



ADDIS ABABA UNIVERSITY

COLLEGE OF HEALTH SCIENCES, SCHOOL OF MEDICINE

DEPARTMENT OF ANATOMY

MORPHOLOGICAL CHANGES OF PLACENTA AND BIRTH WEIGHT OF
FETUS ASSOCIATED WITH MATERNAL ANEMIA IN DESSIE REFERRAL
HOSPITAL, NORTHEAST ETHIOPIA.

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Morphological Changes of Placenta and Birth Weight of Fetus Associated with Maternal Anemia in Dessie Referral Hospital, Northeast Ethiopia.

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DECLARATION

By my signature below, I declare and affirm that this thesis is my own work. I have followed all ethical principles of scholarship in the preparation, data collection, data analysis and completion of this thesis. All scholarly matter that is included in the thesis has been given recognition through citation. I affirm that I have cited and referenced all sources used in this document. Every effort has been made to avoid plagiarism in the preparation of this thesis.

This thesis is submitted in partial fulfillment of the requirement for a Master's degree in Anatomy from the Addis Ababa University at College of Health Sciences, School of medicine. The thesis is deposited in the Addis Ababa University Digital Library and is made available to local, national and international scientific community.

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ACRONYMS AND ABBREVIATIONS

ANC	Antenatal Care
ANOVA	Analysis Of Variance
BW	Birth Weight
CSA	Central Statistical Agency
EDHS	Ethiopian Demographic Health Survey
GA	Gestational Age
HB	Hemoglobin
IDA	Iron Deficiency Anemia
IUGR	Intrauterine Growth Retardation/ Restriction
LBW	Low Birth Weight
SD	Standard Deviation
SPSS	Statistical Package for Social Sciences
UNICEF	United Nations International Children's Emergency Fund
USAID	United States Agency for International Development
WHO	World Health Organization

ABSTRACT

Background: – Placenta is a feto maternal organ composed of maternal component, decidua basalis, and a fetal component, chorion frondosum. The intra-uterine existence of the fetus is dependent on this vital organ. Anemia during pregnancy is considered as a cause of pre-placental hypoxia which can give rise to fetal hypoxia and its complications.

Objective: – To compare the morphological changes of placenta and birth weight of the fetus associated with maternal anemia in Dessie Referral Hospital from February, 2018-October, 2018.

Methods and Materials: – A comparative cross sectional method was conducted from May-June, 2018 at Dessie Referral Hospital. Informed consent was taken from mothers under the study and a total of 96 placentas (48 anemic and 48 non anemic) was collected after delivery at labor room. The diameter and thickness was measured, the number of cotyledons was counted and shape of placenta was noted. Placenta and fetus was weighed in the same scale. Tissue for microscopy was taken from 66 placentas (33 anemic and 33 non-anemic). Ocular micrometer was used to measure the width of intervillous space. EPI data version 4.2.0 was used to enter the data and the data was analyzed by SPSS version 21. One way ANOVA and independent sample t-test was used to compare the mean differences of the groups.

Result: -In pregnancies with maternal anemia, mean placental weight was 544 ± 98 g and for non-anemic mother's it was 502 ± 93 g ($p=0.03$). The mean birth weight in anemic group was 2502 ± 360 g and in non-anemic group 3035 ± 305 g ($p<0.001$). The mean number of cotyledons, was 13.5 ± 1.8 and 17.6 ± 1.1 , ($p<0.001$) in anemic and non-anemic groups, respectively. There was a significant difference in mean placental diameter of anemic 18 ± 1.5 cm and 17 ± 1.5 cm in non-anemic mothers ($p<0.001$). Placenta was thicker in anemic mothers 22.7 ± 2.4 mm than non-anemic mothers 20 ± 0.6 mm, ($p<0.001$). A major shape of placenta in this study was circular followed by oval in both groups. However, 4(8.3%) irregular and 2(2.1%) succenturiate lobe was observed in anemic mothers. Based on the result of microscopic morphology, 75.7% of anemic placentas terminal villi vessels were increased in number, compared to 15.1% in non-anemic ($p=0.001$). Placental calcification was 72.7% in anemic groups compared to 54% in non-anemic groups, however, it was insignificant ($p=0.12$). Eighty one percent of anemic placentas had diffuse syncytial knots compared to 15.1% in non-anemic groups. Intervillous space was wider in anemic compared to non-anemic groups ($p<0.001$).

Conclusion: - Placenta was bigger in anemic mothers than non-anemic mothers with increased in mean placental weight, thickness and diameter. Maternal anemia was associated with decrease in number of cotyledons and birth weight of fetus. Majority of placental shape in this study was circular followed by oval both in anemic and non- anemic mothers. Vessels of terminal villi in anemic placenta were increased in number compared to non-anemic. Calcification of placenta was more prominent in anemic groups compared to non- anemic even though it was not significant. Intervillous space width was more prominent in anemic groups.

Key words: Anemia, Placenta, Birth Weight, Morphology

1. INTRODUCTION

1.1 Background

The placenta is a feto-maternal organ composed of maternal component, decidua basalis derived from the endometrium and a fetal component, chorion frondosum develops from chorionic sac(1). At full term the human placenta consists of: - 1. Fetal surfaces which is shiny, gray and translucent enough, due to the color of the underlying maroon villous tissue; 2. Maternal surface which is, finely granular, mapped into 15-20 cotyledons limited by placental septum. 3. Umbilical cord; insertion is usually central but may vary in some specimens like battledore/marginal, velamentous or eccentric (2, 3).

The intra-uterine existence of fetus is dependent on this vital organ. During the course of pregnancy, it acts as the lungs, gut, kidneys, and liver of the fetus. The placenta also has major endocrine actions that modulate maternal physiology and metabolism and provides a safe and protective milieu in which the fetus can develop(4, 5). Fetal nutrient availability results from several elements. These include the interrelationships of maternal food intake, availability of nutrients in the maternal circulation, and ability of the placenta to efficiently transport substrates to the fetal circulation(6).

Central to this critical organ function is, a multilayered membranous structure that consists of the syncytiotrophoblast, cytotrophoblast, connective tissue villus core and the fetal capillary endothelium, separated by a thin interstitium. This specialized barrier is important in separating the maternal intervillous space and fetal circulation and it is responsible for regulating the rate and selectivity of placental transport. It also plays an essential role in fetal development and health by tightly regulating the exchange of endogenous and exogenous materials between the mother and the fetus(7).

The expelled placenta is a flattened discoid with an approximately circular or oval outline, with an average volume of 500ml (range 200-950ml), average weight of 500g (range 200-800g), average diameter of 185mm (range 150-200mm), and average thickness of 23mm (range 10-40mm). Thickest at its center (the original embryonic pole), it rapidly diminishes in thickness towards its periphery where it continues as the chorion leave(8). Fetal growth and well-being depends on the functional and structural component of the placenta. The architecture of the placenta has been claimed to be changed in maternal diseases like anemia(9).

The placenta is subjected to various defects and diseases just like the other vital organs of the body. Various clinical conditions such as anemia, diabetes, hypertension and others have a detrimental effect on the placenta occasionally resulting in morphological change. This may seriously affect the health and even life of the fetus. Some researchers observed that the placenta has considerable functional reserve capacity; it can repair any damage it suffers with considerable ease. It has also its own compensatory mechanisms which tend to limit the ill-effects of both tissue injury and unfavorable maternal situation like anemia(10). Placenta depicts the most accurate record of prenatal experience of an infant. It undergoes different changes in weight, volume, structure, shape and function continuously throughout the gestation to support the prenatal life(11).

Any gross and microscopic changes of placenta can have a detrimental effect on the growth and development of the fetus. It can be severe enough to produce intra uterine growth retardation and still births. Despite placenta is a functional component between fetus and mother, any pathological incident related to mother or fetus, will affect the structure and function of the placenta. Severe abnormalities of the placenta may lead to adverse fetal outcome and some structural changes may be the consequences of poor fetal condition. Anemia during pregnancy is considered as a cause of pre-placental hypoxia which can give rise to fetal hypoxia and its complications(12).

Fetal vasculature of human placenta adapt in a uniform manner in different form of hypoxic stress like high altitude and maternal iron deficiency anemia. Increased capillarization of term villi is found in hypoxic condition like maternal iron deficiency anemia. Dilatation of capillary sinusoid with accompanying thinning of the villous membrane is the principal adaptation to hypoxia. In maternal anemia there is a relative hypoxia, which may be the main factor for syncytiotrophoblast proliferation leading to increase in syncytium thickness(13).

Anemia is a medical condition defined as low hemoglobin level in the blood. It is a condition in which the hemoglobin content of the blood is lower than normal for a person's age, gender and environment resulting in oxygen carrying capacity of the blood being reduced(14).

According to World Health Organization (WHO), anemia during pregnancy is defined as a hemoglobin concentration of <11 g/dl, which ranges from mild to severe. WHO classify anemia in pregnancy based on hemoglobin level as 10.0-10.9g/dl(mild anemia), 7-9.9g/dl(moderate anemia) and <7g/dl (severe anemia)(14). Pregnancy is a state of increased iron demand which

risks from 2.5mg/day in earlier weeks to 6.6mg/day in third trimester. If demand and supply balance is not met, the women will develop anemia(15).

The cause of anemia is multifactorial; the disease is thought to be mainly caused by iron deficiency in developing countries. In sub-Saharan Africa where iron deficiency is common, the prevalence of anemia has often been used as an alternative for iron deficiency anemia (IDA)(16).

Anemia in pregnancy most commonly results from nutritional deficiency either iron or folic acid. other types during pregnancy include anemia of chronic disease, hemoglobinopathies, hereditary spherocytosis or paroxysmal nocturnal hemoglobinuria, drug induced, and aplastic anemia(15).

Pregnancy anemia can be asymptomatic and may be diagnosed following routine screening. The signs and symptoms are often nonspecific with fatigue being the most common. Women may also complain of lethargy, reduced mental alertness, pallor, dyspnea, weakness, headaches, palpitations, and dizziness(15, 17).

1.2 Statement of the Problem

Anemia during pregnancy affects about half of all pregnant mothers in developing countries and it is the major causes of indirect maternal mortality. for instance, severe anemia contributes the risk of maternal death in cases of hemorrhage(18).

Anemia can directly cause poor growth of fetus in utero due to inadequate oxygen flow to the placental tissue or it is indirect indicator of maternal nutritional deficiency. Globally anemia affects around 32.4million (38.2%) of pregnant women. It is a severe public health problem in Southeast Asia (48.7%) and Africa (46.3%). It is estimated that anemia is responsible for 20% of all maternal deaths in sub-Saharan Africa (19-21).A cross sectional institutional based study conducted in Brazil, at the Hospital Maternidade Oswaldo Nazaré (HMON), Rio de Janeiro showed that the prevalence of anemia among pregnant women was 53.7%(22).

In Ethiopia, anemia prevalence among women aged 15-49 years declined from 27% in 2005 to 17% in 2011. But currently it has increased to 24% in 2016 and women who are pregnant or breastfeeding are more likely to be anemic (29% for both groups) as well; these data suggest that anemia is a public health problem in our country. Increases were observed from 2011 to 2016 in all anemia categories(23).The prevalence of anemia among pregnant mothers attending antenatal clinic in Tikur Anbessa Hospital using a cut off level of hemoglobin<11 g/dl (<33% Hematocrit) was 21.3% and the majority of them were of the mild type (hemoglobin: 10-10.9 g/dl(24).

The adverse consequences of maternal anemia may affect not only the neonate and infant but also increase the risk of non-communicable diseases when the child grows into an adult and the risk of low birth weight in the next generation(25). A study conducted in India showed that, maternal anemia resulted decrease in birth weight of fetuses. For instance the average fetal weight of anemic group was 2376.25 whereas the fetal weight of non-anemic group was 2595 gm.(26).

Maldevelopment of placenta is the leading cause of maternal and perinatal mortality and an important factor of fetal growth retardation. Therefore, there is a need to explore the extent of structural changes of placenta, because severity of these morphological parameters change i.e. placental weight, shape, thickness and diameter is correlated with the efficiency of placenta to support the growth of fetus, and low hemoglobin level is likely to be related to insufficient

functioning of the placenta(12, 27).Morphological changes of placenta due to anemia condition influences placenta's exchange and hemodynamic processes(28).

Despite placenta has a remarkable reserve capacity to withstand noxious environment, it is equally true that some unfavorable changes due to maternal anemia causes adverse effect on placenta which ultimately might compromise the well-being of the fetus(29).As mothers affected by anemia, syncytiotrophoblast of entrapped villi degenerates and the stroma of the villi become markedly fibrotic. With this the villous population decreases further and the nutritional demands of the fetus are not adequately fulfilled resulted in placental insufficiency and low birth weight babies(13).

Anemia causes perivillous fibrin depositions which acts as a barrier between fetal and maternal circulation, thereby reducing the transfer of the essential nutrients to the fetus, these resulted in chronic placental insufficiency; causing low birth weight fetus(26).Since placenta is the mirror of the fetomaternal status, the effect of anemia in pregnancy can be so diverse and detrimental to both mother and developing fetus. That's prompted to carry out on the present study to know whether placenta, an organ acting as a bridge between mother and fetus, is associated with major changes due to anemia.

Even though studies on this area are being carried out by various researchers globally, placental changes in maternal anemia have delayed behind than that of diabetes, hypertension and others. Therefore, the present study was conducted to compare the macro/microscopic changes of placentas and birth weight of fetus between non-anemic and anemic mothers and to confirm, agree or deny the findings of the previous researchers in this field of study.

1.3 Significance of the Study

Placenta is certainly the most important organ, but paradoxically the most poorly appreciated and understood organ. Early examination of placental morphology in postpartum period will improve the skill of clinicians to predict birth outcome and will give a clue for earlier identification of the fetus at risk. Thus, it facilitates preparation for management at least in neonatal and childhood period.

