The impact of gestational diabetes on glycation end products oxidative marker expression in placental tissue

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Objectives   This study was designed to determine whether oxidative stress marker advanced glycation end (AGEs) products measurement can serve as an additional diagnostic marker of gestational diabetes, and its correlation with changes seen in placental tissue.

Methods   A case control study was designed, 60 pregnant women at third trimester between 37 and 41 weeks of gestation and average age between 18 and 45 years old were enrolled in this study. All cases selected for this study underwent elective caesarian section. Thirty healthy pregnant women at the same average of age with the same exclusion criteria were involved as control group. Patients group consist of 30 pregnant women who proved to have gestational diabetes mellitus (GDM) depending on OGTT and on (Soluble Insulin or Metformin) as a medication of use. All patients with family history of diabetes, hypertension, thyroid disease, renal diseases, liver diseases, cardiac problems, smoking were excluded from this study. Placental tissue samples were taken after the delivery and was immediately fixed in 10% formalin solution, prepared for immunohistochemical study for localization of AGEs receptors.

Results   There have been marked and dramatic changes in the shape and size of placental villi in diabetic women with decrease in the number of blood vessels in the core of these villi. Fibrinoid formation is a striking feature of change seen in the placenta. AGEs antibodies showed a marked pronounced presence in trophoblastic cell of the placental villi.

Conclusion   Gestational diabetes do have a recognized impact effects on placental end glycation products. There have been marked and dramatic changes in the shape and size of placental villi in diabetic women. Fibrinoid formation is a striking feature of change seen in the placenta. A marked decrease in the number of vessels in villi core in women with GDM.

Keywords   Oxidative Stress, Gestational diabetes, Placental tissue.

Introduction

Gestational diabetes mellitus (GDM) is a condition in which women without a previous history of diabetes (first recognition during pregnancy) exhibit high blood glucose levels during pregnancy, especially during second and third trimester.[1]

It usually affects 3–10% of pregnancies, 87% of the diabetic pregnancies is due to gestational diabetes. It develops in one of 25 pregnancies worldwide and is associated with complications to both mother and fetus. GDM usually disappears after pregnancy but women with GDM and their children are at an increased risk of developing type II diabetes later in life.[2]

Gestational diabetes caused when the insulin receptors do not function properly due to increase insulin resistance and inadequate β-cells compensation. Pregnant women make hormones that can lead to insulin resistance, there are different hormonal and metabolic changes during the second half of pregnancy which facilitate insulin resistance; one of them is the high plasma level of progesterone during the second part of pregnancy, all women have a sort of insulin resistance late in their pregnancy.[3]

Oxidative stress reflects an imbalance between the systemic manifestation of reactive oxygen species (ROS), (are small, highly reactive, oxygen-containing molecules that are naturally generated in small amounts during the body's metabolic reactions, can react with and damage complex cellular molecules such as fats, proteins, or DNA) and a biological system's ability to readily detoxify the reactive intermediates or to repair the resulting damage.[4] It occurs in a cellular system when the production of free radical moieties exceeds the antioxidant capacity of that system, if cellular antioxidants do not remove free radicals, radicals attack and damage proteins, lipids and nucleic acids. Oxidative tissue and organ damage play roles in DM and its complications.

Hyperglycemia can cause oxidative stress particularly by evidence that several biochemical pathways activated during hyperglycemia can increase the production of free radicals. Oxidative stress is a pathophysiological process leading to multiple outcomes in diabetic pregnancies.[5]

Pregnancy is susceptible to oxidative stress and antioxidant defenses can be altered in response to elevated levels of oxidative stress. In GDM, products of lipid peroxidation may be increased, antioxidant enzyme activities decreased, and the oxygen free radicals may be involved in severe damage of cellular structure.[6]

Advances Glycation End products: are a class of complex - often unstable - reactive compounds formed in excess during aging and diabetes mellitus. AGEs formed in successive steps as the end-stage products of glycation reactions (also known as the Maillard reactions) or a lipid molecule to produce non-enzymatic cross-links, these cross-links known as advanced glycation end (AGE) products.[7,8] The process of AGEs formation is not limited to Maillard reactions because additional pathways are involved that may result in the formation of more carbonyl-containing reactive compounds and further AGEs synthesis.[9,10]

Under physiological conditions, most AGE-modified proteins in plasma undergo rapid plasma clearance; however, the formation of AGE proteins beyond physiological levels or impairment of the AGE elimination system potentially results in the accumulation of AGE in tissues.[11] In pregnant with

