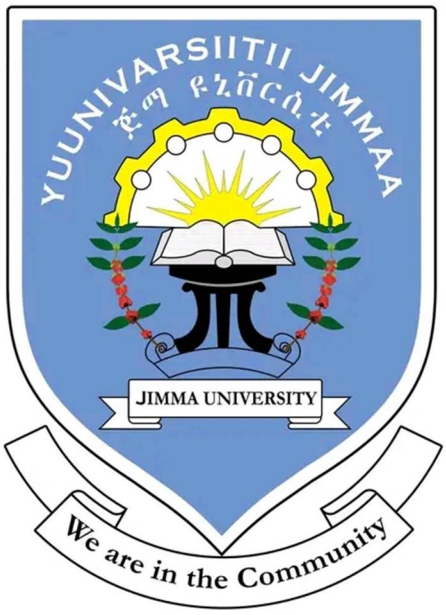
ASSESSMENT OF HEMATOLOGICAL ABNORMALITIES AND ASSOCIATED FACTORS AMONG NEONATES ADMITTED TO NEONATAL INTENSIVE CARE UNIT IN ASSOSA GENERAL HOSPITAL, WESTERN ETHIOPIA.



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INSTITUTE OF HEALTH

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ASSESSMENT OF HEMATOLOGICAL ABNORMALITIES AND ASSOCIATED FACTORS AMONG NEONATES ADMITTED TO NEONATAL INTENSIVE CARE UNIT IN ASSOASA GENERAL HOSPITAL, WESTERN ETHIOPIA.

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ABSTRACT

Background: In neonatal period, babies are extremely vulnerable to a variety of diseases and conditions. The hematopoietic system is one of the systems that can be affected during neonatal period. In sub-saharan Africa, the burden of neonatal anemia ranges from 23 to 66%. Blood loss, RBC destruction and decreased RBC production are the threes general causes of anemia in neonates. Thrombocytopenia is also a common clinical problem in neonatal intensive care unit (NICU), affecting about 20 to 35% of all admitted neonate.

Objective: To assess the hematological abnormalities and associated factors among neonates admitted to NICU in Assosa General Hospital, from February 01 to July 27 2022.

Method: Institutional based cross-sectional study was conducted on a total of 175 neonates admitted to NICU at Assosa General Hospital. A consecutive sampling technique was used to select study participants. Data was collected using structured questionnaire. One ml of venous blood was collected in EDTA microtainer tube for complete blood count performed by Sysmex XN-550 hematological analyser. Blood smear was prepared for peripheral morphology examination. Data was entered into Epidata version 3.1 and exported in to SPSS version 20 for analysis. ANOVA, Kruskal-Wallis test, Mann-Whitney U-test and logistic regression model were used during data analysis. P-values <0.05 was considered as statistically significant.

Result: The overall magnitude of anemia, thrombocytopenia, leukocytosis, and leukopenia in this study was 29.1%, 20.6%, 11.4%, and 4%, respectively. Caesarean mode of delivery (AOR= 3.11; 95% CI: 1.26-7.68) and house hold monthly income below 2850ETB (AOR= 2.63; 95% CI: 1.05–6.63) were found to be associated with anemia in neonate. Sepsis in clinical characteristics of neonate (AOR= 7.23; 95% CI: 1.18–44.16) was a factor significantly associated with leukocytosis.

Conclusion: This study demonstrated the predominant existence of anemia, thrombocytopenia and leucocytosis in neonate admitted to neonatal intensive care unit. Therefore, early diagnosis, monitoring and intervention for hematological abnormalities are required to prevent mortality and long-term implications.

Key words anemia, thrombocytosis, leukopenia, thrombocytopenia, neonate, NICU

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Table of Contents

LIST OF TABLES

LIST OF FIGURES

ACRONYMS AND ABBREVIATION

ANC: Anti-Natal Care

ANOVA: Analysis of Variance

AOP: Anaemia of Prematurity

CBC: Complete Blood Count

EDTA: Ethylene Diamine Tetra Acetic Acid

HCT: Haematocrit

Hgb: Haemoglobin

HSC: Hematopoietic Stem Cells

LBW: Low Birth Weight

MAS: Meconium aspiration syndrome

MCH: Mean Cell Hemoglobin

MCHC: Mean Cell Hemoglobin Concentration

MCV: Mean Cell Volume

MPV: Mean Platelet Volume

MUAC: Midd Upper Arm Circumference

NICU: Neonatal Intensive Care Unit

nRBC: Nucleated Red Blood Cell

PMN: Polymorphonuclear

PNA: Perinatal Asphyxia

RBC: Red Blood Cell

RDS: Respiratory Distress Syndrome

SPSS: Statistical Package for the Social Science

WHO: World Health Organization

CHAPTER ONE: INTRODUCTION

1.1 Background

Hematopoiesis is the process of generating all types of blood cells including formation, differentiation, development and maturation of blood cells to produce and supply the blood system. Hematopoiesis starts with in the yolk sac during the first 2-8 weeks of life and then shift into the liver and spleen temporarily before finally establishing definitive hematopoiesis in the bone marrow after 5^{th} month. All types of blood cells are generated from hematopoietic stem cells (HSC) that have the potential to self-renewal and develop into all line of blood cells [1].

Hematopoietic system can be significantly affected during complications of neonate [2]. The hematological parameter examination is the most commonly requested laboratory test. It gives the physician with qualitative and quantitative information on all type of blood cells that help in diagnosis, therapy, and monitoring of most hematological disorders as well as other diseases [3]. Knowledge of the normal hematological values of neonate and young children is essential for the proper interpretation of test results and understanding of the dynamic changes occurring during that period. In the neonatal period, hematological parameters highly correlates with gestational age, birth weight, blood sampling site, mode of delivery, maternal anemia, iron folate supplement during pregnancy and other factors [4].

The neonatal period is defined as the first 28 days from birth [5]. Neonatal period is a sensitive time in which the neonate has to adapt to absolutely new surroundings and is vulnerable to many problems, which may indeed be life threatening. Millions of neonates are born annually and a large extent of them is admitted to the neonatal intensive care unit (NICU) for different clinical indications. NICU is a specialized unit of a health facility that gives comprehensive and continuous medical service for neonates who are critically sick and/or preterm with low birth weight and it provide care for high-risk neonates [6].

Most of neonatal deaths happen in sub Saharan Africa countries [7]. In the developed countries, the main reason behind morbidity and mortality during neonatal period is intrinsic abnormalities which are usually non-preventable, but in the developing countries the frequent causes are infections, anemia, birth asphyxia and pneumonia predominate [8]. Neonatal mortality rate in Ethiopia is one of the noteworthy in the world. In Ethiopia the most common diagnoses during admission were prematurity/low birth weight and/or Respiratory Distress Syndrome (RDS), neonatal sepsis, asphyxia, Meconium Aspiration Syndrome (MAS), hyperbilirubinemia, congenital anomalies and anaemia [9.10].

Neonatal sepsis can be defined as systemic inflammatory response syndrome in the presence of infection in a neonate [11]. Neonatal sepsis remains one among the primary reasons of admissions infants to neonatal intensive care unit throughout the world [12]. Bacterial infection of neonate significantly alters hematopoietic system such as leukocytosis, toxic granular change of neutrophil and release immature neutrophils to circulation. These changes are among the most necessary markers of neonatal septicaemia. The early diagnosis of neonatal sepsis by clinical examination is imperative. Sepsis Screen is greatly reliable index of early neonatal septicaemia, with much less expenditure and serves as a good guide for initiating antibiotic therapy [13].

Hematological system is the primary to be influenced in perinatal asphyxia, frequently within the first hour of birth, even earlier than other systemic changes occur [14]. World health organization (WHO) has defined perinatal asphyxia as a failure to initiate and sustain breathing at birth [15]. Nucleated red blood cells (nRBC's) have been suggested as a marker of perinatal asphyxia [16]. In normal newborns less than 10 nRBC's are found per 100 white blood cells (WBC's) [17]. Number of nRBCs directly indicates of the degree of oxygen deprivation [16].

Hematological abnormalities are commonly seen in the NICU, thrombocytopenia is the one of the most common but could be missed in the event that not particularly evaluated for [18]. Thrombocytopenia is defined as platelet count $<150 \times 10^3$ cells/µl regardless of the gestational age [19]. Multiple disease processes can cause neonatal thrombocytopenia [20]. The risk factors related with thrombocytopenia were sepsis, extreme low birth weight, intra-uterine growth restriction and birth asphyxia [18].

Hematological parameters like hemoglobin (HGB), white blood cell (WBC), and platelet count are frequently utilized to assess neonates born preterm. [21,22]. Preterm infants encounter a reduction in haemoglobin concentration after birth, with a decline that typically is more sudden and more significant than in term infants, reaching haemoglobin concentration of 7 to 9 g/dl at 3 to 6 weeks of age. Preterm infants are at high risk for developing iron deficiency because unfortunately they could not get the benefit of a full third trimester of gestation. [23].

Understanding the development of the hematopoietic system might be important in the evaluation of neonates. Anaemia refers to decrement in haemoglobin level to a value more than two standard deviations below the mean. During fetal life, red cells reduce in size and increase in number. In late gestation and after birth, erythrocytes gradually shift from the generation of fetal hemoglobin to adult hemoglobin. After delivery, RBC number starts to decrease because of an increase in the accessibility of oxygen and down-regulation of erythropoietin. Erythrocyte number decreases until oxygen delivery is inadequate and erythropoietin production is stimulated again. [23].

1.2 Statement of the problem

Globally, 2.6 million babies die each year before they achieve the age of one month. Moreover, one million of this death occurs in their first day of life. The mortality rate of under-five children has decreased dramatically in recent decades. However, with 7000 neonates dying every day, it was not significantly decreased in neonates [24, 25]. Eight of the ten countries with the greatest rates of neonate mortality are in Sub-Saharan Africa. Ethiopia ranked fifth among the ten countries with highest neonatal death [26-29]. Approximately 70% of these early neonatal deaths were caused by conditions that could have been anticipated or treated with easily accessible and cheap medications [30]. Prematurity, perinatal asphyxia and infections like sepsis, meningitis, and pneumonia account for more than 80% of neonatal deaths [27]. Infections have been one of the leading diagnoses of in neonatal admissions in neonatal intensive care unit throughout the world [12].

Neonatal septicaemia cause significant change in hematopoietic system. During the bacterial infections, bone morrow release elevated number of neutrophils in to the blood circulation enabling neutrophils to migrate at the site of infection. This enhanced production of neutrophils appears basic for the host's immune defence against bacterial infection. As increased number of neutrophil is released, more & more immature cell reaches the circulation, a process called as shift to left. This finding has been found valuable in early diagnosis of bacterial infection. Vacuolation and toxic granulation of neutrophils commonly exist during sepsis, a change never seen in healthy newborn babies [31].

Erythropoiesis is suboptimal in most premature babies relative to their needs, and RBC survival is poor (only 40 to 60 days). The suboptimal erythropoiesis seen in premature infants is caused by the blunted synthetic response of hepatic oxygen sensor to hypoxia in premature infants [32]. The concentration of haemoglobin drop to between 9.5-11 g/dL around 10-12 weeks in healthy term infants [33]. However, hemoglobin concentration drops to 8-10 g/dL in preterm infants weighing 1200-1400 g at 4-10 weeks of age, and 6-9 g/dL in preterm infants weighing less than 1200 g at 4-10 weeks of age. Preterm infants have more sever and earlier onset of anaemia than physiologic anaemia of infancy, which is referred to as anaemia of prematurity (AOP) [34].

Neonatal disease can cause hematological abnormalities commonly thrombocytopenia. Depending on the population surveyed, the prevalence of neonatal thrombocytopenia ranges from 1% in healthy term babies to about one-third of neonates admitted to the NICU [35]. Thrombocytopenia is so common in neonate; it is often overlooked in the hope that it will be improved on its own. However, if thrombocytopenia not treated properly, may have serious implications such as intracranial and pulmonary haemorrhage, particularly in preterm babies [18].

