

Placental Alterations of Gravidas with Vitamin D Deficiency: A Light and Electron Microscopic Study

Original
Article

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ABSTRACT

Introduction: Recently vitamin D showed greater importance in reproduction. Vitamin D affects cellular proliferation and differentiation, angiogenesis, fetal bone formation and immune modulation. There is increased number of pregnant women with vitamin D deficiency worldwide. Vitamin D deficiency (VDD) has been related to pregnancy complications such as preeclampsia, gestational diabetes mellitus and preterm birth.

Objectives: To evaluate histological changes in placentas of vitamin D deficient mothers.

Material and Methods: Twenty placentas of term live births were divided into two groups; normal vitamin D level (control) and VDD (n = 10 in each group). The morphology of placental villi of control group and VDD group were subjected to light microscopic (LM) examination using H&E, Masson's trichrome and PAS stains, and transmission electron microscopic (TEM) examination. Quantitative parameters of the placenta in the selected sections were estimated and statistically analyzed.

Results: LM examination of placenta of VDD group showed collagen fibers deposition in the villous core, stromal edema, increased syncytial knots, irregularities and thickening of basement membrane of fetal capillaries, and deposition of fibrin around the villi. On TEM, syncytiotrophoblast of VDD group exhibited fragmented heterochromatic nuclei, dilated profiles of rough endoplasmic reticulum, disrupted mitochondria and disorganized and separated microvilli. Cytotrophoblasts of VDD group showed irregular nuclear outline among numerous intracytoplasmic vacuoles. Concerning the histopathological grading, the VDD group showed a higher score in trophoblast degenerative knots compared to control. Edema and collagen fibers deposition were present in VDD group in 70% and 60% of the examined sections respectively.

Conclusion: Maternal VDD is likely to be responsible for placental barrier impairment that disrupts transplacental transport and exchange which could be associated with some pregnancy complications and might affect development of the fetus and even future life.

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Key Words: Maternal vitamin D deficiency, placental changes, ultrastructure.

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INTRODUCTION

The placenta is a specialized organ that supports fetal growth and development. Fetal development requires a completely functional placenta, whereas impaired placental function disrupts fetal development and may result in long-term health complications^[1]. During human placental development at 100 days of gestation, the placental structure becomes more complex, forming projections that increase the maternal-fetal contact surface, reaching maximum development at 150 days of gestation with considerable deposition of connective tissue^[2]. Homogenous distribution of vitamin D and vitamin D receptor (VDR) expression in syncytiotrophoblasts, cytotrophoblasts and chorion villus stroma demonstrate the importance of vitamin D in the progression pregnancy^[3].

Epidemiologically, between 18% and 84% of pregnant women worldwide suffer from vitamin D deficiency^[4]. Recent years have witnessed a rising interest in vitamin D importance for reproduction. Vitamin D metabolism during pregnancy is characterized by a physiological increase of vitamin D active form [calcitriol 1,25 (OH)₂D] and

calcidiol subgroup [25(OH)D] in the maternal blood aimed to reaching its optimal level in the fetus^[5]. Circulating vitamin D is converted to 25-hydroxyvitamin D (25(OH)D) via the enzymatic activity of 1 α -hydroxylase^[6]. Although conversion of vitamin D typically occurs in the kidney, the placenta also exhibits abundant expression and activity of 1 α -hydroxylase, which likely indicates a functional importance for placental vitamin D metabolism during gestation. Also, calcidiol freely crosses the placental barrier and appears to be the basic pool of vitamin D in the fetus^[7].

Vitamin D plays a key developmental events including cellular proliferation and differentiation^[8], angiogenesis and vascular function^[9], fetal bone formation and immune modulation^[10]. Accordingly, maternal vitamin D deficiency has been linked to pregnancy complications such as preeclampsia^[11], gestational diabetes mellitus^[12] and preterm birth^[13]. Moreover, gestational vitamin D deficiency is associated with intrauterine growth restriction (IUGR) in infants^[14] and adverse postnatal health outcomes in offspring, including increased rates of asthma, and impaired neurodevelopment^[15].

Although, there is continual worldwide effort for early diagnosis and new regimen of treatment of VDD, there is no established and definite studies remedy for VDD during pregnancy. Therefore, the present study aimed to evaluate the histological changes of placental villi in pregnancy complicated by VDD.

MATERIAL AND METHODS

Case selection

Full term pregnant women at the Obstetrics & Gynecology Department, El-Demerdash Maternity and Child Hospital (Ain Shams University Teaching Hospital), Egypt, from January 2020 till February 2021 were evaluated for eligibility for this study. Patients in this study should apply these criteria: (a) pregnant women with gestational age 38- 40 weeks ; (b) pregnant women with singleton gestation; (c) participant age between 24 and 40 years old ; (d) At delivery blood samples were taken from mothers for assays of 25(OH)D; (e) vitamin D deficiency was defined as a 25(OH)D level below 12 ng/mL, (study group, n = 10) and sufficient vitamin D was defined as 25(OH)D level above 25 ng/mL (control group, n = 10). The exclusion criteria were preterm birth or intrauterine growth restriction (IUGR). Pregnant women with chronic disease (diabetes or hypertension), autoimmune disorders or cardiac dysfunctions, which can influence placental circulation, were also excluded from the present study. Before starting the study, all sharing women signed an informed consent knowing all what will be done with their placentas and blood samples

Tissue collection

The placental tissue was processed for light and transmission electron microscopic (TEM) study. Some specimens were fixed in 10% neutral formalin and processed

for paraffin blocks. Paraffin sections (4-5- μ m thick) were stained with Haematoxylin and Eosin (Hx. &E), Masson's trichrome and periodic acid Schiff (PAS)^[16]. Periodic acid-schiff (PAS) and Masson's trichrome staining were performed to evaluate the glycogen and collagen fibers deposition within the chorionic villi respectively. The sections were examined with an Olympus light microscope in Anatomy department and were photographed.

Other specimens from the placenta were cut into 1m 3 pieces and dipped in 2.5% glutaraldehyde immediately and kept in the refrigerator at 4°C for 2 hrs. After wash with phosphate buffer, they were post-fixed in 1% buffered osmium tetra-oxide. The specimens were dehydrated in ascending grades of ethyl alcohol. Specimens were cleared in propylene oxide followed by propylene oxide and epoxy resin for at least one hour. Finally, the specimens were embedded in gelatin capsules filled with fresh epon. Semithin sections were cut (1 μ m in thickness) using an ultra-microtome and stained with 1 % toluidine blue. Ultrathin sections were obtained and stained with uranyl acetate followed by lead citrate^[17]. The ultrathin sections were examined with JEOL JEM 1010 electron microscope (Jeol Ltd, Tokyo, Japan) in the Electron Microscope Research Laboratory of Faculty of Medicine, El Azhar University, Egypt.

Histopathological grading

The histopathological grading was done by using the image analyzer in image analysis unit, Faculty of Medicine, Ain Shams University. Each examined placental section received a score from 0 to 3, according to criteria outlined by Günyeli *et al.*,^[18] for the following indicators: inflammatory infiltration (Chorionic villitis), trophoblast degenerative knots, presence of villous edema and collagen fibers deposition as shown in (Table 1).

