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Quantitative Morphometric Study of The Chorionic Villi in Hypertensive Mothers

Abdulrhman Saleh Dairi[1], Wagih Gamal Elbarrany[2], Amna Abdul Rahim Moulana[3], Ahmad Sami A Himayda[1], Iyad M.Hakeem[1]

[1]Medical Intern, Faculty of Medicine and Surgery, Umm Al-Qura University, [2]Professor of Anatomy, Faculty of Medicine and Surgery, Umm Al-Qura University, [3]Consultant of Anatomical Pathology, Maternity and Children's Hospital, Ministry of Health, Makkah, Saudi Arabia.

Corresponding Author: Abdulrhman Saleh Dairi, email:askdairi@gmail.com, mobile:+966558306306

ABSTRACT

Background: Several studies have reported that mothers with pregnancy induced hypertension or those suffering from hypertensive disorders have abnormalities in histological features of the placenta. The fetus connection with the mother is through chorionic villi. Besides several other histological features changes in the chorionic villi have also been reported. This lead to the reduced supply of the necessary nutritional elements for the fetus.

The aim of the Study: The principal objective of this study was to evaluate morphometric changes in the placenta of expecting mothers have hypertensive disorders of pregnancy and women without any symptoms of hypertension. As the placenta is capillary-rich region and any physiological change can adversely affect the fetal health.

Patients and Methods: In this study, a total of 84 expecting mothers were recruited. Among these 42 have hypertensive symptoms before pregnancy whereas the other 42 have their blood pressure in normal ranges. Among the 42 hypertensive women, only 13 met the study inclusion criteria,i.e., blood pressure in the range of 140/90 mmHg in the 30th week of the pregnancy. The quantitative morphometric parameters included shape and size of the placenta, damage to the blood vessels mainly in the chorionic villi and an overall number of blood vessels.

Results: A comparative evaluation of placenta from the hypertensive and normotensive expecting mothers showed that blood vessels area in the hypertensive mothers was significantly reduced when compared to normotensive mothers, same holds from the blood vessels in the perimeter areas. These findings have important implications as far as the fetal development among hypertensive mothers is concerned.

Conclusions: Higher blood pressure associated changes in the placenta are manifested in the form of several histological and morphological changes mainly in the chorionic villi structures involved in fulfilling nutritional requirements of the fetus.

Keywords: Placenta, Chorionic villi, Hypertension, Pregnancy.

INTRODUCTION

Foetal development is a well-orchestrated and highly complex developmental process involving the formation of several membranes linking fetus to the mother[1]. The most common fetal membranes are the amnion and chorion ultimately forming the amniotic sac that encircles the fetus and has a major protective function[2]. Mainly the small projections or villi of the chorionic membrane play a significant role in providing fetal to mother contacts an appropriate supply of maternal blood supply to the fetus[3]. Few studies have described the timing of the development of the chorionic villi, but it is believed that the development of the vill ibegins at the end of the second week to the third week of embryonic development. Late in the second week of the development of the embryo, projections of the proliferating cytotrophoblast cells appear to be evaginating into the syncytiotrophoblast in several regions in the placenta known as primary villi [4,5].The formation of the primary villi is followed by the emergence of the mesenchymal core that is embedded into the progressively expanding villus[6]. Presumably, this has been noted to be during the fourth or fifth week of embryonic development even though some studies have pointed out that it is a few days after the formation of the primary villi. The secondary villi, develop into tertiary villus when the blood vessels penetrate the mesenchymal core and the branches that have been newly formed[7].

This happens during the third and the fourth week of pregnancy.

The chorionic villi are differentiated into four critical layers each with a distinct function in the maintenance of the embryo. These include the outer syncytiotrophoblast (multinucleated), the inner layer cytotrophoblast stems cells, stroma and blood vessels[8-10]. As the fetal development

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proceeds, the cytotrophoblast cell layer of the villi gradually begins to disappear [11].This usually happens when the fetus is above six months of age.

The chorionic villi are involved in several vital functions playing a significant role in the health and development of the fetus. Among several others facilitating the transfer of nutrients required by the fetus from them by crossing the placental barrier is of utmost importance[12]. Nutrients and oxygen in the maternal blood diffuse across the walls of the villi to the embryo while various waste products from the fetus diffuse across the chorionic villi into the maternal blood for excretion.