Perinatal asphyxia can cause significant change in hematological profile which can be used to evaluate the severity and consequence of asphyxia [36]. Perinatal asphyxia is one of the leading causes of morbidity and mortality. According to WHO report, birth asphyxia affects about 3% of the 120 million infants born every year in developing countries. Each year, it is estimated that 900,000 of these newborns die [15]. Birth asphyxia has adverse influence on the neonatal brain. While hypoxic-ischemic encephalopathy is the hallmark of severe asphyxia, multisystem failure including the heart, kidneys, and gastrointestinal systems is common in such cases [36]. Even though several study finding indicated the risk of neonatal complication on haematological parameters, studies were not conducted regarding this title at the current study area. Therefore, the aim of this study was to assess haematological abnormalities among neonates admitted to neonatal intensive care unit in Assosa general hospital, B/Gumuz region, Western Ethiopia.

1.3 Significance of the study

Since infants are the windows hope for the future development and existence of a country, it is a great deal to be concerned about their health and safety. During neonatal period, babies are susceptible for many disease conditions. The haematological parameter analysis can provide crucial quantitative and qualitative data enabling the detection, investigation, and monitoring of most hematologic disorders as well as many other diseases.

The findings in the present study will help to generate information about the hematological parameter of sick neonate. Early assessment of hematological parameters will have paramount significance for early detection and management of hematological disorders like anemia and lower the risk of developing further complication and death. Moreover this study will gives a baseline data for further investigation on hematological parameters and associated factors of neonate in the current study area and at a larger scale in the country. Additionally, the result of this finding will be used by governmental and non-governmental organizations and health care providers. The results of this study will be therefore feed-backed to neonatal intensive care unit of the hospital. Physician, nurses and policy makers as well as and Benishangul Gumuz regional heath office administration will also be benefitted in providing appropriate intervention and care for neonate.

CHAPTER TWO: LITERATURE REVIEW

The first 28 days are almost pivotal and hazardous period of human life compared to any other time. Neonatal period is the most vulnerable time for variety of diseases conditions and most of morbidity and mortality are preventable [24.37]. Neonates have low immune system, they are prone to infection and most of the illnesses they acquire usually require critical care. During neonatal period haematological parameters can be affected by several variables. Hematological parameters correlates highly with gestational age, birth weight, blood sampling site, mode of delivery, sepsis, birth asphyxia and other factors [2]. There are some literatures about haematological parameters and neonate with different clinical condition in different countries

According to prospective observational study carried out on 258 neonates admitted to NICU over a period of two years from 2007 to 2009, thrombocytopenia in the study group was 70.5%. This was 93.7% of the high-risk group and 41.7% of the low-risk group in which the difference was found to be statistically significant with $p < 0.05$. Sepsis was the most common main high-risk factor for thrombocytopenia. Other risk factors include low birth weight (LBW) babies; babies born to pre-eclamptic mothers and babies with birth asphyxia. Around 55.9% of cases of thrombocytopenia in the high-risk group had bleeding manifestation. This was in the form of petechial in 40% followed by pulmonary haemorrhage in 33.3% cases, bleeding from multiple sites in 24% [18].

A cross-sectional study was conducted in a total of 94 Neonates with suspected septicaemia in Warangal, Telangana, during the period of two year, from 2014 to 2016. Toxic granulation, leukopenia and immature neutrophil to total neutrophil ratio are among the indirect markers of neonatal infection. When these indirect markers are collectively studied, they can provide an extremely reliable index of neonatal sepsis much earlier and serve as a useful guide for initiating antibiotic therapy. Leucopenia, toxic granulation, increased immature neutrophil to total neutrophil ratio (<0.2) and thrombocytopenia ($<100 \times 10^3 /\mu L$) were 40.9%, 68.18%, 50% and 39% of neonate respectively with positive culture result and 20.8%, 45.8% , 23.66% and 25.3% of neonate with negative culture result. [13]

Another study conducted on haematological profile in 108 clinically suspected cases of neonatal Sepsis, 24 (22.22%) had proven sepsis and 24 (88.89%) probable sepsis group neonates. 75% of cases of proven Sepsis showed morphologic changes in PMN either as toxic granulation, vacuolisation or both. Out of neonates with proven Sepsis 16.6% had leucocytosis, 20.9% had leukopenia, and 62.5% had WBC count within the reference range. However, only 1.75% of the no sepsis neonates had leukopenia. 79.2% of neonates with proven Sepsis, 77.8% neonates with probable Sepsis and 29.8% of neonates with the no sepsis group had elevated band forms counts. 37.5% of neonates with proven Sepsis, 37.1% of neonates with probable Sepsis and 12.3% of the no sepsis group had thrombocytopenia. 29.1% proven sepsis group neonates, 18.52% probable sepsis group neonates and 10.5% no sepsis group neonates showed low levels of haemoglobin and hematocrit [38].

Case control study conducted on premature neonates in Lahore, Pakistan in 2018. A total of 200 study subjects were involved, 100 premature infants and 100 normal infants were taken as healthy control. Significantly low levels of RBCs, platelets, hematocrit, neutrophil counts, and hemoglobin were recorded in preterm infants (3.09±0.18 x 10^6/µL, 165.99±10.96 x 10^3/µL 31.29±3.42 %, 3.88±0.42 x 10^3/µL and 9.26±1.44 g/dL) as compared to full-term healthy infants (4.26±0.27 x 10^6/µL, 314.58±14.16 x 10^3/µL, 39.35±3.16%, 5.26±0.58 x 10^3/µL and 14.19±1.05g/dL) respectively. On the other hands, the levels of WBC was significantly increased in preterm anemic infants, 10.26±0.13 x 10^3/µL as compared to full term healthy infants, 7.33±0.88 x 10^3/µL [39].

According to case control study conducted on neonates with perinatal asphyxia in Gwalior, India in 2016. A total of 200 neonates were involved, 100 cases and 100 controls. A Significant difference is seen in mean values of nucleated RBCs among the cases and controls (p value <0.001). Among cases mean RBC count was 10.85 ± 6.39 x 10^6/µL, while among control group mean was 5.91 ± 3.60 x 10^6/µL. Mean Total Leukocyte count was found 17.06 ± 7 x 10^3/µL among cases while among control mean was 14.14 ± 4.15 x 10^3/µL, which was significant. Similarly, significant difference was found in mean Hb, 16.86 ± 2.48 g/dL among cases while 16.00 ± 2.52 g/dL among control group. Mean haematocrit was 51.01 ± 6.22 % among cases while 49.24 ± 7.98 % among control group, which was insignificant. Similarly, insignificant difference was found in mean platelet count. An association of polychromatia and perinatal asphyxia was found. Among both group 24% cases of birth asphaxia had polychromatia while only 2% had polychromatia among control group which was found significant [36].

Comparative study was conducted on haematological parameters of newborns delivered vaginally versus caesarean section in Menoufiya, Egypt in 2013. A total of 72 neonates were involved, group I included 31 neonates delivered vaginally and group II included 41 neonates delivered by caesarean section. The CBC parameters were examined in full-term neonates by their mode of delivery. The Hb, RBC count, Hct, platelets, total leucocyte count, eosinophils and basophils were found to be higher in vaginally born infants than infants delivered by caesarean section ($P < 0.001$). The mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), red cell distribution width, lymphocytes and monocytes showed no significant differences in these two groups. On comparing the CBC parameters in preterm neonates by their mode of delivery, the Hb, RBC count, Hct, mean corpuscular, MCH, red cell distribution width, platelets, total leucocyte count, eosinophils, lymphocytes and neutrophils were found to be higher in vaginally born infants than infants delivered by caesarean section ($P< 0.001$). The MCHC, basophile and monocyte showed no significant differences between these two groups [40].

According to prospective cohort study conducted on 216 neonates admitted to neonatal care unit of a tertiary care hospital in Addis Ababa in 2015, the most common primary diagnoses at admission to the neonatal care unit were prematurity with respiratory problem (36.6%), neonatal sepsis (22.7%), and asphyxia (16.2%). High case fatality was observed among neonates with the diagnosis of prematurity with respiratory problem (40.5%) and asphyxia (40.0%). Under multivariate analysis, diagnosis of asphyxia was an independent predictor of mortality (AOR =5.817; 95% CI: 1.611–20.977), while gestational age above the mean of the study population (36.6 weeks) was protective of mortality (AOR =0.683; 95% CI: 0.588–0.795) [9].

According to cross sectional study conducted on 192 neonates in Gondar comprehensive specialized hospital, Northwest Ethiopia, in 2019, the Hgb value ranges from 4.2–20 g/dl with a median (IQR) value of 15 g/dL (13.9–16.2 g/dL). The overall prevalence of anemia among newborn babies was 25% with a prevalence of 29.2 % and 20.8% among male and female newborn babies, respectively. Based on severity of anemia, 89.6, 6.3, and 4.2% were mild, moderate and severe anemia, respectively. Of the total anemic newborn babies, 14.5% (7/48) had microcytic hypochromic type of anemia and 85.5% (41/48) had normocytic normochromic anemia type. Based on maternal occupation, 26.9% anemic newborn babies were born from mothers who were a housewife in their occupation. The prevalence of anemia among low and normal birth weight newborn babies was 16.7 and 25.9%, respectively [41].

A facility-based cross-sectional study involving 278 newborns was conducted in Nekemte specialized hospital, Western Ethiopia, from October to November, 2020 with an interview based questionnaire that included maternal socio-demographic and obstetrics characteristics, newborn’s weight and sex. Newborns delivered by caesarean section were 4.17 (95% C.I.: 1.89–9.20, $P < 0.001$) times more likely to be anemic compared to newborns delivered vaginally. Similarly, newborns from mothers with anemia during pregnancy were 3.95 (95% C.I.: 1.97–7.92, $p < 0.001$) times more likely to be anemic than newborns from non-anemic mothers. The risk of developing anemia in newborns born to mothers without iron-folate supplementation during pregnancy was 2.17 (95% C.I.: 1.07–4.41, p=0.032) times higher than that of newborns born to mothers with iron-folate supplementation [42].

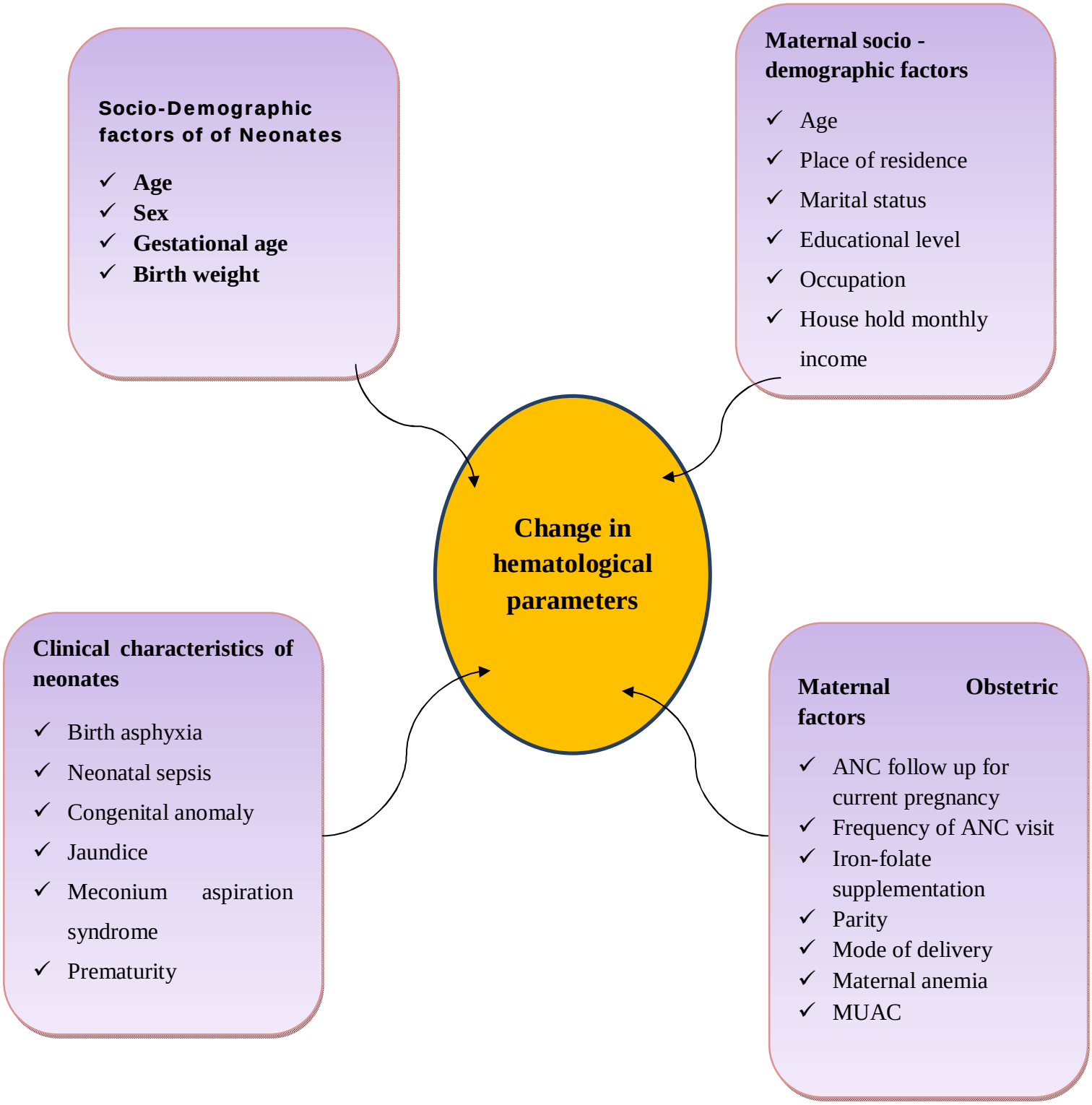

Figure 1: conceptual frame work showing socio demographic factors, clinical and obstetric characteristics influencing hematological parameters of neonate.

