Anemia in pregnancy is a public health problem biology essay

Science, Biology

Chapter 1

INTRODUCTION

Anemia in pregnancy is a public health problem, mainly in developing countries (Al-Hilli, 2010). Anemia is a qualitative or quantitative deficiency of hemoglobin or red blood cells in circulation causing reduced oxygen transport capacity of the blood to tissues and organs (Grewal, 2010). In pregnant women this condition is associated with an increased risk of low birth weight, preterm delivery, perinatal and maternal mortality (Malee, 2008). According to WHO standards, anemia in pregnancy is present when the hemoglobin concentration in the peripheral blood is less than 11gm/dl (Buseri et al., 2008). Global prevalence of anemia in pregnant women is 41. 8%, in South-east Asia it is 48. 2% and in Pakistan 39. 1% pregnant women are anemic (Benoist et al., 2008). In many developing countries, prevalence rates of up to 75% are reported (Broek, 2003). In Pakistan, anemia during pregnancy is supposed to be more common and according to local studies it is approximately 90%, varying slightly geographically. Anemia is high in this area due to certain socio-economic issues and culture. Most of the women are malnourished, ignorant, have multiple pregnancies beginning at an earlier age, have lack of health care and birth control (Khalil et al., 2007). Anemia is the late manifestation of deficiency of nutrients needed for hemoglobin synthesis. Iron deficiency is responsible for more than 90 percent cases of maternal anemia. The incidence of folate deficiency is around 5 percent (though it is often underdiagnosed) and this is almost always the cause of megaloblastic anemia in pregnancy, with vitamin B12

deficiency being rare (Johnston, 2004). Multiparity, poor socioeconomical and educational status are the principal reasons for high prevalence of anemia in our population (Awan, 2004). Anemia in pregnancy can be categorized as mild, moderate or severe, with WHO categorizing mild anemia as hemoglobin level of 10. 0-10. 9gm/dl, moderate anemia as 7-9. 9gm/dl and <7gm/dl as severe anemia (Regil et al., 2011; Grewal, 2010). It is extremely common that severe anemia contributes to maternal morbidity and mortality (Buseri et al., 2008). It is assessed that anemia may be responsible for 40-60 % of maternal deaths in developing countries. It causes deaths from cardiac failure, hemorrhage, infection and pre-eclampsia (Jaleel and Khan, 2008). Severe maternal anemia may impair the oxygen delivery to the fetus and interferes in normal intra-uterine growth, resulting in intrauterine growth retardation, still birth, low birth weight and neonatal deaths. Infants of anemic women are born with reduced iron stores and are at risk of anemia during infancy and increased risk of infant morbidity and mortality (Buseri et al., 2008). Normal fetal growth and survival depends on the appropriate development and function of the placenta (Li et al., 2006). The placenta is an energetic organ which is distinctive in its growth and functions. It is the only organ which is derived from two distinct individuals, the mother and the fetus. The placenta is responsible for the nutritional, respiratory, excretory, immunological and endocrinal functions of the fetus. The abnormalities of the placenta are usually related with placental inefficiency, which could lead to problems in the fetus (Raghunath et al., 2011). Placenta is the most precise record of the infant prenatal experience. After delivery if the placenta is examined thoroughly, it provides much insight into the prenatal

health of the baby and the mother (Verma, 2010). Placenta is the structure where the fetal and maternal tissues come in direct contact without rejection. Maternal blood bathes the surfaces of the chorion, which fill the intervillous space. The placenta is the site of exchange between maternal and fetal circulation (Ross, 2006). The placental tissue can be described as a sum of parenchymal, non-parenchymal and pathological components. The parenchyma consists of villi with their vessels and maternal intervillous space, the non-parenchyma consists of chorionic and decidual plates, fetal vessels of diameter greater than 0. 1 mm and maternal intercotyledonary septa (Chowdhury, 2009). Anemia in pregnancy is related with variable histomorphological changes in placenta, which show a clear reflection for the poor fetal outcome. There is a threshold for the level of hemoglobin and consequently for oxygen transport below which placental function is impaired. This explains the increased frequency of premature births, fetal death and perinatal mortality and morbidity in anemia during pregnancy (Mongia, 2011). A study carried out in Mexico reported that the most frequent lesion observed in placentas of anemic mothers, is infarct (Levario et al., 2003). Infract is characterized by aggregation of the villi with marked narrowing, often obliteration of the intervillous space. The syncytial nuclei show early necrotic change such as pyknosis and there is progressive coagulative necrosis of the villi (Huang et al., 2001). The microscopic changes of the placental components in maternal anemia studied in Bangladesh, confirmed that the pathological areas such as infarcts are significantly increased and villous area is reduced in anemic groups (Begum et al., 2010). Intervillous space is found between chorionic villi and is filled

with mother’s blood (Ramic et al., 2006). In a quantitative study of placental structures in women with pregnancy iron deficiency anemia, conducted in China, a significant increase in absolute volume of the intervillous space is found in the placentas of anemic mothers and a negative correlation between maternal hemoglobin level and absolute volume or surface area of the intervillous space is observed (Huang et al., 2001). In maternal anemia the placenta adapts through thinning of the villous membrane so that diffusion capacity is maintained at normal levels. Morphometric analysis of villous membrane thickness, carried out in Ukraine, revealed that mean thickness of the villous membrane is significantly less in the anemia because of an increase in volume fraction of the fetal capillaries. Consequently, the morphometric diffusing capability of the villous membrane is maintained (Reshetnikova et al., 1995). Syncytial knots are aggregates of syncytial nuclei at the surface of tertiary villi and develop in the third trimester, due to ischemic changes (Loukeris et al., 2010; Saga et al., 2008). Quantitative changes in histological features of full term placenta in anemia, studied in India, revealed that the numbers of syncytial knots are significantly more in the anemic group and there is also a tendency for the number of vasculosyncytial membranes to be more in the anemic group. These changes suggest adaptation to relative hypoxia in anemia (Dhall, 1994). Placental hypertrophy is associated with mild and moderate degree of maternal anemia and the enlargement of placenta appears to be a uniform physiological compensatory growth (Huang et al., 2001). The effects of anemia on placenta and birth weight have been evaluated in Mexico and a trend towards an increase in placenta weight in patients fulfilling the anemia

criteria is observed (Levario et al., 2003). There is need to explore the extent of above mentioned structural changes, because severity of these histomorphological parameters is correlated with the efficiency of placenta to support the growth of a fetus, and this condition is likely to be related to insufficient function of the placenta (Roberts et al., 2008). In West, analysis of histopathological changes in placenta of anemic mothers has been carried out but there are no such studies available in Pakistan where these changes are evaluated by morphometry. In order to address this issue we designed the present study.

