Demonstration of placenta calcification in normal and gestation diabetes mellitus placenta of woman in wasit province/ IRAQ

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Abstract

The placenta is a distinguishing organ, which facilitates the supply of nutrients and oxygen to the fetus during placenta microcirculation. The placenta is impacted by gestational diabetes mellitus and contributes significantly to fetal and maternal mortality and morbidity. This study was designed to showed the general structure of tissue and detected calcification in the normal placenta and placenta of gestation diabetes with some histopathological change. The sample of this study were collect from Obstetrics and Gynecology Department of Al-Batool and Al-Zahra Hospital, Kut city, Wasit region, Iraq. Total 16 placentas were collect from (17-48) years old women. They were divided into two groups, according to age and pathological cases (Normal Gestation NG and Gestation Diabetes mellitus GDM), with eight placentae for each group. The placenta samples were anatomized and taken from parts from the edge of the placenta and the middle of the parenchyma. All these samples were fixed in formalin at 10%. The histological study has been made by utilizing three types of dyes, hematoxylin and eosin to show the general histological structure, van koss and alizarin red stain to detect the deposition of calcium salt in tissue. The findings revealed placenta calcification in NG, slight calcification in young women, and mild calcification with advantage of women's age. In the placenta with GDM, the calcification appeared in the form of large black spots scattered through the chorionic plate at a young age in women and at mid-spots at an advantaged age with some histopathological chanage included fibrinoid necrosis, infarction, syncyial knot, edema and chorangiosis.

Keywords: Calcification, placenta, Gestation Diabetes Mellitus, Pregnancy, Von Kossa, Alizarin Red stain
Introduction

The placental is a highly specialized pregnancy organ that promotes normal fetal growth and development (Gude et al., 2004). The placenta plays a role in the exchange of many substances between maternal and fetal. The primary functions are to deliver nutrients to the fetus and to remove Western products from the fetus via blood circulation (Husain and Mughal, 1992). The human placenta serves as a link between the fetus and the mother, mediating the exchange of oxygen and carbon dioxide, the secretion of a plethora of hormones, cytokines, and growth factors, as well as the transport of nutrients and waste disposal, all of which are essential for the fetus's development (Nelson, 2015). The exchange between mother and fetus occurs via the umbilical cord, which has a single umbilical vein and two umbilical arteries (Rodney et al., 2013). Histologically, the human placenta is composed of three main tissue layers that circle the amniotic sac: amnion, chorion derived from the fetal, as well as the decidua derived from maternal tissue (Haller, 2013). Calcium is a necessary nutrient for many human health functions. It is the most abundant mineral in the body, 99% found in your teeth and bones. In the serum, only 1% can be found (Beto, 2015). There are biological limits to the ability of pregnant women to increase the absorption of calcium. If she does not consume an adequate amount of calcium nutrition, her risk of pregnancy complications such as pre-term delivery, preeclampsia, and longer-term morbidities such as excessive bone loss may be increased (Hacker, Fung, and King, 2012).

There are many changes that may occur during the gestation or pregnancy period, one of which is placental calcification, caused by the accumulation of calcium on the placenta, which is considered a physiological process (Miller et al., 1988). Calcification can harm both the fetus and the mother before 32 weeks of gestation, resulting in fetal death or hemorrhage in the mother (Heerema-McKenney, 2018). Gestational diabetes mellitus is referred to as any degree of glucose intolerance with an onset or first recognition during pregnancy. Any type of diabetes during pregnancy results in different types of placenta abnormalities (Treesh, and Khair, 2015). In placentas with gestational diabetes mellitus, there is an increase in calcium deposition on the maternal placenta surface (Salge, 2012). In gestational diabetes, many complications usually occur in gestational diabetes and effect the fetus and mother such as the birth of a large baby and a large placenta, mothers with gestational diabetes have a risk of miscarriage, preterm labor, and stillbirth. They also have a risk of blood pressure disorders, and babies have an increased risk of mortality and morbidity associated with respiratory distress (El Sawy et al., 2018). Placental calcification is either physiological calcification, metastatic calcification or dystrophic calcification (Grannum, Berkowitz and Hobbins, 1979). The purpose of this study is to detect calcification that is present in the placenta by examination under a light microscope and to determine the difference between the placenta calcification in normal gestation and the placenta with gestational diabetes mellitus with placental changes.
Material and Method

The sample collection

16 samples of placenta were collected from Al-Batool and Al-Zahra hospital in Kut. They were divided into two groups according to age and pathological case, (Normal Gestation NG and Gestation Diabetes Mellitus GDM). Depending on the medical recorder, each group contained eight placentae. These placenta samples were anatomized and taken a piece or roll of the edge of the placenta, a block from the middle of the placenta parenchyma near the place where the umbilical cord is implanted. The samples were kept in 10% formalin for 72 hours. They were cleaned up with water for 2-3 and transported to a number of histological techniques as followed: dehydration, clearing, infiltration, embedding, cutting and staining with hematoxylin and eosin (H&E) stain to show the general structure of the tissue; we also used Alizarin Red and Von Kossa stain to detect the deposit of calcium salts in the paraffin section.

