ADDIS ABABA UNIVERSITY

COLLEGE OF HEALTH SCIENCES

DEPARTMENT OF MEDICAL LABORATORY SCIENCES

Effect of Maternal Iron Deficiency Anemia on Iron Store of Term Newborns

By: Betelihem Terefe

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

Advisors:

1. Aster Tsegaye (BSc, MSc, PhD)
2. Asaye Birhanu (BSc, MLT, MSc)

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Department of Medical Laboratory Sciences, College of Health Sciences, Addis Ababa University, approved by the Examining Board

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Chairman Dep. Graduate Committee	Signature
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Advisor	Signature
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Advisor	Signature
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Examiner	Signature

Abstract

Background: Iron deficiency anemia (IDA) is a severe form of iron deficiency. It most commonly occurs in pregnant women and infants. One of the causes of IDA in early childhood is the acquisition of lower than the normal amount of iron store at birth. The stored iron of the newborn babies, which is the main source of iron during the first six months of their age, is endowed from the maternal circulation. Therefore, knowledge of the effect of maternal IDA on the iron stores of term newborns will be relevant for better management of IDA in infancy.

Objective: The aim of this study was to assess the effect of maternal iron deficiency anemia on iron store of term newborns.

Methodology: A cross-sectional study was conducted from December 13, 2011 to February 20, 2012 in Obstetrics and Gynecology departments of St. Paul's hospital, Selam and Gulelie health centers. A total of 95 pregnant women and their respective newborns that fulfilled the inclusion criteria were included in the study. Blood samples were collected from the mothers and cord of newborns and analyzed for complete blood count and serum ferritin levels using Cell-dyn 1800 and Cobas e 411 analyzers, respectively. Women were classified into three groups as: iron deficient anemic, iron deficient non anemic and non iron deficient non anemic based on hemoglobin and serum ferritin values. All pre-analytical, analytical and post-analytical quality aspects were thoroughly controlled. For statistical analysis MedCalc® software Version 12.1.4 was used.

Result: The median hemoglobin and serum ferritin levels for the pregnant women were 12.2g/dl and 42.1ng/ml, respectively. The median hemoglobin and serum ferritin levels for the newborns were 16.1g/dl and 187.6ng/ml, respectively. Newborns of iron-deficient anemic pregnant women (152.6ng/ml) had significantly lower levels ($p = 0.0008$) of serum ferritin than non iron deficient non anemic pregnant women (225.9ng/ml). Besides, newborns ferritin and hemoglobin levels have significant correlation with hemoglobin ($r_s = 0.256, p = 0.0122; r_s = 0.226, p = 0.0279$), and ferritin ($r_s = 0.366, p = 0.0003; r_s = 0.268, p = 0.0086$) levels of the mothers. Maternal age had an effect on the ferritin status of newborns, where newborns delivered from younger mothers ($\leq$ 24 years) had higher serum ferritin values than the others (> 24 years).

Conclusion: The study demonstrated that maternal iron deficiency anemia has a significant impact on the iron store levels of their newborn.

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Operational definitions

Anemia: Hgb concentration < 11 g/dL for pregnant women as recommended by the WHO and it was adjusted for altitude by subtracting the tabulated factor of 1.1 g/dl for 2400 m suggested by Cohen et al [59].

Iron deficiency: serum ferritin below 50ng/ml, to balance the effect of infection as recommended by the WHO for developing countries [23].

By using the Hgb and serum ferritin values, the pregnant women were classified into:

Iron Deficient Anemic (IDA): both Hgb and ferritin below cutoff

Iron Deficient Non Anemic (IDNA): Hgb normal and ferritin below cutoff

Non Iron Deficient Non Anemic groups (NIDNA): both Hgb and ferritin normal

List of abbreviation

ANC –Antenatal Care

CBC – Complete Blood Count

CV – Coefficient of Variation

ECL – Electrochemiluminescence

Hgb – Hemoglobin

ID – Iron Deficiency

IDA – Iron Deficiency Anemia

IDNA – Iron Deficient Non Anemic

IQR – Inter Quartile Range

K_3EDTA – Tri-potassium Ethylene Diamine Tetraacetic Acid

LIC – Low Income Countries

NIDNA – Non Iron Deficient Non Anemic

OBGY – Obstetrics and Gynecology

WHO – World Health Organization

1. Introduction

1.1 Back ground

Anemia, according to the World Health Organization (WHO), is a state in which the hemoglobin (Hgb) concentration in the blood is lower than levels considered normal for age, gender and physiological state [1].

Anemia is one of the world's most widespread health problems of both developing and developed countries which affects about 1.62 billion people (24.8% of the population) globally [1, 2]. The highest prevalence of anemia exist in low income countries (LIC), bearing more than 90% of the global burden [2, 3]. There are many causes of anemia; by far the primary cause of anemia is iron deficiency (ID) and it is estimated to be the cause for about 50% of all anemia cases [1].

ID is resulted from extensive negative iron balance and it exists in a continuum, from a reduction in body storage iron to iron deficient erythropoiesis and ultimately to iron-deficient anemia [4]. ID can be caused by low dietary intake of iron, poor absorption of dietary iron, or blood loss which leads to loss of iron [5].

Iron deficiency anemia (IDA), most severe form of iron deficiency, results when the body’s iron supply cannot support production of Hgb in adequate amounts to maintain normal functioning of the body [6]. Thus, Iron deficiency anemia is a coexistence of anemia and iron deficiency. It is estimated to affect more than 1 billion people, and cause 841,000 deaths worldwide and about 71% of the global mortality burden is in Africa and parts of Asia [2, 3]. IDA sometimes is interchangeably used with the gross term of anemia [2].

In Ethiopia, anemia according to estimates from WHO is classified as severe public health problem and prevalence rates for preschool age children (75.2%), pregnant women (62.7%), and non pregnant women (52.3%) have been documented [1]. According to the recent Central Statistics Agency (2012) Demographic and Health comprehensive survey, 44% of children aged 6-59 months were anemic, with 21% mildly anemic, 20% moderately anemic, and 3% severely anemic. In addition, 17% of women aged 15-49 are anemic; of which 13% are mildly anemic, 3% are moderately anemic, and less than 1% are severely anemic [7]. IDA has also ranked as one of the significant micronutrient deficiency problems in Ethiopia [8].

Anemia is mostly diagnosed in the laboratory by determination of Hgb. The "gold standard" for identifying iron deficiency is a direct test of bone marrow biopsy with Prussian blue staining. However, bone marrow aspiration is too invasive for routine use [9]. Other assays used for diagnostic workup of iron deficiency includes red cell indices, serum iron, serum total iron binding capacity, serum transferring saturation, serum transferring receptors level and serum ferritin level [10].

Several studies have proven that serum ferritin is the best less invasive test and is very useful and reliable index of iron stores especially during pregnancy with low levels indicating iron deficiency [11, 12]. There is only one limitation with serum ferritin, as it is an acute phase reactant and is not a sensitive indicator of iron stores in those suffering from an infection or inflammation [10]. In recent years, the serum transferrin receptor level has been introduced as promising new tool for the diagnosis of iron depletion [13].

Treatment is given for server anemia, 25mg/dl and 120mg/day for 3 month duration for children less than 2 years and pregnant women, respectively [14].

1.2 Statement of the problem

IDA can occur at all stages of the life cycle, but due to the increased iron requirements of pregnancy and growth, pregnant women and infants are recognized as the most vulnerable groups [15]. Globally, IDA in pregnancy is highly prevalent and the prevalence ranges from 39.9-43.8% [2]. This may be related to high iron requirements during gestation since iron is necessary to cover basal iron losses, the increase in maternal red cell mass, and development of the fetus and placenta [16].

The risk of IDA is particularly high in women who begin gestation with depleted or low body iron stores, a situation common in Africa and most developing countries [17]. Indeed, a majority of women in the reproductive age group in developing countries are anemic even before conception; pregnancy only tends to intensify it further [18]. Moreover, the pregnancy associated anemia in these countries is different from that in the developed world. This is because it is widely prevalent and more severe in degree, frequently coexists with maternal malnutrition, and is of long duration in that it is present since the beginning of pregnancy or antedating it [18, 19].

Under these situations, the competing demands of mother and fetus may disturb the normal maternal-fetal iron homeostasis [4]. The resultant anemia in pregnancy can result in preterm delivery, low birth weight and prematurity of newborns [20]. In addition, the anemia is associated with increased maternal morbidity and mortality and it has been reported that 40% of all maternal perinatal deaths are linked to anemia [21, 22]. In Ethiopia IDA in women of reproductive ages has a prevalence of 17%, which makes it a problem among these subjects [23].

In infancy IDA remains the most common feature worldwide both in developed and developing countries with an estimated prevalence of 20 to 25% [24]. Zinc and Iron are essential

micronutrients in fetus growth and development [8]. IDA in infancy is associated with behavioral and cognitive delays, including impaired learning, decreased school achievement, and lower scores on tests of mental and motor development [25]. Moreover, the effect seems to be long lasting and may be irreversible even after treatment [24, 26]. In addition, IDA may lower resistance to infections and increase absorption of lead and other heavy metals [26-28].

Among the 42% and 17% prevalence of anemia seen in children younger than 5 years in developing countries and developed countries respectively, the highest proportions are seen during periods of rapid growth (6–24 months of age) [27, 28]. Prevalence of anemia among preschool aged children in Africa is found to be 64.6% from which the 8.8% is severe anemia [2]. In Ethiopia, anemia is considered as a severe public health problem in children aged 6-59 months, in which more than half (54%) are anemic and 4% are severely anemic [2, 29]. A study on preschool children (6-60 months) from northern part of Ethiopia revealed anemia prevalence of 42%, and in sub sample of the children, 43% had a depleted serum ferritin level indicating that the anemia was largely due to iron deficiency [30].

One of the causes of IDA in early childhood (6-24 months) is the acquisition of lower than the normal amount of iron store at birth [31]. It is well established that the two main sources of iron for infants 0-6 months are the iron that is acquired and stored during pregnancy and the iron that is ingested through breast milk after birth. [31, 32] All term newborns are assumed to have sufficient iron during the first three months because of the containing of most of the total body iron within the circulating Hgb [33]. But, after three months of age, iron stores are usually mobilized to meet the erythropoietic demands of an expanding total Hgb mass and growth [33]. Since breast milk provides only small amounts (0.2–0.4 mg/L) of iron, which is not sufficient to

meet the demands of growth, the infant will be highly dependent on the stored iron until 6 months [27, 34].

The iron stores of healthy term infant at birth are approximately 75 mg/kg, which is sufficient to cover the needs for growth during the first 6 months of life [35, 36]. However, infants who are born with poor or small iron stores will deplete iron earlier than the 6 months of their life that may predispose them to iron deficiency and anemia in early infancy (three to six months) [31, 33, 34]. This iron store of the newborns is endowed from maternal circulation through a rapid and unidirectional process [33]. Thus, it will be logical to question the effect of maternal iron deficiency anemia extension beyond pregnancy.