PURPOSE OF STUDY

The present study was aimed to evaluate the histopathological parameters of the placenta in maternal anemia in both qualitative and quantitative manner, to assess the effects of anemia on placenta, in our setup.

Chapter 2

REVIEW OF LITERATURE

2. 1 ANATOMY OF PLACENTA

2. 1. 1 Placenta

At full term, the placenta is flattened and circular and has a sponge like consistency. A normal term placenta measures 15-20 cm in diameter, 1. 5 to 3 cm in thickness and weighs 450 to 600 g. It thins out at the edges, where it is continuous with the fetal membranes (Snell, 2012; Rosai, 2011). The placenta is torn from uterine wall approximately 30 minutes after the birth of the child and is expelled from the uterine cavity (Sadler, 2012). Once it is

cast off from the uterus it takes the form of a flattened, round or oval organ. It presents two surfaces and a margin, the surface in contact with the decidua is designated as the maternal surface, and the surface directed towards the fetus is the fetal surface. The maternal surface of freshly shed placenta is dark red in color and oozes blood from the maternal vessels. When held between the finger and thumb it has a sponge like consistency (Snell 1995). The maternal surface of placenta is divided into about 20 irregular segments called cotyledons that are demarcated from each other by the positions of the placental septa (Young et al., 2006). Cotyledons are enclosed by a layer of decidua basalis (Sadler, 2012). Fetal surface of the placenta is entirely covered by the chorionic plate. The chorionic vessels, join towards the umbilical cord, the veins being deeper. The chorion is in turn covered by the amnion (Sadler, 2012).

2. 1. 2Fetal membranes

Fetal membranes extend from the placental margin and consist of amnion and chorion. The amnion, which is the innermost of the membranes, is thin, smooth, shiny and transparent. It has a thickness of about 0. 02 to 0. 5mm. The chorion is thicker, vascular and more opaque than amnion. It contains villi and a scant decidua parietalis. The chorion forms a complete covering that surrounds the embryo, amnion, yolk sac and body stalk (Novak and Woodruff 1979).

2. 1. 3Umbilical cord

The umbilical cord at term measures 55 to 65 cm in length (Rosai, 2011). It is inserted in the placenta in a central or eccentric fashion. Insertion at the

margin is called " Battledore placenta" (Baird, 1957). The bulk of cord is made up of highly mucoid connective tissue known as Wharton’s jelly. Embedded within its substance are umbilical vessels, represented by two arteries and a single vein (De Lia and Bendon, 1997).

2. 2 HISTOLOGY OF PLACENTA

The placenta is the only organ composed of cells derived from two different individuals. The chorion, characterized by the villi, is the fetal part and the decidua basalis, derived from endometrium, is the maternal part.

2. 2. 1Trophoblastic Villi

The trophoblastic villi that arise from the trophoectoderm following formation of the blastocyst, constitute the functional unit of the placenta. During the first trimester, they are composed of an outer syncytiotrophoblastic layer and an inner cytotrophoblastic layer, which surrounds a central mesenchymal core containing primitive fibroblast and scattered macrophages (Hofbauer cells). The syncytiotrophoblast is composed of multinucleated giant cells with abundant eosinophilic cytoplasm. The cytotrophoblast, which is the progenitor of the syncytiotrophoblast, is made up of mononuclear cells with clear cytoplasm and a well-defined cell membrane. In the term placenta, the cytotrophoblast is inconspicuous, and the syncytiotrophoblast is clumped in the form of syncytial knots. The third trophoblastic type is presented by the intermediate trophoblast, also known as interstitial extravillous trophoblast and X cells. This type is present in the villi and in the membranes but is particularly numerous in the extravillous region that forms the deepest structural component of the implantation site

(Rosai, 2011). Each villous has a core of connective tissue containing collagen and fibronectin (Williams et al, 1998). The core of villus also comprises blood vessels that make capillary loops in the smallest villi. The capillaries are without fenestrae and have extensive bands of intermediate filaments. There are no nerves or lymphatics in placental villi. By the third trimester there may be as many as 15 generations of branching villi from the chorionic plate. The first orders of villi that contain only capillaries are intermediate villi. These are 300 to 600 µm long but slender. Terminal villi arise irregularly from intermediate villi. They are short and reduced to about the size of 35 µm in diameter. The dilated fetal capillaries become situated close to the surface and even bulge the surface of syncytial trophoblast in thin regions. Subsequently the diffusion distance between fetal blood in placental capillaries and maternal blood in the intervillous space is significantly reduced in the later placenta enabling oxygenation of fetal blood (Kelly et al., 1984).

Decidua

During the process of implantation, secretion by the syncytiotrophoblast of human chorionic gonadotrophin interrupts the ovarian cycle. This results in growth and proliferation of stromal cells of the endometrial stratum functionalis, at the implantation site into large polyhedral decidual cells. The decidua beneath the developing embryo is known as decidua basalis and with the trophoblast will form the placenta. The decidua overlying the embryo is known as decidua capsularis and the decidual lining of the rest of the uterus is called the decidua parietalis. The decidual cells proliferate and

enlarge greatly, their cytoplasm staining pink due to the presence of numerous mitochondria and intermediate filaments. These large cells have vacuolated cytoplasm that contains glycogen and lipids and a clear nucleus with a prominent nucleolus (McPhee et al., 1993).

