components of the cell are stained by both components of the dye producing variable colors.

Cord blood sample for CBC collected by EDTA can be retained for 8 hours like that of venous blood for any test repetition. Leftover sample from the analysis were stored as a plasma separated from the whole blood and kept in deep freeze for the future other laboratory analysis.

4.7 Statistical Analysis

All the data from the questionnaires and laboratory results were coded and checked for completeness. Then data were entered and analyzed using SPSS-version 23 statistical software for windows. The minimum, maximum, median and percentiles (2.5^{th} and 97.5^{th}) for each hematological parameters with 90% CI reference Intervals were determined. The non-parametric Independent Mann-Whitney U test (Wilcoxon rank-sum test) was used to compare the distribution of the parameters between genders, delivery modes, and gestational age groups.

4.8 Data Quality Assurance

Pre-analytical

All pre-analytical factors, including subject preparation, sample collection and processing, the analytical method, and instrumentation (daily, weekly and monthly preventive maintenances) checkup, must be carefully defined and used for testing. Therefore, samples were collected and processed

according to the standard operating procedure (SOP) and protocols established for both maternal and cord blood.

Analytical

The reliability of the data generated is critical, because both the imprecision and inaccuracy of the method will determine its diagnostic utility. Therefore, in-house or commercial quality control materials were used in the same format as for patient testing, which not only monitors the analytical protocol used during the process but also ensures equivalence of results over the long term.

Post-analytical

For the entire CBC results peripheral blood smear was performed for further investigation of any abnormality related to WBC, RBC and Platelet. The results were recorded and handled appropriately and secured.

4.9 Operational Definitions

Hematological Parameters: refers to measurable or quantifiable characters which include WBC, PLT, RBC, HGB, WBC Diff count (NEU, LYM and MXD), HCT, MCV, MCH, MCHC, RDW, PLT, MPV and PDW.

Apparently healthy: An individual who has no sign and symptoms and history for any disease and negative result for the screening tests.

Reference interval: an interval or set of values that are used by physicians to classify individuals as healthy or ill together with clinical history

Preterm: less than 37 weeks completed (259 days)

Term: 37-41 weeks and 6/7 days (260-294 days)

Post-term: greater or equal to 42 weeks (295 days)

Normal birth weight: 2500-3999 g

Term: 37-41 weeks and 6/7 days (260-294 days)

Cesarean Section (CS): surgical removal of the fetus through incisions in the abdominal wall and the uterus.

Spontaneous Vaginal Delivery: normal delivery the baby passing through the birth canal without any medical interference.

Umbilical Cord Blood: Blood collected shortly after delivery by clamping the cord in two places and severed between the clamps.

Apgar score: is a tool that can be used objectively to define the state of a newborn at given times after birth, traditionally at 1 minute and 5minutes.

Parity: number of previous pregnancies of >28 weeks.

4.10 Ethical Consideration

The study was conducted after getting ethical clearance from the Department Research and Ethics Committee (DREC) of the Department of Medical Laboratory Sciences, College of Health Sciences, Addis Ababa University. An official support letter of request was written to St. Peter Specialized Hospital to obtain approval and carry out the study. The mother of neonates was informed about the aim of the study and assured about the confidentiality of the information and the participation is sole voluntary and none of a single service from the facility missed at the time of unwillingness or withdrawal by signing the consent form. The specimen collected from the participants was analyzed only for the intended purposes.

5: WORK FLOW

5.1 Work Flow

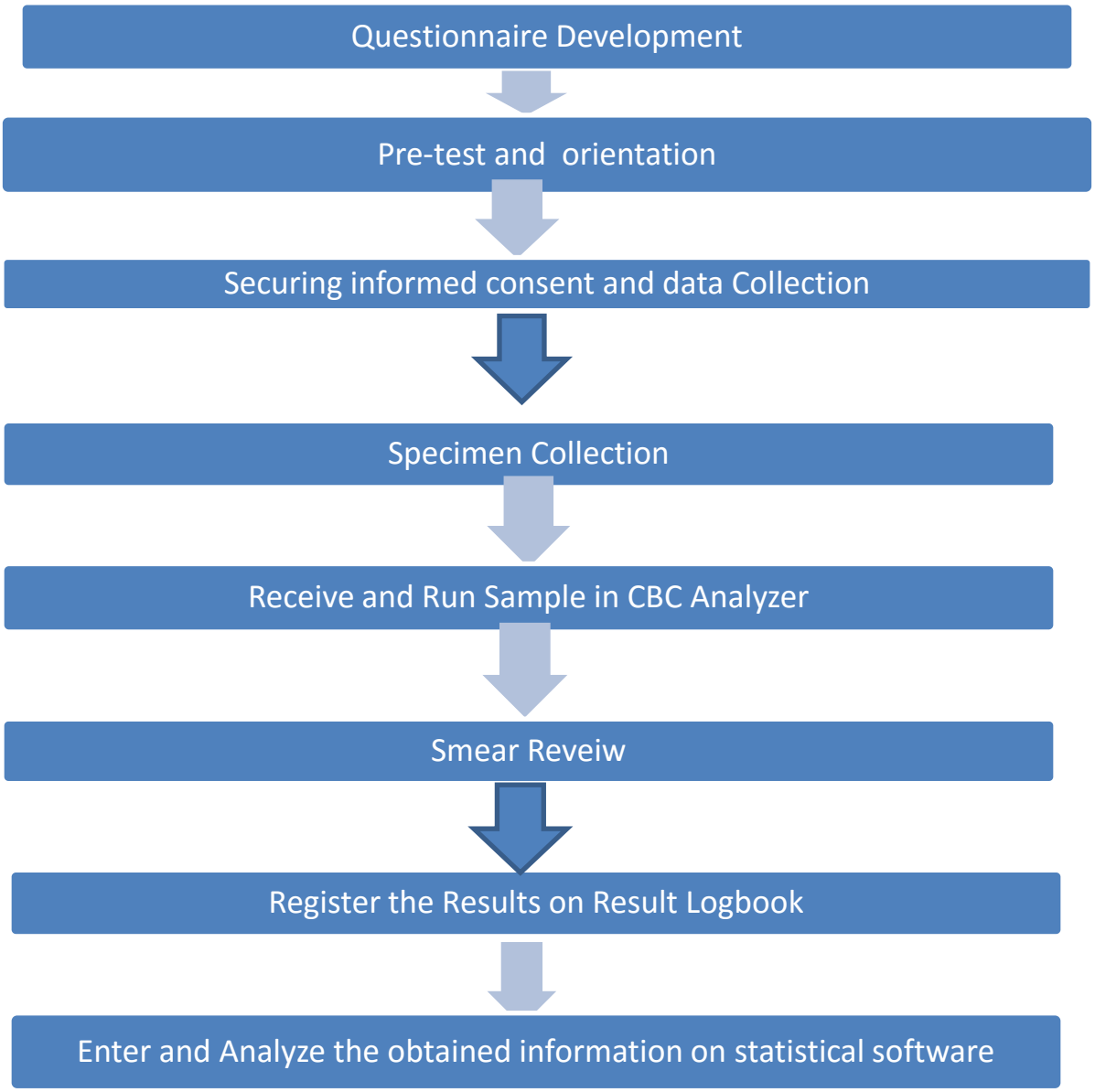

Figure 1 Work Flow Chart

6. RESULT

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,

Table 1 Demographic and Medical 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		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
Habit	Alcohol	Yes	27	19.4
		No	112	80.6
	Smoking	Smoke	0	100
		No	139	100
Maternal Conditions	Medical	Yes	1	0.7
		No	138	99.3
	Obstetric	Yes	2	1.4
		No	137	98.6
	Prescription	Yes	2	1.4
		No	137	98.6

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 2 were for WBC= 12.4 x10^9/L [6.55-19.35], RBC= 4.51 x10^{12}/L [3.55-5.52], HGB= 15.80g/dL [12.41-19.65], HCT= 45.9% [37.9-56.25], MCV=102.10fL [83.90-111.55], MCH= 35.30pg [29.35-39.10], MCHC= 34.3% [32.25-37.40], PLT= 236 x10^9/L [146-438], LYM= 37.5% [16.6-63.0%], MXD= 7.9%[1.65-15.75%], and NEU= 53.7%[30.25-78.35].

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=11.05[6.55-19.35], RBC=4.39[3.55-5.52], and absolute NEU =6.00[2.65-12.8] compared to newborns delivered through SVD with value for WBC=12.90[6.55-19.35], RBC=4.55[3.55-5.52], and absolute NEU =7.55[2.65-12.8] ($p<0.05$). On the other hand, for all hematological parameters there were no statistically significant difference with in gestational age groups [37-39.1 versus 39.2-42 months] ($p>0.05$) (Table 3).

