Iraqi JMS

Published by Al-Nahrain College of Medicine
P-ISSN 1681-6579
E-ISSN 2224-4719
Email: iraqijms@colmed-alnahrain.edu.iq
http://www.colmed-alnahrain.edu.iq
http://www.iraqijms.net
Iraqi JMS 2017; Vol. 15(2)

Histomorphometric and Endothelin-1 Immunohistochemical Study of Human Placental Villi Correlated with Umbilical Cord Coiling Index

Hayder J. Mubarak[1] *PhD*, **Sarah A. Ahmed**[2] *MSc*

[1]Dept. of Human Anatomy, College of Medicine, Al-Nahrain University, Baghdad, Iraq, [2]Dept. of Biology, College of Science, Baghdad University, Iraq

Abstract

Background Umbilical cord coiling index plays a role in predicting the pregnancy outcome and risk of low birth weight. Endothelin-1 binding sites exist on placental stem villi vessels and trophoblastic layer of the villi. Endothelin-1 is involved in regulation of the feto-placental circulation and specific trophoblastic functions.

Objective To investigate the profile of anti-endothelin-1 antibody expression in the human normal placental villi in relation to the coiling indices of the umbilical cords attached to these placentas.

Methods Normal human placentas were collected with inclusion criteria (full term newborns with normal perinatal outcome whose mothers were normal), and classified according to their umbilical cord coiling index into three groups: (N, H, and H), endothelin-1 marker used to investigate the localization of the endothelin-1 in the placental villi of each group.

Results There was a difference between the mean positivity percentage of endothelin-1 immunohistochemical reactivity in normocoiled group in comparison with hypercoiled group and hypocoiled group. There is a difference between the mean number of terminal villi in the three groups and in the perimeter of blood vessels.

Conclusion The pattern of endothelin-1 reactivity is associated with vasodilatation of the villous vascular bed to maximize the exchange function of the placenta as a physiological response to overcome the sequel of obstruction of umbilical vessels in both types of abnormal coiled cords during pregnancy.

Keywords Placental villi, umbilical cord, coiling index, normal pregnancy, endothelin-1, immunhistochemistry.

Citation Hayder J. Mubarak, Sarah A. Ahmed. Histomorphometric and endothelin-1 immunohistochemical study of human placental villi correlated with umbilical cord coiling index. Iraqi JMS. 2017; Vol. 15(2): 198-205. doi: 10.22578/IJMS.15.2.13

List of abbreviations: ET-1 = Endothelin-1, UCI = Umbilical cord coiling index

Introduction

The placenta is arguably the most important organ of the body, but paradoxically the most poorly understood [(1)].

Endothelin has three isoforms, endothelin-1 (ET-1), -2, and -3; endothelin-1 is the most potent and long-lasting vasoconstrictor known, being 100 times more potent than noradrenaline [(2)]. Mature ET-1 is a 21-amino acid peptide, and it is a main member of the endothelin peptide family. Endothelins has two receptors, ETA and ETB. ET-1 and -2 bind to ETA and ETB, while ET-3 only binds to ETB [(3)]. In the full-term human placenta, the immunoreactivity of ET-1 is local to endothelial cells of capillaries of placental microvilli, small-

and medium-sized arteries and veins in addition to placental syncytiotrophoblasts [4]. Several studies also confirmed a broad distribution of ET-1 expression in placentas throughout gestation in which, ET-1 expression is increased along with gestational age through the first trimester to full term [5]. Trophoblasts produce/release ET-1, human placenta also expresses ETA and ETB receptors. ETB is strongly expressed in placental trophoblasts and its expression is increased along with gestational age. In contrast, ETA is weakly expressed in placental trophoblasts [6]. Although the reason for the differential expression of ETA and ETB in placental trophoblasts is not clear, an in vitro binding assay has clearly shown that ET is capable of binding to the isolated trophoblast membranes. ET-1 is also involved in trophoblast invasion and differentiation of trophoblast cells isolated from first-trimester placentas [3].

