Placenta Previa Changes Among Egyptian Women: A Morphological, Histological and Immunohistochemical Study

Original Article

Heshmat Sabet Haroun[1], Mohammed Hany Shehata[2], Motaz Talaat Elghnam[1], Hossam Yehia Sayed[1], Mohamed Zakaria Kotb[1] and Ahmed Hamed Bayoumi[1]

[1]Department of Anatomy and Embryology, [2]Department of Gynecology and Obstetrics, Faculty of Medicine, Cairo University, Egypt

ABSTRACT

Introduction: Placenta previa is abnormally located either very close to or covering the internal os of the uterine cervix and may interfer with normal fetal growth and development. The incidence of placenta previa during second trimester is 5%, but near the end of pregnancy more than 90% of these cases will not be placenta previa as observed by trans-vaginal ultrasonography. Recent evidence has suggested that disproportion between pro-angiogenic and anti-angiogenic factors may have a principal role in the pathogenesis of placenta previa.

Aim of the Work: The present study aimed at studying the morphological features, histological and immunohistochemical changes (vascular endothelial growth factor [VEGF] and connective tissue growth factor [CTGF]) of placenta previa compared to normal placenta.

Materials and Methods: Thirty placentas were brought from term pregnant women undergoing delivery, 15 healthy normally situated control placentas and 15 placenta previa. Gestational age, neonatal birth weight and delivery mode were obtained. Maternal, placental and fetal parameters were statistically assessed. Placentas were then subjected to morphological, histological and immunohistochemical studies.

Results: Placenta previa was associated with higher incidence of cesarean section and lower neonatal birth weight. Histological examination of placenta previa showed morphological changes indicative of increased syncytial knots, villous agglutination and infarcts, distal villous hypoplasia, and vascular mural hypertrophy of stem villi. Placenta previa revealed significant decrease in the expression of CTGF compared to control placentas. Assessment of histomorphometric data were carried out using "ImageJ 1.50i" program. The data were statistically analyzed using IBM SPSS advanced statistics version 26.

Conclusion: Angiogenic factors appeared to have no role in pathogenesis of placenta previa. Moreover, the risk caused by persistent placenta previa necessitates further studies of both umbilico-placental and utero-placental circulations.

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Key Words: CTGF, fetal growth, placenta previa, VEGF.

Corresponding Author: Ahmed Hamed Bayoumi, MD, Department of Anatomy and Embryology, Faculty of Medicine, Cairo University, Egypt, **Tel.**: +20 10 0913 1878, **E-mail:** ahmedhamed81@kasralainy.edu.eg

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INTRODUCTION

Placenta is the foreteller of the upcoming events in pregnancy. It also bears many secrets inside its microscopic structure that can help in revealing the etiology of many obstetric conditions. It also reflects the maternal and fetal conditions, thus placenta can be regarded as a diary of intrauterine environmental changes during pregnancy[1].

Chorionic villi have a tree-like architecture. Its stem villi resemble the major branches of the tree and their progressive division eventually results in the evolution of small, closely packed terminal villi[2,3].

Placental villi are classified into many types that are variable in size, percentage of existence in each stage of pregnancy, structure, location inside the villous tree, and function. These types are the "mesenchymal villi", the "immature intermediate villi", the "stem villi", the "mature intermediate villi" and the "terminal villi"[2].

Placenta previa is a condition in which placenta is abnormally located either close to or covering the internal os of the uterine cervix[1]. Implantation of the placenta in the less vascular lower uterine segment may interfere with normal placentation and fetal development. However, these possible effects are still controversial[4,5].

The incidence of placenta previa during second trimester is 5%, but near the end of pregnancy more than 90% of these cases will not be placenta previa as observed by trans-vaginal ultrasonography. In the third trimester of pregnancy, the incidence of placenta previa decreases to be 0.3-1%. Serial ultrasound examinations during pregnancy have shown that the placenta previa may migrate upwards to escape away from the poorly vascularized area[6,7].

The placental tissue does not move, and the term "placental migration" is a misnomer as the decidua is anchored to the chorionic villi. It is assumed that greater blood flow in the upper uterine segment facilitates

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placental growth and expansion toward the fundus while the less vascularity of the lower uterine segment near to the internal os leads to marginal atrophy, a process called trophotropism[1,7].

Complications of placenta previa include preterm deliveries, fetal or neonatal death, antepartum, intrapartum, and postpartum hemorrhages, sepsis, and hysterectomy[8,9].

The patho-etiology of placenta previa is not fully elucidated as the studies regarding the underlying mechanisms and placental histopathology are relatively rare[10]. However, the molecular regulations of placental angiogenesis and trophoblast cell infiltration have gained attention in the past few years. A recent study has stated that disproportion between pro-angiogenic and anti-angiogenic factors may play a principal role in evolution of placenta previa[11].

The present study aimed at studying the morphological features, histological and immunohistochemical changes (vascular endothelial growth factor [VEGF] and connective tissue growth factor [CTGF]) of placenta previa and at evaluating their effect on maternal and fetal perfusion compared to normally situated placenta.

MATERIALS AND METHODS

Thirty placentas were obtained from term pregnant women undergoing delivery in Obstetrics and Gynecology Department, Kasr Alainy Hospital, Faculty of Medicine, Cairo University, Egypt, after getting written consents from the patients. Fifteen term healthy placentas were used as control while the other fifteen placentas were obtained from pregnant women diagnosed by ultrasonography throughout the third trimester to have placenta previa without the association of any other medical or obstetrical disorders. In each case, the following data were obtained: gestational age, neonatal birth weight (NW) and mode of delivery.