GDM, high AGEs levels might possibly influence the delicate maternal–fetal balance mechanism both in umbilical cord vessels and in the placenta and therefore; alter the pregnancy outcome.[12]

In placenta, receptors for AGEs (RAGEs) evidenced in syncytiotrophoblast and cytotrophoblast of normal pregnancy at term, but RAGE expression appears to start earlier and becomes more intense in the succeeding trimesters.[8] AGEs noticed in the syncytiotrophoblast, endothelial cells, and macrophages of placenta in normal pregnancies. The human placenta is capable of responding to AGEs and the trophoblast of the first trimester placenta does contain receptors to AGEs.[13]

Prevention of AGE-mediated cell toxicity has proposed as a key strategy in preventing the onset of diabetic complications and some age-related pathology. As the existence of a long-term (sustained) hyperglycemic environment is very important for enhanced AGEs formation, it is not surprising that the major medical condition in which they were implicated is diabetes.[14,15]

The goal of this study is to determine whether oxidative stress marker (AGEs) can serve as an additional diagnostic marker of gestational diabetes. This goal can be elucidated through evaluation of the oxidative stress changes in the placental tissue and assessment of the impact of GDM on it.

Materials and Methods

A case control study was carried out on 60 pregnant women in their third trimester between 37 and 41 weeks of gestation and average age between 18 and 45 years old were enrolled in this study. They were diagnosed by their gynecologists after proper physical, biochemical, and gynecological examinations and ultrasound confirmation. All cases selected for this study underwent elective caesarian section (C/S).

Patients group consist of 30 pregnant women who proved to have GDM depending on OGTT and on (Soluble Insulin or Metformin) as a medication of use. Control group enrolled 30 women with no GDM and underwent elective C/S for mal-positioning.

The placental tissue was prepared for paraffin section according to Bancroft et al. as follows[16]:

Fixed with 10% buffered formalin solution for 72 h at room temperature, dehydrated using up graded concentration of ethyl alcohol, cleared by transferring the specimens into xylene two exchange 30 min each to ensure a good tissue transparency. Impregnation and embedding was done by embedding the sections in a labeled baths of a molten paraffin wax (melting point 56°C; two changes were performed 2 h each using an embedding oven. Then the specimens were transferred to be blocked in paraffin wax. Serial sections of 5 µm thickness were cut using the electrical microtome. These sections stained then with hematoxylin and eosin (H&E) staining method for general descriptive purposes.

The non-stained placenta sections were set for immunohistochemical study for localization anti-AGEs products. The tissue sections were de-paraffinized and rehydrated, blocked with peroxidase and serum blocking reagents, treated with AGE antibody and incubated overnight, Incubated with biotinylated secondary antibody and HRP-streptavidin complex respectively. After that was treated with DAB chromogen, stained by hematoxylin as counter stain, dehydrated and covered with cover slips.

Results

The placenta is the most important organ for fetal intrauterine life, it provides the means for physiological exchange between the fetal and maternal circulation.

Placental sections stained with H&E were proceeded for histological analysis, placental tissue in normal full term non-diabetic women showed that, there are large number of villi, these villi have different sizes and shapes, their diameters varied a lot and all bathed in maternal blood, intervillous spaces are wide (Fig. 1).

The core of villi has multiple blood vessels in different sizes and types examining one villi, it shows that it has an irregular outline with two layers of cells. These two layers are an outer syncytiotrophoblast layer occurred as basophilic cells and an inner cytotrophoblast seen as few cells below the syncytiotrophoblast. Each villi have a basal lamina separate the cells from the connective tissue core where branch of umbilical artery and vein are situated (Fig. 2).
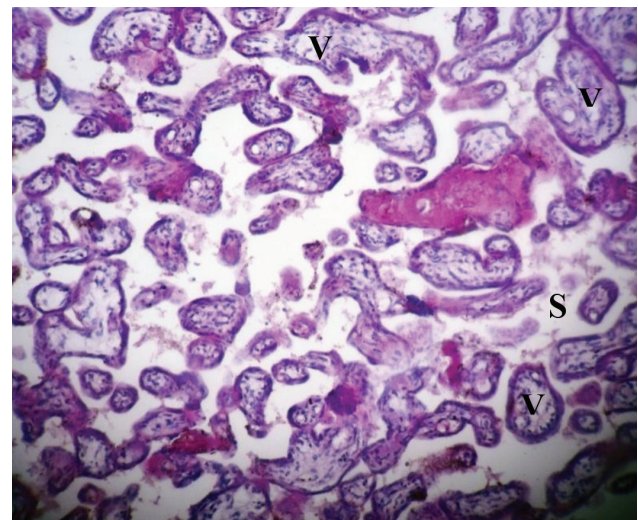