CHAPTER THREE: OBJECTIVES

3.1 General objective

To assess the hematological abnormalities and associated factors among neonates admitted to NICU in Assosa General Hospital, Western Ethiopia, from February 01 to July 27 2022.

3.2 Specific objective

- To assess haematological parameters among neonate admitted to NICU at AGH during study period.
- To determine the prevalence of hematological abnormality among neonate attending NICU at AGH during study period.
- To assess associated factors of hematological abnormality among neonate attending NICU at AGH during study period.

CHAPTER FOUR: MATERIALS AND METHODS

4.1 Study area

The study was conducted among NICU neonate in Assosa general hospital, Assosa, Benishangul gumuz regional state, western Ethiopia. The town is the administrative center of the B/gumuz Regional State, located 663 km away from Addis Ababa, the capital city of Ethiopia. Based on figures from the Central Statistical Agency in 2007, Asosa has an estimated total population of 22,725. It found at latitude & longitude of 10°04′N and 34°31′E respectively with elevation of 1570 meter above sea level & its maximum temperature range from 29.4-38.5 ^{0} c & minimum from 5^{0}c -10^{0}c, also its average annual rain fall is 1236mm. Assosa general hospital is found in Assosa town, it provides comprehensive health care services including neonatal intensive care (NICU) and laboratory services. The hospital is giving service for more than 100 thousand population living in and around Assosa. The hospital laboratory is performing several laboratory investigations including CBC and peripheral morphology examination [43].

4.2 Study design and period

Institutional-based cross-sectional study was conducted among neonate admitted to NICU in Assosa general hospital from February 01 to July 27 2022.

4.3 Population

4.3.1 Source population

All neonates admitted to NICU in Assosa general hospital.

4.3.2 Study population

All neonates admitted to NICU in Assosa general hospital from February 01 to July 27 2022.

4.4 Eligibility Criteria

4.4.1 Inclusion criteria.

- ✓ Neonates attending NICU of Assosa general hospital during the study period whose parent was willing to give informed consent for participation of their baby.

4.4.2 Exclusion criteria

- ✓ Neonates with history of previous blood transfusion or exchange.
- ✓ Neonates with invisible vein there by unable to collect blood
- ✓ Neonates with serious illness thereby puncturing and drawing blood was suspected to be a risk to neonate.

4.5 Sample size determination and sampling technique.

4.5.1 Sample size determination

The sample size was determined using single population proportion formula by considering the following assumptions:-

- ✓ 21% prevalence of anemia from the previous study done in Netherland [44].
- ✓ 95% confidence interval (Z = 1.96)
- ✓ 5% maximum allowable error (w = 0.05)

$$\mathbf{n_o = \frac{Z\alpha/2^2 p(1-p)}{w^2}}$$

Where n_o= initial sample size

P= previous prevalence, 0.21

w= margin of error, 0.05

$Z_{\alpha/2}$= 1.96, the standard normal distribution at 95% confidence level

Using the formula, an initial sample size is 255.

According to data from AGH, 420 NICU admissions were taken place over one year in 2019/20. Since 420 was less than 10,000, the required sample size was calculated using a finite population proportion formula.

$$\mathbf{n = \frac{n_0}{1 + (\frac{n_0}{N})}}$$

Where n_o= initial sample size, 255

N= source population, 420

n= required sample size

According to finite population proportion formula, the required sample size was 159. Adding 10% of the sample size as a contingency for non-response rate, the total sample size is 175.

4.5.2 Sampling technique

Consecutive sampling technique was used to select eligible neonate from NICU admission.

4.6 Measurement variables

4.6.1 Dependent variable

- ✓ Hematological parameters and hematological abnormalities

4.6.2 Independent variables

Neonatal socio-demographic Characteristics ✓ Age ✓ Sex ✓ Gestation age ✓ Birth weight	**Clinical characteristics of neonates** ✓ Perinatal asphyxia ✓ Neonatal sepsis ✓ Jaundice ✓ Congenital anomaly ✓ Meconium aspiration syndrome ✓ Prematurity
Maternal socio –demographic factors ✓ Age ✓ Place of residence ✓ Marital status ✓ Educational level ✓ Occupation ✓ House hold monthly income	**Maternal Obstetric factors** ✓ ANC follow up for current pregnancy ✓ Frequency of ANC visit ✓ Iron-folate supplementation during current pregnancy ✓ Parity ✓ Mode of delivery ✓ Duration of labor ✓ Complication during pregnancy ✓ MUAC ✓ Maternal bleeding ✓ Maternal anemia

4.7 Data collection

Prior to data collection, all parents of study subject was formally informed regarding to the objective and importance of this research in providing scientific information about hematological parameters of neonates and will benefit study participants by early detection of hematological abnormality, if any, for appropriate therapy. All eligible neonates attending NICU during study period was included. All necessary socio-demographic, clinical related and laboratory data were collected and retained appropriately.

4.7.1 Socio-demographic and clinical related data

A structured questionnaire was used to collect socio-demographic and clinical related data through interview and reviewing medical record which was conducted by trained data collector. After data collection, questionnaire was checked for the completeness and blood sample was collected for the analysis of hematological parameter.

4.7.2 Laboratory data

Around One ml of venous blood specimen was collected from dorsal hand veins or the veins of the antecubital fossa by trained NICU nurses from each study participant. Venipunctured specimens was collected into EDTA microtainer (2ml) test tube and processed within 4 hours of collection. The specimen was analysed for complete blood cell count and peripheral morphology examination. Sysmex XN-550 (Sysmex Corporation, Japan) hematological analyser was used to determine complete blood count. Wright's stain was used to stain blood smear to look at morphological characteristics of cells. The peripheral smear was examined by a senior laboratory technologists and principal investigator. Standard operating procedure was strictly followed in each step to maintain quality of the laboratory results.

4.8 Data quality assurance and management

Before the study conducted the data and specimen collectors were oriented to ensure quality of the data. Training/orientation was also given in specimen collection to apply standard operational procedures to minimize pre analytical error and to ensure the quality of test result. An English version questionnaire was translated to Amharic and then it was translated back into English by independent translator to check for consistency. After data collection process, the data was checked for completeness and incomplete or misfiled questioners were identified and corrected. All reagents was checked for their expiry date and prepared according to the manufacturer's instructions. Quality control sample was used. All specimens was analysed in one laboratory with the same haematology analyser and the same trained professionals. The overall data collection, application of standard procedure and accuracy of test results was checked by principal investigator.

4.9 Operational definition

Hematological parameters: are parameters such as RBC parameters (RBC count, Hgb, HCT), RBC indices (MCV, MCH, MCHC), WBC parameters (total WBC and differential counts), platelet count, MPV and peripheral morphology.

Hematological abnormality: is defined by the presence of anemia or polycythemia, or leucocytosis or leukopenia or thrombocytosis or thrombocytopenia,

Neonate: An infant whose age is ≤28 days.

Preterm neonate: Newborn delivered before completed 37 weeks of gestational age.

Low birth weight (LBW): is defined as weight of the new born at birth less than 2500 g.

Thrombocytopenia: is defined as a platelet count <150,000cells/µl.

Maternal anemia: as Hgb <11g/ dL

Leukocytosis: is defined as WBC count > 25000 cells/µl

Leukopenia: defined as WBC count < 5000 cells/µl

Neonatal anemia: is defined as Hgb <13g/dL for term and <15g/dL for preterm neonates.

Moderate anemia: Hemoglobin value between 7 gm/dL-10 g/dL.

Severe anemia: Hemoglobin < 7 g/dL [42, 45].

4.10. Data processing and analysis.

The data was coded and entered in to Epidata version 3.1 and was exported to Statistical Package for the Social Science (SPSS) statistical software version 20 which was used to analyse the data. Descriptive statistics (mean, median, frequency, percentage) were used to summarize the characteristics of study participants and the results were presented in tables and charts. One-way analysis of variance (ANOVA) was used in the analysis to compare the difference in mean of hematologic parameters between different clinical characteristics of neonate. Kruskal-Wallis test with median (IQR) was used for data not normally distributed. In both condition, P value < 0.05 was considered as statistically significant. A binary logistic regression model was used to identify factors associated with hematological abnormalities. The Hosmer-Lemeshow goodness-of-fit test was used to assess the fitness of the model. Independent variables having a p-value less than 0.25 in bi- variable analyses were included in the multi-variable analyses to control confounders. Both crude odds ratio (COR) and adjusted odds ratio (AOR) with the corresponding 95% confidence interval were used to show the strength of association. P-value <0.05 in the multi- variable binary logistic regression model was considered as statistically significant.

4.11 Ethical clearance.

Ethical issues were considered in all steps of research process. Ethical clearance was obtained from the Institutional review board of Institute of Health, Jimma University. Letter of cooperation was written to Assossa general hospital. Assosa general hospital manager was asked for permission to use medical records of neonates. The purpose, benefit and procedure of study were clearly explained and a written informed consent was obtained from parents of the neonate. Parents were also informed that all data will be kept confidential by using codes instead of personal identifier. The specimens collected from the participants were analyzed only for the intended purposes. All laboratory test results during the research process were informed to the clinician for proper management of the neonate.

4.11 Dissemination of the result.

The finding of this study will be submitted to Jimma University, Institute of Health, Faculty of Health Sciences, School of Medical Laboratory Science. The finding of this study will also be submitted to B/G regional health office and Assosa general hospital. The findings of this study will be also published in peer-reviewed scientific journals. The finding will be presented on different scientific forums both in Ethiopia and abroad.

CHAPTER FIVE: RESULT

5.1 Socio-demographic characteristics

A total of 175 neonate and their mothers enrolled to this study; of the participants, 112 (64%) were males. Regarding their age distribution, 111 (63.4%) had less than 72 hours, 55 (31.4%) were between 3 and 7 days and 9 were between 8 and 28 days old. The median (IQR) birth weight of the neonate was 2867 (1995-3200) g. **(Table 1)**

Parent of the neonates were interviewed. About 60 (34.3 %) of them had a monthly family income below World Bank's extreme poverty line of $57 per month or 2850 ETB. Out of 175 respondents, 103 (58.9%) were urban dwellers and 163 (93.1%) were married. All mothers are under the age category of 20-34 years. Regarding their educational status, 29 (16.6 %) had no formal education. About 72 (41.1%) of mothers were housewives. **(Table 2)**

Table 1 : Socio-demographic characteristics of neonates admitted to NICU at Assosa general hospital, Western Ethiopia, February to July, 2022 (n=175).

