

Evaluation of the histology and the oxidative stress status in the placentas of obese mothers

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ABSTRACT

Background: Obesity becomes one of the most universal medical problems that affects women at reproductive period.

Aim: To highlight the placental changes in obese mothers and to determine their relation to the oxidative stress.

Methods: This work is a prospective case- control study in which placentas were obtained from 60 singleton pregnant women who delivered at Al-Khansaa Maternity Teaching Hospital, Mosul, Northern of Iraq, starting from January 2020 to March 2020. The women were classified into three groups: Group 1: includes (control) women who have BMI ranged between 18-24.9 kg/m² (N=20), Group2: (Overweight) includes women who have BMI ranged between 25-29.9 kg/m² (N=20), Group 3: (Obese) includes women with BMI \geq 30 kg/m² (N=20). Two placental biopsies were obtained from maternal side and fetal side of each case and submitted for histological examination via light microscope. In addition, Fresh placental specimens (0.5gm) had been taken from all study groups and frozen until the time of procedure for oxidative stress analysis.

Results: The Histological sections of group 2 and 3 placental discs revealed different histological changes in comparison with those of group 1. These changes include syncytial knotting ,villous hypovascularity, villous fibrinoid necrosis, thickening of trophoblastic basement membrane, cytotrophoblastic hyperplasia, perivillous fibrin deposition ,increase in number of nucleated red blood cells (NRBC), stromal fibrosis, chorangioma ,paucity of vasculosyncytial membrane (VSM), villous edema, features of deciduitis, villitis, and increase the thickening of tunica media of the placental blood vessels. The biochemical analysis of the level of malondialdehyde (MDA) in the placental homogenates of the three study groups revealed a significant increase in homogenates of group 2 and 3 in comparison with that in group 1 indicating the presence of the oxidative stress.

Conclusion: this work reveals a marked increase in the level of MDA in placental homogenates of the obese and overweight groups in comparison with that of control group suggesting the role of the oxidative stress.

Keywords: Placenta, obesity, histological changes, oxidative stress.

تقييم النسيج و حالة الاجهاد التاكسدي لمشيمة الامهات اللواتي يعانين من السمنة

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الخلاصة

الخلفية: السمنة هي واحدة من أكثر المشاكل الطبية انتشارا التي تؤثر على الإنجاب والتي لها نتائج سلبية معقدة على الام والطفل والتي قد تظهر مباشرة او بعد فترة من الزمن .

اهداف الدراسة: تسليط الضوء على التغيرات النسيجية للمشيمة وعلاقتها بالاجهاد التاكسدي في الامهات اللواتي يعانين السمنة. **طرائق العمل :** يعتبر هذا العمل دراسة مراقبة الحالات المستقبلية للكشف عن المشائم المأخوذة من النساء الحوامل اللاتي ولدن في مستشفى الخنساء التعليمي النسائي في مدينة الموصل شمالي العراق. اجريت هذه الدراسة خلال الفترة من كانون الثاني ٢٠٢٠

لغاية اذار ٢٠٢٠ اشتملت هذه الدراسة على فحص المشائم المأخوذة من ٦٠ امرأة. قسمت النساء الى ثلاثة مجاميع اعتمادا على مؤشر كتلة الجسم :

ضمت المجموعة الاولى (مجموعة السيطرة) عشرين امرأة ذات مؤشر كتلة جسم تراوحت بين ١٨-٢٤.٩ كغم/م² والمجموعة الثانية (ذوات الوزن الزائد) ضمت عشرين امرأة ذات مؤشر كتلة جسم تراوحت بين ٢٥-٢٩.٩ كغم/م² في حين ضمت المجموعة الثالثة (النساء البدنيات) عشرين امرأة ذات مؤشر كتلة جسم اكبر او يساوي ٣٠ .