2. 3DEVELOPMENT OF PLACENTA

Approximately 3 days after fertilization, the cells of the compacted embryo divide to form a morula. The inner cell mass of morula forms the embryo proper, and the outer cell mass give rise to the trophoblast, which later take parts in the formation of placenta. When the morula enters the uterus on the 3rd or 4th day of fertilization, a cavity appears, and the blastocyst forms. Cells of the inner cell mass, called the embryoblast, and those of the outer cell mass, are called trophoblast. At the start of the second week, the blastocyst is partly implanted in the endometrial stroma. Trophoblast differentiates into an inner, vigorously proliferating layer, the cytotrophoblast, and an external layer, the syncytiotrophoblast, which erodes maternal tissues. By day 9, lacunae develop in the syncytiotrophoblast. Subsequently, maternal blood comes in the lacunar network and at the end of the 2nd week, an embryonic uteroplacental circulation initiates. The cytotrophoblast, in the meantime, forms cellular columns penetrating into and enclosed by the syncytium. Those columns are called primary villi. At the end of the 2nd week, the blastocyst is entirely inserted. By the start of the 3rd week, the trophoblast is described by primary villi that contain of a cytotrophoblastic core enclosed by a syncytial layer. During further development, mesodermal cells enter the core of the

primary villi and develop toward the decidua. This is called a secondary villus. By the end of the 3rd week, mesodermal cells present in the core of the villus start to differentiate into blood cells and blood vessels, establishing the villous capillary system. The villus is now called a tertiary villus. The capillaries in tertiary villi make contact with the capillaries developing in mesoderm of chorionic plate and in connecting stalk. In the meantime, cytotrophoblastic cells in villi penetrate gradually into the overlying syncytium till they touch the maternal endometrium. Here they create contact with similar extensions of adjacent villous stems, developing a thin outer cytotrophoblast shell. This shell gradually surrounds the trophoblast completely and attaches the chorionic sac firmly to the maternal endometrial tissue. Villi that spread from chorionic plate to decidua basalis are termed stem or anchoring villi. Those villi that branch from the sides of stem villi are terminal villi, over which transfer of nutrients and other elements will ensue. The chorionic cavity turns larger. By the 19th or 20th day, embryo is connected to its trophoblastic shell through a thin connecting stalk. The connecting stalk later grows into the umbilical cord. By the beginning of the 2nd month, trophoblast contains numerous secondary and tertiary villi. Stem villi spread from the mesoderm of the chorionic plate to cytotrophoblast shell. Surface of the villi is made by the syncytium, lying on a layer of cytotrophoblastic cells that cover a core of vascular mesoderm. Capillary system growing in the core of villous stems rapidly comes in connection with capillaries of chorionic plate and the connecting stalk, thus establishing an extraembryonic vascular system. Through spiral arteries maternal blood is delivered to the placenta. Erosion of maternal vessels to release blood into

the intervillous spaces is associated with endovascular invasion by the cytotrophoblast cells. These cells invade the terminal ends of the spiral arteries, where they substitute maternal endothelial cells in the vessels' walls, forming hybrid vessels comprising both maternal and fetal cells. Cytotrophoblast cells also undergo an epithelial to endothelial change. Invasion of spiral arteries by the cytotrophoblast cells alters these vessels from high-resistance, small-diameter vessels to low-resistance, larger-diameter vessels that can deliver increased quantities of maternal blood to the intervillous spaces. Through the next months, many small extensions grow out from stem villi and spread as free villi into the adjacent lacunar or intervillous spaces. Firstly, these newly formed free villi are primitive, but by the commencement of the 4th month, the cytotrophoblastic cells and certain connective tissue cells disappear. Syncytium and endothelial wall of blood vessels are the only layers that distinct the maternal and fetal circulations. Frequently, the syncytium turns out to be very delicate, and large pieces containing numerous nuclei may break off and fall into the intervillous blood. These pieces, called as syncytial knots, arrive in the maternal circulation and degenerate. Disappearance of cytotrophoblastic cells occurs from smaller to larger villi, and even though some always persevere in large villi, they do not contribute in the exchange between the two circulations. In the initial weeks of development, villi cover the entire surface of the chorion. As pregnancy progresses, villi on embryonic pole continue to grow and enlarge, giving rise to the chorion frondosum. Villi on abembryonic pole degenerate, and at the 3rd month, this side of chorion, now termed as chorion laeve, is smooth. The alteration between the abembryonic and embryonic poles of the chorion may

be seen in the structure of decidua, which is shed during parturition. Decidua over the chorion frondosum, decidua basalis, comprises of a layer of large cells, called decidual cells, with rich amounts of lipids and glycogen. The decidual plate, is firmly connected to the chorion. The decidual layer above the abembryonic pole is the decidua capsularis. With development of chorionic vesicle, this layer degenerates. Consequently, the chorion laeve comes into connection with the uterine wall (decidua parietalis) on the opposite side of uterus, and the two fuses, obliterating the uterine lumen. Therefore, the only portion of the chorion contributing in the exchange process is chorion frondosum, which, together with decidua basalis, creates the placenta. Likewise, fusion of amnion and chorion to form amniochorionic membrane obstructs the chorionic cavity. This membrane ruptures during labor. Through the commencement of the 4th month, the placenta has two components: a fetal portion, formed by chorion frondosum and a maternal portion, formed by decidua basalis. On fetal side, placenta is bounded by the chorionic plate and on its maternal side; it is bounded by decidua basalis, of which the decidual plate is merged into the placenta. In junctional zone, trophoblast and decidual cells intermix. This zone, composed of syncytial and decidual giant cells, is rich in extracellular material. At this time, most cytotrophoblast cells have degenerated. Among the decidual and chorionic plates are the intervillous spaces that are filled with maternal blood. They are derivative of lacunae in the syncytiotrophoblast. The villous trees develop into the intervillous blood lakes. During the 4th and 5th months, the decidua forms a number of decidual septa, which extend into intervillous spaces but do not extent the chorionic plate. These septa have a core of the

maternal tissue, but their surface is covered by a layer of the syncytial cells, so, a syncytial layer divides maternal blood in the intervillous lakes from the fetal tissue of the villi. Due to this septum formation, placenta is divided into a number of sections, or cotyledons. As the decidual septa do not extent the chorionic plate, contact among intervillous spaces in the different cotyledons is retained. As a result of the uninterrupted growth of the fetus and expansion of uterus, the placenta also expands. Its increase in surface area roughly equals that of the growing uterus, and during the course of pregnancy it covers approximately 15% to 30% of the inner surface of uterus. The increase in thickness of placenta results from arborization of the existing villi and is not caused by additional infiltration into maternal tissues (Sadler, 2012).