Placentas were subjected to morphological, histological and immunohistochemical studies. Maternal, placental, and neonatal parameters were also statistically assessed.

Ethical declaration

The present study was approved by the Research Ethics Committee of the Faculty of Medicine, Cairo University (Code: MD-181-2020).

Morphological study

Once the placenta was delivered, a quick overall evaluation was done for the shape, site of cord insertion, and presence of thrombi or any other abnormality. The umbilical cord was cut 2 cm away from the placenta, any membrane remnants were cut and eliminated, and loose soft clot was removed from the maternal surface under running tap water. The placenta was then weighed an hour after placental delivery using baby weighing scale. The diameter and thickness at center of the placentas were determined using wooden toothpicks. Whenever the placenta was irregular in shape, the longest axis, and the length of the axis perpendicular to it were measured and their mean was considered as the diameter[12].

Histological study

A full thickness tissue sample from an area 1x1 cm2 near the center of each placenta was excised using scalpel, scissors and toothed forceps, fixed in 10% neutral buffered formalin, dehydrated using alcohols, cleared in xylene, and placed in paraffin wax blocks. Subsequently, sections of 5μm thickness were stained with Hematoxylin & Eosin (H&E) and with Masson's trichrome[13]. Pathological lesions were categorized into vascular/ villous lesions causing mal-perfusion of maternal origin, vascular/ villous lesions causing mal-perfusion of fetal origin, and inflammatory lesions[14,15].

Immunohistochemical study

Paraffin sections of the placenta were immunohistochemically stained for vascular endothelial growth factor (VEGF) to detect angiogenesis and connective tissue growth factor (CTGF) to detect fibrosis. The 5μm paraffin sections were heated in a 10 mm citrate buffer (pH 6.0) for dewaxing and antigen retrieval. After blockage of non-specific site binding and endogenous peroxidase activity with serum, incubation of the sections with monoclonal anti-VEGF antibody (VEGF-A) (555036, 1:50, BD PharMingen, Heidelberg, Germany) and polyclonal anti-CTGF antibody (ab5097, 1:400, Abcam) was done. The sections were then incubated with biotinylated secondary antibodies and avidin-biotin-peroxidase complex. The immunoreactivity was revealed using 3-amino-9-ethylcarbazole and diaminobenzidine tetrahydrochloride as brown chromogens[16,17]. The positive controls of VEGF and CTGF were human colon tissue and human hippocampal protein extracts respectively. Specificity of immune-expression were confirmed via negative controls where the first antibodies were omitted in the automated staining protocol.

All sections were examined under light microscope Optika B-150. Histological changes in placenta previa were recorded, described, and photographed by Amscope microscope digital camera MU1400 (14MP aptina color cmos with ultra-fine color engine).

The range and mean values of maternal age, placental weight, diameter, central thickness, and fetal weight, for both placenta previa and control groups, were recorded and statistically analyzed using IBM SPSS advanced statistics version 26.

Evaluation and assessment of Masson's trichrome, VEGF and CTGF (×400) sections were carried out using “ImageJ” computer image analysis software program version 1.50i to measure stained areas. Measurements were calculated in non-overlapping 15 microscopic fields inside standardized frame of measurement of 85,550 mm. Each of these microscopic field images were divided into RGB stacks and the red stack was used to measure

the area percentage of immune reactivity, then threshold adjustment was done to mask areas of immunoreactivity with red color. Finally, the percentage of these red masked areas was analyzed and calculated in relation to the total microscopic field area.

Student t-test was used to compare between the quantitative variables and Fisher's Exact test was used to compare between the qualitative variables. A *p-value* < 0.05 was considered significant. The Spearman correlation coefficient was used to measure correlation between the sets of variables.

RESULTS

Maternal and neonatal data

The maternal age of normally situated (control) placentas ranged from 19 to 32 years (mean of 26.73 ± 3.59 years) (Table 1). Parity (P) of pregnant women with control placentas ranged from nullipara (P0) to P4 (Table 2). In control group, four women (26.67%) gave a history of previous cesarian sections (Table 3). The number of previous maternal miscarriages was once in three women (20%) and twice in one woman (6.67%) in this control group (Table 4). Neonatal weight in this group ranged from 2900 g to 3700 g (mean of 3213 ± 264.2 g) (Tabble 1).

In placenta previa group, maternal age ranged from 23 to 34 years (mean of 29.93 ± 3.28 years) (Table 1). Parity ranged from P1 to P4 (Tab. 2). In this group, 13 women (86.67%) had a history of previous cesarian sections (Table 3). The number of previous maternal miscarriages in placenta previa group ranged from once in four women (26.67%) to twice in three women (20%) (Table 4). Neonatal weight (NW) in this group oscillated between 2400 g and 3400 g (mean of 2967 ± 264 g) (Table 1).

Morphological results

In the control group, the placental weight ranged from 400 g to 550 g (mean of 490 ± 47.06 g). The diameter of the control placentas oscillated between 17.5 cm and 21 cm (mean of 19.03 ± 1.17 cm). Central thickness of the control placentas ranged from 1.6 cm to 2.6 cm with a mean of 1.97 ± 0.25 cm (Tab. 5). In all control placentas, the umbilical cord was central in attachment (Figuer 1a).