The effect of maternal IDA on iron store of newborns has been studied in different parts of the world, particularly by comparing the serum ferritin level of the mother and their babies [37 – 49]. But the results found are inconsistent and inconclusive. This lack of precise information and considerable differences between results and published reports from different countries can be due to variations in the main characteristics of target population, differences in methodology such as laboratory test, and definition of anemia. In Ethiopia, while high prevalence of maternal IDA has been documented [23] studies on its effect on the iron store of newborns are very limited.

Thus, considering high prevalence of IDA in Ethiopian women, the presence of doubts regarding the relationship between maternal and neonatal iron status, and lack of sufficient data regarding the effect of maternal iron status on iron store of newborns in Ethiopia, this study was carried out to determine iron store of newborns that are born from mothers suffering from IDA.

1.3 Significance of the study

It is well documented that IDA has a long term effect on infants; and one of the cause for IDA during infancy is the acquisition of less amount of iron stores at birth. By utilizing the available methods for detection of IDA, this study has tried to determine the effect of maternal IDA on iron stores of Ethiopian newborns. So, it will be a significant input for the development of intervention strategies to reduce IDA among infants. The study will also suggest whether or not targeting of mothers in the prevention of IDA during infancy is needed. This will in turn enhance effective and more convergent utilization of resources in IDA management. In addition, it may contribute for the development of national anemia policy.

2. Literature review

Effect of Maternal Iron Deficiency Anemia on Newborns iron status: Global situation

Several studies are performed to assess the effect of maternal IDA on the iron store of newborns [37-49]. While many of these studies have demonstrated that iron stores of infants who are born to mothers with IDA is unaffected [37-42, 49], others have shown that maternal IDA can cause depletion of fetal iron stores [43-48]. Thus, data whether or not IDA in pregnant women might lead to a deficient iron store of their babies are still inconclusive.

Wong' and Saha (1990) conducted a study to show the inter-relationship of maternal iron storage with their newborns' by determining the serum ferritin and transferrin concentrations of both mothers and newborns; and no correlation was detected [37]. Similarly, a case control study conducted on Jordanian mothers and their newborns by analyzing the Hgb and plasma ferritin revealed absence of correlation between maternal and cord blood Hgb and ferritin values [38]. On another study done on non-anemic pregnant women and their newborns by determining Hgb, erythrocyte zinc protoporphyrin and ferritin showed that there were no significant differences in mean values of the iron status parameters between neonates born to females with and without iron-deficient erythropoiesis [39].

Likewise a study conducted in the New Zealand on mothers, who had normal iron status or mildly-to-moderately anemic and their newborns showed that there was no statistically significant relationship between maternal and fetal cord blood Hgb, iron, and ferritin levels [40].

In addition, a cross-sectional study carried out on three groups of mothers who were anemic, iron deficient and non-iron deficient and their newborns by determining complete blood count (CBC),

serum iron, total iron-binding capacity, serum ferritin, zinc protoporphyrin, and transferrin saturation demonstrated that there was no significant difference in mean values of any parameter studied between newborns in the three groups [41]. These results are also strengthened by a study performed on Iranian anemic and normal control pregnant women and their newborns [42]. In this latter study Hgb and serum ferritin levels were determined and the result revealed no significant correlations between neonatal and maternal serum ferritin concentration, though significant differences were found between neonatal Hgb levels from normal and anemic mothers [42].

On the other hand, others reported dissimilar results from the above studies. In a study carried out by Singla et al (1996) it was demonstrated that the levels of Hgb, serum iron, transferrin saturation and ferritin were significantly lower in the newborns from anemic women compared to their non anemic counterparts [43]. Likewise a study carried out on cord/maternal blood pairs by detecting iron, ferritin, serum transferrin; Hgb concentration and mean cell volume revealed that maternal iron depletion is associated with decreased cord ferritin and Hgb [44]. Correspondingly, an additional cross-sectional study performed on three groups of Iranian pregnant women who were iron deficient anemic, non-anemic iron deficient and non-anemic non-iron deficient and their newborns revealed significant differences in mean Hgb and serum ferritin values in neonates born to normal control and iron deficient anemic mothers [45].

On another cross-sectional investigation done to determine the relation between maternal anemia and neonatal iron status on three groups of mothers and their babies by determining Hgb, serum iron, total iron binding capacity and serum ferritin showed that there was significantly lower levels of serum ferritin in babies of iron deficient anemic mothers than non anemic, non iron

deficient mothers [46]. In addition a study conducted on anemic and healthy non anemic pregnant women and their newborns showed that maternal ferritin levels had significant correlations with Hgb, iron, and ferritin levels in cord blood [47]. These results are also supported by a study which revealed significance difference in Hgb, red cell indices, and iron profile of neonates born to anemic mothers as compared to controls, particularly in moderate to severe anemia cases [48].

Review of Maternal Iron Deficiency Anemia in Ethiopia

In Ethiopia, the existence of iron deficiency anemia was controversial, most reports attributing its scarcity to the high iron content of the staple diet *teff* in many parts of the country [50, 51] while deficiency of nutrients is still a problem [52,53]. Studies in the 1980's reported the rarity of anemia even in the high risk groups such as pregnant women [49, 54]. Gebre-Medhin and Birgegård (1981) for example analyzed ferritin in serum from 38 Ethiopian and 10 Swedish pregnant women and in cord blood from their newborn infants. The mean ferritin level in the Ethiopian mothers was significantly higher than in the Swedish mothers as well as in a non-pregnant population of apparently healthy Swedish women. The authors stated that the reported rarity of gestational anemia in Ethiopia is due mainly to the good iron state of Ethiopian women, especially those who still eat the traditional staple diet. They also found no correlation between maternal and cord blood ferritin [49].

In addition, in the earlier studies of Gebremedhin et al (1976), no difference in Hgb, heamatocrit, serum iron, transferrin, total iron binding capacity, folate and vitamin B12 was found between pregnant women and controls from Addis Ababa. The authors attributed the rarity of anemia to

the probable lifelong exposure to a very high iron intake combined with hypoxia due to the high altitude [51].

On the other hand, several others [23, 52-54] including a large cross-sectional study, which involves women of reproductive age from nine administrative regions of the country [55], reported different degrees of overall anemia and IDA.

The study of Desalegn (1993) from Jimma town, South West Ethiopia, showed 41.9% overall prevalence of anemia in pregnant women; of which, 74.3% had moderate anemia and 2.5% had severe anemia [53]. Geis et al (2003) reported an overall anemia prevalence of 15.1%, mild anemia 10.4%, moderate anemia 4.2% and severe anemia 0.3%, in pregnant women from Hawassa town (Southern Ethiopia) [54]. Gibson et al (2008) reported higher rates from the rural part of Southern Ethiopia in which 29% had anemia, 13% had iron deficiency anemia, 33% had depleted iron stores, and 74 and 27% had low plasma zinc and retinol, respectively [52]. Haidar et al (2003) on the other hand reported 18.4% prevalence of IDA in pregnant lactating mother in rural Ethiopia [56].

A large scale cross sectional study was conducted in Ethiopia in 270 clustered villages drawn from 9 administrative regions in 2005. A total of 22,861 women of reproductive age (15-49) were involved in the study. The reported prevalence rates were 11,3% clinical anemia, 30.4% anemia, 49.7% iron deficiency and 17% IDA. The majority of anemic women had mild anemia (19.3%), followed by moderate (10.3%) and severe anemia was 0.9%. The study demonstrated a great variation in the prevalence rate among the studied regions. For instance, a high prevalence rate was observed in Afar district region in which 26.7% had clinical anemia, 79.4% anemia, 65.1% iron deficiency and 58% IDA. The lowest prevalence of ID and IDA was reported from

Benshangul Gumuz in which 34.0% had ID and 4.5% IDA. Thus, generalization of a finding from pocket studies in Ethiopia would be misleading [55].

Haidar (2010) conducted a cross-sectional community-based study in 970 women, aged 15-49 years and reported an overall prevalence of anemia of 30.4%, ID 50.1%, IDA 18.1%, and 31.3% deficiency of folic acid. The investigator noted that one in every three women had anemia and deficiency of folic acid while one in every two had iron deficiency, suggesting that deficiencies of both folic acid and iron constitute the major micronutrient deficiencies in Ethiopian women. The data contradicts the old notion that anemia is a rare phenomenon in Ethiopia, and reflects the risk imposed by anemia to the health of women ranging from impediment of daily activities to poor pregnancy outcome [23].

Taken together, although data on the effect of maternal IDA on the iron stores of the newborns is limited [49], the aforementioned studies in Ethiopia revealed the existence of different degrees of IDA in pregnant women in the different regions. On the other hand, higher prevalence rates of anemia 47.2% by Zein (1991) in children aged 0.5-6 years from the north western part of Ethiopia; the highest rate (60%) being observed in children 1-2 years have been reported [57]. The study also showed 20% had microcytic hypochromic red blood cells, a typical morphologic finding in IDA [57, 58]. Adish et al (1999) studied more than 2,000 children aged 6-60 months from the northern part of Ethiopia. Accordingly, anemia was highly prevalent (42%) and in a sub-sample of 230 anemic children, 43% had a serum ferritin of less than 12 μg/l indicating that the anemia was largely due to iron deficiency [30].

One of the causes of IDA in early childhood (6-24 months) is the acquisition of lower than the normal amount of iron store at birth [31]. It is also shown that the iron that is acquired and stored

during pregnancy and the iron that is ingested through breast milk after birth are the two main sources of iron for infants 0-6 months [31, 32]. Thus, although the one published earlier data in Ethiopia revealed that no correlation was found between maternal and cord blood ferritin [49], there is a need to investigate the effect of maternal iron store on their babies.

Hypothesis: Iron store of newborns will be affected by maternal iron deficiency anemia.

3. Objective of the study

3.1 General objective

To assess the effect of maternal iron deficiency anemia on iron store of term newborns delivered at St. Paul's hospital, Selam and Gulelie health centers, Addis Ababa, Ethiopia.

3.2 Specific objectives

- To determine the correlation between ferritin status of mothers and their newborns
- To determine the correlation between maternal hemoglobin with ferritin status of their newborns
- To determine the correlation between maternal ferritin and hemoglobin with the hemoglobin level of the newborn
- To determine the effect of some maternal factors like age, antenatal iron supplementation, and parity on ferritin status of the newborn

4. Methods and materials

4.1 Study site and period

The study was conducted at St. Paul's hospital, Selam and Gulelie health centers from December 13, 2011 to February 20, 2012.

