ADDIS ABABA UNIVERSITY

COLLEGE OF HEALTH SCIENCES

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DEPARTMENT OF ANATOMY

GROSS AND HISTOMORPHOLOGIC STUDY OF UMBILICAL CORD AND ITS VESSELS IN PREECLAMPSIA

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Addis Ababa, Ethiopia

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A thesis submitted to Department of Anatomy, School of Medicine, College of Health Sciences, Addis Ababa University for the partial fulfillment of the requirements for the Degree of Masters of Science (MSc.) in Human Anatomy.

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Declaration

This is to certify that the thesis prepared by Elsabet Mohammed, entitled, **"Gross and histomorphologic study of umbilical cord and its vessels in preeclampsia"** and submitted in partial fulfillment of the requirements for degree of Masters of Science in Human Anatomy complies with the regulation of the University and meets the accepted standards with respect to originality and quality. This thesis has not been presented for degree in any other University and that all sources of materials used for the thesis have been fully acknowledged.

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Abstract

Preeclampsia is the most common medical complication of pregnancy worldwide, occurring in an average of 4% of all pregnancies and accounting for 10% of perinatal and neonatal mortality rate. Histomorphological changes in umbilical vessels are known to affect the critical functions of placenta in the developing fetus. Relationships between umbilical cord abnormalities and preeclampsia have been found to be a big debated issue in recent literature. This study aims to assess gross and histomorphological change of umbilical cord and it's vessels in preeclamptic mothers as compared to low risk mothers at Black Lion Specialized and Gandhi Memorial Hospitals in Addis Ababa, Ethiopia. A case-control study was carried out in fresh specimens of placentas with umbilical cords on seventy-five mothers (50 controls and 25 cases). Gross morphologies were examined by inspection and measuring followed by microscopy examinations of umbilical cord sections. Histological slides were taken from placental, middle, and fetal segments of the placenta. A reduction in the diameter of umbilical cord by a mean of 0.93± 0.29cm was found in the preeclamptic group as compared to non preeclamptic mothers. The luminal diameter and wall thickness of umbilical arteries in preeclamptic mothers were found reduced as compared to those from the non preeclamptic mothers. The reductions were higher and significant in the fetal segment than the middle and placental segments. In case of umbilical veins, there was reduction of umbilical veins' luminal diameter and wall thickness in preeclamptic group at all segments. However, only the luminal diameter of umbilical vein at the placental segment, showed a significant decrement by a mean of 5.43 ±1.16mm. Therefore, the present study collectively found that although the length and site of insertion of umbilical cord did not show a significant difference, preeclamptic mothers have dramatically affected gross as well as histomorphology of the umbilical cord and its vessels as compared to non preeclamptic mothers.

Keywords: Umbilical cord, Preeclampsia, Pregnancy induced hypertension, Umbilical vessels, Histomorphology, Mothers,

List of Abbreviations

AAU - Addis Ababa University

ACOG - American College of Obstetricians and Gynecologist

AGA - Appropriate for gestational age

CHS - College of Health Sciences

CHT - Chronic hypertension

CI - Confidence interval

cm - Centimeter

C/S - Cesarean section

DIC - Disseminated intravascular coagulation

dL - Deciliter

DM - Diabetes mellitus

g - Gram

H & E - Hematoxylin and Eosin

HELLP - Hemolysis, elevated liver enzymes and low platelet count

HICs - Higher income countries

IUD - Intrauterine death

IUGR - Intrauterine growth retardation

L - Liter

LGA - Large for gestational age

LMIC - Low and middle income countries

µL - Microliter

mg - Milligram

mm - Millimeter

mm Hg - Millimeter of mercury

mmol -Millimole

µm - Micrometer

µmol - Micromole

OR - Odds ratio

PE - Preeclampsia

PIH - Pregnancy Induced Hypertension

SGA - Small for gestational age

SOGC - Society of Obstetricians and Gynecologists of Canada

SVD - Spontaneous vaginal delivery

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1 INTRODUCTION

1.1 Background

1.1.1 Umbilical cord

Umbilical cord is an elastic cord that connects the fetus with placenta (Paiker *et al*., 2016). It is an intra-amniotic free floating structure fixed between the placenta and the fetus (Bhavina et al., 2016). It is the lifeline between the fetus and placenta (Kaur *et al*., 2014).

At term, the definitive umbilical cord is pearl-white, 1–2cm in diameter, 50–60cm in length, eccentrically positioned, and contains the right and left umbilical arteries, left umbilical vein, and mucus connective tissue (Dudek, 2014). The two umbilical arteries and one umbilical vein are embedded in a loose proteoglycan rich matrix known as Wharton's jelly which has an abundance of ground substance composed primarily of hyaluronic acid & very few fibers. The cells in this tissue are mainly fibroblasts (Yasoob *et al*., 2014). Umbilical vessels are not supplied by vasa vasorum and thus depend on their own oxygen supply making them more vulnerable to changes in hemodynamic condition (Barnwal *et al.*, 2012). Nerves are not detected in the umbilical cord (Saha *et al*, 2014). As shown in the Figure 1, the site of cord insertion onto the placenta is variable, usually central or eccentric but in about 7% of the cases is marginal (Battledore) or into fetal membranes (Velamentous) (Kaur *et al*., 2014).

Figure 1(a-d): Different sites of umbilical cord insertion taken from (Kaur *et al*., 2014)

Microscopically, the umbilical arteries are composed of double layered muscular wall, with no internal elastic lamina. They contain large amounts of circularly arranged well developed smooth musculature in an outer layer and a less well differentiated inner longitudinal muscle layer which has an abundance of ground substance (Karim *et al*., 2016). The cross-sectional area of umbilical cord artery increases from 8.9mm^2 at 24 weeks of gestation to 13.2mm^2 at 38 weeks of gestation in normal pregnancy (Togni *et al.*, 2007).

The umbilical vein is larger in diameter, and has a thin wall with single layer of disorganized circular smooth muscle and an internal elastic lamina (Karim *et al*., 2016). The diameter of the umbilical cord vein increases from 4.1mm at 20 weeks to 8.3mm at 38 weeks gestation. Togni *et al*. (2007) have reported an increase in the cross-sectional area of the umbilical cord vein from 28mm^2 at 24 weeks of gestation to a maximum of approximately 58mm^2 between 34–38 weeks, followed by a slight decline from the 39th week of gestation. Another variation in the umbilical cord vein is a decrease in the diameter of vessel by approximately 1mm between the placental and fetal ends (Spurway *et al*., 2012). As in figure 2, the larger thin walled single umbilical vein and two thick walled umbilical arteries are shown in a cross sectional image of umbilical cord.

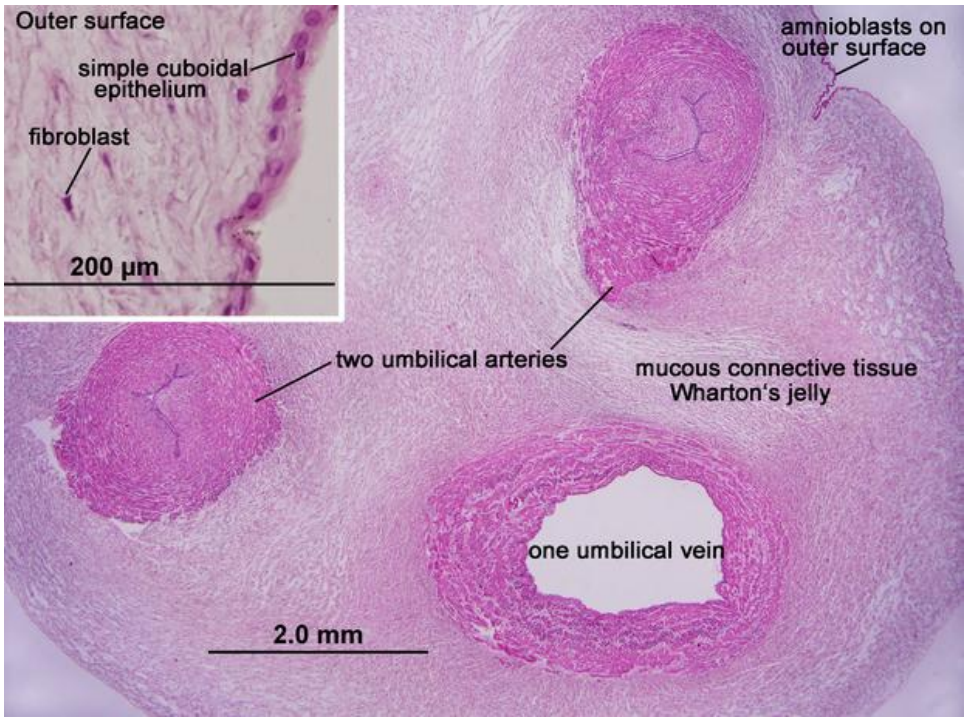

Figure 2: A cross sectional image of umbilical cord showing the major structures. (www.lfp.cuni.cz/Histologie/Preparaty/preparaty2E.php?SYSTEM=Femalereproductivesystem&NAHLED=4). Retrieved on July 21, 2018

The rudimentary umbilical cord is formed during the 4th to 8th weeks of gestation (Spurway *et al*., 2012). A patent opening called the primitive umbilical ring exists on the ventral surface of the developing embryo through which three structures pass: the connecting stalk, the yolk stalk (vitelline duct) and the canal connecting the intraembryonic and extraembryonic cavities. The connecting stalk contains the allantois and the umbilical vessels. As the amnion expands, it squeezes the vitelline duct and connecting stalk together to form the primitive umbilical cord. At week 6, the gut tube connected to the yolk sac herniates in a process called the physiological umbilical herniation, into the extraembryonic coelom. At approximately the end of the third month, the loops are withdrawn into the body of the embryo, and the cavity in the cord is obliterated. When the allantois and the vitelline duct are also obliterated, all that remains in the cord are the umbilical vessels surrounded by Wharton's jelly (Sadler, 2012). Blood flow is

established within the umbilical cord by the end of the 5th week of gestation (Spurway *et al*., 2012).

