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ORIGINAL ARTICLE

Histological Evaluation of Placenta in Relation with Maternal Age in Normal Uncomplicated Pregnancy

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ABSTRACT

Background: The placenta is a fetomaternal structure that determines normal fetal growth and maturation, the function of the placenta depends on the normal differentiation of the villous trophocytoblast and the thickness of the placental barrier. Placental insufficiency associated with abnormal neonatal outcome. The aim of this study is to evaluate the histological structure of the placenta according to maternal age.

Methods: A case control prospective study is done on sixty placentae from women delivered normally at Al-Khansaa obstetric Hospital, the samples of placentae are classified into three groups according to the maternal age: group A: mother age (20-34) years considered as control group, group B: mother age (19-15) years while group C: maternal age 35-50. The placentae are weighted and their diameters include thickness, are measured then pieces from placentae are taken to asses oxidative stress biomarker and other pieces are fixed in formalin solution then processed and prepared for light microscopic examination.

Results: The mean weight and surface area of placentae of mothers over 35 years were decreased while their thickness increased as compared to that of control and young age group, the oxidative stress biomarker increased in this group whereas the histological study revealed thickening of placental barrier, decreased of villus vascularity, increased villus stroma with fibrinoid deposition and increased syncytial knot.

Conclusion: Pregnancies, in advanced maternal age rather than youth, can negatively impact the structure of the placenta due to increased oxidative stress. To potentially help support function in this age group adding antioxidants could be beneficial.

Key words: Placenta; Surface area; Pathological changes; Oxidative stress.

INTRODUCTION

The placenta is the chief organ for the existence of the fetal body. Normal and regular placental function is considered a major factor in controlling intrauterine fetal growth, as nutrient and oxygen supply to the embryo throughout gestation is achieved mainly through the placenta [1, 2].

The function of the placenta depends on the normal differentiation of the villous trophocytoblast and the thickness of the placental barrier [3]. The foetal growth is affected by the quantity and quality of maternal blood poured in the placental intervillus region [4]. The placenta, as any other developing tissue, undergoes remolding and apoptosis of cells [5]. Abnormal cell development leads to placental insufficiency, which has a direct association with abnormal neonatal outcomes such as respiratory distress, growth restriction, and long-term deterioration of health [6, 7]. One of the most common factors that affect the structure and function of the placenta is maternal age [8, 9].

The most suitable age for pregnancy in women is between 20 and 34 years; however, pregnancy with maternal age below 20 (teenager) or above 35 (advanced age) is associated with high risk of complication [10]. Previous research showed that advanced maternal age, as well as teenage mothers, have been associated with a high risk of intrauterine growth retardation, small infants, and prenatal death [11–13].

The exact mechanism by which maternal age affects fetal growth is unclear, but it may be related to the placenta, so in this study we assess the placental weight and structure in different age groups of the mother.

MATERIALS AND METHODS

• **Placental collection**: Sixty full term placentae from normal pregnant women were collected. The inclusion criteria for mothers include maternal weight (60-70 kg), gestational age (38-42 weeks), male baby, normal vaginal delivery, uncomplicated pregnancy (the mothers were normotensive, not diabetic, and had no other endocrine or systemic disorder) and had no previous history of any disease. The placentae are classified into three groups according to the age of the mothers:

Group A: 20 placentae from women aged 20 to 34 years at the time of delivery considered control group. **Group B:** 20 placentas of pregnant women with a 15-19 year age. **Group C:** 20 placentas from pregnant women aged 35 -50 years.

All samples of this prospective study of case and control have been collected from the obstetrics department of the Al-Khansa Teaching Hospital in Mosul city during the period of May 2023 to December 2023. The placentae were cleaned with a towel, the umbilical cords were cut about 4 cm from their insertion site, the diameters of the placentae were measured and recorded, they were weighted, and then the tissue pieces (not less than one gram) were preserved in aluminium foil and stored in the refrigerator to be used later to measure the biomarker of oxidative stress according to the thiobarbituric acid (TBA) technique according to Gilbert et al. [14] other pieces from various parts of each placenta are fixed in 10% formalin solution.