4.2 Study design

The study design was cross sectional.

4.3 Source population

The source population was all pregnant women who came for delivery to Obstetrics and Gynecology (OBGY) department of St. Paul's hospital, Selam and Gulelie health centers and their respective newborns.

4.4 Study population

The study population was comprised of pregnant women who gave birth at OBGY department of St. Paul's hospital, Selam and Gulelie health centers during the study period with their respective newborns fulfilling the inclusion criteria.

4.5 Inclusion and exclusion criteria

Pregnant women with singleton live births, gestational age between 37 and 42 weeks were included in the study and thus, blood sample was collected both from the mothers and the cord of their respective newborns.

Pregnant women having bleeding during pregnancy, or preterm delivery (<37 weeks) were not included in the study. Pregnant women with other cause of anemia have also been excluded.

4.6 Sample size

The sample size was determined using the formula of sample size determination for correlation by taking the correlation coefficient p of the analytical hypothesis as 0.52 from a previous study [43].

$H_o \rightarrow p = 0$
$H_1 \rightarrow p \neq 0$

$$n = \left[\frac{(Z_{\alpha/2} + Z_{\beta})}{[F(Z_0) + F(Z_1)]}\right]^2 + 3$$

$F(Z) = 0.5 \ln [(1+p)/(1-p)]$

Where, n = Sample size

$\alpha = 0.05$

Power = 80%

β = 1- Power = 0.2

$P_o = 0$

$p_1 = 0.52$ [43]

$$n = \left[\frac{(1.96 + 0.84)}{[0 + 0.576]}\right]^2 + 3 = 26$$

Since there were 3 groups the total sample size was 78.

4.7 Sampling method

The study participants were recruited consecutively.

4.8 Variables

A. Dependent variables

- Ferritin and Hgb concentration of newborns

B. Independent variable

- Maternal Hgb, and ferritin concentration
- Newborn gender, birth weight, mode of delivery
- Maternal age, parity, birth spacing, educational level, antenatal care attendance, antenatal iron supplementation, feeding practice

4.9 Data collection

4.9.1 Data collection tools

Interviewer administered questionnaires were used for the collection of demographic characters of study participants and for the assessment of some maternal conditions like feeding practice, birth spacing, antenatal care (ANC) attendance (Annex V).

4.9.2 Laboratory analysis

Laboratory investigations included were analysis of CBC and measurement of serum ferritin for the assessment of iron depletion.

Sample collection, processing and transportation

Five ml of peripheral venous blood was collected from median cubital vein of the pregnant women's arm using a needle into K_3EDTA tube and serum gel separator tube during the process of labor for CBC and serum ferritin concentration determination respectively. For newborn, 5ml of cord blood was collected from the cord, immediately after clamping, into K_3EDTA tube and serum gel separator tube for CBC and serum ferritin concentration determination respectively. Protocol for sample collection (Annex VII A and B) was strictly followed to have safe procedure and reliable specimen.

Serum was obtained by allowing the collected blood to clot at room temperature for 30-45 minutes. After clot formation, the sample was centrifuge for 15-20 minutes at speed of 2500-3000 RPM. The serum was then transferred to another test tube immediately and stored at -20^{o}c until analyzed. Protocols for sample processing and transportation (Annex VII C) were strictly followed.

Analytical method and instrumentation

Cell-dyn 1800 (Abbott Laboratories, Abbott Park, Illinois) was used for the analysis of CBC, which uses electrical impedance method and Modified methemoglobin method for RBCs count and Hgb measurement respectively. The CBC was analyzed immediately after collection at the laboratory of School of Medical Laboratory Science, Addis Ababa University. In cases where analyses was impossible within 4 hours of collection, samples were kept at 2-8^{o}c and were tested within 48 hours. Serum ferritin was analyzed by using Electrochemiluminescence (ECL) with

fully automated Cobas 4 e11 (Roche Diagnostics GmbH, D-68298 Mannheim, Germany) using commercial kits at Ethiopian Health and Nutrition Research Institute laboratory.

4.10 Quality assurance

The pre-analytical, analytical and post-analytical phases were controlled throughout the process of this study.

Pre analytic quality considerations

Protocols for sample collection, processing and transportation were strictly followed to have safe procedure and reliable specimen. The questionnaire was translated into Amharic version and well trained counselor nurses carried out the interview.

Analytical consideration

Calibration of the Cell-dyn 1800 and Cobas e 411 was performed. The Cell-dyn 1800 analyzer was checked for its stability and good performance by carrying out precision test; 20 replicates were tested and the precision was within the acceptable limit (Table 1). Commercial quality control was included in every session of analyses for both CBC enumeration and serum ferritin concentration determination. To this effect, three levels whole blood controls (High, Medium, Low) and plasma control (Low, Normal) were used for CBC and ferritin determinations, respectively.

Table 1. Precision test for Cell-Dyn 1800		
Measurements	**Manufacturer specification (CV)**	**This study' s CV**
White blood cells	≤ 2.5%	1.8%
Red blood cells	≤ 1.7%	0.8%
Hemoglobin	≤ 1.2%	0.8%
Mean cell volume	≤ 1.5%	0.6%
Platelet	≤ 6.0%	2.8%
Mean platelet volume	≤ 6.0%	3.4%

Post analytical considerations

Two individuals performed the data entry and it was checked for agreement. The data were cross checked with the questionnaire and print out results of the analyzers.

4.11 Data analysis

The results of CBC analysis and ferritin determination from maternal blood and cord blood samples were recorded in Microsoft excel sheet. Similarly, all the information on the questionnaires was encoded in Microsoft excel sheet. The excel sheet was then exported to MedCalc® version 12.1.4 software program for statistical analysis. The normality of data distribution was tested by the D'Agostino-Pearson test. Since all of the analytes studied were not normally distributed, non-parametric tests were applied. Frequencies, percentages, medians and interquartile ranges (IQR) were computed to summarize the data. In order to compare quantitative variables between the groups, Mann–Whitney and Kruskal-Wallis tests were applied. Spearman rank correlation coefficient was used for correlation analysis. P value of <0.05 was considered as statistically significant in all analyses.

4.12 Ethical considerations

The proposal of this study was defended in the Department of Medical Laboratory Science and ethical approval was obtained from the research review committee of the department. The OBGY department of St. Paul's hospital, Selam and Gulelie health centers was requested for their permission providing with the letter of ethical approval, to use their respective facilities. An informed (written) consent was obtained from each study participant after clearly explaining about the objective and purpose of the study. Confidentiality of the information was assured by omitting names of the study participants from the questionnaires and protecting Microsoft excel sheets containing data of study participants using passwords. Participation in the study was on voluntary basis. Mothers who were not willing to participate in this study were given the right to do so. The results of anemic and/or iron deficient mothers were communicated to their doctors/nurses.

4.13 Dissemination of results

The results of this study will be presented in the Department of Medical Laboratory Science in front of external examiners, staffs and peers. It will also be presented in different national and international professional association conferences. A copy of the findings will be given to the health institutions included in the study for their utilization. Dissemination of the findings to the remaining scientific community will further be accomplished by submitting an article to reputable national or international journals for possible publication. Furthermore, it will be communicated to organizations which may have the capacity for designing and implementing health policies such as Ministry of Health.

5. Result

5.1 Description of the socio-demographic data of study participants

A total of 95 pregnant women with their newborns were included in this study. The median age of the pregnant women was 24 years (IQR = 21-27 years). As clearly presented in Table 2, more than one-third of the pregnant women (36.8%; n = 35) had educational level above secondary school, while 28.4% (n=27) of the mothers were illiterates. House wives were dominant and accounted for 75.8% (n=72) of the participants.

The majority of pregnant women were primiparous (64.2%; n=61), while the remaining 35.8% (n=34) were multiparous with median birth spacing of 2.75 years (IQR = 2-4 years). Most of the pregnant women (87.4%; n=83) were attending ANC during their pregnancy, of which greater than half (56.6%; n=47) started attending the ANC at their second trimester. Those who have been taking iron during their pregnancy accounted for 57.9% (n=55); and the median iron intake was about 1 month (IQR = 1-2 month) (Table 2).

Table 2. Summary of Socio-demographic and Obstetric characteristics of mothers attending St Paul's Hospital, Selam and Gulelie Health Centers, Addis Ababa

Characteristics	Frequency	Percentage
Maternal age (n=95)		
≤ 24 yrs	53	55.8
> 24 yrs	42	44.2
Maternal education level (n=95)		
Illiterate	27	28.4
Primary school	20	21.1
Secondary school	13	13.7
Above secondary school	35	36.8
Maternal occupation (n=95)		
House wives	72	75.8
Employed	23	24.2
Parity (n=95)		
Primiparous	61	64.2
Multiparous	34	35.8
Birth spacing (n=95)		
≤ 2 years	82	86.3
> 2 years	13	13.7
ANC follow up (n=95)		
Yes	83	87.4
No	12	12.6
ANC follow up starting trimester (n=83)		
First	36	43.4
Second	47	56.6
Iron intake during pregnancy (n=95)		
Yes	55	57.9
No	40	42.1

More than 3/4th, 85.3% (n=81), of the pregnant women reported consumption of vegetables at least three times per week, 27.4% (n=26) consumed meat or poultry at least once weekly and 68.4% (n=65) were consuming fruits at least three times per week. All of the pregnant women participated were coffee/tea consumer, with 88.4 % (n=84) of them consuming it at least once daily (Table 3).

Table 3. Feeding Practice of pregnant women during pregnancy among those attending St Paul's Hospital, Selam and Gulelie Health Centers, Addis Ababa (n=95)

Characteristics	Frequency	Percentage
Consumption of meat/poultry(weekly)		
At least once	26	27.4
Less than once	69	72.6
Consumption of vegetables(weekly)		
At least three times	81	85.3
Less than three times	14	14.7
Consumption of fruits (weekly)		
At least three times	65	68.4
Less than three times	30	31.6
Consumption of coffee/tea(daily)		
At least once	84	88.4
Less than once	11	11.6

Most of the babies were delivered through vaginal delivery (78.9%; n=75) and the proportion of male (49.5%; n=47) and female (50.5%; n=48) newborns were almost equal (Table 4). The babies had median weight of 3100 g (IQR = 2800-3400 g) and a few (12.6%; n=12) had low birth weight.

Table 4. Summary of gender, weight and mode of delivery of newborns among those born at St Paul's Hospital, Selam and Gulelie Health Centers, Addis Ababa (n=95).