النتائج: اظهرت نتائج الفحص النسيجي المرضي لعينات المشيمة المأخوذة من النساء ذوات الوزن الزائد و البدنيات وجود عدة تغيرات نسيجية في المشيمة مقارنة مع مجموعة السيطرة. تضمنت التغيرات المرضية النسيجية وجود العقدة المخلاوية ، زغابات قليلة الأوعية الدموية ، النخر الفيبريني للزغابات ، التثخن في الغشاء القاعدي لطبقة الارومة الغذائية ، فرط تنسج خلايا الطبقة الغذائية من المشيمة ، بالإضافة إلى ترسب الفيبرين حول الزغابات ، زيادة وجود خلايا الدم الحمراء المنواة ، التليف ، وسعة دموية مشيمية، ضعف الغشاء الوعائي المخلاوي ، وذمة زغابية بالإضافة إلى ذلك ، التهاب الزغابات ، التهاب نسيج الساقط ، وزيادة سمك غلالة الأوعية الدموية المشيمية.

اظهر التحليل الكيميائي لمستوى المالونالديهيد لمزيج او مستخلص المشائم للمجاميع الثلاثة وجود زيادة معنوية في المجموعتين الثانية والثالثة مقارنة مع المجموعة الاولى كاشارة الى وجود الاجهاد التاكسدي.

الاستنتاجات: اظهرت هذه الدراسة زيادة ملحوظة في تحليل الاجهاد التاكسدي لدى الأمهات ذوات الوزن الزائد و البدنيات مقارنة بمجموعة السيطرة..

الكلمات المفتاحية: المشيمة، السمنة، التغيرات النسيجية، الاجهاد التاكسدي.

INTRODUCTION

Maternal obesity has a significant relation with an increased risk of adverse maternal and fetal outcome. It also has an effect on mother and her family psychologically and financially. Optimization of women weight is essential for a good pregnancy outcome and it is important to maintain a healthy life for mother and baby as well as to keep the familial unity.

Obesity is usually measured as a ratio between a body weight to height and identified as body mass index, ¹ which is directly related with the amount of fat in the body.

Obesity is defined as a body mass index (BMI) $\geq 30 \text{ kg/m}^2$.²⁻⁴

The presence of excessive amount of adipose tissue produces a significant quantity of pro inflammatory cytokines as Interleukin 6(IL 6), adipokinin and Tumor necrotic factor alpha (TNF alpha) as well as, hormones such as adiponectin and leptin in dysregulated manner that lead to insulin resistance and inflammation.⁵ Moreover, there is an increase in infiltration of macrophages and expressing inflammatory markers which may lead to maternal systemic inflammation.⁶

In addition, maternal obesity has an impact on placental metabolism and fetal redox balance. Changes in cortisol level, hypoxia, nitrate and oxidative stress may precipitate in fetal metabolic programming of the metabolic syndrome.⁷

Oxidative stress reflects the disturbance in the balance between the systemic indicator of reactive oxygen species (ROS) and the ability of biological system to repair the consequential damage or reactive intermediates detoxification.⁸ Although (ROS) can be helpful (the immune system used

them as a method to attack and destruction of pathogens),⁹ but the creation of free radicals and peroxides due to disturbances in the normal cells redox state will cause toxic effects and damage to all cell components.⁸ Oxidative stress is assumed to be implicate in the development of myocardial infarction ,Parkinson's disease, atherosclerosis, cancer, heart failure, Alzheimer's disease, autism.⁹

There are numerous oxidative biomarkers, one of them is the malondialdehyde which is an organic substance produced by lipid peroxidation when lipid was degenerated by oxidation.¹⁰

The reports on the histological changes of placentas in obese mothers and even their relation -if present- to the oxidative stress status has not been studied well yet. So, this study tried to highlight the effect of obesity on the placental oxidative stress and to determine its effect on the placental histology.

Subjects, materials and methods

This is a prospective case-control study that had examined placentas which were obtained from 60 singleton pregnant women who delivered at AL-Khansaa Maternity Teaching Hospital in Mosul province in north of Iraq, starting from 1st of January 2020 to 1st of March 2020. Ethical approval was obtained from Committee of Ethics at Nineva Health Directorate, Mosul, Iraq. Subject's history was obtained from every woman, their gestational age was calculated using the last menstrual period and/ or the early ultrasonographic examination report. Pregnant women with non-gestational diabetes mellitus, essential hypertension, placental abruption, multiple

pregnancy, smoking and Rhesus negative blood group were excluded.