• **Tissue Processing and Staining:** After 24 hour fixation, the tissues were dehydrated with an ascending grade of alcohol, cleaned with xylene, embedded in paraffin, then 5 μ sections were cut and stained with hematoxylin and eosin [15].

• **Quantitative analysis:** The thicknesses of the feto- maternal membrane were measured at 200X magnification using the colour USB 2.0 camera " A3950U" provided with image processing software, which was calibrated to the objective lens of the microscope by a 0.01 mm micrometre " ESM-11 Japan", the measurement was performed on ten randomly chosen fields from each section.

• **Measurement of oxidative stress biomarker:** The generation of free radicles disturbs cell redox with increasing cell damage and lipid peroxidation of the cell membrane, which is associated with the production of toxic substances such as malondialdehyde (MDA) that are considered as biomarkers of oxidative stress [14].

To measure the level of MDA, the previously preserved placental tissues are homogenized by a special device and centrifuged with a cold Tris-EDTA solution for 15 minute , 0.5 ml of homogenized solution treated with cooled peroxidizing agent and placed in a 73 ° C water bath for half an hour, then 0.5 ml of sodium arsenate dissolved in thiobarbituric acid was added and centrifuged for five minute at 3000 rpm to stop peroxidation after that one ml of filter was drowned and mixed with 0.25 m of TBA and distilled water , shacked well , put for 15 minute in boiled water bath and finally spectrometer was used to measure absorbent of each sample at wavelength of 532 nm and at 453 nm after 15 minute [14].

Regarding statistical analysis, the results of descriptive statistics were expressed as mean ± standard error (SE). The significance of variation was measured by GraphPad Prism (version 8.0) using one-way ANOVA followed by Student's t-new man–Keuls multiple comparison test with a level of significance at $P \leq 0.05$.

RESULTS

• Placental weight

Placental weight was measured in grams in all groups. The mean placental weight in group A is approximately 463.68 ±16.13, in group B it is 415.93±13.25 while in group C it is 378.12±.19.35 **(Figure 1)**. There was a significant variation ($p < 0.001$) in the mean placental weight in group C compared to other groups; however, there were also significant differences ($p < 0.05$) differences between group A and B.

• Thickness and surface area of the placenta

The thickness of the placenta was measured in millimetres by a graduated needle inserted near their centre, while the surface area (SA) of the placenta has been calculated using the following formula [16]:

$$\mathbf{SA} = \frac{\pi D1D2}{4} \quad , \quad (\pi = 3.14)$$

D1: Largest diameter (length),

D2: The lowest diameter (widths) perpendicular to the length measured in centimeters (cm).

The mean values of the thickness and surface area of placentae in group C were significantly ($p< 0.01$) differ compared to other groups; however, there were non-significant ($p>0.05$) variation in surface area between group A and B **(Table 1)**

• Placental Barrier Thickness

There is a significant ($p <0.001$) increase in the thickness of the feto-maternal membrane in the group of mothers aged 35-50 years, the mean thickness of the placental barrier in this group is about 5.09±2.67m compared to the control group (mother age 20-34) and the young mother (15-19) in whom the thickness of the placental barrier are 2.02±3.45 and 1.98±3.55 m respectively and there is no significant change between these two groups **(Figure 2)**.