Characteristics	Frequency	Percentage
Gender		
Male	47	49.5
Female	48	50.5
Weight		
Low birth weight	12	12.6
Normal birth weight	83	87.4
Mode of delivery		
Vaginal delivery	75	78.9
Cesarean section	20	21.1

5.2 Hematological and ferritin status of pregnant women and their newborns

The median Hgb and serum ferritin levels for the pregnant women were 12.2 g/dl (IQR = 10.9-12.8g/dl) and 42.1 ng/ml (IQR = 27.23-73.88ng/ml), respectively (Table 5). The table also summarizes the median and the inter-quartile ranges for the red cell indices parameters of the pregnant women. The median Hgb and serum ferritin levels for the newborns were 16.1 g/dl (IQR = 15.0-17.2g/dl) and 187.6ng/ml (IQR = 139.25-258.98ng/ml) respectively. As expected the values for all the parameters shown in the table were significantly higher in the newborns as compared to their mothers' values (Table 5).

Table 5. Hematological profile and ferritin status of pregnant women and their newborns at St Paul's Hospital, Selam and Gulelie Health Centers, Addis Ababa (n=95)

Parameters	***Median (IQR)[a]***	
	Mothers	**Newborns**
Hemoglobin (g/dl)*	12.2 (10.9-12.8)	16.1 (15.0-17.2)
Mean cell volume (fl)*	90.0 (87.95-93.23)	105.5 (102.8-109.78)
Mean cell hemoglobin (pg)*	30.7 (30.13-31.78)	37.3 (36.3-38.2)
Mean cell hemoglobin concentration (%)*	34.2 (33.9-34.78)	35.0 (34.3-35.88)
Red cell distribution width (%)*	14.1 (13.5-14.9)	16.3 (15.63-17.08)
Serum ferritin (ng/ml)*	42.1 (27.23-73.88)	187.6 (139.25-258.98)

[a]IQR, 25th to 75th quartiles

* $P < 0.0001$ compared to their mothers by Mann-Whitney test.

Based on Hgb status of pregnant women and using Hgb cut off of 11g/dl after altitude correction, 28.4% (n=27) of the participants were anemic and only mild (55.6%, n=15) and moderate (44.4%, n=12) cases were identified. Based on serum ferritin values cut off of 50ng/ml, 56 pregnant women (58.9%) were iron deficient while the other 39 were non-iron deficient (41.1%). Based on both the Hgb and serum ferritin levels, 27 pregnant women (28.4%) were classified as iron deficient anemic (IDA), 29 pregnant women (30.5%) as iron deficient non anemic (IDNA) and the rest 39 pregnant women (41.1%) as non iron deficient non anemic (NIDNA).

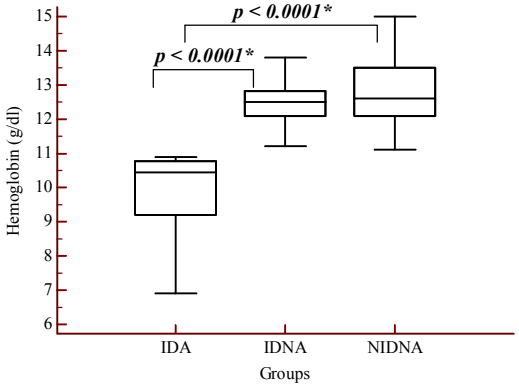
Table 6 shows the median values with their respective IQR values of hematological profile and ferritin status of pregnant women belonging to the 3 categories.

Table 6. Hematological profile and ferritin status of pregnant women based on the groups [a] at St Paul's Hospital, Selam and Gulelie Health Centers, Addis Ababa (n=95)

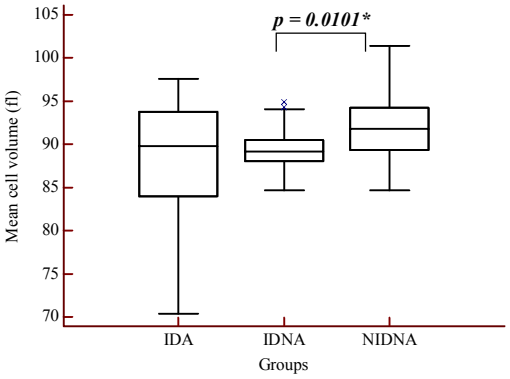
Parameters	Group Median (IQR)[b]			P value*
	IDA(n=27)	IDNA (n=29)	NIDNA (n=39)	
Hgb (g/dl)	10.45 (9.2-10.78)	12.5 (12.1-12.83)	12.6 (12.1-13.5)	**< 0.0001**
MCV (fl)	89.8 (83.95-93.8)	89.2 (88.05-90.48)	91.8 (89.33-94.2)	**0.0342**
MCH (pg)	30.6 (29.33-31.58)	30.61 (30.18-30.9)	31.6 (30.23-33.0)	**0.0297**
MCHC (%)	33.9 (33.15-34.3)	34.3 (34.1-34.7)	34.5 (33.9-35.18)	**0.0047**
RDW (%)	14.75 (14.13-15.35)	13.6 (13.18-14.2)	14.3 (13.5-15.0)	**0.0013**
Ferritin (ng/ml)	23.05 (16.96-31.25)	38.89 (26.74-43.72)	86.59 (66.53-120.95)	**< 0.0001**

Hgb=Hemoglobin; MCV=Mean Cell Volume; MCH=Mean Cell Hemoglobin; MCHC= Mean Cell Hemoglobin Concentration; RDW=Red Cell Distribution Width. [a]IDA=Iron deficient anemic; IDNA= Iron deficient non anemic; NIDNA= Non iron deficient non anemic. [b] IQR, 25th to 75th quartiles. ∗ Data are from the Kruskal-Wallis test.

Note: The difference for those analytes having a *p* value of < 0.05 is indicated in figure 1 below.



a)



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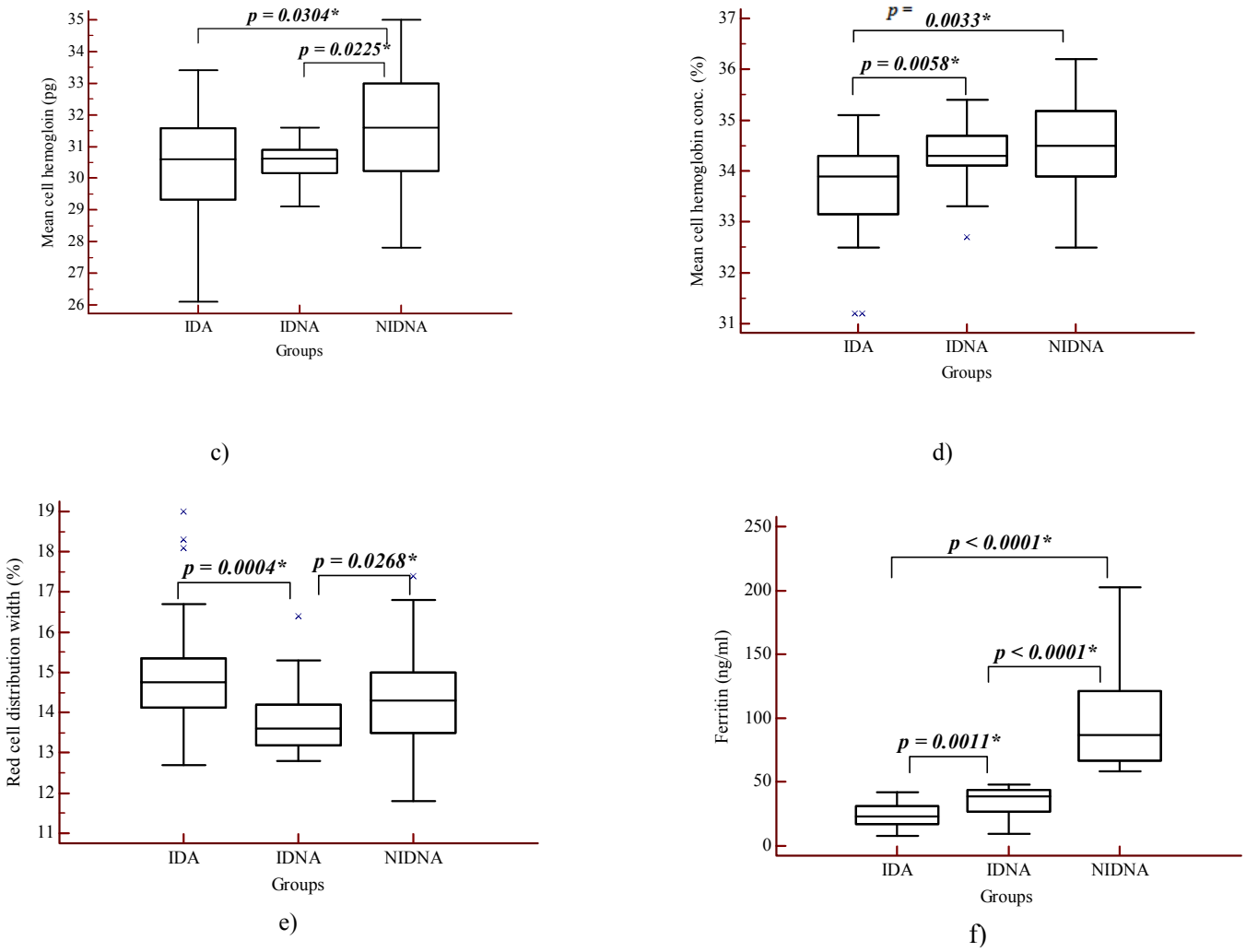

Figure 1. Box plots of hematological profile and ferritin parameters in pregnant women according to anemia and iron status of the women. *[a]IDA=Iron deficient anemic; IDNA= Iron deficient non anemic; NIDNA= Non iron deficient non anemic. * P values are from the Mann-Whitney test.*

In the newborns data a significant difference was shown only in Hgb and ferritin values between the groups (Table 7 and figure 2). As shown in Figure 2a, newborns born from mothers having IDA had significantly lower Hgb values than those from non iron deficient non anemic mothers (P=0.0084). The iron stores of newborns born from non anemic non iron deficient mothers, as

measured by ferritin, was significantly higher compared to those born from iron deficient mothers (both IDNA and IDA groups; P values 0.0108 and 0.0008, respectively) (Figure 2b).