Conventionally, maternal health status or changes in the physiological functions of the mother has very debilitating effects on the growth and development of the baby[13]. Principally, mothers facing health complications have problems with the proper movement of nutrients and maternal protective immune molecules from the fetus to the mother or otherwise[4]. Chorionic villiare the primary conduit through which the exchanges take place. Furthermore, the fetal waste material transfer also occurs from villi. Specifically, hypertension in expecting mothers expose the chorionic villi to significant morphological changes thus exposing the fetus to several clinical challenges. It has been reported that pregnancy complications such as hypertension or even gestational diabetes are often reflected in the form of changes in placenta[14]. Reduced fetal weight is considerably is the ultimate outcome among the mothers who are hypertensive as compared to the non-hypertensive groups[15].

Since these pregnancy complications affect the morphology of the chorion villi, there have been many efforts done by researchers to study structural changes in chorionic villi[16,17]. The villi take a different form, shape and even measurement when exposed to these pregnancy risks. Destruction of the placenta is reflected in the form of deformities in the area of chorionic villi. Other structures such as the Hofbauer cells, eosinophilic histiocytes found within the placenta reduce in number at the destruction of the placenta [18]. The Hofbauer cells have granules within the mesoderm of the chorionic villi, and this means that any destruction of the placenta translates into a destruction of these cells [4].This observation prompted us to study choronic villi morphology at quantitative levels among the hypertensive expecting mothers.

PATIENTS AND METHODOLOGY

This study was approved by Ethical Research Committee of the University of Umm Al-Qura in Makkah. In the present study, expecting mothers with a history of hypertension were recruited from Maternity and Children's Hospital in Makkah during the period of early February 2017 to late April 2017. The inclusion criteria for this study was an expecting mother having a blood pressure higher than 140/90 mmHg in the30th week of the pregnancy. The selection of this time frame is based on the data that pregnant women in the 30th week of their pregnancy are at the risk of developing pre -eclampsia where the chorionic villi in the placenta would be damaged[19]. Among 42 expecting mothers screened 13 met our inclusion criteria, i.e., having blood pressure ranges as described above.

Eighty-four placenta samples were obtained, 42 of which were hypertensive and 42 non-hypertensives, each sample was taken immediately after delivery. The specimen was prepared and subjected to sectioning (1 micron each). The specimens were stained with Haematoxylin and Eosin, their images were scanned and digitalized using computerized image analysis system comprising a high-resolution digital camera attached to a microscope and to an IBM-compatible computer. The samples were examined under a microscope with magnification X100 and photographed.

Both the control and hypertensive expecting mothers were subjected to various analyses including blood vessel number, the area of the blood vessels, and the villus areas. The selection of these parameters was based on the fact that any damage to the placenta, or the chorionic villi, is reflected in the form of damaged blood vessels in the chorionic villi. The study also has taken into consideration the morphometric evaluations,i.e., shape and the size of organs, any kind of damage to the blood vessels within the chorionic villi which could affect the surface area of the blood vessels. The size of the vessels undergoes a systematic reduction in terms of their sizes. This is equally notable in the villus area, a parameter that has been used to study the changes in the field of the chorionic villi. It is logical to note that if there is any form of damage on the surface area of the chorionic villi, there would be considered fundamental changes in the cross-sectional area of the villus.

The information collected from the hypertensive mothers were then compared to that of the non-hypertensive mothers based on the above-described parameters. The study sought to outline if there are fundamental structural changes of the chorionic villi of the non-hypertensive mothers compared to the hypertensive pregnant women. The data was further subjected to statistical analyses by using the Statistical Package for Social Scientists version 24(SPSS). Data were summarized as means and standard deviations. Normal Q-Q plots were constructed and Shapiro-Wilk test was used to assess the normality of data. A p- value greater than 0.05 indicated that the distribution of the data is significantly different from the normal distribution. When normality assumption was fulfilled, unpaired t-test was used to compare the means across hypertensive and normal pregnant women.

When such assumption was violated, the t-statistic was boot strapped and 95% BCA confidence intervals using 10000 samples. This is better than using non- parametric tests such as Mann-Whitney as it allows for the calculation of the 95% confidence interval for the difference between both groups. Six variables of interest were compared across normal and hypertensive pregnant women which are:

- Blood vessel number
- Blood vessels area
- Perimeters
- Villous area.
- Number of blood vessels / villous area
- Blood vessels area/ villous area
- Blood vessels perimeter/ villous area

The remaining variables were summarized as means and standard deviations as they were not assessed in both groups. Data were compared in 42 normal pregnant women and 42 hypertensive pregnant women.