Variable	Category	Frequencies (n=175)	Percentages (%)
Age (Neonate)	<72 hours	111	63.4
	3-7 days	55	31.4
	8-28 days	9	5.1
Sex (Neonate)	Male	112	64.0
	Female	63	36.0
Gestational age	Pre-term	61	34.9
	Term	114	65.1
Birth weight	<2500 g	58	33.1
	≥2500 g	117	66.9

Table 2: Socio-demographic characteristics of mothers of neonate admitted to NICU at Assosa general hospital, Western Ethiopia, February to July, 2022 (n=175).

Variable	Category	Frequencies (n=175)	Percentages (%)
Residence	Rural	72	41.1
	Urban	103	58.9
Marital status	Married	163	93.1
	Unmarried	12	6.9
Educational status	No formal education	29	16.6
	Primary school	38	21.7
	Secondary school	51	29.1
	College and above	57	32.6
Occupation	Employed	67	38.3
	House wife	72	41.1
	Daily labourer	36	20.6
Household monthly income	<2850ETB	60	34.3
	≥2850ETB	115	65.7

5.2 Maternal obstetric and neonatal clinical characteristics

The majority of mothers, 118 (67.4%) had antenatal care check-up. About 117 (66.9%) mothers had taken iron folate supplementation during pregnancy. About 15 (8.6%) mothers had bleeding experience during their pregnancy. Of the total neonate, 83 (47.4%) were born from multigravida mother. About 159 (90.9%) mother had duration of labor less than 24 hours. Most of the babies, 114 (65.1%) were delivered through spontaneous normal vaginal delivery. Most of the neonate 78 (44.6%) had clinical diagnosis of sepsis. Regarding the maternal MUAC, majority of mothers 150 (85.7%) had MUAC level above 23cm. (**Table 3)**

Table 3: Maternal obstetric and neonatal clinical characteristics of neonate admitted to NICU at Assosa general hospital, Western Ethiopia, February to July, 2022 (n=175).

Variable	Category	Frequencies (n=175)	Percentages (%)
ANC follow up	Yes	118	67.4
	No	57	32.6
Frequency of ANC visit	1 visit	5	4.2
	2-3 visit	44	37.3
	4 and above	69	58.5
Iron-folate supplementation	Yes	117	66.9
	No	58	33.1
Parity	Primipara	92	52.6
	Multipara	83	47.4
Mode of delivery	Vaginal delivery	114	65.1
	Caesarean section	61	34.9
Duration of labor	<24 hours	159	90.9
	≥24 hours	16	9.1
Complication during pregnancy	Yes	36	20.6
	No	139	79.4
Type of complication	APH	7	19.4
	Pre-eclamsia	4	11.1
	Ecclamsia	10	27.8
	Obstructed labour	8	22.2
	PPH	4	11.1
	Malaria	3	8.3
Maternal anemia	Hgb < 11g/dl	16	9.1
	Hgb ≥ 11g/dl	156	90.9
Bleeding during pregnancy	Yes	15	8.6
	No	160	91.4
MUAC	<23 cm	25	14.3
	≥23 cm	150	85.7
Clinical characteristics of neonate	Neonatal sepsis	78	44.6
	Perinatal asphyxia	40	22.9
	Meconium aspiration syndrome	28	16.0
	Prematurity	29	16.6

5.3 Hematological parameters and magnitude of hematological abnormalities

The range of WBC count was 3.57 - 56.9×10^3 cells/μL, with a median (IQR) value of 14.69(10.79 – 17.88) × 10^3 cells/μL. Leukocytosis was detected in 20 (11.4%) neonate whereas; leukopenia was detected in 7 (4%) neonate. The range of platelet count was 36 - 450 × 10^3cells/μL, with a median (IQR) value of 218 (160 - 303) × 10^3cells/μL. Thrombocytopenia was detected in 36 (20.6%) neonate. Hemoglobin level ranges from 6.5 – 22 g/dL, with a mean ± SD value of 15.35 ± 3.29 g/dL. The overall magnitude of anemia was found to be 29.1%. Among anemic neonate, 28 (21.2%) were males and 23 (31.9%) were from rural dwellers. The prevalence of anemia among preterm and term was 32.8% and 27.2%, respectively**. (Table 4, Figure 2)**

Table 4: Hematological parameters of neonate admitted to NICU at Assosa general hospital, Western Ethiopia, February to July, 2022 (n=175).

Parameters	Ranges	Median (IQR)	Mean ±SD
WBC (10^3/μl)	3.57-56.90	14.59 (10.79-17.88)*	-
Neu (10^3/μl)	1.47-30.60	8.08 (5.32-10.90)*	-
Lymph (10^3/μl)	0.91-37.87	3.65 (2.42-4.84)*	-
Mon (10^3/μl)	0.20-4.46	1.68 (1.14-2.37)*	-
Eos (10^3/μl)	0-1.53	0.80 (0.10-0.20)*	-
Bas (10^3/μl)	0.01-1.88	0.70 (0.40-0.15)*	-
RBC (10^6/μl)	2.22-6.56	4.53 (3.90-5.13)*	-
MCV (fl)	56.3-128.0	109.30 (102.40-115)*	-
MCH (Pg)	17.1-41.1	32.2 (33.3-36.60)*	-
MCHC (%)	28.0-37.8	32.4 (31.40-33.30)*	-
RDW (%)	12.1-21.6	17.1 (16.00-18.20)*	-
Platelet (10^3/μl)	36-450	218.0 (160.0-303.0)*	-
MPV (fl)	7.8-13.1	-	10.05 ± 0.94**
Hgb(g/dl)	6.5-22.0	-	15.33 ± 3.26**
HCT (%)	19.4-68.3	-	47.22 ± 10.36 **

Note: ** data presented as mean ± standard deviation, * data presented as median with interquartile range.

Abbreviations: SD, Standard Deviation; IQR, Inter Quartile Range; WBC, white blood cell; Neu, neutrophil; Lym, lymphocyte; Mon, monocyte; Bas, basophil; Eos, eosinophil; RBC, red blood cell; Hgb, hemoglobin; HCT, hematocrit; MCV, mean cell volume; MCH, mean cell hemoglobin; MCHC, mean cell hemoglobin concentration; RDW, red blood cell distribution width; MPV, mean platelet volume.

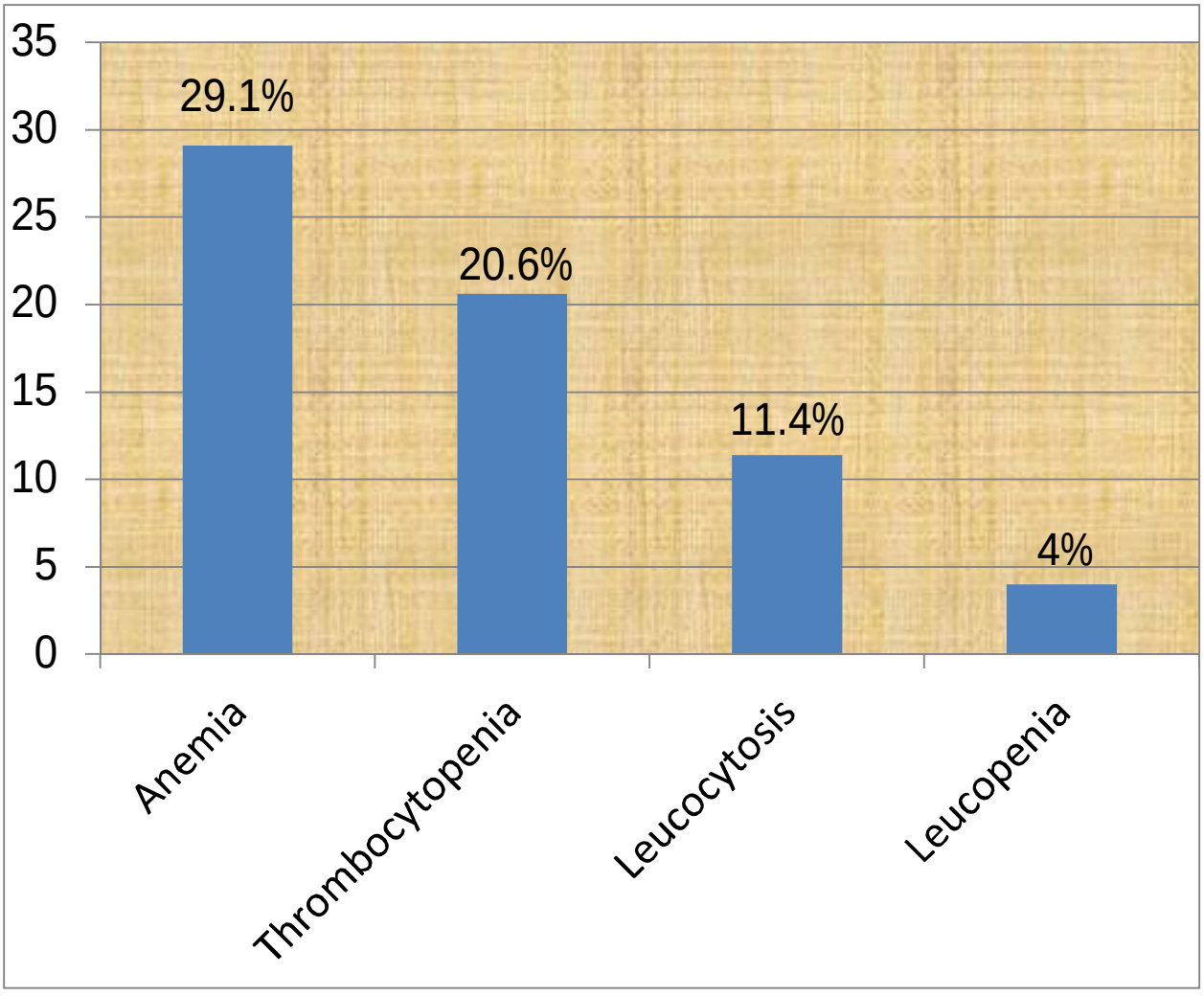

Figure 2: Magnitude of hematological abnormalities among neonate admitted to NICU at Assosa general hospital, Western Ethiopia, February to July, 2022 (n=175).

5.3.1 Severity of anemia

The severity of anemia was classified based on their Hemoglobin levels. Hemoglobin value between 7-10 gm/dL was categorized under moderate anemia. Whereas, haemoglobin value < 7 gm/dL was considered as severe anemia. Accordingly, among 51 anemic neonate, 1 (2%) were found to be severely anemic, whereas 10 (19.6%) and 40 (78.4%) were moderately and mildly anemic, respectively. **(Figure 3)**