The umbilical arteries were initially paired ventral branches of the dorsal aorta. However, during the fourth week each artery acquires a secondary connection with the dorsal branch of the aorta, the common iliac artery, and loses its earliest origin. After birth, the proximal portions of the umbilical arteries persist as the internal iliac and superior vesical arteries, and the distal parts are obliterated to form the medial umbilical ligaments (Sadler, 2012).

A paired umbilical veins were originating in the chorionic villi and run on each side of the liver. They carry well-oxygenated blood from the placenta to the sinus venosus. As the liver develops, the umbilical veins lose their connection with the heart and empty into the liver. The right umbilical vein and proximal part of the left umbilical vein disappear, so that the left vein is the only one to carry blood from the placenta to the liver. A large venous shunt, the ductus venosus develops within the liver and connects the umbilical vein with the inferior vena cava. This vessel bypasses the sinusoidal plexus of the liver. After birth, the left umbilical vein and ductus venosus are obliterated and form the ligamentum teres hepatis and ligamentum venosum, respectively (Sadler, 2012).

The main function of the umbilical cord is to circulate blood between the embryo and the placenta (Schoenwolf *et al.*, 2009). The deoxygenated fetal blood leaves the fetus through two umbilical arteries that pass through the umbilical cord .When they reach the fetal surface of the placenta, these vessels divide into many branches which enter the chorionic villi. The oxygenated blood returns to the fetus via the venules and veins in the chorionic villi. These join to form the umbilical vein in the umbilical cord (Lateef, 2011). The umbilical cord regulates blood flow, in both directions, thus allowing for mother to child immunological communication, oxygenation of fetal blood, fetal nutrition and in utero cardiac function of the fetus (Olaya and Bernal, 2014). It has been estimated that by 31 weeks, the umbilical cord must carry 70 quarts of blood per day, moving at 4 miles an hour. This remarkable organ also must participate in fetal growth milestones. Additionally, it may act as an assist pump to the fetal heart (Alam *et al*., 2014).

1.1.2 Preeclampsia

Preeclampsia is a multisystem disorder of unknown etiology, unique to pregnancy (Duley, 2003). Hypertension is one of the common complications that occur during pregnancy. Hypertension in pregnancy is defined as: a systolic blood pressure ≥ 140mmHg or a diastolic blood pressure ≥ 90mmHg. These measurements are based on the average of at least two measurements, taken using the same arm, and several hours apart (Institute of Obstetricians and Gynecologists, 2016). Hypertension can be further defined as mild, moderate or severe. Mild Hypertension: Diastolic blood pressure 90-99mmHg and systolic blood pressure 140–149mmHg. Moderate Hypertension: Diastolic blood pressure 100–109mmHg, and systolic blood pressure 150–159mmHg. Severe Hypertension: Diastolic blood pressure 110mmHg or greater, and systolic blood pressure 160mmHg or greater (Institute of Obstetricians and Gynecologists, 2016).

According to report of the National High Blood Pressure Education Program, the classification of hypertensive disorder of pregnancy is as follows: Chronic hypertension, Gestational hypertension, Preeclampsia-eclampsia and Preeclampsia superimposed on chronic hypertension (Superimposed preeclampsia) (NHBPE, 2000).

Chronic hypertension is defined as hypertension that was either present before conception or detected before the 20th week of gestation and did not resolve in the early postpartum (Peters and Flack, 2004). Gestational hypertension is diagnosed when de novo hypertension occurs after 20 weeks gestation, without any features of preeclampsia (Lowe *et al.*, 2009). Superimposed preeclampsia is development of new signs and/or symptoms of preeclampsia after 20 weeks of gestation, in a woman with chronic hypertension (Brown *et al.*, 2001).

Preeclampsia is a syndrome that chiefly includes the development of new onset hypertension in the second half of the pregnancy (American College of Obstetricians and Gynecologists, 2013).
A diagnosis of preeclampsia can be made when hypertension arises after 20 weeks gestation and is accompanied by one or more of the following: renal involvement (significant proteinuria (dipstick proteinuria subsequently confirmed by a spot urine protein/ creatinine ratio ≥ 30mg/mmol), Serum or plasma creatinine ≥ 90µmol/L, oliguria), hematological involvement

(thrombocytopenia, hemolysis, disseminated intravascular coagulation), liver involvement (raised serum transaminases, severe epigastric or right upper quadrant pain), neurological involvement (convulsions (eclampsia), hyperreflexia with sustained clonus, severe headache, persistent visual disturbances (photopsia, scotomata, cortical blindness, retinal vasospasm), stroke), pulmonary edema, fetal growth restriction, placental abruption (Lowe *et al.*, 2009).

Preeclampsia can range from mild to severe. Preeclampsia in the absence of severe manifestation has been characterized as mild preeclampsia. Sever preeclampsia is characterized by systolic blood pressure of 160mm Hg or higher, or diastolic blood pressure of 110mm Hg or higher on two occasions at least 4 hours apart while the patient is on bed rest. Additionally, women who have met the basic criteria for preeclampsia, along with new evidence of thrombocytopenia, impaired liver dysfunction, renal insufficiency, pulmonary edema, or visual loss or cerebral disturbance, also are considered as having severe disease (American College of Obstetricians and Gynecologists, 2013). Eclampsia is the convulsive phase of preeclampsia, when the seizures cannot be attributed to other causes (Peters and Flack, 2004).

Some pregnant women present with a specific constellation of laboratory findings – hemolysis, elevated liver enzymes, and low platelet count – that has been labeled as "**HELLP syndrome**." It is often considered a preeclamptic subtype (American College of Obstetricians and Gynecologists, 2013).

The pathogenesis of preeclampsia is not fully elucidated but much progress has been made in the last decades. The placenta has always been a central figure in the etiology of preeclampsia because the removal of the placenta is necessary for symptoms to regress (Phipps *et al.*, 2016). Preeclampsia is caused by placental dysfunction (incomplete spiral artery remodeling in the uterus that contributes to placental ischemia (Phipps *et al.*, 2016)) followed by the release of factors by the diseased placenta into the maternal circulation, inducing widespread endothelial dysfunction that heralds the classic manifestations of the disease (Sircar *et al*., 2015). Primi parity, previous preeclamptic pregnancy, chronic hypertension or chronic renal disease or both, history of thrombophilia, multi-fetal pregnancy, in vitro fertilization, family history of

preeclampsia, DM, obesity and advanced maternal age (older than 40) are risk factors for preeclampsia (American College of Obstetricians and Gynecologists, 2013).

The umbilical cord vessels are the connection between the placenta and the fetus. They are influenced by vascular and hemodynamic functions during fetal life. Many studies have demonstrated morphological and histological differences in the umbilical cord of preeclamptic pregnancies (considered to be a consequence of uteroplacental hypoperfusion), such as thicker umbilical arteries (tunica intima and media) with no changes in thickness of the umbilical cord vein, and umbilical cord artery vasoconstriction due to excess of thromboxane. Preeclampsia has also been associated with thinner or leaner umbilical cords, as well as with decreased cross-sectional cords area (thickness), antenatal umbilical coiling index (hypocoiling), amount of Wharton's jelly, and venous blood flow volume (Olaya-C *et al*., 2016). Junek *et al*. reported increased thickness of the tunica media and intima in the arteries and an increased rate of duplication of the internal elastic lamina in preeclamptic cords. Inan *et al*. reported reduced luminal areas and reduced vessel wall thickness in both artery and vein in preeclampsia compared to normal pregnancies and pregnancies complicated with chronic hypertension (Koech *et al*., 2008).

1.2 Statement of the problem

A complex and coordinated interaction between maternal, placental and fetal factors influences the normal fetal growth. Any structural variations and pathological changes in umbilical cord have the potential to lead to IUGR, adverse pregnancy outcome and even stillbirth (Kotrannavar *et al*., 2016).

Hypertensive disorders during pregnancy are major causes of maternal and fetal morbidity and mortality, affecting 5%– 10% of pregnancies (Wang *et al*., 2013).In severe cases hypertension results in fetal complication like IUGR, SGA, prematurity and still birth (Bhavina *et al*., 2016).

Preeclampsia is the most common medical complication of pregnancy worldwide, occurring in 3% to 5% of all pregnancies and carrying a perinatal and neonatal mortality rate of 10% (Maynard and Karumanchi, 2011). In Africa, preeclampsia occurs in 10% of pregnancies, which is significantly higher than the global average of approximately 2% (Vata *et al*, 2015).According to WHO multi country survey, eclampsia occurs in 1.0–2.0% of pregnancies (Abalos *et al*., 2014). Severe preeclampsia occurs in 0.6% to 1.2% of pregnancies in the western countries (Sibai, 2011).

Preeclampsia remains one of the top five causes of maternal and perinatal mortality worldwide. Using data from 29 LMICs participating in the WHO multi country Survey on maternal and neonatal health, the odds of maternal death associated with the diagnosis of preeclampsia (compared with no preeclampsia) was 3.73 (95% CI 2.15–6.47) and with eclampsia (vs. no eclampsia) (OR 42.4, 95% CI 25.1–71.4) (Abalos *et al*, 2014). It is estimated that preeclampsia claims the lives of more than 70,000 women per year and more than 500,000 of their fetuses and newborns; this is equivalent to the loss of 1600 lives per day (Firoz *et al*., 2011). More than 99% of these losses occur in low and middle income countries, particularly those on the Indian subcontinent and sub Saharan Africa (Khan *et al.*, 2006).

The contribution of preeclampsia to the overall preterm birth rate is substantial: 8–10% of all preterm births result from hypertensive disorders (Slattery *et al*., 2008). Small for gestational age

babies (mainly because of IUGR arising from placental disease) are common, with 20–25% of preterm births and 14–19% of term births in women with preeclampsia being less than the tenth centile of birth weight for gestation (Rasmussen and Irgens, 2006).

The Ethiopian National Emergency Obstetric and Newborn Care showed that preeclampsia contributed for the complication of approximately 1% of all deliveries and 5% of all pregnancies. Moreover, 16% of direct maternal mortality and 10% of all maternal mortality (direct and in direct) was due to preeclampsia/eclampsia (Gaym *et al*., 2011). On the basis of research data the incidence rate of preeclampsia in Dilla University Referral Hospital was found to be 2.23% (Vata *et al*, 2015).