Table 1: Histopathological grading of the placental sections

	0	1	2	3
Chorionic villitis	None or 1-3 cells in < 3 fields	1- 3 cells per field or in > 3 fields	4-6 cells per field or in > 4 fields	>6 cells in at least 4 fields
Trophoblast degenerative knots	None or 1-5 cells in< 3 fields	≤ 5 cells per field in at least 3 fields	5-10 cells per field in at least 4 fields	>10 cells in at least 4 fields
villous edema	None	Present in 10% of fields in at least 3 fields	10-30% of fields in at least 3 fields	> 30% of fields in at least 3 fields
Collagen fibers deposition	None	Present in 10% of fields in at least 3 fields	Preset in 10-30% of fields in at least 3 fields	Present in > 30% of fields in at least 3 fields

Villous edema and collagen fibers deposition were estimated through measuring area percentage of the examined fields using Leica Q win V3 image analysis software (Leica microsystems, Germany) at image analysis unit. In Masson's trichrome sections, the stained areas were measured in high power fields (x400) from 20 sections of each group.

Placental barrier assessment and statistical analysis

With respect to the ultrastructural findings of placental barrier terminal chorionic villi were examined (height and number of syncytiotrophoblast microvilli and thickness of fetal capillaries basement membrane). Five fields of vision from each ultrathin sections were selected randomly and investigated at x10,000 magnification. The microscopic

analyses were performed by two operators blindly. The results were expressed as mean \pm SD from each group. The data were subjected to analysis of variance using T-test. Results were considered statistically significant when P value \leq 0.05 and highly significant when P value \leq 0.001^[19].

RESULTS

Light microscopic results

Hematoxylin and eosin-stained sections

The human control placenta sections comprised numerous densely packed terminal chorionic villi (Figure 1a). Each chorionic villous contained a core of fetal mesenchymal cells, fetal capillaries and was covered by two layers of trophoblast (Figure 1b). Syncytiotrophoblast (Sy) was composed of syncytium of cells (one mass of multinucleated cytoplasm that lacked definite cell boundaries). The cytotrophoblast consisted of cuboidal cells with rounded nuclei separated from each other by well-defined cell boundaries (Figure 1c). The villous core contained thin-walled fetal capillaries surrounded by fetal mesenchymal cells of various shapes. The narrow intervillous spaces were filled with maternal blood cells (Figures 1 b,c).

Placentas of VDD group showed intervillous fibrin deposition in between the chorionic villi (Figure 1d). Scattered areas of edema were detected in the villi core (Figure 1e). The placentas of VDD appeared studded by the syncytiotrophoblastic cells with darkly stained cytoplasm and small dark nuclei forming syncytial knots. The syncytial knots occupying variable areas of the chorionic villi (Figure 1f). The cytoplasm of fetal mesenchymal cells showed multiple vacuoles. Inflammatory cells infiltrate were observed in the chorionic villi core (Figure 1f).

Masson's Trichrome stained sections

The control placentas showed collagen fibers in the vascular mesenchyme of the villi core stained light blue with Masson's trichrome (Figures 2 a,b).

The placentas of VDD showed an apparent increase of irregularly arranged collagen fiber bundles in the less vascular villi involving both stem and terminal chorionic villi. Meanwhile, abundant collagen fibers were deposited around fetal capillaries in the villous core (Figures 2 c,d).

Periodic acid-schiff stained sections

Sections of the human control placentas showed PAS-positive reaction in the syncytiotrophoblast cells and the basement membrane of the fetal capillaries (Figures 3 a,b).

The placentas of VDD PAS-positive reaction in the syncytial knots were observed, while the basement membrane of fetal capillaries showed irregularities and thickening (Figures 3 c,d).

Electron microscopic results

Electron microscopy of the control placenta revealed

that the chorionic villi were covered by syncytiotrophoblast (Sy) which exhibits a relatively dense cytoplasm (Figure 4a). The Sy had a lot of long, cylindrical and regular microvilli (Figures 4 a,b). The heterochromatic nuclei of Sy were surrounded by mitochondria and rough endoplasmic reticulum (rER) (Figure 4b). The deep layer was formed by cytotrophoblast (Cy) which contained paler cytoplasm (Figure 4a). The Cy had some rER cisternae along with a few mitochondria surrounding euchromatic nuclei (Figure 4c). The villi core was harbored fetal capillaries that lined by flat endothelial cells. The fetal capillary basement membrane was externally surrounded by concentric fine reticular fibers (Figure 4c).

The perivascular fetal mesenchymal cells had euchromatic nuclei and long pseudopodia-like processes on each side of the cells (Figure 5).

Electron microscopic examination of the VDD exhibited some fragmented heterochromatic nuclei of Sy with chromatin clumps (Figure 6a). Other Sy nuclei showed irregular outline with areas of glycogen granules, dilated profiles of the rER and disrupted mitochondria. The microvilli of the Sy were disorganized, separated and some had terminal club ends (Figure 6b). Also, the cytotrophoblast nuclei were heterochromatic with irregular nuclear outline among numerous intracytoplasmic vacuoles (Figure 7). Aggregation of irregular electron dense particles in Cy cytoplasm were detected (Figure 7). The fetal mesenchymal cells with irregular nuclear outline and peripheral condensation of chromatin were noted. The fetal mesenchymal cell processes contained pleomorphic vesicular structures. Irregularities and thickening of basement membrane of fetal capillaries appeared externally surrounded by numerous reticular fibers (Figure 8). Moreover, there were stromal macrophages with their lysosomes and inclusion bodies that contained an electron dense material (Figure 9). Within the chorionic villous core, abundant collagen fiber bundles were deposited (Figures 7,8).

Histopathological grading

The histopathological grading for placental changes included four parameters: cellular inflammatory infiltration (Chorionic villitis), trophoblast degenerative knots, presence of villous edema and collagen fibers deposition (Table 2).

Control group scored 0 in all parameters, as the majority of the examined sections showed minimal findings in all criteria. For chorionic villitis and trophoblast degenerative knots 70% of the examined sections showed none or 1-3 dead cells in less than 3 fields. Likewise, 60% and 75% of the examined placenta sections showed no villous edema and collagen fibers deposition respectively.

In VDD group scored 3 in all parameters, as the majority of the examined sections showed findings indicative for score 3 in all criteria. Regarding trophoblast degenerative knots, it was graded higher score (score 3). In this

group, 60% of the examined sections showed more than 6 degenerative knots cells in at least 4 fields of the total examined fields. In chorionic villitis infiltration scoring, it scored 3 as 40% of the examined sections showed more than 6 cells in at least 4 fields of the total examined fields. Concerning edema and collagen fibers deposition criteria, VDD group scored 3 as these criteria were present in 70% and 60% of the examined sections respectively.

