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)

The Profile of Matrix Metalloproteinase-9 in Relation to Coiling Index of Human Umbilical Cord

Hiba A. Abdul Sattar[1] *MSc,* **Hayder J. Mubarak**[2] *PhD*

[1]Dept. of Medical Laboratory Techniques, Osol Aldeen University College, Baghdad, Iraq, [2]Dept. of Human Anatomy, College of Medicine, Al-Nahrain University, Baghdad, Iraq

Abstract

Background Fetal blood flow through the umbilical cord vessels associated with abnormal coiling of the cord can have serious deleterious effects on the health of the fetus and newborn. Matrix metallopeptidase-9 (MMP-9) is a class of enzymes that are involved in the degradation of the extracellular matrix collagen and other proteins.

Objective To investigate the profile of MMP-9 immunohistochemical reactivity in the umbilical cord with variable coiling indices.

Methods In this study, 60 umbilical cords with inclusion criteria (full term newborns with normal perinatal outcome whose mothers were normal) collected from labor rooms of Ibn Al-Balady Hospital in Baghdad. The cords were used for immunohistochemistry matrix metaloprotenase-9 study.

Results The results of Immunohistochemical study showed variability in mesenchymal tissue reactivity among the three groups of the umbilical cords in different regions (sub amniotic region, perivascular region and central region) of the umbilical cords. The results obtained were evaluated by using the Aprio image scope analysis software.

Conclusion The immunohistochemical reactivity could indicate that the MMP-9 localization has role in degradation of collagen and proteins to maintain a harmless perivascular pressure in association with hypercoiling and hypocoiling of the cord and that could preserve the normal vascular blood flow to a certain limit.

Keywords Umbilical cords, human, coiling index, matrix metalloprotienase-9, normal pregnancy, immunohitochemistry.

Citation Hiba A. Abdul Sattar, Hayder J. Mubarak. The profile of matrix metalloproteinase-9 in relation to coiling index of human umbilical cord. Iraqi JMS. 2017; Vol. 15(2): 165-174 . doi: 10.22578/IJMS.15.2.9

List of abbreviations: ECM = Extracellular matrix, MMPs = Matrix metalloproteinase

Introduction

Abnormalities of the umbilical cord can lead to a major fetal morbidity and mortality [(1)]. The umbilical cord is protected by amniotic fluid, Wharton's jelly, helical patterns, and coiling of vessels [(2)]. The umbilical vessels' coiling develops as early as 28 days after conception and is existent in about 95% of fetuses by 9 weeks of pregnancy [(3)].

Umbilical cord coiling index can be defined as number of coils per one centimeter of length of the cord. Normal index is one coil for a length of five centimeters. If the numbers of coils are more per centimeter it is called as hypercoiled and less than it is called as hypocoiled umbilical cord [(4)].

Most published studies define hypocoiled (undercoiled) or hypercoiled (overcoiled) umbilical cords as below the 10th and above the 90th percentiles, respectively [5,6].

Umbilical coiling may serve to improve cord hemodynamics, as arterial pulsations transmitted to the vein may assist pump blood back up the cord from the placental capillary bed. Both abnormally coiled cords (hypercoiled and hypocoiled cords) are associated with an increased risk of adverse perinatal outcome. Abnormal cord coiling in its two shapes has been reported to be associated more frequent with preeclampsia and gestational diabetes [2,5].

Elastin and collagen are the important component of extracellular matrix (ECM). It is known that activities of collagenolytic enzymes like matrix metalloprotenase-9 are an essential factor regulating the degradation of collagen [7]. Matrix metalloproteinase (MMPs) are able to break down all components of ECM. They are involved in many remodeling processes of the connective tissue [8,9].

MMPs constitute a family of enzymes with 25 members identified to date, which are all extracellular of two subgroups: some membrane-bound or type MT-MMPs which are anchored to the plasma membrane, six MMPs have been assigned to this subgroup, and predominantly pericellular or soluble MMPs (chiefly 1, 2, 3, 8 and 9) in that gelatinase A is (MMP-2) while gelatinase B is (MMP-9). MT1-, MT2-, MT3-, MT4-, MT5- and MT6-MMP (MMP-14, -15, -16, -17, -24 and -25 respectively) [10]. MMPs are calcium and zinc-binding endopeptidases i.e. requiring Ca^{2+} and Zn^{2+} for their enzymatic activity [11].