Table 7. Hematological profile and ferritin status of newborns by iron status of their mothers [a] at St Paul's Hospital, Selam and Gulelie Health Centers, Addis Ababa (n=95)

Parameters	*Group Median (IQR)[b]*			P value*
	IDA(n=27)	IDNA (n=29)	NIDNA (n=39)	
Hgb (g/dl)	15.6 (14.8-16.88)	16.2 (14.75-17.03)	16.78 (15.7-18.08)	**0.0384**
MCV (fl)	105.1 (101.98-109.33)	105.9 (103.35-109.85)	105.0 (102.58-109.85)	0.7388
MCH (pg)	36.7 (35.93-38.0)	37.7 (36.53-38.33)	37.18 (36.33-38.28)	0.4064
MCHC (%)	35.0 (33.9-35.45)	35.2 (34.3-35.93)	35.0 (34.33-35.9)	0.3875
RDW (%)	16.1 (15.63-16.4)	16.3 (15.7-17.73)	16.44 (15.6-17.18)	0.2193
Ferritin (ng/ml)	152.6 (102.73-213.05)	161.5 (138.1-233.05)	225.9 (173.18-306.48)	**0.0017**

Hgb=Hemoglobin; MCV=Mean Cell Volume; MCH=Mean Cell Hemoglobin; MCHC= Mean Cell Hemoglobin Concentration; RDW=Red Cell Distribution Width. [a]IDA=Iron deficient anemic; IDNA= Iron deficient non anemic; NIDNA= Non iron deficient non anemic. [b] IQR, 25th to 75th quartiles. ∗ Data are from the Kruskal-Wallis test.

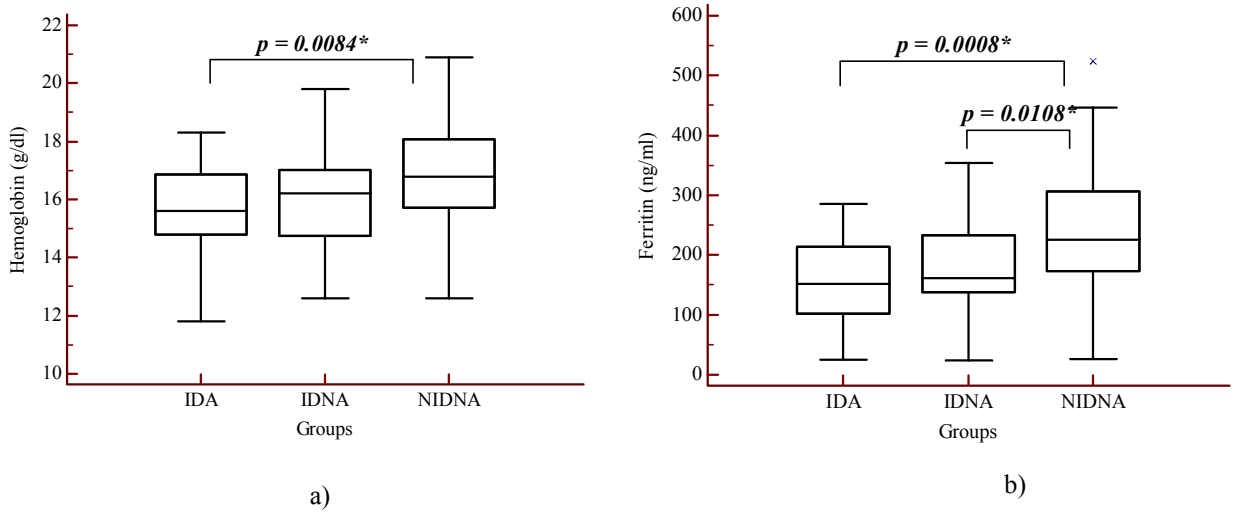

Figure 2. Box plots of hematological profile and ferritin parameters in newborns according to anemia and iron status of the women. *IDA – Iron Deficient Anemic (n = 27), IDNA – Iron Deficient Non Aneminc (n =29), NIDNA – Non Iron Deficient Non Anemic (n = 39). P values are from the Mann-Whitney test; only significant values are shown (P<0.05)*

5.3 Correlations between pregnant women and newborns laboratory parameters

The newborns ferritin level has significant correlation with Hgb ($r_s = 0.256, p = 0.0122$), and ferritin ($r_s = 0.366, p = 0.0003$) levels of their mothers (Table 8). In addition, the newborns Hgb had significant correlation with Hgb ($r_s = 0.226, p = 0.0279$) and ferritin ($r_s = 0.268, p = 0.0086$) levels of their mothers (Table 8); additionally the newborns Hgb has found to show significant correlation with pregnant women MCH and MCHC (Table 8).

Table 8. Spearman's correlation coefficients (r) comparing hematological profile and ferritin status of pregnant women and their respective newborns at St Paul's Hospital, Selam and Gulelie Health Centers, Addis Ababa (n=95)

Newborns parameters	Pregnant women's parameters r (p-value)					
	Hgb	MCV	MCH	MCHC	RDW	Ferritin
Hgb	0.226[a]	0.067	0.242 [a]	0.367 [c]	-0.012	0.268 [b]
MCV	0.042	0.016	-0.029	-0.086	-0.065	0.124
MCH	0.159	0.010	0.122	0.175	-0.068	0.115
MCHC	0.161	0.172	0.310[b]	0.438 [c]	-0.022	0.033
RDW	0.008	-0.183	-0.159	0.030	0.041	0.006
Ferritin	0.256 [a]	0.112	0.105	0.078	-0.183	0.366[c]

Hgb=Hemoglobin; MCV=Mean Cell Volume; MCH=Mean Cell Hemoglobin; MCHC= Mean Cell Hemoglobin Concentration; RDW=Red Cell Distribution Width. [a] *p-value < 0.05,* [b] *p-value < 0.01,* [c] *p-value < 0.001*

5.4 Effect of Maternal factors on the ferritin status of newborns

The effect of some maternal factors on the value of newborns ferritin was determined by using Mann-Whitney test (Table 9).

Table 9. Results of Mann-Whitney test, performed to see effect of maternal factors on the values of newborns ferritin at St Paul's Hospital, Selam and Gulelie Health Centers, Addis Ababa (n=95)

Variables	P-value
Maternal age	**0.0393**
Parity	0.3357
Birth spacing	0.9224
ANC follow up	0.2674
ANC follow up starting trimester	0.1205
Iron intake during pregnancy	0.9010

Note: The difference for those analytes having a *p* value of < 0.05 is indicated in figure 3 below.

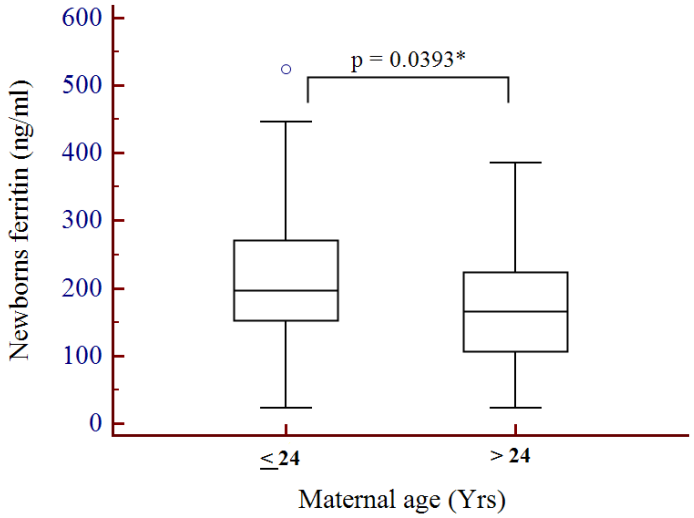

Figure 3. Box plots indicating the effect of maternal age on newborns ferritin level **Data from the Mann-Whitney test*

6. Discussion

This study aimed to show the effect of maternal iron status on the iron store of term newborns born at three health facilities in Ethiopia. Based on the findings of Hgb and ferritin levels, the mothers were grouped into 3 (IDA, IDNA and NIDNA).

The present study showed that all the hematological and ferritin parameters studied were markedly higher in newborns than in their mothers. The higher ferritin levels in newborns can be explained by the existence of active transfer of iron across placenta from mother to the fetus [60]. As well, it can be due to the upregulation of transferrin receptor synthesis in the case of iron deficiency, which enables placenta to compete more effectively for circulating transferrin iron with erythroid marrow of the pregnant mothers intending for adequate iron supply of the growing fetus [31, 39, 43].

In this study, the median Hgb and ferritin concentrations were significantly different in newborns delivered from IDA, IDNA and NIDNA pregnant women. The Hgb and ferritin values showed a significant decrement with the course of pregnant women iron deficiency status. The ferritin values of newborns delivered from IDA and IDNA pregnant women were significantly lower than that of newborns delivered from NIDNA pregnant women. These results suggest that maternal ferritin status does have influence on the iron store of the newborn and indicate that maternal iron deficiency anemia accounted for reduced iron stores in these newborns. Of note, maternal iron deficiency even without the development of a full blown anemia had a significant influence on the iron store of newborns. This will remain a significant challenge since laboratory markers for iron store measurements are unaffordable; this warrants strengthening of intervention measures.

It is well known that the main source of iron during 0-6 months of infancy is the iron that is stored during the pregnancy [36]. It is also evident that 70% of the iron in the infant's Hgb during the first year of life, and 40% in the second year, is still maternal in origin [61]. Additionally, Cord serum ferritin has been shown to be associated with the level of serum ferritin during infancy [62]. Longitudinal study carried out on Norwegian children from birth to 2 years showed a positive correlation of cord ferritin with serum ferritin at 6, 12, and 24 months (p= 0.45, 0.31, and 0.16 respectively; P <0.05 for all) [62]. Thus, the significantly lower levels of ferritin in newborns delivered from IDA and IDNA pregnant women may not be sufficient to meet the erythropoietic demands of expanding total Hgb mass and growth of the baby, which starts around three month of age. Consecutively, this may predispose them to early infancy iron deficiency and/or anemia, suggesting the need for strengthening strategy to improve the maternal iron status.

However, the ferritin levels between newborns delivered from IDA and IDNA pregnant women were not statistically different. This can be due to the absence of severe anemia cases in the studied pregnant women.

Moreover comparison of the newborns Hgb concentration between the groups revealed that Hgb concentration of newborns born to IDA pregnant women were significantly lower than that of newborns born to NIDNA pregnant women, implying that maternal Hgb concentration does have an impact on the Hgb level of the newborn. In addition to fetal storage iron at birth, the heme iron recycled from senescent red blood cells will be stored as ferritin and serve as main source of iron during the first several months of life [31]. The significantly lower Hgb in newborns born to IDA pregnant women may result in decreased amount of recycled heme iron resultantly

decreasing its contribution for the iron pool. Subsequently, this may predispose them to iron deficiency and anemia in early infancy.

Several studies, with differing results, have been conducted elsewhere to assess whether maternal iron status influences fetal iron stores at birth or not. Some of the studies have found similar results with the present study showing an adverse influence of maternal iron deficiency on the acquisition of iron by the fetus [43-48].

The study carried out by Singla et al, was comparable with this study and they found out that the levels of Hgb, serum iron, transferrin saturation and ferritin were significantly low in the cord blood of anemic women than non anemic ones [43].

Another consistent result with our study was that by Sweet et al, which showed an association between maternal iron depletion and decreased cord ferritin and Hgb [44]. This finding was further corroborated by similar findings by Kumar et al and El-Farrash et al which showed that concentrations of Hgb, iron, and ferritin were significantly lower in the cord blood of anemic mothers than control mothers [47, 48].