Placental barrier assesment

Placental barrier was constituted of the endothelium

and the trophoblast layers together the fetal mesenchymal tissue of the core of the chorionic villi. There were statistically significant differences between control group and VDD group regarding the placental barrier parameters. Regarding to the thickness of basement membrane of the fetal capillary, its mean range was significantly greater in VDD group ($361.6 \pm 11.3 \mu\text{m}$) compared to control group ($161.5 \pm 6.5 \mu\text{m}$). In VDD group, the mean of microvilli number (17.8 ± 2.8) showed highly significant reduction in comparison to control group (42.6 ± 3.3) (Table 3)

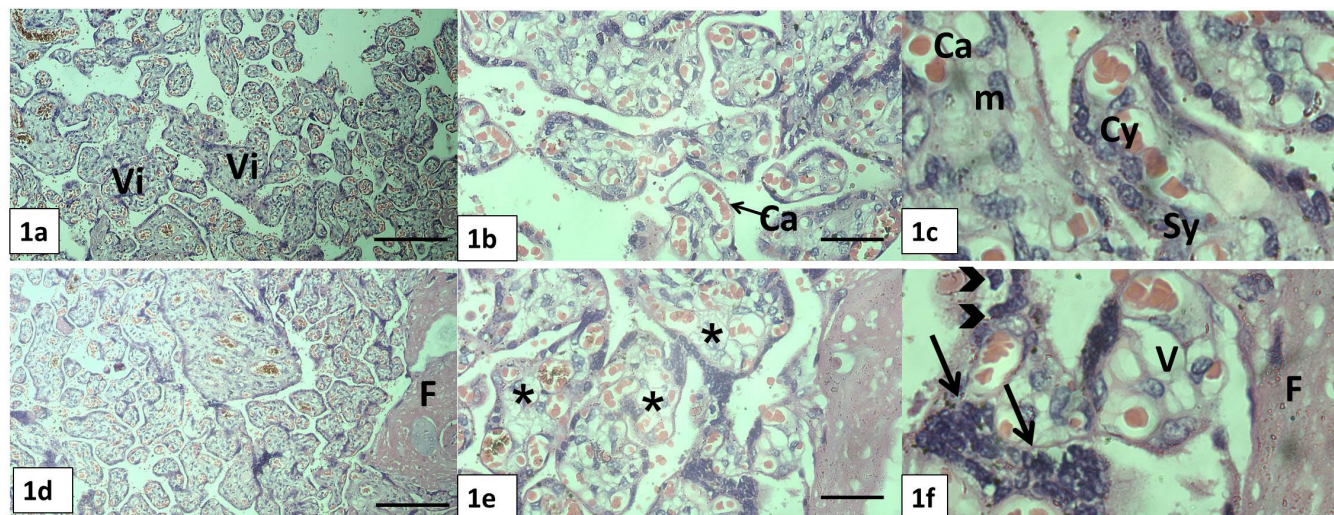


Fig. 1: Photomicrographs of placental villi sections of control group (a, b and c) and of VDD group (d, e and f) showing (a) numerous and densely packed chorionic villi (Vi). (b) the chorionic villi core that contains fetal capillaries (Ca) and mesenchymal connective tissue. (c) The multinucleated syncytiotrophoblast cells (Sy) and cytotrophoblast cells (Cy) with rounded nuclei covering the chorionic villi. Note the perivascular mesenchymal cells (m) appear near the fetal capillaries (Ca). (d) intervillous fibrin deposition inbetween the chorionic villi (F) (e) scattered areas of edema in the villous core (*). (f) Numerous Sy nuclei accumulate forming syncytiotrophoblastic knots (black arrows). The cytoplasm of fetal mesenchymal cells appears with multiple vacuoles (V). Inflammatory cells infiltrate (arrow head) are noted. [H & E stain: scale bar: (a & d) x100, (b & e) x400 and (c & f) x1000].

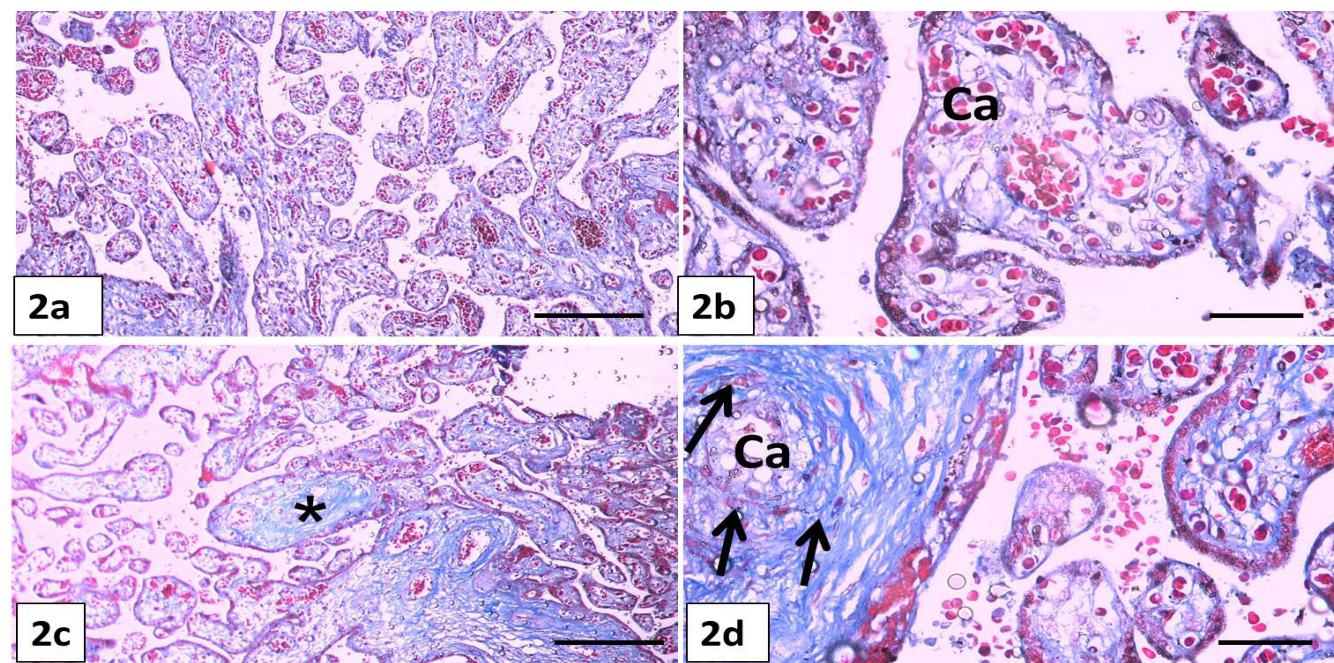


Fig. 2: Photomicrographs of placental villi sections of control group (a and b) and of VDD group (c and d) showing: (a & b) blue coloured fine collagen fibers in the villous core around fetal capillaries (Ca). (c) an apparent increase of irregularly arranged collagen fiber bundles (*) in the less vascular villi involving both stem and terminal villi. (d) abundant collagen fibers deposition (arrows) around fetal capillaries (Ca) in the villous core. [Masson's trichrome stain: Scale bar: (a & c) x100, (b & d) x400].