In placenta previa group, placental weight (PW) varied from 400 g to 550 g (mean of 470 ± 59.16 g). The diameter of the placenta previa varied from 17 cm to 20 cm (mean of 18.47 ± 1.26 cm), the central thickness from 1.5 cm to 2.6 cm (mean of 1.95 ± 0.32 cm) (Table 5). The site of insertion of the umbilical cord into placenta previa was central in nine placentas (60%) and marginal in six placentas (40%) (Figuer 1b). Comparing the two groups of placentas, the incidence of marginal insertion of the umbilical cord was significantly higher in placenta previa group (*p-value*= 0.017) (Table 6).

In the placenta previa, retroplacental blood hemorrhage was observed in two placenta previa specimens (13.33%) (Figuer 1c, Table 7).

Histological results

Light microscopic examination of the control placental specimens stained with H&E showed that the amniotic membrane was composed of amniotic epithelium and amniotic connective tissue (CT); being separated from the chorionic plate by a spongy layer. The chorionic plate was formed of chorionic CT and extra-villous cytotrophoblasts entangled in a fibrinoid material (Langhans fibrinoid) underneath it (Figuer 2a). Deep to the chorionic plate, various types of chorionic villi were identified with dominance of the terminal villi that exhibited high degree of capillarization and average intervillous spaces. In addition, the syncytial knots were observed in less than 30% of the existing terminal villi, appearing as basophilic masses bulging from the surface of the terminal villi (Figuers 2b, 2c).

The basal plate near the maternal surface of control placentas usually lost its typical layering that was kept in some placentas. This typical layering of placenta, from fetal to maternal side, was formed of: Rohr`s fibrinoid, extra-villous cytotrophoblast within Nitabuch`s fibrinoid, and decidual cells (Figuer 2d).

The current study reported increased number of syncytial knots projecting from the terminal villi in 10 placenta previa specimens (66.67%) (Figuer 3a, Table7). Four placenta previa specimens (26.67%) revealed aggregated RBCs (thrombi) of the chorionic plate / stem villous (Figuer 3b, Table 7).

Massive deposition of peri-villous fibrin, villous agglutination, and collapsed intervillous spaces were observed either alone or associated with villous infarction. Villous infarction occurred in the form of loss of the basophilic nuclear appearance of the syncytium, pyknosis, and karyorrhexis giving the look of "ghost villi". Increased intervillous fibrin was detected in 12 specimens of placenta previa (80%) of which nine specimens (60%) revealed changes related to villous infarction (Figuer 3c, Table 7).

In 10 placenta previa specimens (66.67%), distal villous hypoplasia was detected in the form of prevalence of small caliber villi, occasional filiform villi, and wide intervillous space (Figuer 3d, Table 7). Other four placenta previa specimens (26.67%) showed vascular mural hypertrophy of the stem villi with ultimate obliteration of the vascular lumen (Figuer 3e, Table 7).

Apart from vascular mural hypertrophy of stem villi, the number of variable pathological lesions observed in sections of placenta previa was counted as 0-6 findings. There was a significant negative correlation between the total number of placental lesions in each specimen (after exclusion of vascular mural hypertrophy) and the NW in placenta previa group (Correlation Coefficient = -0.635 with a *p- value* = 0.011).

Four placenta previa specimens (26.67%) revealed combined lesions related to maternal and fetal mal-perfusion, 10 placenta previa specimens (66.67%)

exhibited lesions related to maternal mal-perfusion of which two specimens (13.33%) showed combined vascular and villous lesions and eight specimens (53.33 %) revealed villous lesions only. One placenta previa specimen (6.67%), in this work, was free from lesions (Table 8).

Masson's trichrome stained sections were used to assess the content of collagen fibers, that were stained blue, and their mean area percentage. Histological sections of control placentas showed collagen fibers distribution inside the villi (Figuer 4a) while the collagen fibers in placenta previa were not augmented (Figuer 4b). The data obtained from the image analysis software program (Image J, version 1.48) were statistically analyzed, revealing that there was non-significant difference between the two groups (*p-value* =0.219) where the mean (±SD) area percentage of collagen fiber content in normally situated placenta was 21.13 ± 2.34 while that of placenta previa was 19.05 ± 3.01.

Immunohistochemical results

VEGF and CTGF stained sections of placenta were used to assess angiogenesis and fibrosis respectively.

VEGF was minimally expressed in both placenta previa and control placentas and there was was non-significant difference in area percentage expressing VEGF between two groups (*p-value* =0.490) where the mean (±SD) area expressing VEGF in normally situated placenta was 0.31 ± 0.04, while that of placenta previa was 0.27 ± 0.06 (Figuers 5a, 5b).

CTGF was faintly expressed in both placenta previa and control placentas and there was a statistically significant difference in area percentage expressing CTGF between two groups (*p-value* = 0.05) where the mean (±SD) area expressing VEGF in normally situated placenta was 14.15 ± 1.53 while that of placenta previa was 6.99 ± 0.83 (Figuers 6a, 6b).