RESULTS

Descriptive statistics:

Descriptive statistics in normal pregnant women:

Table 1 summarizes the mean values of different parameters measured among the examined 42 pregnant women. The measured parameters were the placental barrier diameter of placental barrier in µ, the number of Hofbauer cells, the endothelial area, the endothelial number, the endothelial area/blood vessel area ratio, the mean endothelial area/blood vessels ratio, the endothelial area/villous area ratio, the barrier/perimeter ratio, the mean barrier/blood vessel area, and the Hofbauer cells/villous area ratio.

	Valid N	Mean
Barrier (µ)	42	128.944
Hofbauer cells	38	2.79
Endothelial Area	16	3109.355
Endothelial Number	16	4.75
Endothelial area/Blood vessel area	16	12.174
Endothelial area/Blood vessels perimeter	16	462.95
Endothelial area/Villous area	16	.395
Barrier/Perimeter	42	.119
Barrier/Blood vessel Area	42	.00296
Hofbauer cells/Villous Area	39	.00572

Descriptive statistics in hypertensive pregnant women:

As regards the hypertensive pregnant women, the parameters measured were the number of knots, the knots area, the mean knots/area ratio, and the mean knots area/villous area ratio, the Fibrin area, and the fibrin/villous. Table 2 illustrates the values of the measured parameters in details.

	Valid N	Mean
Knots number	31	1.90
Knots area	31	9603.64
Knots/area	31	.000294
Knots area/Villous area	31	.0368
Fibrin Area	5	25542.163
Fibrin/Villous Area	6	.04698

Normality assumption

The data collected from the recruited samples were non-parametric. Q-Q plots and Shapiro-Wilk test showed that the different studied variables, namely blood vessel area, blood vessel perimeter, number of blood vessels, villus area, number of blood vessels/villous area, blood vessel area/villous area, and blood vessels perimeter/villous area, were not normally distributed; with probability values ranging from <0.001 to 0.042. The collected data were not normally distributed; therefore, the t-test was performed through boot strapping to compare the different variables across normal and hypertensive pregnant women.

Comparison results

Results of comparison – detailed in table 3 - showed that the blood vessel area in normal pregnant women was significantly larger than that of hypertensive pregnant women (p=0.001). Similarly, the perimeter of blood vessels was significantly higher among normal women than hypertensive women 531.14±259.40 (p=0.002). The villous area was also significantly larger among normal pregnant women (p<0.001). On the other side, the number of blood vessels/villous area ratio was significantly higher in hypertensive women (p<0.001). The same was noted for the number of blood vessels which significantly higher among hypertensive when compared to normotensive patients (p=0.011).

The only two parameters which were not significantly different between normal and hypertensive pregnant women were the blood vessel area/villous area ratio (p=0.114) and blood vessel perimeter/villous area ratio (p=328).

	Hypertensive	Normal	P value
Blood vessel area	$14.7\ X10^{3}$	$107.3\ X10^{3}$	0.001
Blood vessel perimeter	531.14	1959.23	0.002
Villous area	$35.8X10^{4}$	$98.9X10^{4}$	<0.001
No. of blood vessels/Villous area**	$19.5X10^{-6}$	$3.7X10^{-6}$	<0.001
Blood vessel Area/Villous area	.0882	.1287	0.114
Blood vessel perimeter/Villous area	$2.6X10^{-6}$	$2291.1\ X10^{-6}$	0.328
Number of blood vessels**	6	3	0.011