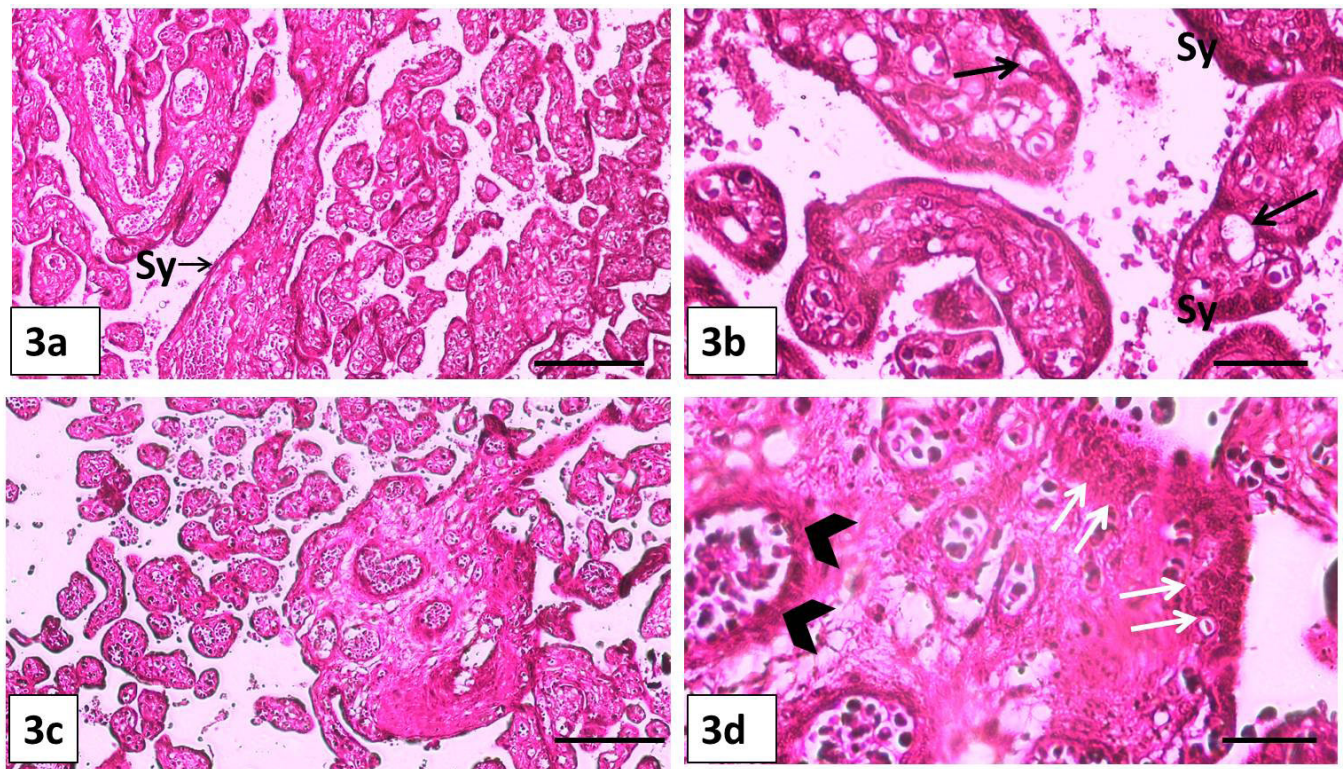


Fig. 3: Photomicrographs of placental villi sections of control group (a and b) and of VDD group (c and d) showing: (a & b) PAS-positive reaction in the syncytiotrophoblast cells (Sy) and the basement membrane of the fetal capillaries (black arrow). (c & d) irregularities and thickening of basement membrane of fetal capillaries (arrow head) and PAS-positive reaction of syncytiotrophoblast knots (white arrows). [PAS reaction: Scale bar: (a & c) x100, (b & d) x400].

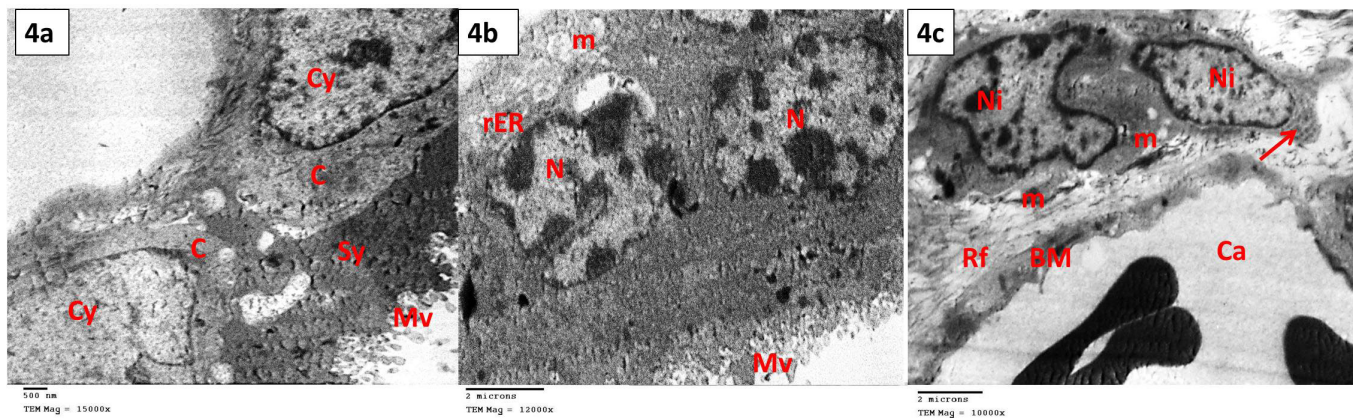


Fig. 4: Electron photomicrographs of placental villi ultrathin sections of control group showing: (a) the trophoblastic layer is formed of Sy with relatively dense cytoplasm, and a lot of long, regular cylindrical microvilli (Mv) on the surface. The Cytotrophoblast cells (Cy) form a deep layer and its cytoplasm (C) appear paler. (b) The Sy nuclei (N) are heterochromatic and its cytoplasm contains mitochondria (m) and rER cisternae. (c) The Cy nuclei (Ni) are euchromatic, its cytoplasm is harboring some rER cisternae (arrows) and few mitochondria (m). The basement membrane (BM) of the fetal capillary (Ca) is externally limited by concentric fine reticular fibers (Rf). (Uranyl acetate& lead citrate; (a): X15,000, (b):12,000 & (c):10,000)