• Histological evaluation

The histological study of placenta revealed that about 92% of placenta from group A, 85% of group B and 10% of group C revealed a normal structure, they had a random distribution of chorionic villi with regular lumen capillaries and cuboidal trophocytoblasts with rounded nuclei , the basement membrane between capillary and trophopblast is thin **(Figure 3)**. However, 90% of placenta from group C and 8%, 15% of group A and B respectively showed different histological changes

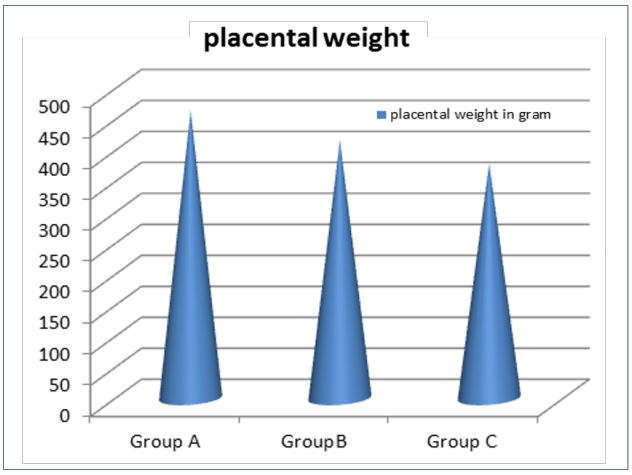

Figure 1. Histogram showing placental weight (wt in gram) in different groups of mothers.

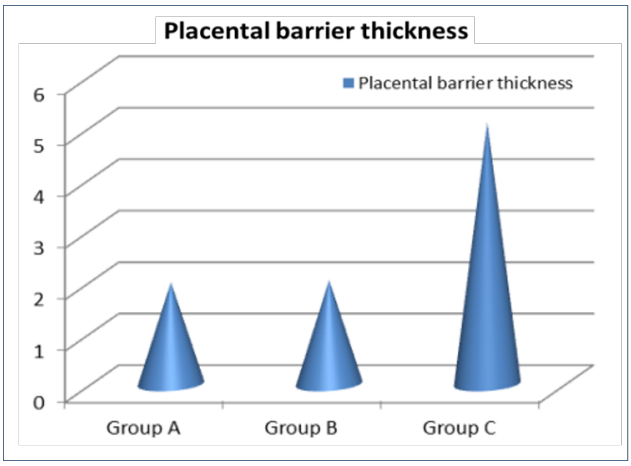

Figure 2. Histogram shows placental barrier thickness in different groups of mothers.

which include irregular, few capillaries with thickness of basement membrane between the capillaries and trophopblast, increase of stromal thickness of tertiary villi , deposition of fibrinoid between the villi and increase frequency of syncytial knots **(Figure 4, 5 and 6)**.

• Malondialdehyde Analysis

The mean level of MDA in placentae of mothers of advanced age was 1.89 ± 1.07 nmol / gm, which is significantly ($p < 0.01$) higher than that of the control (0. 68 ± 1.23) and that of the mothers of adolescents (0. 65 ± 1.56) in whom there was no significant variation ($p > 0.05$) between them **(Figure 7)**.

Table 1. Comparison of different parameters between HS and CT techniques.

Parameters	Group A (Mean± SE)	Group B (Mean± SE)	Group C (Mean± SE)
Placental surface area (cm^2)	221.72±31.26*	221.68±41.36*	173.27±65.17*
Placental thickness (mm)	22.36±4.12*	22.31±2.2*	23.12±5.31*

* Significant differences ($p<0.01$).