Table 2 Medians, Minimum, Maximum and 95th 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	6.57	19.25	0.787
	F	72	12.4	5.8	23.5	6.14	23.17	
	Combined	139	12.4	5.80	23.5	6.55	19.35	
RBC (x10^{12}/L)	M	67	4.59	3.54	5.70	3.59	5.59	0.075
	F	72	4.44	3.10	5.70	3.37	5.30	
	Combined	139	4.51	3.10	5.70	3.55	5.52	
HGB (g/dL)	M	67	15.9	12.3	20.0	12.44	19.79	0.374
	F	72	15.7	10.8	20.6	11.06	18.79	
	Combined	139	15.8	10.8	20.6	12.41	19.65	
HCT (%)	M	67	46.0	36.0	60.2	37.89	57.75	0.861
	F	72	45.45	36.4	61.8	37.31	56.27	
	Combined	139	45.9	36.0	61.8	37.90	56.25	
MCV (fL)	M	67	102.0	81.9	115.5	82.11	112.84	0.171
	F	72	102.3	85.4	123.3	88.54	113.48	
	Combined	139	102.1	81.9	123.3	83.90	111.55	
MCH (Pg)	M	67	35.1	28.6	39.7	28.81	39.35	0.139

	F	72	35.3	27.8	41.1	31.60	38.71	
	Combined	139	35.3	27.8	41.1	29.35	39.10	
MCHC (g/L)	M	67	34.6	32.6	37.5	32.81	37.22	0.386
	F	72	34.3	30.	37.9	31.03	37.74	
	Combined	139	34.3	30.2	37.9	32.25	37.40	
PLT (x10^9/L)	M	67	230.0	145.0	469.0	145.70	466.20	0.369
	F	72	241.5	141.0	433.0	145.95	418.15	
	Combined	139	236.0	141.0	469.0	146.00	438.00	
%LYM	M	67	36.6	4.8	71.2	10.82	64.55	0.274
	F	72	37.8	14.7	76.0	19.40	66.35	
	Combined	139	37.5	4.8	76.0	16.60	63.00	
%MXD	M	67	8.3	1.5	17.8	1.71	15.84	0.807
	F	72	7.85	0.0	17.3	0.00	16.64	
	Combined	139	7.9	0.0	17.8	1.65	15.75	
%NEU	M	67	54.7	22.5	88.4	26.91	84.13	0.227
	F	72	53.4	18.6	82.3	29.74	75.37	
	Combined	139	53.7	18.6	88.4	30.25	78.35	
#LYM	M	67	4.4	0.8	7.7	1.85	7.70	0.188
	F	72	4.6	1.7	10.6	1.78	10.44	
	Combined	139	4.5	0.8	10.6	1.85	8.30	
#MXD	M	67	1.0	0.0	2.4	0.14	2.26	0.657
	F	72	1.0	0.0	2.5	0.00	2.42	
	Combined	139	1.0	0.0	2.5	0.10	2.40	
#NEU	M	67	6.8	1.9	15.7	2.04	15.42	0.684
	F	72	6.3	2.5	11.2	2.75	10.79	
	Combined	139	6.6	1.9	15.7	2.65	12.85	
RDW-CV	M	67	16.0	12.9	18.3	12.97	18.30	0.01
	F	72	15.0	12.0	19.0	12.00	19.00	
	Combined	139	15.6	12.0	19.0	12.00	19.00	
PDW-CV (%)	M	67	11.2	9.1	16.5	9.31	16.22	0.199
	F	72	10.8	8.6	15.8	8.60	14.81	
	Combined	139	11.0	8.6	16.5	9.10	15.65	
MPV(fL)	M	67	9.4	7.0	11.3	7.41	11.23	0.913
	F	72	9.35	8.0	12.5	8.08	12.34	
	Combined	139	9.4	7.0	12.5	8.05	11.76	

Table 3 Shows Independent (2 Groups) Mann-Whitney U Test In 95% Confidence Interval Level with Significance Level 0.05

Parameters	P-Value By Sex	P-Value By Delivery Mode	P-Value By Gestational Age
WBC	0.787	0.007	0.366
RBC	0.075	0.018	0.852
HGB	0.374	0.110	0.904
HCT	0.861	0.193	0.707
MCV	0.171	0.117	0.990
MCH	0.139	0.094	0.832
MCHC	0.386	0.519	0.540
RDW-CV	0.01	0.318	0.197
PLT	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	0.420	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	0.913	0.858	0.503

This study also tried to compare the established cord blood RI with that of provided by Sysmex Corporation for 0-24 hours old newborns. Accordingly, the current result shows lower RI for WBC, RBC, HGB, HCT, and MCV (both the upper and lower limit). While lower limit was noted for MCH, higher upper limit for MCHC, RDW and PLT (Table 4).

Table 4 Median with 95th Percentile Reference Interval for umbilical cord hematological value in parallel with Sysmex Kx-21 normal range for newborns (0-24hrs)

Median with 95th Percentile Reference Interval for umbilical cord hematological value in parallel with Sysmex Kx-21 normal range for Newborns (0-24hrs)		
Parameter	Median [2.5-97.5]	Reference range
WBC ($x10^9$/L)	12.4 [6.6-19.3]	9.0-30.0
RBC ($x10^{12}$/L)	4.5 [3.6-5.5]	4.1-6.7
HGB (g/dL)	15.8[12.4-19.7]	15.0-24.0
HCT (%)	45.9[37.9-56.2]	44-70
MCV (fL)	102.1[83.9-111.6]	102-115
MCH (Pg)	35.3[29.4-39.1]	33.0-39.0
MCHC (g/L)	34.3[32.3-37.4]	32.0-36.0
RDW-CV	15.6 [12.0-19.0]	11.8-15.6
PLT ($x10^9$/L)	236 [146-438]	140-385
#NEU	6.6 [2.7-12.9]	6.0-26.0
#LYM	4.5 [1.9-8.3]	2.3-10.8
#MXD	1.0 [0.1-2.4]	0.1-3.6

Table 5 displays comparison of the current findings with other studies from Sudan, Nigeria, South India, Saudi Arabia, and Greece. Most studies present their findings as Mean±SD and hence the comparison was made accordingly. The mean WBC of the current study was lower than those reported from Sudan, Nigeria, South India, Saudi Arabia but much higher (12.4 versus $7.2x10^9$/L) than Greece. Whereas mean RBC, HGB, HCT were higher than all except Saudi Arabia but mean MCV was lower than all shown in Table 5, and RDW was lower than Nigeria; Mean PLT was slightly lower than values from Sudan but slightly higher than those reported by the rest.

Table 5 Mean±SD or Median Comparison with Previous Studies from Africa

Parameters	Our Study		Sudan	Nigeria	South India	Saudi Arabia		Greece
	Mean±SD	Median	Mean±SD	Mean±SD	Mean±SD	Mean±SD/Median		Mean±SD
WBC($x10^9$/L)	12.4±3.38	12.6	12.3±4.17	13.1±5.2	15.9±5.1	16.7±9.3	16.1	7.2±3.4
RBC($x10^9$/L)	4.51±4.49	4.5	4.34±0.6	4.05±0.55	4.10±0.40	5.6±10.7	5.1	2.46±0.82
HGB(g/dL)	15.8±1.64	15.8	14.4±1.55	13.9±1.5	14.9±1.7	17.7±2.0	17.7	8.8±2.9
HCT (%)	46.1±4.62	45.9	44.1±5.14	44.8±5.78	44.6±5.3	53.2±6.3	53.2	25.9±8.8
MCV (fL)	101.2±5.97	102.1	105.5±5.14	110.4±11.88	108.1±4.8	105.1±7.2	106.0	105±6
MCH (Pg)	35.1±1.97	35.3	33.5±1.99	32.6±4.13	36.0±1.7	35.4±5.2	35.5	35.8±3.1
MCHC (g/L)	34.5±1.17	34.3	33.1±1.19	29.8±1.64	33.3±0.8	33.6±6.6	33.2	34.3±7.3
RDW-CV(%)	15.4±1.60	15.6	NA	19.8±4.26	NA	NA	NA	12.1±1.6
PLT($x10^9$/L)	245.5±69.78	236	261± 83.16	225.1±72.21	215±67	238.4±60.8	234.0	160±59
LYM%	38.2±10.96	37.5	NA	NA	35.9±12.2	26.5±10.2	25.2	36.7±1.29
MXD%	8.0±3.44	7.90	NA	NA	13.3±6.3	10.5±8.8	8.8	16.4±2.55
NEU%	53.9±10.84	53.7	NA	NA	50.3±12.2	62.8±11.9	64.5	46.9±1.8
MPV (fL)	9.5±0.90	9.4	NA	NA	NA	NA	NA	8.0±0.7
PDW-CV (%)	11.5±1.85	11.0	NA	NA	NA	NA	NA	NA

NA stands for not available

7. DISCUSION

Cord blood is a blood collected from long and helical cord that connects the fetus with the mother for substances exchange. After delivery, the cord is clamped in 1 minute into two places to separate the fetus from the mother to establish Hematological reference interval (6, 8, 64).