Matrix metallopeptidase-9 (MMP-9), also known as 92 kDa type IV collagenase, 92 kDa gelatinase or gelatinase B (GELB), is a matrixin, a class of enzymes that belong to the zinc-metalloproteinases family involved in the degradation of the extracellular matrix [12].

In this study, the profile of MMP-9 immunohistochemical reactivity in the umbilical cord with variable coiling indices will be investigated.

Methods

This study is a cross sectional study, done in the period between (February 2016 and October 2016) in the Department of Human Anatomy, College of Medicine, Al-Nahrain University.

One centimeter piece of twenty (normociled, hypocoiled and hypercoiled) umbilical cords was taken from a full term, healthy (not have hypertension, diabetes mellitus, any other gynaecological problems or major diseases, nonsmoker and normal vaginal delivered) women who admitted to the Obstetric Ward of Ibn Al-balady Hospital in Baghdad and transferred immediately to be fixed with 10% formalin and processed for paraffin blocks.

The total number of the cords used in this study was 60; these include 20 cords for each of the three coiling indices (namely; hypercoiled, hypocoiled and normocoiled cords).

Each of the umbilical cords collected was examined grossly for its length, coiling pattern (presence or absence of segmental variability in the coiling density) and the umbilical coiling index was calculated.

Measurement of the length done by tape measure with consideration of the umbilical stump of the cord that remains attached to the umbilicus of the baby.

An umbilical coiling index of less than (0.17) and more than (0.37) was accepted as being hypocoiled and hypercoiled respectively, between (0.17 and 0.37) was normocoiled [1].

Serial paraffin sections of 4-5 µm thickness were cut using the electrical microtome and set on positive charged slides (Fisher Scientific, USA) used for the immunohistochemical studies. In the present study, (Expose Mouse and Rabbit Specific HRP/DAB detection IHC Kit (ab80436) from (abcam, UK) was used. Slides; for three types of umbilical cords, immunohistochemical studies had been examined using a light microscope (Olympus BX41, Japan). Assessment of immunohistochemical staining was achieved by applying Aperio positive pixel count algorithms program (from Aperio Image Scope software v11.1.2.760 (Aperio Technologies Inc, USA),

which can be used to analyze digital slides. The Aperio positive pixel count algorithm can be used to quantify the amount of a specific stain present in a slide image. These inputs have been pre- configured for brown color quantification in the three intensity ranges (weak positive, positive, and strong positive).

For statistical analyses, we used the SPSS software program software, version 20. The data are expressed as mean and standard error of the mean (SEM). Analysis of variance (ANOVA) was used to examine the statistical significance differences between the mean percentages of the subamniotic, perivascular and central region (for normocoiled, hypercoiled and hypocoiled cords). Also, it was used for testing the statistical significance differences between the mean percentages of the subamniotic, perivascular and central region within the same type of umbilical coiling cords. P value ≤ 0.05 denoted a statistically significant difference.

Results

Analysis of variance (ANOVA) of the counted mean values (percentage of positive reactivity) of matrix metalloproteinase-9 antibody immuno-histochemical reactivity in the mesenchymal tissue of the umbilical cord obtained by the application of the Aperio positive pixel count algorithms program (Aperio Image Scope software) showed a significantly more intensity in hypercoiled and hypocoiled cords compared to the reactivity in normocoiled cords ($p<0.05$). This significant variability was considered when comparing the subamniotic, perivascular, and central regions of each of umbilical cords.The light microscopic examination of the immunohistochemical reactivity of the umbilical cords did not show accurate measurable different between the regions of different coiling umbilical cords (Figure 1).

The counting of the mean value of the number of positive pixels in the normocoiled cords was significantly higher in the subamniotic region compared to perivascular and central regions. Non-significant variability was obtained between the counted mean values of perivascular and central regions of the noromocoiled cords (Figure 2).

