Original Research Article

The Evaluation of Placental Vascular Generation and Placental Apoptosis in Preterm and Post-date Placentae in Relation to Apgar Score at Birth

Zainab Hassan Hashim[*]   Huda Rasheed Kareem   Haider Abdulrasool Jaafar
College of Medicine, Al-Nahrain University, Baghdad, IRAQ

[*]E-mail: zainab_hashim@yahoo.com

Abstract

The placenta is a fetal organ with different functional values as a metabolic, excretory, and respiratory in addition to endocrine functions.To evaluate the vasculature and apoptosis of human placenta atdifferent ages (preterm and post-date) in relation to APGAR score of the newborn infants at birth.

A total of 50 normal human placentae,delivered by elective cesarean section,were used.These placentae were divided into 3 groups according to the gestational ageinto:(15) Preterm placentae, (20) Term placentae(as control) and (15) Post-date placentae.Regional placental vascular study was achieved by using latexcasting technique, while the detection of the apoptotic cells in the placental tissues was done via the insitu direct DNA fragmentation Assay.

The terminal villi of the preterm placentae group showed asignificant reduction in theirnumbersand lengthsP value(0.0035), (0.045), respectively, and no significant difference in their diametersin comparison to the control group. While the terminal villi of the post-date placentae revealeda significant increase in their numbers (P value <0.001) with no significant difference in their lengths and diameters.The terminal villi of preterm and post-dateplacentae revealed a significant reduction in the numbers of the apoptotic cells (P value (0.0053) and (0.0004) respectively.The APGAR score of preterm and postdate placentae reported significant decreasein their values (P value < 0.001 for both). As well as, there were significant changes in the numbers of apoptotic cells among these three groups related to theirApgar scores.

Conclusions: it was revealed that the vascular pattern of placenta by latex casting technique and apoptotic cells numbers by tunel test were reflectedfor both the maturity of placenta and physiological status of baby which was assessed by Apgar score.

Key words:placenta, Casting technique, Tuneltest, and Apgar score.

<u>الخلاصة</u>

المشيمة هي عضو الجنين ذات اهمية وظيفية مختلفة باعتبارها مصدر للتمثيل الغذائي، طرح الفضلات،كما انها تعمل كجهاز تنفسي بالإضافة إلى وظائف الغدد الصماء. أنها تحتوي على الأوعية الدموية للأم والجنين معا والتي تكون جنبا إلى جنب. فإنها تحصل على أعلى تدفق للدم نسبة الى اجهزة الجنين الاخرى (٤٠٪ من تدفق قلب الام) و في نهاية الحمل، تتنافس المشيمة مع الجنين لأخذ المواد الغذائية من الام حيث تستهلك جزء كبير من الجلوكوز والأكسجين.الهدف من الدراسة هو تقييم توالد الأوعية الدموية و الموت المنظم للخلايا حسب عمر المشيمة البشرية (قبل عمر ٣٧ أسبوعا،والأكثر من ٤٠ أسبوعا) وعلاقتهما مع تقييم الوليد عند الولادة(احراز ابغار).

تم استخدام عينه من خمسون مشيمة إنسان طبيعيه ولدت بولادة قيصريه وقسمت إلى ٣ مجاميع:المشيمة قبل عمر ٣٧ أسبوعا(الخدج), المشيمة بين ٣٨ إلى ٤٠ أسبوعا كمجموعة ضابطة والمشيمة أكثر من ٤٠ أسبوعا.والعينة لكل مجموعه تم تحديدها من موقع٢ سم من طرف المشيمة. والتقييم لكل مجموعة يتم بواسطة: تقنيه الصب لدراسة الأوعية الدموية المشيميه باستخدام الحقن بمادة اللاتكس,استخدام الصبغات الكيميائنسيجية مناعيه كعنصر قياس جهد التاكسد و صبغه TUNEL نوع التالقي (لقياس تجزؤ الحمض النووي مباشرة) .