It is understood that established hematological reference interval is specific for the subgroup of the population whose measurements are greatly affected by several factors including age, sex, ethnicity, race, altitude, physiological conditions (pregnancy), condition of assay and variations in instrumentation (1, 26). Therefore to make the right clinical decision for the observed or measured laboratory results, establishing suitable reference interval is demanding(12). The aim of this study was to establish umbilical cord blood hematological parameters reference interval for newborns in Addis Ababa for interpretation of laboratory results.

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 hematological parameters except RDW ($p<0.05$, which was a consistent finding with a finding from Greece that had shown RDW, in addition to WBC, NEU & PLT had statistically significant gender difference (46) . Other previous studies from South India, Nepal, Iraq, Korea, Nigeria, Iran, Sudan and Saudi Arabia on the other hand, conclude there were no statistically significant differences by gender at this early life (45, 47, 51, 53-57).

In the current study all the measured values were located within interval compared to reference value given for Sysmex KX-21 hematology analyzer newborns (0-24hrs) reference interval by Sysmex Corporation Kobe, Japan. 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 (65).

In the current study, we found higher total WBC compared to studies from Taiwan, Nepal, Greece, Iraq, and Korea (44, 46, 51, 53, 54). However, the reference values from South India(47), Saudi Arabia(57), Pakistan(49, 50), and Nigeria(45) are slightly higher than our findings. On the differential part, we found higher neutrophil reference value compared to Taiwan, Pakistan and Korea (44, 49, 54). However, we found lower lymphocyte and mixed parts when we come across with most of the studies included in this paper may be due to nutrition and ethnic variation (66-68).

In the current study, there were higher RBC, HGB and HCT RIs compared to RIs determined in Taiwan, South India, Nepal, Greece, Iraq, Korea, Pakistan, Nigeria and Sudan(44-47, 49, 51, 53, 54, 56). Our findings for both MCH and MCHC were also higher than those from Taiwan, South India, Nepal, Iraq, Nigeria and Sudan (44, 45, 51, 53, 56). MCV reference value in this study is surprisingly lower from all the studies included in this paper. The reference value for RDW in our finding is lower than Saudi Arabia, Nigeria and Iran, but higher from that of Greece (45, 46, 55, 57).

In the present study, the PLT reference interval was higher than RIs from Taiwan, Nepal, Korea and Nigeria(44, 45, 53, 54); however, still it is lower than intervals determined in South India, Saudi Arabia, Iraq, Pakistan, Iran and Sudan(47, 49, 51, 55-57). On the other hand, the platelet indices (MPV=8.1-11.8 and PDW=9.1-15.7) RI 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)(46, 55). There may be possible reasons that can be mentioned that causes such variations in our findings and previous studies. The first reason is due to is the presence of biological variation within or between individuals (for example variation between black and white populations, altitude) for nearly all laboratory measurements(23, 68). The second reason is that the presence of quantitative (size, number) and qualitative (type, shape, contents) differences in the developmental changes during fetal hematopoiesis and, so, correlate with gestational age (5, 24, 27). Although, in this study the gestational age is limited from 37 to 42 weeks (Term), however sometimes gestational weeks are approximate and ultrasound results are not always feasible. The third reason is the method of deliveries. In the current study, the majority of the results showed higher values. This is due to 93 (66.9%) babies were delivered by SVD rather than CS which was also supported by previous studies from Sudan (*Younis et al*) , Egypt (*El Gendy et al*) and Taiwan (*YH Chang et al*)(44, 56, 58). SVD born neonates have higher values due to the fact that birth is a stressful event that accompanied by hormonal activity associated with physical changes to help the process and nuclear factor kappa B-regulated inflammatory pathways that in turn affect hemogram of the fetus (69-71).

The fourth reason is sample collection, processing and analysis may greatly affect reference interval establishment as described by different text books and literatures (1, 16, 26, 64, 72). For example, in the current study lower platelet RI values were noted compared to some studies that were mentioned earlier this paragraph; this may be the effect of EDTA-K_2 anticoagulant, i.e., dilution effect (44, 72).

All in all, umbilical cord blood hematological reference intervals vary from country to country as evidenced by the current study as well underscoring the need for locally derived values to contribute to appropriate patient care, future cord blood banking, and transplantation services.

8. STRENGTH AND LIMITATION

8.1 Strength

Since this study is pioneer of its kind with regards to hematological reference intervals in cord blood, it could fill the gap of lacking reference range for Ethiopian neborns who need early care. It can also be used as baseline information for researchers interested in this area.

8.2 Limitation

This study firstly had limitation with relatively small sample size as compared to previous studies, even though sufficient to establish reference intervals. The other limitation is lack of much literatures for comparison of RDW and platelet indices (MPV, PDW). The last to mention as limitation is unaffordability or lack of molecular technologies to screen for congenital anomalies or other malignancies that may influence hematological parameters. However, term infants from apparently healthy mothers were included to minimize such interferences.

9. CONCLUSION AND RECOMMENDATION

9.1 Conclusion

Most of the hematological parameters in this study showed differences from similar studies done in other countries including company values. The values obtained from our study provide reference intervals for some hematological parameters in healthy newborns of Addis Ababa and its surrounding special weredas.

9.2 Recommendation

The hematological reference interval for Ethiopian cord blood need to be confirmed by larger number of samples from different centers of throughout the country which might play important role in neonatal care and transplantation medicine (stem cell transplantation from cord) to establish Umbilical Cord Blood Bank (UCBB). It is also recommended that laboratories to incorporate cord blood analysis to their routine test lists and laboratory handbooks.

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Annex

Annex I – Standard Operating Procedure (SOP)

SOP for Sysmex KX-21 Hematology Analyzer

1. **Purpose**: To determine complete blood count using Sysmex® automated haematology analyser KX-21
2. **Principle:**

The Sysmex KX-21N is a quantitative automated hematology analyzer for in vitro diagnostic use for determining 18 hematological parameters. Examination of the numerical and/or morphologic findings of the complete blood count are useful in diagnosis of such disease states as anemias, leukemias, allergic reactions, viral, bacterial, and parasitic infections. The Sysmex KX-21N analyzer directly measures the WBC, RBC, HGB, HCT, and PLT, LYM #, MIXED #and NEUT #. The remaining parameters are calculated or derived MCV, MCH, MCHC, MPV, RDW-CV and RDW-SD, and differential percentages LYM%, MIXED%, NEUT%. The KX-21N counts and sizes red blood cells (RBC) and platelets (PLT) using electronic resistance detection. Hematocrit (HCT) is measured as the ratio of the total RBC volume to whole blood using cumulative pulse height detection. Hemoglobin (HGB) is converted to methemoglobin, and read photometrically at 555 nm. White blood cells (WBC) are analyzed by direct current and discriminated into a three-part differential using Particle Distribution Analysis (PDA). The resulting WBC histogram is discriminated into lymphocyte, neutrophil and mixed cell populations. The mixed cell population contains monocytes, basophils and eosinophils.

3. **Abbrevations**
    - ✓ **EDTA:** Ethylene diamine tetra acetate
    - ✓ **LCD:** liquid crystal display

4. Materials

Reagents:

- ✓ Diluent: CELLPACK (20 L)
- ✓ WBC/HGB lyse: STROMATLYSER-WH (500 mL X 3)
- ✓ Detergent: CELLCLEAN(50 ml)

Reagent preparation

Reagent stability and storage:

- ✓ Leave the reagent at room temperature (15 - 30°C) for at least 24 hours before using
- ✓ Use reagents within the expiration date

- ✓ CELLPACK expires after 60 days of opening the seal
- ✓ STROMATLYSER-WH expires after 90 days of opening the seal

Supplies

- ✓ Disposable gloves
- ✓ EDTA vacationer tubes (purple top)
- ✓ Thermal paper

Instrument

- ✓ Sysmex®KX-21 analyser

5. Sample

- ✓ **Type**: whole blood collected according to the SOP described in blood collection for haematology and CD4 count
- ✓ **Amount required:** A specified amount of sample, corresponding to the amount of EDTA anticoagulant, is collected from the vein.
- ✓ **Transport and storage:** Ambient temperature (15-25 ^{0}C)
- ✓ **Stability:** within 8 hours of sample collection

6. Special safety precautions:

- ✓ Wear protective clothing
- ✓ Follow infection prevention principle during sample handling
- ✓ Wear gloves for handling blood or serum
- ✓ Decontaminate working area with 0.5% bleach solution
- ✓ Change gloves when they become contaminated
- ✓ Wash hands after handling specimens
- ✓ CELLCLEAN is a strong alkaline detergent; take care not to have it adhere to the skin or clothes. If the skin or clothes should come in touch with it, flush it away using plenty of water. Otherwise, it can damage the skin or clothes

7. Quality control: Quality Control checks are performed to monitor an instrument's performance over time. EIGHTCHECK-3WP is the quality control material recommended by Sysmex to monitor the performance of the KX-21 analyzer. This product is supplied with three control levels; at least two levels should be run every 8 hours of operation or in accordance to regulations applicable to your laboratory. Quality control material should be run after component replacement or after a service call.