2. 4PHYSIOLOGY OF PLACENTA

The placenta is versatile organ carrying out several functions during intrauterine life, many of which are vital.

2. 4. 1Circulation

The single umbilical vein in the cord transports oxygenated blood from placenta to fetus. Blood is returned to the placenta by two umbilical arteries, which are distal branches of hypogastric arteries. Cotyledons obtain their blood through 80 to 100 spiral arteries that enter the intervillous space at regular intervals. Pressure in these arteries forces the blood into intervillous space and bathes the small villi of villous tree in oxygenated blood. When the pressure decreases, blood flows back from chorionic plate towards decidua, where it enters into the endometrial veins. Collectively, the

intervillous space of a mature placenta contains approximately 150 ml of blood. This blood transfers along the chorionic villi, which has a surface area of 4 to 14 m^2. Placental diffusion does not take place in all villi, however, only in those that have fetal vessels in close interaction with the covering syncytial membrane. The syncytium, in these villi, often has a brush border consisting of several microvilli, which significantly increases the surface area and consequently the exchange rate between fetal and maternal circulations (Sadler, 2012).

The placental barrier

The placental membrane, which separates fetal and maternal blood, is originally composed of four layers, endothelial lining of fetal vessels, connective tissue in villous core, cytotrophoblastic layer and the syncytium. From the fourth month on, the placental membrane thins because the endothelial lining of vessels comes in close contact with syncytial membrane, significantly increasing the rate of exchange. The placental membrane, at times called the placental barrier, is not a true barrier, as many substances pass through it easily. Because the maternal blood in the intervillous space is separated from the fetal blood by chorionic derivative. Normally, there is no mixing of maternal and fetal blood. However, small numbers of fetal blood cells infrequently escape across microscopic defects in the placental membrane (Sadler, 2012).

Functions of Placenta

The main functions of the placenta are discussed below.

Exchange of respiratory gases

The placental membrane is highly permeable to respiratory gases. Fetal hemoglobin has a higher affinity for oxygen and a lower affinity for carbon dioxide than maternal hemoglobin. This will therefore favour transfer of oxygen to the fetus and carbon dioxide to the mother (Gude et al., 2004).

Exchange of water and minerals

Water transfer across the placenta is dependent upon hydrostatic and osmotic pressure. It is presumed to move across the placenta passively, and its transfer may be facilitated by a water channel forming integral protein expressed in the trophoblast (Stulc, 1997). Sodium and chloride levels in fetal and maternal blood are similar, whereas potassium, calcium and phosphate levels are higher in fetal blood (Shennan and Boyd, 1987). Potassium, magnesium, calcium and phosphate are all transported across the placenta actively, whereas sodium and chloride transfer may occur passively (Stulc, 1997).

Transmission of maternal antibodies

Immunological competence initiates to develop late in the first trimester, at that time fetus makes all the constituents of complement. Immunoglobulins consist almost totally of maternal Immunoglobulin G, which begins to be transported from mother to the fetus at around 14 weeks. In this way, the fetus gains passive immunity against numerous infectious diseases (Sadler, 2012).

Transport of carbohydrates

Glucose is the main carbohydrate transported across the placenta from mother to fetus. As the fetus is capable of very little gluconeogenesis, this glucose must be derived from the maternal circulation. Transport of glucose across the placenta is generally via protein-mediated facilitated diffusion, and a number of glucose transporters (GLUTs) are involved. Uptake of maternal glucose occurs initially across the microvillous membrane of the syncytiotrophoblast. Once inside the syncytiotrophoblast cytoplasm, glucose can be transported out of the syncytiotrophoblast, e. g. via the basement membrane towards the fetal capillary endothelial cells, which also have membrane-located glucose transporters. However, the rate-limiting step for maternal-fetal glucose transfer is thought to be within the syncytiotrophoblast (Bauman et al., 2002). The facilitative glucose carrier GLUT1 has been located at term both in the maternal blood-facing and fetal capillary-facing membranes of the placental tissues, and is thought to be responsible for a major component of glucose transport across term placenta . At term, GLUT3 is localised to the endothelial cells lining the fetal capillaries and is thought to be important for regulating glucose levels between these cells and fetal blood (Illsley, 2000). GLUT4 is present in placental stromal cells and may be important for transporting glucose and conversion to glycogen in these cells in response to insulin in the fetal circulation (Xing et al., 1998). GLUT8 has been found to be expressed in human placenta at term, but may be less important in early pregnancy (Limesand et al., 2004). The human placenta produces large amounts of lactate, and lactate is also transported by the placenta (Piquard et al., 1990).

Transport of amino acids

Amino acids are required by the fetus for protein synthesis, but they can also be metabolised by the fetus. Transport of amino acids to the fetus during pregnancy occurs via the microvillous and basal membranes of the syncytiotrophoblast. The ratio of most amino acids in fetal compared with maternal plasma is generally greater than 1 indicating active, i. e. energy-requiring, transport of amino acids from mother to fetus(Yudilevich and Sweiry, 1985). A number of heterodimeric amino acid and monomeric transporters are thought to be expressed in placenta (Cariappa et al., 2003).

Transport of lipids

Both free fatty acids and glycerol can readily cross the membranes of the placental syncytiotrophoblast. They can do so by simple diffusion, as they are lipophilic. However, they can also cross via the action of membrane-bound and cytosolic fatty acid binding proteins (Haggarty, 2002). The placenta is able to preferentially transport long chain polyunsaturated fatty acids, and the fetal blood is enriched in these compounds compared with maternal blood (Dutta-Roy, 2000). Although the placenta can synthesise cholesterol, under normal circumstances cholesterol is derived from maternal blood. The placental trophoblast contains enzymes and transporting proteins that are involved in the handling of bile acids, biliary pigments and xenobiotics (Marin et al., 2003).