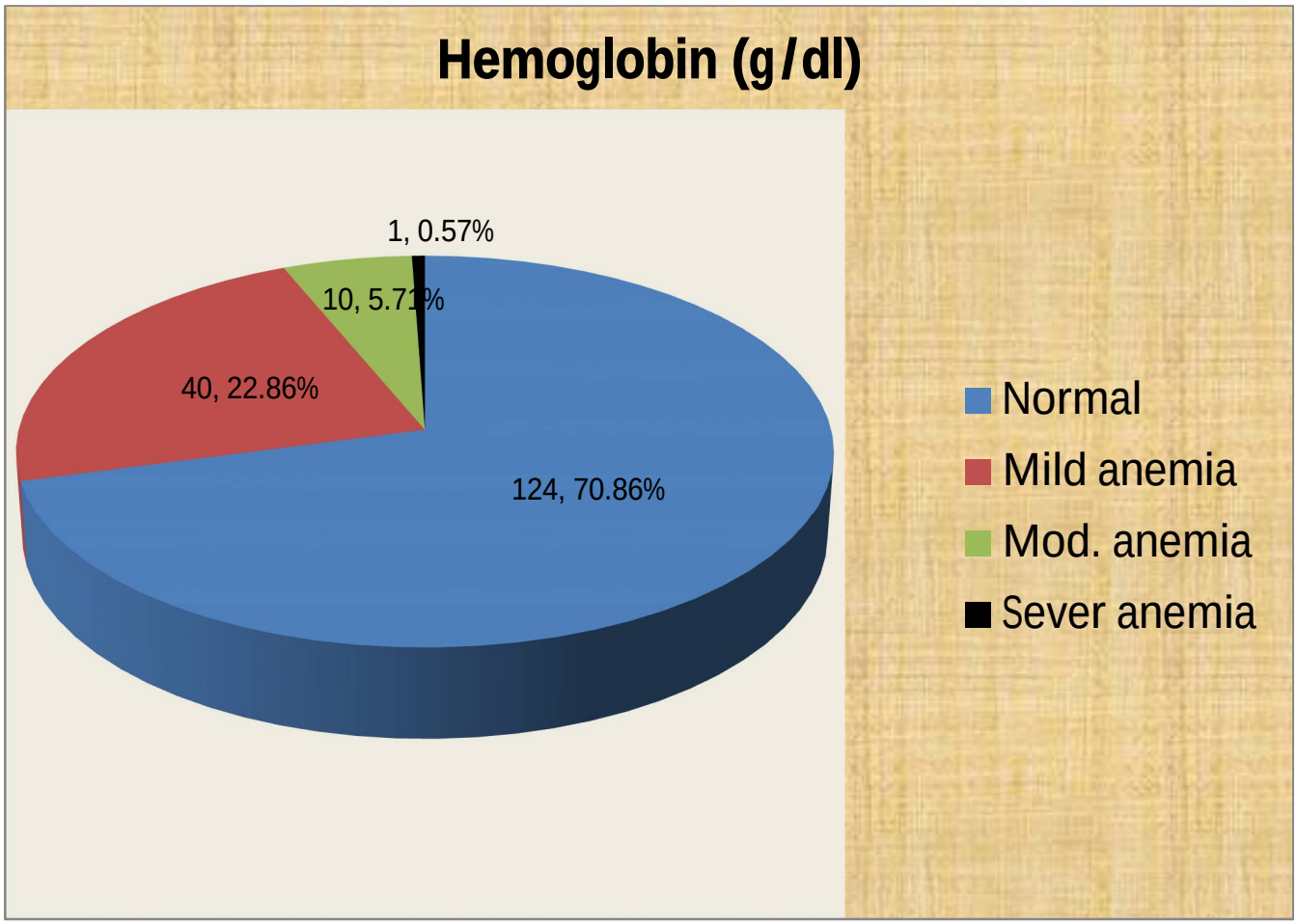

Figure 3: Hemoglobin profiles among neonate admitted to NICU at Assosa general hospital, Western Ethiopia, February to July, 2022 (n=175).

5.4 Hematological abnormalities and their associated factors

5.4.1 Anemia and associated factors

First, a bivariable logistic regression analysis was performed for all independent variables. Associations between dependent and independent variables resulted in a p-value of <0.25 in bivariable analysis were identified as candidate for multivariable logistic regression. Backward LR method was used during multivariable logistic regression. Based on the analyses, birth weight less than 2500g, family monthly income less than 2850 ETB, not having ANC follow up, not taking iron-folate supplement, caesarean mode of delivery, having more than 24 hours in duration of labor, being anemic mother and having bleeding during pregnancy were identified as candidate for multivariable logistic regression. However, only having family monthly income below 2850 ETB (AOR= 2.63; 95% CI: 1.05–6.63) and caesarean mode of delivery (AOR= 3.11; 95% CI: 1.26-7.68) have remained associated factors with anemia in the multi-variable logistic regression analyses. ANC follow up and iron-folate supplement were dropped to solve multicollinearity problem (VIP>10) **(Table 5).**

Table 5: Logistic regression analyses of anemia and explanatory variables among neonate admitted to NICU at Assosa general hospital, Western Ethiopia, February to July, 2022 (n=175).

Variables	Categories	Anemia		COR (95% CI)	P-value	AOR (95% CI)	P-value
		Yes	No				
Birth weight	≥2500 g	30	87	1.00			
	<2500 g	21	37	0.61 (0.31-1.20)*	0.15		
HH monthly income	≥2850 ETB	40	75	1.00		1.00	
	<2850 ETB	11	49	2.38 (1.13-5.07)*	0.025	2.63 (1.05-6.63)*	0.040
Duration of labor	<24 hours	49	110	1.00			
	≥24 hours	2	14	3.12 (0.68-14.25)*	0.142		
Mode of delivery	Vaginal delivery	42	72	1.00		1.00	
	Caesarean section	9	52	3.37 (1.51-7.53) *	0.003	3.11 (1.26-7.68)*	0.014
Maternal anemia	Hgb ≥11g/dl	44	115	1.00			
	Hgb <11g/dl	7	9	0.49 (0.17-1.40)*	0.184		
Bleeding during pregnancy	No	44	120	1.00			
	Yes	7	8	0.43 (0.15-1.27)*	0.126		

Note: COR: Crud Odds Ratio, AOR: Adjusted Odds Ratio, CI: Confidence Interval,

*: Statistically significant association (p-value <0.25 for COR whereas, p<0.05 for AOR)

5.4.2 Leukocytosis, leukopenia and their associated factors

Both bi-variable and multi-variable binary logistic regression analyses were done to assess associated factors for leukocytosis. The variables that were statistically associated with leukocytosis was sepsis in clinical characteristics of neonate (AOR= 7.23; 95% CI: 1.18–44.16) **(Table 6).** Both bi-variable and multi-variable binary logistic regression analyses were done to assess factors associated with leukopenia. However, any of the variables did not show statistically significant association in the multi-variable binary logistic regression model.

Table 6: Logistic regression analyses of leukocytosis and explanatory variables among neonate admitted to NICU at Assosa general hospital, Western Ethiopia, February to July, 2022 (n=175).

Variables	Categories	Leukocytosis		COR (95% CI)	P-value	AOR (95% CI)	P-value
		Yes	No				
Sex	Male	10	102	1.00			
	Female	10	53	0.52 (0.20-1.33)*	0.171		
Residence	Urban	6	97	1.00			
	Rural	14	58	0.26 (0.09-0.70)*	0.008		
Clinical diagnosis of neonate	Sepsis	5	73	3.04 (0.81-11.42)*	0.099	7.23(1.18-44.16)*	0.032
	PNA	5	35	1.46 (0.38-5.59)	0.582		
	MAS	5	23	0.96 (0.25-375)	0.951		
	Prematurity	5	24	1.00		1.00	
Educational status	No formal Education	0	29	1.64 (0.68-10.33)	0.892		
	Primary school	14	24	0.13 (0.04-0.43)*	0.001		
	Secondary school	2	49	1.85 (0.34-10.55)	0.489		
	College & above	4	53	1.00			
Household monthly income	≥2850ETB	10	105	1.00			
	<2850ETB	10	50	0.48 (0.19-1.22)*	0.121		
Complication during pregnancy	No	19	120	1.00			
	Yes	1	35	5.54 (0.72-42.89)*	0.101		

Note: COR: Crud Odds Ratio, AOR: Adjusted Odds Ratio, CI: Confidence Interval,
*: Statistically significant association (p-value <0.25 for COR, whereas p<0.05 for AOR)
Abbreviations: PNA: Perinatal Asphyxia, MAS: Meconium Aspiration Syndrome,

5.4.3 Thrombocytopenia and associated factor

To determine the association between the independent variables and thrombocytopenia, both bivariable and multivariable binary logistic regression was done. Based on the analyses, Being female in sex, being preterm in gestational age, PNA and MAS in clinical diagnosis and having complication during pregnancy were identified as candidate for multivariable logistic regression. However, none of the variables found to be significantly associated with thrombocytopenia in multivariate logistic regression analysis.

5.5 Hematological abnormalities based on clinical characteristics of neonate

Anemia was observed in 22 (43.14%) neonate with clinical diagnosis of sepsis and 13 (25.49%) neonates in each clinical diagnosis of meconium aspiration syndrome and prematurity. Majority thrombocytopenia, 16 (44.44%) was detected in neonate with clinical diagnosis of sepsis followed by MAS, 11 (30.56%). Leukopenia was detected in 5 (71.43%) and 2 (28.57%) neonates with clinical diagnosis of sepsis and perinatal asphyxia respectively (**Table 7).** Statistically significant association was seen between neonatal sepsis with toxic granulation and NuRBC ($p<0.05$) (**Figure 4)**.

Table 7: Hematological abnormalities based on clinical characteristics of neonate admitted to NICU at Assosa general hospital, Western Ethiopia, February to July, 2022.

Hematological abnormalities	Sepsis	PNA	MAS	Prematurity	Total
Anemia	22 (43.14%)	13 (25.49%)	3(5.88%)	13 (25.49%)	51
Thrombocytopenia	16 (44.44%)	3 (8.33%)	11 (30.56%)	6 (16.67%)	36
Leukocytosis	5 (25%)	5 (25%)	5 (25%)	5 (25%)	20
Leukopenia	5 (71.43%)	2 (28.57%)	0 (0%)	0 (0%)	7

Abbreviations: PNA, Perinatal Asphyxia; MAS, Meconium Aspiration Syndrome.

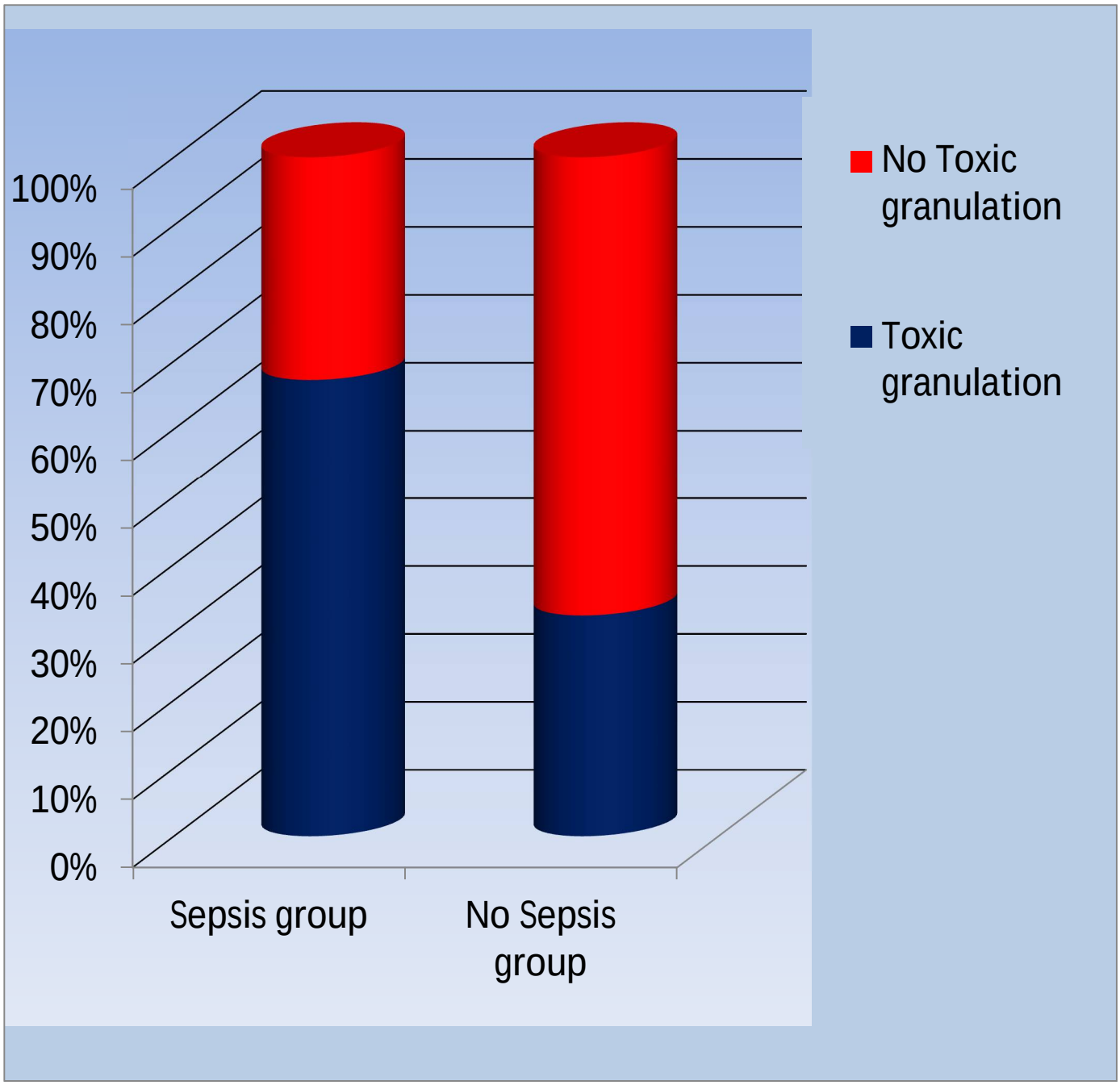

Figure 4: Association between neonatal sepsis and toxic granulation among neonate admitted to NICU at Assosa general hospital, Western Ethiopia, February to July, 2022 (n=175).