Examination of placenta in postpartum period provides much insight to the prenatal health of the baby and the mother. Especially in mothers who had no previous antenatal checkup, a thorough examination of the placental morphology will help in the early diagnosis of the fetal complications, soon after parturition and guide post-partum management of mother and newborn especially in a rural setup. It also gives evidence that, correcting anemia in antenatal period is important to prevent adverse maternal and fetal outcomes as a result of anemia.

It is helpful to decide whether anemia that endangered the wellbeing of the fetus is an acute or a chronic process that aid for medico legal investigation of cases during perinatal morbidity/mortality. As well as, it will provide information for the future care of mother and her offspring.

It is very helpful to scrutinize the exact etiopathogenesis/effects of anemia on placenta; this will give an evidence for clinicians for early supplementation of iron and folic acid to prevent maternal anemia during pregnancy and will strengthen evidence for continuing iron supplementation policy.

It will show the association of anemia, placental changes and birth weight of fetus.

Moreover, few researches are conducted in India, Europe and America regarding this topic. No similar study was conducted in Ethiopia, therefore; this research will offer a base line data for future researchers in Ethiopia and across as well.

2. LITERATURE REVIEW

The placenta forms a functional unit between the mother and the fetus. Therefore, any pathological event that concerns the mother or the fetus will influence the normal function of the placenta, occasionally resulting in morphological change. Severe abnormalities of the placenta may lead to adverse fetal outcome. However, placental lesions are not necessarily the cause of unfavorable outcome, and some structural changes may be the consequences of poor fetal condition(30). Morphology of placenta is characterized by its shape, diameter, thickness, weight, lobes/cotyledons and its micro architecture.

2.1 Altitude, Placental Morphology and Birth Weight of Fetus

Placental growth and development appear remarkably well protected at high altitude, with the modifications observed likely to benefit placental exchange by increasing the surface area and reducing the path length of diffusion. Compared to the placental abnormalities seen in fetal growth restriction at low altitude, the relative protection of placental morphology at high altitude is due to differences in the timing of the insults on placental vs. fetal growth. For instance, the more chronic nature of the growth restriction present, the more possibly portions of the vasculature or other organ systems affected(31).

A study conducted in Saudi Arabia on 20 normal pregnant women living permanently at high altitude (3100 m) and 20 normal pregnant women living permanently at low altitude (500 m) , the mean birth weight of fetus was significantly greater at low altitude(3375±537g) compared to high altitude(3207±524 g)(P <0.001) respectively. The mean placental weight was also significantly greater at low altitude compared to high altitude (p<0.025) (32).

Another study conducted in Bolivia, on 68 pregnancies (24 from low altitude, 44 from high altitude) from mean altitudes of 400 m and 3600 m respectively. The mean birth weight at low altitude of 3.31 kg (range 2.60-4.03 kg) was significantly heavier than high altitude (mean 3.01 kg; range (2.13-3.90 kg)(33). Among studies conducted at high altitude, the most consistent findings are alterations in the uterine vasculature and blood flow and an increase in fetal capillary density in the placenta in association with fetal growth restriction. On average, there is a 100 g decrease in birth weight per 1,000m elevation in altitude(31).

2.2 Shape of Placenta

The shape of the placenta is determined by the persistent area of chorionic villi finally left on chorionic sac; usually this is a circular area, giving the placenta a discoid shape. The definitive shape of the human placenta is a result of the disappearance of villi from all but a circumscribed locus on the chorion(34).

Deviation from the round or oval shape such as an irregularly shaped, bilobed, or multilobed placenta can be attributed to disturbed implantation or uterine abnormalities, but it can be assessed only in the clinicopathological context(30). In the study conducted in North Bengal Medical College and Hospital, Darjeeling, India there was no difference in the shape of placenta that is circular in 67% and oval in 33% of cases in both, anemic and non-anemic groups at term(10).

On the contrary, a research conducted by AL-Hakeem, out of 50 placentas from anemic mothers 40 of them were regular in shape(27 circular and 13 oval),but 10 of the placentas were irregular in shape, Whereas, all 50 placentas of non-anemic mothers were regular in shape (36 circular and 14 oval)(35).

2.3 Weight of Placenta

The weight of placenta is used in the determination of feto placental ratio. The weight of the placenta gives an idea of the amount of substances that is exchanged between the mother and fetus. According to the research conducted in Norway, the mean placental weight was highest in pregnancies with maternal hemoglobin concentrations <9 g/dl, and placental weight decreased by increasing maternal hemoglobin concentrations. In pregnancies with hemoglobin concentrations <9 g/dl, mean placental weight was 701.2 ± 160.6 g, followed by 678.1 ± 150.2 g for hemoglobin concentrations 9–13.5g/dl and 655.5 ± 147.7 g for hemoglobin concentrations >13.5 g/dl (ANOVA, $p < 0.001$)(36).

The mean placental to birth weight ratio was highest in pregnancies with maternal hemoglobin concentrations <9 g/dl (0.203 ± 0.036). There was no difference in mean placental to birth weight ratio between pregnancies with hemoglobin concentrations 9–13.5 g/dl and >13.5 g/dl (0.193 ± 0.040) and (0.193 ± 0.043) respectively)(36).

There was a high placental weight but low birth weight in pregnancies with low hemoglobin concentrations. Thus, in these pregnancies the placental to birth weight ratio was high. If the

growth of the placenta and fetus were proportional across maternal hemoglobin concentrations, one would expect similar placental to birth weight ratio in pregnancies with low, normal and high hemoglobin concentrations(36).

Biswas et al., reported that the weight of placentas in anemic mothers ranges from 220-500 g (mean 382 g, mode 450 g) and the mean feto-placental ratio was 6.1:1. But in non-anemic mothers placental weight ranges from 250-500 g (average 370.3 g, mode 450 g) and their feto-placental ratio was 7:1(26).

Similarly, a study conducted in North Bengal Medical College and Hospital, Darjeeling, India reported that the mean placental weight at term in non-anemic group was found to be 466.7 g, but in anemic group it was 502.2g, this was statistically significant ($p < 0.025$)(10). In addition, another study conducted in Karad tertiary care hospital the mean placental weight of anemic mothers was larger 512.8 ± 89.8 g than normal mother's 459.4 ± 78.8 g, this was statistically significant ($p = 0.001$)(37).

On the contrary, another study conducted in India, on cases of anemia during pregnancy showed a significant reduction in the placental weight, with a feto-placental ratio of 5:1 and an increase in the number (25%) of ill-defined cotyledons (38). The birth weight, placental weight and number of placental cotyledons were significantly reduced in the severely anemic mothers (hemoglobin ≤ 6 g/dl) and direct relationships with the maternal hemoglobin levels also reported(39).

Kiran et al., also documented the weight of placenta is decreased as the level of hemoglobin decreased as follows: hemoglobin concentration of normal ≥ 11 g/dl (581.67 ± 83.97 g), mild anemia 10.0-10.9 g/dl (545.95 ± 73.24 g), moderate anemia 7-9.9 g/dl (499.15 ± 87.52 g) and severe anemia < 7 g/dl (373.60 ± 83.48 g) placental weight respectively(12).

Another research conducted in India; on morphological parameter of placenta found that the mean placental weight was 480 g in the non-anemic group and 350 g, 300g and 250 g respectively in the mild, moderate and severe anemia cases. The mean placental volume was seen to be 360 cc, 320 cc, 300 cc and 250 cc in non-anemic, mild anemic, moderate anemic and severely anemic women, respectively(40).

2.4 Diameter of Placenta

The diameter and thickness of placenta gives an idea about the size of the placenta which may intend to give indirect information about the fetal-placental ratio. Similarly, it also affect the

amount of nutrients, oxygen and carbon dioxide that will pass from the mother to the child and vice versa(41).

A normal term placenta has 15-25 cm and 2-3 cm diameter and thickness respectively. A study conducted by AL-Hakeem found that, the mean diameter of placenta was (16.26) cm in comparison with non-anemic group which was 16.79 cm, whereas, the mean central thickness of placenta area was 24.272 mm in anemic mothers and 28.204mm in non-anemic mothers (35).

Another study conducted in Bangladeshi, revealed that, the mean $\pm$ SD diameters of the placenta were 15.60 ± 0.74 cm; 18.041 ± 1.32 cm and 18.80 ± 1.96 cm in non-anemic, mild anemic and moderate anemic respectively. Diameter of the placenta was greater in anemic groups than the control groups and was statistically significant ($p < 0.001$) between non-anemic and mild anemic and between non anemic and moderate anemic women(42).

They also observed that the mean $\pm$ SD in thickness of placenta between groups were 2.14 ± 0.26 cm, 2.10 ± 0.16 cm and 2.19 ± 0.13 cm in non-anemic, mild anemic and moderate anemic women respectively. Thickness of the placenta was greater in non-anemic groups than anemic groups and was not statistically significant ($p > 0.1$)(42). On the contrary, another research conducted in India revealed; the mean placental thickness of anemic mothers was thicker (1.9 ± 0.2 cm) than that of non-anemic mothers (1.79 ± 0.1 cm)(10).

2.5 Number of Cotyledons

As the chorionic villi invade the decidua basalis, decidual tissue is eroded to enlarge the intervillous space. This erosion produces several wedge shaped areas of decidua, placental septa that project toward the chorionic plate. The placental septa divide the fetal part of the placenta into irregular convex areas called cotyledons(1).

When the placenta encountered by anemia, it causes significant morphological and structural changes of the terminal villi. A study conducted in India showed the numbers of cotyledons in anemic mothers were decreased with the severity of anemia; the number of cotyledons in non-anemic mothers was 18 and in anemic mothers it was 15, 12 and 9 in mild, moderate and severe anemic mothers respectively. Average number of cotyledons in anemic and non-anemic groups was 18 and 12 respectively(40).

Another study conducted on placenta in maternal anemia showed reduced number of cotyledons and increase in incidence of ill-defined cotyledons(43). Similarly, a study on the same area revealed that, out of 60 placentas 30 anemic, and 30 non-anemic; 17% of non-anemic placentas

developed ill-defined cotyledons, and 20% of anemic placentas developed ill-defined cotyledons and none of non-anemic mothers developed succenturate lobe but 3% of anemic mothers developed succenturate lobe(10).

2.6 Placental Histology

Light microscopic studies of the histological sections of placentas under different magnifications revealed variations in villous structures at different regions of the same placenta, and also from one placenta to the other. Structures of the basal plates, chorionic plates and intervillous spaces also varied. These variations were observed in both anemic and non-anemic group of placentas(26).

A study conducted in India on morphological changes of placenta due to anemia observed, placenta of maternal anemia showed villi without complete trophoblastic lining, increased intravillous and perivillous fibrin deposition, increased syncytial knotting and more avascular villi compared to non-anemic mothers(26).

According to a prospective case control study conducted by Lelic et al., revealed, hypoxia, as a consequence of maternal sideropenic/iron deficiency anemia, lead to significant increase of terminal villi blood vessels in 1cm³ of placental tissue and their total volume (44).

Another study conducted by Baske, abnormalities of placental villous blood vessels was not detected in both non-anemic and anemic mothers. But presence of features like fibrinoid necrosis, hyalinization or fibrosis was significantly higher in placental villi of anemic mothers. Occurrence of syncytial knots, calcified patches and stromal fibrosis were also seen to be drastically frequent in anemic than non-anemic mothers(29).

Similarly a study conducted by Soni and Nair , on histological changes of placenta in maternal anemia reported, in non-anemic group each villous had minimal number of small capillaries but in anemic group capillaries per villi were seen to be increased in number and dilated with severity of anemia. Syncytial knots are occasionally scattered in non-anemic mothers but occurrence of fibrosis and syncytial knot tends to increase as the severity of anemia increased. Cytotrophoblastic proliferation (more than one layer of cytotrophoblast in the villi) was not found in any of non-anemic groups, whereas in anemic group cytotrophoblastic proliferation increases with severity of anemia increases(45).

According to Baske, incidence of syncytial knot formation has been significantly increased in anemic group (83% in anemic group compared to 10% in non-anemic group). It was reported

that 30% of anemic placentas forming new syncytiotrophoblast, in cases where the tissue suffers from ischemic damage as a result of low oxygen tension compared to 13% in non-anemic groups. Although there had been a tendency to increase placental calcification with advancing maturity, there was no initial difference in calcification between mature and post mature placenta. Thus, Incidence of calcification showed a significant increase in anemia (40% in comparison to the non-anemic value of 10%). Regarding to intervillous space, 50% has intervillous thrombosis in anemic mothers compared to 30% presence of intervillous thrombosis in non-anemic mothers. Sixty percent of intervillous space of placenta in anemic mothers were increase in width compared, to 6.6% of non- anemic placenta increase in intervillous space width (29).

A study conducted in Pakistan reported that, intervillous space was wider in anemic groups as compared to non-anemic groups. For instance, the Mean $\pm$ SD intervillous space width was 15.98 $\pm$ 3.82 μ m, 28.29 $\pm$ 11.07 μ m, 47.51 $\pm$ 16.84 μ m, and 49.06 $\pm$ 10.7 μ m in non-anemic, mild anemic, moderate anemic and severe anemic groups respectively(46).

The number of syncytial knots was also remarkably increased in placentas at hemoglobin concentration 7-9.9 g/dl and <7g/dl (20.05 $\pm$ 4.96 and 23.87 $\pm$ 4.70 respectively) where at normal hemoglobin level it was 11.27 $\pm$ 2.96(12, 47).