Umbilical vessels are critical to fetal blood supply by serving as the exchange point between fetal and maternal blood. Several studies have shown that morphologic variations of the umbilical cord are associated with adverse perinatal outcomes. Umbilical blood vessels are not innervated and regulation of blood flow to the placenta must depend on structural changes and the effect of vasoactive factors. Failure to achieve these adaptations may result in reduced fetoplacental perfusion. Maternal hypertension is frequently associated with an abnormal blood flow pattern in umbilical vessels which is probably due to imbalance between vasoconstrictors and vasodilators. Recently, a relationship between umbilical cord abnormalities and preeclampsia has been found in the literature. Assessment of umbilical cord and its component can be used as early screening tool for fetal growth and it provides useful information about pregnancy at risk and help in averting poor perinatal outcome. The subclinical presence of vascular changes at term may also predict vascular diseases in early childhood like early onset of systemic arterial hypertension (Barnwal *et al*., 2012). To date, little is known about the umbilical cord and its vessels from healthy and PE pregnancies and its relation to the measurements of the umbilical cord and its vessels. Even though umbilical cord examination is crucial; it is mostly neglected by health professionals and researchers worldwide. Especially in Ethiopia, there have been no studies on the sequential structural changes along the length of umbilical vessels and how these are related to the severity of PIH. To this end, this study is set to assess the presence of any gross and histological changes of umbilical cord and its vessels in preeclampsia compared to low risk mothers in two governmental hospitals in Addis Ababa.

1.3 Significance of the study

Fetus proper growth and development depend on balance between fetal, placenta, and maternal unit. The umbilical cord is the nexus between the fetus and the placenta, thus the umbilical vessels' configuration may be an indicator of fetal status. Umbilical cord examination reflects what had happened with it, when it was in mother's womb and what is going to happen with fetus in future and so in future pregnancy; fetus adverse outcome can be prevented. The present study has been undertaken to explore morphological and structural changes of umbilical cord and its vessels in preeclampsia to provide a theoretical basis for fetal development. It also helps the clinicians to give timely management of mother and infant, thus to prevent maternal or fetal adverse outcomes. Furthermore, it provides an informational support to health workers concerning the baby's overall development. This study could also serve as a baseline for further studies to be carried out on histomorphometric and immunohistochemical studies of umbilical cord in the same disease or other diseases.

2 LITRATURE REVIEW

Preeclampsia is the most common pathological syndrome associated with pregnancy which is known to affect the vascular system of mother as well as the fetus. The following are gross and histomorphometric characteristics of umbilical cord in clinical terms.

2.1 Length and diameter of umbilical cord

Most umbilical cords have been reported to be 50 - 60cm, with only very few to be abnormally short or long. To date, some authors have agreed that excessively short and long umbilical cords correlate with a variety of fetal problems such as intrapartum distress and/or demise (Rogers *et al.*, 2003; Krakowiak *et al.*, 2004; Baergen *et al.*, 2001), while some authors have denied any relation to fetal distress and poor fetal outcome from problems of umbilical cord length (Dippel, 1964; Sinnathuray, 1965).

Several studies have investigated the relation of pregnancy induced hypertension with the length of umbilical cord. In a morphometric analysis of umbilical cord in normal vs. hypertensive pregnancies in a population of Lucknow, Uttar Pradesh, India, no statistical difference was found with respect to its length with values of 31.46± 10.49cm in the controls as compared to 31.03±10.94cm, in the cases (Paiker *et al*., 2016). On the other hand, a study conducted in Pakistan have reported the mean length of umbilical cord in preeclamptic cases to be shorter than that of the controls with respective values of 51.6±2.01cm and 57.4±1.17cm, although this was not statistically significant (Yasoob *et al*., 2014). Among the pregnancy induced hypertensive cases, a study done in Nairobi, Kenya reported that there was no difference in cord length (p= 0.108) between mild and severe PIH (Koech *et al*., 2008). In line with this, a study done in India have found that the mean cord length of umbilical cord in gestational hypertension and preeclampsia were, respectively 30.81±11.8cm and 29. 5±10.38cm, with no statistically significant difference (Bhavina *et al.*, 2013).

Regarding diameter of the umbilical cord, several studies have also investigated its relationship with pregnancy induced hypertension. A comparative study done in India found that there was a significant reduction in the cord diameter in PIH mothers as compared to controls with the mean values of 9.65mm at placental side and 8.67mm at fetal side for the control group and 7.69mm at placental side and 6.82mm at fetal side for the PIH group (Sonawane and Rathod, 2016). In line with this, a study conducted in Pakistan reported that there was statistically significant reduction (*P=0.05*) in the mean umbilical cord diameter at proximal and distal ends with the mean values of 2±0.23, and 1.3±0.25cm for preeclamptic group and 2.7±0.2, 2.08±0.22cm for the control group. The difference in umbilical cord diameter at the middle of the cord was not statistically significant (Yasoob *et al*., 2014). On the other hand, a study conducted in India among mothers with PIH, reported that there is no statistically significant difference in umbilical cord diameter in all (fetal, middle, and maternal) segments of the umbilical cord with respective values of 1.16±0.15cm, 1.17±0.16cm and 1.17±0.23cm for gestational hypertension and 1.22±0.24cm, 1.22±0.24cm and 1.16±0.22cm for preeclamptic mothers (Bhavina *et al*., 2013). In line with this, another study from India also reported that there was no statistically significant difference in umbilical cord diameter at the fetal end with the mean being 1.36±0.39cm and 1.30±0.65cm for the control and preeclamptic mothers respectively (Paiker *et al*., 2016).

2.2 Site of insertion of umbilical cord

There are several studies that have reported no statistically significant difference on site of insertion of umbilical cord among preeclamptic cases and controls. One of the studies done in India, among 75 cases with PIH, 33.33% had central and 30.67% eccentric insertion of umbilical cord. In control group, 48% had central and 28% had eccentric insertion of umbilical cord. Marginal insertion of umbilical cord was found in 21.33% cases of study group as compared to only 8% cases of control group (Kaur *et al*., 2014). Another study done in Pakistan also reported that there was no statistically significant difference in insertion site of umbilical cord in cases and controls. In preeclamptic group out of 10 umbilical cords included in the study, 70% were attached centrally, 20% were battledore and 10% were velamentous. In controls out of 20 umbilical cords, 80% were attached centrally, 10% were battledore and 10% were velamentous (Yasoob *et al*., 2014). In a morphometric analysis of umbilical cord in normal vs. hypertensive

pregnancies in population of Lucknow, Uttar Pradesh, India, the site of insertion in both groups (controls and gestational hypertension) showed almost equal number of centric and eccentric insertions leading to the conclusion that no significant difference could be seen between the two groups (Paiker *et al*., 2016).

2.3 Histomorphometry of umbilical cord arteries

With regard to histomorphometry of umbilical cord arteries, several studies reported alteration in luminal diameter and wall thickness of umbilical cord arteries with pathologic changes in preeclamptic cases.

A study conducted in Pakistan found that the luminal diameter of umbilical artery was reduced in preeclamptic cases with the value of 0.8±0.017mm in the first umbilical artery and 0.62±0.028mm in the other artery. In controls it was 0.93±0.071mm in the first umbilical artery and 0.92±0.085mm in the second umbilical artery (Yasoob *et al*., 2014). In line with this, a study conducted in Saudi Arabia observed that arterial measurement showed significant reduction in the luminal arterial area (p=0.032) and luminal arterial index (p=0.004) in the preeclamptic vessels compared to the controls (Almasry *et al*., 2016). On the other hand, a study conducted in Chandigarh, India reported that in preeclamptic mothers luminal area was not significantly changed as compared to controls (Barnwal *et al*., 2012). In line with this, a comparative study done in Turkey have found that there was no significant difference in arterial luminal area between preeclamptic mothers who had normal Doppler and controls with respective value of 0.31±0.05mm^2 and 0.40±0.05mm^2. However, a statistically significant reduction in arterial luminal diameter was observed in preeclamptic patients who had an abnormal Doppler with a value of 0.25±0.06mm^2 in comparison to normal and hypertensive groups (Inan *et al*., 2002). In contrary to the above studies, a study done in Argentina reported that lumen area of umbilical arteries was significantly higher in the preeclamptic patients as compared to controls with a respective value of 8.4±1 and 3.4±2 (Blanco *et al.*, 2011).

Regarding wall thickness of umbilical artery, a comparative study done in Turkey reported that there was statistically significant reduction in umbilical artery wall thickness among

preeclamptic mothers who had abnormal Doppler compared to controls. The difference was not statistically significant in preeclamptic mothers who had normal Doppler compared with controls with the mean values of 597.08±18.02µm, 547.58±12.01µm and 426.66±19.22µm for the controls, preeclamptics with normal Doppler and preeclamptics with abnormal Doppler respectively (Inan *et al*., 2002). In line with this a research done in Israel have found that the wall thickness of umbilical arteries was thinner in the study group as compared to the control group with the respective value of 0.36±0.13mm in preeclampsia and 0.44±0.16mm in controls, although this was not statistically significant (Sharony *et al*., 2016). On the other hand, a study conducted in Chandigarh, India reported that wall thickness of umbilical arteries was significantly increased (617.11± 48.90 and 652.83±32.91µm in the first and second artery) with p value <0.05). The thickness of arteries was increased by 20% in preeclamptic group as compared to control group (Barnwal *et al*., 2012). In line with this, a study done on severe preeclampsia complicated by HEELP syndrome in Turkey have found that the mean arterial wall thickness was significantly increased (p value <0.001) with the respective value of 95.3±8.06 in cases and 71.1±7.73 in controls *(Balsaka et al*., 2015). A study done in Argentina also reported that the outer layer area and inner layer area of umbilical arteries were significantly higher in the preeclamptic patients (Blanco *et al.,* 2011). Although it was not statistically significant a study conducted in Pakistan reported that in preeclamptic mothers the mean wall thickness of the first and the second umbilical artery was 0.61±0.031mm and 0.62±0.016mm respectively. In controls it was 0.54±0.036mm in the first artery and 0.60±0.014mm in the other artery (Yasoob *et al*., 2014).

