Structural Changes in the Chorionic Villi of Placentae of Arabic Patients with Preeclampsia: An Electron Microscopic Study

Sami Algaidi*[1], Hazem A. Sayed [1], Moustafa Abu Alnaja[2]

[1]Faculty of Medicine, University of Taibah, Saudi Arabia
[2] Faculty of Medicine, King Abdul-Aziz University, Saudi Arabia
*algaidi@hotmail.com

Abstract: Genetic variations between different races play a major role in the susceptibility of some races to specific diseases. There are western studies on the structural changes in the placenta in cases of preeclampsia. However, there is no similar study on Arabic patients from the Middle East. Furthermore, very few studies correlate histological changes and the severity of the disease. The present study aims at throwing a light on the structural changes in the chorionic villi of placentas in Arabic patients with preeclampsia using electron microscope to compare the changes with other international studies. Electron microscopic examination of preeclamptic placenta revealed multiple chorionic infarcts with many abruptions and intervillous adhesions. The nuclei of the chorionic vascular endothelium showed deep indentations with interrupted intercellular junctions and thickened folded basement membrane. Also the trophoblastic cytoplasm of the affected villi showed many secondary lysosomes and vacuoles of different shapes and sizes. No difference was found between these changes and other studies. However, further studies are required to investigate the correlation between these changes and the severity of the disease.
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1. Introduction

The formation of the human placenta begins by the eighth day post fertilization, after initial implantation of the blastocyst [1]. After gentle erosion between the epithelial cells of the surface endometrium, the invading trophoblasts burrow deeper into the endometrium. This process of erosion and invasion into the endometrium is carried out actively by the trophoblast cells [2]

In human placenta, the trophoblastic layer is essential to feto-placental exchanges. This specialized layer, which covers the surface of the chorionic villious tree, consists of two distinct layers: The syncytiotrophoblast (STB) layer, which is in contact with maternal blood in the maternal intervillous spaces, and the cytotrophpblast (CTB, Langhans cells) layer, which is separated from the STB by a basal lamina [3].

Chorionic villi can first be distinguished in the human placenta on about the 12th day after fertilization .Mesenchymal cords, invade the solid trophoblastic columns forming the secondary villi. After angiogenesis occurs from the mesenchimal cores, the resulting villi are termed tertiary villi. By about the 17th day, fetal blood vessels are functional, and a placental circulation is established. The feto-placental circulation is completed when the blood vessels of the embryo are connected with the chorionic blood vessels [4].

The electron microscopic studies of the fine structure of the placenta shows prominent microvilli on the syncytial surface corresponding to the so-called brush border described by the light microscope. Associated pinocytotic vacuoles and vesicles related to the absorptive and secretory placental functions are numerous. The inner layer of the villi, the cytotrophpblast and the associated basal lamina are more prominent during the first trimester of gestation [5].

Later in gestation, however, the layer of cytotrophoblasts inside the syncytium is no longer continuous, with only scattered cells present at term, creating a narrower barrier that facilitates transport of nutrients and oxygen to the fetus [5].

By scanning electron microscope, the extra cellular fibrillar matrix of placental villi appears as a continuous network of isolated collagen fibrils and small fibrillar bundles interwoven each other. This is the sort of collagenous fibrillar skeleton which forms the axis of chorionic villi [6].

The connective tissue layer outside the fetal blood capillaries in the stroma of placental villi contains phagocytic cells called Hofbauer cells [7]. Mesenchymal derived machrophaes (Hofbauer cells) express vascular endothelial growth factor and other angiogenic factors and are thought to initiate vasculogenesis in the placenta. Later in placental development, additional capillaries form by branching angiogenesis from pre-existing capillaries [8-10].

Normal fetal growth and survival depend on the proper development and function of the placenta. This is achieved by maintaining a maternal-fetal interference for the exchange of blood gases, nutrients and waste products [11].

Preeclampsia is one of the most important complications of pregnancy, substantially contributing to maternal morbidity and mortality. About 7% of pregnancies are complicated by preeclampsia and its only cure is delivery [12]. Although the etiology of preeclampsia has not been fully understood, increasing evidences suggest that pathophysiological changes of trophoblast cell in the placental bed and failed conversion of maternal endometrial spiral arteries may be a leading cause in the pathogenesis of preeclampsia [13].