2.7 Maternal Anemia and Birth Weight of Fetus

The linear relationship between maternal hemoglobin and different components of fetal anthropometry indicates that fetal growth is compromised in maternal anemia, particularly when it is moderate and severe anemia. A hospital based research conducted in India revealed, the effect of maternal anemia on fetal birth weight had high correlation coefficient between maternal hemoglobin and birth weight($r=-0.708$, $P<0.001$)(48).

A study conducted in North Bengal Medical College and Hospital, Darjeeling, India revealed, mean birth weight of fetus at term in non-anemic group was 2.589 kg, but in anemic group it was decreased to 2.182 kg. This decrease of 407 g is highly significant ($p <0.001$), but the age of mothers included under the study was 17-30 years(10). Another study conducted in India indicated that the incidence of IUGR and LBW among anemic pregnant mothers was 6.7%, and 28.3% respectively(49).

Similarly, a study conducted by Biswas et al., Birth weights of fetus among maternal anemia groups ranged from 1250-3050 g (mean 2376.25 g, mode 2250 g), where as in non-anemic mothers birth weights of fetus ranged from 1750-3500 g (mean 2595 g, mode 2750 g), and they documented, mean fetal weight in maternal anemia group was less than non-anemic group ($p = 0.01$); however, researchers did not consider factors which might contribute low birth weight of fetus(26).Rasmussen concluded that severe maternal anemia <8 g/dl is associated with birth weight values 200–400 g lower than women with >10 g/dl hemoglobin values, but researchers generally have not excluded other factors that might also have contributed to both LBW and the severity of the anemia(50).

On the contrary, a study conducted in Brazil reported that, birth weight of fetus showed similar results 3375.9 ± 506.9 g and 3300.2 ± 458.4 g of fetus from anemic and non-anemic mothers respectively. The birth weight of fetus from anemic mothers based on gestational age showed 90.9% were appropriate for gestational age and 9.1% were large for gestational age, and regarding new born from non-anemic mothers, 4.8% were small for gestational age, 80.9% were appropriate for gestational age and 14.3% large for gestational age, with no difference in frequency between anemic and non-anemic mothers(22).

3. OBJECTIVES

3.1 General Objective

- To compare the morphological change of placenta and birth weight of the fetus associated with maternal anemia in Dessie Referral Hospital from February, 2018- October, 2018.

3.2 Specific Objectives

- ❖ To compare macro-architecture of placenta (weight, shape, thickness, diameter and number of cotyledons) in anemic and non-anemic mothers.
- ❖ To determine the micro-architecture of placenta in anemic and non-anemic mothers.
- ❖ To compare the birth weight of fetus in anemic and non-anemic mothers.

4. MATERIALS AND METHODS

4.1 Study Design and Study Period

A comparative cross-sectional study design was conducted from May, 2018–June, 2018.

4.2 Study Area

The study was conducted at Dessie Referral Hospital which is found in Amhara region North East Ethiopia. Dessie town is Northeast of 401 Km from Addis Ababa the capital of Ethiopia and 478 km far from Bahir dar which is the capital city of Amhara regional state. The town has an elevation of 2470 meter above sea level with north latitude and east longitude of 11°8' and 39°38' respectively. The Hospital is found in Dessie town serving 2.4 million peoples including neighboring zones. It has more than five wards including the obstetrics and gynecology ward and the hospitals monthly delivery report is above 500 mothers. There are 411 health care workers in the hospital, among those 242 are nurses, 42 General Practitioner, 59 laboratory technician workers, 44 midwives and 23 others (Emergency surgeon, health officer, specialists, anesthetics and pharmacists).

4.3 Source Population

- All term pregnant mothers who attend their delivery at Dessie Referral Hospital.

4.4 Study Population

- Term anemic and non-anemic mothers who attend their delivery at Dessie Referral Hospital during our data collection period.

4.5 Eligibility Criteria

4.5.1 Inclusion Criteria

Group I

- Anemic (mild, moderate and severe) pregnant mothers during our data collection period aged 20-35 years, diagnosed clinically and hematologically.

Group II

- Non-anemic pregnant mothers aged 20-35 years having no signs and symptoms of anemia with their hemoglobin level recorded to be more than or equal to 11g/dl at any time during pregnancy.

4.5.2 Exclusion Criteria

Any pathological condition which affect placenta as well as the fetus, for instance, Pregnant mothers who experience any complication during pregnancy like gestational hypertension, chronic hypertension, pre-existing diabetes mellitus, maternal under nutrition, intrauterine fetal death, precampsia, eclampsia, gestational diabetics mellitus, chronic intrauterine infection, fetal hydrops, Retroviral Infections, multiple pregnancies, placenta accereta, placenta percreta, placenta previa, abruption placenta, Rh Isoimmunization, incomplete delivery of placenta, maternal smoking, diagnosed single umbilical artery ,mothers whose hemoglobin is less than 11g/dl during 1st and 2nd trimester but corrected at the time of data collection, pre and post term pregnancies was excluded from this study.

4.6 Sample Size Determination and Sampling Method

- The desired sample size was calculated by using Open Epi, version 3.0, using the difference of means formula, comparison with equal number of cases and controls(n1=n2).

$$n_1 = \frac{(\sigma_1^2 + \sigma_2^2)(Z_{\alpha/2} + Z_{\beta})^2}{(\mu_1 - \mu_2)^2}$$

- Therefore, the variable of interest arethe following: -

Variables	Mean		Mean difference	Standard deviation		n1=n2	Total sample size
	Anemic(n1)	Non-anemic(n2)	n1-n2	Anemic	Non anemic		
Placental weight	502.16	466.67	35.5	±50.7	±20.6	19	38
Placental thickness	1.90	1.79	0.11	±0.2	±0.1	33	66
Placental diameter	19.38	18.56	0.82	±1.1	±1.7	48	96
No. of cotyledons	12.90	17.46	-4.56	±1.9	±0.1	2	4
Birth weight of babies	2.18	2.58	-0.4	±0.3	±0.29	9	18

Where: -

- n_1 = Sample size for anemic / complicated mothers ($n_1=48$)
- n_2 = Sample size for controls / non-anemic mothers ($n_2 = 48$)
- $Z_{\beta} = 0.84$ for 80% power
- $Z_{\alpha/2} = 1.96$ for 95% confidence level(two sided)
- $\mu_1 - \mu_2$ = Mean difference of the two groups

Note: - The final sample size for gross morphology was the value of placental diameter since it gives the largest sample value which was 48 cases and 48 controls = 96 placentas. However, for microscopic morphology due to budget limitation, 66 placentas (33 anemic, and 33 non-anemic) was used, which was the 2nd largest sample value during our sample size calculation. A recent literature which was published on June 2017 for determining the sample size was used(10).

4.7 Sampling Technique and Procedure

Purposive sampling technique was employed to conduct this research; during our data collection period the number of mothers delivered in Dessie Referral Hospital were 550. From those 550 mothers delivered in the hospital, the sample was taken purposively till the total sample size was achieved. In the meantime, for controls, tissues of 33 placentas out of 48 were taken by lottery method for microscopic morphology. For cases proportional allocation by their level of anemia 14 placentas for mild, 13 placentas for moderate and the whole 6 placentas for severe anemic mothers tissues was taken accordingly.

4.8 Variables

4.8.1 Dependent Variables

- Gross morphology: - weight, shape, thickness, diameter, and number of cotyledons
- Microscopic morphology
- Birth weight of fetus

4.8.2 Independent Variables

- Maternal anemia:-mild, moderate and severe
- Socio demographic characteristics: -Age, residence, educational level, sex of fetus, mode of delivery and parity.

4.9 Operational Definitions

- **Placental morphology:** -the form and structure of placenta such as: shape, diameter, thickness, weight, cotyledons and its micro architecture.
- **Anemia during pregnancy:** - maternal hemoglobin values less than 11g/dl or maternal Hematocrit less than 33%.
- **Mild anemia:**– mothers whose hemoglobin level is between 10 g/dl -10.9 g/dl
- **Moderate anemia:**- mothers whose hemoglobin level is between 7g/dl-9.9 g/dl
- **Severe anemia:**– mothers whose hemoglobin level is less than 7g/dl
- **Birth weight:** – body weight of babies immediately after birth.

4.10 Data Collection Tool and Procedure

4.10.1 Materials

To conduct this study, the materials used were as follows: - weight scale, flat tray, measuring cylinder, wooden block, gloves, towels, plastic sheet, sponge, blade holder and blade, wooden ruler, embedding cassette, tissue block, Hematoxylin and eosin, glass slide with its cover, paraffin wax, Xylene, needle, digital camera, alcohol and formalin solution.

4.10.2 Data Collection Procedure

After getting ethical approval from institutional review board of Addis Ababa University, letter of cooperation was written by Anatomy Department to Dessie Referral Hospital and South Wollo zone health office. Next, it was communicated with Head of Obstetrics and Gynecology department and other concerned bodies. After getting permission from them, informed consent was taken from the mother postpartum period. Data was collected by 2 BSc Midwifery staffs who work in delivery room and I was supervisor of them. Data collection was accomplished within 1 month period.

For each case, a preliminary history was elicited from the mother and her clinical sheets regarding her current and past Medical, Surgical, Obstetrics and Gynecologic histories which affect the morphology of placenta. Then, the fresh placenta was collected as soon as delivery and checked for its completeness; secondly umbilical cord was cut 5cm away from its site of insertion and trimming of a membrane. Then it was washed by running water, cleaned up by towel and labeled with code numbers. After doing this the following placental parameters was observed and measured.

- A. Shape:** - Shape of the placenta was noted after proper inspection. Each placenta was categorized as circular, oval, and irregular in shape.
- B. Diameter:** -After putting the placenta on a flat tray, the maximum diameter was measured with a non-stretched scale graduated in centimeters (cm). Then a second maximum diameter was taken at right angles to the first one. The mean of the two measurements was considered as the diameter of the placenta expressed in centimeter(51).



Figure 1: Maximum diameter measured on the maternal surface in two axes at right angle to each other at Dessie Referral Hospital, Northeast Ethiopia, May-June, 2018.

- C. Thickness:** - With a long needle, placental thickness was measured at five points of each placenta. Each placenta was placed on fetal surface and divided arbitrarily into three equal zones by drawing two circles on the maternal surface. One thickness was measured from the center of the central zone, two from middle and two from peripheral zones. Finally, the mean of all five measurements was calculated and considered as thickness of the placenta(52).

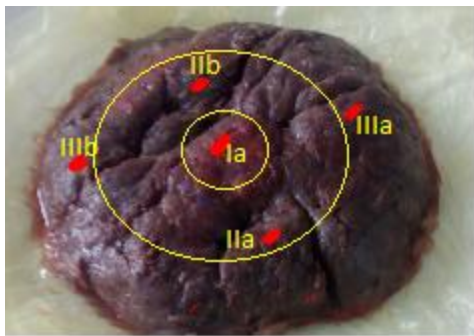


Figure 2: Method of selecting sites from different zones of placenta for measurement of placental thickness. 1 represents the site in the arbitrary central zone; 2a and 2b the sites in the middle zone; 3a and 3b represent the peripheral zones at Dessie Referral Hospital, North East Ethiopia, May-June,

D. Weight: - weight of each placenta was recorded in grams by using a weighting machine scale after removal of membranes, umbilical cord and blood clots inside it.



Figure 3: Method of weighting placenta by standardized weighting machine at Dessie Referral Hospital, Northeast Ethiopia, May-June, 2018.

E. Number of Cotyledons: - After measuring and inspecting the above parameters placenta was fixed by 10% formalin for 24 hours to make the placental septum separation visible to count the cotyledon. Each formalin-fix placenta was taken on both hands; then gentle pressure was applied on the central part of the fetal surface with thumbs of both hands while holding the periphery of the placenta with the other fingers. As a result, the

cotyledons on the maternal aspect become prominent. Then the placenta was put on a flat tray with maternal side facing upward by placing a wood block on the fetal side. Then counting was started from the left side of one end of the placenta going rightward and again turning back to the left in a manner of loop. This counting procedure was repeated until the other end of the placenta is reached. Then, the total number of cotyledons was recorded.

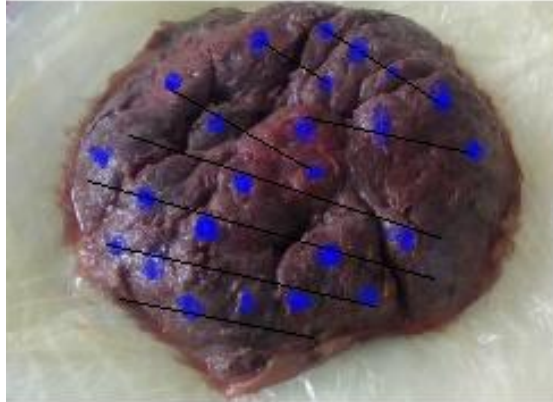


Figure 4: Method of counting placental cotyledons from one end to the other end in a looped manner at Dessie Referral Hospital, Northeast Ethiopia May-June, 2018.

F. Birth weight: - Birth weight of fetuses was noted from the clinical sheets.

G. Placental Histology:-After performing the above parameters placental tissue for microscopy was taken as follows:-

Group I (Non-anemic):- Comprised of 33 placentas from mothers having no sign and symptoms of anemia and their hemoglobin level were recorded to be more than 11 gm/dl.

Group II (Anemic):- Comprised of 33 placentas from anemic mothers (hemoglobin level < 11 gm/dl).

Full thickness of fresh placental tissue for microscopy was taken from the following sites:-

- I. Margins –peripheral area
- II. Centre of the placenta
- III. Between center and margin
- IV. Pathologic area, if any

Then, after taking the tissue it was labeled and kept in 10% formalin for fixation. Each selected tissue again was cut in small pieces of 5 mm × 2mm. Finally, tissue processing was processed as routine (**Annex IV**).