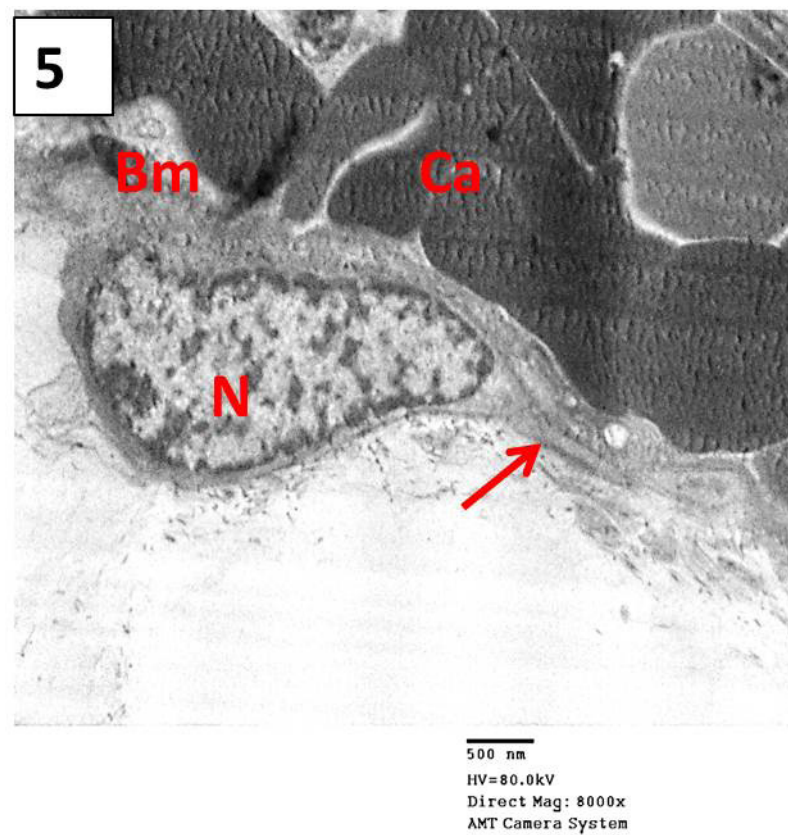


Fig. 5: Electron photomicrograph of placental villi ultrathin section of control group showing the perivascular fetal mesenchymal cells with long pseudopodia-like processes (arrow). The mesenchymal cell has euchromatic nucleus (N). Notice the basement membrane (BM) of the fetal capillary (Ca). (Uranyl acetate& lead citrate; X 8000)

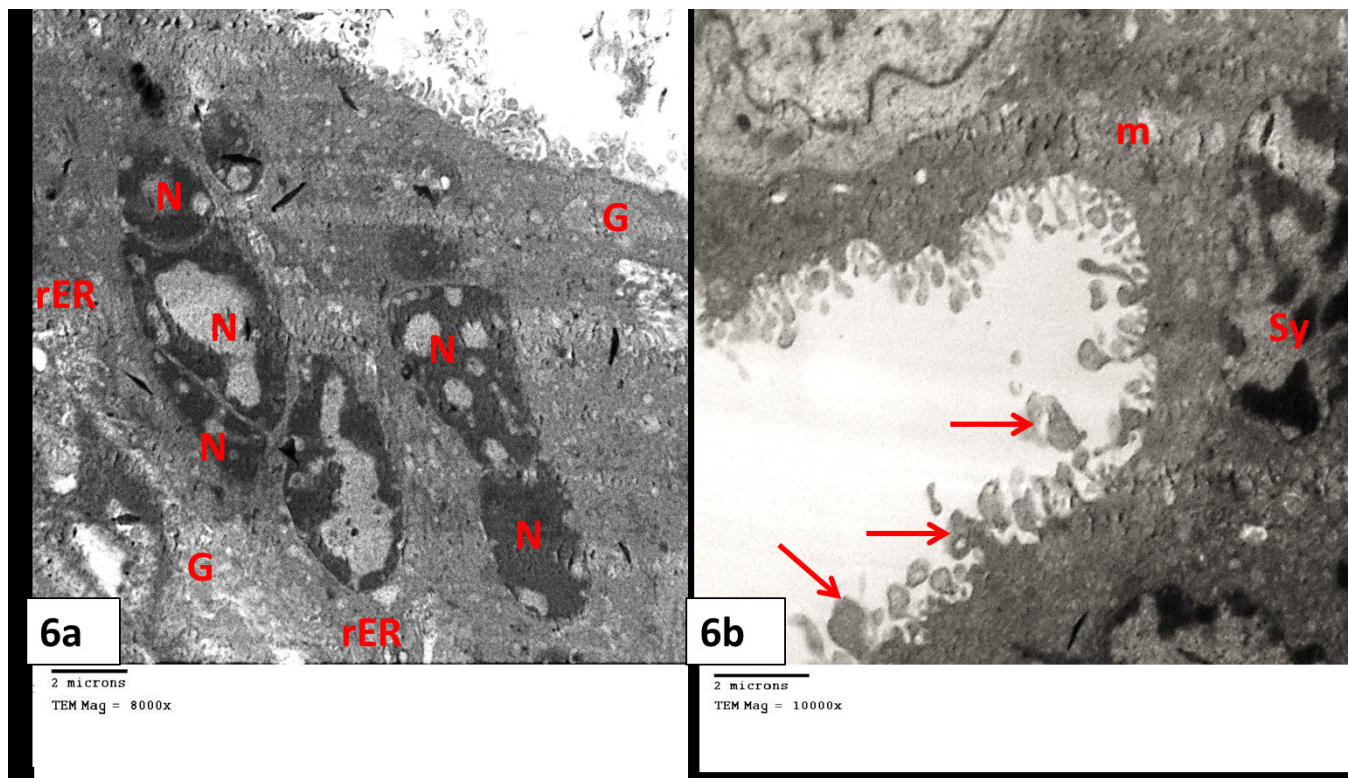


Fig. 6: Electron photomicrographs of placental villi ultrathin sections of VDD group showing (a) the fragmented Sy nuclei (N) with chromatin clumps. Areas rich in glycogen granules (G) are detected with dilated profiles of the rER. (b) The microvilli of the Sy (arrow) appear disorganized, separated and some has terminal club ends. Notice disrupted mitochondria (m). (Uranyl acetate& lead citrate; (a): X 8000 & (b): X10000)