Protective functions of the placenta

The placenta can act to protect the fetus from certain xenobiotics that could be circulating in maternal blood. Xenobiotic molecules can cross the placenta

by simple diffusion and placental transport systems. However, there are a number of protective features of the human placenta, which can help reduce placental transfer of potentially toxic substances. These features include export pumps in the maternal-facing membrane of the syncytiotrophoblast, including multidrug resistance protein 1 (MDR1), several members of the multidrug resistance-associated protein (MRP) family, placenta-specific ATP-binding cassette proteins (ABCP), breast cancer resistance protein (BCRP) and mitoxantrone resistance-associated protein (MXR) (Marin et al., 2003). In addition, the placenta contains a number of cytochrome P450 enzymes that can metabolise drugs and other xenobiotics(Pasanen, 1999). The placenta generally forms a barrier against transmission of many bacteria from mother to fetus. However, some bacteria, some protozoa, and a number of viruses can be transmitted across the placenta. For example, although the majority of human immunodeficiency virus (HIV) infection is transmitted from mother to baby around the time of birth (Soilleux and Coleman, 2003). Other viruses that can infect the fetus include cytomegalovirus, rubella, polio, varicella, variola and coxsackie viruses. The bacterium that causes syphilis can also be transmitted across the placenta, as can the protozoal parasite that causes toxoplasmosis. It has also been postulated that viral infection of trophoblast may be related to poor pregnancy outcomes (Arechavaleta-Velasco et al., 2002).

Hormone production

The placenta is devoid of nerves, and therefore any communication between it and the mother or fetus would normally occur via blood-borne substances.

Endocrine, paracrine and autocrine factors that are produced by the placenta include oestrogens (produced in conjunction with the fetal adrenal gland and possibly fetal liver), progesterone, chorionic gonadotrophin, placental lactogen, placental growth hormone, a number of growth factors (including epidermal growth factor, insulin like growth factors I and II, platelet-derived growth factor), cytokines, chemokines, eicosanoids and related compounds, vasoactive autacoids, pregnancy-associated proteins of placental origin, corticotrophin-releasing hormone, gonadotrophinreleasing hormone, thyrotrophin-releasing hormone and many others. Progesterone is produced by the human placenta and is released into both maternal and fetal circulations. Progesterone inhibits uterine contraction. The corpus luteum also produces progesterone, but by about the 9th week of pregnancy it has atrophied, and then the placenta is responsible for the production of most of the circulating progesterone. At around this time, the placenta also becomes the main source of circulating estrogens, which include estrone, estradiol and estriol. Estrogens act as specialized growth hormones for the mother’s reproductive organs, including breasts, uterus, cervix and vagina (Page, 1993). Human chorionic gonadotrophin (hCG) is produced by the trophoblast and secreted predominantly into the maternal circulation. Cytotrophoblast cell fusion and the functional differentiation of villous trophoblast are stimulated by hCG, as well as by estradiol and glucocorticoids (Malassine and Cronier, 2002). Human placental lactogen is synthesised by the syncytiotrophoblast and released into both maternal and fetal circulations. In the fetus, human placental lactogen acts to modulate embryonic development, regulate intermediary metabolism and stimulate the

production of insulin-like growth factors, insulin, adrenocortical hormones and pulmonary surfactant (Handwerger and Freemark, 2000). It may also be involved in angiogensis (Corbacho et al., 2002). Placental growth hormone is secreted by the placenta into the maternal circulation and may play a role in maternal adjustment to pregnancy, control of maternal insulin-like growth factor I (IGF-I) levels, and placental development via an autocrine or paracrine mechanism (Lacroix, 2002). Both human placental growth hormone and human placental lactogen act to stimulate maternal IGF production and modulate intermediary metabolism, resulting in an increase in the availability of glucose and amino acids to the fetus (Handwerger and Freemark, 2000). Insulin-like growth factors I and II (IGF-I and -II) are produced by fetal tissues and play an important role in feto-placental growth throughout gestation (Nayak. and Giudice, 2003). The placenta and extraplacental membranes produce a large number of cytokines, chemokines, eicosanoids and related factors, and some of these may be involved in parturition (Keelan et al., 2003). Eicosanoids may also be involved in control of blood flow in the placenta, along with many other locally produced autacoids (Gude et al., 1998). Indeed, there are numerous vasoactive autacoids that are produced by the placenta including endothelins, adrenomedullin, nitric oxide and many others(Grabbau et al., 1997; Al-Ghafra et al., 2003; Gude et al., 1994). There are many pregnancy-associated proteins of placental origin, and not all of these have been well studied. One that has been studied is pregnancy-associated plasma protein A, which is produced by the placenta and belongs to the metzincin superfamily of metalloproteinases. It is an insulin-like growth factor binding

protein-4 proteinase, and its levels may be reduced in first trimester when a fetus with Down’s syndrome is present (Fialova and Malbohan, 2002). The placenta produces large amounts of acetylcholine (King et al., 1991). Although the functions of placental acetylcholine are not clear, it has been postulated that non-neuronal acetylcholine may play a role in cell proliferation, differentiation, organization of the cytoskeleton and the cell—cell contact, cell migration and immune functions (Wessler et al., 2003).

2. 4. 4Placental transport mechanisms

Almost all materials are transported through the placental membrane by one of the following four major transport mechanisms:

Simple diffusion

It is characteristic of substances moving from areas of higher to lower concentration until equilibrium is established.

Facilitated diffusion

It requires a transporter but no energy. It is through electrical gradient.

Active transport

It is passage of ions or molecules across a cell membrane.

Pinocytosis

It is a form of endocytosis in which the material being engulfed is a small amount of extracellular fluid. This method is usually reseved for large molecules. Some proteins are transferred very slowly through the placenta by pinocytosis (Moore et al., 2012).