The counting of the mean value of the number of positive pixels in the hypocoiled cords showed significant variability between the subamniotic, perivasclar, and central regions. The highest intensity was shown in perivascular region, and the least was in the central region (Figure 3).

The counted mean value of the number of positive pixels in hypercoiled cords was significantly higher in the perivascular region compared to both subamniotic and central regions. Non-significant variability was shown between the values of subamniotic and central regions of these cords (Figure 4).

The mean values of MMP-9 antibody reactivity in the subamniotic region showed highest intensity in hypocoiled cords and the least in normocoiled cords (Figure 5).

The counted mean values of MMP-9 antibody reactivity in the mesenchyme of the perivascular region obtained showed highest intensity in hypercoiled cords and the least in normocolied cords (Figure 6).

The mean values of the reactivity in the mesenchyme of the central region showed highest intensity in hypercoiled cord and the least in normocoiled cords (Figure 7).

Discussion

The Dynamic of Adverse Effect of Hypercoiling of Umbilical Cord

It was concluded that hypercoiling results in mechanical compression and diminished the blood flow in umbilical vessels as a result of the forceful obstruction of the umbilical circulation. Hypoxia subsequently resultant is described as (cord accident), that represent unconventional behavior with an outcome similarly happening in cases of cord prolapsed or true knots [13,14].