- ✓ **Execute quality program**
    - Press [SELECT] key in the Ready status. The Select Menu screen appears
    - Using [↑] key or [↓] key, move the cursor to select "2: Quality Control."
    - Press [ENTER] key. The QC Chart screen appears
- ✓ **Select QC file**
    - Press [SAMPLE No.] key on the QC Chart screen
    - The cursor comes to right of the FILE No. Use the numeric keys to enter a FILE No.
    - Press [ENTER] key. The QC Chart screen for the selected QC file appears.
- ✓ **Erase all:** To start quality control newly, the control data in the QC File has to be erased. When a control blood lot has changed, use the menu of "3: Erase All" to erase all control data in the QC File.
    - Display the QC Chart screen for the QC File you want to erase.
    - Press [3] key to select "3. Erase All." The Erase All Confirmation message appears.
    - Using [←] key or [→] key, move the cursor to select "Yes" or "No." [Yes]: Erases the plot display on the QC Chart screen and displays the second Erase All Confirmation message. [No]: Cancels the erasing of the plot display on the QC Chart screen and returns to the QC Chart screen.
    - Using [←] key or [→] key, move the cursor to select "Execute" or "Cancel." [Execute]: Completely erases QC data and returns to the QC Chart screen. [Cancel]: Cancels QC data erase and returns to the QC Chart screen.
    - Press [ENTER] key to execute the selected process.
- ✓ **Set TARGET/LIMIT values**
    - Display the QC Chart screen for the QC File you want to set.
    - Press [2] key to select "2: Settings."
    - Using [↑] key or [↓] key, move the cursor to select control parameters. Eight parameters can be set.
    - Using [←] key or [→] key, move the cursor to select "TARGET" or "LIMIT."
    - Enter the set values using the numeric keys.
    - Press [ENTER] key.

- When settings are completed, press [SELECT] key. The Setting Confirmation message appears.
- Using [←] key or [→] key, move the cursor to select "Cont.," "Set," or "Cancel." [Cont.]: Returns to the QC File Setting screen. And the setting operation can be continued. [Set]: Updates the settings and returns to the QC Chart screen. [Cancel]: Cancels the changed settings and returns to the QC Chart screen.
- Press [ENTER] key to execute the selected process.

✓ Execute **X** control

- Display the QC Chart screen for the QC File in which to enter QC data.
- Press [1] key to select "1: QC Analyze." The **X** Control Analysis screen appears.
- Confirm that "Ready" is displayed for QC analysis.
- Mix control blood sufficiently.
- Remove the cap while taking care not to allow blood to scatter.
- Set the control blood container to the sample probe and press the start switch in that status. When the buzzer sounds two times - beep, beep, - and "Analyzing" is displayed on the X Control Analysis screen, remove the control blood container. Then, automatic analysis is executed.
- Under the "X1" column on the Analysis Result screen, the 1st time analysis result is displayed. The analysis result comprises three screens which can be changed over by pressing [←] key or [→] key.
- If the data is acceptable, press [1] key to confirm. Press [2] key to reject. Press [3] key and select "3: Print" to print the contents of analysis result on the built-in printer.
- The 2nd analysis result is displayed under "X2" on the Analysis Result screen, the mean values of the 1st and the 2nd analyses under " **X**" and the comparison result with control limits under the "Judgment" column. The Analysis Result screen, as in the 1st analysis, can be changed over using [←] key or [→] key. The data confirmation message is displayed and you are asked to decide whether or not to accept the analysis result as QC data.
- A parameters in which the mean of the 1st and the 2nd analyses fell outside the control limits is indicated with +□ □or –□ □under the "Judgment" column, while the alarm sounds and the QC error message appears.

- Set the data using [1] key or [2] key. [1: OK]: QC data is accepted and the plot is entered to the QC Chart. This QC data (mean) is kept in the stored data also. [2: NG]: Cancels the 2nd analysis result. And the system remains to be ready for the 2nd analysis again.
- When the built-in printer is specified for the output device, the set data (mean) is printed on the built-in printer.

8. Procedures

- ✓ **Inspection of reagents:** Check to see that the reagents needed for the number of the samples to be processed for the day are available (1 Liter of CELLPACK for 30 samples and 50 mL of STROMATLYSER-WH for 47 samples in whole blood analysis mode are enough)
- ✓ **Inspection of the instrument:** Inspect the connection of tubing and cords to see that there are no broken tubes and the power cord is properly plugged in the outlet
- ✓ **Inspection of waste:** If waste is found to have collected in the trap chamber on the left side of the unit and the waste tank, discard the waste.
- ✓ **Inspection of printer paper:** Open the front cover and check if printer paper needed for processing the samples for the day is available
- ✓ **Turn on the power and wait for the result of self-check**
    - **Permissible background count**
        - WBC 0.3 [$X10^3$/mL] or less
        - RBC 0.02 [$X\ 10^{6/}$mL] or less
        - HGB 0.1 [g/dL] or less
        - PLT 10 [$X\ 10^3$/mL] or less
- ✓ **Selecting whole blood mode**
    - Confirm the “READY” status on LCD
    - Press [MODE] key to display the Change Mode screen
    - Press **[←]** or **[→]** key to select "Whole Blood (WB)."
    - Press [ENTER] key to changeover the analysis mode and return to the Analysis screen
- ✓ **Inputting Sample/ID number**

- Press [SAMPLE No.] key in the Ready status. In the system status area on the LCD screen, the next sample No. turns to the reverse display and the system is waiting for Sample No. input (in the "Not Ready" status)
- The cursor appears under sample No. Input sample No. using the numeric keys
- Press [ENTER] key. This will fix the sample No. and the status becomes Ready, namely, ready for analysis

✓ **Analyzing samples**

- Mix the sample sufficiently
- Remove the plug while taking care not to allow blood scatter
- Set the tube to the sample probe, and in that condition, press the “START” switch
- The buzzer sounds two times - "beep, beep" and when the LCD screen displays "Analyzing," remove the tube. After that, the unit executes automatic analysis and displays the result on the LCD screen. Then the unit turns to the “READY” status, becoming ready for analysis of the next samples.

✓ **Display and printing of analysis result**

- The result of each analysis is displayed on the LCD screen
- The display screen of analysis result consists of three pages, and pages are turned over by using **[←]** or **[→]** key
- Analysis result can be printed out on the built-in printer

9. Limitations

✓ Some abnormal samples may give incorrect results by automated cell counting methods e.g. Cold agglutinin, platelet aggregation, erythroblastosis, nucleated RBC, leukocytosis and etc.

✓ Hemoglobin measurements may be falsely elevated due to the influences of abnormal samples including leukocytosis above 100,000/uL, lipemia or abnormal proteins in blood plasma. The effect of lipemia and abnormal proteins may be removed by plasma replacement or plasma blank procedures

10. Expected values

Parameter	Range for Female	Range for Male	Parameter	Range for Female	Range for Male
WBC	3.1-10.3(X 10^3µL)	2.6-8.8 (X 10^3µL)	**Mxd%**	1.3-25.9 (%)	1.9-24.6 (%)
RBC	3.2-4.6 (X 10^3µL)	3.6-5.3 (X 10^3µL)	**Neut%**	43.7-77.1 (%)	38.3-69 (%)
Hgb	9.9-13.6 (g/dL)	11.3-15.7 (g/dL)	**Lym#**	0.9-2.8(X 10^3µL)	0.8-2.7(X 10^3µL)
Hct	30.2-42.3 (%)	32.6-47.5 (%)	**Mxd#**	0.1-1.6(X 10^3µL)	0.1-1.5(X 10^3µL)
MCV	78.6-102.2 (fL)	80.3-103.4 (fL)	**Neut#**	1.6-6.9(X 10^3µL)	1.2-5.3(X 10^3µL)
MCH	25.2-34.7 (pg)	26-34.4 (pg)	**RDW-CV**	10.6-15.7 (fL)	10.8-14.9 (fL)
MCHC	31.3-35.4 (g/dL)	31.8-36.3 (g/dL)	**PDW**	9.4-18.1 (fL)	9.8-18.0 (fL)
PLT	128-434 (x10^3µL)	134-377(x 10^3µL)	**MPV**	8.5-12.4 (fL)	8.1-12.4 (fL)
Lym%	15-45.8 (%)	17.5-47.9 (%)	**P-LCR**	14.3-44 (%)	(%)

11. Clinical Utility

- ✓ Low level of Hgb seen in patient with anemia and the different red cell parameters give for the possible type of etiology.
- ✓ Elevated white blood cell count may indicate infection.
- ✓ Decreases in white blood cell count may occur with disease progression or may indicate bone marrow suppression from ARV therapy.
- ✓ Total lymphocyte count: After a patient is on ARV therapy, a decrease in Absolute lymphocyte count may reflect bone marrow suppression from treatment.
- ✓ Total lymphocyte count of < 1,200/ml has been correlated with a CD4 count of less than 200/ml. However, the total lymphocyte count alone shouldn't be used in asymptomatic patients when deciding whether to start ARV therapy.
- ✓ An increase in neutrophils may be due to an acute bacterial infection or hematological malignancies such as Myeloid Leukemia.
- ✓ An increase in eosinophils may be due to a parasitic infection or an allergic Reaction.
- ✓ An increase in lymphocytes may be due to viral infections or chronic infections

Procedures for Cord Blood Collection

A. All the needed equipment for the procedure including needle and syringe put in place within safe and easily reachable materials like tray and trolley, ensuring that all the items are clearly visible.