Fig. 1 **Cross section through a placental tissue showing large number of villi (V) of different sizes and shapes all bathed in maternal blood, intervillous spaces (S) wide, H&E, 10×, control group.**

Fig. 2 **Section through a placental tissue at higher magnification showing core of villi with multiple blood vessels (V) in different sizes and types, syncytial knots (K), Syncytiotrophoblast layer (S), cytotrophoblast layer (C), H&E, 40×, control group.**

Small aggregations of darkly stained areas are seen in large numbers, these are called syncytial knots. The syncytial knots site of aggregation seems to be in association with size of the villi, syncytiotrophoblast usually as uninterrupted cytoplasmic mass that covers the surface of villi and contain multiple nuclei, these cells are usually basophilic and cytotrophoblasts are seem as few cells and large below this layer (Figs. 1 and 2).

In pregnant women with gestational diabetes, the villi have shown a dramatic change with a different histological feature than that of the normal. The villi showed a striking feature of changing their shape from being irregular rounded to cylindrical thin shaped villi, two types of villi changes have been seen in diabetic women; first type of villi was seen as long cylindrical bended villi (Fig. 3). The other type was seen as very large villi with wide core and less vascularity (Fig. 4). The number of syncytial knots was also increased as compared with the normal non-diabetic placenta.

Immunohistochemical results

For the expression of AGEs in human placental tissue (AGEs marker) was used in this study, immune localization of AGEs in human placental tissue of the control group (non-diabetes) showed that the reaction was mainly confined to the blood vessels especially large one (Figs. 5A and 5B). Detection for the AGEs reaction in cytotrophoblast and syncytiotrophoblast was difficult. Intervillous spaces, blood lakes, area surrounded blood vessels and syncytial knots showed a highly positively reaction of the AGEs demonstrated by the present of fine small brownish granules which was easily seen filling the spaces (Fig. 6).

Demonstration of AGEs receptors in human placental tissue of diabetic women showed the appearance of a positively stained brownish reaction inside the villi especially on its periphery and intense reaction was seen in between intervillous spaces (Fig. 7).

Examination the villi at higher power showed that the reaction mainly presented as fine small brownish granules most prominent staining area was observed as very limited small peripheral areas of the villi (Fig. 8). Fine small granules easily seen filling the apical rim of cytoplasm of syncytiotrophoblast (Fig. 9).

The remaining part of the cells especially the basal area showed no reactivity, other component of the placental tissue (blood vessels, intervillous spaces and blood lakes) also showed a higher positive reactivity plus the appearance of strong reaction in the syncytial knots (Figs. 9 and 10).
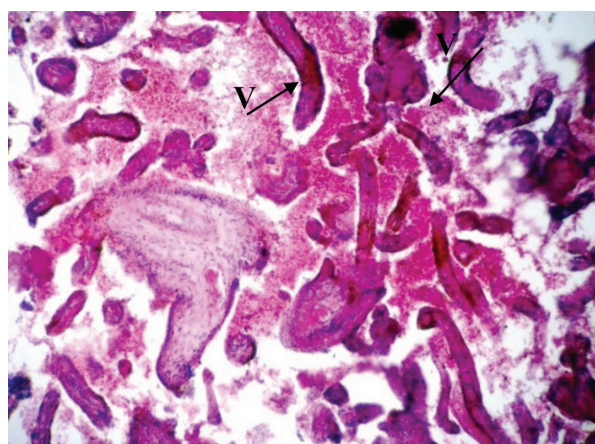