The umbilical cord is the lifeline that attaches the placenta to the fetus [7]. The umbilical cord is not directly linked to the mother's circulatory system, instead it joins the placenta [8]. The attachment of the umbilical cord is generally eccentric, and to a less extent central, with a rare variety of being marginal. However, the umbilical cord, occasionally, is inserted into the chorionic membranes, to the outer surface placenta and this formal is known as velamentous cord insertion [9]. The coiling of the umbilical vessels grows as early as 28 days after conception and is present in about 95% of fetuses by 9 weeks of conception [10]. Umbilical cord coiling index (UCI) is well-defined as the total number of coils divided by the total length of the cord in centimeters [11]. The difference in coiling was described as an antenatal marker identifying fetus at risk. Majority of the studies on UCI had been done postnatally. Although UCI can be calculated antenatally by ultrasonography, limited data is available as to its accuracy [12]. The umbilical cords with abnormal coiling, were studied and showed that fetal death, fetal intolerance to labor, intrauterine growth retardation and choriomnionitis were associated with abnormal coiling [13]. In these abnormal cords, umbilical vein thrombosis, chorionic vessels thrombosis and umbilical cord structure was observed which can induce chronic (in the form of growth retardation) or acute (labor intolerance and fetal death) effects on fetal health [14].

This study was done to correlate the morphometric and the endothelin-1 immunohistochemical criteria of the placental tissues with the umbilical cord coiling index.

Methods

A sample of 30 normal human placentas were obtained from a full term, healthy (have no hypertension, diabetes mellitus, any other gynecological problem or major diseases), nonsmoker and normal vaginal delivered women admitted to the Obstetric Ward of Al-Bnouk Private Hospital. The gestational age was determined by ultrasound examination with the aid of history taken from those women prior to delivery to ensure a full-term pregnancy, which was measured as about 40±2 week, from the first day of the last menstrual period. The umbilical cords coiling indices of these placentas were examined. After being expelled, each of the placentas with their umbilical cords collected were examined grossly to ensure that they have an eccentric cord insertion with no abnormality or infraction, then the umbilical cord length and the umbilical coiling index were calculated. The placental samples were divided into three groups: (10 placentas with normocoiled coiling index, 10 placentas with hypercoilied coiling index, and 10 placentas with hypocoilied coiling index). Samples of placentas were selected from peripheral region (2 cm from placental margin near cord insertion). Placental samples were cut through the whole thickness of the placenta from fetal to maternal surfaces. Only the fetal sides of these sections were included in this study. Paraffin blocks were prepared for histological, and

immunohistochemical study [15]. Sections were cut at (5 µm) thickness serially.

The following variables were assessed for each section obtained from the regions of the three types of a sample.

1. The number of terminal villi per high power field.
2. The mean perimeter of the blood vessels in the terminal villi per high power field.

The 2nd variable was assessed by morphometric-image analysis system namely, Image J. After the random selection of a field has been done, the measurement of the perimeter of nine different sizes intravillous blood vessels; three large, three medium and three small vessels selected indiscriminately for each section, were obtained. So, each section will have 9 values for this parameter and later on the mean of these values will be obtained to be considered as the perimeter of blood vessels in terminal villi per power field which is calculated in µm. Immunohistochemical staining for Anti-ET-1 antibody was provided by USBiological with code no.15111257. It contains small molecule of synthetic endothelin-1 conjugated to bovine serum albumin, it is mouse monoclonal anti-human endothelin-1. It used with immunohistochemistry detection kit from Santa Cruz Biotechnology, Inc, evaluation of anti- ET-1 marker was done with aperio scope image analysis software v11.1.2.760, ten fields were randomly selected for each group (normocoiled, hypercoiled, and hypocoiled) and examined at power 100X to measure the percentage of the positive pixels. The percentage of the positive pixels count (number of positive excluded the weak positive pixels/number of total), is selected as it measures the anti- ET-1 in terminal villi tissue.

Statistical analysis of data was done by using Analysis of variance (ANOVA) test applied to look for statistical significance between the means of different groups for each variable. The statistical analysis of data done by using the SPSS software version 20 setup in a personal computer.