2. 5PATHOPHYSIOLOGY OF ANEMIA

During pregnancy, plasma volume increases by 50 per cent, red cell mass increases by up to 25 per cent & there is a consequent fall in hemoglobin concentration, hematocrit and red cell count because of hemodilution, sometimes called physiologic anemia of pregnancy (Frey, 2003). The adaptive physiological hypervolemia aids to maintain the blood pressure in presence of diminished vascular tone, facilitates fetal and maternal exchange of respiratory gases, metabolites and nutrients and protects the mother from hypotension, through reducing the risks related to hemorrhage at delivery. Increased maternal and fetal production of estrogen and progesterone increases the plasma volume. Progesterone increases the aldosterone production. Both aldosterone and estrogen increase plasma renin activity, augmenting renal sodium absorption and water retention through the renin-angiotensin-aldosterone system. The concentration of plasma adrenomedullin rises during pregnancy, and relates considerably with blood volume. Red blood cell volume decreases during the first 8 weeks, rises to the prepregnancy level by 16 weeks, and go through a further increase to 30% above prepregnancy volume at term. Raised erythropoietin level and erythropoietin effects of placental lactogen, progesterone and prolactin result in an increase in red blood cell volume. The reduction in blood viscosity from the lower hematocrit reduces resistance to the blood flow, as a compensatory mechanism. Though, if the Hb concentration falls <10 g/d, other causes of anemia should be considered (Grewal, 2010). The implications of anemia in pregnancy stem from adverse effects of decreased tissue oxygen delivery. Oxygen is carried in the blood as physical solution in

plasma and as combination with hemoglobin. Arterial blood contains only 0. 3 ml of oxygen, in each 100 ml of blood at a partial pressure of 100 mm Hg and temperature of 37°C. This insignificant quantity reveals tension of oxygen in blood and acts as a pathway for supply of oxygen to hemoglobin and for the transfer of oxygen to cells. Majority of the oxygen carried in blood is in combination with hemoglobin. As blood leaves the lung, hemoglobin reversibly binds to four molecules of oxygen which equals to 1. 37-1. 39 ml/g of hemoglobin. Consequently, the oxygen quantity of the blood is the amount of oxygen contained in red cell plus the quantity dissolved in the plasma. Therefore, the oxygen content of the blood when PO2 is 100 mm Hg and hemoglobin level is 15 g/ dl, is 20 ml. In anemia when hemoglobin level falls by 50% (7. 5 g/dl), oxygen content falls to 10 ml/dl. Total quantity of oxygen in arterial blood delivered to the tissues is a function of cardiac output. When anemia occurs, the cardiac output increases as a compensatory mechanism to conserve oxygen delivery to tissues. Sudden cardiac failure can result due to too much strain on the myocardium; therefore increase in CO should be <10 l/min. The cardiovascular system of the patient must be healthy enough to compensate the increases in cardiac output. Therefore, the risk of anemia will depend on the magnitude of fall in tissue oxygen content and on the type and severity of simultaneous medical illnesses. Apart from an increase in cardiac output, the oxygen delivery is significantly affected by the relationship among the saturation of hemoglobin with oxygen and the partial pressure of oxygen in blood, best described by oxygen dissociation curve. The hemoglobin molecule has particular characteristics which permit the oxygenation of one subunit of hemoglobin

molecule to facilitate 300 times greater oxygen affinity of the other subunits. Likewise release of oxygen from the first hemoglobin subunit will facilitate the release of further oxygen. " This augmentation of oxygen uptake and release is the reason of the sigmoidal shape of oxygen dissociation curve." The sigmoid shape is significant as the rapid descent allows a great fraction of oxygen to be released to tissues with a modest fall in the partial pressure of oxygen. The partial pressure of oxygen in the blood at which the hemoglobin is 50% saturated designated as P50 is 26. 6 mm Hg. The P50 is a predictable measure of hemoglobin affinity for oxygen. Increased temperature and rise in the hydrogen ion and 2, 3- diphosphoglycerate (DPG) concentrations decrease the affinity of hemoglobin for oxygen, leading to an increase in P50 and rightward shift of the curve therefore facilitating the unloading of oxygen at peripheral tissues. A fall in P50 shows a left shift in the oxygen dissociation curve and an increased affinity of hemoglobin for oxygen, so that a lower than- normal oxygen tension saturates hemoglobin in the lung and the following release of oxygen to the tissues ensues at a lower-than-normal capillary oxygen tension. Therefore, in anemia rightward shift of oxygen dissociation curve is observed. Changes in 2, 3-DPG are most significant and frequently seen in chronic anemia particularly sickle cell anemia, and chronic hypoxemia. 2, 3-DPG is a product of anaerobic metabolism with a regular intraerythrocyte level of 15 µmol/g of hemoglobin. It binds with β-chains when hemoglobin is deoxygenated, therefore increasing oxygen availability. Increase in 2, 3-DPG level in red blood cells, is seen in case of maternal anemia. Thus, though tissue oxygenation is not compromised during chronic or physiological anemia as a result of

compensatory adaptations, these may possibly be compromised in acute onset or severe anemia leading to severe consequences like right tissue hypoxemia, heart failure, angina, etc (Grewal, 2010).

2. 6HISTOMORPHOLOGY OF PLACENTA IN MATERNAL ANEMIA

The histomorphological findings of placenta in anemic mothers which are an adaptation to maternal hypoxia can correlate with the poor fetal outcome giving a documentary evidence and explanation against false implications of neonatal deaths (Munjal, 2011). Placental development influenced by anemia and hypoxia, e. g. causes abnormal trophoblast invasion and release of hypoxia inducible factor (Begum et al., 2010). When pregnancy is complicated by maternal anemia, many pathological changes, such as infraction, intervillous thrombosis, fetal vessel hemolysis, occur which reduces the functional villous mass and there is a concomitant increase in the intervillous space (Begum et al., 2010; Huang et al., 2001). Anemia, even moderate is associated with compensatory placental hypertrophy which results in increase in placental weight (Levario et al., 2008). Severe maternal anemia caused highly significant placental enlargement. Anemia causes inadequate oxygenation of the fetoplacental unit, which in turn, evokes a physiological response which results in compensatory placental hypertrophy. Some placentas weighed in excess of 700 g (Beischer et al., 1970). Placental infarcts are the most common and conspicuous lesions observed by the pathologist. They represent villous tissue that has died because of deficient intervillous (maternal) circulation. Infarcts are firm, condensed, dead areas of villous tissue that often encompass the entire thickness of the placenta.