However, there are studies contrary to the present study which showed that iron accretion in the fetus was independent of maternal iron status [37-41]. This can be due to the use of different methods to classify the groups of pregnant women.

For example, the findings of Hadipour et al contradict with the present study in that did not found differences in serum ferritin values between newborns delivered from anemic and non-anemic mothers [42]. This can be due to difference in cut off value for serum ferritin, in which Hadipour et al used cut off value $\leq$ 10ng/ml which has low sensitivity [42] while we used a

cutoff value of 50ng/ml, to balance the effect of infection as recommended by the WHO for developing countries [23].

Paiva et al also found different result from our study, which showed the absence of significant difference in the mean values of Hgb, serum ferritin, transferrin saturation and zinc protoporphrin between newborns born from anemic, iron deficient and non iron deficient women [41]. This difference can be due to the use of different methods for classification of the groups. Paiva et al had used a combination of hematological and biochemical parameters; Hgb, ferritin, transferrin saturation and zinc protoporphrin, but in this study only Hgb and serum ferritin were used [41].

In this study, newborns ferritin level has significant correlation with Hgb, and ferritin levels of pregnant women. In addition, the newborns Hgb had significant correlation with Hgb and ferritin levels of pregnant women. Several investigators have determined the correlation between Hgb and ferritin parameters of newborns and their mothers; however, the results vary from study to study probably it can be due to the use of different cut off values of ferritin.

Kumar et al, for example, has showed maternal ferritin levels had significant correlations with Hgb levels (rs = + 0.488; $p < 0$ .001), and ferritin (rs = + 0.440; $p < 0.001$) in cord blood [47]. Singla et al has also found that maternal serum ferritin was significantly correlated with cord blood Hgb (r_s = + 0.390, $p < 0.01$), and cord serum ferritin (r_s = + 0.523. p < 0.001) [43]. The relatively lower correlation observed in this study than the two studies by Kumar et al and Singla et al may be due to the absence of any severe anemia cases in our study, while there were severe anemia cases in the two studies.

Even if, it is explained that the higher values of ferritin in newborns than in pregnant women is due to the natural mechanism available to ensure adequate iron supply to the growing fetus, the result of the present study and other similar studies have showed that the fetus is not gifted with unlimited ability to extract iron from the mother. Rather, it takes up iron in direct proportion to the levels available in the mothers as indicated by the relationship of maternal iron deficiency anemia and newborns ferritin and Hgb and by the positive correlation of maternal ferritin and Hgb levels with newborns ferritin and Hgb levels. Thus, it seems that placental iron transport mechanisms are not inviolate, and in maternal iron deficiency anemia, these mechanisms might fail, leading to an insufficient iron supply to the fetus.

Newborns ferritin value was not affected by the majority of maternal factors (parity, birth spacing, ANC follow up, iron supplementation during pregnancy, ANC follow up starting trimester). However, it was affected by maternal age and newborns delivered from younger mothers' age $\leq$ 24 years were having higher ferritin levels.

In contrary to this study, Hay et al have found a significantly higher cord serum ferritin ($p=0.02$) concentration in infants born to mothers who had taken iron supplements during pregnancy than those infants born to mother who had not taken [62]. The difference might be due to the presence of iron supplementation of women in Hay et al study according to their SF values. Whereas, in our study the basis for iron supplementation was mainly Hgb value. Moreover, in this study it has been shown that 57.9% of the studied mothers were taking iron supplementation and the median intake of the iron was only 1 month. This is lower than the recommended iron supplementation by the Ethiopian Ministry of Health, which is 6 months [14].

7. Conclusion and recommendations

7.1 Conclusion

- The median hemoglobin and ferritin concentrations were statistically different in newborns delivered from IDA, IDNA and NIDNA pregnant women ($p < 0.0001$).
- Ferritin values of newborns delivered from IDA pregnant women (152.6ng/ml) were significantly lower than that of newborns delivered from NIDNA pregnant women (225.9ng/ml) ($p = 0.0008$).
- Newborns ferritin level had significant correlation with hemoglobin ($r_s = 0.256, p = 0.0122$), and ferritin ($r_s = 0.366, p = 0.0003$) levels of pregnant women.
- Newborns hemoglobin concentration had significant correlation with hemoglobin concentration ($r_s = 0.226, p = 0.0279$) and ferritin ($r_s = 0.268, p = 0.0086$) levels of their mothers.
- Based on these findings we can conclude that maternal iron deficiency anemia may affect the iron stores of newborns.
- Maternal age is also found to affect the iron store of newborns.

7.2 Recommendations

- Prevention of iron deficiency during pregnancy is better to be strengthened.
- It is advisable to propose strategies for intervention of iron deficiency in the infants born from iron deficient mother.
- Nutrition education may be beneficial to improve the dietary intake of pregnant mothers.
- It is worthwhile that term infants born from iron deficient anemic mothers should be screened for their iron store status at birth.
- Further investigations including larger population of pregnant women with different stages of anemia should be performed to verify the interrelation between maternal iron deficiency anemia and iron store of newborns.
- Follow up studies from birth to at least six months of age on newborns delivered form maternal iron deficiency anemia are needed to investigate the long term effect of maternal iron deficiency anemia.

8. Limitations of the study

- The dietary data were based on qualitative information and, thus, could not estimate the precise assessment of the nutrient intake.
- Absence of C-reactive protein assessment.

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10. Annexes

Annex I. Subject information sheet (English version)

ADDIS ABABA UNIVERSITY
COLLEGE OF HEALTH SCIENCES
DEPARTMENT OF MEDICAL LABORATORY SCIENCES

Subject information sheet for mothers whose venous and cord blood is to be used in the study of the effect of maternal iron deficiency anemia on iron store of newborns

You are invited to participate in a study to be conducted by Msc student at Addis Ababa University, 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 is 'Effect of maternal iron deficiency anemia on iron store of term newborns'. It is aimed at assessing the effect of maternal iron deficiency anemia on the iron store status of the newborn.

Participation in this study is exclusively voluntarily. If you are not interested to participate or if you once decide to participate and withdraw yourself 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 participant of the study?

As a participant of this study, you are expected to agree that 5ml blood will be collected from your vein, before delivery during the first labour and cord immediately and 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 an identification code will be used in such conditions.

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

You will spend 25-30 minutes until the specimen is collected, the questionnaire is filled and the consent form is signed.

What are the risks of participating in this study?

Specimen collection will have no effect on the labor and the newborn and pose no 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 secrete?

All information that you give and the results from your specimen will be used for this study only. Only limited numbers of professional 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 determining of the effect of maternal iron deficiency anemia on the iron store of newborns that can be useful for identify newborns who are at risk of developing iron deficiency early in childhood. You will also obtain all the results of the analysis for free and communicated to your physician for the appropriate management.

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 question for further explanations about the study. You can get the results of the analysis.

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

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

Betelihem Terefe

Department of Medical Laboratory Sciences

College of Health Sciences

Addis Ababa University

Mob: +251-91-2-40-94-18

Email: betch.nym@gmail.com

For additional information, please contact Addis Ababa University, College of Health Sciences, Department of Medical Laboratory Sciences at:

Telephone - +251-1-12-75-51-70

Agree to participate? ☐Yes ☐No

Annex II Subject information sheet (for pregnant mothers, Amharic version)

አዲስ አበባ ዩኒቨርሲቲ
የጤና ሳይንስ ኮሌጅ
የሕክምና ላቦራቶሪ ሳይንስ ት/ክፍል
ቻነብሰ ጡር እናቶች እና ቻእትብት ላይ የደም ናሙና በመውሰድ የነብሰ ጡር እናቶች ደም ማነስ በሚወለዱት ህጻናት የአይረን ክምችት ላይ ያለውን ተጽዕኖ ለማወቅ በሚሰራው ጥናት ላይ ለሚሳተፉ እናቶች የተዘጋጀ መረጃ

በአዲስ አበባ ዩኒቨርሲቲ፣ ጤና ሳይንስ ኮሌጅ የሕክምና ላቦራቶሪ ሳይንስ ት/ክፍል በማስተርስ ዲግሪ ተማሪ የመመረቂያ ጥናት ላይ እንዲሳተፉ ተጋብዘዋል። እባክ- በዚህ ጥናት ለመሳተፍ ቻመስማማት- በፊት ቻዚህ ቀጥሎ የሚገኘውን ምንባብ በጥሞና ያንብቡና ግልጽ ያልሆነል- ትን ማንኛውንም ሃሳብ ይጠይቁ።

መግቢያ

የጥናቱ ርዕስ 'የነብሰ ጡር እናቶች ደም ማነስ በሚወለዱት ህጻናት የአይረን ክምችት ላይ ያለው ተጽዕኖ' ነዉ። አላማውም የነብሰ ጡር እናቶች ደም ማነስ በሚወለዱት ህጻናት የአይረን ክምችት ላይ ያለው ተጽዕኖ ማወቅ ነው።

እርስ- በዚህ ጥናት ላይ የሚኖር- ት ተሳትፎ ሙሉ በሙሉ በበጐ ፈቃደኝነት ላይ የተመሠረተ ነው። በዚህ ጥናት ውሰጥ ላለመሳተፍ ወይም ለመሳተፍ ቻወሰኑ በኋላ ለማቋረጥ የሚወስኑ ቢሆንም እንኳን በዚህ ሆስፒታል ውሰጥ የሚሰጠው ማንኛውም አገልግሎት አይቋረጥም። በጥናቱ ለመሳተፍ የሚስማሙ ቻሆነ የስምምነት ቅጹ ላይ በጹሑፍ ወይም በጣት ፊርማ- ን ማስቀመጥ ይጠበቅብ- ታል። ቻፈለጉ ይህንን የመረጃ ቅጽ አንድ ቅጅ ለራስ- ሊያስቀሩ ይችላሉ።

የጥናቱ ተሳታፊ በመሆኔ የሚጠበቅብኝ ምንድን ነው?