Graphical illustration of the results

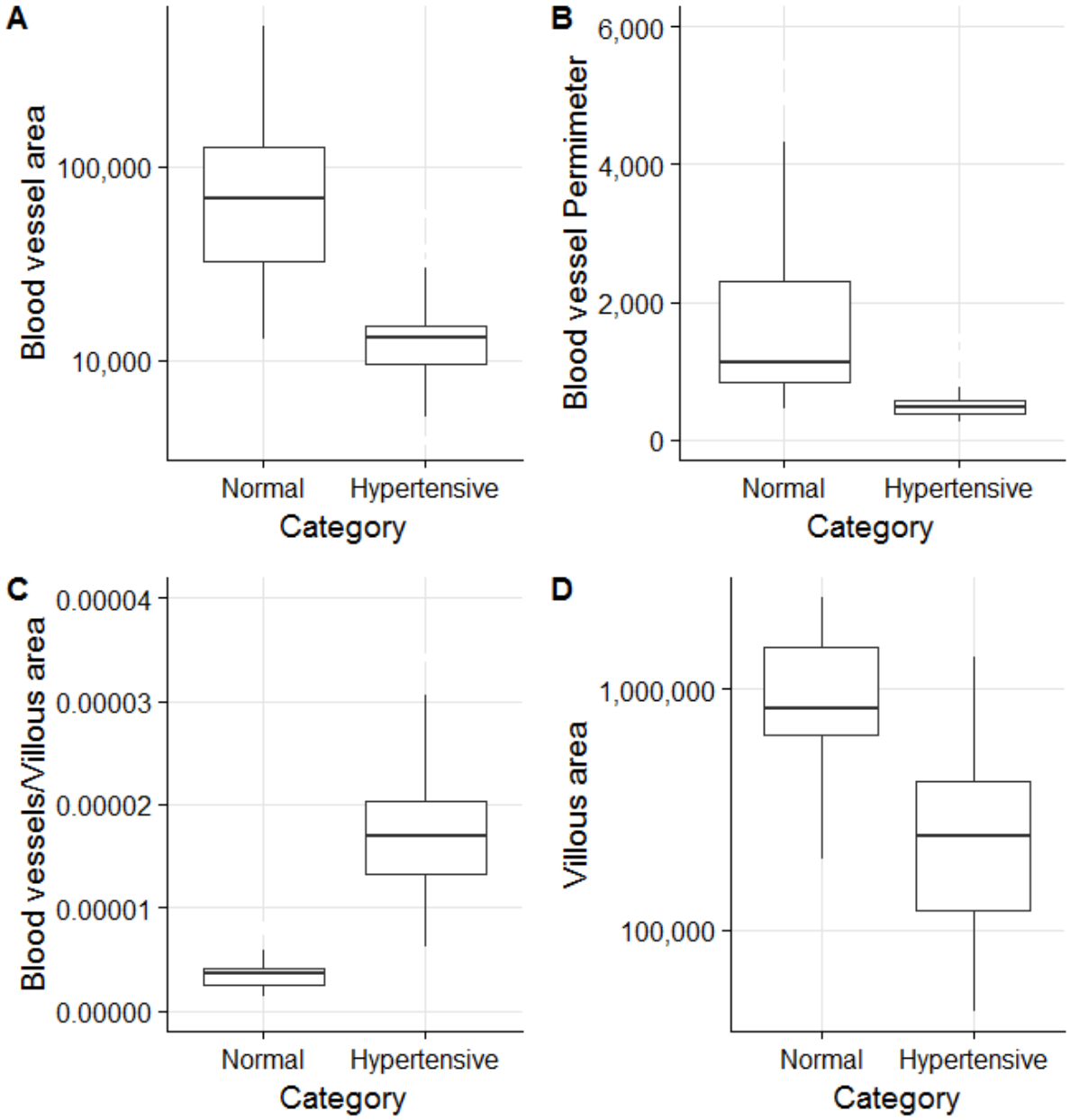

Figure 1. Comparison of various parameters across normal and hypertensive pregnant women
Y- axes were log transformed in Figures A and D