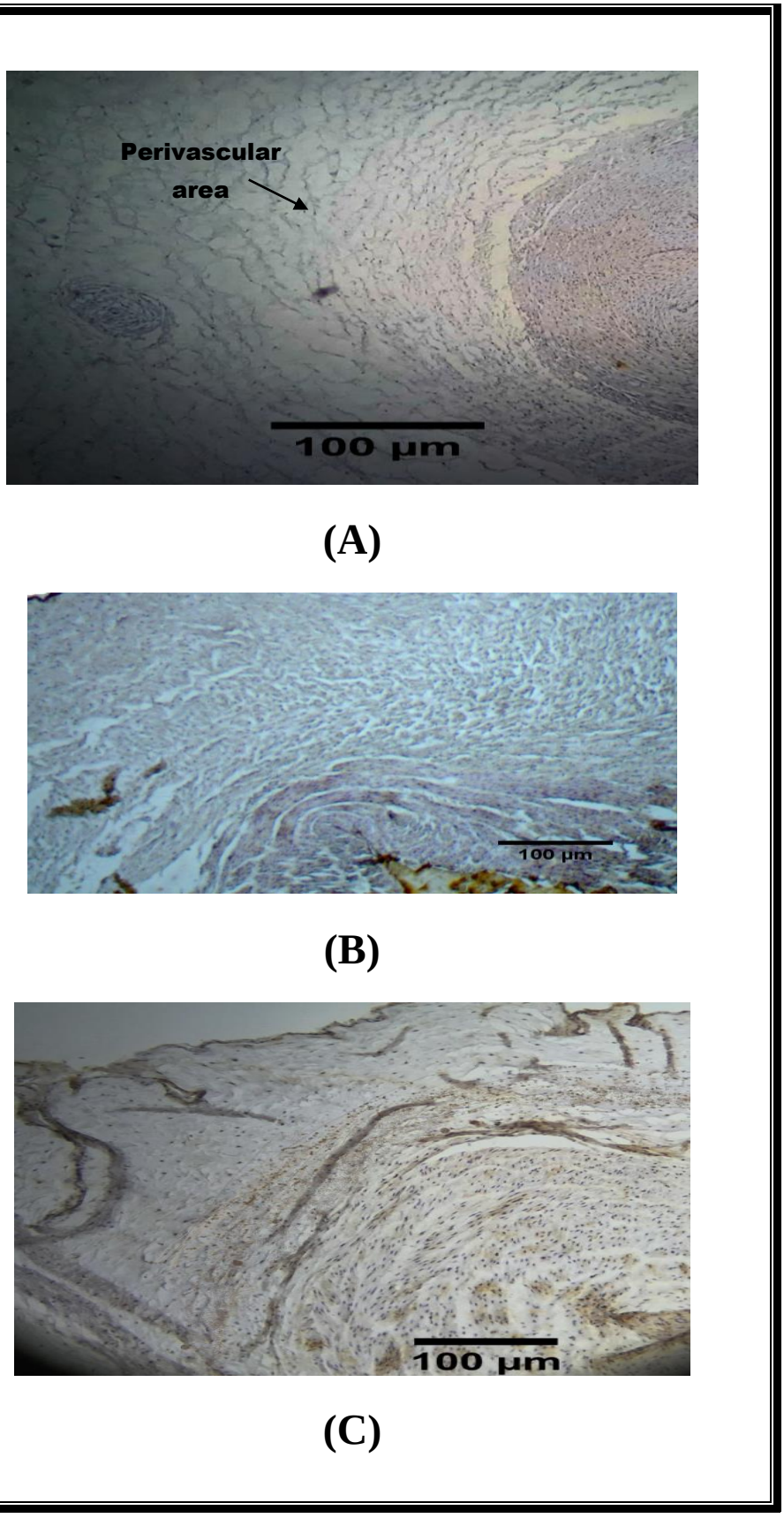

Figure 1. (A) Perivascular region of normocoiled cord, (B) Perivascular region of hypocoiled cord and (C) Perivascular region of Hypercoiled cord

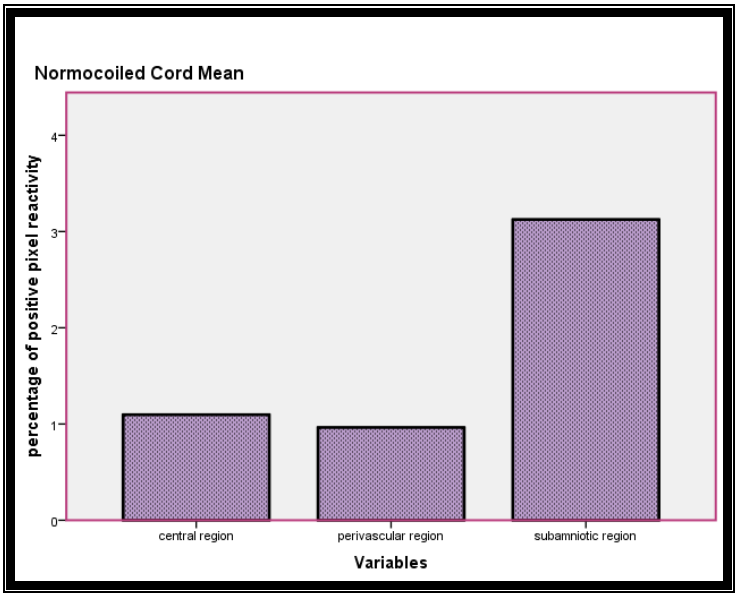

Figure 2. The mean value of the number of percentage positive pixels reactivity in the normocoiled between the mean values of perivascular, subamniotic and central regions

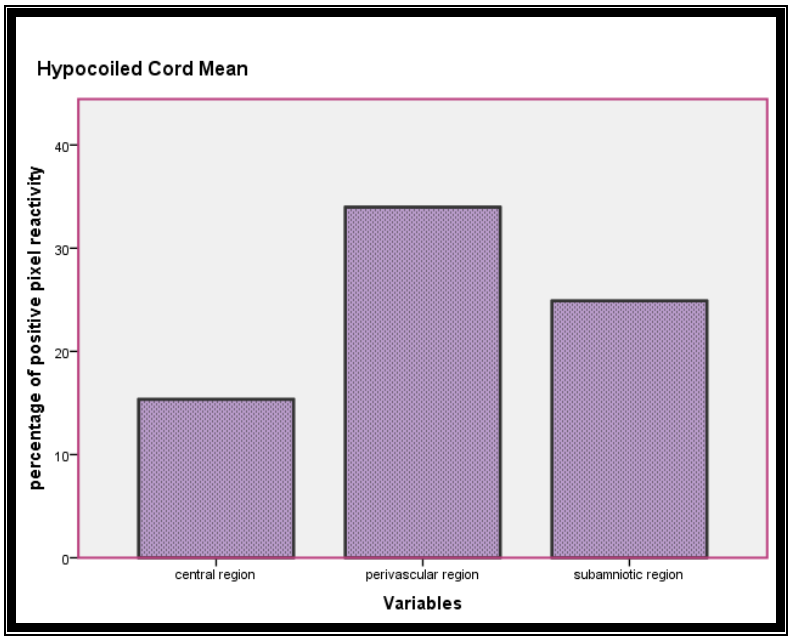

Figure 3. The mean value of the number of percentage positive pixel reactivity in the hypocoiled cords between the mean values of subamniotic, perivasclar, and central regions