Maximum possible fields were examined for each slide to assess the following parameters:-

- ❖ Abnormalities of blood vessels within villi
 - ✓ Increase in number of terminal villi vessels (when >6 vessels per villi)
- ❖ Abnormalities of intervillous space
 - ✓ Intervillous thrombosis
 - ✓ Intervillous space width
- ❖ Placental calcification
- ❖ Abnormality of cytotrophoblast in villi and presence of syncitial knots; for instance, focal (> 30% of 1 full thickness slide) and Diffuse (>=2 full thickness slide) (29).
- Width of Intervillous space was measured in 5 random fields per each slide under 100X objective with ocular micrometer and mean was calculated.

4.11 Data Quality Assurance

Data was collected and recorded on the checklist by 2 BSc midwifery staff members working in delivery room. They were trained by the principal investigator for 2 days concerning on the placental gross morphology, measurements, how they take tissue from it and appropriate disposal of the placenta. Finally, the collected data was checked for completeness by the principal investigator.

4.12 Data Processing and Analysis

Data was entered using EPI-data Version 4.2.0 and exported to SPSS Version 22 for analysis. Descriptive statistics like frequency, ratio, mean and standard deviation was computed to describe the study variable and was presented by tables and graphs. Association of dependent and independent variables was confirmed by chi-square test. Comparisons of morphology of placenta (weight, shape, diameter, thickness, number of cotyledons and histology) and birth weight of fetus in anemic and non-anemic mothers was analyzed using independent t-test and one way ANOVA. Differences $p < 0.05$ was considered statistically significant.

4.13 Ethical Considerations

The proposal was submitted to Research and Ethics committee of Department of Anatomy School of Medicine, College of Health Sciences, Addis Ababa University. Following approval by the committee it was submitted to Institutional Review Board (IRB). After which, letter of cooperation was written by Department of Anatomy to Dessie Referral Hospital and South wollo zone health bureau. Each study participant was adequately informed about the objective, benefit and risk of the study. Individual verbal informed consent was obtained from every study participant and those who agree were included in the study. Then, giving due respect, confidentiality, and appropriate disposal of placenta was observed/done by the data collectors and the supervisors. The raw data obtained is secured by password, locked in to a locker and is accessed only by the principal investigator.

5. RESULTS

5.1 Socio Demographic and Related Characteristics

Among 96 mothers who participated in this study, 48 mothers were normal hemoglobin level with mean ($\pm$ SD) of 13.8 ± 0.7 g/dl. Of 48 cases, maximum number of cases were mild anemia which was 23 cases (47.9%) with the mean ($\pm$ SD) hemoglobin level of 10.3 ± 0.2 g/dl, 19 (39.6%) cases were moderately anemic mothers with the mean ($\pm$ SD) hemoglobin level of 8.9 ± 0.8 g/dl and 6 (12.5%) of mothers were severely anemic with the mean ($\pm$ SD) hemoglobin level of 4.8 ± 0.9 g/dl. The study revealed, the mean ($\pm$ SD) age of mothers was 27 ± 3.9 years and 64 (66.6%) of the mothers age was within 25-29 years. The mean ($\pm$ SD) body mass index of mothers (BMI) was 20.9 ± 2.1 (Kg/m²) and 22 ± 3.0 (Kg/m²) in anemic and non-anemic mothers respectively. In this study 22 (45.8%) and 33 (66.8%) of anemic and non-anemic mothers live in urban area respectively. Regarding their mode of delivery 73 (76%) of mothers delivered spontaneous vaginal delivery and 23 (24%) of mothers delivered by cesarean section. Out of 96 deliveries 52 (54.1%) of babies were males and 44 (45.9%) of them were females.

The mean ($\pm$ SD) gestational ages of mothers were 38.4 ± 0.4 and 39.1 ± 1 for anemic and non-anemic mothers respectively.

Table 1: Socio demographic and related characteristics of mothers delivered in Dessie Referral Hospital, Northeast Ethiopia, May-June, 2018.

Socio demographic and related variables		Anemic		Non-anemic	
		Frequency	Percent	Frequency	Percent
Occupation	Housewife	32	66.7%	25	52.02 %
	Employed in government institution	7	14.6%	10	20.8%
	Employment in private sector	2	4.2%	4	8.3%
	Merchant	7	14.6%	9	18.75%
	Total	48	100.0%	48	100.0%
Educational level	Can't read and write	21	43.8%	3	6.3%
	Can read and write	10	20.8%	16	33.3%
	Primary	7	14.6%	14	29.2%
	Secondary	6	12.5%	6	12.5%
	Higher education	4	8.3 %	9	18.5%
	Total	48	100.0%	48	100.0%

As the figure shown below, 26(54.2%) of anemic mothers were multiparous followed by 12(25 %) primiparous. Out of 48 non-anemic mothers 21(43.8%) were multiparous followed by 20(41.7%) primiparous and 4 (8.3%) nulliparous.

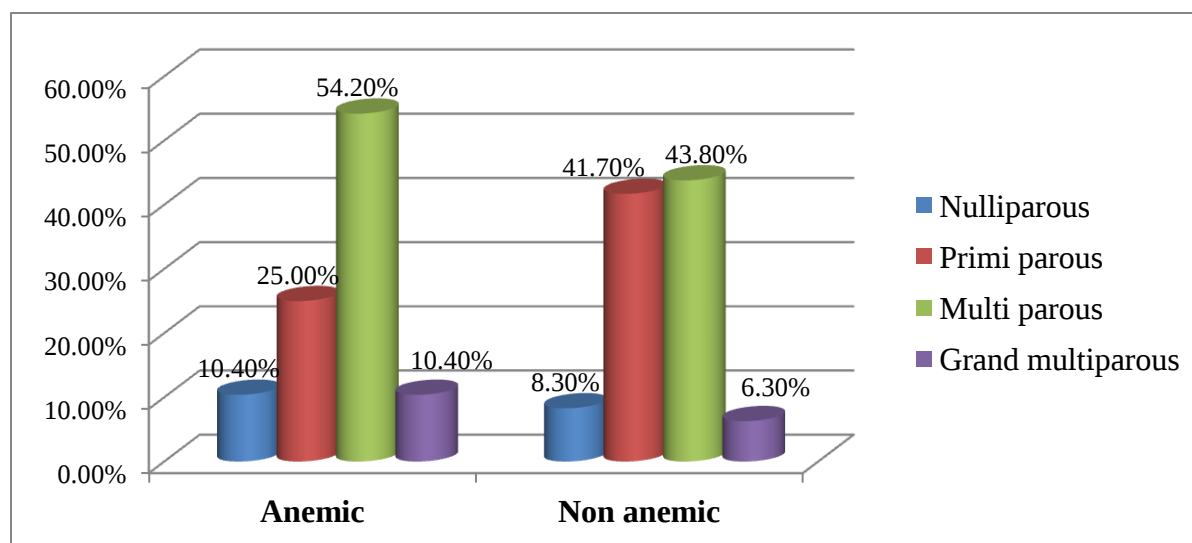


Figure 5; Number of parity distribution in anemic and non-anemic mothers at Dessie Referral Hospital, Northeast Ethiopia, May-June, 2018.

5.2 Placental Morphology and Birth Weight of Fetus

As the figure shown below 26(54.2%) of anemic mothers placenta was circular in shape followed by 16(33.3%) oval. Of non-anemic mothers 31(64.6%) was circular in shape followed by 16(33.3%) oval.

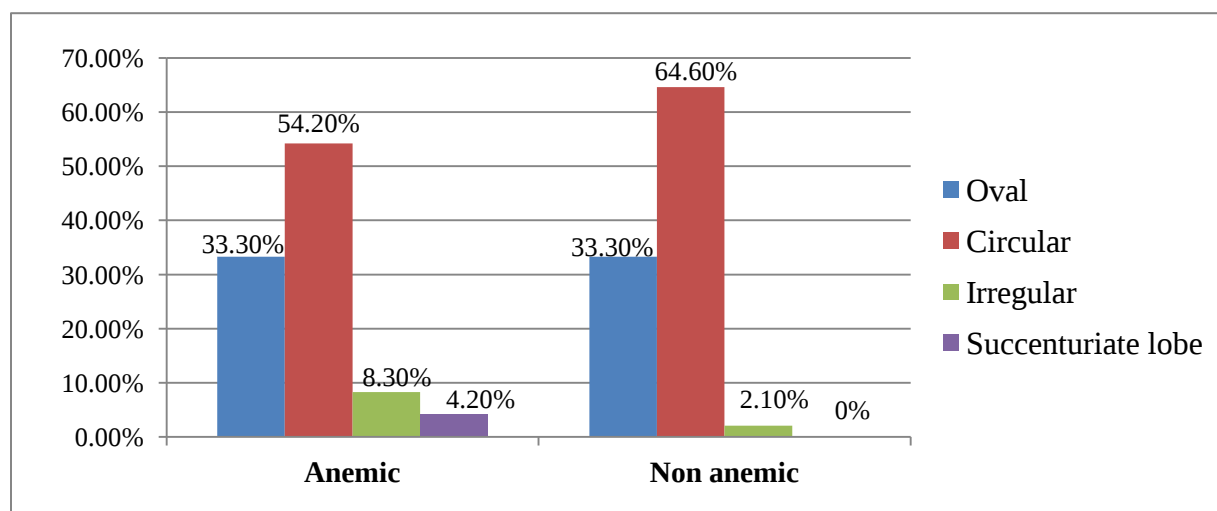


Figure 6: Placental shape both in anemic and non-anemic mothers at Dessie Referral Hospital, Northeast Ethiopia, May-June, 2018.

Table 2; Chi square distribution of placental shape with maternal status at Dessie Referral Hospital, Northeast Ethiopia, May-June, 2018.

Maternal status	Placental Shape				Total	P-value
	Oval	Circular	Irregular	Succunturiate lobe		
Anemic	16(33.3%)	26 (54.2%)	4 (8.3%)	2 (4.2%)	48 (100%)	0.268
Non-anemic	16 (33.3%)	31 (64.6%)	1 (2.1%)	0 (0.0%)	48 (100%)	
Total	32 (33.3%)	57 (59.4%)	5 (5.2%)	2(2.1%)	96 (100%)	

Placental shape has no significant association with maternal status ($p = 0.268$, which is >0.05).

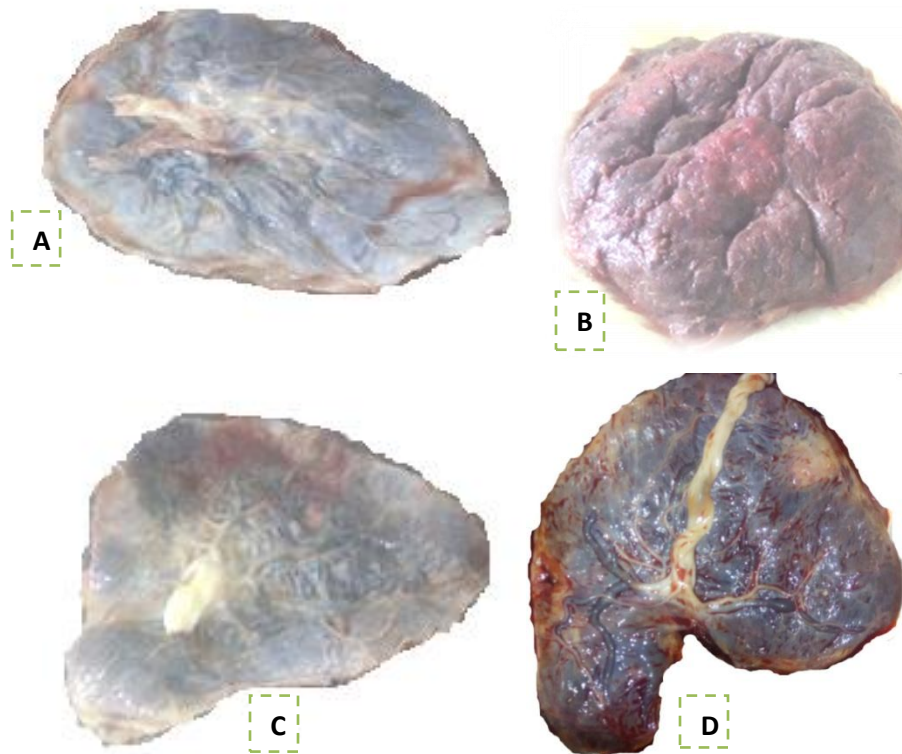


Figure 7: Variety shapes of placenta (A.Oval, B. Circular, C. Irregular, D, Succnturiate lobe) at Dessie Referral Hospital, Northeast Ethiopia, May-June, 2018.

The mean placental thickness of anemic mothers was 22.7 ± 2.4 mm, and in non-anemic mothers it was 20 ± 0.6 mm. There was statistically significant difference between the mean placental thickness of the two groups ($t=5.742$, $p < 0.001$).

In case of mean number of cotyledons, in anemic mothers it was 13.5 ± 1.8 as compared to non-anemic mothers 17.6 ± 1.1 . Based on the result of the two groups by independent sample t- test ($t=-13.153$, $p < 0.001$) revealed there was statistically significant difference in the mean number of cotyledons between the two groups.

The mean placental diameter of non-anemic mothers was 17 ± 1.3 cm; where as in anemic mothers it was 18 ± 1.5 cm which was larger than non-anemic. The statistical significance difference between the two comparison groups was ($t= 3.14$, $p=0.002$), indicates that there was a significant difference in placental diameter of anemic and non-anemic mothers.