CHAPTER SIX: DISCUSSION

Total of 175 neonates were included in this study. Male neonates constituted for more than half (64%) of our study population which is comparable to a study from Iran (51.3%) [46]. However, constituent of male was found to be higher than reports from Indonesia (48.6%), Nigeria (42.4%), Nekemte (49.3%), and Gondar (50%) in Ethiopia [47,48,41,42]. In our study, a great majority of neonates were born at term (65.1%) which is comparable to a study from Turkey (73.6%) and Indonesia (85.7%) [47,49]. However the constituent of neonate born at term in our study was higher than that of studies reported from Iran (34.1%) [50] and Saudi Arabia (51.9 %) [51]. Neonates with low birth weight constituted for 33.1 % of our study population which was significantly lower than study from India (62.5%) [52]. However, the constituent of neonates born with low birth weight in this study was higher as compared to study from Indonesia (20.7%) [47] and Nigeria (13.6%) [48]. The possible reason for lower constituent of neonate with LBW in study from Indonesia and Nigeria may be the difference in sample size (Indonesia=135, Nigeria=132) and study population, newborns before age of 24 hours are included.

Out of the 175 neonates included in the study, 51 (29.1%) anemia, 36 (20.6%) thrombocytopenia, 20 (11.4%) leukocytosis and 7 (4%) leukopenia were detected. The prevalence of anemia in this study compared to WHO public health limits indicates a moderate public health problem [53]. Due to reduced oxygen-carrying capacity; anemia has a still serious public health implication that leads to neonatal morbidity and mortality [54]. Caesarean mode of delivery and family monthly income below 2850 ETB were risk factors which have been associated with anemia in neonate.

The prevalence of neonatal anemia in our study is consistent with studies from Rio de Janeiro in Lagos Nigeria (35%), Bosnia (29.3%), Gondar in Ethiopia (25%) and Brazil (32.6%) [55-58]. The observed prevalence of in our finding is lower than the studies in Ghana (57.3%), [59]. The possible reason for the lower prevalence in our study may be due to difference in sample size and study population. A study in Ghana was conducted on a sample of 1154, about half of the mothers of whom had HIV or malaria infection.

In addition, the observed prevalence of anemia in our finding is lower than that of studies in Nigeria (65.6%) and Benin (61.1%) [60,61]. The possible reason for the lower prevalence observed compared to the study in Benin and Nigeria may be the study was conducted among newborn only delivered from malaria-infected mothers, however, all neonates admitted to NICU were included in our study. Malaria parasites transmitted to the fetus through congenital and destroyed the fetal RBC [62]. This leads to lower hemoglobin value and subsequently increases prevalence of newborn anemia in Benin study as compared to our study. In addition, sample size difference may be another contributing factor for the discrepancy in report from Benin; it was prospectively studied for over one-year with 617 mothers and 656 newborns.

Moreover, anemia prevalence in this study was lower compared to the study reported from Iran (53%) [63]. The possible reason for this discrepancy might be attributed to variation in study participants based on the mode of delivery. In Iran, all study participants were born through caesarean section, whereas the majority of our study participant was born through normal vaginal delivery. During caesarean section, there may be accidental incision of the placenta, results bleeding leading to anemia as compared to normal vaginal delivery.

The results of the current study revealed a higher prevalence of neonatal anemia than studies in New York, USA (21%) and Netherlands (21%) [64,65]. The lower prevalence in New York and Netherland could be due to the difference in the socio- demographics and economic status of the mothers. The results of the current study also revealed a higher prevalence of neonatal anemia than studies in southern Malawi (23.4%) [66]. The possible reason for this discrepancy may be due to the use of a lower Hgb limit (<12.5g/dl) in the Malawian study. In addition, the results of the current study revealed a higher prevalence of neonatal anemia than studies in Nepal (5.7%) and Addis Ababa (9%) [67,68]. The difference between our study and studies from Nepal and Addis Ababa could be due to the small sample size in both studies compared to our study. In this study, 175 study participants were included, whereas a study from Addis Ababa and Nepal were conducted among 89 and 114 study participants respectively.

In the current study, thrombocytopenia and anemia were more common in preterm and low-birth-weight infants. Prematurity is one of the most frequent reasons of death of infants in worldwide [69]. Preterm newborns are not complete their full gestational age. Baby get majority of iron store during the last trimester of pregnancy. Due to this premature babies had negative iron balance which leads development of anemia [70]. Furthermore, in the preterm newborn, kidney is immature that cannot produce sufficient amount of erythropoietin, which results in development of anemia as compared to full-term babies [71]. In contrast to this study, study done in India reported there was no difference in prevalence of anemia among preterm from term neonate [72]. The possible reason for this discrepancy may be defined as haemoglobin cut-off value, 13 g/dL for both preterm and term neonates which might not be the reality, especially in the first 2–3 weeks of postnatal life.

According to this study, the risk of anemia is more likely in neonate born from family having less than 2850ETB monthly income (AOR= 2.63; 95% CI: 1.05–6.63) than non anemic neonate. Moreover, the risk of neonatal anemia is more likely in neonate born through caesarean section (AOR= 3.11; 95% CI: 1.26-7.68) than non anemic neonate. This finding is in line with several studies in Egypt, Pakistan, South Korea, Netherlands, and Poland. [73-77]. The possible reason for higher risk of anemia in neonate born in caesarean mode of delivery may be attributed to the following causes: Caesarean section is associated with weak force and duration of placental transfusion, which can lead to anemia in newborns. The decrease in placental transfusion during caesarean section may be due to the lack of uterine pressure, the effect of anaesthesia, and immediate clamping of the umbilical cord [78]. In contrast to our study, studies in Turkey and Iraq found no significant association between mode of delivery and newborn hemoglobin levels [79, 80]. A possible explanation could be there was only seven newborns delivered by caesarean section in the study carried out in Turkey and the difference in study population compared to the study carried out in Iraq, only full term neonates was included and difference in type of specimen, cord blood was used immediately after clamping of the babys' umbilical cord in the labor room.

In the current study, the second common hematological abnormalities were thrombocytopenia. Thrombocytopenia is a frequent challenge for neonatologists, as it affects 22 to 35% of infants admitted to the neonatal intensive care unit. Multiple diseases can cause neonatal thrombocytopenia [81]. The incidence of thrombocytopenia in neonates varies significantly depending on the population studied. According to this study, 36 (20.6%) neonates in NICU were thrombocytopenic.

The observed incidence of thrombocytopenia in our study was comparable to a report from India (16.7%), Iran (17.9%) and Ontario (22 %) [82-86]. However, the incidence of thrombocytopenia in our study was significantly lower than study from Nigeria 53% [87]. The possible reason for the higher prevalence in study from Nigeria may be explained by the variability of the types and magnitude of the risk factors involved in the development of thrombocytopenia in which neonates admitted NICU only with surgical disorders are involved.

In the current study, the third common hematological abnormality was leukocytosis, which was observed in 20 (11.4 %) neonates. The observed prevalence of leukocytosis in our finding is higher than that another study (6.1%) [88]. This difference may be explained by the variability of the types and magnitude of the risk factors and type of study design in which retrospective cohort study was done among neonates only with Very low birth weight. In the current study, leukocopenia was observed in 7 (4 %) neonates. The observed prevalence of leukopenia in our finding is lower than that of studies in Iran (28.5%) [89]. The possible reason for the higher prevalence of leucopenia in study from Iran may be the study was conducted among newborn only delivered from preeclamptic mothers, however, all neonate admitted to NICU at Assosa general hospital were included in the current study.

Overall, this study yield important insights into the hematological parameters and associated factors of neonate attending NICU at Assosa general hospital. The results of this study can be used by health professionals and policy makers to plan for improvements at this age. The main strength of this study was that it is a prospective study; one of the very few studies in sick neonates admitted to the neonatal intensive care unit across the world and assessed various contributing factors for hematological abnormalities neonate. However, it had to be interpreted with limitations. One limitation of this study is that we cannot determine a cause-effect relationship due to the cross-sectional nature of our study design. A relatively small sample size was used. Another limitation is that neonates clinically diagnosed as Sepsis was not confirmed by culture.

CHAPTER SEVEN: CONCLUSION AND RECOMMENDATIONS

7.1 Conclusion

In the current study anemia was the major hematological abnormality, followed by thrombocytopenia, leukocytosis and leukopenia among neonate admitted to neonatal intensive care unit. Caesarean mode of delivery and monthly family income below 2850 ETB were significantly associated with anemia. Sepsis in clinical characteristics of neonate was linked to leukocytosis. Therefore, hematological parameter test should be routinely used and properly interpreted in management of sick neonate to prevent neonatal mortality and long-term implications.

7.2 Recommendation

To the clinicians

Strict monitoring of CBC profile of neonate should be considered for proper management of sick neonate. Close attention should be given to anemia and thrombocytopenia. Health-care providers should also pay attention to pregnant women and neonate with the risk factors.

To the researchers

A longitudinal study should be conducted using a large sample size to identify specific etiology and cause-effect relationships between risk factors and hematological abnormalities. It might be very important to have similar studies in different health care setting.

To the Policymakers

Policy makers should pay strong attention to sick neonates. Guidelines for the management of sick neonate should consider screening of hematological parameters into the routine assessment to enable early detection and treatment of neonatal disease. Guideline and strategies should be designed in manner to reduce the magnitude of hematological abnormalities, particularly anemia, and their risk factors.

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ANNEXES

Annex-I: Information sheet

Information sheet (English version)

Title of the research project: Assessment of haematological abnormalities and associated factors among neonates admitted to NICU in Assosa general hospital, Assosa, Western Ethiopia.

Name of principal investigator: Mulatu Molla (MSc candidate at Jimma University)

Introduction: This study will assess haematological profile among neonate admitted to NICU in Assosa general hospital. Your baby can be selected, so that, you are being invited to give the informed consent on behalf of your baby for participation in this study. Your baby will be enrolled only when a voluntary informed consent is given. The research team includes one principal investigator and two advisors from Jimma University. The research proposal has been approved by ethical review committee of institute of health, Jimma University. Please read the complete information listed below and you can raise any question regarding this study for more information.

Purpose of the research project: This study will assess haematological parameters among neonate attending NICU with variety of clinical setting. We will assess haematological parameter with regard to clinical diagnosis of the neonates. This study will also determine magnitude of haematological abnormalities and associated factors. The finding will provide scientific information about haematological parameter of neonate attending NICU in Assosa general hospital and will help in early diagnosis of haematological abnormalities, if any, during research process. Therefore, we are inviting you to give a voluntary permission to participate your baby in this study.

Risks associated with the study: There will be no added risk to the neonate due to the research. There may be a minor discomfort during blood specimen collection. All appropriate precaution will be taken during blood specimen collection from your baby. Specimen collection will be performed by trained professional.

Procedures and what will be expected from you for participation: First of all, you are expected to have complete information about purpose and procedure of this project. If you are volunteer for participation of your baby, you need to give your consent. After consent, 1 ml of blood specimen will be collected from your baby and the specimen will be used for the intended purpose only.

Incentives and compensation; There will be no payment or compensation for participation of your baby in this study. But you will get the result of your baby for free. If any abnormal result in haematological parameter is detected, it will be notified to paediatrician.