The epithelial lining of chorionic villi undergoes continuous renewal like most epithelia, and the syncytiotrophoblast is maintained by fusion with underlying cytotrophoblasts [14]. In disorders like preeclampsia, cytotrophoblasts invasion is limited to the superficial deciduas, and few arterioles are normally branched due to abnormalities in the adhesion molecule switching by invasive cytotrophoblasts, suggesting that this subpopulation of trophoblast cells fails to differentiate properly [15].

It is postulated in preeclampsia that hypoxia/reperfusion injury disrupts syncytial architecture and promotes increased release of soluble syncytial factors including cytokines, eicosanoids, peroxides, the anti-angiogenic factors soluble fms-like tyrosine kinase (sFlt)-1 and endoglin, as well as syncytiotrophoblast microparticles (STBM). These factors are suggested to pathologically activate the maternal endothelium leading to maternal proteinuria and hypertension, clinical hallmarks of preeclampsia [16]. In addition to preeclampsia [17], there is a strong evidence that race plays a major role in several pregnancy-related disorders such as anemia [18], asthma [19], nausea and vomiting [20], diabetes [21] and obesity-related disorders[22]. As discussed above, there is an increasing evidence suggest that pathological changes in trophoblastic cells may be a leading cause in pathogenesis of preeclampsia. However, up to our best knowledge, there is no epidemiological or histopathological studies on Arabic patients. Therefore, the present study was designed to investigate the morphological and the ultrastructural changes in trophoblastic cells and the blood vessels of the chorionic villi of placentae from Arabic patients with preeclampsia, and to compare the findings with other studies.

2. Material and Methods

Diagnosis of preeclampsia:

Fifty placentae of full-term normal pregnancies (control group) and twenty preeclampsia cases (study group) were obtained from cases admitted at Department of Obstetrics and Gynecology in Maternity and Children Hospital. Written consent was obtained from all participants, and the method was approved by ethical board of University of Taibah. Preeclampsia was diagnosed according to the following criteria [23]:

1- Blood pressure: two separate readings taken at least 4 hours apart of 140/90 or more after 20 weeks gestation.
2- Proteinuria: 300 mg of protein in a 24-hour urine sample or $\geq$ +1 dipstick.

Tissue preparation:

After delivery the placenta was examined from the maternal aspect to avoid sampling from areas of obvious infarctions. Full thickness samples were taken from the central part.

For electron microscopic investigations, some of pieces of specimens were fixed in 2.5% glutaraldehyde at 4°C for 2hours then rinsed with cacodylate buffer solution, post fixed in 5% osmium tetraoxide and dehydrated in graded concentrations of ethanol and finally were embedded in epoxy resin, then semi thin sections were prepared and stained with toluidine blue. Ultrathin sections were prepared for transmission electron microscopic study.

The other pieces of the specimens were dried and mounted on a specimen stub using epoxy resin or electrically-conductive double-sided adhesive tape, and sputter coated with gold or gold/palladium alloy before examination by scanning electron microscopic study to detect the morphological changes in the chorionic villi of the placentae with preeclampsia.

3. Results

In semithin sections (Figure 1), the wall of the umbilical vessels of preeclampsia is thicker than that of the normal placenta with heavy deposition of collagen fibers. The endothelial cells showed deeply indented nuclei with numerous cytoplasmic vacuoles. The cytoplasm of the trophoblastic cells showed many vacuoles.

Scanning electron microscopic examination (Figure 2) revealed that the chorionic villi of the preeclamptic placenta appear smaller than that of normal placenta. The affected placenta showed multiple chorionic infarcts and many abruptions. The fibrin skeleton of the affected chorionic villi was thickened, rough and irregular with intervillous adhesions when compared with the normal chorionic

villi.

A transmission electron microscopic study (Figure 3) of the chorionic villi of preeclamptic placenta showed rounded up deeply indented nuclei of the endothelial cells. The intercellular junction was interrupted specially near basement membrane which was folded and thickened. The interstitial tissue was narrowed with dense deposition of collagen fibers when compared with that of normal placenta. The trophoblastic cells of the affected villi showed enlarged and rounded apical microvilli and a thickened interrupted basement membrane. The trophoblastic cytoplasm contained many secondary lysosomes and vacuoles of different sizes and shapes.