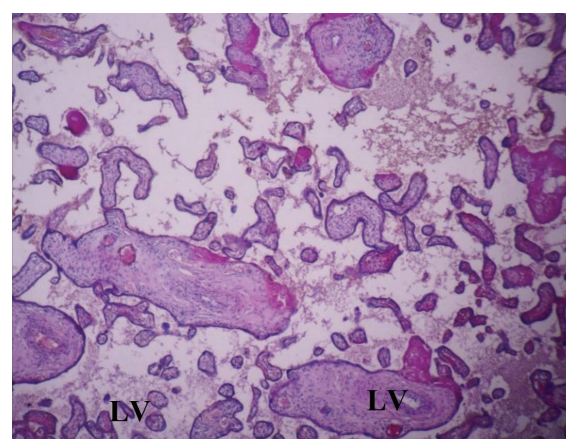Fig. 3. **Section through a placental tissue showing long cylindrical bended villi (V), H&E, 10×, diabetic group.**

Fig. 4 **Section through a placental tissue showing very large villi (LV) with wide core and less vascularity, H&E, 4×, diabetic group.**
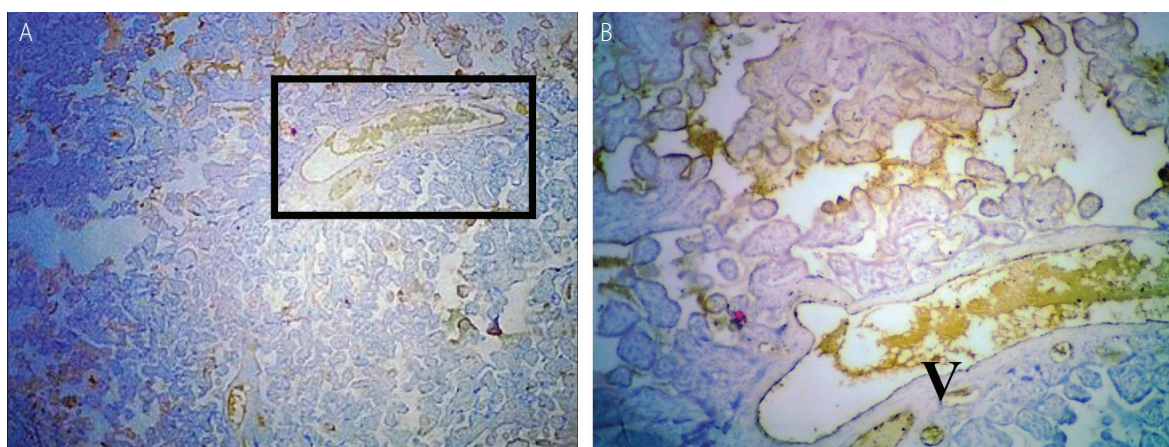

Fig. 5 **Section through a placental tissue showing: (A) immune localization of AGEs reaction at the intervillous spaces with specific intense reaction of the blood vessels, 4×. (B) A higher magnification of the selected area showing the intense reaction at the blood vessel (V). Anti-AGEs marker, 40×, control group.**
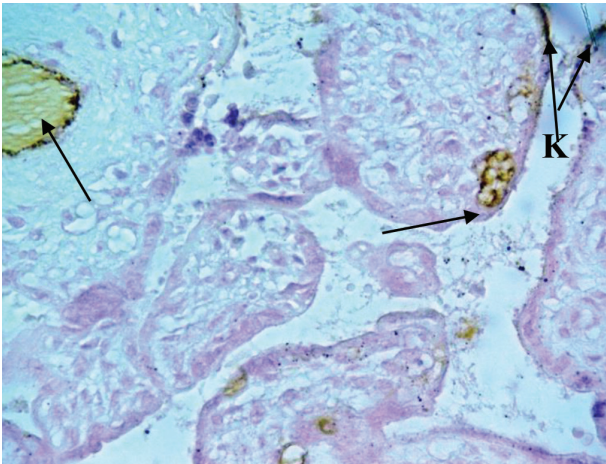
Fig. 6 Section through a placental tissue showing the same above reaction at a higher magnification with clear intense reaction of the blood vessel (→) and showing reaction at syncytial knots (K), anti-AGEs marker, 100×, control group.
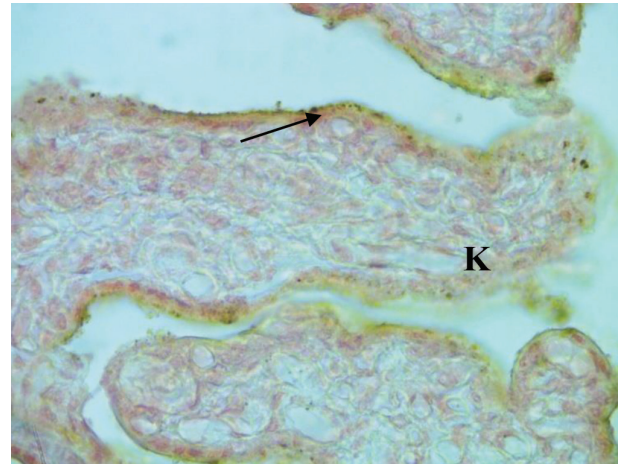