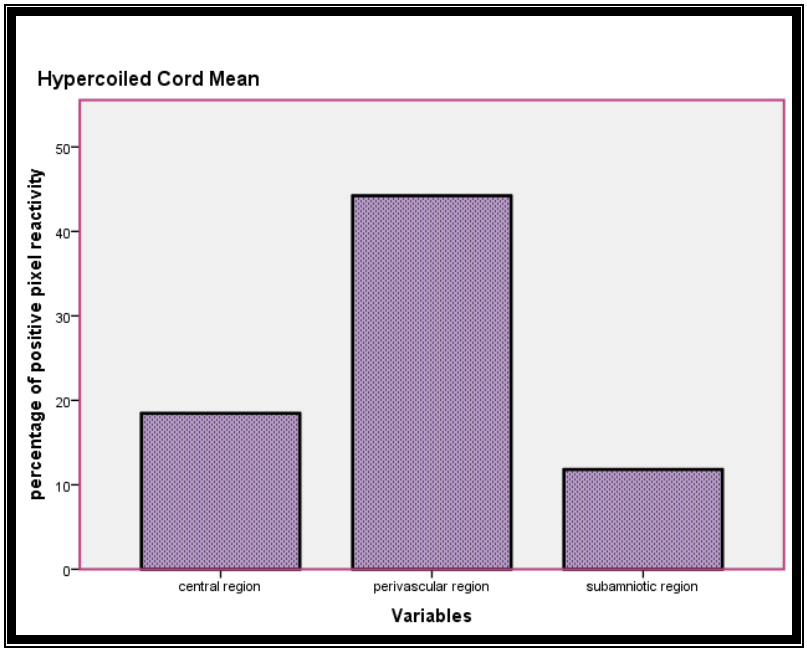

Figure 4. The mean value of the number of percentage positive pixel reactivity in the hypocoiled cords between the mean values of subamniotic, perivasclar, and central regions

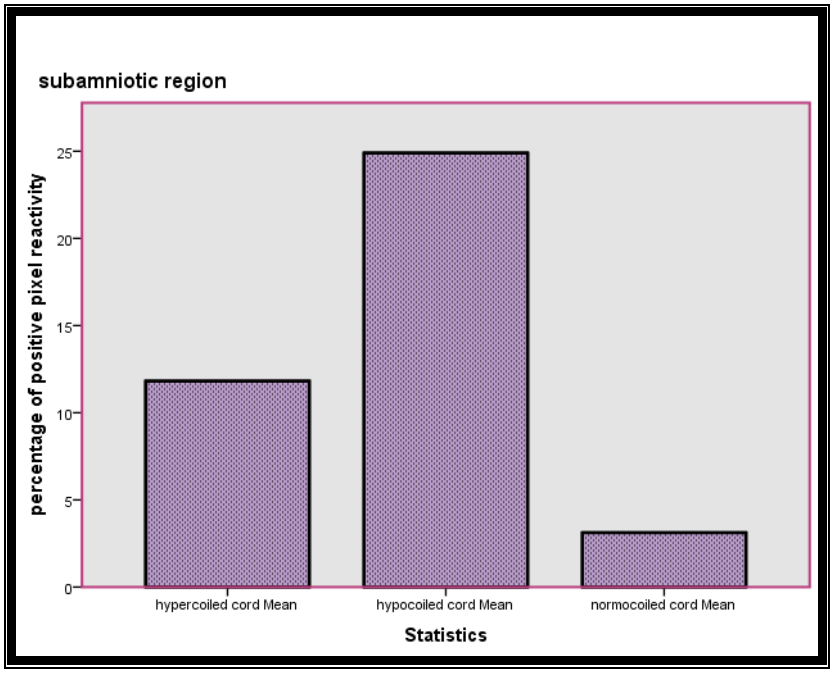

Figure 5. The mean values of percentage of positive pixel reactivity reactivity of the subamniotic region in hypocoiled, hypercoiled and normocoiled cords