في الدراسة الحالية، كان هناك انخفاض كبير في عدد وأطوال الزغابات في المشيمة الباكر مقارنة بالمشيمة المكتملة العمر كمجموعة ضابطة Pvalue (0.0035)، (0.045) على التوالي، في حين لا يوجد فرق كبير في قطر الزغب بين هاتين المجموعتين. أيضا كان هناك زيادة كبيرة في عدد الزوائد للمشيمة المتقدمة بالعمر ، في حيث لا يوجد فروق ذات دلالة إحصائية في أبعاد محطة الزغب للمشيمة المتقدمة في العمر. خلايا من محطة الزغب شهدت انخفاض كبير في عدد الخلايا التي تعاني من الموت المنظم في المشيمة الباكر والمشيمة المتقدمة بالعمر. ايضا وجد فرق كبير بين احراز ابغار من المشيمة الباكر والمشيمة المتقدمة بالعمر،كذلك كانت هناك تغييرات كبيرة في الخلايا الطرفية المتعرضة للموت المنظم من المشيمة الباكر، والمشيمة المتقدمة بالعمر والمشيمة المكتملة العمر مع احراز ابغار من نفس المجموعة،P value (0.0118)، (<٠.٠٠١)،(<٠.٠٠١) على التوالي. الدموية للمشيمة باستعمال تقنية القوالب وعدد خلاياي تعاني الموت المنظم والتي تكشف بطريقة صبغةTUNEL تعكس حالة نضج المشيمة والحالة الفسلجية للوليد.

الكلمات المفتاحية: المشيمة،تقنية الصب ، صبغهTUNEL، و احراز ابغار .

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Introduction

The exchange of nutrients and waste products between the maternal and fetal circulatory systems are regulated by the growth and function of the placenta that operates at optimum efficiency [1].Placenta contains maternal and fetal vascular beds that are juxtaposed. It receives the highest blood flow of any fetal organ (40% of cardiac output) and, toward the end of pregnancy, competes with the fetus for maternal substrate, consuming the major fraction of glucose and oxygen taken by the gravid uterus [2]. The age of the pregnancy can be divided into Full term birth whichis the delivery of an infant after 37 weeks of gestation, Preterm birth in which delivery of an infant weighing between 500 and 2500 gm after 20 weeks and before 37 completed weeks of gestation and post-date birth that persists beyond 40 weeks from the onset of the last normal menstrual period [3].APGAR score is a simple and replicable method that quickly and summarily assess the health of newborn children immediately at birth [4].
Aim of the study is to evaluate the vasculature of intermediate and terminal chorionic villi and syncytiotropho-blastapoptosis in different human placental ages (preterm, post-date) in relation to Apgar score at birth.

Materials and Methods

A sample of 50 normal human placentae that delivered by elective cesarean section all of them were primigravida aged from twenty to thirty four years old with BMI<30Kg/m² with no history of gestational diabetes and hypertention, smoking or drug taken.These samples were taken after informed consent that signed by all participants. The samples were divided into 3 groups according to the gestational age that confirmed by LMP and last U/S report in to (15)Preterm placentae with gestational age of 24 weeks to less than 37 weeks, (20) Term placentae with gestational age from 38 to 40 weeks(as control) and (15)Postdate placentae with gestational age more than 40 weeks to 42 weeks. The placental weight and birth weight were measured for the three groups. Mean ± SD of preterm placental and birth weight were (0.82±0.10) and (2.34±0.16) respectively, for term were (1.38±0.21) and (2.89±0.26) respectively, and for posterm were (1.42±0.23) and (3.23±0.26) respectively.
Placenta of each group will be studied by using: Casting technique: for regional placental vascular study. Placenta from the three groups selected for study by this technique, were milked off excess blood; washed thoroughly with tab water to remove hematomas and blood clots from both fetal and maternal surfaces, fetal membranes were excised from their attachments to placental margin. The umbilical cord vessels were (cleaned and recognized into two arteries and single vein), cannulated with intravascular cannula, tap water was pushed

into each cannula to perform irrigation of the placenta then the casting material was pushed into the cannula. The casting material that used was (latex) which is a polymer that infiltrates the tissue without changing its volume. The placenta then was transferred to 10% formaldehyde and kept overnight to perform hardening and fixation of placental tissue, each age group was then studied for intra cotyledon vascular pattern study: including vessels that enter the cotyledon tissue, and their generations into (terminal divisions of fetal capillaries in chorionic villi and their dimensions) in peripheral cotyledons.Tunel study utilizes Terminal deoxyribonucleotidyl-Transferase (TdT) to catalyze incorporation of fluoresce in -12-dUTP at the free 3'-hydroxyl ends of the fragmented DNA. The fluorescin-labeled DNA can then be observed by the fluorescent microscope. *In Situ* Direct DNA fragmentation Assay Kit provided by abcam code number ab66108 that used in this study was optimized for cytological examination.