Fig. 9 Section through a placental tissue showing multiple villi with the same reaction as mentioned above with clear syncytial knot (K), anti-AGEs marker, 100×, diabetic group.
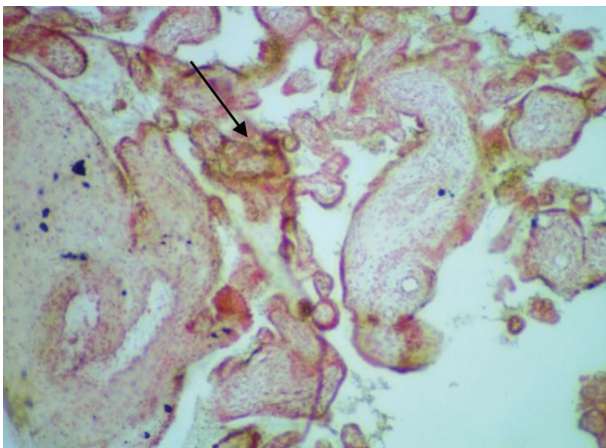

Fig. 7 Section through a placental tissue of diabetic group showing the immune localization of the reaction as brownish pigmentation. Anti-AGEs marker, 40×, diabetic group.
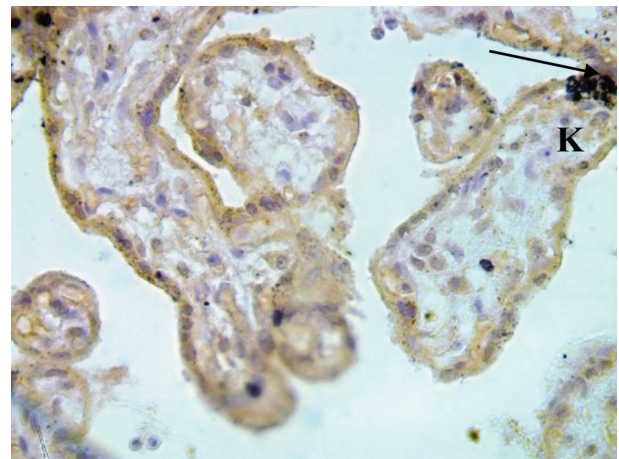

Fig. 10 Sectional through multiple villi showing the immune reaction in both cells and blood vessel, with intense reaction of the knots (K), AGEs marker, 100×, diabetic group.

Discussion

The role of advanced glycation end products in progression and complications of diabetes was thoroughly studied and these studies concluded that, the biochemical process of advanced glycation appears to be enhanced in the diabetes mellitus as a result of not only hyperglycemia but also other stimuli such as oxidative stress and lipids.[17]

Advanced glycation ends interact with glycosylated proteins and lipids, thereby changing the structure and effect of proteins and inducing abnormal changes that occur most readily in small vessels, leading to vasculopathy in diabetics, when this occurs in the placental blood vessels of GDMs, it would affect placental function and consequently fetal growth and development.[18]

When Ling et al.,[19] investigating diabetic rats, they found that hepatocytes, splenocytes, renal acinar and intercapillary endothelial cells, testicular Leydig cells and red blood cells, all showed accumulation of AGEs, leading to the corresponding pathology, they also recorded that persistently raised blood glucose was important in the rapid accumulation of AGEs in diabetic rat cells.
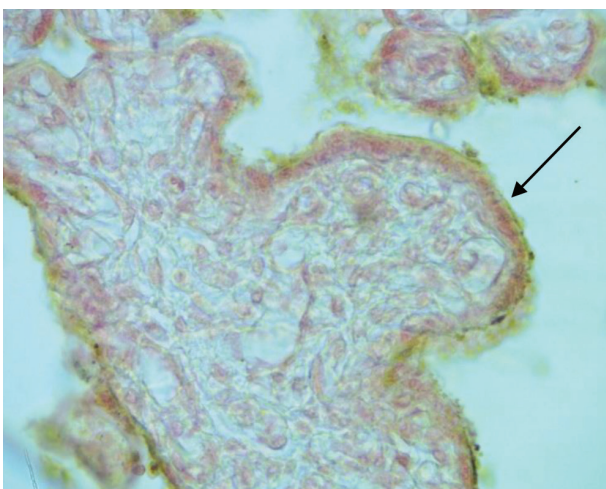

Fig. 8 Section through a placental tissue showing villi at higher-powered showing intense reaction at the apical cytoplasm of the cell of villi appearing as small and fine brownish granules (→), anti-AGEs marker, 100×, diabetic group.