Regarding the mean birth weight of babies who were delivered from non-anemic mothers was 3035 ± 305 g. But in anemic mothers the mean birth weight of babies was 2562 ± 360 g which was smaller than babies delivered from non-anemic mothers. The statistical significance between the two comparison groups ($t = -6.938$, $p < 0.001$) indicates that there was a significant difference in the mean birth weight of fetus between them.

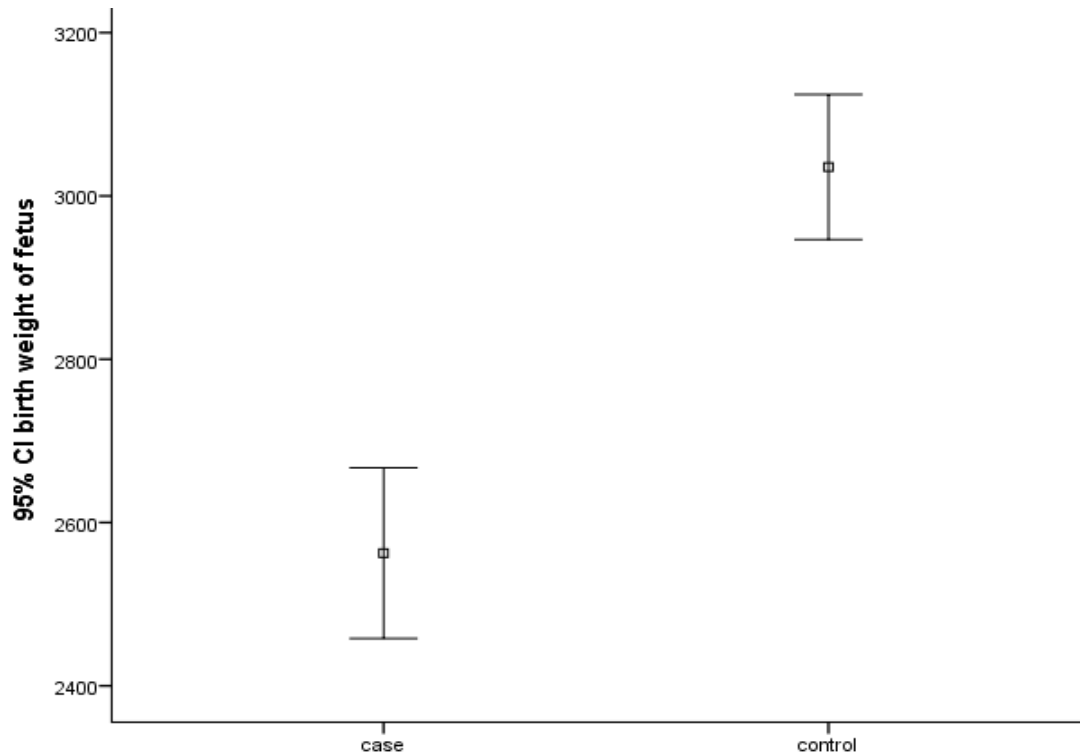


Figure 8:- Error bar graph shows the mean birth weight of babies both in anemic(case) and non-anemic(control) mothers at Dessie Referral Hospital, Northeast Ethiopia, May-June, 2018.

The mean placental weight of anemic mothers was 544 ± 98 g, and in non- anemic mothers it was 502 ± 93 g. The statistical significance between the two groups was ($t = 2.162$, $p = 0.03$). This indicates that anemic mothers placenta was significantly larger in weight compared to non-anemic mothers. In this study, the placental-Fetal ratio and placental coefficient of anemic and non-anemic mothers' was $1:4.7(0.212)$ vs. $1:60(0.165)$ respectively.

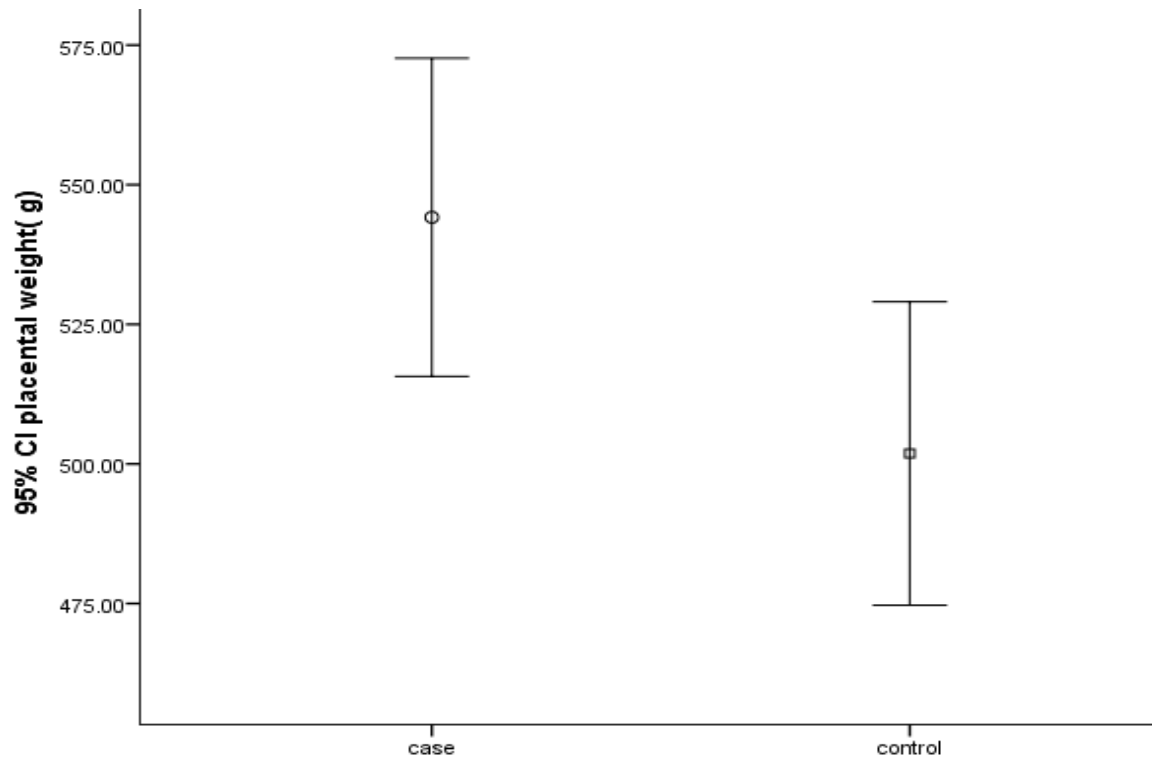


Figure 9:- Error bar graph shows mean placental weight of anemic and non-anemic mothers in Dessie Referral Hospital, Northeast Ethiopia, May- June, 2018.

Table 3 : Independent sample t- test of placental morphology and birth weight of fetus both in anemic and non-anemic mothers, at Dessie Referral Hospital, Northeast Ethiopia, May- June, 2018.

Variables	Anemic (case)	Non-anemic (control)	t- statistic	p- value
Placental weight	544 ±98 g	502±93 g	2.162	0.03
Placental diameter	18±1.5 cm	17±1.5 cm	3.14	0.002
Placental thickness	22.7±2.4 mm	20±0.6 mm	5.742	< 0.001
Number of cotyledons	13.5±1.8	17.6±1.1	-13.153	< 0.001
Birth weight of fetus	2562±360 g	3035±305 g	-6.938	< 0.001

As the table shown below, in non-anemic mothers the mean birth weight of babies delivered by cesarean section was 3166±333 g, which was larger when compared to babies delivered by

spontaneous vaginal delivery (2991±287 g). However, it has no significant difference between the two modes of delivery ($t = -1.755$, $p = 0.085$). Similarly, in anemic mothers the mean birth weight of babies delivered by cesarean section was larger (2636±233 g) when compared to babies delivered by spontaneous vaginal delivery (2437±351 g). But statistically it has no significance difference between the mean birth weight of the two modes of delivery ($t = -1.006$, $p = 0.323$).

Table 4: Comparison of mean birth weight of fetus with their mode of delivery both in anemic and non-anemic groups of mothers using independent sample t- test in Dessie Referral Hospital, Northeast Ethiopia, May- June, 2018.

Maternal Status	Birth weight of Babies(Mean ± SD)		t – statistics	P- value
	SVD	C/S		
Anemic	2540±389 g	2636±233 g	-1.006	0.323
Non anemic	2991±287 g	3166±333 g	-1.755	0.085

SVD= spontaneous vaginal delivery, C/S= cesarean section, SD- standard deviation

Based on the findings of this study the mean birth weight of male babies delivered from anemic mothers was (2611±327 g); which was relatively larger than mean birth weight of female babies' (2500±397g). However, there was no statistically significant difference between birth weight of the two sex groups ($t = 1.062$, $p = 0.294$). Similarly, in non-anemic mothers mean birth weight of male babies' was (3080±256 g); which was relatively larger than mean birth weight of female babies (2986±350g). But there was no statistically significant difference between the mean birth weights of female and male babies in non-anemic mothers ($t = 1.055$, $p = 0.297$).

Table 5: Independent sample t- test to compare mean birth weight of babies with their sex both in anemic and non-anemic groups of mothers in Dessie Referral Hospital, Northeast Ethiopia, May- June, 2018

Maternal status	Birth weight of fetus (Mean ±SD)		t- statistics	P- value
	Male	Female		
Anemic	2611±327 g	2500±397 g	1.062	0.294
Non-anemic	3080±256 g	2986±350 g	1.055	0.297

SD –standard deviation

The current study revealed that placental weight of anemic mothers who were delivered by spontaneous vaginal delivery (541±105 gram) was less than mothers delivered by cesarean

section (553±69 gram), There was no significant difference between the two modes of delivery in anemic mothers ($t= -0.361, p=0.71$). Similarly in non-anemic mothers the mean placental weight delivered by spontaneous vaginal delivery was 491±71g, and by cesarean section it was 531±140g. There was no statistically significant difference between the two modes of delivery in non-anemic mothers ($t= -0.996, p=0.35$)

Table 6: Independent sample t- test to compare the mean placental weight of both anemic and non-anemic group of mothers with their mode of delivery at Dessie Referral Hospital, North East Ethiopia, May- June, 2018.

Maternal status	Placental weight (Mean ± SD)		t- statistic	p – value
	SVD	(C/S)		
Anemic	541±105 g	553±69 g	-0.361	0.71
Non-anemic	491±71 g	531±140 g	-0.966	0.35

SVD - spontaneous vaginal delivery, C/S - cesarean section, SD- standard deviation

Based on the findings of this study, mean placental weight of non-anemic mothers who delivered female baby was (506±78 g); which was relatively larger than mean placental weight of mothers who delivered a male baby (497±107g). However, there was no statistically significant difference between placentas of the two sex groups ($t= -0.358, p=0.722$). Similarly, in anemic mothers mean placental weight of mothers who delivered female baby was (559±123 g); which had relatively larger placental weight than anemic mothers who delivered male baby (532±73g). There was no statistically significant difference between the mean placental weight of having delivered female and male baby in anemic mothers also ($t= -0.899, p = 0.375$).

Table 7: Independent sample t- test to compare the mean placental weight of both anemic and non anemic groups of mothers with sex of their fetus at Dessie Referral Hospital, Northeast Ethiopia, May-June, 2018.

Maternal status	Placental Weight(Mean ±SD)		t- statistic	p- value
	Male	Female		
Anemic	532±73 g	559±123 g	- 0.899	0.375
Non-anemic	497±107 g	506±78 g	-0.358	0.722

SD- standard deviation

In the current study; the mean placental weight increases as the level of hemoglobin decreases from normal to moderate anemic mothers, then it decreases when the level of hemoglobin

reaches at severe anemia level (Table, 8). There was a significant difference in the mean placental weight between groups (ANOVA, $F=8.974$, $P< 0.001$). Using multiple comparison of groups there was a significant difference in mean placental weight of (non-anemic vs. moderate anemic mothers $p<0.001$) but there was no significant difference between (non-anemic vs. mild anemic $p=0.983$) and (non-anemic vs. severe anemic mothers $p=0.675$).

Based on this study, it was found that placental thickness increases as the level of hemoglobin decrease still it reaches at severe anemia, but decreases in severe anemic mothers (Table, 8), with the significance level of (ANOVA, $F=38.4$, $p< 0.001$). Based on multiple comparison between groups, there was significant difference between non-anemic with mild ($p<0.001$), moderate ($p<0.001$) and severe anemic ($p=0.02$) mothers.

In the case of mean number of cotyledons, as the level of hemoglobin decreases the number of cotyledons also decreases; for instance, 17.6 ± 1.1 , 14.3 ± 1.9 , 13 ± 0.9 and 11 ± 1.1 for non-anemic mild, moderate and severe anemic mothers respectively, with the significance level between groups (ANOVA, $F= 86.8$, $p<0.001$). By multiple comparison of non-anemic with different anemia levels there was a statistically significant difference between (normal vs. mild $p < 0.001$), (normal vs. moderate $p<0.001$), (normal vs. severe $p <0.001$).

As the level of hemoglobin increases the mean placental diameter also increases from non-anemic to moderate anemic mothers but decreased in severe anemic mothers (Table, 8), with the significance difference between groups (ANOVA, $F=13.29$, $p<0.001$). Based on multiple comparison of non-anemic with anemic mothers there was a statistically significant difference between non-anemic to moderate anemic mothers ($p<0.001$). But it was insignificant between (non-anemic vs. mild $p=0.525$) and (non-anemic vs. severe $p= 0.538$) mothers.

The mean birth weight of fetus decreases as the level of hemoglobin decreases (Table, 8): the significance difference between the mean birth weight of groups was (ANOVA, $F=28.5$, $p<0.001$). Based on the multiple comparison of non-anemic with anemic mothers there was a statistically significant difference in birth weight of fetus between non-anemic to different hemoglobin levels of anemic mothers as follows: (non-anemic vs. mild $p=0.001$), (non-anemic vs. moderate $p<0.001$), and (non-anemic vs. severe $p<0.001$).