Body mass index (BMI) of each mother was calculated by dividing the weight in (Kg) by height in square meter (m^2). The weight was obtained according to Davis *et al.* by subtracting 12.5 kg from the current weight.^{4,11} According to their BMI, the women were classified into three groups as the follows:

1. Group1:(control group) which includes 20 women with BMI ranged between 18-24.9 kg/m^2
2. Group2:(over weight group) which includes 20 women with BMI ranged between 25-29.9 kg/m^2
3. Group3:(obese group) which includes 20 women with BMI ≥ 30 kg/m^2 .

Two biopsies of placental tissue were obtained, from maternal side (decidua) and fetal side (chorionic plate), and were prepared for histological examination after staining with (H&E) using light microscope.¹²⁻¹⁴ While analysis of the oxidative stress parameter (MDA) was done as follows:^{15,16}

Fresh placental specimens (0.5 gm) were taken from all groups involved in this study and were frozen until the time of procedure of biochemical analysis. Each specimen was applied in inspection tube of homogenizer device, then cold Tris-EDTA buffered solution (10 ml) was added, then was put in centrifuge for 15-60 seconds within each accelerated speed from 400- 1100 round per minute (rpm.), then 0.5 ml of homogenizer solution was mixed with 0.5 ml of cooled peroxidation solution in a test tube with well shaking, then was put in water bath for half an hour at 73°C. After that the reaction of peroxidation was stopped via addition of 0.5 ml of sodium arsenate that was dissolved in trichloroacetic acid and was put in the centrifuge for 5 min. at 3000 rpm. Then it was filtrated and 1 ml of the filtrate was put in another test tube. Adding of 0.25 ml of both thiobarbituric acid (TBA) and distilled water was done with well shaking. Then transferring it to water bath for 15 min at 100°C. Using spectrophotometer, the absorbance of each sample of all study groups was read twice, firstly at 532 nanometer (nm) and after 15 mints. at 453nm.

Statistical analysis of data was done using excel program and statistical package for social sciences (SPSS) version 3. To compare the frequencies of different placental lesions between two groups, chi square (χ^2) was used, where P value <0.05 was considered as significant.¹⁷

RESULTS

This study has examined placentas from 60 women who were categorized into three groups.

The light microscopical examination of sections obtained from the fetal surface of placental disk of control group (Group1) ,using H&E preparation ,showed that each chorionic villous appeared as a vascular stroma lined or covered with multinucleated syncytiotrophoblastic layer with indistinct cell boundaries and dark stained nuclei with occasional inconspicuous cytotrophoblastic cells (Figure 1). While the microscopical examination of sections of the maternal surface of this group showed normal appearance of maternal decidual cells (Figure 2).

On the other hand, the present study revealed that the histopathological examination of placental sections obtained from overweight mothers (Group 2) and obese mothers (Group 3) showed several placental changes compared to those of control group. Table 1.

The most frequent feature among these sections is the presence of syncytial knotting in 3 (15%) out of 20 placentas of group 2 and 13 (65%) out of 20 placentas of group 3 (Figure 3). In addition, features of hypovascular villi are noticed in 4 (20%) in sections of group 2 and 10 (50%) of group 3 sections (Figure 3).

Moreover, villous fibrinoid necrosis occurred in 3 (15%) placental sections of overweight mothers while it occurs in 10 (50%) of placental sections of obese mothers (Figure 4), while thickening of trophoblastic basement membrane occurred in 2 (10%) of group 2 and 9 (45%) of group 3 sections. (Figure 4).

Also, the histopathological examination of placental sections obtained from overweight mothers (group 2) revealed presence of perivillous fibrin deposition which appeared in 4 (20%) whereas in obese group it appeared in 10 (50%) (Figure 5). Features of stromal fibrosis were noticed in (40%) of obese sections (Figure 5). In addition cytotrophoblastic hyperplasia occurs in 2 (10%) of group 2 sections and in 8 (40%) of group 3 sections (Figure 6).