Confidentiality

All information will be kept confidential. Unauthorized access to research materials that contain personal information will be strictly restricted. The record and result of study participant will be kept locked in cabinet. Specific research serial number and codes will be used instead of personal identifiers.

Right to refuse or withdraw: You have full right to withdraw your baby from the study at any time and there will be no discriminated in health services due to refusal.

Agreement

If you are volunteer for the participation of your baby, you will be kindly requested to put your signature of agreement on the informed consent format.

Contact information

For more information you can contact.

Mr Mulatu Molla (BSc), Tel: +251-916-399236, Email:mulatumolla222@gmail.com

Dr Tilahun Yemane (MD, MSc), Tel: +251-917-804067, Email: yemanetilahun@yahoo.com

Mr Wondimagegn Adissu (BSc, MSc), Tel: +251-961-928402, Email: wondimagegn.adissu@ju.edu.et

Information sheet Amharic version

የጥናቱ ርዕስ፤ በአሶሳ አጠቃላ ሆስፒታል የጨቅላ ህጻናት ፅኑ ህመምተኞች ክፍል የሚገኙ ህጻናት የደም ህዋሳት ዝርዝር ማወቅ፡፡

የዋና ተመራማሪ ስም፤ ሙላቱ ሞላ (እጩ ኤም ኤስ ሲ ተመራቂ፤ ጅማ ዩኒቨርሲቲ)

መግብያ፤ ይህ ጥናት በአሶሳ አጠቃላ ሆስፒታል የጨቅላ ህጻናት ፅኑ ህመምተኞች ክፍል የሚገኙ ህጻናት የደም ህዋሳት ዝርዝር ለማወቅ የሚሰራ ነዉ፡፡ ይህ የመረጃ ቅጽ የተዘጋጀዉ ከላይ በተጠቀሰው ጥናት የእርሰዎን ልጅ ለማሳተፍ ስለፈለግን ስለጥናቱ ጠቅላላ ማብራርያ ለመስጠት እንድረዳን እና የእርሰዎን በጎ ፈቃድ ለመጠየቅም ጭምር ነው ። በመሆኑም ልጅዎ በጥናቱ የሚሳተፈዉ በራስዎ በጎ ፈቃድ ብቻ መሆኑን በትህትና እንገልፃለን፡፡ በጥናቱ ስራ ላይ አንድ ዋና ተመራማሪ ይኖራል እንድሁም ሁለት አማካሪዎች ከጅማ ዩኒቨርሲቲ ይኖራሉ። ይህ ጥናት በጅማ ዩኒቨርሲቲ ስነ ምግባር (Ethical) ግምገሚ ኮሚቴ ታይቶ ፍቃድ የተሰጠዉ ነዉ፡፡ ከዝህ ቀጥሎ ስለጥናቱ የተዘረዘሩ ሙሉ መረጃ እንድያነቡ እና ያልገበዎት ማነኛዉንም ጥያቀ ማንሳት እንደምችሉ በትህትና እንጠይቃለን፡፡

የጥናቱ ዓለማ፤ የዝህ ጥናት ዓላማ በአሶሳ አጠቃላ ሆስፒታል የጨቅላ ህጻናት ፅኑ ህመምተኞች ክፍል የሚገኙ ህጻናት የደም ህዋሳት ዝርዝር ማወቅ ነዉ፡፡ የጥናት ዉጤት የህጻናቱን የደም ህዋሳት ዝርዝር በሚመለከት ሳይንሳዊ መረጃ ይሰጣል፡፡ በጥናቱ ስራ ላይ ህጻናት ማነኛዉም አይነት የደም ችግር እንዳለባቸዉ የምያሳይ ዉጤት ስኖር ወድያዉኑ በማሳወቅ ህጻናቱንም ተጠቃሚ ይደረጋሉ፡፡ በመሆኑም በዝህ ጥናት ላይ የእርሰዎን ልጅ እንድሳተፍ የእርሰዎን በጎ ፈቃድ እንጠይቃን፡፡

ከጥናቱ ጋር የተያያዙ ስጋቶች፤ ከዝህ ጥናት የተነሳ ልጅዎ ላይ የሚደርስ ተጨማሪ ጉዳት አይኖረም፡፡ ናሙና ሲዎሰድ ትንሽ የህመም ስሜት ልኖር ይችላል፡፡ ናሙና የሚወሰደዉ በሰለጠኑ ባለሞያዎች ስሆን ሁሉም የጥንቃቀ እርምጃዎች የሚተገበሩ ይሆናል፡፡

የጥናቱ ቅደም ተከተል፤ መጀመርያ ጥናቱን በተመለከተ ሙሉ መረጃ እንድያገኙ ከተደረገ በኋላ ፍቃደኝነትዎ ይጠየቃል፡፡ ፍቃደኛ መሆነዎ ስረዳደጥ ብቻ ከልጅዎ አንድ ኤም ኤል የደም ናሙና ይወሰዳል፡፡ የደም ናሙናዉም ለጥናቱ አላማ ብቻ የሚዉል ይሆናል፡፡

ጥቅማጥቅም፤ ልጅዎ በዚህ ጥናት ውስጥ በመሳተፉ በጥሬ ገንዘብ የሚደረግ ክፍያም ሆኔ ካሳ እንደማይኖር እንገልጻለን። ነገር ግን የልጅዎን ዉጤት በነፃ መዉሰድ ይችላሉ። በምርመራ ላይ በሽታ የምያመላክት ዉጤት ከተገኘ ወድያዉኑ የህጻኑ ሀኪም እንድያዉቃ ይደረጋል።

ሚስጢራዊነት፤ ማንኛዉም ለጥናቱ የተሰበሰበ መረጃ ምስጢራዊነቱ የተጠበቀ ይሆናል። የጥናቱ መረጃዎች በሙሉ የምቀመጡት ቁልፍ ባለዉ ሳጥን ዉስጥ ሲሆን ጥናቱን ከሚያስከሄዱት ባለሙያዎች በስተቀር ማንም አካል መረጃዉን እንድያገኝ አይፈቀድም። መረጃዎች የምሰበሰቡት የጥናቱ ተሳታፊ ማንነት በሚገልጥ መልክ ሳይሆን ለጥናቱ ተብሎ በሚሰጥ ስውር ቁጥር ይሆናል።

ያለመቀበል ወይም ጥሎ የመዉጣት መብት፤ በዚህ ጥናት ዉስጥ የልጅዎ ተሳትፎ ሙሉ በሙሉ በእርሰዎ ፈቃደኝነት ላይ የተመሰረተ እንደመሆኑ መጠን ተሳተፎዉን ያለመቀበልም ሆነ በማንኛውም ጊዜ የልጅዎ ተሳትፎ የማቋረጥ መብታችሁ ሙሉ በሙሉ የተጠበቀ ነዉ። ልጅዎትን በጥናቱ ባለማሳተፈዎ ወይም ከጥናት በማገወለልዎ ምክንያት የህክምና እርዳታ ላይ የምደርስ ተፅዕኖ አይኖርም።

ስምምነት፤ በመጨረሻ ፍቃደኛ ስሆኑ ብቻ በተዘጋጀዉ የስምምነት ቅፅ ላይ ፊርማዎን እንድያስቀንጡ ይጠበቃል።

አድራሻዎች፤ ለተጨማሪ መረጃም ሆኔ ሀሳብ የሚከተሉትን አድራሻዎች ይጠቀሙ::

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Annex II: Consent form

Consent form (English Version)

This Consent form is organized for the research project in partial fulfilment of the requirements for Masters of Science in Clinical Laboratory Science speciality in Hematology and Immunohematology**.** It is planned to assess hematological parameters and associated factors among neonates admitted to NICU in Assosa general hospital**.** Please read carefully and express your open willing by putting a mark in the box below.

I have informed enough and understand about the aim and procedures of the research planned to assess hematological parameter and associated factors among neonates attending NICU in Assoa general hospital. I have been told there will be no added risk to the neonate due to this research. I have been informed blood specimen will be taken for research purpose only and then I can get result of my baby for free. I understood specimen will be collected by experienced professionals according to standard. In addition I have been told all the information collected throughout the research process will be kept confidential. I understood that, if I refuse to give consent, my baby will not be discriminated in current and future medical service due to refusal. Therefore I give my consent free from coercions for participation of my baby in this study.

Agree ☐

Disagree ☐

Card number_____

Name of parent or legal guardian: _____

Signature: _____

Name of investigator: _____

Signature_____

Date_____

Consent form (Amharic Version)

በአሶሳ አጠቃላ ሆስፒታል የጨቅላ ህጻናት ፅኑ ህመምተኞች ክፍል የሚገኙ ህጻናት የደም ህዋሳት ዝርዝር ለማወቅ የታቀደ ጥናት ሙሉ መረጃ በማግኘቴ የጥናቱን ዓላማ እና አካሄዱን ተረድችያለሁ። ከዝህ ጥናት የተነሳ ልጄ ላይ የሚደርስ ተጨማሪ ጉዳት እንደማይኖር ተገልፆልኛል። የደም ናሙና እንደምወሰድና ናሙናዉ ለጥናቱ አላማ ብቻ እንደምዉል ተነግሮኛል። የልጄን ዉጤት በነፃ መዉሰድ እንደምችል አዉቅያለሁ። ናሙና የሚወሰደዉ ደረጃዉን በጠበቀ መልክ ልምድ ባላቸዉ ባለሞያዎች እንደምሆን አዉቅያለሁ። በተጨማሪም ማንኛዉም ለጥናቱ የተሰበሰበ መረጃ ምስጢራዊነቱ የተጠበቀ እንደምሆን ተረድቸያለሁ። ልጄን በጥናቱ ለይ ባለማሳተፈ ወይም ከጥናት በማገወለለ ምክንያት የህክምና እርዳታ ላይ የምደርስ ተፅዕኖ እንደማይኖር ተገልፆልኛል። በመሁኑም ያለምንም አስገዳጅ ሁኔታ ፍቃደኛ መሆኔን እገልፃለሁ።

እስማማለሁ ☐

አልስማማም ☐

ካርድቁጥር_____

የወላጅወይምህጋዊሞግዚትሰም_____ፊርማ_____

የተመራማሪዉስም_____

ፊርማ_____ቀን_____

Annex III: Data collection sheet

Data collection sheet (English version)

This questionnaire is organized for the research project in partial fulfilment of the requirements for Masters of Science in Clinical Laboratory Science speciality in Hematology and Immunohematology. It is planned to assess hematological parameters and associated factors among neonates admitted to NICU in Assosa general hospital.

Research serial number:_____ Card number_____

Part I: Socio-demographic and clinical characteristics of neonates admitted to NICU.

1. Age (days):_____

2. Sex: A. Male B. Female

3. Gestation age at birth (weeks) _____

4. Weight at birth (g) _____

5. What was the clinical diagnosis of neonate? _____

Part II: Maternal socio-demographic factors of neonates admitted to NICU.

1. Age of the mother (years) _____

2. Place of residence: A. Rural B. Urban

3. Marital status: A. Married B. Unmarried C. Divorced D. Windowed E. Others_____

4. Educational level:

A. No formal education B. Primary school C. Secondary school D. College and above

5. Occupation: A. Employed B. House wife C. Daily labourer D. Student E. Other_____

6. House hold monthly income A. <2850EB B. >/=2850EB

Part III: Maternal Obstetric factors of neonates admitted to NICU.