For test slides routine steps of deparaffinization and rehydration were carried, followed by permeabilization of specimen this done by the use of protinase K (DAKO ready to use protinaseK) this step is specifically used for paraffin embedded tissues. Apoptotic cells were stained in bright yellow color; these were seen with non-apoptotic red stained cells and a green color seen at connective tissue and blood within the blood vessels.

The Apgar score is determined by evaluating the newborn baby on five simple criteria on a scale from zero to two, then summing up the five values thus obtained backronym (Appearance, Pulse, Grimace, Activity, and Respiration). Foreach criterion, the infant is given a score of 0, 1 or 2. The scores are added up and the total sum is their Apgar score. Apgar score was measured for each infant by aid of pediatrician.

Statistical Analysis

The results were studied by SPSS statistical tests using 2 tailed unpaired T tests and ANOVA test.

Results

The vascular generations in casted placental tissue that stained with H&E revealed a significant reduction in the numbers and lengths of terminal vessels of peripheral preterm placentae (4 ± 0.63), (3.5 ± 1.21) respectively,in comparison to the numbers and lengths of terminal vessels of peripheral term placentae (5 ± 0.63),(5 ± 1.81) respectively; P value (0.0035), (0.0456) respectively,where as no significant changes between diameter of terminal vessels of these two groups. On the other hands there was significant increase in the numbers of terminal vessels of peripheral postdate placentae (7 ± 0.77) and those of peripheral term placentae (5 ± 0.63), P value (< 0.001), whereas no significant differences in the lengths and diameters of terminal vessels of peripheral postdate placentae (5.5 ± 1.32), (0.03 ± 0.027) respectively, and those of peripheral term placentae (5 ± 1.81), (0.1 ± 0.16) respectively, as shown in table 1

Table1:comparison betweendimensions of arteries in terminal villi in casted model of preterm, term and postdate at peripheral placentae by unpaired T test

	Generations of vessels of cotyledon	Length of terminal vessel Mean ±SD	Diameter of terminal vessel Mean ±SD		Generations of vesselsof cotyledon	Length of terminal vessel Mean ±SD	Diameter of terminal vessel Mean ±SD
preterm	4±0.63	3.5± 1.21	0.1± 0.075	postdate	7±0.77	5.5 ±1.32	0.03± 0.027
term	5±0.63	5±1.81	0.1±0.16	Term	5±0.63	5±1.81	0.1±0.16
P value	0.003533	0.04564	1	P value	< 0.001	1	0.160177

Significant at p≤ 0.05

In comparison between numbers of apoptotic cells in the peripheral region of preterm placentae (19.5 ± 10.1) and term placentae (26 ± 8) there were significant differences between these two groups, P value (0.005).

Moreover, there was significant change between numbers of apoptotic cells in the peripheral region of postdate placentae (19.7 ± 5.9) and those of term placentae (26 ± 8), P value (0.0004), as illustrated in table 2.

Table 2:comparison between numbers of apoptotic cells in the peripheral region of placentae of preterm, term and postdate by tunel test by unpaired T test

	No. of apoptotic cells Mean ± SD		No. of apoptotic cells Mean ± SD
Preterm	19.5 ± 10.1	Postdate	19.7 ± 5.9
term	26 ± 8	Term	26 ± 8
P value	0.005395	P value	0.000405

Significant at p≤ 0.001

In table 3, there were significant differences between APGAR score of the preterm (4.8 ± 0.74),term (8.2 ± 0.74), andposterm infants (5.6 ± 0.916), P value ≤ (0.001)

Table 3:comparisons between APGAR score of the preterm, term and posterm infants by Anova test

Age groups	APGAR score Mean ± SD
Preterm	4.8 ± 0.74
Term	8.2 ± 0.74
Posterm	5.6 ± 916

Significant at p≤ 0.001

As demonstrated in table 4 there were significant changes between Apgar score of the preterm (4.8 ± 0.74), term (8.2 ± 0.74) and posterm infant (5.6 ± 0.916) withnumbers of apoptotic cells of the preterm (19.5 ± 10.1), term (26 ± 8)and postdate placentae (19.7 ± 5.9) respectively, P value (< 0.001) for all groups.