በዚህ ጥናት ለመሳተፍ የሚስማሙ ቻሆነ 5 ሚ.ሊ. የደም ናሙና ቻመውለድ- በፊት በመጀመሪያው ምጥ ላይ ቻክንድ- እና በሚወልዱበት ጊዜ ቻእትብት ላይ እንደሚወሰድ እና ለጥናቱ እንዲውል መስማማት ይጠበቅብዎታል። ቻተወሰደዉ ናሙና ላይ የሚገኙ መረጃ- ች ቻዚህ ሆስፒታል ውጭ ለሚገኙና ለሥራው አግባብነት ላላቸው ሰ- ች ቢነገር የማይቃወሙ መሆኑን መስማማት ይጠበቅብ- ታል። ይሁን እንጂ ይህ ዓይነቱ መረጃ የእርስ- ን ማንነት የሚገልጹ መረጃ- ችን ማለትም ስም፣አድራሻና፣ የስልክ ቁጥር የመሳሰሉትን መረጃ- ችን አይጨምርም። ይልቁንም ለዚህ ጥናት አገልግሎት ብቻ የሚውል እርስ- ን ለማወቅ የሚያስችል መለያ ቁጥር ጥቅም ላይ እንዲውል ይደረጋል። በተጨማሪም ስለ እርስ- አጠቃላይ የጤና ሁኔታ ለሚቀርቡ አንዳንድ ተጨማሪ ጥያቄ- ች መልስ መስጠት ይጠበቅብ- ታል።

በዚህ ጥናት መሳተፍ ምን ያህል ጊዜ ይፈጃል?

የተዘጋጀዉን መጠይቅ ለመሙላት፣ የስምምነት ቅጹ ላይ ለመፈረምና ናሙና ለመስጠት ቻ25-30 ደቂቃ ያስፈልጋል።

በዚህ ጥናት መሳተፍ የሚያስቻትላቸው ችግሮች ምንድን ናቸው?

ናሙና በሚሰበሰብበት ወቅት ምንም አይነት ችግር በምጥ በህጻኑ ላይ አያስቻስትብ- ትም እንዲሁም ምንም አይነት የህመም ስሜት አያስቻትልብ- ትም። ስለዚህም የሚያጡት ነገር ቢኖር መጠይቁን ለመሙላት የሚያጠፉት ጊዜ ነው።

የሕክምና መረጃዬ በሚስጥር ተጠብቆ መቆየት የሚችለው እንዴት ነው?

ስለራስ- የሰጡት ማንኛውም መረጃና ቻትወሰደው ናሙና ላይ የተገኘው የላቦራቶሪ ውጤት የሚውለው ለጥናቱ አላማ ብቻ ነው። ይህን ማህደር ሊያገኙ የሚችሉት የተወሰኑ የጥናቱ ተባባሪ ሰራተኞች ብቻ ናቸው። ቻዚያም በላይ ስለእርስ- ያለውን ማንኛውንም መረጃ የተለየ የይለፍ ቃል ባለው የኮምፒውተር የመረጃ ማህደር ውስጥ እንዲቀመጥ ይደረጋል።

በዚህ ጥናት መሳተፍ የሚያስገኛቸው ጥቅሞች ምንድናቸው?

ይህ ጥናት የማስተርስ ዲግሪ መመረቂያ እንደመሆኑ መጠን ለተሳታፊዎች ገንዘብ አይሰጥም። ነገር ግን የእርስ- ተሳትፎ ደም ማነስ ካለባቸው የሚወለዱ አዲስ ህጻናት ለወደፊቱ ለደም ማነስ የተጋለጡ መሆን አለመሆናቸውን ለማረጋገጥና ለወደፊቱ ተፈላጊውን እርዳታ እንዲሰጣቸው ለማድረግ ይረዳል። ቻፈለጉም የሁሉንም የላቦራቶሪ ውጤቶች በነፃ ያገኛሉ።

በዚህ ጥናት ተሳታፊ በመሆኔ መብቶቼ ምንድን ናቸው?

በጥናቱ ውስጥ ያለ- ትን ተሳትፎ በማንኛውም ጊዜ የማቋረጥ ሙሉ መብት- የተጠበቀ ቻመሆኑም በላይ ራስ- ን ቻጥናቱ በማግለል- ምክንያት የሚቀርብ- ት ምንም አይነት የሆስፒታል አገልግሎት አይኖርም። ቻዚህም በተጨማሪ ጥናቱን በተመለቻት ማንኛውንም አይነት ጥያቄ የመጠየቅና ገለፃ የማግኘት መብት አለ- ት። የላቦራቶሪ ምርመራ ውጤቱንም በነፃ ማግኘት ይችላሉ።

ጥያቄ ካለኝ ወይም ችግር ቢያጋጥመኝ ምን ማድረግ ይገባል?

ይህን ጥናት በተመለቻት ወይም ቻዚህ ጥናት ጋር በተዛመደ መልቻ ስለሚያጋጥሙ ድንገተኛ አደጋ- ች ወይም ጥያቄ ካለ- ት በሚቻትለው አድራሻ ይጠቀሙ።

ቤተልሔም ተረፈ

የሕክምና ላቦራቶሪ ሳይንስ ት/ክፍል

የጤና ሳይንስ ኮሌጅ

አዲስ አበባ ዩኒቨርሲቲ

ሞባይል +251-91-2-40-94-18

ኢሜይል betch.nym@gmail.com

ለተጨማሪ መረጃ- ች የአዲስ አበባ ዩኒቨርሲቲ የሕክምና ላቦራቶሪ ሳይንስ ት/ክፍል ይጠይቁ።

ስልክ - +251-1-12-75-51-70

ለመሳተፍ ይስማማሉ?

☐ እስማማለሁ ☐ አልስማማም

Annex III Consent Form (English version)

Code number _____

Name of study subject _____

I have been informed about the study which is aimed at assessing the effect of maternal iron deficiency anemia on the iron store status of the newborn. For this study blood is required from me and cord. The aims of the study were 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 the 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 me and the cord for the investigation. In addition, I have had the opportunity to ask questions about it and received clarification to my satisfaction. I have also been informed that the benefit of participation is to get the results of the analysis measured for free via the counselor nurse.

Participant's Signature/ finger print _____
Name of deponent _____ signature _____
(for mothers unable to read)

Name of Counselor nurse _____ Signature_____

Date _____

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

Betelihem Terefe

Department of Medical Laboratory Sciences

College of Health Sciences

Addis Ababa University

Mob: +251-91-2-40-94-18

Email: betch.nym@gmail.com

For additional information, please contact Addis Ababa University, College of Health Sciences, Department of Medical Laboratory Sciences at:

Telephone- +251-1-12-75-51-70

Annex IV - Consent form (Amharic version)

የስምምነት ቅጽ

የሚስጥር ቁጥር_____
የተሳታፊዋ ስም_____

እኔ ስሜ ቻላይ የተጠቀሰው ተሳታፊ 'የነብሰ ጡር እናቶች ደም ማነስ በሚወልዱት ህጻናት የአይረን ክምችት ላይ ያለው ተጽዕኖ ለማወቅ ስለሚሰራው የህክምና ጥናት በቂ ገለጻ ተደርጎልኛል። ለጥናቱም ቻእኔ እና ከእትብት ላይ የተወሰደ የደም ናሙና እንደሚያስፈልግ ተገልፆልኛል። የጥናቱን አላማ- ችም ተረድቻለሁ።

በመጠይቁ ላይ የገለፅኳቸው መረጃ- ች በሙሉ በሚስጥር የተጠበቁ እንደሚሆኑ ተነግሮኛል። በጥናቱ ላይ ያለመሳተፍና ማንኛውንም መረጃ ያለመስጠት እንዲሁም በማንኛውም ጊዜ ቻጥናቱ ራሴን የማግለል መብቴ የተጠበቀ እንደሆነ ተገልፆልኛል።

ስለዚህ ለዚህ ጥናት መረጃና የስምምነት ቃሌን የሰጠሁት በአጠቃላይ ሁኔታውን በመረዳትና በፍጹም ፈቃደኝነት ነው። ቻእኔ እና ከእትብት ላይ የሚወሰደው ናሙና ለምርምር እንደሚውልም ተረድቻለሁ። በተጨማሪም ጥያቄ ለመጠየቅ ተፈቅዶልኝ ለማወቅ የፈለኩትን ያህል ማብራሪያ አግኝቻለሁ። የዚህ ጥናት ተሳታፊ በመሆኔ የማገኘው ጥቅም የሁሉንም ምርመራ ውጤት በነፃ ማግኘት እንደሆነ ተረድቻለሁ።

የተሳታፊዋ ፊርማ/የጣት አሻራ_____
የምስክር ስም _____ **ፊርማ**_____
(የስምምነት ቅጹን ማንበብ ለማይችሉ ተሳታፊ- ች)

የአማካሪ ነርስ ስም_____ **ፊርማ** _____
ቀን_____

ይህን ጥናት በተመለቻተ ወይም ቻዚህ ጥናት ጋር በተዛመደ መልኮ ስለሚያጋጥሙ ድንገተኛ አደጋ- ች ወይም ጥያቄ ካለ- ት በሚቻተለው አድራሻ ይጠቀሙ።

ቤተልሔም ተረፈ

የሕክምና ላቦራቶሪ ሳይንስ ት/ክፍል

የጤና ሳይንስ ኮሌጅ

አዲስ አበባ ዩኒቨርሲቲ

ሞባይል +251-91-2-40-94-18 **ኢሜይል** betch.nym@gmail.com

ለተጨማሪ መረጃ- ች የአዲስ አበባ ዩኒቨርሲቲ የሕክምና ላቦራቶሪ ሳይንስ ት/ክፍል ይጠይቁ።

ስልክ - +251-1-12-75-51-70

Annex V- Questionnaire

ADDIS ABABA UNIVERSITY

COLLEGE OF HEALTH SCIENCES

DEPARTMENT OF MEDICAL LABORATORY SCIENCES

Questionnaire for data collection from mothers who's venous and cord blood is to be used in the study of the effect of maternal iron deficiency anemia on iron store of term newborns

1. Introduction
   1.1 Subject identification number _____ Tel. no. _____
   1.2 Age of the mother (in years) _____ 1.3 Gestational weeks _____
2. Information about the newborn
   2.1 Weight (in grams) _____ 2.2 Gender male ☐ female☐
3. Educational level
   ☐ Unable to write and read ☐ Secondary School
   ☐ Primary school ☐ Above secondary school
4. Occupation
   ☐ House wife ☐ Employed
5. Parity
   ☐ Primiparous (woman with her first pregnancy/delivery)
   ☐ Multiparous (woman who has given birth two or more than two times)
   If you are multiparous or how much is your average previous child birth spacing?
   ☐ < 2 years ☐ > 2 years
6. Mode of current delivery
   ☐ Normal spontaneous delivery ☐ Cesarean section
7. Have you visited health care centers during your pregnancy to get antenatal care?
   ☐ Yes ☐ No
   a. *If the answer is yes, how many times did you visit the health care centers?*
      ☐ *One time* ☐ *Three times*
      ☐ *Two times* ☐ *Four times*
   b. *At what period of your trimester do you visit the health care centers?* _____
8. During this pregnancy, have you taken any iron tablets or iron syrups?
   ☐ Yes ☐ No
   a. *If the answer is yes, for how many days did you take the tablets or syrups during the whole pregnancy?* _____
9. Nutritional information → How often do you consume the following food items?