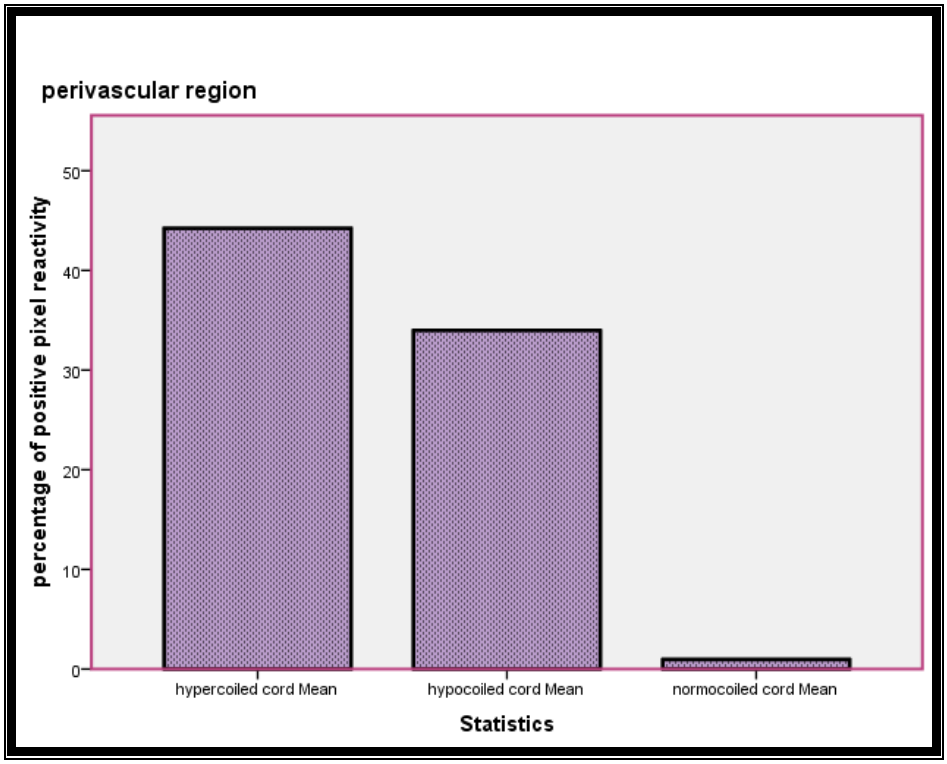

Figure 6. The mean values of percentage of positive pixel reactivity reactivity of the perivascular region in hypocoiled, hypercoiled and normocoiled cords