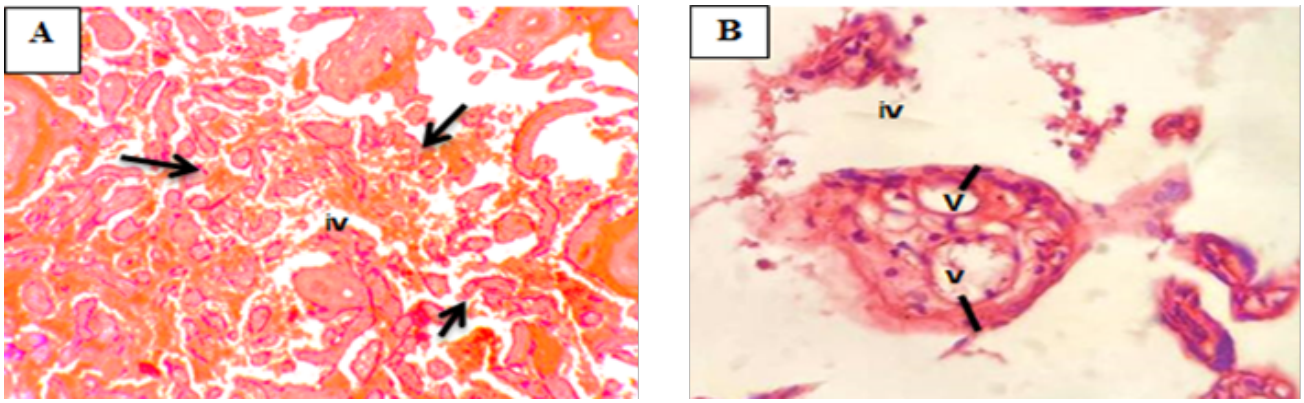

Figure 3. Photomicrographs of the normal placenta show the tertiary villi (arrows) floating in the intervillous space (IV), the thin placental barrier (black lines) , regular thin wall vessels (v), HE , A;100X, B; 400X

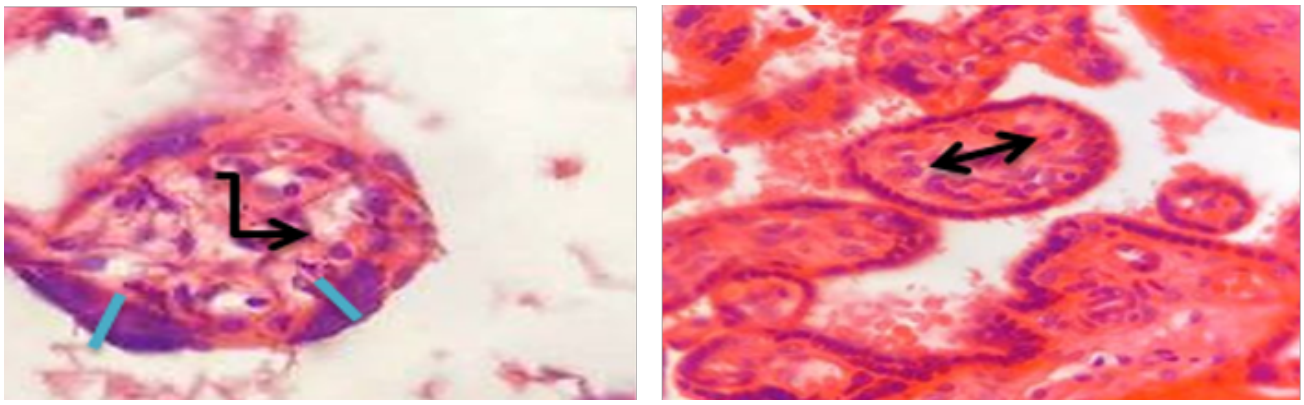

Figure 4. Photomicrograph of the placenta show thickening of placental barrier (green lines), decrease vascularity (curved arrow), increase stromal thickness (bihead arrow) H E , 400X