B. Perform hand hygiene of the phlebotomist

C. Put on well-fitting gloves

D. After delivery of the newborn, double-clamped the umbilical cord and cut.

E. Remove any blood from the surface of the cord with gauze

F. Insert the needle just above the clamp that remains on the cord

G. After collecting sufficient amount of blood, withdraw the needle gently.

H. The tube was labeled with the medical registration number and date

I. Discard the used needle and syringe or blood-sampling devices into a puncture resistant container.

J. Dispose the used gloves appropriately and perform hand hygiene

SOP for Peripheral Morphology

Purpose: This Procedure provides instructions on how to differentiate White blood cells and look at morphology of white blood cells, red blood cells and Platelets.

Principle:

A drop of blood is spread on a slide and then fixed, stained, and examined under microscope. In this way, red blood cells, leukocytes, and platelets may be studied. The Romanowsky stains contain eosin Y which is an acidic anionic dye, as well as azure B and other thiazine dyes, which are basic cationic dyes. When these dyes are diluted in buffered water, ionization occurs. Eosin stains the basic components of blood cells; for example, hemoglobin stains pink-red, and other methylene blue–derived dyes stain the acidic components of the cells. Nucleic acids and nucleoproteins stain various shades of mauve-purple and violet, the granules of basophils stain dark blue-violet, and the cytoplasm of monocytes and Lymphocytes stain blue or blue-grey. The staining reactions of Romanowsky stains are pH dependent, which is why the stains are diluted in buffered water with a specific pH.

Abbreviation:

WBC- White blood cells

RBC- Red blood Cells

DIFF- Differential Cell count

PLT- Platelet

QC- Quality Control

EQA- External Quality Assessment

CBC- Complete blood cell count

Mono- Monocyte

Neut - Neutrophil

Eos - Eosinophil

Baso - Basophil

Gran- Granulocyte Absolute Count

%Gran- Granulocyte Percentage

%LYM- Lymphocyte count percentage

EDTA- Ethylene diamine tetra acetate

Reagents:

- Romanowsky stains (Eg.May-Granuwald-Giemsa, Leshmanin stain)
- Geimsa stain (powder)
- Absolute Methanol
- Glycerol
- Methyl Alcohol

Reagents preparation:

Glycerol (Glycerine) ----------250ml

Methanol (Methyl Alcohol) ----------250 ml

Step Action

1. Weigh the Gimesa on a pieces of clean paper by using Analytical/Beam Balance and transfer to dry brown bottle of 500ml capacity which contains few glass beads,
2. Using Dry Cylinder, Measure the methanol and add to the stain. Mix well methanol is toxic and highly flammable, therefore handle with care and use well away from an open flame.
3. By Using the same cylinder, Measure the glycerol and add to the stain, Mix well
4. Place the bottle containing the stain in to a water bath at 50-60 0C or if not available place at 37 0C for up to 2hrs. To facilitate the stain and dissolve easily, mix well at intervals.
5. Label the bottle and mark it Flammable and Toxic, store at room Temperature in the dark. For Several Months

Reagents stability and storage:

Giemsa stain solutions are stable for several months if it is kept at Room Temperature in the dark.

Supplies:

- Disposable gloves
- Microscopic slides
- Syringe/Lancet
- Alcohol 70%
- Reagent bottles
- Cotton swab
- Glass beads

Sample:

Anti-coagulated Whole Blood

- Minimum of 100Ul
- Keep 8 hrs. at Room Temperature
- Limitations: Hemolysed sample, High volume of Anti-Coagulant and inadequate volume of samples i.e. less than 2/3
- Discarded after 24 hours.

Special Safety and precautions: Apply universal biological safety precautions

Maintenance: N/A

Calibration and calibrator preparation: N/A

Quality Control

- Repeat on a selected slides

Control preparation:

-Prepare your own IQC Slide according to the QA Guide line.

Note:

Repeat counts on selected slides on subsequent days because this will give an indication of the range in the variation of the results (include note on reagent Quality). WBC Should have a blue nucleus with a lighter staining cytoplasm and the red cells with pinkish color and pale central pallor.

Procedure

1. Make a thin smear in a microscopic slide and fix with absolute Methanol for 10-15 min. Transfer the slide into a stain jar containing Methanol solution and allow staining for 5 minutes.

2. Transfer slides without washing or drying into a jar containing Giemsa stain solution for 10 minutes
3. Transfer the slides into a jar containing buffered water and rapidly wash then allow it to stand undisturbed for 2 minutes.
4. Air Dry the slides
5. Examine the smear by using 10 x objectives to scan check
6. Then focus and count the cells by using 100 x objectives
7. Count a total of 100 Leukocytes and record the number of each type of leukocyte seen as percentage.
8. Report the percentages, Number and Morphology of Cells counted

Expected Values

Analyte	Reference Range	
	Male	Female
Neu	45-75%	45-75%
Lym	15-45%	15-45%
Mon	2-10%	2-10%
Eos	1-6%	1-6%
Baso	0-1%	0-1%

Limitations:

- Poorly made smear
- Dirty glassware
- Incorrect buffer PH
- Films prepared from old blood etc.

Clinical Utility

- Elevated white blood cell count may indicate infection.
- Decreases in white blood cell count may occur with disease progression or may indicate bone marrow suppression from ARV therapy.

- Total lymphocyte count: After a patient is on ARV therapy, a decrease in
- Absolute lymphocyte count may reflect bone marrow suppression from treatment.
- Total lymphocyte count of <1,200/ml has been correlated with a CD4 count of <200/ml. However, the total lymphocyte count alone shouldn't be used in asymptomatic patients when deciding whether to start ARV therapy.
- An increase in neutrophils may be due to an acute bacterial infection or hematological malignancies such as Myeloid Leukemia.
- An increase in eosinophils may be due to a parasitic infection or an allergic reaction.
- An increase in lymphocytes may be due to viral infections or chronic infections
- Microcytic hypochromic red cells associated with iron deficiency, sideroblastic, thalassemia and anemia of chronic disease
- Macrocytic normochromic red cells associated with megaloblastic anemia and folate deficiency
- Normocytic and normochromic red cells with decreased number and polychromasia associated with hemolytic anemia and hematological disorders.
- Thrombocytopenia can be shown by the presence of giant platelet, platelet aggregates and platelet satelitism.

Source: St. Peter Specialized Hospital Laboratory SOP for Sysmex Hematology Analyzer Ver. No. 3 Jan 2016 and Manual Differential Count Ver. No. 2 Jan 2015

Annex II – Subject Information Sheet (For Pregnant Mothers, English Version)

Addis Ababa University

College of Health Sciences

Department of Medical Laboratory Sciences

Subject Information Sheet for Mother Whose Cord Blood is to be used in the Establishment Of Hematological Reference Values

You are invited to participate in a study to be conducted by MSc student at Addis Ababa, College of Health Sciences, Department of Medical Laboratory Science. Please read the following statements and ask any unclear points before you agree to participate.

Introduction

The topic of this study ***"Establishing Reference Interval for Umbilical cord Blood Hematological Parameters of Newborns Delivered in St. Peter's Specialized Hospital from January 1 to March 31, 2019***." The aim of the study is to establish reference values that can be used as a baseline for clinical decisions associated with anemia, thrombocytopenia and infection in newborns.

Participation in this study is exclusively voluntarily. If you are not interested to participate or if you once decide to participate and withdraw at any time, there will be no consequences and you will get all the services provided in the hospital with no problems. If you decide to participate, you have to sign on the consent form and you may obtain a copy of this information sheet.

What is expected from me as a participant of the study?

As a participant of this study, you are expected to agree that 2-3mL blood will be collected from the cord immediately after your delivery before the expulsion of the cord. In addition, you are expected to give answers for some questions about your health and socio-demographic conditions. You need to know that your results might be discussed with other appropriate individual out of this hospital. But your name, address and phone number will not be disclosed and rather than identification code will be used in such conditions.

How much time will I spent to participate in this study?

You will spend 20-25 minutes until the specimen is collected, the consent form is signed and the questionnaire is filled.

What are the risks of participating in this study?

The sample collection will pose minimal pain on you and the only thing you spend is just your time to fill the questionnaire.

How my information is to be kept in secret?

All information that you give and the results from your sample will be used for this study only, only limited numbers of professionals will have access to the information. All the information will be encoded in a computer and saved with password protection.

What are the benefits from participation?