A study conducted in Iraq reported separation of the muscular layer of umbilical arteries from the surrounding Wharton's jelly due to presence of strong vasoconstriction which may lead to narrowing of the lumen in preeclamptic cases. In addition there was a significant increase in the thickness of the basement membrane of the endothelial cells that line the arterial lumen, with some histological changes in the arterial wall such as contraction of smooth muscle cells which have irregular shapes in their nuclei (losing their wave like appearance) and increase in the inter cellular fluid associated with edema resulting in irregular space between smooth muscle cells (Karim *et al*., 2016). In line with this, a study conducted in Turkey reported that microscopic examination showed a widening under the epithelium of the artery and between the muscle

layers and separation with contraction of the muscle cells with wave like appearance of the nucleus and morphologically hypoplastic appearing umbilical arteries among preeclamptic cases with abnormal Doppler studies (Inan *et al*., 2002). On the other hand, a study done in Saudi Arabia observed that light microscopic examination of the human umbilical cord sections taken from preeclamptic group showed the endothelium of umbilical vessels with areas of discontinuation and contained pyknotic darkly stained nuclei. The smooth muscle layer of umbilical vessels showed some areas of thickening. Pale stained areas of separations between smooth muscle layers and in the subendothelial connective tissue of the umbilical vessels and within Wharton's jelly were detected (Almasry *et al*., 2016). A study conducted in Pakistan also reported that there were thickening of vessels wall and narrowing of lumen with thrombosis and fibrinoid necrosis in preeclamptic group as compared to controls (Yasoob *et al*., 2014).

2.4 Histomorphometry of umbilical cord veins

Several studies have investigated the relation of pregnancy induced hypertension with histomorphometric changes of umbilical veins. A study done in Haikou, China reported that preeclampsia cases had significantly decreased lumen diameter compared to controls for all segments (placental, middle and fetal) of the umbilical vein (P = 0.001 for all).It also found that luminal diameter of the umbilical vein gradually decreased from the placental to middle to fetal segments for both preeclampsia cases and controls (P = 0.001) (Lan *et al*., 2018). In line with this, a study conducted in Kenya has found that the luminal diameter of umbilical vein was significantly reduced in cases than in controls for all segments of the umbilical vein with a p value of 0.011, 0.000 and 0.024 for the placental, middle and fetal segments respectively Koech *et al*. (2008) . On the other hand a comparative study done in Turkey reported that there was a significant reduction in the luminal area of umbilical vein in both preeclamptic groups (with normal and abnormal Doppler) as compared to controls with a respective value of 1.96±0.21mm^2, 1.10±0.22mm^2 and 2.97±0.29 mm^2 (Inan *et al.*, 2002). Yasoob *et al*. (2014) also reported that the umbilical veins in PIH had a smaller luminal area as compared to the controls. In contrast to the above studies, a study in India reported that the umbilical vein lumen was seen to be significantly enlarged (7.10±1.29mm^2 vs. 2.41±0.66mm^2) in preeclamptic mothers than low risk mothers (Barnwal *et al*., 2012). This relationship was also reported form a study done in

Saudi Arabia where, compared to the control group, luminal venous area ($p<0.001$) as well as the luminal venous index ($p=0.005$) was significantly higher in the preeclamptic group (Almasry *et al*., 2016). Although statistically not significant a histomorphometric study conducted in Argentina also reported that in umbilical veins lumen area was higher in the preeclamptic mothers (Blanco *et al.*, 2011).

Regarding wall thickness of umbilical vein, a comparative study done in Turkey reported that there was no statistically significant difference in umbilical vein wall thickness among preeclamptic group who had normal Doppler and control groups. However, there was a statistical significant reduction in umbilical vein wall thickness in preeclamptic group who had abnormal Doppler studies as compared to controls with the mean being 471.75±33.27µm, 437.75±10.79µm and 398.58±11.54µm, for the controls, preeclamptics with normal Doppler and preeclamptics with abnormal Doppler studies respectively (Inan *et al*., 2002). This was in line with a study done in India where there was a significant reduction in wall thickness of umbilical vein in preeclamptic mothers as compared to low risk mothers with a mean of 308.76±25.47µm and 385.73± 27.38µm respectively (Barnwal *et al*., 2012). A study done in Iraq also found that there was significant reduction (p value <0.001) in vein wall area in preeclamptic group compared to controls with the respective mean values of 687.2+11.4 and 1224.6+31.4 (Karim *et al*., 2016).On the other hand, Sharony *et al*. (2016) found that wall thickness of umbilical vein section was 0.22±0.12mm in preeclampsia and 0.27±0.14mm in controls. The wall thickness of umbilical veins was thinner in the study group compared to the control group, but the differences did not reach statistical significance. In contrast to the above studies, a study done on severe preeclampsia complicated by HEELP syndrome in Turkey found that wall thickness of umbilical vein was significantly increased (p value 0.007) in the HELLP group as compared to control group with respective value of 78.6±8.84 and 63.0±10.7 (Balsaka *et al*., 2015). In line with this, a study done in Haikou, China reported that preeclampsia cases had significantly increased tunica media thickness and wall thickness compared to controls for the fetal segment on the umbilical vein ($P = 0.001$). However, it was not significant for the placental and middle segment of the umbilical vein (Lan *et al*., 2018). Koech *et al*. (2008) also demonstrated that the umbilical vein showed marked changes in PIH. The vein wall was markedly thicker in cases than in controls. The apparent increase was due to the tunica media and the tunica intima. Yasoob *et al*.

(2014) also reported that morphological modifications of the umbilical cord veins were detected in pregnancy induced hypertension. The umbilical veins in PIH had a greater wall thickness as compared to the controls. On the other hand a histomorphometric study conducted in Argentina reported that in umbilical veins, wall area was higher in the preeclamptic mothers, although this is not statistically significant (Blanco *et al.*, 2011).

Morphological modifications of the umbilical cord veins were detected in pregnancy induced hypertension. In preeclamptic group 3/10 (30%) of umbilical veins showed disruption in endothelium and basement membrane. The tunica media of 3/10 (30%) umbilical veins were showed hypertrophy. There were 3(30%) umbilical veins, which showed mild thrombosis in their walls. Regarding fibrinoid necrosis, 2/10 (20%) of the umbilical veins wall displayed partial necrosis 0.2±0.42, while 1/10 (10%) depicted complete necrosis in their walls (Yasoob *et al*., 2014). On the other hand, a study done in India found that the endothelium, subendothelium and muscle layer of vein were observed to have completely joined each other (Barnwal *et al*., 2012)

3 OBJECTIVES

3.1 General objective

This study aims to assess gross and histomorphological change of umbilical cord and it's vessels in preeclamptic mothers as compared to non preeclamptic mothers at Gandhi Memorial and Black Lion Specialized Hospitals.

3.2 Specific objectives

- To compare diameter and length of umbilical cord in preeclamptic mothers with non preeclamptic mothers.
- To compare insertion site of umbilical cord in preeclamptic mothers with non preeclamptic mothers.
- To compare histomorphometry of umbilical cord vessels of preeclamptic mothers with non preeclamptic mothers.

4 MATERIALS & METHODS

4.1 Study design and study period

A case-control study was conducted from June 2017 to January 2018 GC.

4.2 Study area

The study was conducted at Gandhi Memorial and Black Lion Specialized Hospitals, Addis Ababa, Ethiopia. Addis Ababa is the capital and largest city of Ethiopia. It has 33 hospitals, of which 5 are managed under the health bureau of the city administration. Four are managed by the federal ministry of health (one of the hospitals, St Paul General Specialized Hospital is affiliated with a medical school), one university hospital (Black Lion Specialized Hospital is under AAU) and the rest are either privately owned or owned by non-governmental and other governmental organizations. Black Lion Specialized Hospital is a tertiary referral hospital and the largest of all public hospitals in Addis Ababa. The hospital has a total 800 beds, 80 of which are currently being used by department of Obstetrics and Gynecology. About 3000 deliveries are attended each year and 60% of this is operative delivery. Gandhi Memorial Hospital is a university affiliated regional hospital and the only 'women only' hospital in the city. It has stayed on service for more than 50 years providing service for the highest number of women in Addis with its 100 beds. Average number of deliveries per year is about 7000 of which 305 comprise of operative deliveries.

4.3 Source population

The source population was all term pregnant mothers in Gandhi Memorial and Black Lion Specialized Hospitals.

4.4 Study population

The study population was all term pregnant mothers who fulfill the inclusion criteria and attended their delivery at Gandhi Memorial and Black Lion Specialized Hospitals during data collection time.

4.5 Eligibility criteria

4.5.1 Inclusion criteria

Non preeclamptic and preeclamptic mothers with gestational age of 37-42 weeks who gave birth at Black Lion and Gandhi hospitals were included.

4.5.2 Exclusion criteria

Pregnant women who did experience of any complication during pregnancy like multiple pregnancies, pre and post term pregnancies, gestational hypertension, chronic hypertension, superimposed preeclampsia, intrauterine fetal death, chronic intrauterine infection, chorioamnionitis and those with diabetes mellitus were excluded from the study.

4.6 Sample Size Determination

The desired sample was calculated using difference of means formula.

n_1 or $n_2 = \left(\frac{r+1}{r}\right)\left(\frac{(\sigma_1{}^2 + \sigma_2{}^2)(z_{\alpha/2} + z_\beta)^2}{(\mu_1 - \mu_2)^2}\right)$ where,

n_1 or n_2 = Sample size in each group

r = Ratio of low risk to preeclamptic = 1:1; as used by (Koech *et al*., 2008).

$\sigma_1{}^2$ and $\sigma_2{}^2$ = Variance of length of umbilical cord of low risk mothers vs. preeclamptic mothers from a study done in Nairobi, Kenya (Koech *et al*., 2008).

z_β = 0.84 for 80% power

$z_{\alpha/2}$ = 1.96 for 95% confidence level

$\mu_1 - \mu_2$ = Difference of means of length of umbilical cord of low risk mother vs. PE mothers (Koech *et al.*, 2008).