1. ANC follow up: A. Yes B No (If No, jump to #3)

2. Frequency of ANC visit:

A. 1 visit B. 2-3 visit C. 4 and above

3. Iron-folate supplementation during current pregnancy: A. Yes B. No

4. Parity: A. Primipara B. Multipara

5. Mode of delivery: A. Vaginal delivery B. Caesarean section

6. Duration of labour (hours) _____

7. Complications during pregnancy and labour (if No jump question #8): A. Yes B. No

8. Types of pregnancy and labour complication (if yes for question number 7):

A. APH

B. Pre-eclamsia

C. Eclamsia

D. Twin pregnancy

E Obstracted labour

F. Others_____

9. Maternal anaemia: A. Yes B. No

10. Maternal bleeding during pregnancy: A. Yes B. No

11. MUACA. <23cm B. >/=23cm

Data collection sheet (Amharic version)

ይህ መጠይቅ በክሊኒካል ላቦራቶሪ ሄማቶሎጂ እና ኢሚኖሄማቶሎጂ ሳይንስ ለሁሌተኛ ዲግሪ መመረቅያ ፅሁፍ የተዘገጋጀ ነዉ። ጥናቱ ከ ታህሳስ 23 እከ ሚያዝያ 22 2014 ዓ.ም በአሶሳ አጠቃላይ ሆስፒታል የጨቅላ ህጻናት ፅኑ ህመምተኞች ክፍል የሚገኙ ህጻናት የደም ህዋሳት ዝርዝር ለማወቅ የታቀደ ነዉ።

የጥናቱ መለያ ቁጥር_____ካርድ ቁጥር _____

ክፍል አንድ፤ በጨቅላ ህጻናት ፅኑ ህመምተኞች ክፍል ዉስጥ የሚገኙ ህፃናት ማህበራዊ እና ህክምና ነክ ሁኔቶች

1. ዕድሜ (በቀን) _____

2. ፆታ ሀ. ወንድ ለ .ሴት

3. ህፃኑ እስከምወለድ የወሰደዉ የእርግዝና ጊዜ ስንት በሳምንት ነዉ?_____

4. ህፃኑ ስወለድ የነበረዉ ክብደት በግራም (gram)_____

5. ህፃኑ በጨቅላ ህጻናት ፅኑ ህመምተኞች ክፍል እንድገባ ያደረገዉ በሽታ ወይም ምክንያታ ምንድነዉ?_____

ክፍል ሁለት፤ በጨቅላ ህጻናት ፅኑ ህመምተኞች ክፍል ዉስጥ የሚገኙ ህፃናት የእናታቸዉ ማህበራዊ ሁኔቶች

1፣ የእናቱ/ቷ እድሜ (በአሜት) ----------

2. የመኖሪያ አድራሻ ሀ. ከተማ ለ. ገጠር

3. የትዳር ሁኔታ

ሀ. ያገባች ለ. ያላገባች ሐ. የተፋታች መ. ባሏ የሞተባት ሰ. ለላ (ጥቀስ)--------

4. የትምህርት ደረጃ

ሀ. ያልተማረች ለ. የመጀመሪያ ደረጃ የጨረሰች ሐ.ሁለተኛ ደረጃ የጨረሰች መ. ኮሌጅና ከዚያ በላይ

5. የሥራ ሁኔታ

ሀ. ተማሪ ለ. ሰራተኛ ሐ.የቤት እሜበት መ. የቀን ሰራተኛ ሰ. ለላ (ጥቀስ)----------

6. የቤተሰብ ወራዊ ገቢ (በኢትዮጵያ ብር) ሀ. ከ2850 ብር በታች ለ. 2850 ብር እና ከዝያ በላይ

ክፍል ሶስት፤ በጨቅላ ህጻናት ፅኑ ህመምተኞች ክፍል ዉስጥ የሚገኙ ህፃናት የእናታቸዉ የማዋለድ ህክምና ሁኔቶች

1, ቅድሜ ወሊድ ክትትል ስለማድረግ ሀ. ያደረገች ለ. ያላደረገች

2. ቅድሜ ወሊድ ክትትል ድግግሞሽ ሀ. አንድ ጊዜ ለ. ከ2-3 ጊዜ ሐ. 4 እና ከዝያ በላይ

3. አይረን ፎሌት ስለመዉሰድ ሀ. የወሰደች ለ. ያልወሰደች

4. የእርግዝና ሁኔታ ሀ. የመጀመርያ ለ. ከአንድ በላይ የወለደች

5.ህጻኑየተወለደበትመንገድ ሀ. በምጥ ለ. በቀዶጥገና

6. የእናቱ/ቷ የደም ማነስ ሁኔታ ሀ. አለባት ለ. የለባትም

7. ኤምዩኤሲ (በሴንቲሜትር) ሀ. ከ23 ሴ/ሜ በታች ለ. 23 ሴ/ሜ እና ከዝያ በላይ

Annex IV: Laboratory procedure

Blood specimen collection

Materials and reagents

- Glove
- Soap
- Towel
- Tourniquet
- Syringe
- 70% alcohol
- Test tube with EDTA anticoagulant
- Gauze pads or cotton
- Marker
- Rack

Procedure

1. Assemble the necessary materials and equipment.
2. Perform hand hygiene (If using soap and water, dry hand with towel)
3. Identify the right patient and immobilize the baby
4. Put the tourniquet on the baby above the venipuncture site
5. Put on well-fitting glove
6. Remove plastic sleeve from the syringe
7. Disinfect the collection site and allow to dry
8. Insert the needle into the vein and draw blood, after required amount of blood has been collected release the tourniquet
9. Place dry gauze over the venipuncture site and slowly withdraw the needle
10. Ask the parent to continue applying mild pressure
11. Dispose the needle in sharp container.
12. Fill test tube and mix tube well
13. Labelling test tubes
14. Remove and dispose gloves appropriately and perform hand hygiene.

Complete blood count (CBC)

Principle of sysmex XN-550 hematological analyzer

Hydrodynamically focused DC detection method

The RBC detector counts RBC and PLT via the hydrodynamically focused DC detection method. Red blood cells and platelets are simultaneously counted and separated by their physiological size variation. Inside detector the sample nozzle is positioned in front of the aperture and in line with the center. After diluted sample is forced from sample nozzle into the conical chamber, it is surrounded by front sheath reagent and passes through the aperture center.

The direct current resistance is detected by particular detector as blood cells suspended in the diluent pass through the aperture. This resistance causes an electrical pulse change proportional to the size of the blood cell. These electrical data are converted into graphical displays of volume distribution curves, or histograms. After passing through the aperture the diluted sample is sent to the recovery tube. This prevents the blood cell in this area from drifting back, and prevents the generation abnormal blood call pulses.

RBC is calculated as particle count between 2 discriminators (Lower discriminators (LD), 25 to 75 fl and Upper discriminators (UD) 200 to 250 ft). Platelet is also calculated as particle count between 2 discriminators LD and UD which are automatically set up in range of 2 to 6 fl and 12 to 30 fl respectively.

Flow cytometry method using semiconductor laser

Cytometry is used to analyze physiological and chemical characteristics of cells. Flow cytometry is used to analyze those cells and particles as they are passed through extremely small flows. A blood sample is aspirated, diluted to the specified ratio and labelled. The sample is then fed into the flow cell by the sheath flow mechanism. A semiconductor laser beam is emitted onto the blood cells passing through the flow cell. When laser beam is emitted to blood cell particles, light scattering occurs and scattered light is received by the photodiode.

Forward scattered light provides information on blood cell size and lateral scattered light provides information on the cell interior (nucleus). When light is emitted to fluorescent material, such as stained blood cells, light of longer wavelength than the original light is produced. The intensity of the fluorescent light increases as the concentration of the stain becomes higher. By measuring the intensity of the fluorescence emitted, you can obtain information on the degree of blood staining. Fluorescence light is emitted in all directions; this instrument detects the fluorescent light that is emitted sideways.

WDF channel is a channel primarily for classifying WBC. By flow cytometery method 2-dimentional scattergram is drawn, with X-axis representing the intensity of side scattered light and the Y-axis representing the intensity of side fluorescence.

SLS-Haemoglobin Method:

SULFOLYSER is added to the red blood cells, and haemoglobin is converted into SLS-haemoglobin. Concentration of SLS-haemoglobin is measured as light absorbance.

Sysmex XN-550 reagents

- **CELLPACK DCL:** It is a diluent used to dilute aspirated analysis samples in order to measure an RBC count, hemoglobin concentration and platelet count.
- **SULFOLYSER:** is a reagent for the automated determination of haemoglobin concentration of the blood.
- **Lysercell WDF:** is a reagent used in combination with Flourocell WDF. By hemolysing red blood cells with Lysecell WDF and dyeing the white blood cell componenets with Flourocell WDF.
- **Fluorocell WDF:** is to be used to label the leukocytes in diluted and lysed blood sample for determination of WBC differential.
- **CELLCLEAN:** it is a strong alkaline detergent used to remove lysing reagents, cellular residuals and blood proteins remaining in the hydraulics of the instrument.

Specimen requirements

Type of specimen

Blood specimens should be collected either by venipuncture for processing in whole blood mode or micro sampling by skin puncture for capillary mode processing. For micro sampling the blood can be obtained from the earlobe, finger or from the heel of an infant. Ideally, large drops of blood should exude slowly but spontaneously, and only the very gentlest squeezing is permissible.

Conditions of collection

Venous blood should be collected into EDTA test tube and processed within 4 hours of collection. If specimens cannot be processed within 4 hours, they should be refrigerated at 2 - 8ºC. Before processing refrigerated specimens should be allowed to warm up to room temperature (minimum 15 minutes), then mixed, preferably by rotation, for at least 2 minutes.

Required sample volume:

- ✓ 1 mL or more of whole blood (for a 13 mm diameter sample tube)
- ✓ 500µL or more of whole blood (for a micro tube)
- ✓ Aspirated sample volume: Approximately 50 µL

Preparation of blood film

Materials and reagents

- ❖ Clean microscope slides
- ❖ Well-mixed EDTA blood sample
- ❖ Pipette
- ❖ pencil
- ❖ Gloves
- ❖ Waste and sharps disposal containers

Procedures

- Place a small drop of well mixed EDTA blood 1.0 cm far from the end of the glass slide
- The spreading slide is placed in front of the drop of blood at an angle of about 30^{o} - 40^{o} to the slide and then is moved back to make contact with the drop
- The drop will spread out quickly along the line of contact of the spreader with the slide
- The spreader is advanced with a smooth steady motion so that a thin film of blood is spread over the slide
- Label with patients ID
- Allow the smear to air-dry

Wright staining and examination

Principle

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 colours 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 an orange to pink colour. 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 colours.

Procedure

- Place the air-dried smear film side up on a staining rack
- Cover the smear with undiluted filtered stain and leave for 1 minute
- Add equal volume of distilled water (i.e., the same number of drops as the stain)
- Mix by blowing until a metallic sheen appears.
- Allow the diluted stain to act for 3-5 minutes
- Wash off the stain with running tap water
- Wipe the back of the slide clean and stand it in a rack for the smear to dry.
- Examine gross morphology by 40x and use the 100x objective for studying the fine details of the cell morphology.

Annex V: Laboratory result report formats

CBC result reporting form

Research serial #_____ Card number_____

Test	Result	Remark
RBC	× 10^6/uL	
HGB	g/dl	
HCT	%	
MCV	Fl	
MCH	Pg	
MCHC	g/dl	
RDW	%	
WBC	× 10^3/uL	
Neutrophil	× 10^3/uL	
Lymphocyte	× 10^3/uL	
Monocyte	× 10^3/uL	
Eosinophil	× 10^3/uL	
Basophil	× 10^3/uL	
PLT	× 10^3/uL	
MPV	fl	

Peripheral blood morphology report form

Research serial number_____ Card number_____

WBC Series_____

_____

RBC Series _____

_____

PLT Series_____

_____

Possible conclusion _____

Annex VI: Declaration

I the undersigned, declare that this thesis is my work and has never been presented for any degree or other purposes at Jimma University or any other institution of higher learning. I also declare that, when other people work has been used, it has been carefully acknowledged and referenced per the requirements. Therefore, I agree to accept responsibility for the scientific, ethical, and technical conduct of this research paper.

Name of the student: Mulatu Molla
Signature: _____ Date: _____/_____/_____

Place of submission: School of Medical Laboratory Sciences, Faculty of Health Sciences,

Institute of Health, Jimma University.

Date of submission: _____/_____/_____

This research thesis submitted with the approval of university advisors and examiners.

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1. Dr. Tilahun Yemane (MD, MSc). Signature: _____Date: _____/_____/_____

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Examiners:

1. Name_____ Signature_____ Date_____/____/____

2. Name_____ Signature_____ Date_____/____/_____