The present study revealed that the histopathological examination of placental sections obtained from overweight mothers (group2) showed presence of nucleated red blood cells (NRBC) in fetal capillaries and this finding was identified in 4 (20%) while it occurred in 7 (35%) of the obese group sections. (Figure 6), while features of chorangiomas was found in 1 (5%) in group 2 and 4 (20%) of group 3 sections. (Figure 6).

On the other hand, paucity of vasculosyncytial membrane VSM was found in 15% of overweight sections while it occurred in 35% of sections of obese mothers. (Figure 6).

Features of villous edema were shown in 2(10%) out of 20 sections of overweight mothers and in 4 (20%) of obese group sections. (Figure 7). This study observed features of villitis in 1 (5%) in overweight group and 4(20%) out of 20 placental sections of obese mothers. (Figure 8), while deciduitis was noticed in 4 (20%) in group 2 and 7(35%) of group 3 sections. (Figure 8).

This work reveals thickening of tunica media of placental vessels in 2 (10%) of group 2 and 5 (25%) of group 3 (Figure 9).

Analysis of oxidative stress (MDA level):

The mean concentration of MDA in placental homogenates of all study groups revealed that level of MDA in obese group was 2.46 ± 0.06 n mol/gram, while MDA concentration in control group was 0.55 ± 0.05 n mol/gram. On the other hand, the level of MDA concentration in homogenates of overweight group was 1.55 ± 0.24 n mol/gram. Table 2. Histogram 1.

Table 1. Histological finding of placentas in all study groups.

GROUPS	Control group Mean \pm SE	Overweight group Mean \pm SE	Obese group Mean \pm SE
Oxidative stress			
MDA concentration n mol/gram tissue	0.55 ± 0.05 a	1.55 ± 0.24 b	2.46 ± 0.06 c

The rows with similar letters mean no significant difference among groups ($p \leq 0.05$)

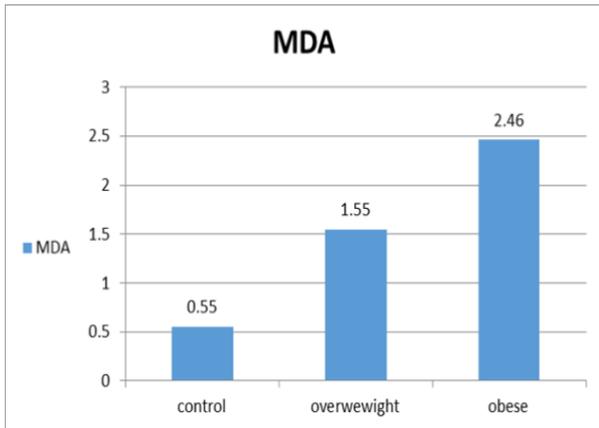
The rows with different letters mean significant difference among groups ($p \leq 0.05$)

Table 2. The level of MDA in placental homogenates of the three study groups.

Groups	Group 1 (Control) N=20 No. (%)	Group 2 (overweight) N=20 No. (%)	Group 3 (Obese) N=20 No. (%)
Syncytial knotting	2 (10%) a	3 (15%) b	13 (65%) c
Hypovascular villi	1 (5%) a	4 (20%) b	10 (50%) c
Villous fibrinoid necrosis	1 (5%) a	3 (15%) a	10 (50%) b
Thickening of trophoblastic basement membrane	1 (5%) a	2 (10%) a	9 (45%) b
Cytotrophoblastic hyperplasia	0 (0%) a	2 (10%) b	8 (40%) c
Perivillous fibrin deposition	1 (5%) a	4 (20%) b	10 (50%) c
Nucleated RBCs	1 (5%) a	4 (20%) b	7 (35%) c
Stromal fibrosis	0 (0%) a	1 (5%) a	8 (40%) b
Chorangiosis	1 (5%) a	1 (5%) a	4 (20%) b
Paucity of VSM	1 (5%) a	3 (15%) b	7 (35%) c
Villous edema	1 (5%) a	2 (10%) b	4 (20%) c
Villitis	0 (0%) a	1 (5%) b	4 (20%) c
Deciduitis	1 (5%) a	4 (20%) b	7 (35%) c
Thickening of tunica media of vessels	1 (5%) a	2 (10%) b	5 (25%) c

The rows with similar letters mean no significant difference among groups ($p \leq 0.05$)

The rows with different letters mean significant difference among groups ($p \leq 0.05$)



Histogram 1. The value of MDA concentration in placental homogenates of the three study groups.