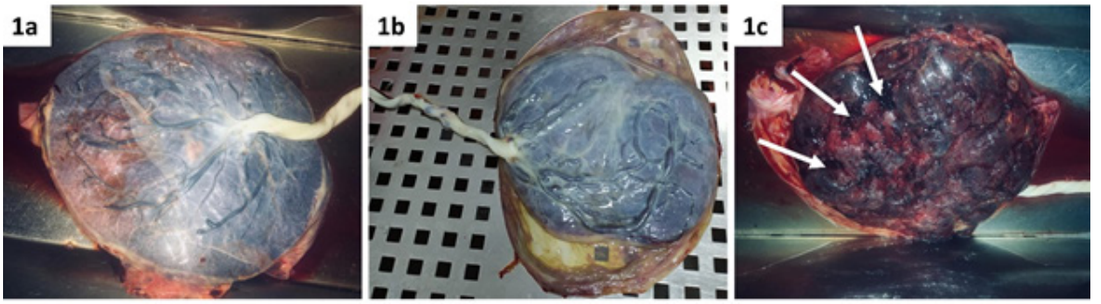

Fig. 1a: A photograph of fetal surface of a discoid term control placenta showing centric insertion of the umbilical cord. **Fig. 1b:** A photograph of fetal surface of a discoid term placenta previa showing marginal insertion of the umbilical cord. **Fig. 1c:** A photograph of maternal surface of a term placenta previa showing retroplacental blood clot (arrows).

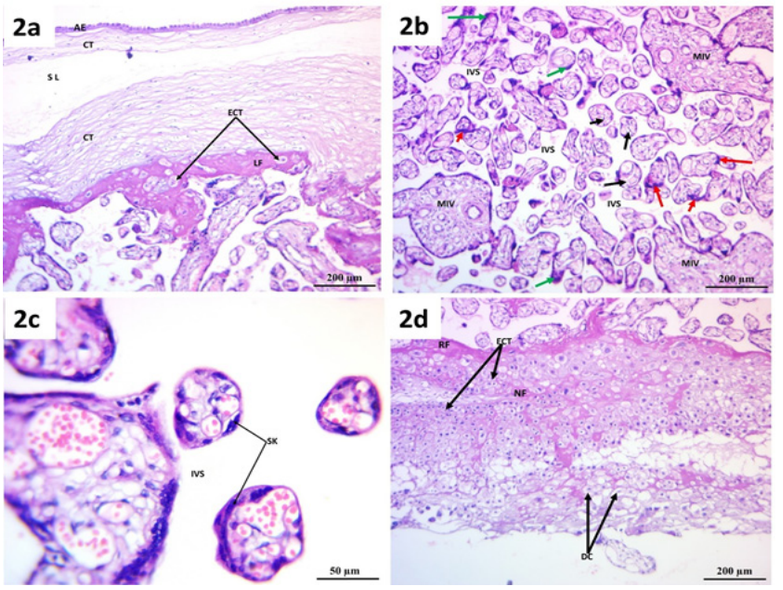

Fig. 2a: A photomicrograph of a section of term placenta of the control group showing normal amnion formed of amniotic epithelium (AE) resting on connective tissue (CT) with an underlying spongy layer (SL) separating the chorionic plate from the amnion. The chorionic plate is formed of connective tissue (CT) and extravillous cytotrophoblasts (ECT) within Langhans fibrinoid (LF). (H&E X 100) **Fig. 2b:** A photomicrograph of a section of term placenta of the control group showing terminal villi (black arrows) and intervillous space (IVS) inbetween. Syncytial knots are seen bulging from the terminal villi (green arrows). Syncytial bridges (red arrows) appeared connecting adjacent villi. Mature intermediate villi (MIV) are also seen at the periphery of the field. (H&E X 100) **Fig. 2c:** A photomicrograph of a section of term placenta of the control group showing terminal villi and intervillous space (IVS) inbetween. Syncytial knots (SK) are seen bulging from the terminal villi. (H&E X 400) **Fig. 2d:** A photomicrograph of a section of term placenta of the control group showing the basal plate formed of Rohr`s fibrinoid (RF), extravillous cytotrophoblasts (EVT), Nitabuch`s fibrinoid (NF), and decidual cells (DC). (H&E X 100)