Since this study is MSc student research, there will not be payments for participants. But your participation is important for the establishment of reference values that can be useful for the correct interpretation of hematological parameters in newborns.

What are my rights as a participant of this study?

You have the right to withdraw yourself from the study at any time and all the services provided in the hospital will not be discontinued. You are also welcomed if you have any questions for further explanations about the study. You may also get the results of the analysis.

What can I do if I have a problem or a question?

Please direct any questions or problem you may encounter during this study to:

Ammanuel Angelo
Department of Medical Laboratory Sciences
College of Health Sciences, Addis Ababa University
Mob: +251921452169
Email: angelo.amanuel19@gmail.com
Advisors: Dr Aster Tsegaye 0911696085; Dr Girma Deribe (OBS/GYN)

For additional information, please contact Department of Medical Laboratory Sciences, Addis Ababa University, Institutional Review Board (IRB) office;

Tel: +2511911107099
P.O Box: 9086, Addis Ababa, Ethiopia

Agree to participate? Yes No

Annex III – Subject Information Sheet (For Pregnant Mothers, Amharic Version)

አዲስ አበባ ዩኒቨርሲቲ

የጤና ሳይንስ ኮሌጅ

የሕክምና ላቦራቶሪ ሳይንስ ት/ክፍል

<u>ከእትብት ላይ ደም ተወስዶ ለሚሰራው አጠቃላይ የደም ምርመራ (CBC) ውጤት የማወዳደሪያ ዋጋ ጥናት ለሚሳተፉ እናቶች የተዘጋጀ መረጃ</u>

አዲስ አበባ ዩኒቨርሲቲ፤ የጤና ሳይንስ ኮሌጅ፤ የሕክምና ላቦራቶሪ ሳይንስ ት/ክፍል በማስተርስ ዲግሪ ተማሪ የመመረቂያ ጥናት ላይ እንዲሳተፉ ተጋብዘዋለው። እባክዎ በዚህ ጥናት ለመሳተፍ ከመስማማትዎ በፊት ከዚህ ቀጥሎ የሚገኘውን ምንባብ በጥሞና ያንብቡና ግልፅ ያልሆነልዎትን ማንኛውም ሃሳብ ይጠይቁ።

መግቢያ

የጥናቱ ርዕስ በእትብት የደም ናሙና ላይ የሚሰራ የአጠቃላይ ደም ምርመራ (CBC) ውጤት ማወዳደሪያ ነው። አላማውም አጠቃላይ ደም ምርመራ ውጤቶች ማወዳደሪያ ዋጋ ማግኘት ሲሆን ጥቅሙም ጨቅላ ህፃናትና አዲስ ለተወለዱ ህፃናት የሚኖራቸውን የእነዚህን አጠቃላይ ደም ምርመራ ውጤቶች ዋጋ በትክክል ለመተርጎም ይጪላል።

እርስዎ በዚህ ጥናት ላይ የሚኖርዎት ተሳትፎ ሙሉ በሙሉ በበጎ ፈቃደኝነት ላይ የተመሰረተ ሲሆን በዚህ ጥናት ዉስጥ ላለመሳተፍ ሆነ ለመሳተፍ ከወስኑ በኋላ ለማቋረጥ የሚወስኑ ቢሆንም እንኳን በዚህ ሆስፒታል ውስጥ የሚገኝ ማንኛውም አገልግሎት አይቋረጥም። በጥናቱ ለመሳተፉ ከፈለጉ የስምምነት ቅጹ ላይ በፅሁፍ ወይም በጣት ፊርማ ማረጋገጥ ይኖሩበታል። ከፈለጉም ይህን የመረጃ ቅፅ አንድ ቅጂ ለራስዎ መውሰድ ይችላሉ።

በጥናቱ ተሳታፊ በመሆኔ የሚጠበቅብኝ ምንድን ነው?

በዚህ ጥናት ላይ ለመሳተፍ የሚስማሙ ከሆነ 2-3ሚ.ሊ የደም ናሙና በሚወልዱበት ጊዜ ከእትብት ላይ እንደሚወሰድ እና ለጥናቱ እነደሚዉል መስማማት ይጠበቅቦታል። ከተወሰደው ናሙና ላይ የሚገኙ መረጃዎች ከዚህ ሆስፒታል ውጭ ለሚገኙናለስራዉ አግባብነት ላላቸው ሰዎች ቢነገር የማይቃወሙ መሆኑን መስማማት ይጠበቅቦታል። የስልክ ቁጥር የመሳሰሉትን መረጃዎችን አይጨምርም። ይልቁንም ለዚህ ጥናት አገልግሎት ብቻ የሚዉል እርስዎን ለማወቅ የሚያስችል መለያ ቁጥር ጥቅም ላይ እንዲዉል ይደረጋል። በተጨማሪም ስለ እርስዎ አጠቃላይ የጤና ሁኔታ ለሚቀርቡ አንዳንድ ተጨማሪ ጥያቄዎች መልስ መስጠት ይጠበቅቦታል።

በዚህ ጥናት መሳተፍ ምን ያህል ጊዜ ይፈጃል?

የተዘጋጀውን መጠይቅ ለመሙላት የስምምነት ቅጹ ላይ ለመፈረም ከ20-25ደቂቃ ያስፈልጋል።

በዚህ ጥናት መሳተፍ የሚያስከትላቸዉ ችግሮች ምንድን ናቸው?

ናሙና በሚወሰድበት ጊዜ ምንም አይነት የህመም ስሜት አያስከትልብዎትም ስለዚህም የሚያጡት ነገር ቢኖር መጠይቁን ለመሙላት የሚያጠፉት ጊዜ ነው።

የህክምና መረጃዬ በሚስጢር ተጠብቆ መቆየት የሚችለዉ እንዴት ነዉ?

ስለራስዎ የሰጡት ማንኛውም መረጃና ከተወሰደው ናሙና ላይ የተገኘው የላቦራቶሪ ውጤት የሚውለው ለጥናቱ አላማ ብቻ ነው። ይህን ማህደር ሊያገኙ የሚችሉት የተወሰኑ የጥናቱ ተባባሪ ሰራተኞች ብቻ ናቸው። ከዚያም በላይ ስለእርስዎ ያለውን ማንኛውም መረጃ የተለየ የይለፍ ቃል ባለው ኮምፒዩተር የመረጃ ማህደር ውስጥ እነዲቀመጥ ይደረጋል።

በዚህ ጥናት ላይ መሳተፍ የሚያስገኛቸው ጥቅሞጭ ምንድን ናቸው?

ይህ ጥናት የማስተርስ ዲግሪ ተማሪ መመረቂያ እንደመሆኑ መጠን ለተሳታፊዎች ገንዘብ አይከፈልም፤ ነገር ግን የእርስዎ ተሳትፎ አዲስ የሚወለዱ ህፃናትን ለመርዳትና በህፃናቱ ላይ የተገኘውን የአጠቃላይ ደም ምርመራ ውጤቶችን ለመተርጎም ይጠቅማል።

በዚህ ጥናት ተሳታፊ በመሆኔ መብቶቼ ምንድን ናቸው?

በጥናት ውስጥ ያልዎትን ተሳትፎ በማንኛውም ጊዜ የማቋረጥ ሙሉ መብትዎ የተጠበቀ ከመሆኑም በላይ ራስዎን ከጥናቱ በማግለልዎ ምክንያት ምንም አይነት የሆስፒታሉ አገልግሎት አይቋረጥብዎትም። ከዚህም በተጨማሪ ጥናቱን በተመለከተ ማንኛውም ጥያቄ የመጠየቅና ገለፃ የማግኘት መብት አሉዎት።የላቦራቶሪ ምርምራ ዉጤቱንም በነፃ ማግኘት ይችላሉ።

ጥያቄ ካለኝ ወይም ችግር ቢያጋጥመኝ ምን ማድረግ ይገባል?

ይህን ጥናት በተመለከተ ወይም ከዚህ ጥናት ጋር በተዛመደ መልኩ ስለሚያጋጥሙ ድንገተኛ አደጋዎች ወይም ጥያቄ ካልዎት በሚከተለው አድራሻ ይጠቀሙ።

አማኑኤል አንጀሎ

የህክምና ላቦራቶሪ ሳይንስ ት/ክፍል፤

የጤና ሳይንስ ኮሌጅ አዲስ አበባ ዩኒቨርሲቲ

ሞባይል፡ +251921452169 ኢሜይል፡ angelo.amanuel19@gmail.com

ጥናት አማካሪዎች፡ ዶ/ር አስቴር ፀጋዬ 0911696085 ፤ ዶ/ር ግርማ ደርቤ 0930387777

ለተጨማሪ መረጃ የአዲስ አበባ ዩኒቨርሲቲ ህክምና ፋከልቲ ኢንስቲትዩሽናል ሪቪዩ ቦርድ ይጠይቁ።

ስ.ቁ፡ +2511911107099

ፋክስ፡ +251115511513099

ፖ.ሳ.ቁ፡ 9086

አዲስ አበባ ፤ ኢትዮጵያ

ለመሳተፍ ይስማማሉ?