Results

ET-1 reactivity

There was a difference between the mean positivity percentage of ET-1 in normocoiled group in comparison with hypercoiled group and hypocoiled group. The highest positivity percentage was found in normocoiled group compared to hypercoiled group and hypocoiled group. Slightly higher value in hypocoiled group was observed in comparison to hypercoiled group. Table-1 shows the mean positivity percentage of ET-1 in terminal villi in the three groups. Table-2 shows that the difference between mean positivity percentage of ET-1 in terminal villi in the three groups was statistically significant (P value < 0.05).

The mean number of the terminal villi

Table 3 shows the mean number of terminal villi in the three groups. There is a difference between the mean number of terminal villi in the three groups. Table-4 shows that the difference between mean numbers of terminal villi in the three groups was statistically significant (P value < 0.05).

Table 1. The mean positivity percentage of ET-1 in termimal villi among the three groups

Groups	Mean	No.	Std. Deviation
Normocoiled	80.86	10	9.45
Hypercoiled	20.56	10	3.07
Hypocoiled	30.78	10	2.83

Table 2. ANOVA test between the mean positivity percentage of ET-1 in the terminal villi among the three groups

Groups	Mean positivity percentage of ET-1	p- value
Normocoiled	80.86	
Hypercoiled	20.56	0.000
Hypocoiled	30.78	

* Significance was accepted at p- value < 0.05

Table 3. The mean number of the terminal villi among the three groups

Groups	Mean	No.	Std. Deviation
Normocoiled	13.94	10	0.86
Hypercoiled	12.01	10	1.17
Hypocoiled	11.06	10	1.59

Table 4. ANOVA test between the mean number of the terminal villi among the three groups

Groups	Mean number of the terminal villi	p- value
Normocoiled	13.94	
Hypercoiled	12.01	0.041
Hypocoiled	11.06	

* Significance was accepted at p- value < 0.05

The mean blood vessels perimeter in terminal villi

Table 5 shows the mean perimeter of the blood vessels of terminal villi in the three groups. There is a difference between the mean perimeter of blood vessels in hypocoiled group in comparison with that of hypercoiled group and normocoiled group. The highest value of perimeter of blood vessels was found in hypocoiled group compared to that of hypercoiled group and normocoiled group, a slightly higher value in hypercoiled group was observed in comparison to normocoiled group. Table-6 shows that the difference between mean perimeter of blood vessels of the terminal villi in the three groups was statistically significant (P value < 0.05).

Discussion

Anti-endothelin-1 immunohistochemical of placental villi reactivity in response to umbilical cord coiling

The positive anti-endothelin-1 reactivity was seen in most of villous vascular endothelial cells of normocoiled group, this reactivity was weaker in these placental tissues of hypercoiled and hypocoiled groups.

Table 5. The mean perimeter of blood vessels (µm) of the terminal villi among the three groups

Groups	Mean	No.	Std. Deviation
Normocoiled	81.15	10	9.21
Hypercoiled	89.70	10	14.23
Hypocoiled	103.81	10	17.57

Table 6: ANOVA test between the mean number of the terminal villi among the three groups

Groups	Mean perimeter of blood vessels (μm) of the terminal villi	p- value
Normocoiled	81.15	
Hypercoiled	89.70	0.041
Hypocoiled	103.81	