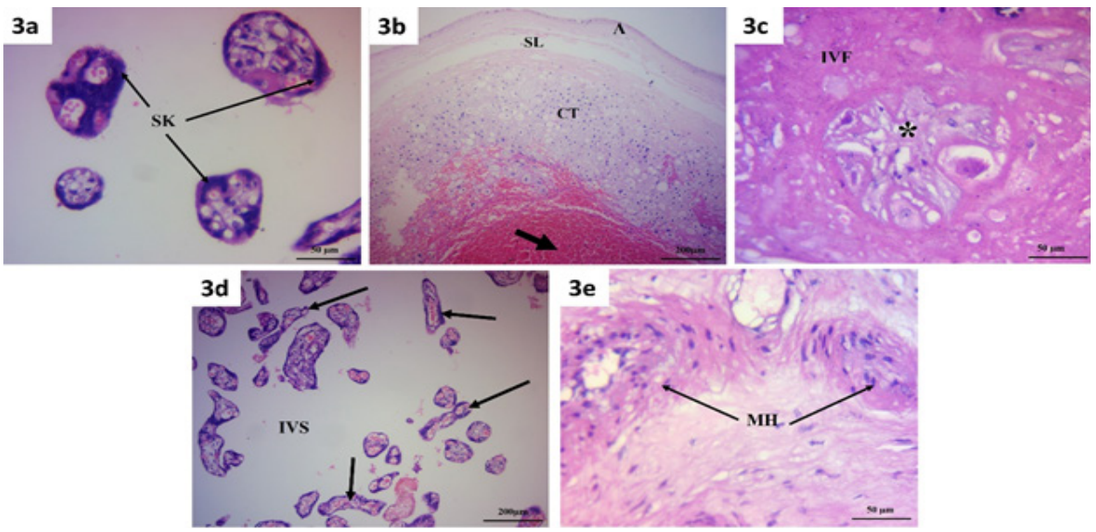

Fig. 3a: A photomicrograph of a section of term placenta previa showing increased syncytial knots (SK). (H&E X 400) **Fig. 3b:** A photomicrograph of a section of term placenta previa showing chorionic plate aggregated RBCS (arrow). (H&E X 100) A: amniotic membrane - SL: spongy layer - CT: connective tissue. **Fig. 3c:** A photomicrograph of a section of term placenta previa showing villous infarction manifested with massive intervillous fibrin deposition (IVF) and ghost chorionic villous (asterisk). (H&E X 400) **Fig. 3d:** A photomicrograph of a section of term placenta previa showing distal villous hypoplasia in the form of few filiform villi (arrows), small caliber villi, and wide intervillous space (IVS). (H&E X 100) **Fig. 3e:** A photomicrograph of a section of term placenta previa showing vascular mural hypertrophy (MH) with narrowing and obliteration of the vascular lumina. (H&E. X 400)

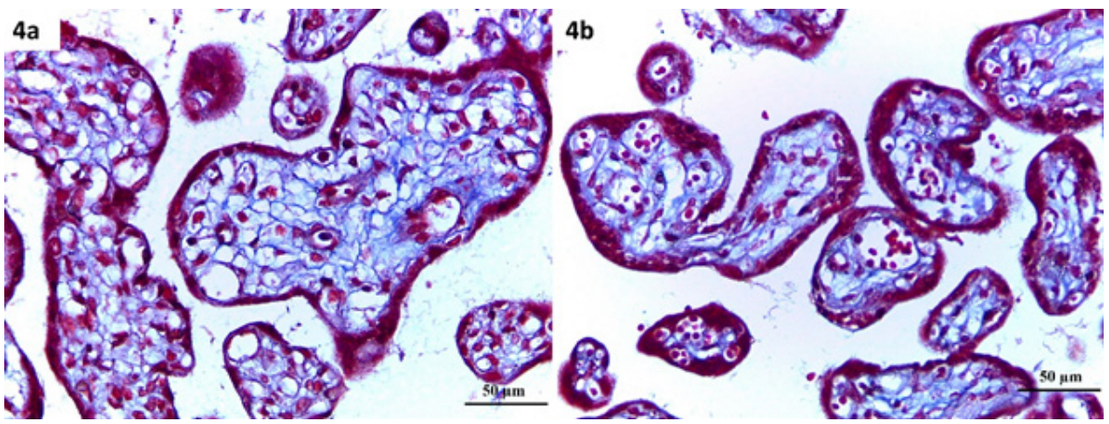

Fig. 4a: A photomicrograph of a section of control placenta showing collagen fibers distribution (blue color) (Masson's trichrome X 400)**Fig. 4b:** A photomicrograph of a section of term placenta previa showing no augmentation in collagen fibers distribution inside placental villi (blue color). (Masson's trichrome X 400)

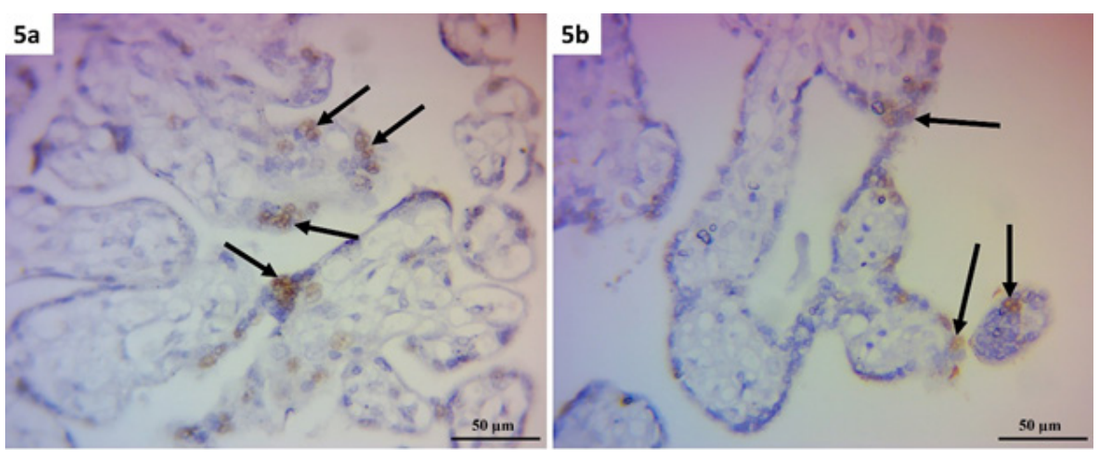

Fig. 5a: A photomicrograph of a section of control placenta showing mild VEGF expression (arrows). (VEGF X 400) **Fig. 5b:** A photomicrograph of a section of placenta previa showing mild VEGF expression (arrows). (VEGF X 400)