Table 8: One way ANOVA result on morphologic characteristics of placenta and birth weight of fetus at different maternal hemoglobin levels in Dessie Referral Hospital, Northeast Ethiopia, May- June, 2018.

Variables	Group I (Non-anemic)	Group II (Anemic)			ANOVA	p- value
		Mild anemic(n=23)	Moderate anemic(n=19)	Severe anemic(n=6)		
Placental weight	502±93 g	510±41 g	612±116 g	455±39 g	8.974	0.001
Placental diameter	17±1.3 cm	17.5±1.1 cm	19±1.2 cm	16±1.0 cm	13.89	0.001
Placental thickness	20.0±0.6 mm	22±1.5 mm	23.8±2.4 mm	18±1.2 mm	38.4	<0.001
No. of cotyledons	17.5±1.1	14.3±1.9	13±0.9	11±1.1	86.8	<0.001
Birth weight of fetus	3035±305 g	2721±261 g	2536±318 g	2033±314 g	28.5	<0.001

Table 9: Multiple comparison (post-hoc) of placental variables between non-anemic to anemic mothers at Dessie Referral Hospital, Northeast Ethiopia, May-June, 2018.

Variables	Control vs. Mild	Control vs. Moderate	Control vs. Severe
Placental weight	0.983	<0.001	0.675
Placental diameter	0.018	0.003	0.004
Placental thickness	<0.001	<0.001	0.02
No. of cotyledons	<0.001	<0.001	<0.001
Birth weight of fetus	0.001	<0.001	<0.001

ANOVA- Analysis of Variance,

Control- Non-anemic

4.3 Microscopic Morphology

Based on the table shown below, in the present study 72.5 % of anemic terminal villi vessels were dilated, whereas in non-anemic placentas the dilated terminal villi vessels were 24.2%. Terminal villi vessel dilation was increased as severity of anemia increases. Based on the result of this study, 75.7% of anemic placentas had increase in number of terminal villi vessels, but in non-anemic placentas vessel proliferation accounts 15.15%. Placental calcification in anemic mothers accounted 72.7% when compared to non- anemic placentas which were accounted 54.5%. Of anemic placentas 51.5%had intervillous thrombosis, but it was not observed in non-anemic placentas. The intervillous space of anemic placenta was wider compared to non-anemic placenta. For instance, the width of intervillous space was (19.3±1.8µm) in non-anemic,(27.5±6.4µm)mild anemic, (33.0±5.5µm)moderate anemic, and(40.3±3.4µm)severe

anemic placentas respectively. In anemic placenta 48.5% of cytotrophoblast proliferation was observed compared to non-anemic which was 3%. Diffuse syncytial knots was 81.8% in anemic placentas, whereas in non-anemic placentas it was 15.1%.

Table 10; Distribution of microscopic findings in anemic and non- anemic mothers placenta at Dessie Referral Hospital, Northeast Ethiopia, May-June, 2018.

Microscopic findings	Anemic (N=33)		Non- anemic (N=33)	
	Frequency	Percent	Frequency	Percent
Vascular dilation	24	72.7 %	8	24.2%
Vascular proliferation	25	75.7%	5	15.1%
Calcification	24	72.7%	18	54.5%
Intervilous thrombosis	17	51.5%	0	0%
Cytotrophoblast proliferation	16	48.5%	1	3.0%
Syncytial knots	27	81.8%	5	15.1%

Based on the table shown below, terminal villi vascular dilation was significantly increased in maternal anemia with the level of significance ($p < 0.001$).

Table 11: Chi square distribution of terminal villi vessels dilation in anemic and non-anemic mothers at Dessie Referral Hospital, Northeast Ethiopia, May-June, 2018.

Maternal status	Terminal villi vascular dilation		Total	χ^2 - statistic	p-value
	Yes	No			
Non anemic	8(24.2%)	25(75.8%)	33(100%)	15.52	0.001
Anemic	24(72.7%)	9(27.3%)	33(100%)		
Total	32(48.5%)	34(51.5%)	66(100%)		

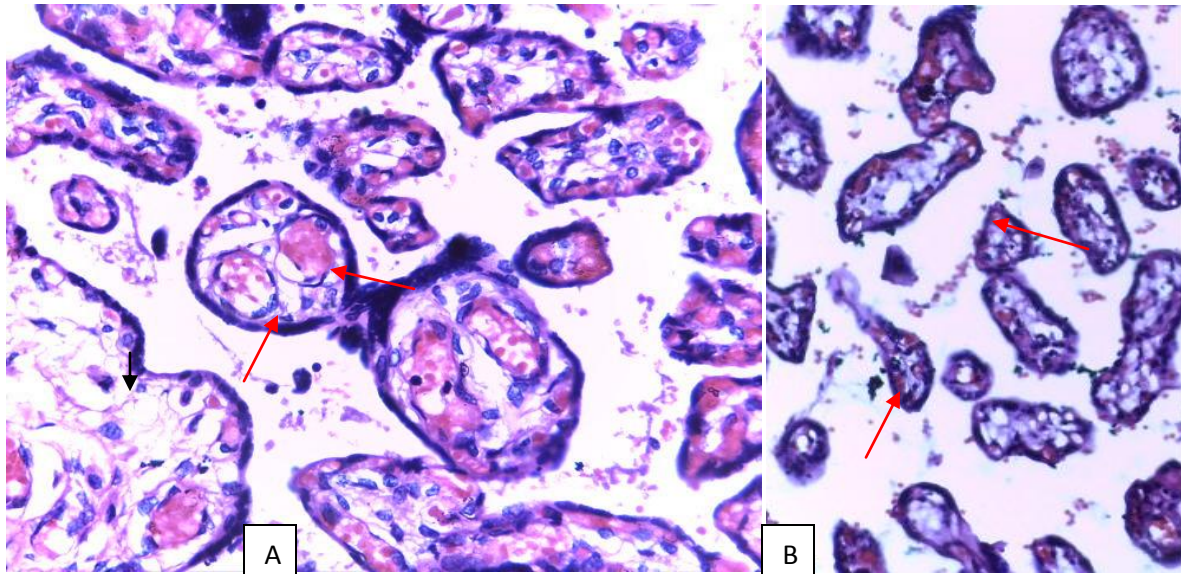


Figure 10: Photomicrograph showing markedly increased dilated and congested terminal villi vessels (arrows) in anemic placenta (A) than non-anemic placenta (B) at Dessie Referral Hospital, Northeast Ethiopia, May-June, 2108. Stain used Hematoxyline and Eosin, 40X.

Vascular proliferation in the terminal villi of anemic placenta was prominent, and it was significantly increased in anemic placenta ($p < 0.001$).

Table 12: Chi square distributions of terminal villi vascular proliferations in anemic and non-anemic mother's placenta at Dessie Referral Hospital, Northeast Ethiopia, May-June, 2018.

Maternal status	Vascular proliferation		Total	χ^2 - statistic	p- value
	Yes	No			
Non-anemic	5(15.2%)	28(84.8%)	33(100%)	24.44	<0.001
Anemic	25(75.2%)	8(24.2%)	33(100%)		
Total	30(45.5%)	36(54.5%)	66(100%)		

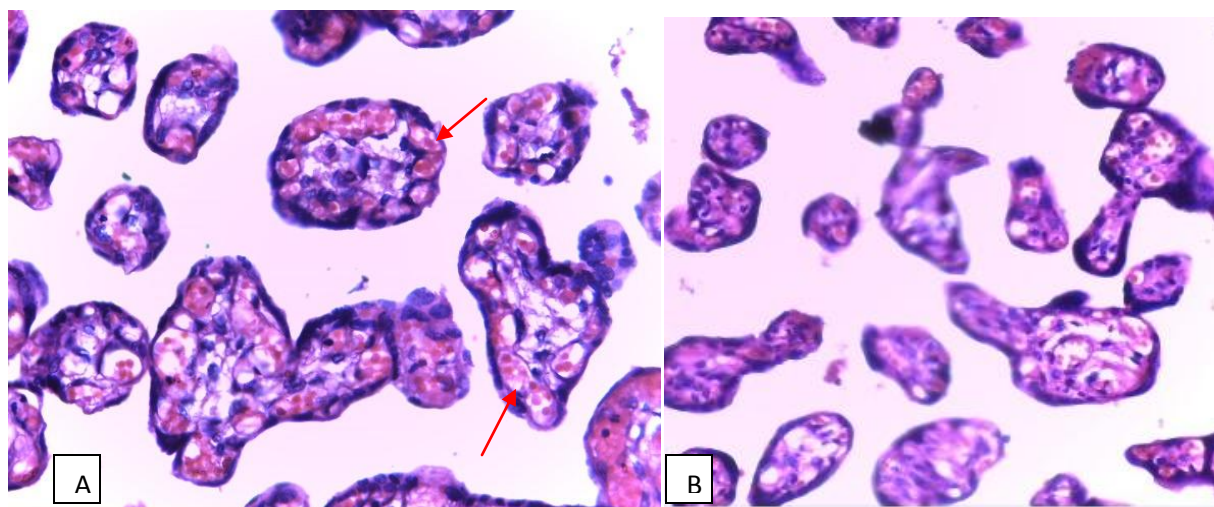


Figure 11: Photomicrograph showing increase in number of capillaries (proliferation) in the terminal villi of placenta (arrows), in anemic placenta (A) than non-anemic placenta (B) at Dessie Referral Hospital, Northeast Ethiopia, May-June, and 2018. Stain used Hematoxyline and Eosin, 40X.

As shown on the table below, even though, placental calcification was prominent in anemic than non-anemic placenta, it was not significantly higher, ($p=0.12$).

Table 13: Chi square distributions of placental calcification in anemic and non- anemic mothers at Dessie Referral Hospital, Northeast Ethiopia, May-June, 2018.

Maternal status	Calcification		Total	χ^2 - statistic	P-value
	Yes	No			
Non-anemic	18(54.5%)	15(45.5%)	33(100%)	2.35	0.12
Anemic	24(72.7%)	9(27.3%)	33(100%)		
Total	42(63.6%)	24(36.4%)	66(100%)		

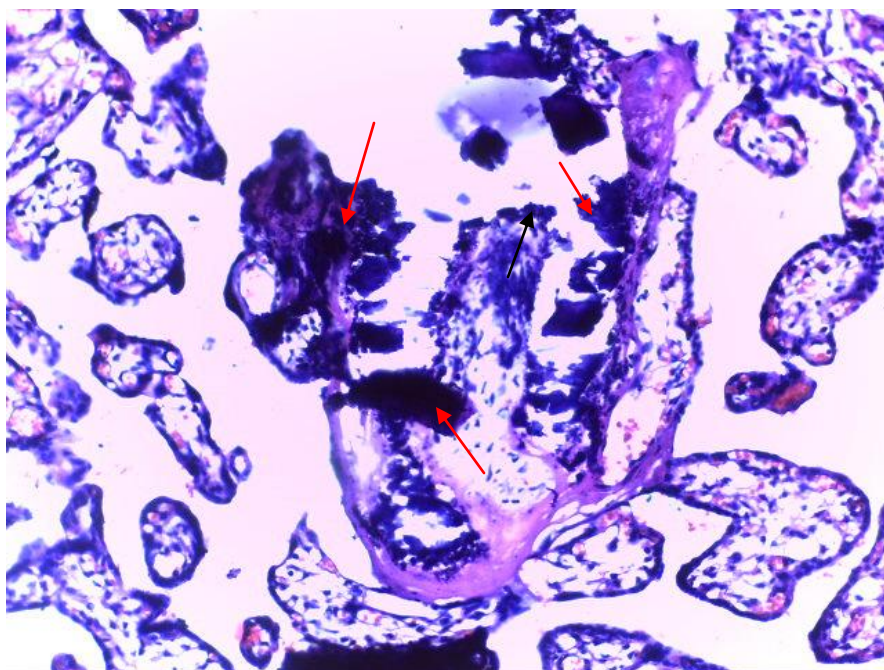


Figure 12: Photomicrograph showing extensively calcified placenta (arrows) in anemic group of mothers at Dessie Referral Hospital, Northeast Ethiopia, May-June, 2018. Stain used H and E, 10X.

In this study, intervillous space was wider in anemic placenta as compared to non-anemic placenta. There was a significant difference in intervillous width of anemic and non-anemic groups (ANOVA= 64.03, $p < 0.001$).

Table 14: One way ANOVA shows Width of intervillous space in anemic and non- anemic mothers' placenta at Dessie Referral Hospital, Northeast Ethiopia, May-June, 2018.

Maternal status	Mean $\pm$ SD(μ m)	ANOVA	p-value
Non-anemic	19.3 $\pm$ 1.8	64.03	< 0.001
Mild anemic	27.5 $\pm$ 6.4		
Moderate anemic	33.0 $\pm$ 5.5		
Severe anemic	40.3 $\pm$ 3.4		

μ m= micrometer

In the current study, intervillous thrombosis was significantly high in anemic mothers' placenta, but in non-anemic placenta it was not observed, ($p < 0.001$).

Table 15: Chi square distributions of intervillous thrombosis in anemic and non-anemic mothers' placenta at Dessie Referral Hospital, Northeast Ethiopia, May-June, 2018.