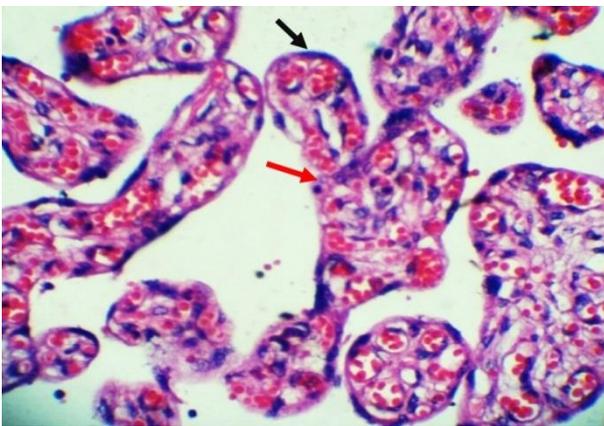


Figure 1. A photomicrograph of a placental tissue obtained from control group with normal villous architecture. The vascular stroma lined with multinucleated syncytiotrophoblastic layer with indistinct cell boundaries and dark stained nuclei (black arrow) with occasional cytotrophoblastic cells (red arrow) (H&E×250).

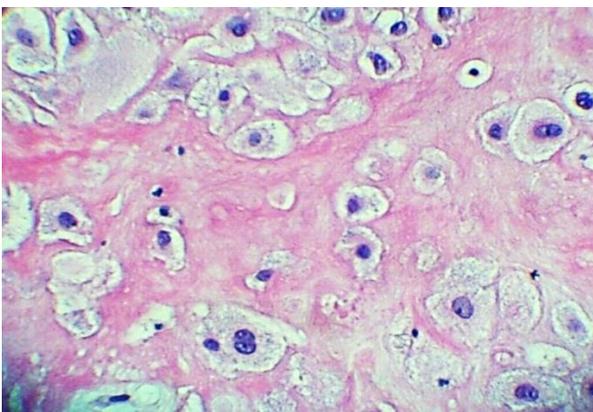


Figure 2. A photomicrograph of a placental tissue obtained from a woman of control group with normal decidual architecture (H&E×400).

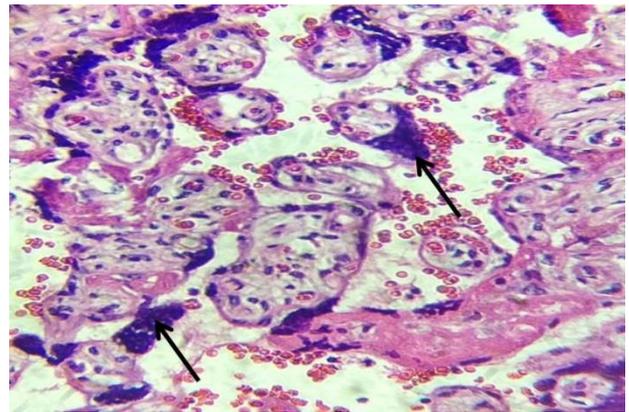


Figure 3. A photomicrograph of a placental tissue obtained from a woman of overweight group with features of hypovascularity and syncytial knotting (arrow). (H &E×400).