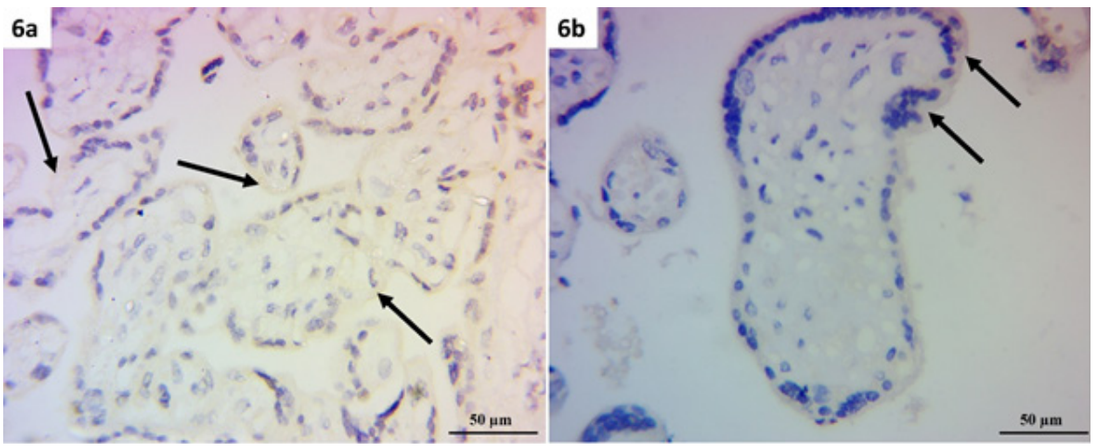

Fig. 6a: A photomicrograph of a section of control placenta showing mild CTGF expression (arrows). (CTGF X 400) **Fig. 6b:** A photomicrograph of a section of placenta previa showing mild CTGF expression (arrows). (CTGF X 400)

Table 1: Maternal age and neonatal weight of both control and placenta previa groups.-

Parameter	Control		Placenta previa		*P-value*
	Range	Mean (±SD)	Range	Mean (±SD)	
Maternal age	19-32 years	26.73 ± 3.59 years	23-34 years	29.93 ± 3.28 years	0.017*
Neonatal weight	2900-3700 g	3213 ± 264.2 g	2400-3400 g	2967 ± 264 g	0.016*

* Means significant

Table 2: Parity in both control and placenta previa groups.

Parity	Control no. (%)	Placenta previa no. (%)
0	5 (33.33%)	Zero (0%)
1	3 (20%)	2(13.33%)
2	4 (26.67%)	10 (66.67%)
3	2 (13.33%)	1 (6.67%)
4	1 (6.67%)	2 (13.33%)

Table 3: Number of cesarean sections in both control and placenta previa groups.

Cesarean sections	Control no. (%)	Placenta previa no. (%)
0	11 (73.33%)	2 (13.33%)
1	3 (20%)	2 (13.33%)
2	1 (6.67%)	8 (53.33%)
3	Zero (0%)	1 (6.67%)
4	Zero (0%)	2 (13.33%)

Table 4: Number of miscarriages in both control and placenta previa groups.

Miscarriages	Control no. (%)	Placenta previa no. (%)
0	11 (73.33%)	8 (53.33%)
1	3 (20%)	4 (26.67%)
2	1 (6.67%)	3 (20%)

Table 5: Placental parameters of both control and placenta previa groups.

Parameter	Control		Placenta previa		*P-value*
	Range	Mean (±SD)	Range	Mean (±SD)	
Placental weight	400-550 g	490 ± 47.06 g	400-550 g	470 ± 59.16 g	0.315
Placental diameter	17.5-21 cm	19.03 ± 1.17 cm	17-20 cm	18.47 ± 1.26 cm	0.213
Central placental thickness	1.6-2.6 cm	1.97 ± 0.25 cm	1.5-2.6 cm	1.95 ± 0.32 cm	0.85

* Means significant

Table 6: Incidence of central and marginal cord insertions.

Site of cord insertion	Control Placentas	Placenta previa	*P-value*
Central cord insertion	100%	60%	0.017*
Marginal cord insertion	Zero%	40%	

* Means significant

Table 7: Incidence of morphological and histopathological findings in placenta previa.

Pathological findings	Number of specimens	Percentage
Retroplacental heamorrhage	2	13.33%
Increased syncytial knots	10	66.67%
Chorionic plate/stem villous thrombi	4	26.67%
Increased intervillous fibrin	12	80%
Villous infarction	9	60%
Distal villous hypoplasia	10	66.67%
Vascular mural hypertrophy	4	26.67%

Table 8: Categories of histopathological lesions detected in placenta previa and their incidence.

Category of histopathological lesion	Number of cases	Percentage
Combined maternal and fetal mal-perfusion lesions	4	26.67 %
Combined vascular and villous lesions related to maternal mal-perfusion	2	13.33 %
Villous lesions related to maternal mal-perfusion	8	53.33 %
No pathological lesions detected	1	6.67 %

DISCUSSION

The persistence of placenta previa in its primary site of implantation and its potential effect on the fetus has remained a field for research for many decades. The detailed description of its morphology and histological structures and the knowledge of their difference from those of the normally situated placenta have a crucial role to clarify its effect on the fetus[12].

The current study showed that the mean maternal age of placenta previa group was significantly older than that of the control group by more than three years and this is consistent with the results reported by King *et al*.,[18] and Sheibak *et al*.,[19] who reported maternal age difference of one year or more between the two groups. On the other side, Xie *et al*.,[11] and Han *et al*.,[20] reported a mean maternal age difference of few months only.