Table 4:comparison between Apgar score of the preterm, term and postdate infant and numbers of apoptotic cells of the preterm, term and post-date placentae by tuneltest respectively by unpaired T test

Age groups	No. ofapoptotic cells Mean ± SD	APGAR score Mean ± SD	P value
Preterm	19.5 ± 10.1	4.8 ± 0.74	< 0.001
Term	26 ± 8	8.2 ± 0.74	< 0.001
Postdate	19.7 ± 5.9	5.6 ± 0.916	< 0.001

Significant at p≤ 0.001

Discussion

Casting models of placental vascular structure provides knowledge of the placental vasculature, thus its critical to understand normal fetal growth and development as well as a variety of pregnancy- related diseases, such as PE and FGR [5].

Vascular pattern in this study showed:Each generation of chorionic vessels showed tendency to run into territories, each derived

from umbilical artery these territories were determined by blunt dissection of placenta according to their vascular supply.

In the present study showed that the chorionic vessels give to another generation called the intracotyled on vessels, these are smaller arteries branched from the chorionic vessels perfuse the cotyledons. Detailed investigation at the level of terminal artery measurements was done since it's the site of fetal-maternal exchange of gases, nutrition and wastes product as mentioned by Arts [6].

In the present study, there were significant reduction between numbers and lengths of terminal vessels of preterm placentae compared to term placentae. Differences in chorionic arterial branching patterns between term and preterm placentas arise from differences in placental size. Preterm placentas showed microvascular regression and extreme hypovascularity in peripheral areas [7]. Whereas no significant changes between diameter of terminal vessels of these two groups. The unique property of the uterine circulation during pregnancy, namely that the diameter of the vessels increases, rather than decreases was observed as they approach their target organ [8].Thus, by mid-pregnancy the diameter of the arcuate arteries exceeds the diameter of the uterine vessels, and by term the diameter some are twice the diameter[9].

In the present study, there was significant increase in the number of terminal vessels that enter cotyledon, whereas no significant changes between lengths and diameters of terminal vessels of peripheral postdate placentae and those of peripheral term placentae. This agrees with authors who found that the placenta doesnot undergo a true aging change during pregnancy [10]. The persisting belief in placental aging has been based on confusion between morphological maturation and differentiation and aging, a failure to appreciate the functional resources of the organ as a reason for increased neonatal mortality and low Apgar score [10].

In the current study, there were significant reduction in the number of apoptotic cells of peripheral region in preterm and postdate placentae compared to term group .This could be part of normal turnover of trophoblastic tissue occur during pregnancy. This is in agreement with other studies which are suggested that increased apoptotic cells formation is part of normal turnover of trophoblastic tissue of human placenta as apoptosis is a physiological event in normal placental tissue, and its amount changed throughout normal pregnancy[11].

Moreover, further studies reported that by the 3rd trimester the placenta is in a normoxic environment(greater than 6%) and little proliferation of cytotrophoblast is observed. Alteration in placental function by external factors such as hypoxia and reactive oxygenspecies can lead to significant increase in placental apoptosis [12].

In this study, there were significant differences between Apgar scores of the preterm and posterm infants compared with those of term groups.Apgar scores 7 and above are generally normal, 4 to 6 fairly low and 3 and below are generally regarded as critically low [13].

A low score on the one-minute test may show that the neonate requires medical attention [13] but does not necessarily indicate a long-term problem. An Apgar score that remains below 3 at later times—such as 10, 15, or 30 minutes—may indicate longer-term neurological damage, including a small but significant increase in the risk of cerebral palsy. However, the Apgar test's purpose is to determine quickly whether a newborn needs immediate medical care [10].

Furthermore, the Apgar score has its own limitations. A number of factors that may influence an Apgar score such as drugs, trauma, congenital anomalies, infections, hypoxia, hypovolemia, and preterm birth. Up to date, there are few consistent dataon the significance of Apgar score in preterm infants. Because elements of the score such as tone, color and reflex irritability partially

depend on the physiological maturity of the infants, situation may lead to a healthy preterm infant with no evidence of asphyxia receiving a low score only because of immaturity [14].

Conclusion

Preterm and postdate placentae are associated with low 5-minute Apgar score of newborn infants.

Casting of the placenta with intravascular latex injection provides excellent method to study placental vascular changes with aging.

Peripheral region that revealed an increase in the generations of blood vesselswhich showed maturity changes lately in pregnancy (in postdate group).

Apoptotic cells formation is part of normal turnover of trophoblastic tissue of human placenta as apoptosis is a physiological event in normal placental tissue, and its amount changed throughout normal pregnancy.

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