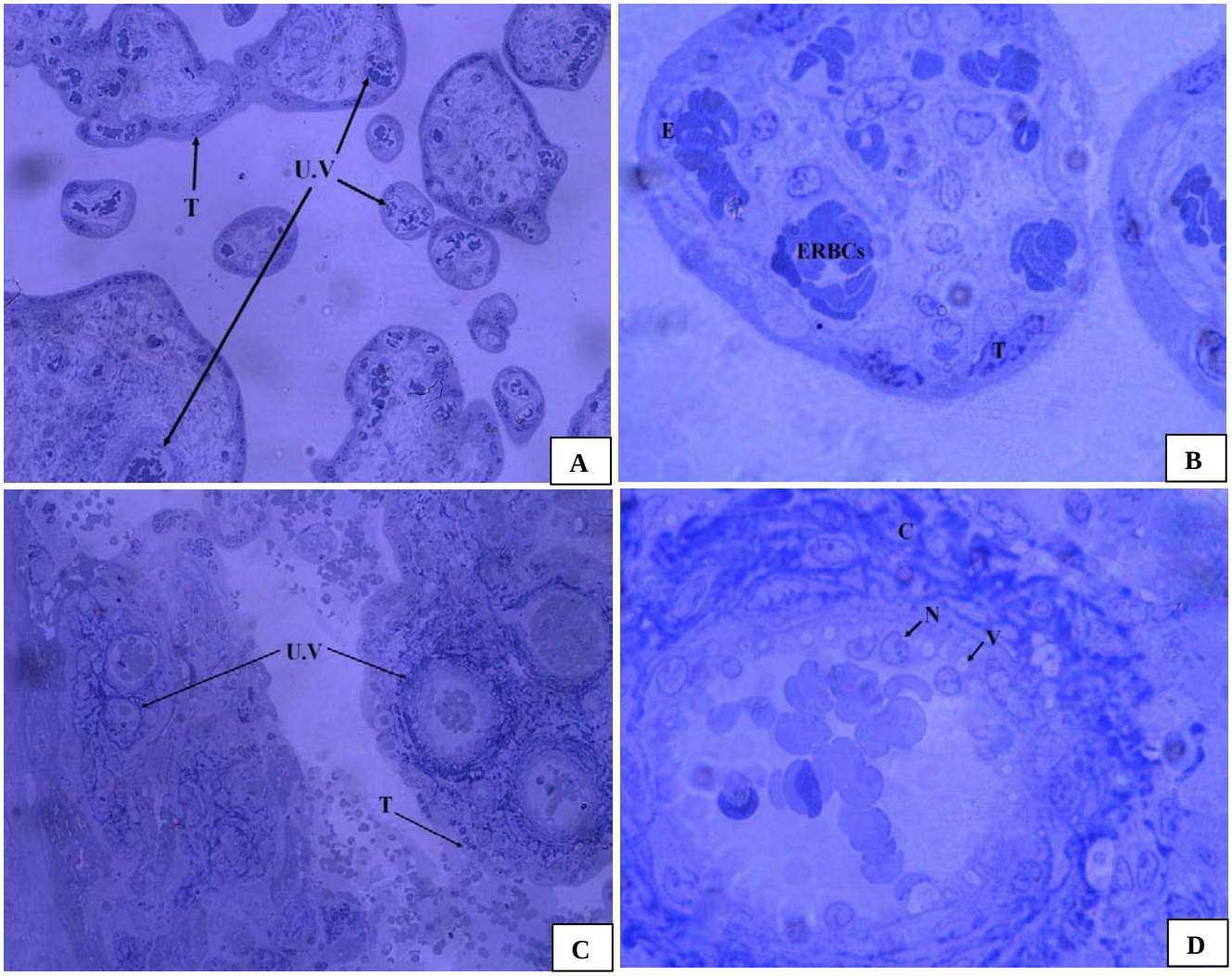

Figure 1:

(A) A photomicrograph of an axial semi-thin section (Toluidine blue, ×100) from chorionic villi of the normal placenta showing multiple umbilical vessels (U.V) with trophoblast cells that are arranged in a single layer at the surface of the chorionic villi (T).

(B) A photomicrograph of an axial semi-thin section (Toluidine blue, ×1000) from chorionic villi of the normal placenta showing flattened endothelial cells (E) of the umbilical vessels filled with fetal red blood cells (FRBCs) and the trophoblast cells that are arranged in a single layer covering the chorionic villi (T).

(C) A photomicrograph of an axial semi-thin section (Toluidine blue, ×200) from chorionic villi of the pre-eclamptic placenta showing excessive thickening of the umbilical vascular wall (U.V) and multiple vacuoles in the cytoplasm of the trophoblast cells (T).