* Significance was accepted at p- value < 0.05

ANOVA of the counted mean values of endothelin-1 antibody reactivity was evaluated collectively in groups of terminal villi using the Aperio Image Scope software. The counting of the mean value of the positivity percentage of normocoiled group was found to be significantly higher than that with hypercoiled and hypocoiled groups. Differential localization of endothelin (A) and (B) binding site in human placenta was investigated [16] on the bases that ET may have a role in the placenta by acting through its receptors to affect foetoplacental blood flow and other aspects of placental functions. It was concluded that specific high-density ET-1 binding sites were localized throughout the villous tree. Moderate to low density binding was found in the extravillous and villous trophoblast respectively. ET-A binding sites were found to be predominated in blood vessels in distal regions of villous tree. The proportion of ET-A/ET-B receptors varies between different regions of the placental vascular bed. ETB receptors have previously been considered only to occur on some vascular endothelial cells and may mediate vasodilatation [17]. ET-A receptors mediate the ET-induced arterial vasoconstriction [18]. It was established that hypercoling and hypocoiling of the cord could be associated with poor perinatal outcome. Both cord types were suggested to be less flexible or more prone to kinking and torsion, these criteria make them less tolerant to withstand the stress of pregnancy and labour resulting in the compression of these vessels [19,20]. Accordingly, the significant decrease of anti-endothelin-1 reactivity of the terminal villi investigated in the results of this study could be related to the need of eliminating the vasoconstriction effect of endothelin on the ET-A binding sites predominated in blood vessels in distal regions of villous tree. This reactivity is consequently associated with maximum vasodilatation of the villous vascular bed to maximize the exchange function of the placenta as a physiological response to overcome the sequel of obstruction of umbilical vessels in both types of abnormal coiled cords during pregnancy. This conclusion is supported by the reports that adaptations of fetal tissues (as the placenta) occur in response to failure of nutrients supply to overcome the deficit in fetal demands [21]. The results of this study agree with the finding of Jewsbury et al. (2007) [22] that vascular adaptation occurs with altered hemodynamic conditions arise due to variability of vasoconstrictor substances as a result of a pathophysiologic state. In support to this conclusion was the report that the increased ET associated with preeclampsia as supposed to be involved in impaired placental blood flow [23]. The relative density or proportion of vascular and villous ET receptor subtypes in placentae were similar in placentae obtained from pregnancies exhibiting preeclampsia compared with normal term controls [24].

Placental morphometry in relation to coiling index

The villous size, perimeter and vascularity were found be decreased in association with diseases decreasing the surface area available for gas exchange per villus [25]. Also, it was concluded that placental morphometric measures related to materno-fetal exchanges

as area and number of terminal villi and their respective villous vessels varied in associated with maternal diseases [26]. The morphometric evaluation of the placental tissues involved in this study was directed toward investigating the possible changes in parameters that could be considered as an accommodative response of placenta to improve the exchange function with hypercoiled and hypocoiled cord. The number of the terminal villi was found in this study to be significantly higher in normocoiled group compared to that of hypercoiled and hypocoiled groups. The terminal villi of the hypercoiled group showed statistically significant higher number compared to hypocoiled group. It was reported that placental nutrient transfer is regulated by blood flow and morphometric characteristics of the placenta [27]. This report is supportive to the morphometric results, the higher number of TV in normocoiled group found in this study is a normal morphometric criterion indicating normal placental transfer that is associated with normal blood flow in the placenta with normocoiled cords. The defected blood flow in the placenta with abnormal coiled cords is associated with defected placental transfer function, which is associated with the less number of placental villi. Hypocoiling was found to be associated with larger perimeter of villous vessels in the morphometric results of this study, statistically non-significant large perimeter of the villous blood vessels as found in hypercoiled group compared to normocoiled group. In conclusion, it seems that dilatation of the terminal villous blood vessels is a criterion associated with abnormal umbilical cord coiling. In support, it was hypothesized that abnormal placental morphology is an indication of deformed uteroplacental and foetoplacental vascular pathology that associated with negative effects on placenta and fetal development [28]. The results of this study illustrated that the consequences of altered umbilical cord coiling index on the placenta is an attribute for the remedial effect of the placenta in response to hypercoiling in form of both maintenance of the number of villi as possible and vasodilatation of villous blood vessels. The hypocoiled group showed vasodilation of the villous capillaries only, vasodilatation is more marked in hypocoiled group than hypercoiled group. It seems that vasodilation is the only mechanism to increase the uteroplacental blood flow in hypocoiled. Hypercoiling causes distress to fetoplacental blood flow in association with external compression, while hypocoiling was considered as a representation for an intrinsic abnormal development. The hypercoiling with external compression on the umbilical vessels reduces the blood flow to the placenta, a phenomenon that is not taking place in hypocoiled cord. This fact may be the attribute to the larger lumen of the placental villi vasculature found in this study, voluminous blood flow in the villous capillaries of the hypocoiled group (compared to hypercoiled group) is added to the vasodilation effect of diminished endothelin-1 receptors resulting in more capacious vascular lumen.