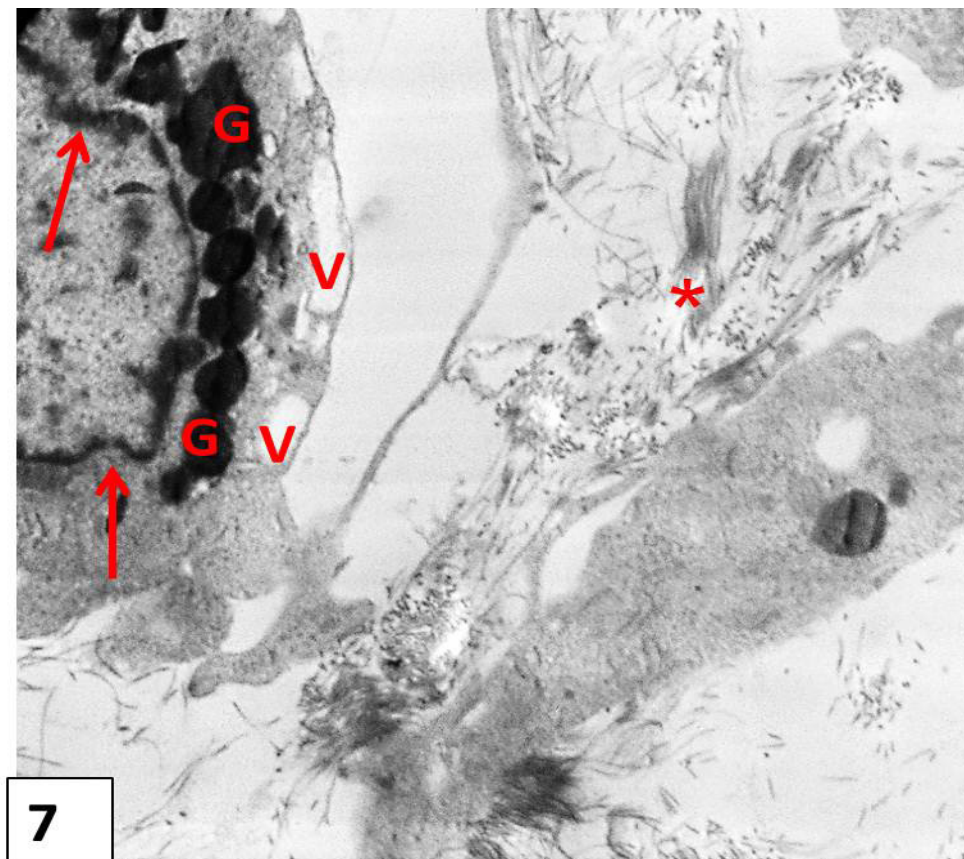


Fig. 7: Electron photomicrograph of placental villi ultrathin section of VDD group showing irregular outline Cy nucleus (arrow) with numerous intracytoplasmic vacuoles (V) and aggregation of irregular electron dense particles (G). Notice the collagen fibers deposition (*) in the villous core. (Uranyl acetate& lead citrate; X 10000)

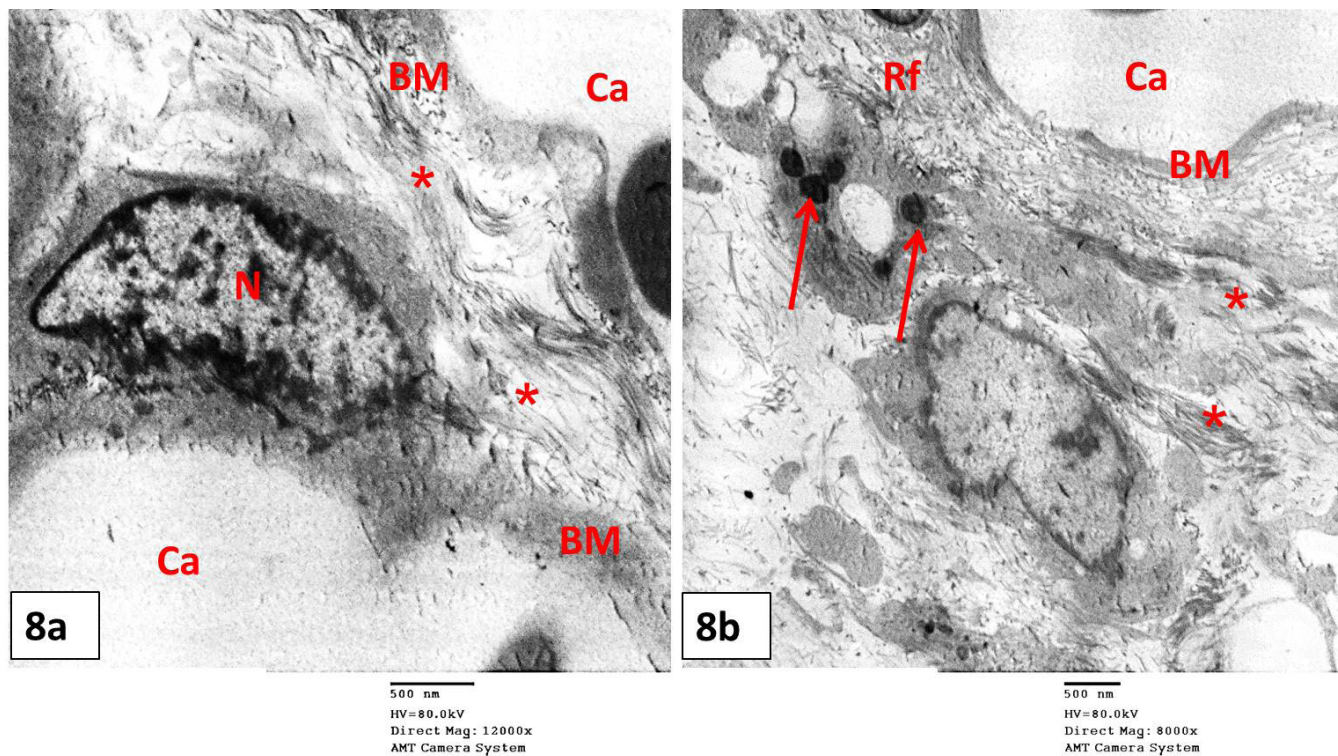


Fig. 8: Electron photomicrographs of placental villi ultrathin sections of VDD group showing (a) the fetal mesenchymal cell with irregular nuclear outline (N) and peripheral condensation of chromatin. (b) The fetal mesenchymal cell processes contains pleomorphic vesicular structures (arrow). Irregularities and thickening of basement membrane (BM) of fetal capillaries (Ca) are noted and externally surrounded by numerous reticular fibers (Rf). Notice the collagen fibers deposition (*) in the villous core. (Uranyl acetate& lead citrate; (a): X12000 & (b): X 8000)