እስማማለሁ አልስማማም

Annex IV – Consent Form (Pregnant Mothers, English Version)

Code Number----------

I have been informed about the study which is aimed at establishing reference values from cord blood. For this study blood is required from the cord. The aim of the study was explained to me.

I am also informed that all the information contained within the questionnaire is to be kept confidential. Moreover, I have been well informed of my right to keep hold of information, decline to cooperate and make myself withdraw from this study.

It is therefore, with full understanding of the situation that I gave the informed consent voluntarily to the researcher to use the blood taken from the cord for the investigation. In addition, I have also been informed that the benefit of the participation is to get the results of the analysis measured for free via the counselor.

Participant's signature/ finger print----------

Name of deponent (mother unable to read) ----------Signature----------Date--------

Name of Counselor----------Signature----------Date----------

Please direct any questions or problem you may encounter during this study to:

Ammanuel Angelo

Department of Medical Laboratory Sciences

College of Health Sciences

Addis Ababa University

Mob: +251921452169

Email: angelo.amanuel19@gmail.com

Advisors: Dr Aster Tsegaye 0911696085; Dr Girma Deribe (OBS/GYN)

For additional information, please contact Department of Medical Laboratory Sciences, Addis Ababa University, Institutional Review Board (IRB) office;

Tel: +2511911107099

P.O Box: 9086, Addis Ababa, Ethiopia

Annex V – Consent Form (For Pregnant Mothers, Amharic Version)

የስምምነት ቅጽ (ለእናት)

የምስጢር ቁጥር ----------

እኔ ስሜ ከላይ የተጠቀሰው ተሳታፊ በእትብት ላይ ስለሚሰራው የአጠቃላይ ደም ምርመራ (CBC) ውጤት ማወዳደሪያ ጥናት በቂ ገለፃ ተደርጎልኛል። ለጥናቱም ከእትብት የተወሰደ የደም ናሙና እንደሚያስፈልግ ተገለፆልኛል። የጥናቱን አላማዎችንም ተረድቻለው።

በመጥይቁ ላይ የገለፅኳቸው መረጃዎች በሙሉ በምስጥር የተጠበቁ እንደሚሆኑ ተነግሮኛል። በጥናቱ ላይ ያለመሳተፍና ማንኛውም መረጃ ያለመስጠት እንዲሁም በማንኛውም ጊዜ ከጥናቱ እራሴን የማግለል መብቴ የተጠበቀ መሆኑን ተገለፆልኛል። ስለዚህ ለዚህ ጥናት መረጃና የስምምነት ቃሌን የሰጠሁት በአጠቃላይ ሁኔታውን በመረዳትና ፍፁም ፈቃድኝነት ነው። ከእትብት ላይ የሚወሰደዉ ናሙና የልጁ/ጅቷ ጤና ሁኔታ ለማወቅ እና ለምርምር እነደሚውልም ተረድቻለው። በተጨማሪም ጥያቄ እንድጠይቅ ተፈቅዶልኝ ለማወቅ የፈለጉትን ማብራሪያ አንግቻለሁ። የዚህ ጥናት ተሳታፊ በመሆኔ የላቦራቶሪ ምርምራ በነፃ ማግኘት እንደሆነ ተረድቻለው።

የተሳታፊዋ ፊርማ/ የጣት አሻራ----------

የምስክር ስም---------- ፊርማ---------- ቀን----------

(የስምምነት ቅጹን ማንበብ ለማይችሉ ተሳታፊዎች)

የአማካሪ ስም----------ፊርማ---------- ቀን----------

ይህን ጥናት በተመለከተ ወይም ከዚህ ጥናት ጋር በተዛመደ መልኩ ስለሚያጋጥሙ ድንገተኛ አደጋዎች ወይም ጥያቄ ካልዎት በሚከተለው አድራሻ ይጠቀሙ።

አማኑኤል አንጀሎ

የህክምና ላቦራቶሪ ሳይንስ ዲፓርትመንት

የጤና ሳይንስ ኮሌጅ፤ አዲስ አበባ ዩኒቨርሲቲ

ሞባይል፡ +251921452169 ኢሜይል፡ angelo.amanuel19@gmail.com

ጥናት አማካሪዎች፡ ዶ/ር አስቴር ፀጋዬ 0911696085 ፤ ዶ/ር ግርማ ደርቤ 0930387777

ለተጨማሪ መረጃ የአዲስ አበባ ዩኒቨርሲቲ ህክምና ፋከልቲ ኢንስቲትዩሽናል ሪቪዩ ቦርድ ይጠይቁ።

ስ.ቁ፡ +2511911107099 ፋክስ፡ +251115511513099 ፖ.ሳ.ቁ፡ 9086፣ አዲስ አበባ፤ ኢትዮጵያ

Annex VI – Assent Form (Pregnant Mothers, English Version)

Code Number----------

I have been informed about the study which is aimed at establishing reference values from cord blood. For this study blood is required from the cord. The aim of the study was explained to me.

I am also informed that all the information contained within the questionnaire is to be kept confidential. Moreover, I have been well informed of my right to keep hold of information, decline to cooperate and make myself withdraw from this study.

It is therefore, with full understanding of the situation that I gave the informed consent voluntarily to the researcher to use the blood taken from the cord for the investigation. In addition, I have also been informed that the benefit of the participation is to get the results of the analysis measured for free via the counselor.

Participant's signature/ finger print----------

Name of deponent (mother unable to read) ----------Signature----------Date--------

Name of Counselor----------Signature----------Date----------

Please direct any questions or problem you may encounter during this study to:

Ammanuel Angelo

Department of Medical Laboratory Sciences

College of Health Sciences

Addis Ababa University

Mob: +251921452169

Email: angelo.amanuel19@gmail.com

Advisors: Dr Aster Tsegaye 0911696085; Dr Girma Deribe (OBS/GYN)

For additional information, please contact Department of Medical Laboratory Sciences, Addis Ababa University, Institutional Review Board (IRB) office;

Tel: +2511911107099

P.O Box: 9086, Addis Ababa, Ethiopia

Annex VII -Assent form (Amharic Version)

የስምምነት ቅጽ (ለልጁ/ጅቷ)

የምስጢር ቁጥር ----------

እኔ ስሜ ከላይ የተጠቀሰው ተሳታፊ በእትብት ላይ ስለሚሰራው የአጠቃላይ ደም ምርመራ (CBC) ውጤት ማወዳደሪያ ጥናት በቂ ገለፃ ተደርጎልኛል። ለጥናቱም ከእትብት የተወሰደ የደም ናሙና እንደሚያስፈልግ ተገለፆልኛል። የጥናቱን አላማዎችንም ተረድቻለው።

በመጥይቁ ላይ የገለፅኳቸው መረጃዎች በሙሉ በምስጥር የተጠበቁ እንደሚሆኑ ተነግሮኛል። በጥናቱ ላይ ያለመሳተፍና ማንኛውም መረጃ ያለመስጠት እንዲሁም በማንኛውም ጊዜ ከጥናቱ እራሴን የማግለል መብቴ የተጠበቀ መሆኑን ተገለፆልኛል። ስለዚህ ለዚህ ጥናት መረጃና የስምምነት ቃሌን የሰጠሁት በአጠቃላይ ሁኔታውን በመረዳትና ፍፁም ፈቃድኝነት ነው። ከእትብት ላይ የሚወሰደዉ ናሙና የልጁ/ጅቷ ጤና ሁኔታ ለማወቅ እና ለምርም እነደሚውልም ተረድቻለው። በተጨማሪም ጥያቄ እንድጠይቅ ተፈቅዶልኝ ለማወቅ የፈለጉትን ማብራሪያ አንግቻለሁ። የዚህ ጥናት ተሳታፊ በመሆኔ የላቦራቶሪ ምርምራ በነፃ ማግኘት እንደሆነ ተረድቻለው።

የተሳታፊዋ ፊርማ/ የጣት አሻራ----------

የምስክር ስም---------- ፊርማ---------- ቀን----------

(የስምምነት ቅጹን ማንበብ ለማይችሉ ተሳታፊዎች)

የአማካሪ ስም----------ፊርማ---------- ቀን----------

ይህን ጥናት በተመለከተ ወይም ከዚህ ጥናት ጋር በተዛመደ መልኩ ስለሚያጋጥሙ ድንገተኛ አደጋዎች ወይም ጥያቄ ካልዎት በሚከተለው አድራሻ ይጠቀሙ።

አማኑኤል አንጀሎ

የህክምና ላቦራቶሪ ሳይንስ ዲፓርትመንት

የጤና ሳይንስ ኮሌጅ፤ አዲስ አበባ ዩኒቨርሲቲ

ሞባይል፡ +251921452169 ኢሜይል፡ angelo.amanuel19@gmail.com

ጥናት አማካሪዎች፡ ዶ/ር አስቴር ፀጋዬ 0911696085 ፤ ዶ/ር ግርማ ደርቤ 0930387777

ለተጨማሪ መረጃ የአዲስ አበባ ዩኒቨርሲቲ ህክምና ፋከልቲ ኢንስቲትዩሽናል ሪቪዩ ቦርድ ይጠይቁ።

ስ.ቁ፡ +2511911107099 ፋክስ፡ +251115511513099 ፖ.ሳ.ቁ፡ 9086፣ አዲስ አበባ፤ ኢትዮጵያ

Annex VIII – Questionnaire (For Pregnant Mothers, English Version)