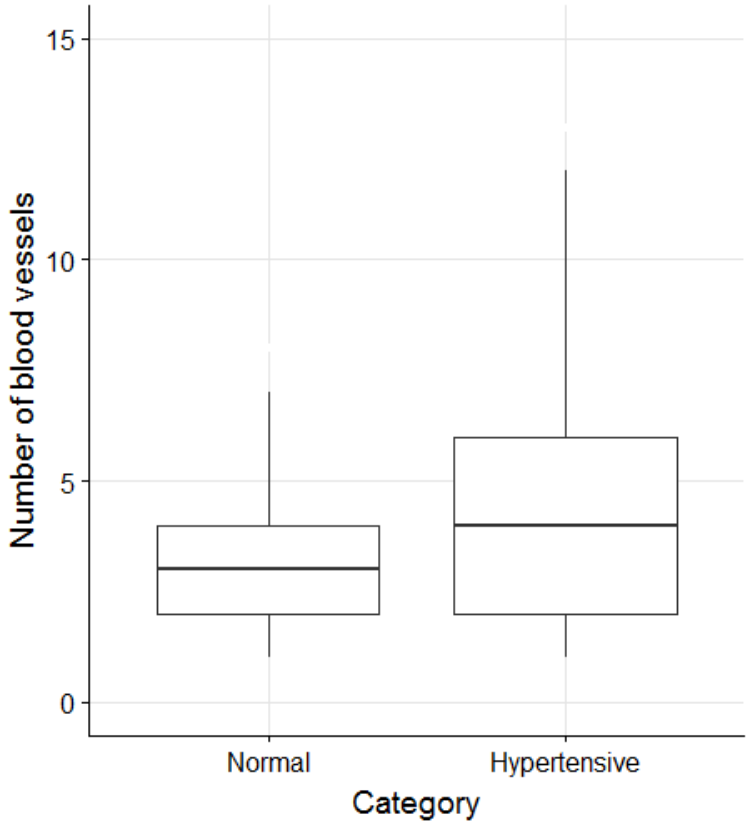

Figure 2. Number of blood vessels across normal and hypertensive pregnant women

DISCUSSION

Hypertension during pregnancy does not only hamper the physiological functions of the body but also induces structural deformities in various organs of the body including placenta, Thus having deleterious effects on both the expecting mothers and the fetus[20]. Particularly, structural damage to membranous part of any organ is detrimental to the physiological functioning of the body. Preeclampsia, the most prevalent disorder among hypertensive mothers, is linked to structural damages to the placenta[21]. Several inflammatory and non-inflammatory mediators are reported to play a major role as the preeclampsia in hypertensive mothers is concerned.

This study focused mainly on the structural and physical changes in placenta and especially chorionic villi of hypertensive expecting mothers. The deformities observed in the chorionic villi of the studied mothers corroborated with several previous reports and recommend adopting effective measures to control such changes mainly among hypertensive expecting mothers [22,23]. It is important to keep in mind that any deformity in the chorionic villi is linked to a decreased supply of essential nutrients to the fetus, and the observed changes in hypertensive mothers may lead to issues that had been explored earlier or to be defined by further controlled studies.

The present study showed that chorionic villi of hypertensive expecting mothers were significantly smaller when compared to normotensive expecting mothers. Potentially this could be due to the reason that higher pressure builds up within the placental capillaries damages the tissues surrounding the organ. Our study also confirmed the previous findings that indicated that damaged chorion villi were smaller in size when compared to the normal one [24,25]. Moreover, the peripheral cells of the villi become more and more asymmetric as the tissues within the cells collapse. Compared to the healthy chorion villi, the area will be smaller and irregular.

The reduction of the area of the chorion villi cannot be comprehensively explained through the observation of the blood vessels area. As suggested by other studies, there is a need to perform highly controlled studies on the perimeter of the blood vessels on the villi and the blood vessel number [26] and our study touched this unique avenue.

High blood pressure, as discussed by Al-Jameil[27], exerts its deleterious impacts on the placenta of expecting mothers. Naturally, this

abnormality was reflected in the chorionic villi which are directly projected from the placental membrane. Any deletions on the membranes of the placenta eventually delete the villi on the surface of the placenta leading to loss of the mass of area of the villi. Perhaps this can be used to explain the variance in terms of the cross section are or the perimeter of the chorion villi. The deleterious effects of the increased blood pressure have also been established to be responsible for the damage of the blood capillaries that surround the microvillus at the placenta. This explains the reduction in the area of the chorionic villi and the blood capillaries in the chorionic villi. A broad spectrum of lesions in the chorion villi have been observed amongst the hypertensive mothers. These villous lesions are attributable to the reduced maternal uteroplacental flow of blood in preeclampsia. As described by Lodhe andMane[4], this is due to maternal vasospasm. Such findings have been further affirmed by other studies suggesting that placental pathology, conventionally worsens as the blood pressure of an individual rises [27].

At the end of the pregnancy period. There is sloughing off of the villi near the end of gestation. This sloughing explains why the number of blood vessels per villous area was significantly higher in hypertensive women that the number of blood vessels per villous areaamong the control group[15]. It also explains the reason behind the significantly higher number of blood vessels among hypertensive pregnant women than those with normal blood pressure during pregnancy.

CONCLUSION

Hypertension is a global problem in both males and females. Particularly when occur during pregnancy and expose expecting mothers to the risk of developing eclampsia [28].

This quantitative morphometric study of the chorionic villi in hypertensive mothers showed that the high blood pressure during pregnancy reduced significantly the size of chorionic villi, blood vessels area, as well as blood vessels perimeter, predisposing hypertensive pregnant women to eclampsia and its associated ailments. Furthermore, hypertension increased the number of blood vessels and the number of blood vessels per villous ratio. It did not seem to affect either the blood vessel area per villous area ratio or the blood vessel perimeter per villous area ratio.

The damaging effect of the hypertension to the placenta exposes the chorionic villi several physical changes that eventually affect the functionality of these blood vessels. As established in this study, these structural changes in the chorionic villi are as a result of the high blood pressure build-up within the placenta. This study thus affirms emphatically that while the chorionic villi of the control group of mothers remain intact in terms of size, shape and even size, the pressure build-up at the placenta for the mothers with hypertension reduces the size of the chorionic villi thus predisposing them to eclampsia and associated ailments.

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