Our research recorded a higher parity among placenta previa cases, and this result is in line with that reported by Jung *et al*.,[10] who reported more incidence of placenta previa in parous women without mentioning number of parity. Xie and colleagues[11] also stated that placenta previa group has a higher incidence of miscarriage than control group and this is compatible with the results of this work. This is possibly because higher parity and higher incidence of miscarriage are usually associated with increased maternal age.

The current study also revealed that placenta previa group has a higher incidence of cesarean section than that of the control group and this is in harmony with the outcomes announced by Kawashima *et al*.,[21] Xie *et al*.,[11] Jung *et al*.,[10] and King *et al*.[18]. This can be clarified by the concept that cesarean section is one of the most important risk factors of placenta previa[1]. Also, it can be interpreted by our findings that the placenta previa group has older maternal age than the control group, and that the older the maternal age, the higher the possibility to perform cesarean section.

The present study revealed that the weight and diameter of placenta previa were less than those of the control placentas and this runs in harmony with the results of Weiner *et al*.,[12] and Han *et al*.[20] who mentioned that the mean weight of placenta previa is lighter than that of control placentas. This could be attributed to the low vascularity of the lower uterine segment that leads to abnormal placentation and increased intervillous spaces accompanying distal villous hypoplasia.

On the contrary, Heidari *et al*.,[22] Jung *et al*.,[10] and Sheibak *et al*.[19] claimed that placenta previa was heavier than normal placenta. They believed that the cause was the theory of trophotropism (placental migration) where the upper end of the placenta grows upwards, on the other hand they ignored the rest of the migration theory that states, "the less vascularity of the lower uterine segment near the internal os leads to marginal atrophy of the lower end of the placenta" and this makes their explanation a doubtful one.

On comparing the central thickness of the placenta previa and control groups, there was no significant difference. This observation confirms the results reported by Jung and co-workers[10].

This study has clarified that the placenta previa has a statistically significant higher incidence of marginal cord insertion than the control placentas. This outcome is in agreement with the results reported by Weiner *et al*.,[12] and Jung *et al*.[10]. This could be explained according to the "placental migration theory" due to passive shifting of the site of the cord insertion.

The current study reports a statistically significant lower neonatal birth weight (NW) in the placenta previa group as compared to normally situated placenta group. This is consistent with the reports of Ananth *et al*.,[23] Rosenberg *et al*.,[24] Kawashima *et al*.,[21] Raisanen *et al*.,[5] Kirbas *et al*.,[25] Weiner *et al*.,[12] Xie *et al*.,[11] and Han *et al*.[20]. However, it is important to admit that some of the previously mentioned studies included preterm delivered neonates. The design of this work overcame the obstacle of preterm deliveries. Burton and Jauniaux[26] declared that placental and fetal growth are linked to each other. These authors also stated that fetal growth is dependent on uteroplacental blood flow, fetoplacental blood flow and integrity of villous trophoblast development; and this was compatible with the data announced from this study. On the contrary, Harper *et al*.,[27] Yeniel *et al*.,[28] Kassem and Alzahrani[4], and Sheibak *et al*.,[19] stated that there is no correlation between placenta previa and neonatal weight.

The current study also demonstrated that the villous lesions affecting utero-placental blood flow causing maternal mal-perfusion were the most detectable lesions followed by vascular changes affecting feto-placental blood flow causing fetal mal-perfusion then vascular lesions linked to the maternal mal-perfusion. Inflammatory lesions and villous lesions linked to the fetal mal-perfusion were not detected in this work. Absence of placental beds in some placenta previa specimens was an obstacle toward identification of placental vascular changes of maternal origin in these beds causing a possible underestimation of vascular lesions linked to maternal mal-perfusion. The current findings regarding placental lesions are in agreement to those of Jung *et al*.,[10] who reported an increased incidence of maternal mal-perfusion lesions, especially increased intervillous fibrin and villous infarction, in placenta previa specimens. Also, Weiner *et al*.,[12] described high frequency of maternal mal-perfusion lesions and significantly increased fetal mal-perfusion lesions in placenta previa specimens. However, it is worth noting that the inclusion of placenta previa specimens of women with pre-eclampisa, gestational diabetes, and thrombophilia might affect the validity of results reported by Weiner *et al*.[12].

In the current work, the placenta previa specimens revealed the absence of any inflammatory lesions. This could be accepted on the basis that placenta previa is the result of abnormal placentation and abnormal site of implantation without any inflammatory cause. Therefore severe inflammatory lesion in placenta previa could be considered as an outcome of a superimposed condition.

The pathological findings of vascular mural hypertrophy in stem villi of placenta previa specimens, observed in the present study, is not mentioned by the society for pediatric pathology[14] nor by Amsterdam placental workshop group criteria[15]. Benirschke *et al*.,[2] mentioned that the vessels of the stem villi may occasionally show mural hypertrophy in cases of old thrombus. Burton and Jauniaux[26] correlated between changes in smooth muscles of stem villi vessels with absent or reversed end-diastolic blood flow in umbilical artery.

This study showed no significant difference in collagen content in villi of placenta previa compared to control placenta and although non of the previous studies commented on the collagen content in placenta previa, this finding can be explained on the basis of the nature of placenta previa as a non-inflammatory condition[1] and this was confirmed by the absence of any inflammatory lesions in the placenta previa specimens in this work.