Therefore, n_1 or $n_2 = \left(\frac{1/1+1}{1/1}\right)\left(\frac{(1.96+ 0.84)^2(8.5^2 + 9.9^2)}{(33.1-28.3)^2}\right)$

n_1 or n_2 = 115.87 = 116

But, a purposive sample size of 75 mothers (50 controls and 25 preeclampsia cases) were enrolled in this study as it was used by previous studies; Koech *et al*., (2008) enrolled 36 mothers (18 cases and 18 controls), Barnwal *et al*., (2012) included 60 mothers(30 cases and 30 controls), Blanco *et al.*, (2011) conducted a study on 29 mothers (9 cases and 20 controls), Balsaka *et al*., (2015) included 40 mothers (20 cases and 20 controls), Yasoob *et al*., (2014) enrolled 50 mothers (30 cases (divided in to 3 groups with respect to severity of the Disease) and 20 controls. And also the cost for the calculated sample size was too much and there was also shortage of term preeclamptic cases.

4.7 Sampling procedure

The study subjects were collected by using systematic random sampling technique until the required sample size was reached in case of controls. To determine sampling frame, the total number of low risk mothers who delivered in May 2017(one month before the data collection) were 420 divided by the total sample size. K= N/n, N=420, n=50, k=420/50=8.4 ~8. With this interval, every 8^{th} mothers' placenta with attached umbilical cord was selected starting from the randomly selected newly delivered eligible mothers. Due to shortage of preeclampsia cases all the available cases that fulfill the criteria during the study period were included in this study. For histomorphometry 30 umbilical cords (15 cases and 15 controls) were selected by lottery method).

4.8 Variables of the study

4.8.1 Independent variables

Preeclampsia was the independent variables of this study.

4.8.2 Dependent variables

The dependent variables include the diameter, length and site of insertion of umbilical cord, and wall thickness, luminal diameter and histopathological changes of umbilical cord vessels.

4.9 Operational definitions

PE mother: Pregnant women who were diagnosed by the physician for preeclampsia & written on card before or during delivery.

Non preeclamptic mother: Pregnant women who were diagnosed by the physician as low risk & written on her card before or during delivery.

Gestational age: The period b/n conception & birth which was written by the physician on mother's card during delivery.

SVD: A mode of delivery which include vaginal delivery with or without instrument.

Term pregnancy: gestational age between 37 to 42 weeks

Site of insertion was considered as follows:

Central: when it was attached at the center or within 2 cm of the center of the placenta.

Eccentric: when the cord was inserted at any point between the central and marginal point of attachment.

Marginal: When the cord was attached at or within 2cm of the placental margin

Velamentous: It was recorded when the umbilical cord was inserted on the fetal membranes.

4.10 Materials and Apparatus

To conduct this study flat tray, towels, gloves, blade, blade holder, meter, ruler, tissue holder (capillary pipette), measuring cylinder, formalin, plastic embedding cassette, microtome blade, glass slides, cover slips, electronic microscope and digital camera were used.

4.11 Data Collection Procedure

The mothers clinical data; age, parity, BP, and neonatal birth weight was retrieved from the patients chart. The placenta with attached membranes and umbilical cords were collected soon after delivery. Then fresh sample of umbilical cords were observed for any gross abnormalities and the following gross and histological parameters were measured:

4.11.1 Length

The length of the umbilical cords was measured from the cut end of the cord up to its placental attachment with the non- elastic measuring tape. Then 5 cm stump, the part of the umbilical cord which remains with newborn after the cut end, was added to each measurement made as was done by Paiker *et al*., (2016).

4.11.2 Site of insertion

First the center of the placentas was located. Then insertion of each umbilical cord was measured from the center of the placenta up to insertion point. The insertion of the umbilical cord was recorded as "central" when it was attached at the center or within 2 cm of the center of the placenta. When the cord was attached at or within 2cm of the placental margin, it was recorded as a "marginal" or "peripheral" insertion. "Eccentric" position was recorded when the cord was inserted at any point between the central and marginal point of attachment. "Velamentous" insertion was recorded when the umbilical cord was inserted on the fetal membranes (Saha *et al*., 2014).

4.11.3 Diameter

The umbilical cords were cut two centimeter away from placental end. On the cut surface of the umbilical cord, the maximum diameter was measured with a metallic scale. Then the second maximum diameter was taken at right angles to the first one. Lastly, the mean diameter of the umbilical cord was calculated from these two measurements (Saha *et al*., 2014).

4.11.4 Histological preparation

Umbilical cord was divided into three parts of equal length as placental, middle and fetal segments. One cm thick transverse sections from each of these segments were then taken for histological processing. The working method for all of the histological section preparations followed the routine paraffin procedure and Hematoxylin and Eosin staining as indicated in Appendix III and IV.

4.12 Microscopy and photomicrography

The stained slides were studied under light microscope. Unbiased morphometric study was performed using a Leitz Dialux 20 (Germany) binocular microscope attached with Ocular Micrometer. Entire sections including the arteries and the vein were digitalized and used for histomorphometry at 10× objective magnification. The same investigator performed all analyses blinded. The following measurements were obtained: (a) arterial measurements: luminal arterial area, wall thickness and any histopathologic changes (b) venous measurements: luminal venous area, wall thickness and any histopathologic changes. Photomicrographs of selected samples of umbilical cord sections from both the case and control groups were taken under a magnification of 4× objective by using Leica DM 750.

4.13 Data quality assurance

The data was collected and recorded on the checklist by the investigator. Study of histological slides was done by the same investigator blindly. Standard daily quality control protocols were performed. Validity of working reagents and instruments were carefully monitored. Finally the collected data was checked for completeness.

4.14 Data processing and analysis

All data were analyzed with SPSS-23 and expressed as mean ± SD. Independent samples t-test and one-way ANOVA were used to compare parameters between groups and among segments, respectively. Pearson correlation analysis was used to analyze correlations. $P<0.05$ was considered statistically significant.

4.15 Ethical considerations

This study was carried out after ethical clearance was obtained from Departmental Research Ethics Review Committee (DRERC) of the Department of Anatomy and Department of Gynecology and Obstetrics, School of Medicine, AAU. Participants were adequately informed about the objective, benefit & risk of the study and informed consent was obtained from all subjects involved in the study. Due respect, confidentiality and appropriate disposal of placenta and umbilical cord was done.

4.16 Dissemination and Utilization of Results

The results of this study will be presented to the department of Anatomy, Addis Ababa University. They will also be communicated to all concerned bodies like Black Lion Specialized and Gandhi Memorial Hospitals, advisors and to relevant stakeholders. The findings of this study would be published in peer reviewed national or international journal for the public.

4.17 Limitation of the study

In interpreting the findings of this study, certain limitations need to be considered. This study was done on small number of participants because of cost, time and shortage of preeclamptic cases. This may have an impact on the generalization of the present study to a wider population. The other limitation is that histochemical and or molecular studies were not carried out because of cost and time.

5 Result

The age of the participants studied ranged from 15 to 45, with means of 26.06±3.79 in low risk mothers and 26.52±3.40 in preeclamptic mothers.

Table 1: Age categories in low risk and preeclamptic mothers

Age(year)	Low risk (%)	Preeclamptic (%)
15-25	46	52
26-35	54	44
36-45	0	4

Majority of the participants were nulliparous, accounting for 46% among the low risk and 60% among the preeclamptic participants.

Table 2: Parity distribution among controls and cases

Parity	Low risk (%)	Preeclamptic (%)
Para-0	46	60
Para-1	34	20
Para-2	8	12
Para≥3	12	8

In this study, the blood pressure of the preeclamptic mothers was found to be higher than those of the low risk mothers. Specifically, their diastolic pressure (95.80±11.69) was significantly raised as compared with those of the controls (75.20±5.04). Similarly, although statistically not significant at p value < 0.05, the systolic pressure was also raised. The mean gestational age was 39.04±5.65 and 40.89±10.34 in low risk and preeclamptic mothers respectively. Regarding mode of delivery, in this study the rate of C/S delivery was higher in the preeclamptic group than the low risk group, being 48% in the preeclamptic group while only 8% of the low risk group delivered by C/S.

Table 3: Clinical data of the study population

Variable	Controls (mean ± SD)	Preeclampsia cases (mean ± SD)	P value
Systolic pressure (mmHg)	112.60±6.32	141.88±23.62	0.093
Diastolic pressure (mmHg)	75.20±5.04	95.80±11.69	0.008*
Gestational age (weeks)	39.04±5.65	40.89±10.34	0.238

*: statistical significant difference at $p < 0.05$

In this study majority of the newborns had normal birth weight in both groups, although the mean birth weight was lower in preeclamptic group, being 2764gm for preeclamptics and 3135.7gm for controls.

Table 4: Weight categories among the newborns of low risk and preeclamptic mothers

Weight (gm.)	Controls (%)	PE (%)
1000-1499	0	4.0
1500-2499	4.0	20.0
2500-3999	92.0	72.0
≥4000	4.0	4.0

Although it is not statistically significant at p value <0.05, the umbilical cord length is lower in the preeclamptic group than the low risk groups.

In this study, the umbilical cord diameter is significantly lower in the preeclamptic group than in the low risk mothers with p value of 0.024, and the mean values of 0.52±0.14cm in PE and 1.14±0.26cm in control group.

Table 5: Gross examination of the umbilical cord in preeclamptic and control groups

UC parameter		Mean ± SD	P Value
Length (cm)	Control	53.86±14.98	0.964
	PE	47.52± 13.54	
Diameter (cm)	Control	1.14±0.26	0.024*
	PE	0.52±0.14	

*: statistical significant difference

Among preeclamptic mothers, umbilical cord insertion site was central in 8%, and marginal in 16%, while the rest had eccentric insertion site (76%). In the non preeclamptic participants, the insertion sites were again central in 18%, and marginal in 6%, while the majority had eccentric insertion (76%). The difference was not statistically significant.