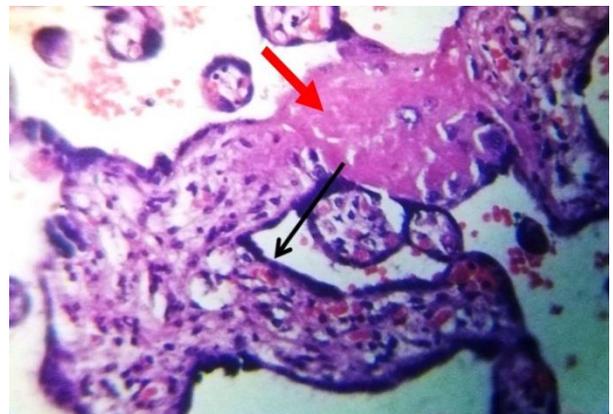


Figure 4. A photomicrograph of a placental tissue obtained from an obese woman showing villous with thickening of basement membrane (arrow) and fibrinoid necrosis (red arrow) (H&E×400).

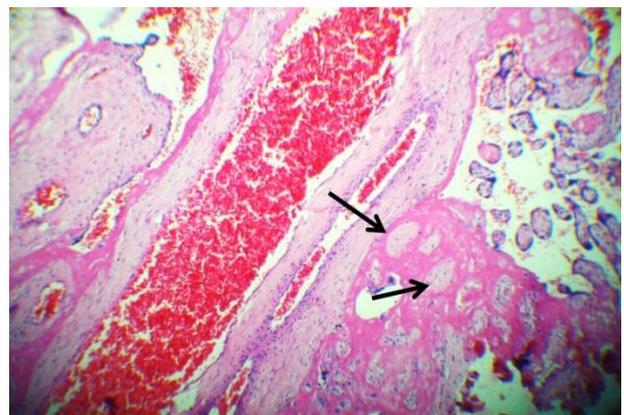


Figure 5. A photomicrograph of a placental tissue obtained from a woman of the obese group .Fibrotic avascular villi are embedded in a sea of fibrin (perivillous fibrin deposition) (arrows). (H&E×160).



Figure 6. A photomicrograph of a placental tissue obtained from a woman of overweight group with features of nucleated red blood cells (arrows) with cytotrophoblastic hyperplasia (green arrows) , chorangiomas and paucity of VSM (red arrow) (H&E×400).

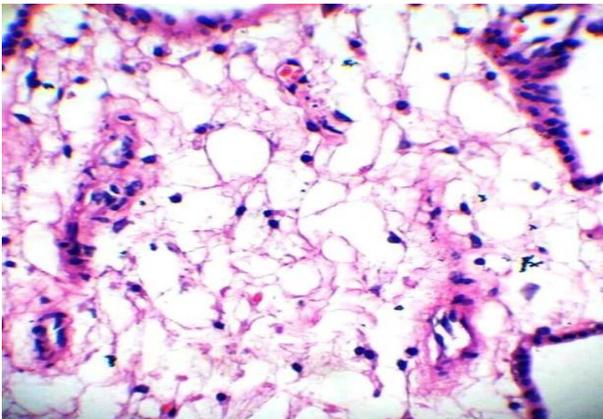


Figure 7. A photomicrograph of a placental tissue obtained from an obese mother. Villous edema is noticed. (H&E×400).

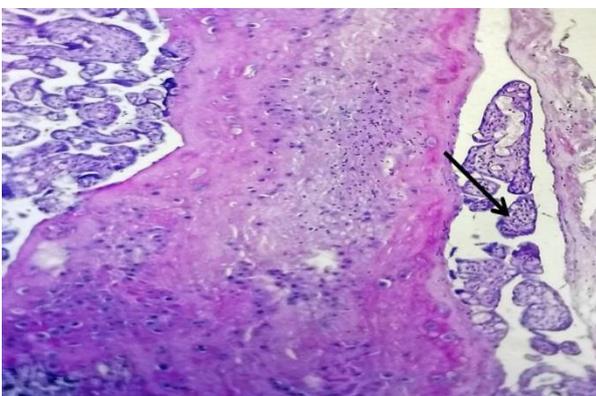


Figure 8. A photomicrograph of a placental tissue obtained from a woman in the overweight group. Features of decidualitis and villitis (arrow) are seen (H&E×160).