The conclusion interpreted in this study agree with the arguments that abnormal cord coiling (hypocoiling or hypercoiling) is not sufficient to cause adverse fetal outcomes, instead, placental changes could be involved. This conclusion is supported by the contribute of previous study [29].

Acknowledgments

Regard and gratefulness should be presented to the staff members Department of Human Anatomy at the College of Medicine, Al-Nahrain University for their assistance and cooperation.

Authors contributions

Ahmed: The M.Sc. candidate performing the laboratory research work and performing production of the results. Dr. Mubarak: The advisor of the M.Sc. research performing the interpretation of the results.

Conflict of interest

The authors disclose no any financial and personal relationships with other people or organizations that inappropriately influence (bias) our work.

Funding

The research working funding was provided by the authors.

References

1. Burton G, Fowden A. The placenta: a multifaceted, transient organ. Philos Trans R Soc Lond B Biol Sci. 2015; 370(1663): 20140066. doi: 10.1098/rstb.2014.0066.
2. Alonso D, Radomski W. The nitric oxide-endothelin-1 connection. Heart Fail Rev. 2003; 8(1): 107-15.
3. Wang Y. Vascular biology of the placenta. In: Colloquium series on integrated systems physiology: from molecule to function. Morgan & Claypool Life Sciences; 2010. p. 1-98. http://dx.doi.org/10.4199/C00016ED1V01Y201008ISP009.
4. Bilban M, Barth S, Cervar M, et al. Differential regulation of endothelin secretion and endothelin receptor mRNA levels in JAR, JEG-3, and BeWo choriocarcinoma cell lines and in human trophoblasts, their nonmalignant counterpart. Arch Biochem Biophys. 2000; 382(2): 245-52. doi: 10.1006/abbi.2000.2016.
5. Barros J, Bairos V, Baptista M, et al. Immunocytochemical localization of endothelin-1 in human placenta from normal and preeclamptic pregnancies. Hypertens Pregnancy. 2001;20(1):125-37. doi: 10.1081/PRG-100104179.
6. Chakraborty C, Gleeson L, McKinnon T, et al. Regulation of human trophoblast migration and invasiveness. Can J Physiol Pharmacol. 2002; 80(2): 116-24. https://doi.org/10.1139/y02-016.
7. Mathew J. Timing of umbilical cord clamping in term and preterm deliveries and infant and maternal outcomes: a systematic review of randomized controlled trials. Indian pediatr. 2011; 48(2): 123-9.
8. Benirschke K, Burton G, Baergen R. Pathology of the human placenta. Berlin Heidelberg: Springer; 2012 p. 309-75.
9. Rocha J, Carvalho J, Costa F, Meireles I, et al. Velamentous cord Insertion in a singleton pregnancy: an obscure cause of emergency cesarean - a case report. Case Rep Obstet Gynecol. 2012; 2012: 308206. doi: 10.1155/2012/308206.
10. Chitra T, Sushanth Y, Raghavan S. Umbilical coiling index as a marker of perinatal outcome: an analytical study. Obstet Gynecol Int. 2012; 2012: 213689. doi: 10.1155/2012/213689.
11. Mittal A, Nanda S, Sen J. Antenatal umbilical coiling index as a predictor of perinatal outcome. Arch Gynecol Obstet. 2015; 291(4): 763-8. doi: 10.1007/s00404-014-3456-5.
12. Ezimokhai M, Rizk D, Thomas L. Abnormal vascular coiling of the umbilical cord in gestational diabetes mellitus. Arch Physiol Biochem. 2001; 109(3): 209-14. doi: 10.1076/apab.109.3.209.11593.
13. Diwakar RK, Naik MM, Jindal MM. Umbilical cord coiling: case report and review of literature. BJR Case Rep. 2017; 2: 20150152. http://dx.doi.org/10.1259/bjrcr.20150152.
14. Machin G, Ackerman J, Gilbert E. Abnormal umbilical cord coiling is associated with adverse perinatal outcomes. Pediatr Dev Pathol. 2000; 3(5): 462-71. doi: 10.1007/s100240010103.
15. Suvarna K, Suvarna K, Layton C, et al. Bancroft's Theory and practice of histological techniques. 7th ed. Churchill Livingstone; 2013. eBook ISBN: 9780702058172.
16. Min X, Kang W, Ya-Jun W, et al. Expression of endothelin-1 and endothelin receptors mRNA in placental villous from normal pregnancy induced hypertension. Med Life Sci. 2006; 8(1): 49-51.
17. Rauh A, Windischhofer W, Kovacevic A, et al. Endothelin (ET)-1 and ET-3 promote expression of c-fos and c-jun in human choriocarcinoma via ETB receptor-mediated Gi-and Gq-pathways and MAP kinase activation. Br J Pharmacol. 2008; 154(1): 13-24. doi: 10.1038/bjp.2008.92.
18. Mazzuca MQ, Khalil RA. Vascular endothelin receptor type B: structure, function and dysregulation in vascular disease. Biochem Pharmacol. 2012; 84(2): 147-62. doi: 10.1016/j.bcp.2012.03.020.
19. Ohno Y, Terauchi M, Tamakoshi K. Perinatal outcomes of abnormal umbilical coiling according to a modified umbilical coiling index. J Obstet Gynaecol Res. 2016; 42(11): 1457-63. doi: 10.1111/jog.13092.
20. Jessop F, Lees C, Pathak S, et al. Umbilical cord coiling: clinical outcomes in an unselected population and systematic review. Virchows Arch. 2014; 464(1): 105-12. doi: 10.1007/s00428-013-1513-2.
21. Godfrey RW, Dodson RE. Effect of supplemental nutrition around lambing on hair sheep ewes and lambs during the dry and wet seasons in the US Virgin Islands. J Anim Sci. 2003; 81(3): 587-93.
22. Jewsbury S., Baker P. and Wareing M. Relaxation of human placental arteries and veins by ATP-sensitive potassium channel openers. Eur J Clin Invest. 2007; 37(1): 65-72. doi: 10.1111/j.1365-2362.2007.01749.x
23. LaMarca B. Endothelial dysfunction; an important mediator in the Pathophysiology of Hypertension during Preeclampsia. Minerva Ginecol. 2012; 64(4): 309-20.
24. Dieber-Rotheneder M, Stern C, Desoye G, et al. Up-regulation of the endothelin receptor A in placental tissue from first trimester delayed miscarriages. J Soc Gynecol Investig. 2006 Sep;13(6):435-41. doi: 10.1016/j.jsgi.2006.05.006.
25. Chaikitgosiyakul S, Rijken MJ, Muehlenbachs A, et al. A morphometric and histological study of placental malaria shows significant changes to villous