Maternal status	Intervillous thrombosis		Total	χ^2 - statistic	p- value
	Yes	No			
Non-anemic	0	33(100%)	33(100%)	22.89	<0.001
Anemic	17(51.5%)	16(48.5%)	33 (100%)		
Total	17(25.8%)	49(74.2%)	66(100%)		

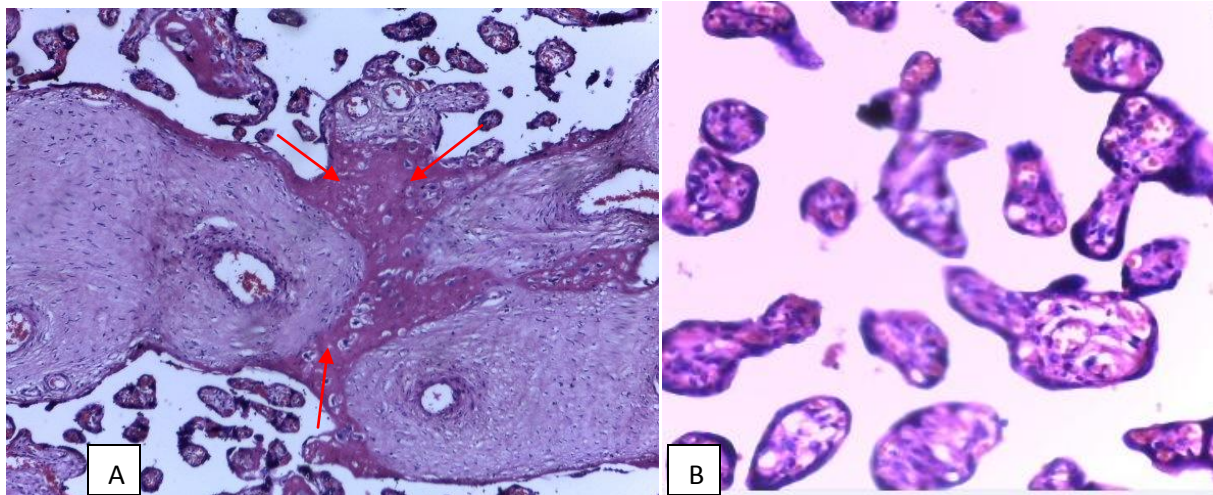


Figure 13: Photomicrograph showing intervillous thrombosis (arrows) in placentas of maternal anemia (A), and without thrombosis in the control placenta (B) at Dessie Referral Hospital, Northeast Ethiopia, May-June, 2018. Stain used H and E, 100X.

Cytotrophoblast proliferation was prominent in anemic placentas, but in non-anemic placenta the proliferation was rare. It was a significantly high in anemic placentas ($p = 0.001$).

Table 16: Chi square distribution of cytotrophoblast proliferation in anemic and non-anemic mothers placenta at Dessie Referral Hospital, Northeast Ethiopia, May-June, 2018.

Maternal status	Cytotrophoblast proliferation		Total	χ^2 - statistic	p-value
	Yes	No			
Non-anemic	1(3%)	32(97%)	33(100%)	17.82	0.001
Anemic	16(48.4%)	17(51.61%)	33(100%)		
Total	17(25.8%)	49(74.2%)	66(100%)		

Based the table shown below, most of anemic placentas had diffuse syncytial knots. But in non-anemic focal syncytial knots was prominent. Moreover, diffuse syncytial knot was significantly increased with severity of anemia ($p < 0.001$).

Table 17: Chi square distribution of syncytial knots in anemic and non-anemic mothers' placenta at Dessie Referral Hospital, Northeast Ethiopia, May-June, 2018.

Maternal status	Syncytial knots		Total	χ^2 - statistic	p-value
	Diffuse	Focal			
Non-anemic	5(15.1%)	28(84.8%)	(100%)	29.02	<0.001
Anemic	27(81.8%)	6 (18.2%)	33(100%)		
Total	32(48.5%)	34(51.5%)	66(100%)		

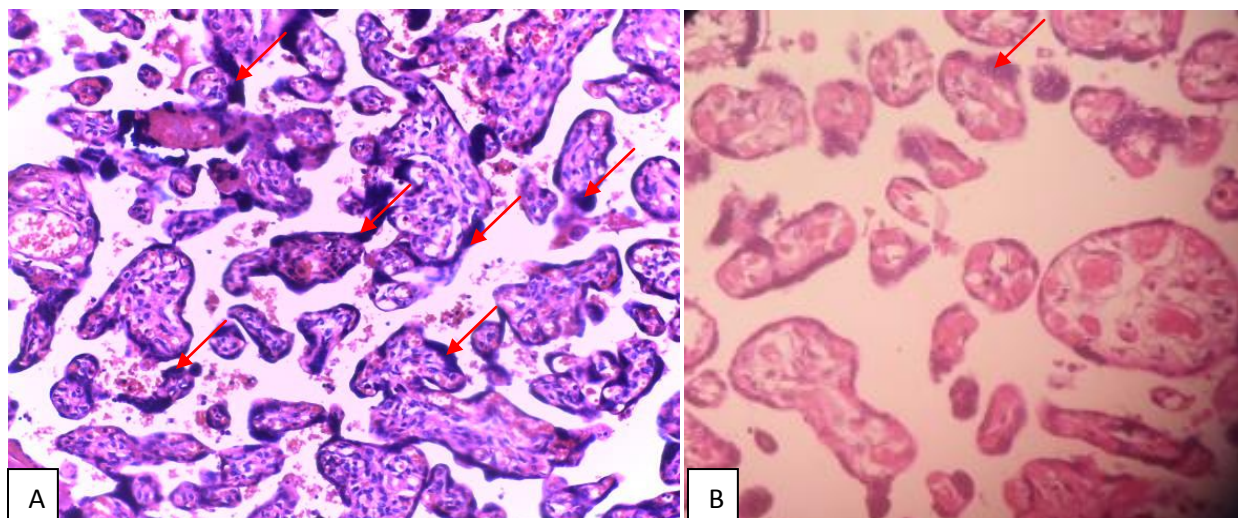


Figure 14; Photomicrograph showing diffuse syncytial knots (red arrows) (A) in anemic placenta and Focal syncytial knots in non-anemic placenta (B) at Dessie Referral Hospital, Northeast Ethiopia, May-June, 2018. Stain used H and E, 40X.

6. DISCUSSION

The placenta is a functional component between mother and fetus. Any unpleasant incident related to mother or fetus may seriously distress the structure and function of placenta and even life of the fetus. The weight of the placenta has a significant importance and much information can be gathered by proper weight recording. In the current study the mean placental weight of anemic mothers was (544 ± 98 g) significantly larger than that of non-anemic mothers (502 ± 93 g) ($t = 2.162$, $p = 0.03$). This is in line with other studies conducted in India (502 ± 50.7 vs. 466 ± 20.7 g, $p < 0.025$), (512.8 ± 89.8 g vs. 459.4 ± 74.8 g, $p = 0.001$) (10, 37). This mean placental enlargement in anemic mothers may be due to adequate compensatory capacity of anemic placentas to satisfy the intrauterine environment. An increase in placental weight in anemic mothers also confirmed by Biswas et al., even though there was no statistical significance difference between the two group (382g vs. 370.3g, $p = 0.4$) (26).

In this study there was an increase in placental weight as the hemoglobin level decreases from non-anemic to moderate anemia. But when the hemoglobin levels of the mother reaches at severe anemia the placental weight was decreased (Table, 8). This result is in contrary to other studies conducted in Norway; In pregnancies with hemoglobin concentrations < 9 g/dl, mean placental weight was 701.2 ± 160.6 g, followed by 678.1 ± 150.2 g for hemoglobin concentrations 9–13.5 g/dl, and 655.5 ± 147.7 g for hemoglobin concentrations > 13.5 g/dl (36). This discrepancy may be due to the use of different cut off point for anemia severity, and ‘it may be suggested that higher placental weight in pregnancies with low hemoglobin concentrations is only pregnancies with optimal potential for placental growth are successful. Hence, it is possible that women with low hemoglobin concentrations have higher risk of placental atrophy and miscarriage’.

Decrease in weight of placenta when the level of hemoglobin decreases was also revealed (480g(non-anemic) vs. 350g(mild), 300g(moderate) and 250g(severe) mothers respectively, and 581.67 ± 83.97 g(non-anemic) vs. 545.95 ± 73.24 g (mild), 499.15 ± 87.52 g (moderate) and 373.60 ± 83.48 g (severe) mothers respectively (12, 40). This inconsistency may be due to the difference in mode of delivery, discrepancy in collection and processing techniques and difference in inclusion and exclusion criteria of study participants.

It is also evident that placental weight maintains more or less a constant relation with the fetal weight. In this study the placental-fetal weight ratio (P/F ratio) was varied among anemic and non-anemic mothers. For instance, the mean placental fetal ratio in this study was (0.212 vs.

0.166) in anemic and non-anemic mothers respectively. This result is in line with other studies conducted in Norway (0.203 vs. 0.193) and India (0.160 vs. 0.142) in anemic and non-anemic mothers respectively **(10, 36)**. The high placental to birth weight ratio with low maternal hemoglobin concentrations may be due to the differences in placental growth relative to fetal growth across different maternal hemoglobin concentrations.

Placental shape is usually described as flattened discoid with an approximate circular margin. Even though it was statistically insignificant, in the current study placentas of anemic mothers were 4(8.3%) irregular in shape and 2(2.1%) succenturiate lobe; which were somewhat deviates from its typical shape. This finding is parallel with other findings conducted by AL-Hakeem, in which 10 out of 50 placentas were irregular in shape**(35)**. On the other hand another researcher reported on 60 mothers (30 anemic and 30 non-anemic) there was no difference in the shape of placenta in which 67% and 33% of both anemic and non-anemic placenta was circular and oval in shape respectively. This inconsistency may be due to the fact that the difference in the number of study participants and discrepancy in severity of anemia cases under the study**(10)**.

The number of cotyledons shows discrepancy in anemic and non-anemic mother's placenta. Mongia et al, stated that, average number of cotyledons in non-anemic and anemic was 18 and 12 respectively**(40)**. Similarly, another researcher revealed that maternal anemia showed reduced number of cotyledons and increase in incidence of ill-defined cotyledons than non-anemic mothers**(43)**. Our study finding is in concurrence with the above results in which the average number of cotyledons was 13.5 ± 1.8 in anemic and 17.6 ± 1.1 in non-anemic mothers respectively. The reduction in number of cotyledons in anemic mothers may be due to the fact that the increase in number of ill-defined cotyledons.

The linear relationship between maternal hemoglobin and different components of fetal anthropometry indicates that fetal growth is compromised in maternal anemia, particularly when it is moderate and severe anemia. In the current study birth weight of fetus in non-anemic group was significantly larger (3035 ± 305 g) than anemic mothers (2562 ± 360 g) with ($p < 0.001$). This result is comparable with others (2589 g vs. 2182g, $p < 0.001$) and (2595 g vs. 2376 g $p < 0.001$) in non-anemic and anemic mothers respectively **(10, 26, 50)**. This reduction in birth weight of fetus in anemic mothers may be due to the fact that a reduction in the exchange surface of the placenta which may be due to long lasting hypoxia of placenta causes direct deterioration of fetal growth.

The diameter and thickness of placenta gives an idea to the size of placenta. In the current study the mean diameter of placenta in non-anemic and anemic mothers was (16.4 ± 1.0 cm vs. 17.6 ± 1.1 cm, $p < 0.001$) respectively. This result is comparable with another studies conducted in India in which the mean placental diameter of anemic mothers was wider than non-anemic mothers (19.38 ± 1.1 cm vs. 18.56 ± 1.7 cm) **(10)**. This may be due to the fact that the compensatory increment in the size of anemic placentas increases its diameter.

Decrease in placental diameter in anemic mothers also reported by AL-Hakeem, the mean diameter of placenta in anemic mothers was 16.26 cm in comparison with non-anemic group which was 16.79 cm **(35)**. This discrepancy may be due to the difference in inclusion of study participants and difference in genetic and environmental factor.

In our study as the level of hemoglobin decreases the diameter of placenta increases till it reaches at the level of severe anemia such; in non-anemic (17 ± 1.3 cm), mild (17.5 ± 1.1 cm), moderate (19 ± 1.2 cm) and severe (16 ± 1.0 cm) respectively with ($t = 13.9$, $p < 0.001$). This result was in line with other studies conducted in Bangladeshi with the diameter of normal (15.60 ± 0.74 cm); mild (18.041 ± 1.32 cm) and moderate (18.80 ± 1.96 cm), $p < 0.001$) even though they couldn't incorporate severe anemia cases **(42)**. The decrease in diameter of placenta in severe anemic cases in the current study may be due to placental insufficiency as a result of irresistibility of the disease process.

The thickness of placenta gives an idea about the size of placenta which may intend to give indirect information about the fetal-placental ratio. In the current study the mean placental thickness of non-anemic mothers was thinner than anemic mothers with the significance level of ($p < 0.001$). This result is in line with other studies conducted in India, in which the mean placental thickness of anemic mothers was thicker (1.9 ± 0.2 cm) than non-anemic mothers (1.79 ± 0.1 cm) **(10)**. This may be due to the fact that the adequate compensatory hypertrophy due to anemia tends to increase its thickness as well.

In this study as the level of hemoglobin decreases the mean placental thickness was 20.6 ± 0.6 mm, 22 ± 1.5 mm, 23.8 ± 2.4 mm and 18 ± 1.2 mm in non-anemic, mild, moderate and severe mothers respectively and it was significant ($p < 0.001$). This result is not comparable with other studies conducted in Bangladesh in which 2.14 ± 0.26 cm, 2.10 ± 0.16 cm and 2.19 ± 0.13 cm in non-anemic, mild anemic and moderate anemic mothers with ($p = 0.1$) **(42)**. This controversy may be

due to the fact that discrepancy in method of data collection and processing techniques and difference in the inclusion and exclusion criteria of study participants.