(D) A photomicrograph of an axial semi-thin section (Toluidine blue, ×1000) from chorionic villi of the pre-eclamptic placenta showing deep indentation in the nuclei of the vascular endothelium (N) with many vacuoles in their cytoplasm (V) and heavy deposition of collagen fibers in the wall of the blood vessels.

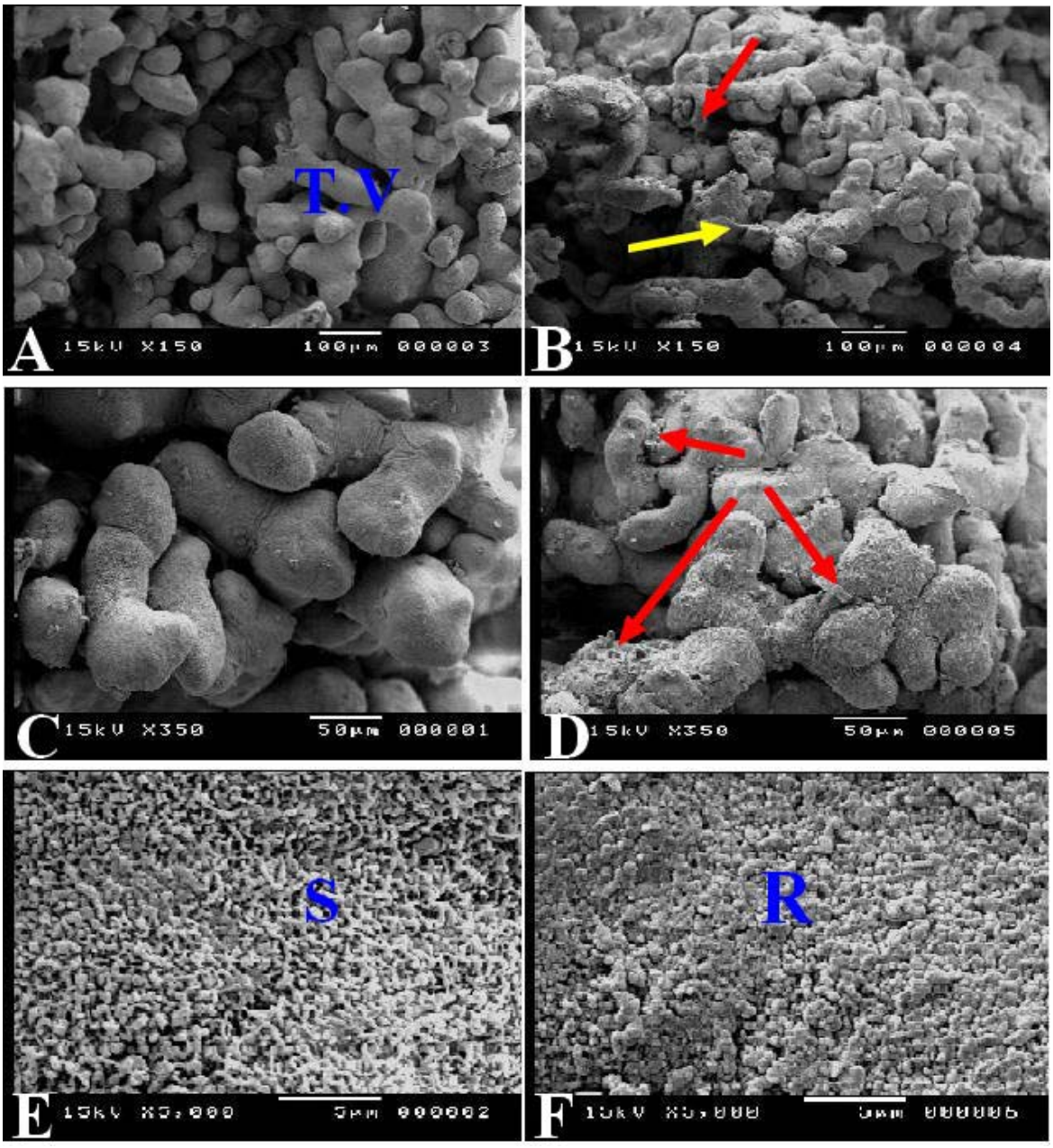

Figure 2:

(A) A scanning electron micrograph (×150) of chorionic villi of the normal placenta showing smooth floating tertiary villi (TV).

(B) A scanning electron micrograph (×150) of chorionic villi of the preeclamptic placenta showing apparently smaller tertiary villi with multiple infarcts (red arrow) and many abruptions (yellow arrow).