Frequently, they involve the base of the placenta and are particularly common at the placental edge. Generally, small marginal infarcts at term usually represent atrophy of placental tissue rather than true infarcts, although they are histologically indistinguishable. When infarcts are found away from the placental margins, and particularly when they are randomly distributed, conditions of malperfusion almost invariably exist. Infarcts in any location in first and second trimester placentas are always abnormal. Macroscopically, infarcts are firmer than the surrounding villous tissue and have a granular surface. Early infarcts are initially dark red and congested and can be distinguished from normal tissue by their firmness and by their lack of a spongy texture. As they age, infarcts become yellow, then tan-gray, and finally white and firm (Baergen, 2011). Histologically, the early infract is characterized by aggregation of the villi in the affected side with marked narrowing, often obliteration of the intervillous space. The villous fetal vessels are dilated and congested, while the syncytial nuclei show early necrotic change such as pyknosis and there is progressive coagulative necrosis of the villi. The fetal erythrocytes trapped in the vessel of the infracted villi undergo hemolysis (Huang et al., 2001). Regional under perfusion results in focal placental infarcts, frequently near the margin, and usually in a pyramidal shape with the base beside the maternal floor. These infarcts are relatively common at term or after term and are associated with senescence. Though placental infarcts are common, numerous infarcts or the infarcts that make more than 5% of placental mass are considered pathologic and may impact the fetal oxygen/nutrient state. The infarcts tend to be small, multiple, and more central in the placental mass are

characteristic of severe, chronic, uteroplacental vascular insufficiency (Roberts, 2008). Syncytiotrophoblastic knots or syncytial knots are aggregates of syncytial nuclei at the surface of terminal villi (Loukeris et al., 2010). Syncytial knots develop in third trimester, due to relative ischemic changes and reduction of mesenchyme which may possibly cause knots formation of the elastic syncytial layer (Saga et al., 2008). Numbers of syncytial knots are increased in placentas of anemic mothers. These changes suggest adaptation to relative hypoxia in anemia (Dhall, 1994; Reshetnikova et al., 1995). Increased syncytial knotting is widely accepted as a diagnostic indicator of placental ischemia. Even though most knots are artifacts, evaluation is still useful because they represent a characteristic deformation of the terminal villi. In hypoxia, the low oxygen levels induce branching angiogenesis, resulting in clusters of richly capillarized, short, highly branched and notched terminal villi, showing increased syncytial knots or Tenney-Parker changes. The highly branched, netlike capillary beds are easy to perfuse, as their structure provides less flow resistance than comparably large capillary beds composed of longer, less-branched capillaries (Baergen, 2011). Evaluation of the width of the intervillous space gives important hints. Intervillous space is found among chorionic villi and is filled by mother’s blood (Ramic et al., 2006). Absolute volume of the intervillous space is found to be increased in the anemic group and there was a significant negative correlation between maternal hemoglobin level and absolute volume or surface area of the intervillous space (Huang et al., 2001). Villous membrane is the diffusion barrier between maternal and fetal circulations. In maternal anemia mean thickness of the villous membrane is significantly reduced

because the placenta adapts through thinning of the villous membrane so that diffusing capacity is maintained at normal levels (Reshetnikova et al., 1995). Villous membrane comprises five layers, namely: trophoblast, trophoblast basement membrane, villous core supporting tissue, capillary endothelial basement membrane and endothelium. In many cases, fetal capillaries are so close to the trophoblast that their basement membranes fuse, reducing the diffusion barrier to only three layers (Young et al., 2006).

Chapter 3

MATERIALS AND METHODS

3. 1SETTING

This study was conducted in Army Medical College, National University of Sciences and Technology in collaboration with Department of Obstetrics and Gynecology, Military Hospital, Rawalpindi, Pakistan.

3. 2DURATION OF STUDY

December 2011 to November 2012

3. 3SAMPLE SIZE

A total of 75 placentas were included.

3. 4 GROUPS

Placentas were divided into control group (Group A, n: 15) and study group (Group B, n: 60). Cases included in control group (Group A) had normal hemoglobin levels i. e. ≥11g/dl and the study group (Group B) was composed of placentas from anemic mothers (hemoglobin <11g/dl). Study

group was subdivided into 3 groups according to severity of anemia i. e. mildly (Group B1), moderately (Group B2) and severely (Group B3) anemic group. The severity of anemia among the mothers in the study group was judged by the criteria suggested by WHO and is as follows; Mild10. 0-10. 9g/dlModerate7. 0-9. 9g/dlSevere <7. 0g/dl(Regil et al., 2011)

3. 5SAMPLING TECHNIQUE

Non probability convenience sampling method

3. 6SAMPLE SELECTION

3. 6. 1Inclusion criteria:

Placentas of the full term mothers having hemoglobin level below 11gm/dl

3. 6. 2Exclusion criteria:

The placentas of the mothers having following disorders were not included in the study. Gestational diabetes mellitusA known case of diabetes mellitus prior to pregnancyPregnancy induced hypertensionEclampsiaAsthmaAntepartum hemorrhage (Begum et al., 2011)

3. 7STUDY DESIGN

Case control study

3. 8SAMPLE COLLECTION PROCEDURE

Permission of the ethical committee was taken. Placentas were collected immediately after expulsion and washed in tap water to remove blood clots. Placentas were preserved in 10% formal saline. Placenta of each patient was given a laboratory number and record was maintained. 3. 9GROSS

MORPHOLOGY (Kaplan and Altshuler, 2009)Placentas were examined for following parameters; Shape of the placenta was assessed. Weight of the placenta was taken without umbilical cord. Dimensions were taken.