There are conflicting reports regarding the role of VEGF in cases of placenta previa. The current study has revealed weak VEGF expression in both placenta previa and control groups. Wehrum *et al*.,[29,30] reported that placenta previa centralis cases are not associated with difference in systemic maternal levels of VEGF when compared to controls. Moreover, Wang *et al*.,[31] stated that there is a significant decrease in serum level of VEGF in placenta previa cases. On the contrary, Xie *et al*.,[11] mentioned that VEGF staining had increased in vascular endothelial cells and trophoblasts of placenta previa specimens. They claimed that VEGF was one of the factors responsible for pathogenesis of placenta previa[11].

Wehrum *et al*.,[30] hypothesized that it is possible that hypoxia stimulates invasion at the site of placenta previa

in the early stages of villous development until the fetal oxygen needs are fulfilled then, a decrease in expression of VEGF occurs to prevent myometrial invasion. One of the present results that support the weak VEGF expression is the presence of terminal villous hypoplasia in placenta previa specimens that means a defect in terminal villi formation. This is due to defective elongation of capillary loops inside the villi, which might reflect a decrease in the levels of VEGF.

CTGF modulates many pathways and its expression corresponds to angiogenesis and fibrosis[32,33]. We couldn't find any previous studies on CTGF expression in placenta previa. Yet, the milder CTGF expression of placenta previa samples compared to that of control group in this study confirms the other results of this work; that connective tissue, VEGF and angiogenesis are not increased in placenta previa specimens.

CONCLUSION

Angiogenic factors appeared to have no role in pathogenesis of placenta previa. The risk caused by persistent placenta previa necessitates further studies of both the umbilico-placental and utero-placental circulations. Modern imaging techniques like magnetic resonance imaging and three-dimensional Doppler could also help to follow the development of the placental and fetal circulation. Moreover, these techniques help in early detection of maternal or fetal mal-perfusion.

CONFLICT OF INTERESTS

There are no conflicts of interest.

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الملخص العربى

المشيمة المتقدمة في السيدات المصريات: التغيرات المورفولوجية و الهستولوجية والهستوكيميائية المناعية

حشمت ثابت واصف هارون[١]، محمد هاني شحاتة[٢]، معتز طلعت الغنام[١]، حسام يحيى سيد[١]، محمد زكريا قطب[١]، أحمد حامد بيومي[١]

[١]قسم التشريح وعلم الأجنة، [٢]قسم أمراض النساء والتوليد، كلية الطب البشري، جامعة القاهرة

مقدمه: يشير مصطلح "المشيمة المتقدمة" إلى وجود المشيمة في مكان غير مناسب إما قريبة جدًا من أو على الجزء الداخلي من عنق الرحم. قد يتداخل نمو المشيمة في الجزء السفلي من الرحم ذي التغذية الدموية الضعيفة مع النمو الطبيعي للمشيمة والجنين.

هدف البحث: صُممت الدراسة الحالية لتحديد التغيرات المورفولوجية والنسيجية والهيستوكيميائية المناعية التي يمكن أن تحدث في المشيمة المتقدمة عند مقارنتها بالمشيمة الموجودة في مكانها الطبيعي.

مواد و أساليب العلاج: أجريت هذه الدراسة على ٣٠ مشيمة تم إحضارها من نساء حوامل خاضعات للولادة في قسم أمراض النساء والتوليد بمستشفى قصر العيني. تم استخدام خمسة عشر مشيمة صحيحة المكان كعنصر تحكم بينما تم إحضار الخمسة عشر مشيمة الأخرى من نساء حوامل تم تشخيصهن بإصابتهن بالمشيمة المتقدمة دون أي اضطرابات أخرى.

النتائج: أظهرت نتائج الدراسة الحالية ما يلي:

- ازدادت نسبة حدوث المشيمة المتقدمة مع التقدم في سن الأم والولادات المتكررة ومع التاريخ المرضي لولادات قيصرية سابقة.
- لم يكن وزن وقطر المشيمة المتقدمة أصغر بكثير من تلك الموجودة في المشيمة المتواجدة في مكانها الطبيعي.
- كان وزن الأطفال حديثي الولادة في حالات المشيمة المتقدمة أقل بكثير من وزن الأطفال في حالات المشيمة المتواجدة في مكانها الطبيعي؛ في حالات الحمل الغير مصابة بأمراض أخرى.
- تواتر التغيرات النسيجية التي تم تحديدها في المشيمة المتقدمة ، في هذا العمل ، كان بالترتيب التالي من الأكثر تكراراً إلى الأقل: التغيرات الزغبية المتعلقة بسوء ضخ دم الأم ، الإصابات الوعائية المتعلقة بسوء تدفق الدم الجنيني والإصابات الوعائية المتعلقة بسوء ضخ دم الأم.
- بشكل غير متوقع، لم يكن هناك دور لعامل نمو بطانة الأوعية الدموية في ثبات المشيمة المتقدمة في موقعها أثناء الحمل.

الاستنتاج: يجب مراعاة هذه التغيرات في المشيمة المتقدمة وربطها بتقييد نمو الجنين داخل الرحم أثناء متابعة حالات المشيمة المتقدمة ، باستخدام تقنيات التصوير الحديثة. علاوة على ذلك ، يجب إجراء الدراسات التي تتضمن التعبير عن بروتينات موت الخلايا المبرمج ومضاداتها لتوضيح آلية هجرة وثبات المشيمة في حالات المشيمة المتقدمة.