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Original

AGEs in placental tissue of GDM

Placentas of GDM pregnancies are larger than normal and show decreased formation of terminal villi and increased numbers of intermediate villi compared with those from normal pregnancies. These vascular changes are likely to affect placental vascular resistance and vascular volume, leading to metabolic changes in the feto-placental microvascular and macrovascular endothelium.[20]

Placental villi of the diabetic group in this study showed marked changes in size and shape with appearance of fibrinoid associated with decrease vascularity in the villi affected by fibrinoid formation; this may lead to placental dysfunction. Placental dysfunction in diabetes may lead to oxygen-free radicals and its metabolites passing from the maternal to fetal circulation, resulting in fetal diseases.[21]

Oxidative stress is increased in hyperglycemic state by increased glucose auto-oxidation and protein glycation, which up regulate the production of oxidative factors, hyperglycemia might result in the non-enzymatic glycation of proteins called AGE products, which can interfere with signal transduction and thus change the soluble levels of cytokines, hormones, and free radicals, and these proteins can alter the function of the glycated proteins. In addition to its function as a ROS scavenger involving, the amelioration of oxidative damage and the proinflammatory state present in high-risk pregnancies.[20]

The AGEs mechanism of action contribute to diabetic complications including; formation of cross-links between key molecules in the basement membrane, which will lead to alter the cellular structure. AGEs can alter properties of the matrix proteins collagen, laminin through intermolecular covalent bounds and these in turn leads to increase connective tissue core matrix.[22] Histological and histochemical changes in placenta of diabetic pregnant females and its comparison with normal placenta was analyzed in a study and the histochemical analysis revealed that reactivity for glycogen was much stronger in diabetic placenta. About 20% cases showed glycogen in traces and 80% cases moderate to severe reactivity. The Sudan Black reactivity gratings were mild in 20%, moderate in 20% and strong in 60% among the diabetic placenta.[23]

Decrease in the vessels seen in the core of villi of women with GDM was recorded in this study; AGEs directly participate in diabetes-associated complications in pregnancy by impairing placental function. These complications is due to defects in the blood vessels of the villi, changes in these blood vessels are in two types: (a) endothelial cell changes, (b) smooth muscle changes in the wall of blood vessels, represent that AGEs directly cause endothelial damage by proapoptotic activation of caspases, thereby supporting the importance of protein AGE modifications as a critical initiating factor in diabetic vasculopathy. This means that AGEs are involved in specific damage and dysfunction of the endothelium, leading to preeclampsia complications found to be two to four times more frequent in diabetic pregnant women than in normal pregnancies.[24]

The Konishi et al.,[25] study showed that, AGEs modulate changes in the trophoblastic layer, which adversely affect implantation of the embryo and placentation, leading to abnormal fetal conditions. The pathogenicity of AGEs is broad. It can affect cell signaling and transduction through interaction with receptors.

There is a major adaptation in the maternal metabolism throughout pregnancy. Several differences were identified in placental villi in women with GDM; one of them was fibrinoid formation. Fibrin presences in the villi core is not indicator of villous degeneration, it might be explained that the syncytium overlying small intervillous fibrinoid despite degenerate only in limited cases.[26]

Fibrin deposition may lead to tissue matrix degradation, which will lead to further fibrin deposition. Several studies have emphasized the possibility that these small intervillous. Fibrinoid is the result of immunological process affecting syncytial cell and in response to such alteration fibrinoid will be formed as an immunological protective barrier or immune absorptive sponge.[27]

From this study the following conclusions were recorded:

- There have been marked and dramatic changes in the shape and size of placental villi in diabetic women.
- Fibrinoid formation is a striking feature of change seen in the placenta.
- A marked decrease in the number of vessels in villi core in women with GDM.
- AGEs antibodies showed a marked pronounced presence in trophoblastic cell of the placental villi.

Conflicts of Interest

None. ■

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