Addis Ababa University

College of Health Sciences

Department of Medical Laboratory Sciences

Questionnaire for Data Collection from Mothers Whose Cord Blood is to be used in the Establishment of Hematological Parameters Reference Values

1. Introduction

   Subject identification number ---------- MRN ----------

   Age of the mother (in years) ----------

   Residential Place---------- Tel: ----------

2. Educational level

   ☐ Unable to write and read ☐ College diploma/degree and above

   ☐ Read and Write

   ☐ Primary (1-8)

   ☐ High School (9-12)

3. Occupation

   ☐ Student ☐ House wife

   ☐ Employed (government, NGO) ☐ Jobless

   ☐ Private work ☐ Other (specify)

4. Marital status

   1. Single 2. Married 3. Divorced 4. Widowed

5. How many children previously delivered?

   ☐ This is my first pregnancy ☐ 1 child ☐ 2 and above

6. If you delivered for question 5, on how many interval?

   ☐ 1 year ☐ 1 year 6 month ☐ 2 year ☐ 2 year and above

7. If yes for question 5, what was the mode of previous delivery?

   ☐ Normal spontaneous delivery ☐ Induced vaginal delivery

   ☐ Vaginal delivery with forceps ☐ Cesarean section

8. Did you drink alcohol during pregnancy?

   ☐ Yes ☐ No

9. If your answer for question 8 is ‘yes’, how often do you drink alcohol?

☐ Daily ☐ Every weekend ☐ occasionally

10. Did you smoke cigarettes during pregnancy?

☐ Yes ☐ No

11. If your answer is 'yes' for question 10, specify pack number smoked per day----------

12. Did you chew khat during pregnancy?

☐ Yes ☐ No

13. If your answer for question 11 is 'yes', how often do you chew chat?

☐ Daily ☐ Every weekend ☐ occasionally

14. Have you been sick for the last 3 months?

☐ Yes ☐ No

If yes, when ---------- describe illness ----------

15. Are you taking any prescribed medication

☐ Yes ☐ No

If yes, specify the name? ----------

End of interview

Thank you!

Annex IX – Questionnaire (For Pregnant Mothers, Amharic Version)

አዲስ አበባ ዩኒቨርሲቲ

የጤና ሳይንስ ኮሌጅ

የሕክምና ላቦራቶሪ ሳይንስ ት/ክፍል

ከእትብት ላይ ደም ተወስዶ ለሚሰራው አጠቃላይ የደም ምርመራ ሁኔታ የማወዳደሪያ ዋጋ ጥናት ለሚሳተፉ እናቶች የተዘጋጀ መጠይቅ

1. መግቢያ

   መለያ ቁጥር---------- ካርድ ቁጥር ----------

   የእናት እድሜ (በአመት)--------

   መኖሪያ አድራሻ ---------- ስልክ ቁጥር ----------

2. የትምህርት ደረጃ

   ሀ. ማንበብና መፃፍ የማትችል መ. ሁለትኛ ደረጃ (9-12ኛ)

   ለ. ማንበብና መፃፍ የምትችል ሠ. ኮሌጅ ሰርቲፊኬት/ዲፕሎማ/ድግሪ እና ከዛ በላይ

   ሐ. መጀመሪያ ደረጃ (5-8ኛ)

3. የስራ ሁኔታ

   ሀ. ተማሪ መ. የቤት እመቤት

   ለ. ተቀጣሪ (የመንግስት/የግል) ሠ. ስራ የሌላት

   ሐ. የግል ረ. ሌላ

4. የትዳር ሁኔታ

   ሀ. ያገባ ለ. ያላገባ ሐ. የፈታ መ. የሞተባት

5. ከአሁን በፊት ምን ያህል ልጆችን ወልደዋል?

   ሀ. ይህ የመጀመሪያዬ እርግዝና ነው ለ. አንድ ሐ. ሁለት መ. ሶስትና ከዚያ በላይ

6. ለ 5ኛው ጥያቄ ከዚህ በፊት ወልደው ከሆነ በምን ያህል ጊዜ ልዩነት?

   ሀ. በ1 አመት ለ. በ1 አመት ከ 6ወር ሐ. በ2 አመት መ. ከ2 አመት በላይ

7. ለ5ኛው ጥያቄ ወልደው ከሆነ በምን አይነት መንገድ ወለዱ?

   ሀ. በተፈጥሮ ምጥ ሐ. በመድሐኒት የታገዘ ምጥ

   ለ. በመሳሪያ የታገዘ ምጥ መ. በቀዶ ጥገና

8. አልኮል ይጠጣሉ?

   ሀ. እጠጣለሁ ለ. አልጠጣም

9. ለ 8ኛው ጥያቄ መልሰዎ እጠጣለሁ ከሆነ በየስንት ጊዜዉ ይጠጣሉ?

   ሀ. በየቀኑ ለ. በሳምንቱ መጨረሻ ቀናት ሐ. አልፎ አልፎ

10. ሲጋራ ያጨሳሉ?

    ሀ. አጨሳለሁ ለ. አላጨስም

11. ለ10ኛው ጥያቄ መልስዎ አዎ ከሆነ፤ ምን ያህል እሽግ በቀን ያጨሳሉ? --------

12. ጫት ይቅማሉ?

    ሀ. እቅማለሁ ለ. አልቅምም

13. ለ11ኛው ጥያቄ መልስዎ እቅማለሁ ከሆነ በየስንት ጊዜዉ ይቅማሉ?

    ሀ. በየቀኑ ለ. በሳምንቱ መጨረሻ ቀናት ሐ. አልፎ አልፎ

14. በአለፈው 3ወር ዉስጥ ታመው ነበር?

    ሀ.አዎ ለ. አልታመምኩም

    አዎ ከሆነ መልስዎ መች?----------ህመሙ ምን እንደነበር ይግለጹ----------

15. መድሐኒት በመውሰድ ላይ ነዎት?

    ሀ. አዎ ለ. አይደለም

    ከወሰዱ የመድሐኒቱን ስም? ----------

መጠይቁን ጨርሰዋል

አመሰግናለሁ!

Annex X- Pregnant Mother and Newborn Medical and Diagnostic Information Sheet

Addis Ababa University

College of Health Sciences

Department of Medical Laboratory Sciences

Code Number ----------

Physical examination and Medical History the Mother

Weight (in Kg) ---------- Hypertension ----------

Height (in meter) ---------- Diabetes Mellitus ----------

Gestational Week ---------- Psychological Disorder ----------

Body Temperature (^{0}C) ---------- Obstetric Problems ----------

Laboratory & Diagnostic Test Results

Blood Type (ABO & Rh) ---------- Syphilis ----------

HGB ----------g/dL HIV ----------

Hepatitis B ---------- Malaria ---------

Ultrasound ----------

Information about the Newborn

Gender Male Female Fetal Life (hr.) ----------

Current Delivery Mode ----------Weight (in Kg) ---------- Appearance (color) ----------

Pulse Rate/min ---------- Umbilical cord appearance ----------

Breathing Rate/min ----------

Initial & Sign. ---------- Date ----------

Sample Collector Initial & Sign. ---------- Date ----------

Annex-XI **Laboratory Request and Result Report Form for Cord Blood**

Addis Ababa University

College of Health Sciences

Department of Medical Laboratory Sciences

Code: ----------

Blood Type (ABO & Rh) ---------- Hepatitis B ------- Syphilis ------- HIV -------

Complete Blood Count (CBC)

WBC ----------	/uL	LYM --------- %	PDW-CV -------%
RBC ----------	/uL	MXD --------- %	
HGB ----------	g/dL	NEU ---------- %	
HCT ----------	%	#LYM ----------	
MCV ----------	fL	#MXD ----------	
MCH ----------	Pg	#NEU ---------	
MCHC ----------	g/L	PLT --------- /uL	
RDW-CV ----------	%	MPV -------- fL	

Nucleated RBC ----------

WBC Morphology ----------

RBC Morphology ----------

Platelet Morphology ----------

Lab Tech Initial & Sign. ---------- Date ---------- (G.C)

PI Sign. ---------- Date ---------- (G.C)

Declaration

I, the undersigned, declare that this M.Sc. thesis is my original work, has not been presented for a degree in this or any other university and that all sources of materials used for the thesis have been duly acknowledged.

M.Sc. candidate: Ammanuel Angelo (B.Sc.)

Signature: _____

Date of submission: _____

This thesis has been submitted with my approval as university based advisor.

Advisor: Aster Tsegaye **(MSc, PhD)**

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.