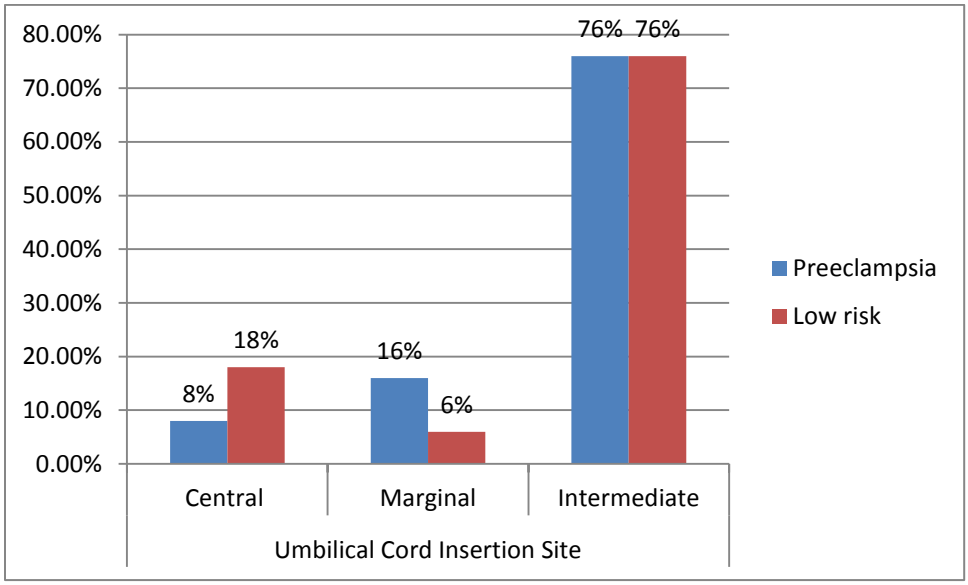

Figure 3: Site of insertion of umbilical cord among preeclamptic and low risk mothers

Histological examination of the umbilical cord under light microscope using H&E staining showed that luminal diameter of the umbilical artery gradually decreased from the placental to fetal segments in the preeclampsia cases ($p = 0.003$). The luminal diameter of the umbilical artery at fetal end was significantly lower in the preeclamptics than the low risk group ($p<0.05$), with mean values of 40±15µm for preeclamptic group and 87.33 ± 67.7µm for controls.

Although it is not statistically significant at p value <0.05, the luminal diameter of the umbilical artery at both placental and middle segments was lower for the preeclamptics than the low risk group.

Similarly the umbilical artery wall thickness at fetal end of the cord was significantly lower for the preeclamptic group than the low risk group with a mean of 630.0 ± 59.16µm. Although statistically not significant at p value <0.05, the umbilical artery wall thickness of middle and placental segments was lower for preeclamptic group than the low risk. No histopathologic evidence of inflammation, thrombosis, or fibrinoid necrosis of umbilical arteries was observed.

Table 6: Morphological changes of umbilical arteries

Measurements	**Fetal (mean ± SD**	**Middle (mean ± SD**	**Placental (mean ± SD**	**P Value (ANOVA)**
Luminal diameter (µm)				
Controls	87.33 ± 67.7	130.3 ± 218.0	84.66 ± 66.64	0.597
PE	40.00 ± 15.69	71.33 ± 27.22	80.00 ± 45.35	0.003*
P Value (t- test)	0.027*	0.066	0.542	
Wall thickness (µm)				
Controls	640.0 ± 103.4	635.0 ± 181.9	620.0 ± 97.37	0.913
PE	630.0 ± 59.16	600.0 ± 86.60	610.0 ± 76.06	0.540
P Value (t- test)	0.013*	0.234	0.253	

*: statistical significant difference

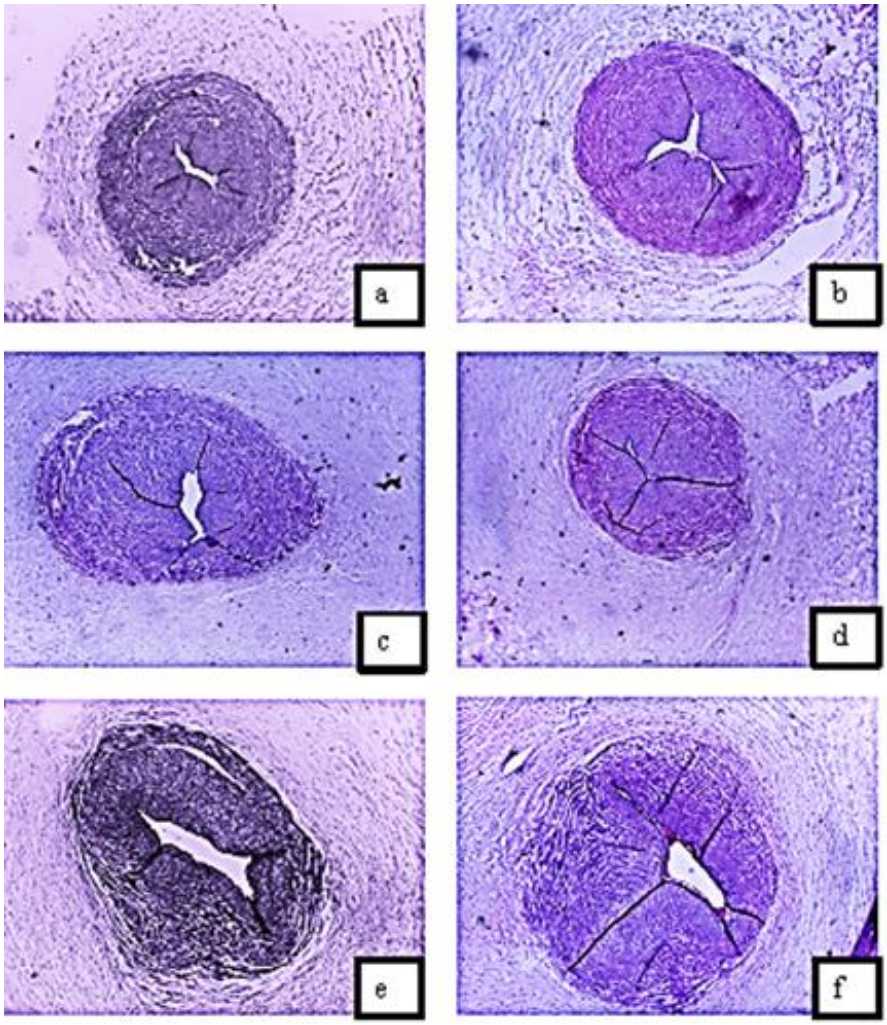

Figure 4: Photomicrographs of Fetal (a & b), Middle (c & d) and Placental (e & f) segments of umbilical arteries from control (a, c & e) as compared to preeclamptic mothers (b, d & f). H & E stained sections (×4)

The luminal diameter of umbilical vein of preeclamptic mothers was found to be reduced in all segments of the umbilical cord. Specifically, at placental end of the umbilical cord, it was significantly reduced with a mean of 543.3 ± 116.3µm for preeclamptic mothers as compared to 620.0 ± 254.1µm for controls.

This study also found that the wall thickness of the umbilical vein in all segments of the cord were reduced in the preeclamptic group than in the controls, although not statistically significant

at p value <0.05. No histopathologic evidence of inflammation, thrombosis, or fibrinoid necrosis of umbilical veins was observed.

Table 7: Morphological changes of umbilical veins

Measurements	**Fetal (mean ± SD**	**Middle (mean ± SD**	**Placental (mean ± SD**	**P Value (ANOVA)**
Luminal diameter (µm)				
Controls	493.3 ± 190.7	526.7 ± 123.6	620.0 ± 254.1	0.201
PE	453.3 ± 120.2	480.0 ± 202.5	543.3 ± 116.3	0.259
P Value (t- test)	0.239	0.212	0.022*	
Wall thickness (µm)				
Controls	595.0 ± 111.8	491.6 ± 113.2	473.3 ± 90.36	0.006*
PE	476.6 ± 97.49	403.3 ± 67.39	458.3 ± 88.47	0.061
P Value (t- test)	0.768	0.142	0.564	

*: statistical significant difference

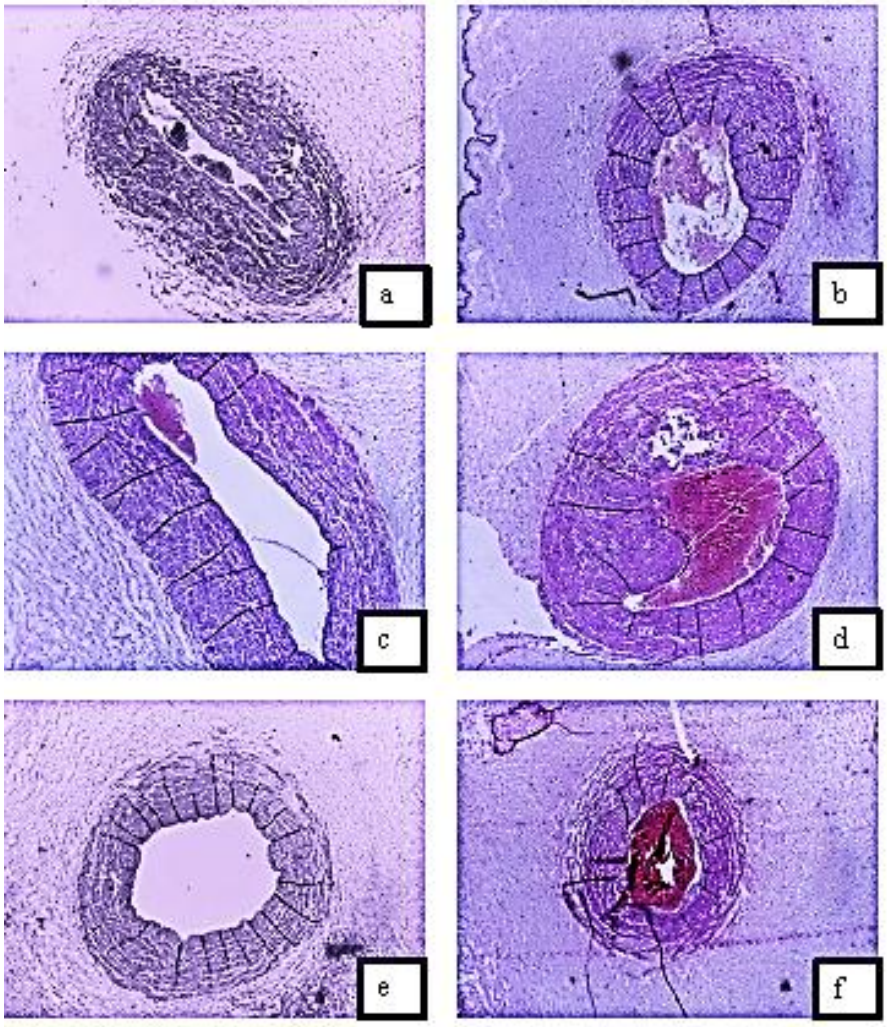

Figure 5: Photomicrographs of Fetal (a & b), Middle (c & d) and Placental (e & f) segments of umbilical veins from control (a, c & e) as compared to preeclamptic mothers (b, d & f). H & E stained sections (×4).