architecture in both Plasmodium falciparum and Plasmodium vivax infection. Malar J. 2014; 13: 4. doi: 10.1186/1475-2875-13-4.

26. Calderon IM, Damasceno DC, Amorin RL, et al. Morphometric study of placental villi and vessels in women with mild hyperglycemia or gestational or overt Diabetes Res Clin Pract. 2007; 78(1): 65-71. DOI: 10.1016/j.diabres.2007.01.023.

27. Fowden AL, Ward JW, Wooding FP, et al. Programming placental nutrient transport capacity. J Physiol. 2006; 572(Pt 1): 5-15. doi: 10.1113/jphysiol.2005.104141.

28. Yampolsky M, Salafia CM, Shlakhter O, et al. Modeling the variability of shapes of a human placenta. Placenta. 2008; 29(9): 790-7. doi: 10.1016/j.placenta.2008.06.005.

29. Predanic M, Perni S, Chasen S, et al. Ultrasound evaluation of abnormal umbilical cord coiling in second trimester of gestation in association with adverse pregnancy outcome. Am J Obstet Gynecol. 2005; 193(2): 387-94. doi: 10.1016/j.ajog.2004.12.092.

Correspondence to Sarah A. Ahmed
E-mail: saabio248@yahoo.com
Received Nov. 14th 2016
Accepted Mar. 30th 2017