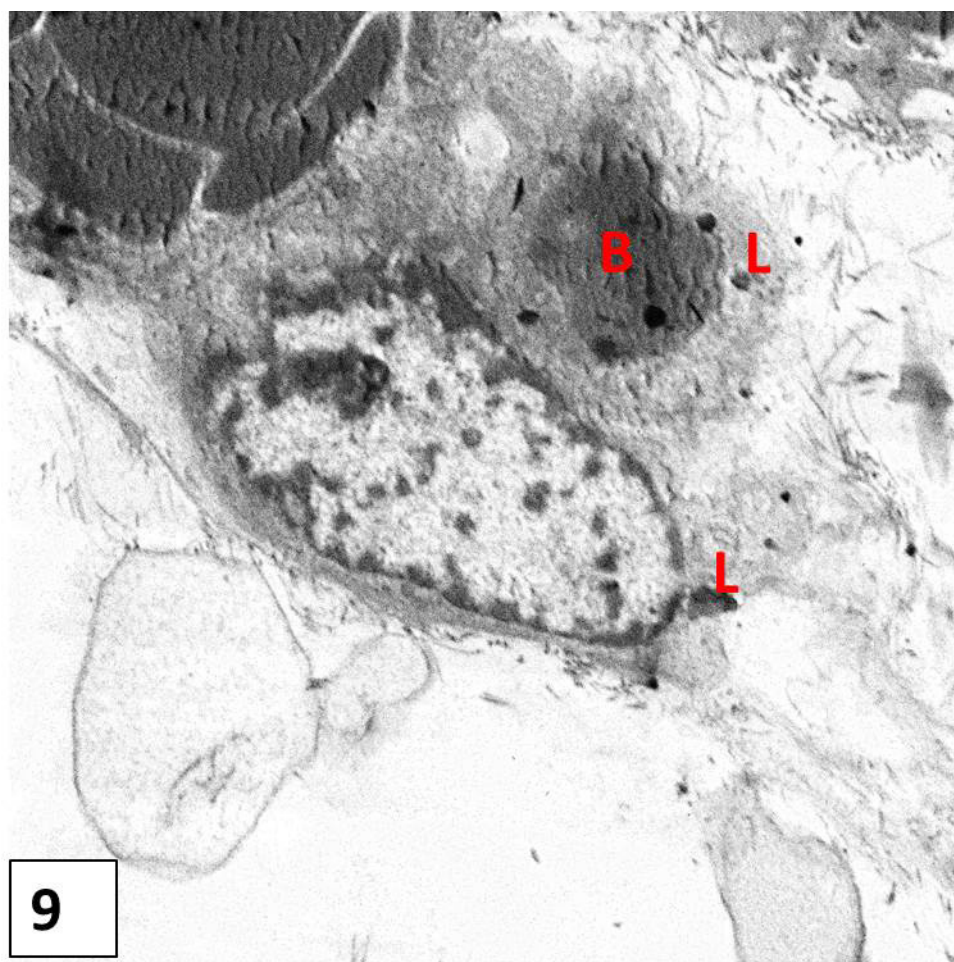


Fig. 9: Electron photomicrograph of placental villi ultrathin section of VDD group showing stromal macrophage with their lysosomes (L) and inclusion bodies (B) that contain an electron dense material. (Uranyl acetate& lead citrate; X 10000)

Table 2: Histopathological scoring system for the placenta frequency distribution (%) of each component examined in five different field/section

Component	Chorionic villitis				trophoblast knots				Villous edema				Collagen fibers deposition			
	0	1	2	3	0	1	2	3	0	1	2	3	0	1	2	3
Grading range	0	1	2	3	0	1	2	3	0	1	2	3	0	1	2	3
Control	70%	20%	10%	0	70%	30%	0	0	60%	20%	20%	0	75%	20%	5%	0
VVD	10%	30%	20%	40%	5%	10%	25%	60%	0	20%	10%	70%	10%	10%	20%	60%

Table 3: Comparison between the control and VVD groups according to placental barrier parameters

	Thickness of basement membrane of fetal capillaries in μm	Microvilli number (per 10 μm)	Microvilli height in μm
Control Group (n = 10)	161.5 \pm 6.5	42.6 \pm 3.3	0.36 \pm 0.03
VDD Group (n = 10)	361.6 \pm 11.3	17.8 \pm 2.8	0.19 \pm 0.02
<i>P-value</i>	0.0375*	0.0012**	0.023*

p: *p value* for comparing between the studied groups

*: Statistically significant with control group at $p \leq 0.05$

**: Highly statistically significant

DISCUSSION

Human trophoblasts express the vitamin D receptor that responds to vitamin D. Vitamin D regulates the synthesis of hormones involved in pregnancy and influences on the placenta by several pathways including the implantation^[20], modulates immune function^[10], alters angiogenic factors (placental growth factor (PIGF), vascular endothelial growth factor (VEGF)) and antiangiogenic factors (soluble VEGF receptor-1)^[21]. During critical periods of placental development, the VDD affects maturation of vascular system and angiogenesis in preeclampsia patients^[22]. Despite of these findings, very scanty studies are found assessing the impact of VDD on ultrastructure of placenta morphology.

It is agreed that the normal upper limit of 25(OH)D is 100 ng/ml^[23]. The Institute of Medicine (IOM) in U.S. has confirmed that the current measurements of the adequacy and deficiency of 25(OH) D used in laboratories have not been proved by accurate scientific studies. It suggests that because no central authority has established, the reports of deficiency and lab ranges may be skewed or overestimated. The Institute of Medicine defined deficiency is ≤ 12 ng/ml and insufficiency = 12–20 ng/ml^[24]. While the vitamin D Council defined deficiency: 0–40 ng/ml^[25]. In the current study, vitamin D was considered deficient ≤ 20 ng/ml.

In the present study, the VDD group revealed serious histo-pathological alteration, in the form of inflammatory cells infiltration, stromal macrophages with their lysosomes and inclusion bodies that contained an electron dense material. These findings are supported by studies of Ao *et al.*^[26] who demonstrate a significant correlation between VDD and increased incidence, or exacerbation, of infectious diseases and inflammatory autoimmune diseases, such as multiple sclerosis, systemic lupus erythematosus, and rheumatoid arthritis. These findings could be explained by Tambllyn *et al.*^[27] who stated that VDD could aggravate an already excessive inflammatory response. Also, It has been proved that T and B cells, monocytes/macrophages and dendritic cells express the vitamin D-activating enzyme and vitamin D 1 α -hydroxylase (CYP27B1)^[28].

In the current study, another striking feature of the placentas of the VDD group was prominent PAS-positive reaction in the syncytiotrophoblast knots. Also, areas of glycogen granules and dilated profiles of the rER were observed in Sy cytoplasm. This finding might indicate hormonal imbalance. This was in accordance with Verstuyl *et al.*^[29] who stated that VDD during pregnancy is associated with the atypical actions of hormones, being linked with gestational diabetes mellitus, insulin resistance and preeclampsia. Glycogen, a large macromolecule bound by α ^[1,4] glycosidic bond, is a major storage form of glucose and is usually present as cytoplasmic granules. Glycogenesis occurs with glucose entering the cell through glucose transporters (GLUT-1) which are tissue-specific. The entry of glucose into the cells is facilitated by two transport systems: insulin-dependent and insulin-independent glucose transport system^[30].

In the current work, Placentas of VDD group showed intervillous fibrin deposition in between the chorionic villi. Also, Aggregation of irregular electron dense particles in Cy cytoplasm were detected. This finding might indicate response of cytotrophoblast to placental separation. Studies with fibrinogen in pregnant mice have shown that fibrin and fibrinogen are essential components in maintaining the foetal-maternal interaction and the attachment of the placenta to the uterine wall by reducing the bleeding at the moment of the spreading of cytotrophoblasts and of maternal vessel remodelling^[31,32]. Moreover, fibrin stimulates trophoblast proliferation through growth factor binding and integrin upregulation^[33] and contributes to placental repair^[32].