6 Discussion

The state of vascular system of mother and of the placenta is known to exert a great influence on intrauterine development of the fetus. Accordingly, preeclampsia is the most common pathological syndrome associated with pregnancy which is known to affect the vascular system of the mother as well as the fetus including the umbilical vessels (Barnwal *et al*., 2012).

In the present study the mean length of umbilical cord was 47.52± 13.54cm in preeclamptic mothers and 53.86±14.98cm in low risk mothers. Even though, the difference was not statistically significant there were a reduction in the length of umbilical cord in preeclamptic cases. This study was in consistent with studies done in India and Pakistan where there were no statistically significant difference in cord length between controls and gestational hypertension/preeclampsia, respectively as 31.46± 10.49cm and 31.03±10.94cm (Paiker *et al*., 2016), and 57.4±1.17cm and 51.6±2.01cm (Yasoob *et al*., 2014).

This study found that the mean umbilical cord diameter was 0.52±0.14cm in preeclamptic mothers and 1.14±0.26cm in controls. Preeclamptic cases had significantly (p=0.024) reduced umbilical cord diameter than low risk mothers. This is in line with study done by Yasoob et al., (2014) where the umbilical cord diameter was reported significantly reduced in the case group as compared to the control group at the proximal and distal levels (p=0.05). Other studies done in India had also found reduced mean umbilical cord diameter in the preeclamptic group compared to the controls, although was not significant statistically (Bhavina *et al*., 2013; Paiker *et al*., 2016). Changes in the composition of Wharton jelly, such as the glycosaminoglycan, water content and extracellular matrix components were suggested to be the main reasons of the reduction in the diameter of the umbilical cord (Inan *et al*., 2002).

In this study majority of umbilical cords had eccentric insertion site in both preeclamptic mothers and low risk mothers and the difference was not statistically significant. This is in agreement with results from previous studies (Yasoob *et al*., 2014; Kaur *et al*., 2014; Paiker *et al*., 2016). In this study, although the difference was not statistically significant marginal insertion of umbilical cord was higher (16%) in preeclamptic cases than controls (6%).This is in line with a study done

by Kaur *et al*., (2014) where marginal insertion of umbilical cord was found in 16 (21.33%) cases of study group as compared to only 2 (8%) cases of control group, but the statistical difference between two groups was not significant.

The umbilical cord vessels are the connection between the placenta and the fetus. They are influenced by vascular and hemodynamic functions during fetal life (Olaya-C *et al.*, 2016). In this study, the umbilical arteries in preeclampsia had a smaller luminal diameter as compared to the controls. The difference was significant at the fetal end of the cord (p value<0.05), which is a main position for controlling fetal blood flow. Luminal diameter of the umbilical artery gradually decreased in the placental, middle and then fetal segments for preeclampsia cases. This is in agreement with a study done in India where there was narrowing of the lumen of the umbilical artery with thrombosis and fibrinoid necrosis in preeclamptic cases (Yasoob *et al*., 2014). Other studies done in Turkey and Saudi Arabia have also reported significant narrowing of the diameter of the lumen of the artery in preeclamptic group as compared to controls (Inan *et al*., 2002; Almasry *et al*., 2016). Although the difference was not statistically significant reduction in luminal diameter, had also reported (Barnwal *et al*., 2012; Karim et al., 2016). Such luminal reduction may be due to separation of the muscular layer of the arteries in the PE group from the surrounding Wharton's jelly because of presence of strong vasoconstriction which may subsequently lead to narrowing of the lumen (Karim *et al*., 2016). In the contrary, Blanco *et al.* (2011) had reported that lumen area of umbilical arteries were significantly higher in the preeclamptic mothers as compared to the controls. However such difference may be due to small sample size (9 cases and 20 controls) used by Blanco *et al.* (2011).

In the present study the preeclamptic cases had thinner arterial wall thickness as compared to those of the controls. The difference was, however, significant (P<0.013) only at the fetal end. This is in line with a study done in Turkey where the umbilical artery wall thickness was significantly reduced in preeclamptic mothers in comparison to the controls (Inan *et al*., 2002). Comparable results were also found by Sharony *et al*. (2016), although the difference was not statistically significant. Such finding was partially explained by the presence of contracted smooth muscle cells that were smaller than their normal size suggesting for a predominant hypoplastic mechanism. The first response to hypoxemia is vasoconstriction of the vessels. If

hypoxemia continues, it may cause morphological changes such as hypoplasia (Inan *et al*., 2002). In the contrary, the present study was in contrast to some other studies (Blanco *et al.*, 2011; Barnwal *et al*., 2012; Yasoob *et al*., 2014; Balsaka *et al*., 2015) who founds that wall thickness of umbilical arteries was significantly increased in cases than controls. This difference may be explained by differences in the study population and methodology.

In this study preeclampsia cases had decreased venous luminal diameter in all segments of the cord, although the difference was significant at p value <0.05 only for the placental end of the cord. This is consistent with a study done in China where preeclampsia cases had significantly decreased lumen diameter compared to controls for all segments of the umbilical vein (Lan *et al*., 2018). Other studies have also reported the luminal area was significantly less in cases than in controls (Inan *et al*., 2002; Koech *et al*., 2008; Yasoob *et al*., 2014).Although the difference was not significant at p value <0.05 Blanco *et al.* (2011) have also reported a decrease in luminal diameter in cases than controls. The smaller lumen may also suggest a state of chronic vasoconstriction (Koech *et al*., 2008). Inan *et al*., (2002) found that the umbilical veins of patients with PIH show contracted smooth muscle cells, which is a characteristic of vasoconstriction. Schonfelder *et al.* (2004) demonstrated presence of nitric oxide synthase (NOS) in smooth muscle of the umbilical vein but none in the artery. On the other hand, preeclampsia was found to be associated with a total loss of NOS protein expression and a significant decrease in mRNA. This reduction in nitric oxide may therefore lead to vasoconstriction of the umbilical vein and result in a smaller lumen in PIH. However, two other studies found a significantly increased venous luminal diameter in Preeclamptic group (Barnwal *et al*., 2012; Almasry *et al*., 2016). The difference may be explained by difference in study population.

In the present study, umbilical veins in Preeclampsia had a thinner wall thickness as compared to the controls for all segments of the veins, although not statistically significant at p value <0.05. This is in line with other studies where there was insignificant reduction (Sharony *et al*., 2016) and significant reduction (Inan *et al*., 2002; Barnwal *et al*., 2012; Karim *et al*., 2016) of umbilical vein wall thickness in preeclampsia. Such findings were suggestive of hypoplastic effect via various factors (Barnwal *et al*., 2012). However, other studies (Koech *et al*., 2008; Yasoob *et al*., 2014; Balsaka *et al*., 2015; Lan *et al*., 2018) reported that increased wall thickness

of umbilical vein in the cases than in the controls. This may be again due to the differences in the study population and methodology.

7 Conclusion

The present study collectively found that although the length and site of insertion of umbilical cord did not show a significant difference, preeclamptic mothers had affected morphology and histology of the umbilical cord as compared to controls. The diameter of the umbilical cord was significantly reduced. There were decrement in the wall thickness and luminal diameter of both umbilical arteries and veins in the preeclamptic mothers as compared to controls. However, the reductions in the diameters were found statistically significant only in the fetal segment of umbilical arteries and placental segment of umbilical veins.

8 Recommendations

The following recommendations as per the findings from this study are made:

- Routine umbilical cord examination and measurements by clinicians during postpartum period may add useful information to the knowledge of gross morphological abnormalities in PE cases.
- This study found that umbilical cord vascular abnormalities are common in preeclamptic cases, so that detection of umbilical cord irregularities by imaging like ultrasound may provide useful information about pregnancy at risk and help in averting poor perinatal outcome.
- This study also can be used as a baseline for further large scale (with a larger sample of participants) gross, histomorphological as well as immunohistochemical studies to be conducted in the same condition or other clinical scenario.

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Annex

I. English version consent form

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

Title of the study: Gross and histomorphometric changes of umbilical cord and its vessels in preeclampsia at Black Lion and Gandhi hospitals, Addis Ababa, Ethiopia.

Verbal Consent Form

Dear study participant, I am----------. I am doing research for partial fulfillment of the requirement for the degree of master in Human Anatomy at Addis Ababa University. I would like to ask your permission to give your placenta to conduct a study on a change of umbilical cord length, diameter, insertion site, wall thickness and luminal diameter of umbilical cord vessels and any histopathologic change of umbilical cord vessels in preeclampsia. Your permission is important in order to do research on your umbilical cord and will help policy makers to design strategies to prevent and control maternal and child morbidity and mortality secondary to preeclampsia. All findings from your umbilical cord will be kept strictly confidential. You are not obligate to give permission without your interest. If you feel discomfort to give permission, please feel free to dropout at any time you want. Could I have your permission to continue?