Fetal membranes

Fetal membranes were inspected for the following macroscopic features; Intact/tornMembrane insertion site (% marginal, % circummarginate, % circumvallate)Color (normal/green/brown/yellow/gray/combined color)

Umbilical cord

Umbilical cord was separated close to its placental insertion and following parameters were noted; Knots (nil/true/false)Number of vessels (3/2)Site of cord insertion (central/marginal/eccentric/velamentous)Color (normal/green/brown/yellow/gray/combined color)

Placental parenchyma

Parallel transverse sections about 2cm apart were made and following parameters were noted; Color (normal/green/brown/yellow/gray/combined color)Appearance of placental tissue (congested/very pale/unremarkable)InfarctsInfarcts were noted and confirmed on microscopic examination. Extent of placental infarct was recorded in terms of percentage of infarcted area (5-25%, 25-50%, > 50%) and their pattern of distribution (focal/multifocal/diffuse). CalcificationCalcification was noted on gross examination.(Kaplan and Altshuler, 2009)

3. 10SECTIONS FOR HISTOLOGY

Three representative sections were taken i. e. one from close to the insertion of umbilical cord (A), one from periphery (C) and one halfway between A and C (B). Additional sections were taken from pathological area, if any. One complete circular section was taken from umbilical cord (D) (Fig 3. 1) (Saga and Minhas, 2008; Begum et al., 2010).

3. 11TISSUE PROCESSING

3. 11. 1 Specimen Processing

Representative sections were enclosed in properly labeled plastic cassettes with perforated walls. These sections were placed in LEICA TP1020 (Germany) automatic tissue processor and following processing schedule was observed;

A

Umbilical cord

D

B

C

Placental parenchyma

Fig 3. 1: Schematic diagram of cross section of placenta showing sampling sites

3. 11. 2Dehydration

Dehydration was done in ascending series of alcohol. 80% alcohol1 hour95% alcohol1 hourAbsolute alcohol – I1 hourAbsolute alcohol – II1 hour

3. 11. 3Clearing

Two changes of xylene were used. Xylene – I2 hoursXylene –II2 hours

3. 11. 4Impregnation

Paraffin with melting point 56-58 c was used for this purpose. First change2 hoursSecond change2 hours

3. 11. 5Embedding

Paraffin embedding centre (LEICA EG 1160- Germany) was use for this purpose. Filtered paraffin with melting point 56-58 c was used for embedding. Blocks were made using plastic cassettes. Each cassette was filled with molten paraffin wax and tissue was placed in the center of the cassette. The cassette was then allowed to cool on the cold plate of the paraffin embedding centre.

3. 11. 6Sectioning

Paraffin blocks were placed in block holder of rotary microtome LEICA RM 22. 5 (Germany) and 3-4um thick sections were cut. These sections were floated in warm water bath at 45 c and were taken separately on two different slides, which were albumin coated. Slides were further dipped horizontally in 70% alcohol bath to remove any creases. The slides were kept in a slanting position for about half an hour to drain excess of water. The section was then dried on hot oven at 60c for about 15- 30 minutes

Staining

Haematoxylin and Eosin (H&E) stain was used in LEICA autostainer (Germany) XL(Appendix V).

3. 12HISTOPATHOLOGICAL PARAMETERS

The slides were examined by trainee and final diagnosis was given by consultant histopathologist (supervisor). Histopathological findings were divided into the following two parameters;

Quantitative Parameters

Intervillous spaceVillous membrane thicknessSyncytial knot hyperplasia

Qualitative Parameters

Placental infarct

3. 12. 1Intervillous space

Width of intervillous space was determined. This measurement was done in 5 random fields per slide A, B and C, under 100X objective and mean was calculated. For this purpose ocular micrometer was used which was calibrated with standard stage micrometer in following manner; Calibration of ocular micrometer: The calibration of ocular micrometer was done with a stage micrometer. The stage micrometer is a microscopic slide with a 1-milimeter long scale etched on the surface. This 1 millimeter was divided into 100 divisions so that each division was equal to 0. 01mm (10µm). This stage micrometer was placed on the stage of the microscope and focused under 100X objective (oil immersion). The ocular micrometer or eye piece reticule was placed in the eyepiece and aligned with the stage micrometer. The numbers of divisions of ocular micrometer corresponding with that of stage micrometer were noted and then the value of one division of ocular micrometer was calculated; 100 divisions of ocular micrometer = 12

divisions of stage micrometer100 divisions of ocular micrometer = 120µmThus 01 divisions of ocular micrometer = 1. 2µmThe ocular micrometer scale was superimposed on the intervillous space. Number of divisions from outer surface of one terminal villous to the outer surface of other terminal villous multiplied by 1. 2 was taken as the actual width of intervillous space.

3. 12. 2Villous membrane

Identification of villous membrane, composed of syncytiotrophoblast and capillary endothelium with intervening stroma, was done. Thickness of villous membrane was determined. This measurement was done in the region of syncytiotrophoblast and capillary endothelium without intervening nucleus and with minimal stroma in cross section of five random terminal villi per slide in A, B and C regions under 100X objective and mean was calculated (Saga et al., 2008). For this purpose ocular micrometer was used which was calibrated with standard stage micrometer in the same manner as mentioned above. The numbers of divisions of stage micrometer corresponding with that of ocular micrometer under 10X objective was noted and value of one division of ocular micrometer was calculated which was equal to 12µm. The ocular micrometer scale was superimposed on the villous membrane at the area of syncytiotrophoblast and capillary endothelium without intervening nucleus and with minimal stroma. Number of divisions from inner surface of capillary endothelium to the outer margin of cytoplasm of syncytial cell, multiplied by 1. 2 was taken as the actual thickness of villous membrane.

3. 12. 3Syncytial knots

Number of syncytial knots was counted. These were identified as clusters of nuclei lying by the periphery of tertiary villous. This counting was done randomly in 5 high power fields per slide from region A, B and C using 40X objective.

3. 12. 4Placental infarct

Identification of placental infarct was done. Placental infract was composed of aggregate of the necrotic villi with obliteration of the intervillous space. Presence or absence of placental infarcts was confirmed on microscopic examination under 10X objective. All the data was recorded in a structured proforma (Appendix I).

3. 13DATA ANALYSIS PROCEDURE:

Data analysis was computer based using SPSS version 17. Mean and standard deviation were calculated for quantitative variables. Percentage was calculated for qualitative variables. Quantitative variables were compared through analysis of variance (ANOVA) while qualitative variables were compared through chi-square test. Pearson correlation test was used for calculating relationship between hemoglobin level and other variables. p value less than 0. 05 was considered as significant (P <0. 05).