1 = Once or more than once /day 2 = 1-3/week 3 = Once or twice /month 4 = Occasionally(holiday, wedding, ceremony etc) 5 = Never	
Meat, Poultry, Fish	
Orange, Pineapple, Guava, Banana, Mango, Melon	
Carrot, potato, beet root, pumpkin, tomato, cabbage	
Tea and coffee	

Annex VI Questionnaire (Amharic version)

አዲስ አበባ ዩኒቨርሲቲ
የጤና ሳይንስ ኮሌጅ
የሕክምና ላቦራቶሪ ሳይንስ ት/ክፍል

ቻነብሰ ጡር እናቶች እና ቻእትብት ላይ የደም ናሙና በመውሰድ የነብሰ ጡር እናቶች ደም ማነስ በሚወለዱት ህጻናት የአይረን ክምችት ላይ ያለውን ተጽዕኖ ለማወቅ በሚሰራው ጥናት ላይ ለሚሳተፉ እናቶች የተዘጋጀ መጠይቅ

1. *መግቢያ*
   ***1.1 የመለያ ቁጥር*_____ስልክ ቁጥር_____**
   1.2 የእናት እድሜ (በአመት) _____ 1.3 የእርግዝና ሳምንታት_____
2. **የተወለደውን/ችውን ህጻን የተመለቻተ መረጃ**
   ***2.1* ክብደት (በኧ.ግ.) _____ *2.2* ጾታ □ ሴት □ ወንድ**
3. **የትምህርት ደረጃ**
   ☐ **ማንበብ መፃፍ የማትችል** ☐ **ሁለተኛ ደረጃ**
   ☐ **አንደኛ ደረጃ** ☐ **ቻሁለተኛ ደረጃ በላይ**
4. **የስራ ሁኔታ**
   ☐ **የቤት እመቤት** ☐ **ተቀጣሪ**
5. **ቻአሁን በፊት ምን ያክል ልጆችን ወልደዋል?**
   ☐ **ይህ የመጀመሪያዬ እርግዝና ነው** ☐ **ቻሁለት በላይ**

ሁለትና ቻሁለት በላይ ቻወለዱ በአማካኝ በልጆች- መካቻል ምን ያክል የእድሜ ልዩነት አለ
☐ ≤ 2 **አመት** ☐ > 2 **አመት**

6. **በምን አይነት መንገድ ወለዱ?**
   ☐ **በተፈጥሮ ምጥ** ☐ **በቀዶ ጥገና**
7. **በእርግዝና- ወቅት ጤና ጣቢያ በመሄድ ክትትል ያደርጉ ነበር?**
   ☐ **አ-** ☐ **የለም**
   መልስ- አ- ቻሆን ለምን ያክል ጊዜ ወደ ጤና ጣቢያ ተመላለሱ?
   ☐*አንዴ* ☐*ሁለቴ* ☐*ሶስቴ* ☐*አራቴ*
   በስንተኛው የእርግዝና ወቅት ወደ ጤና ጣቢያ ተመላለሱ?
   _____
8. **በዚህኛው እርግዝና- የአይረን እንክብል ወይም ሲረፕ ወስደዋል?**
   ☐ **አ-** ☐ **የለም**
   መልስ- አ- ቻሆን ለምን ያክል ጊዜ የአይረን እንክብሉን ወይም ሲረፑን በጠቅላላ የእርግዝና ጊዜ- ወሰዱ? _____
9. **የአመጋገብ ሁኔታ - ምን ያክል ጊዜ ቻዚህ በታች የተዘረዘሩትን ምግቦች ይመገባሉ?**

1 = በቀን አንዴ ወይም ቻዚያ በላይ 2 = በሳምንት ቻአንድ እስቻ ሶስት ጊዜ 3 = በወር አንዴ ወይም ሁለት ጊዜ 4 = አልፎ አልፎ (በሰርግ፣ በበአላት፣ ወዘተ) 5 = ተመግቤ አላውቅም	
ስጋ ፣ ዶሮ፣አሳ	
ብርቱካን ፣ ፓፓዬ፣ ዘይቱን፣ ሙዝ፣ ማንጎ፣ ሀባብ	
ካሮት ፣ ድንች ፣ ቀይ ስር ፣ ዱባ ፣ ቲማቲም ፣ ጥቅል ጎመን	
ሻይ ፣ ቡና	

Annex VII Protocols

A. Venous blood collection

1. Equipments were assembled including needle and syringe. All the needed equipments for the procedure were collected and placed within safe and easily reachable materials like a tray or trolley, ensuring that all the items are clearly visible.
2. Hand hygiene of the phlebotomist was performed
3. Patient identification and preparation was carried out.
4. The vene puncture site was selected and palpation of the area to locate the anatomic landmarks was performed.
5. Tourniquet was applied about 4–5 finger widths above the selected vene puncture site.
6. Well fitting gloves were put on.
7. Disinfection of the site was done using 70% alcohol for 30 seconds and it was allowed to dry completely for approximately 30 seconds.
8. The vein was anchored by holding the patient's arm and placing a thumb below the venepuncture site.
9. The needle was entered into the vein swiftly at approximately 30 degree angle against the arm.
10. After collecting sufficient amount of blood the tourniquet was released before withdrawing the needle.
11. The needle was withdrawn gently and then the patient will be given a clean gauze or dry cotton-wool ball to apply to the site with gentle pressure.
12. The needle was removed from the syringe and the specimen tubes will be filled with the blood.
13. The tubes were immediately inverted several times to mix the blood with the anticoagulant.
14. The tube was labeled with the patient identification number and date.
15. The used needle and syringe or blood-sampling device were discarded into a puncture resistant container.
16. The used gloves were disposed appropriately and hand hygiene will be performed.

B. Cord blood collection

1. Equipments were assembled including needle and syringe. All the needed equipments for the procedure were collected and placed within safe and easily reachable materials like a tray or trolley, ensuring that all the items are clearly visible.
2. Hand hygiene of the phlebotomist was performed
3. Patient identification was carried out.
4. Well fitting gloves were put on.
5. After delivery of the infant the umbilical cord was double-clamped and cut.
6. Any blood from the surface of the cord was removed with gauze.
7. The needle was inserted just above the clamp that remains on the cord.
8. After collecting sufficient amount of blood the needle was withdrawn gently.
9. The needle was removed from the syringe and the specimen tubes were filled with the blood.
10. The tubes were immediately inverted several times to mix the blood with the anticoagulant.
11. The tube was labeled with the patient identification number and date.
12. The used needle and syringe or blood-sampling device was discarded into a puncture resistant container.
13. The used gloves were disposed appropriately and hand hygiene was performed.

C. Specimen processing and transport

Blood serum preparation

Materials and Equipment

- Human blood sample.
- Evacuated collection tubes (with K_3-EDTA anticoagulant (6ml) (13 x 100 mm))
- Test tubes
- Bench top centrifuge
- Disposable transfer pipettes
- Tube rack
- Personal protective equipments
- Biohazard boxes/bags
- Permanent marker

Procedure

A. Whole blood was drawn into serum gel separator evacuated tube tube(s).
B. The whole blood was incubated in an upright position at room temperature for 30-45 min (not longer than 60 min) to allow clotting.
C. The whole blood was centrifuged for 15-20 min at speed of 2500-3000 RPM.
D. Serum was carefully aspirated at room temperature and pooled into another test tube.
E. Serum was inspected for turbidity and if it is turbid it was centrifuged and aspirated again to remove remaining insoluble matter.

Specimen transportation

Blood specimen tubes were transported upright and secured in a screw cap container or in a rack in a transport box. Enough absorbent paper was around them to soak up all the liquid in case of spillage.

i. Packaging of specimen for transport

- ✓ All specimens were collected in a container that is watertight and leak proof. The cap was correctly and securely closed.
- ✓ The containers were kept in an upright position in a rack during transport.
- ✓ The rack holding the containers were put in a cold box.

- ✓ There was an adequate cushioning material inside the box so as to absorb shocks during transport, and adequate absorbing material to absorb any spillage.

ii. Proper specimen handling during transport

- ✓ The outer container (cold box) was handled gently with care. Throwing or dropping of the transport box is prohibited.
- ✓ Good personal hygiene was maintained during transporting these boxes. Hands were washed after each session of work, when contaminated or soiled, or after removal of gloves (Staff must not touch mouth, eyes, nose and mucosal membranes prior to hand washing and definitely not with gloved hands)

iii. Handling of specimen leakage and spillage

- ✓ Leaking specimens were hazardous to all staff involved in their handling. Such specimens were rejected or discarded according to the laboratory practice.
- ✓ When leakage of specimen content within or outside of the cold box is encountered during transport, the spill was decontaminated as soon as possible according to the Spill Clean-up Procedure

iv. Spill Clean-up Procedure

- ✓ Gloves and the appropriate personal protective equipments were worn.
- ✓ The spill was covered with cloth, gauze or paper towels to contain it.
- ✓ Freshly prepared solution of 1 in 5 dilution of domestic bleach was poured over gauze/paper towel.
- ✓ After 30 minutes, the materials were cleared away. A dustpan was used to collect any broken glass or sharps and deposit it into a puncture-resistant container. All the materials was discarded as clinical waste and the dustpan was disinfected after use.

D. Specimen rejection criteria

I. Specimen rejection criteria for CBC

- Wrong study participant identification
- Wrongly labeled specimens
- Inadequate specimen volume
- Dilution with fluids
- Underfilled specimens
- Overfilled specimens
- Inappropriate collection tube/use of wrong anticoagulant
- Hemolysis
- Lipemic specimens (can be centrifuge, mark total volume, remove plasma, replace with saline, perform Hb)
- Clotted specimen
- If specimen is > 48 hours old (from the time of draw)

II. Specimen rejection criteria for serum ferritin

- Wrong study participant identification
- Wrongly labeled specimens
- Inadequate specimen volume
- Dilution with fluids
- Inappropriate collection tube/use of wrong anticoagulant
- Hemolysis
- Lipemic specimens (can be centrifuge, mark total volume, remove plasma, replace with saline, perform Hb)

Declaration

I declare that this thesis (**Effect of Maternal Iron Deficiency Anemia on Iron Store of Term Newborns)** is my own original work, that it has not been submitted for any degree or examination in any other university, and that all the sources I have used have been duly acknowledged.

Name of the candidate: **Betelihem Terefe**

Signature ---------- Submission date -------/-------/---------- Place: Addis Ababa

This thesis has been submitted for examination with my approval and university advisor.

Name of the Advisors:

Aster Tsegaye (BSc, MSc, PhD)

Signature ---------- Date -------/-------/---------- Place: Addis Ababa

Asaye Birhanu (BSc, MLT, MSc)

Signature ---------- Date -------/-------/---------- Place: Addis Ababa