1. Yes, continue.
2. No, skip to the next subject.

II. Amharic version consent form

አዲስ አበባ ዩኒቨርሲቲ

የህክምናና ጤና ሳይንስ ኮሌጅ

ይህን መጠይቅ በጋንዲ እና በጥቁር አንበሳ ሆስፒታሎች ከእርግዝና ጋር በተያያዘ የደም ግፊት በሽታ ያለባቸዉ እናቶችን የእትብት ቁመት፣ ዲያሜትር፣ ከእንግዴ ልጅ ጋር የተጣበቀበት ቦታ እንዲሁም የእትብት የደም ስሮች የቅርፅ ለውጥ ለማጥናት የተዘጋጀ ነው።

የፈቃደኝነት ቅፅ

ጤና ይስጥልኝ----------እባላለሁ። እዚህ የተገኘሁት ይህንን ጥናት በአዲስ አበባ ዩኒቨርሲቲ በአናቶሚ ትምህርት ለሁለተኛ ዲግሪ ማሙያ ጥናት ለማካሄድ ነው፡፡ በዚህ ሆስፒታል በወለድሽዉ እትብት ላይ የሚታየውን የቁመት፣ የዲያሜትር፣ ከእንግዴ ልጅ ጋር የተጣበቀበት ቦታ እንዲሁም የእትብት የደም ስሮች የቅርፅ ለውጥ ለመረዳት እንፈልጋለን። በመሆኑም ጥናቱን እንድናካሂድ ፈቃድዎን እንጠይቃለን። ፈቃድዎን ከሰጡንና ጥናቱ ከተካሄደ ከእርግዝና ጋር ተያይዞ በሚከሰተው የደም ግፊት በሽታ የተነሳ የሚታየውን የእትብት እና የእትብት ደም ስሮች ለውጥ በመለየት በእናቶች እና ህፃናት ላይ የሚከሰተውን የህመም፣ የሞት እና የጤና ችግር ለመቅረፍ የሚያስችሉ ፖሊሲዎችን ለመቅረፅ የሚያስችል መረጃ ለመስጠት ያስችላል። ከእርሶ የሚገኘውን መረጃ በሚስጥር እንጠብቃለን። ጥናቱን የምናካሂደው የእርሶን ሙሉ ፈቃደኝነት ስናገኝ ብቻ ነው።

ጥናቱ እንዲካሄድ ፈቃደኛ ነዎት?

- ☐ አዎ፥ ይቀጥሉ
- ☐ አይደለሁም፥ ያቋርጡ

III. **Checklist**

Age		
Parity		
Gravidity		
Abortion history		
Height		
Weight (pre pregnancy)		
GA (working GA) 37 – 42 WKS		
BP		
Low risk		
PE	Mild	
	Sever (severity sign)	
Eclampsia		
Mode of delivery		
Birth weight		
Sex		
UC color	White	
	Blue	
	Pink	
	Meconium stained	
UC length/cm		
UC insertion	Central	
	Marginal	
	Intermediate	
	Velamentous	
	Other	
UC diameter/cm		
Histopathology		

- Central insertion will be recorded when it attached at the center or within 2 cm of the center of the placenta
- Marginal insertion will be recorded when the cord attached at or within 2 cm of placental margin
- Intermediate insertion will be recorded as a position between the central and marginal point of attachment
- Velamentous insertion will be recorded when the cord inserted on the fetal membranes

IV. Tissue processing procedure

The working method for all of the histological sections followed the routine paraffin procedure as follows:

1. **Fixation:** The tissue was fixed in 10% formalin solution for 24 hrs.
2. **Washing**: The preserved tissue was washed in running tap water several times.
3. **Dehydration:** Then, they passed through upgraded alcohol as follows:-
   70% alcohol - 1hour
   90 % alcohol - 1hour
   Absolute alcohol I - 1hour
   Absolute alcohol II - 1hour
   Absolute alcohol III - 1hour
4. **Clearing:** Clearing of tissue was done in:-
   Xylene I - 1hour
   Xylene II - 1 hour
5. **Infiltration:** Tissue was infiltrated with:-
   Paraffin wax I - 1 and 1/2 hrs.
   Paraffin wax II - 2 and 1/2 hrs.
   Paraffin wax III - overnight.
6. **Embedding:** This step was carried out using an embedding center, where a mould is filled with molten wax and the specimen placed in to it. The paraffin blocks of tissue were made with the help of embedding cassettes.
7. **Sectioning:** The serial paraffin sections of 5µm thickness were cut by rotator microtome and floated in water bath having temperature 45-50 degree Celsius. The sections were made spread on the slide smeared with adhesive solution (mixture of equal amount of glycerol and egg albumin). The slides were dried on hot plate having temperature 50 degree Celsius.
8. **De paraffining of sections:** The slides were put in:-
   Xylene I - 5min
   Xylene II - 5min
9. **Rehydration:** The slides were put in descending grades of alcohol:-
   Absolute alcohol - 2min
   90% alcohol - 2min
   70% alcohol - 2min
   50% alcohol - 2min

The slides were then washed in running tap water for 2min and taken for routine H & E staining.

10. Staining with hematoxylin and eosin

- Stained with hematoxylin for 10 minute.
- Washed in running tap water until section become blue.
- Stained in 1% eosin for 7- 10 min.
- Washed in running tap water (5 min)
- Dehydrated in:-

  70% alcohol - 3min

  95% alcohol - 3 min

  Absolute alcohol I - 3min

  Absolute alcohol II - 3min

- Clear in:-

  Xylene I - 5min

  Xylene II - 5min

	Department of Anatomy Title: Department Research Ethics Review Committee (DRERC)

Meeting No:DRERC/01/09 Date: June 01, 2017

Protocol Title: Gross and Histomorphological study of umblical cord and its vessels in preeclampsia-Eclampsia		
Principal Investigator: Institute:	Elsabet Mohammed Yesuf	
Elements Reviewed	☐ Attached	☐ Not attached
Decision of the meeting:	☒ Approved	☐ Approved with Recommendation
	☐ Resubmission	☐ Disapproved

1. Obligation of the PI-
   i. Should comply with the standard international and national scientific and ethical guidelines
   ii. All amendments and changes made in protocol and consent form needs DREC approval
   iii. The PI should report Serious Adverse Event(SAE) within 10 days of the event
   iv. End of the study, including thesis work and manuscript should be reported to the DREC

2. To IRB ☐

Follow up report expected in
3 Months_____ 6 Months ____ 9 Months _____ one year _____

Acting Secretary, DREC: DrGirmaSeyoum (PhD)

Signature

Date: 01/06/2017

Department Stamp

ADDIS ABABA UNIVERSITY Dept. of Anatomy Medical Faculty A.A.

DEPARTMENT OF ANATOMY
FACULTY OF MEDICINE
ADDIS ABABA UNIVERSITY

P.O. Box 9086
Addis Ababa, Ethiopia

አናቶሚ ክፍል
ሕክምና ፋካልቲ
አዲስ አበባ ዩኒቨርሲቲ

Tel: 251-115-537967
Fax: 251-115-513099

anat 87/09
Date: June 26, 2017

TO: Gandhi Memorial Hospital,
Addis Ababa

FROM: Ato Girmay Amare (MSc),
Acting Head Department of Anatomy,
CHSs, AAU

SUBJECT: **Request for cooperation for MSc Research work of Elsabet Mohammed Yesuf,**

Elsabet Mohammed is working her MSc Thesis entitled: Gross and histomorphometric changes of umbilical cord and its vessels in preeclampsia-eclampsia at Black Lion and Gandhi Memorial Hospitals.

Therefore, the Department of Anatomy requests your cooperation in performing her study. I also want to thank you in advance for your support and cooperation.

With regards

DEPARTMENT OF ANATOMY
FACULTY OF MEDICINE
ADDIS ABABA UNIVERSITY

P.O. Box 9086
Addis Ababa, Ethiopia

አናቶሚት/ክፍል
ሕክምና ፋካልቲ
አዲስ አበባ ዩኒቨርሲቲ

Tel: 251-115-537967
Fax: 251-115-513099

R.no. anat 86/09

Date: June 26, 2017

TO: Obstetrics and Gynecology Department,
CHSs, AAU

FROM: Ato Girmay Amare (MSc),
Acting Head Department of Anatomy,
CHSs, AAU

SUBJECT: **Request for cooperation for MSc Research work of Elsabet Mohammed Yesuf,**

Elsabet Mohammed is working her MSc Thesis entitled: Gross and histomorphometric changes of umbilical cord and its vessels in preeclampsia-eclampsia at Black Lion and Gandhi Memorial Hospitals.

Therefore, the Department of Anatomy requests your cooperation in performing her study. I also want to thank you in advance for your support and cooperation

With regards

አዲስ አበባ ዩኒቨርሲቲ፣ ጤና ሣይንስ ኮሌጅ
ሕክምና ት/ቤት
የፅንስና ማህፀን ት/ክፍል

Addis Ababa University
College of Health Sciences
School of Medicine
Department of Obstetrics & Gynecology

ቀን :-ኃምሌ 18, 2009

Ref:- MF/Gyn/009/2009

ለጥቁር አንበሳ ስፔሻይዝድ ሆስፒታል

ለጋንዲ መታሰቢያ ሆስፒታል

አዲስ አበባ

ጉዳዩ' ትብብር ስለመጠየቅ፡፡

ኤልሳቤጥ መሃመድ የተባሉ የአናቶሚ ትምህርት ክፍል የመጨረሻ ዓመት የማስተርስ ተማሪ ሲሆኑ ለምርምር ሥራ ይረዳቸው ዘንድ "Gross And Histomorphological study of umbilical cord and its vessels in preeclampsia- Eclampsia" በሚል ርዕስ ለሚሰሩት የምርምር ሥራ ከአዲስ አበባ ዩኒቨርሲቲ፣ጤና ሣይንስ ኮሌጅ፣የሕክምና ት/ቤት የአናቶሚ ትምህርት ክፍል "DRERC" Ethical Clearance የተሰጣቸው በመሆኑ ሥራቸውን መሥራት እንዲችሉ መረጃ እንዲሰጣቸው ትብብር እንዲደረግላቸው በትህትና እንጠይቃለን፡፡

ከሰላምታ ጋር

Dept. of Obs-Gyn, Faculty of Medicine

ከአብዱ መንገሻ (ዶ/ር)
የማህፀንና ጽንስ ሕክምና ዘርፍ ት/ክፍል
ሕክምና ትምህርት ቤት
ጤና ሣይንስ ኮሌጅ
አዲስ አበባ ዩኒቨርሲቲ

ግልባጭ

- ኤልሳቤጥ መሃመድ

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