Methodology of Statistics

Computer Assisted digital image analysis (Digital morphometric study)

Slides were photographed utilizing a Canon digital camera placed on a Mejia microscope with a 1/2 X photo adaptor, using a 10X objective. The result images were analyzed on an Intel Core I3-based computer utilizing Video Test Morphology software (Image J) with a specific built-in routine for area, percent area measurement, and object counting.

Result and Discussion

Histological result at normal gestation (NG)

The histological result by using hematoxylin and eosin stain appeared as if the placenta has three main tissue layers: amnion and chorion derived from fetal tissue, the amnion is a single layered epithelium, and the amniotic mesenchyme layer, amnion is an avascular tissue consisting mainly of two types of cells, columnar cells and cuboid epithelial cells, and showed more columnar epithelial cells in the amnion like space. The intermediate spongy layer, which lies between the amnion and the chorion. A chorionic is a thick mass of connective tissue consisting of The chorionic mesenchyme is adjacent to the connective tissue layer of the amnion (Figure 1). A third layer is the basal plate that is derived from maternal tissue. It is the only vascularized tissue of the placenta and contains both external and deep vascular elements such as spiral blood vessels and contains extra villous trophoblast (EVT) and anchoring villi embedded in the basal layer (Figure 2). These facts were agreed upon by others (Harirah et al. 2012), (Gill 2012) and (Haller 2013).

The histological examination of placenta sections of women with normal gestation NG stained by calcium-detecting stains (van Koss and Alizarin red stain) appeared to show slight calcification at the first age of women (17-29) years old (Figure 3 A). Statistically, this ratio of calcification is estimated as (0.022± 0.721) (Figure 3 B). While calcifications appeared moderate in a group of 40–48-year-old women with vascular congestion (Figures 4 A). The ratio of
calcification is estimated as (0.236 ± 0.598) seen in (Figure 4 B) (chart 1). These facts are similar to (Goswami, 2012) and (Cristina et al., 2019).

Calcification in the human placenta is common and recognized as a natural component of this organ's development and aging (Chen et al., 2011). The hazard of calcification increases with an increase in the pregnancy period in the parenchyma, which typically happens in the late and later placenta. Calcification can happen with or without other placental pathologies. The clinical definition, if any, is usually not clear (Hamzah and Yar, 2021).

(Figure 1): photomicrograph of the placenta demonstrating that the amnion is single-layered epithelium with more columnar epithelial cells (black arrow), an intermediate spongy layer (green arrow), and the mesenchymal amnion (blue arrow) and chorion (yellow arrow) (H & E stain 100).
(Figure 2): The histological section of placenta shows decidua layer (yellow arrow) and anchoring villi (green arrow), spiral blood vessel (red arrow), Extra villous trophoplast (blue arrow) and Hafbauer cell (black arrow) (H&E stain 100x).

(Figure 3 A): photomicrograph of the placenta at (17 years) showing the calcification in small red point (arrow) in mesenchyme of chorion (Alizarin Redstain100x).

(Figure 3 B): surface plot of placenta at (17 years) showing the percentage of calcification for tissue.
Calcification in placenta with Gestation Diabetes Mellitus (GDM)

According to the case history in the medical record, the women suffering from GDM, the result of histological examination of the placenta section of the current group stained by van Koss and Alizarin red stain, showed severe calcification in the placenta of women in the age group of 17-20 years old (Figure 5 A) who suffer from gestational diabetes mellitus (GDM), the ratio of calcification placenta of severe calcification was estimated (1.661±1.138) (Figure 5 B). However, calcification appears moderate in women aged 40 to 48 years old (Figure 6 A). The statistical percentage of placenta calcification in moderate cases was (0.295±0.982) (Figure 6 B). The calcification levels in the (GMD) group were barely estimated as grade III in the first age group of patient women and were estimated as grade II in advantaged age women. In this study, the effect of GDM on placental calcification is significant. We note an increase in calcium deposition in the chorionic villi compared with the previous group (chart 1).

Similar results were cited by (Edu et al., 2016) who indicated diffuse calcifications in chronic villous in cases of GDM. And (Pala et al., 2016) showed increased calcification of the villus and increased vascularity in the placenta with GDM. Also (Salge, 2012), note the increased deposition of calcium on the surface of the placenta in the case of GDM.