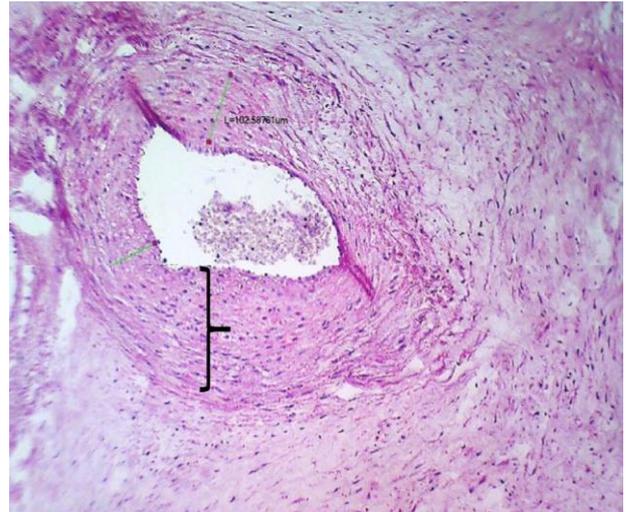


Figure 9 .A photomicrograph of a placental tissue obtained from obese group with an increase in the thickening of tunica media of placental vessels (black bracket). (H&E×100)

DISCUSSION

Practically, pathology of placenta is totally became an identical specialty after years of uncertainty due to different views of experts. Pathologists, with different interests and training skills. Found that there is closely relationship of placental histological lesions with clinical features in both mothers and fetal outcomes.¹⁸

The microscopical examination of placentas that enrolled in this work identified several histological lesions among placentas of obese and overweight groups in comparison with those of control.

Among these lesions, a significant increase in the frequencies of syncytial knots was noted in placentas of obese group and overweight group compared with those of control. Such finding was consistent with Huppertz study in 2008 who considered the syncytial knots as a placental adaptive response.¹⁹

In addition, this study revealed a significant increase in the thickening of trophoblastic basement membrane in placentas of obese group, a finding that also related to placental hypoxia.

Gill *et al.* in 2011 and Sankar *et al.* in 2012 reported that any increase in the thickening of trophoblastic basement membrane leads to reduction of placento-fetal blood circulation and accumulation of syncytial knots.^{20,21}

Another striking feature that have been shown in current work was presence of a significant number of hypovascular villi in placentas of obese and overweight mothers in comparison with those of control group. The hypovascular villi are defined as the villi that contain small non-dilated capillaries in

their stroma and sometimes devoid of capillaries.²² This finding is in accordance with that of Hayward *et al.* in 2013, who was attributed that to the frequent abnormal spiral arteries modifications and alteration of placental vascular function.²²

The villous fibrinoid necrosis was found significantly in placentas of obese mothers when compared with those of overweight and control groups. Benirscheke and Kaufmann in 2000 described that this lesion may result from several coagulation cascades after injury of syncytiotrophoblasts.²³

In fact, this study revealed that syncytial injury was observed in placental sections of obese mothers when compared to those of control group.²⁴

The four preceding histological lesions (syncytial knots, hypovascular villi, thickening of trophoblastic membrane, and villous fibrinoid necrosis) are encompassed under umbrella of accelerated villous maturation (AVM), Which occurs as a result of maternal vascular malperfusion, and abnormal flow of spiral artery that is characterized by high velocity and low volume maternal blood.⁽¹⁸⁾ AVM is represented by short hypovascular villi with increased intervillous fibrin, and syncytial knots in addition with increasing thickness of trophoblastic basement membrane.

AVM was found in the placentas of obese and overweight women.^{13,25}

In fact, AVM may be due to the placental angiogenesis as a result of hypoxia and placental insufficiency.²⁶

Moreover, this study reveals a significant increase of cytotrophoblastic hyperplasia in placental sections of women in obese group and overweight group in comparison with women of control group. Cytotrophoblastic cells normally disappear in term placenta of non-complicated pregnancies. Persistence of cytotrophoblastic cells partially occurs due to hypoxial condition in placental tissue in complicated pregnancies such as maternal diabetes, Rhesus incompatibility, pre-eclampsia, and fetal growth restriction.²⁷⁻²⁹