Light microscopic studies of the micro architecture of placentas under different magnifications revealed variations from one placenta to the other. In the current study, structure of intervillous space, chorionic villi parts and capillaries were varied in anemic and non-anemic group of mothers. In the placentas of maternal anemia cases, it was observed that 72.7% of terminal villi capillaries were dilated compared to 24.2% of terminal villi capillaries dilation in non-anemic placentas. Dilation of terminal villi vessels was significantly increased with severity of anemia in current study ($p=0.001$). This result was in line with a study conducted by Soni and Nair, in which anemic placental capillaries per villi were seen to be increased in number and dilated with severity of anemia **(45)**. This may be due to the fact that compensatory response for hypoxia as a result of maternal anemia.

In the current study, increase in number of capillaries per villi in anemic placentas was 75.7%, compared to 15.1% in non-anemic placentas. Capillary number per villi was significantly increased with severity of anemia ($p < 0.001$). This finding was in line with other studies conducted by Lelic et al., in which hypoxia as a consequence of maternal anemia, lead to significant increase of terminal villi blood vessels **(44, 45)**. This may be due to hypoxic placenta as a result of maternal anemia increase in terminal villi vessels as a compensatory response

Regarding intervillous space width of placenta, intervillous space of anemic placenta was wider than non-anemic placenta. There was a statistically significant difference between groups (ANOVA=64.03, $p<0.001$). This finding was comparable with a study conducted in Pakistan in which width of intervillous space in anemic placenta was wider, compared to non-anemic placenta and it was statistically significant between groups ($p<0.05$) **(46)**. This finding was also parallel with the study conducted in India, which 60% of anemic placenta had wider intervillous space compared to 6.6% in non-anemic placenta **(29)**. The increment in width of intervillous space in anemic placenta may be due to the fact that the reduction in number and size of villi with a resultant increase in intervillous space.

In this study, 51.5% of anemic placenta had intervillous thrombosis, compared to nothing was observed in non-anemic placentas, and space thrombosis was significantly increased with severity of anemia ($p<0.001$). This finding was not comparable with other study conducted in India, in which 50% of anemic placenta had intervillous thrombosis; compared to 30%

intervillous thrombosis in non-anemic placentas (29). This may be due to the discrepancy in inclusion and exclusion criteria of study participants. The dominance of intervillous thrombosis in anemic placentas may be due to mixing of fetal and maternal blood in the space of villi resulted from rupture of villus at the site of syncytial thinning causes release of thromboplastic substance from syncytial damage.

Cytotrophoblast proliferation in anemic placenta was 48.5%, compared to non-anemic placenta which accounts 3%. This result was supported by Baske, in which 30% of placentas in anemic mothers developed cytotrophoblast proliferation, compared to 13% in non-anemic groups (29). Another study revealed that, cytotrophoblastic proliferation was not found in any of non-anemic placentas, whereas in anemic placentas cytotrophoblastic proliferation was increased with severity of anemia increases (45). This may be due to the fact that the tissue suffers from ischemic damage as a result of low oxygen tension, in maternal anemia.

Regarding syncytial knot formation, even though, syncytial knot was evident in non-anemic placentas, the degree of knot formation was significantly increased in anemic placentas ($p < 0.001$). For instance, 81.8% of anemic placenta was developed diffuse syncytial knots, whereas, in non-anemic placentas 15.1% was diffuse knots. This result was in line with a study conducted in India, in which incidence of syncytial knot formation in anemic group was 83% compared to 10% in non-anemic group. The reason behind the increase in diffuse syncytial knots in maternal anemia may be due to the effort being made to form new villi as a compensatory mechanism so as to increase an effective surface area for exchange (29, 45).

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7. CONCLUSIONS

Based on this study, the mean placental weight, thickness and diameter was larger in anemic mothers than non-anemic mothers. It was also observed that maternal anemia resulted decrease in birth weight of fetus and number of cotyledons and it further decreased according to its severity. Majority of placental shape in both groups of mothers was circular followed by oval. On the microscopic morphology, it was observed that, the chorionic villi capillaries were increased in number and it was dilated in anemic placentas, compared to non-anemic placentas. Intervillous space and thrombosis were significantly increased in anemic placentas. Cytotrophoblast proliferation and syncytial knot formation were significantly increased in anemic compared to non-anemic placentas.

8. LIMITATIONS OF THE STUDY

The current study was limited to small number of study participants and there was also budget limitation to conduct the microscopic morphology of the whole sampled placentas. Even though this study attempted to compare anemic and non-anemic maternal placenta and their baby's birth weight, specific types of anemia was not identified and studied.

9. RECOMMENDATIONS

- ✓ South Wollo zone health office should enhance efforts on prevention of maternal anemia during pregnancy.
- ✓ Clinicians should carry out routine placental examination and measurement during post-partum period; hence, this will provide better evidence for clinical decisions.
- ✓ Prompt anatomic placental interpretation should carry out on sick mothers and babies before referral to pathologists.
- ✓ Researchers should conduct large scale study using the current study as a baseline data on the same or different clinical conditions effect on placenta.
- ✓ Large scale study should be conducted on the effects of specific types of anemia on placenta.

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ANNEX
Annex: I

English version information sheet and consent form

Department of medical Anatomy, School of Medicine, College of Health Sciences, Addis Ababa University.

Title of the study: -Morphological changes of placenta and birth weight of fetus Associated with maternal anemia in Dessie Referral Hospital, North East Ethiopia.

Written Consent Form

Dear study participant, Good morning/afternoon, my name is----- I am one of the data collector for the study being conducted by Addis Ababa University, School of Medicine and Health Sciences, Department of Anatomy, on morphological changes of placenta and birth weight of fetus associated with maternal anemia. For this study, your permission is important in order to conduct research on your placenta and it will help policy makers to design strategies to prevent and control maternal and child morbidity and mortality secondary to anemia. Your name will not be written in this form and will never be used in connection with your placenta. All findings from your placenta will be kept strictly confidential. You are not obligate to give permission without your interest. Your decline/refusal to participate will not affect any of the services you should obtain from the hospital. If you feel discomfort to give permission, please feel free to dropout at any time you want. If you have any question/concern time you can communicate me or the IRB on the following addresses;-

Principal investigator address -----0929397251

IRB address -----0911124774

Could I have your permission to continue?

1. Yes, signature_____

2. No, skip to the next subject.

Informed consent certified by investigator

Name _____Signature_____

Date of permission given _____Time_____

Amharic version information sheet and consent form

አዲስ አበባ ዩኒቨርሲቲ

የህክምናና ጤና ሳይንስ ኮሌጅ

ይህ መጠይቅ በደሴ ሪፈራል ሆስፒታል ከእርግዝና ጋር በተያያዘ የደም ሜዳ በሽታ ያለባቸውንና የለባቸውን ወላጅ እናቶች የእንግዲህ ልጅ የቅርፅ፣ የክብደት፣ የወፍረት፣ የስፋት፣ የኮቲለደን መጠን እና በአይን የሚይዝ ውስጣዊ ክፍሎች ለወጥ እንዲሁም የህፃናትን ክብደት ለወጥ ለማጥናት የተዘጋጀ ነው፡፡

የፈቃደኝነት ቅፅ

ወደ ተሳታፊዎችን ጤናማነት ደህና ዋሉ/አደሩ፡-----
እባላለሁ፡፡

እኔ እዚህ የተገኘሁት ይህንን ጥናት በአዲስ አበባ ዩኒቨርሲቲ በአናቶሚ ትምህርት ክፍል ለሀላተኛ ዲግሪ ማህያ ጥናት መረጃ ለመስጠት ነው፡፡ በዚህ ሆስፒታል በወላጅነት የእንግዲህ ልጅ ላይ የሚታየው የቅርፅ፣ የክብደት፣ የወፍረት፣ የስፋት እና የኮቲለደን ቁጥር እንዲሁም የህፃናትን የክብደት ለወጥ ለመረዳት እንፈልጋለን፡፡

ለዚህ ጥናት መካሄድ የርስዎ ፈቃደኝነት ወሳኝ በመሆኑ ጥናቱን እንደናካሄድ ፈቃደኝነት እንጠይቃለን፡፡ ስለዚህ ፈቃዱን ከሰጠኛል ጥናቱ ከተካሄደ ከእርግዝና ጋርተያያዘ በመክሰተው የደም ሜዳ በሽታ የተነሳ የሚታየው የእንግዲህ ልጅ የመጠን ለወጥና የህፃናትን ክብደት ለወጥ በመለየት በእናቶች እና ህፃናት ላይ የሚከሰተውን የህመም፣ የሞት እና የጤና ችግር ለመቅረፍ የሚያስችሉ ፖሊሲዎችን ለመቅረፅ የሚያስችሉ መረጃዎችን ለመስጠት ያስችላል፡፡ ከእርስዎ ከተወለደው የእንግዲህ ልጅ የሚገኘውን መረጃ በመስጠት እንጠብቃለን፡፡ ከዚህ ጥናት ጋር በተያያዘ በማንኛውም ቦታ ጊዜ ስንም እንደሚመዘገብና እንደሚጠቀስ ልንገልፅልዎ እንወዳለን፡፡ ጥናቱን የምናካሂደው የርስዎን ሙሉ ፈቃደኝነት ስንገኝብዎት ነው፡፡ እርስዎ በዚህ ጥናት ባለመተባበር በሆስፒታሉ ውስጥ በሚገኙት ማንኛውም አገልግሎት ጭን እንደሚፈጥርበዎት ልንነግርዎ እንወዳለን፡፡ ፈቃደኝነትዎን ለመስጠት ሆነ ላለመስጠት ወሳኔው የዕርስዎ ብቻ ነው፡፡ ጥያቄ ወይም ችግር ካለ የጥናቱን ባለቤትና የተቋማት የጥናትና ምርምር ገምጋሚ ቦርድ በመክተል አድራሻዎች ማስገባት ይችላሉ፡፡

የጥናቱ ባለቤት ስልክ ቁጥር-----0929397251

የተቋሙ የጥናትና ምርምር ገምጋሚ ቦርድ ስልክ ቁጥር----- 0911124774

በወላጃት የእንግዲህ ልጅ ላይ ጥናቱን እንደናካሂድ ፈቃደኝነት ?

1. አዎ፡-----ፊርማ----- 2.

አይደለሁም-----

የጠያቂው ስም -----ፊርማ-----መጠይቁ የተሞላበት ቀን -----

ስዓት -----

Annex: II

I. Socio demographic characteristics

S/no	Variables	Response
1	Maternal age	-----Years
2	Sex of fetus	1. Male 2. Female
3	Residence	2. Urban2. Rural
4	Educational level	1. Cannot read and write 2. Can Read and write 3. Primary 4. Secondary 5. Higher education
5	Occupation	1. Non employed 2. Housewife 3. Employed in government institution 4. Employed in private sector 5. Merchant 6. If others specify_____?

II. Check list related to mother and newborn

S/no	G A	Parity	Level of HB	Mode of delivery(SVD/C/S)	Placenta l shape	Number of cotyledo ns	Placenta weight(g m)	Placental diameter(cm)	Placental thickness (mm)	BW of Fetus	BMI
1											
2											
-											
96											

Annex: III

III. Checklist related to placentalHistopathology

Slide Code	<u>A</u> Abnormalities of blood vessels within the villi		<u>B</u> Calcification	<u>C</u> Intervillous space		<u>D</u> Cytotrophoblast proliferation	<u>E</u> Syncitial knots	
	Lumen dilation (Y/N)	Sign of Angiogenesis (Y/N)	Y/N	Thrombosis (Y/N)	Dilation (µm)	(Y/N)	Focal Y/N	Diffuse Y/N
1								
01								
001								
0001								
-								
-								

Annex: IV

I. Method of processing

- 1. Fixation:-** The tissue was preserved/fixed in 10% formalin solution
- 2. Dehydration:** - The preserved tissue was washed in running tap water for 4-6 minutes. Then, they passed through upgraded alcohol as follows:- 70% alcohol – 1hour, 85 % alcohol – 1hour, 96% alcohol – 1hour, Absolute alcohol I– 1hour, Absolute alcohol II – 1hour.
- 3. Clearing:** –Clearing of tissue was done in xylene,-1hour in xylene -I, then after in xylene II for 1 hour.
- 4. Infiltration:** -Tissue was infiltrated with paraffin wax I, for 1and 1/2 hrs, paraffin wax II for 2 and 1/2 hrs and paraffin wax III for overnight.
- 5. Embedding:** - The cleared tissue was put in molten wax (melting point 56-58degree Celsius) for 12 hours in cryostat. The paraffin blocks of tissue were made with the help of embedding cassettes.
- 6. Sectioning:** – The serial paraffin sections of 5 micron thickness was cut by rotator microtome and floated in water bath having temperature 45-50 degree Celsius. The section was made spread on the slide smeared with adhesive solution (mixture of equal

amount of glycerol and egg albumin). The slide was dried on hot plate having temperature 50 degree Celsius.

7. **Deparaffinisation of sections:** – The slide was put in xylene II, changes each for 5-10 min in order to remove the extracellular and intracellular wax.
8. **Rehydration:** - The slide was put in descending grades of alcohol i.e. absolute, 90 %, 70 % and 50% alcohol for 2 min for each. The slide was then washed in running tap water for 2 minute and then taken for routine H & E staining.

II. Method of Staining

1. Stained with Hematoxyline for 10 minute.
2. Washed in running tap water until section become blue.
3. Stained in 1% eosin for 7- 10 min.
4. Washed in running tap water (5 minute)
5. Dehydrated through 70 % and 95% Alcohol 3 minutes for each, then absolute alcohol I and Absolute alcohol II for 1 and 1/2 hour for each.
6. Cleaned by - Xylene I and XyleneII,5 minutes for each.

9. Mounted – By DPX