(C) & (D) scanning electron micrograph (×350) of chorionic villi of the normal and pre-eclamptic placenta, respectively, showing increased fibrin depositions with inter-villous thrombosis (red arrows in fig. D) in the preeclamptic placenta.

(E) & (F) scanning electron micrograph (×5000) of chorionic villi of the normal and pre-eclamptic placenta, respectively, showing smooth and regular framework of the normal villi (S in fig. E) while in the preeclamptic villi the framework become irregular, rough and thickened due to excessive fibrin deposition.

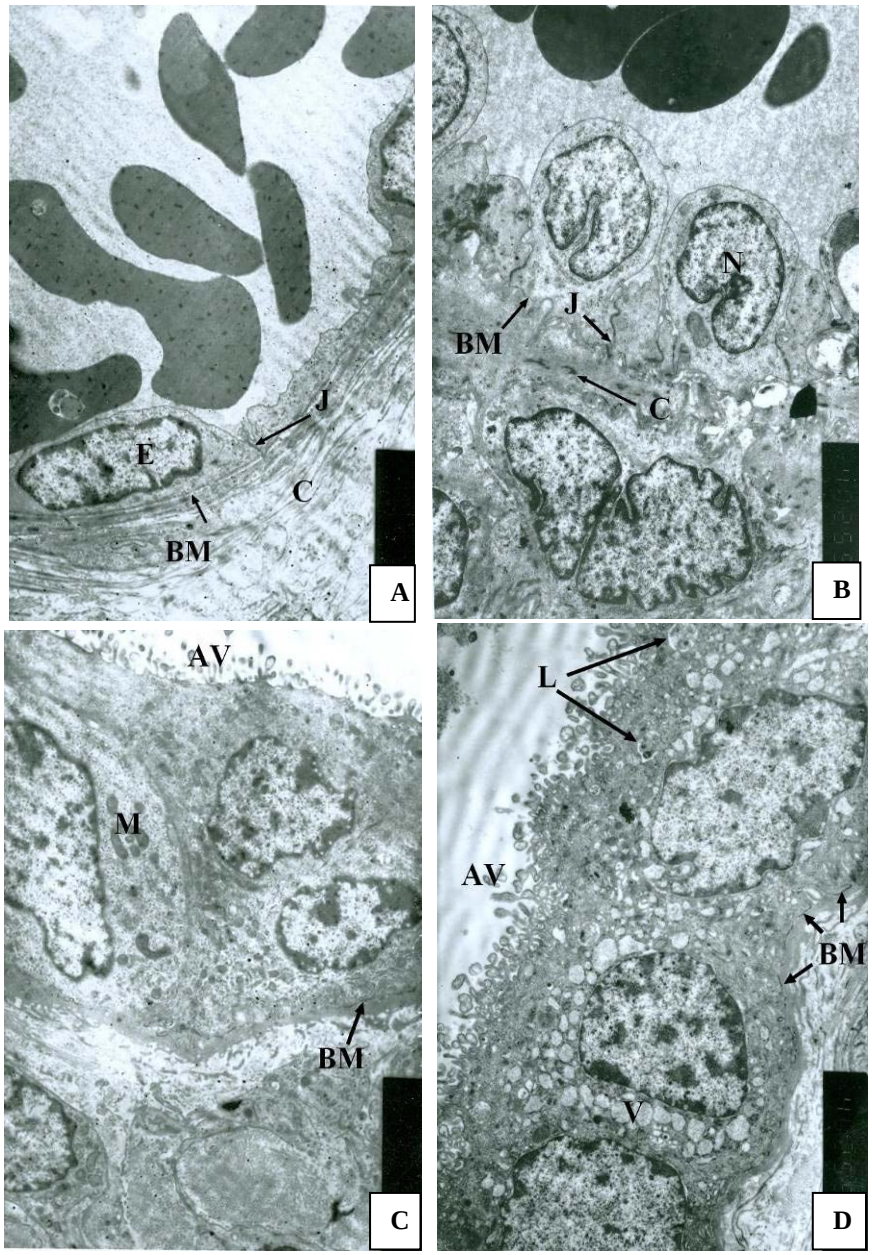

Figure 3:

(A) A transmission electron micrograph (×4000) from the umbilical vessels in the chorionic villi of normal placenta showing flat endothelium (E) with intact intercellular junctions (J) and unfolded basement membrane (BM). Fine collagen fibers (C) are seen in the interstitial tissue.