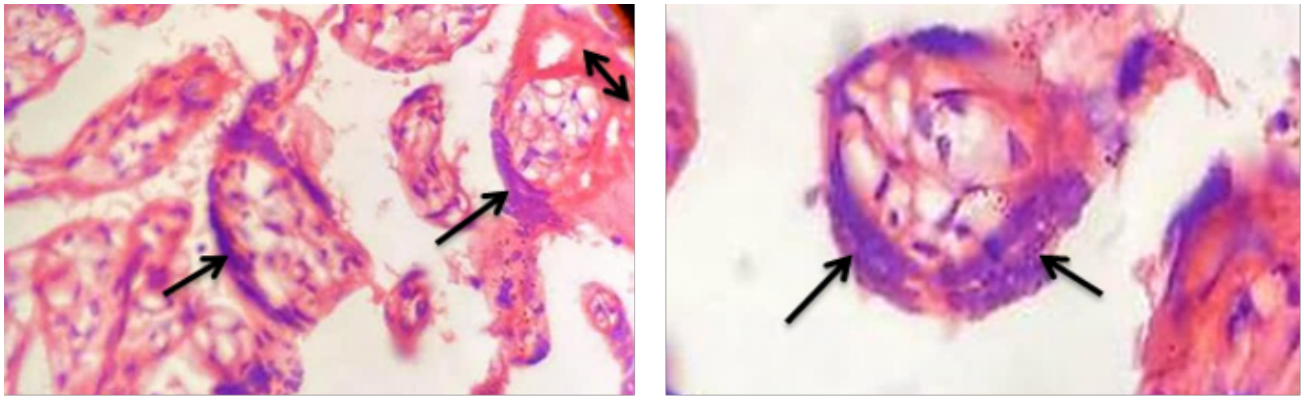

Figure 5. Photomicrographs of placental tissue show intervillous fibroid deposition (bihead arrow), increase the syncytial knot (arrows). HE, 400X.

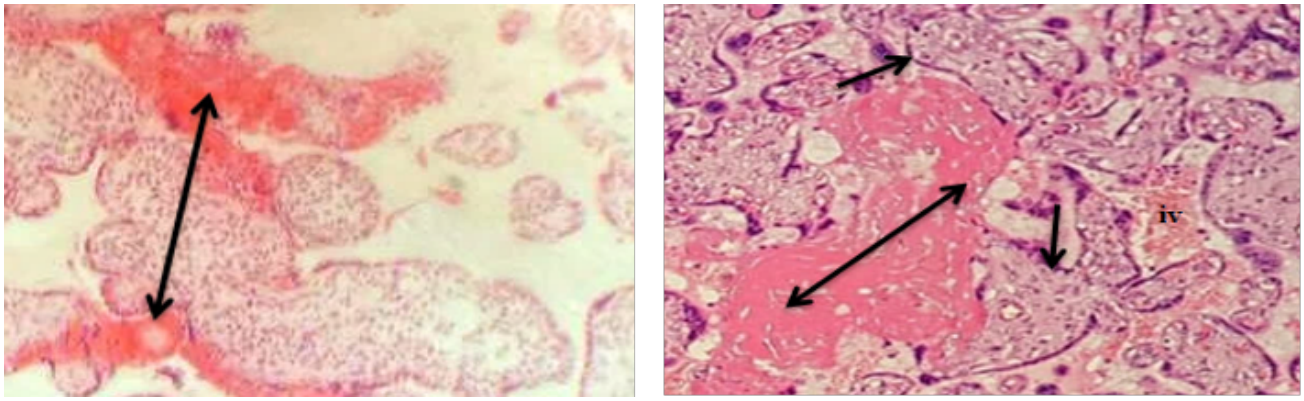

Figure 6. Photomicrograph of placental tissue showing terminal villi (arrows), intervillous space (iv), intervillous fibroid deposition (bihead arrow). H E, 400X.

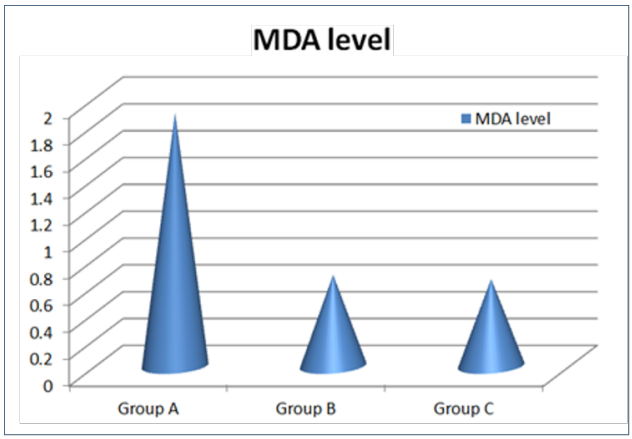

Figure 7. Histogram showing MDA levels of different age groups mothers.