In this study, we found increased syncytial knots, chorangiosis, and fibrinoid necrosis (Figure 7, 8). Similar results were cited by (Petersen et al., 2017), who studied the risks of caesarean section in cases of GDM at the age of 38 and noted an increase with increased syncytial knots and chorangiosis. Also, (Heorman et al., 2012) estimated the 19 placentas of women with GDM and noted changes in placental structures such as chorangiosis and sclerotic villi in some of them.
(Cinderella, 2003) described chorangiosis as a vascular change including the peripheral chorionic villi in the placenta. It occurs due to long-term, low-grade hypoxia in placental tissue, and is correlated with conditions such as diabetes mellitus, gestation hypertension, and intrauterine growth restriction (IUGR). And (Elsennawy, 2016) defined infarction as It is distinguished by the deposition of fibrin on the basal lamina, which extends into the dividing space that traps the chorionic villi. in addition

In this study, they found infarction that extends from the basal layer to the chorion layer and obstructs the villi (Figure 9). In addition, hemorrhage adjacent to the basal layer and congested blood vessels, another abnormality found in the chorionic villous of the placenta with GDM is villous edema (Figure 10).

This is supported by researchers (Memon et al., 2015) and (Elsennawy, 2016) who studied the differences between the normal placenta and the placenta with GMD and noted the differences between the two. They noted infarction, hematoma, and chorangiosis. In addition to (Gheorman et al., 2012) pointed to abnormal histological change in placenta diabetes are: edema, infarction, and chorangiosis.

According to (Mumtaz, 2000), GMD is caused by a hormone secreted by the placenta that prevents the body from effectively using insulin. Glucose builds up in the blood instead of being consumed by the cells.

In gestational diabetes, many changes occur in the placenta due to diabetes. These changes are directly related to the intrauterine blood sugar level, which leads to reduced blood circulation between the mother and the placenta. For example, clinical complications accelerate the process of calcification due to stress mechanisms similar to those reported by (Salge et al., 2012). Maternal hyperglycemia may be determined to be linked with vascular proliferation and intrauterine hypoxia (Calderon et al., 2007), another abnormality found in the chorionic villous of the placenta with GDM is an increase in the number of blood vessels as a result of prenatal low-grade chronic hypoxia. Furthermore, edema was observed in placenta diabetes. In this regard, many studies have said that the increase in edema is due to the increased deposition of mucopolysaccharides in the villi and that the mucopolysaccharides consist of hyaluronic molecules that retain water, which is probably the appearance of edema in the villous stroma due to the abnormal deposits of mucopolysaccharides (Gheorman et al., 2012) and (Petersen et al., 2017).
(Figure 5 A): photomicrograph of the placenta at (18 years) showing severe calcification in the intermediate villous area in a large red spot (arrow) patient with Gestational Diabetes Mellitus (GDM) (Alizarin Red 100X).

(Figure 5 B): surface plot of placenta at (18 years) showing the percentage of calcification for tissue.

(Figure 6 A): photomicrograph of the placenta at (45 years) showing mild calcification spread in stem villi as form point black (black arrow) and in stroma of chorionic villi (red arrow) patient with GDM (Alizarin Red stain 100X).

(Figure 6 B): surface plot of placenta at (45 years) showing the percentage of calcification for tissue.
(Chart 1): chart showing the relative proportions of calcification of the two types of placenta, placenta in normal gestation for young age NG1, placenta in normal gestation for old age NG2, placenta with gestation diabetes mellitus in old age GDM1, placenta with gestation diabetes mellitus in young age GDM2.

(Figure 7): photomicrograph of the placenta at (18 years) showing chorangiosis (blood vessel more than 5) with congestion capillary patient with GDM (H&E 200x).

(Figure 8): photomicrograph of the placenta at (18 years) shows syncytial knot (black arrow) and fibroid necrosis (blue arrow) patient with GDM (H&E 100x).
Conclusion

Histologically, the Hematoxylin and Eosin stain shows the general structure of the placenta, which is composed of amnion and chorion derived from fetal tissue and a decidua layer derived from maternal tissue. The alizarin red stain and the von Kossa stain show different levels of placental calcification in normal and diabetic pregnancy. The calcification is slight at the young age of a woman and moderate calcification at the adventage age of a woman in placenta with NG. There was severe calcification in the placenta with GDM at a young age and moderate in the adventage age of the woman. The calcification of the placenta in GDM is greater than the calcification of the placenta in NG. The morphological changes that can be observed in the placenta with GDM are: edema, chorangiogis, syncytial knots, infarction and fibrinoid necrosis.
References