This study revealed a significant elevation of number of nucleated red blood cells (NRBCs) in fetal capillaries of placentas in obese and overweight groups in contrast to those of control group. In fact, NRBCs are rarely appeared. This finding is similar to that of Rincon *et al.* in 2019, who found that the erythropoietin value and NRBCs are higher in newborns of obese and overweight mothers compared with mothers who have normal BMI. These findings may ensure the theory that the increased pregnant BMI may result in intrauterine hypoxemia.^{30,31}

The delayed villous maturation (DVM) including (chorangiosis, paucity of VSM and stromal

fibrosis).¹⁸ DVM was frequent in obese and overweight women compared to women who have normal BMI. This finding was consistent with those of previous reports.^{32,33}, this lesion is attributed to the insulin resistance and hypoxia. Insulin resistance was more frequent in obese women which lead to increased proliferation of villi in early gestation period that lead to increased placental stroma and decreased numbers of vasculosyncytial membrane (VSM) layers with increase in its thickness. The thickness of VSM was gradually reduced which is necessary to ensure placental function in the final stage of pregnancy.^{34,35}

This study revealed a significant increase in villous edema in sections of overweight and obese groups in comparison with those of control group. This lesion may be a result of fetal vascular malperfusion and weak blood circulation in the villous tree. Rosaddo-yeppez *et al.* in 2019 revealed a high occurrence of villous edema in sections of placentas of obese women.³⁶ This lesion comprises insufficient fetal vascular perfusion and is related to an increase in the morbidity of neonates.³⁷ Villous edema may cause either fetal anemia, CNS injury or perinatal death,³⁸ also it is correlated with severe term fetal acidemia.³⁹

This study revealed a marked increase in the frequency of deciduitis and villitis in placentas of obese and overweight women. Deciduitis and villitis are defined as an infiltration of large number of lymphocytes, neutrophils and macrophages in decidual and villous tissue. These findings were in agreement with those of other studies, which reported a strong relation between obesity of women with increased placental expression to chemotactic cytokines.^{35,40} in addition, Denison *et al.* in 2010 revealed the relation between obesity and inflammation of placenta, and an increase in the pro-inflammatory cytokines in mother circulation.⁴¹ Obesity makes a physiologic condition of low-grade chronic inflammation with increased level of inflammatory circulating markers such as CRP, IL-6, IL-8 and TNF- α . These markers were related with metabolic dysregulation, diabetes and atherogenesis.⁴²

The biochemical analysis of the placental homogenates of three study groups was performed in order to clarify the role of oxidative stress in the placental insufficiency of obese and overweight mothers in comparison with that of control. Level of MDA was measured in the placental homogenates of different groups. There was a significant elevation in the concentration of MDA in homogenates of placentas in groups of obese and overweight women when compared to that of control group. This result is similar to that of Malti

and Nace in 2014, they reported an inequality of status between oxidants and antioxidants.^{43,44}

Moreover, Myatt and Cui in 2004 proposed that the oxidative stress in placentas of obese mothers is counteracted by antioxidant property.⁴⁵

Pro inflammatory cytokines elevation and greater production of reactive nitrogen and oxygen species as a result of increased fatty acids may reduce the function of mitochondria.⁴⁶ Which in turn affects mitochondrial fatty acid oxidation.⁴⁷ On the other hand, mitochondrial function may play a role in controlling of many cellular actions such as cellular signaling and apoptosis.⁴⁸

This study shows several lesions such as villitis, deciduitis, AVM, elevation in number of NRBCs and perivillous fibrin deposition, all may lead to oxidative stress.⁴² At the same time these findings may be attributed to oxidative stress. Inflammatory conditions as well may cause oxidative stress, lipid peroxidation, and damage of protein and DNA which subsequently lead to cell death.⁴⁹⁻⁵¹

In conclusion, this work reveals a marked increase in the level of MDA in placental homogenates of the obese and overweight groups in comparison with that of control group suggesting the role of the oxidative stress. In addition, The placental changes of mothers may start when the BMI reach 25 Kg/m² as the frequency of various histological changes is markedly increased in placentas of overweight mothers and considerably similar to those shown in obese group.

Study recommendation: an administration of antioxidant drugs during pregnancy to decrease the influence of oxidative stress on placental tissue and function.

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