DISCUSSION

Pathological changes in the placenta can affect the utero-placental circulation, resulting in a restriction of the growth of the fetus [17]. The placenta is exposed to the effect of cytokines, many hormones, and other factors present in the circulation of both the mother and fetus and can induce molecules that affect both of them independently [18]. The placenta undergoes structural changes throughout pregnancy, particularly in blood vessels, to accommodate the need for the embryo [19]. These physiological changes may be affected by some factors that may interrupt the growth of their cells and, accordingly, their function. Maternal age may affect placental function, this belief was based on previous studies that indicated a relationship between mother's age and the health of the fetus. Some researchers indicated that teenage pregnancy is associated with high risk of abortion, premature labor, small for date baby and congenital anomaly [20–22]. while others consider that advanced maternal age is a cause of abnormal fetal outcome [23, 24]. The present study showed that placental weight is significantly reduced in advanced age mothers and teenage mothers compared to control with more reduction in advanced age mothers and this result was concomitant with that obtained by Asgharnia etal who observed that placental weight in mothers less than 18 years was reduced compared to those with 25 years [25]; however, our result disagreed with that reported by other researchers who said that placental weight increased with advanced maternal age (over 35 years) [26, 27] this differences in the result may be attributed to the method of delivery and the placental weight procedure. The surface area of the advanced-age mother placentae decreased, while the thickness increased significantly compared to other groups. Elchalal et al. suggested that increased placental thickness may be related to macrosomia, small for date infant, and increased perinatal mortality [28].

In this work, microscopical examination reveals several histopathological changes, particularly among placentae of mothers aged over 35 years; these include increased thickening of the feto-maternal membrane, increased frequency of syncytial knots, and hypovascular chorionic villi that contain small, contracted blood vessels and thick stroma, these findings were consistent with other authors [29, 30]. Huppertz et al. considered the increase of the syncytial knot as the adoptive response of placenta [31]. While Sankar et al. correlate the thickening of the placental membrane with the reduction of placental circulation and the accretion of the syncytial knot [32]. Some authors considered the syncytial knot as a degenerative change and related its formation to the age process [33]. Napso et al. denoted that placental phenotybe, as a gene and protein expression for placental growth, had been modified in advanced maternal age and this may alter placental function [34]. Furthermore, the elevation of the oxidative stress biomarker observed in this study may be responsible for the pathological changes of placentae in mothers over 35 years age. Other workers observed the association of increased oxidative stress and apoptotic changes in the placenta with increased maternal age [35, 36], and another said that oxidative stress and increased lipid peroxidation are related to many pathological changes in the placenta [37].

The present study showed structural and morphological changes in the placenta of older mothers than in adolescents, indicating that abnormal outcomes of the fetus observed by other researchers in these two groups may be related to the nutrition and society of teenage women, while in advanced age they may be related to structural changes and increased oxidative stress, which may explain the method of perinatal care to improve the fetus' health.

CONCLUSION

Pregnancies, in advanced maternal age rather than youth, can negatively impact the structure of the placenta due to increased oxidative stress. To potentially help support function in this age group adding antioxidants could be beneficial.

ETHICAL DECLARATIONS

- **Acknowledgements**

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support during this work.

- **Ethics Approval and Consent to Participate**

  The study was carried out according to the ethical committee of the medical college of the University of Ninevah, informed consent was obtained from all mothers who enrolled in the study.

- **Consent for Publication**

  Non.

- **Availability of Data and Material**

  The datasets are available from the corresponding author upon reasonable request.

- **Competing Interests**

  The author declares that there is no conflict of interest.

- **Funding**

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- **Authors' Contributions**

  Eman Ghanim Sheet contributed significantly, directly, and intellectually to the work and consented it to be published.

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