(B) A transmission electron micrograph (×4000) from the umbilical vessels in the chorionic villi of preeclamptic placenta showing rounded up an deeply indented nuclei (N) of the vascular endothelium. The intercellular junctions (J) are discontinuous near the basement membrane (BM) which is folded and slightly thickened. There is narrowing in the interstitial tissue with dense deposition of collagen fibers(C).

(C) A transmission electron micrograph (×4000) from the trophoblast cells in the chorionic villi of normal placenta showing apical microvillus border (AV) and thick basement membrane (BM). The cytoplasm of the trophoblast cells contains many mitochondria.

(D) A transmission electron micrograph (×4000) from the trophoblast cells in the chorionic villi of preeclamptic placenta showing more rounded and enlarged apical microvilli (AV) and thick interrupted basement membrane

(BM). There are numerous secondary Lysosomes (L) and cytoplasmic vacuoles (V) of different size and shape.

4. Discussion

A key event in normal placentation is invasion of the maternal spiral arteries in the decidua and myometrium by fetal cytotrophoblasts [24].

Zhou *et al.* [25] reported that this process is defective in preeclamptic placentas. Normally trophoblast cells transform from an epithelial phenotype to an endothelial phenotype as they invade the maternal deciduas and myometrium in a process termed pseudovasculogenesis. The migrating trophoblasts transform the maternal spiral arterioles that supply maternal blood to the placenta from small caliber resistance vessels to large caliber capacitance vessels allowing adequate maternal blood flow to the placenta. In preeclampsia this process is disordered and the fetal trophoblasts fail to properly invade the maternal myometrium and spiral arterioles [26].

Normally, early placental development occurs in a hypoxic environment and the placenta becomes increasingly oxygenated with time [27]. It has been theorized that this failure of conversion of spiral arterioles results in persistent placental hypoxia, placental insufficiency, maternal endothelial dysfunction and ultimately the clinical syndrome of preeclampsia [24].

In the present study the scanning microscopic examination revealed that the chorionic villi of the preeclamptic placenta were aberrantly smaller with many abruptions and multiple infarcts when compared with those of the normal placentas which is consistent with the hypoxia theory. These results were in accordance with that of Peilin Zhang *et al.* [28] who performed a placental examination for changes with preeclampsia, and reported poor correlation between these changes with the clinical manifestations of pregnancy induced hypertension. However, no correlation was considered between race, histological changes and clinical manifestation. These changes were also reported in a review article by Seth Guller [16] who explained how hypoxia/reperfusion injury disrupts the syncytial architecture and results in the previously mentioned changes.

The affected chorionic villi also showed more roughened fibrillar skeleton with increased fibrin deposition and intervillous thrombosis. The same results were reported by Seth Guller [16] and Salafia *et al.* [29] who revealed that there were aberrantly high levels of intervillous fibrin which is a critical component of physiological repair and differentiation of the placental villi [30].

The semi and ultra-thin section examinations of the affected villi showed criteria of fibrinoid necrosis of the umbilical blood vessels such as increased fibrin depositions with excessive thickening of the vascular wall. The endothelial cells revealed deeply indented nuclei and multiple cytoplasmic vacuoles. This fibrinoid necrotic picture was reported by Peilin Zhang *et al.* [28] after examination of (350) preeclamptic placentas and stated that there were two types of vascular changes which are characteristic of pregnancy induced hypertension, namely maternal atherosis and fibrinoid medial necrosis.

The trophoblast cells showed more rounded and enlarged apical villi (brush border) with signs of apoptosis as increased cytoplasmic vacuoles and numerous secondary Lysosomes. These changes were in harmony with those described by Hung *et al.* [31]. The author studied the possible etiological factors of preeclampsia *In vitro* and concluded that hypoxia and reperfusion, not hypoxia alone, could induce apoptosis in the syncytiotrophoblasts.

As previously discussed, race was not taken into consideration when investigating preeclampsia. However, a study by Goodwin and Mercer [17] reported a positive association between race and severity of hypertension, hemolysis, elevated liver enzymes and low platelets syndrome. Therefore, future studies should consider race when studying preeclampsia. Also, epidemiological studies of Arabic patients is required to investigate the possibility of an association between severity of preeclampsia, histological changes and race.

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Corresponding author
Sami Algaidi
Faculty of Medicine, University of Taibah
Saudi Arabia.
algaidi@hotmail.com

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