In normal pregnancies the optimal structure of chorionic villi ensures the proper nutrients delivery to the fetus. On the other hand, the chorionic villi of VDD group showed several structural alterations including villous oedema, fibrosis of the villous stroma, and thickening of the basement membrane of fetal capillaries. This might disrupt vascular integrity and increase distance between foetal capillaries and the intervillous space. This finding might reduce O₂ transfer from the maternal to the fetal blood. Moreover, the present study demonstrated an increase in syncytial knots, and hypervacuolized syncytiotrophoblast. These observations were similar to changes associated with adverse pregnancy outcomes, including fetal growth restriction and preeclampsia, defined as feature of maternal vascular malperfusion. Increased syncytial knots appear to arise in response to hypoxic or hypoxia-reperfusion injury to the placenta^[34]. Also, Parks^[34] added that vitamin D protects endothelial cells against oxidative stress and reduce the effects of exposure factors linked with preeclampsia. Finally, vitamin D administration improves vascular elasticity and thickness of the tunica media and intima of blood vessels^[35].

The critical function of the placenta is a specialized barrier that composed of the fetal capillary endothelium and syncytiotrophoblast, separated by a thin interstitium. This multilayered membranous barrier separates fetal circulation and the maternal intervillous space and is responsible for selective placental transport^[36]. Some significant changes in the placental barrier in VDD were detected, including the thickened fetal capillaries basement membrane, disorganized and club ends apical microvilli of Sy, and intracytoplasmic Sy vacuoles that contribute to thickened placental barrier. This finding might disrupt transplacental transport and exchange. The placental barrier thickening is found in women with gestational diabetes mellitus^[37], or who have eclampsia or preeclampsia^[38], or who smoke^[39]. It is unclear whether the histological alterations in the placental terminal villi are indirect or direct effect of low physiological level of vitamin D. Unfortunately, the mechanisms leading to morphological alterations in the human placenta are still to be unveiled. It is also known that microRNAs (miRNAs) molecules are involved in the physiological regulation of

major processes of placentation^[40]. The miRNA molecules play a role in numerous diseases that are involved in post-transcriptional gene expression and modulating pathways that control organ function and differentiation^[41]. Therefore, it might be anticipated that aberrant placental changes in VDD are due to dysfunction of miRNA expression. Recent published studies have explored a causal relationship between preeclampsia and miRNA expression^[42]. The functional roles of miRNAs include controlling trophoblast differentiation, proliferation, invasion, migration, apoptosis, angiogenesis and cellular metabolism such as intrauterine growth restriction and preeclampsia^[43,44].

At the ultrastructural level, VDD group showed dilation of ER cisternae and architectural disruption of the mitochondria. This finding might enhance vacuole formation in Sy, which leads to widespread intracytoplasmic vacuolization and altered metabolic exchange across the placenta. ERs and mitochondria are the most vulnerable organelles, which are liable to hypoxia^[45]. These observations were ongoing with Lokeswara *et al.*^[46] who reported that nutritional deficiencies may lead to altered plasma membrane, endoplasmic reticulum stress, mitochondrial dysfunction, and DNA methylation, miRNA expression, and changes in gene expression, thus inducing a series of inappropriate cellular deaths such as autophagy dysfunction, necrosis and apoptosis resulting in abnormal trophoblast invasion.

The fetal mesenchymal cells with irregular nuclear outline and peripheral condensation of chromatin were noted in VDD group. Moreover, there was chorionic villous core fibrosis where collagen fibers were deposited. It has been demonstrated that upregulation of miRNA-222 enhances mesenchymal stem cells apoptosis in preeclampsia patients in response to hypoxia, by targeting BCL2 (B-cell lymphoma 2)^[47]. Over the last several decades, 1,25(OH)₂D regulate cell cycle progression and have pro-differentiation and anti-proliferative activity in a variety of cell types, including mesenchymal, vascular endothelial, immune cells, keratinocytes, chondrocytes, osteoblasts, and neural cells. The differentiation effects by changes in the expression of growth factors and cytokines, and the proliferation effects are mediated by the induction of cell-cycle inhibitors that prevent the transition from the G1 to the S phase of cell cycle^[48]. Thus, the effects of VDD on cell differentiation and proliferation are complex.

CONCLUSION

Maternal VDD is likely to be responsible for placental barrier impairment that disrupts transplacental transport and exchange which could be associated with some pregnancy complications and might affect development of the fetus and even future life.

CONFLICT OF INTERESTS

There are no conflicts of interest.

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الملخص العربي

تغيرات في مشيمة الحوامل مع نقص فيتامين د: دراسة بالمجهر الضوئي والمجهر الإلكتروني

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خلفية البحث : حديثا ظهر لفيتامين د اهمية كبيرة في التكاثر حيث يؤثر على نمو الخلايا وتنوعها وتكوين الاوعية الدموية وتشكيل عظام الجنين وتحور المناعة. هناك زيادة ملحوظة في عدد الحوامل المصابين بنقص فيتامين د عالميا. وقد لوحظ ان هناك علاقة بين نقص الفيتامين والمضاعفات المصاحبة للحمل مثل تسمم الحمل والحمل السكري والولادة المبكرة.

الغرض من الدراسة: لتقييم التغيرات النسيجية في المشيمة للامهات المصابة بنقص في فيتامين د. **المواد وطرق البحث:** تم دراسة عدد ٢٠ مشيمة لمواليد مكتملي الولادة وتم تقسيمهم الى مجموعتين: مجموعة ضابطة لا تعاني من نقص الفيتامين د ومجموعة اخرى تعاني الامهات من نقص فيتامين د وعدد كل مجموعة ١٠. تم فحص شكل زغابات المشيمة للمجموعتين بالمجهر الضوئي باستخدام صبغات الهيماتوكسيلين والايوسين وصبغة Masson's trichrome وصبغة PAS كما تم الفحص بالمجهر الإلكتروني النافذ. وتم عمل قياسات لبعض المعايير التي تخص المشيمة في المقاطع المختارة وتحليلها احصائيا.

النتائج: لوحظ بفحص المجهر الضوئي وجود تغييرات في المشيمة التي تعاني نقصا في فيتامين د مثل ترسب الالياف الكولاجينية وتورم النسيج الاساسي وزيادة العقد اللاصقة وزيادة سمك وتعرجات الغشاء القاعدي للشعيرات الدموية الجنينية وترسبات الفيبرين على الزغابات.

بينما كشف المجهر الإلكتروني النافذ في العينة المصابة بنقص فيتامين د وجود تجزأ في أنوية الخلايا مع اتساع في الشبكة الإندوبلازمية الخشنة و الميتوكوندريا المشوهة وعدم انتظام الزغابات الميكروسكوبية الدقيقة وتباعدها.

كما اظهرت في خلايا cytotrophoblasts تعرجات للغشاء النووي و تكوين فجوات داخل السيتوبلازم . وصلت نسبة التورم الى ٧٠ ٪ وترسيب الالياف الكولاجين الى ٦٠ ٪ في العينات المشيمة مع نقص فيتامين د .

الخلاصة: من المحتمل أن يكون نقص فيتامين د للحوامل مسؤولا عن ضعف الحاجز المشيمي الذي يعطل النقل والتبادل عبر المشيمة والذي يمكن أن يؤدي الى بعض مضاعفات الحمل وقد يؤثر على نمو الجنين و الحياة المستقبلية للجنين.