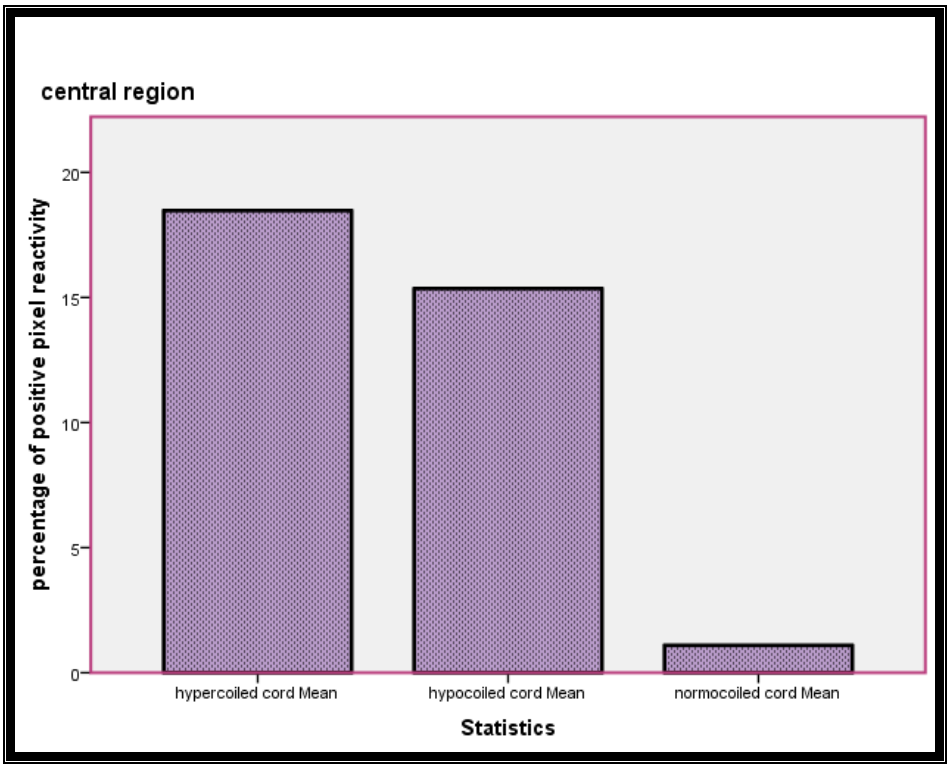

Figure 7. The mean values of percentage of positive pixel reactivity reactivity of the central region in hypocoiled, hypercoiled and normocoiled cords

In this study, normal fetal outcome with normal Apgar score was an inclusion criterion for the cord samples collected. The immune-histochemical matrix metalloproteinase-9 (MMP-9) reactivity demonstrated could be considered as an illumination of sequel of hypercoiling that is well tolerated by the fetus, the exaggeration of coiling could be associated with optimization of the sequel to the extent that disturb the umbilical circulation. This conclusion is supported by many reports documenting that (cord accident) including that of hypercoiling is dependent on the duration and degree of this circulatory obstruction, it may occur acutely or may be intermittent and causes chronic circulatory obstruction that may be associated with many intrapartum complications and adverse prenatal outcomes (as still stillbirth, intrauterine growth retardation, non-reassuring fetal heart tracing, low Apgar scores, and meconium staining) [15-18].

The Dynamic of Adverse Effect of Hypocoiling of Umbilical Cord:

It was concluded that the increased amount of hyaluronan in the umbilical cords of trisomy 21 fetuses would affect the morphology of the umbilical cord resulting in hypocoiling [19]. This conclusion was formulated depending on the fact that Wharton's jelly (a connective tissue responsible for the mechanical properties of umbilical cord) is composed of collagens, glycosaminoglycans, proteoglycans, and other microfilaments [20,21]. Hyaluronan is the major glycosaminoglycans in extracellular matrix of the umbilical cord; it inhibits angiogenesis and therefore has an influence on the growth of umbilical vessels and coiling formation resulting in hypocoiling [19].

The hypocoiled umbilical cord losses the advantage of coiling that make the cord as a semierectile organ which is more resistant to snarling torsion, stretch, and compression and consequently resulting in impair umbilical circulation [22,23].

The mesenchymal tissues in the hypocoiled cords are associated with much collagen accumulation; this massive collagenous buildup provided substantial mass effects that interfere with the normal coiling status.

The role of MMP-9 in the Mesenchyme of Abnormally Coiled Cords:

It is well known that the collagenolytic enzymes including matrix metalloprotenases MMPs are of vital importance in regulation of collagen degradation; MMPs degraded all components of the extracellular matrix and are involved in connective tissue remodeling [8,24]. Accordingly, the results of this study enlighten the cause-effect relationship of hypercoling and hypocoiling of the cord that could be associated with perinatal outcome. This relationship is affected by the counter effect of matrix metalloproteinase digestion of collagen and other ECM proteins resulting in reduction of the tissue masses around the umbilical vessels to relieve the stressful compressiom of umbilical circulation.

From the interpretation of discussing the dynamic of the adverse effect of hypercoiling and hypocoiling of the umbilical cord, it could be concluded that the forceful compression of the umbilical vessels circulation in hypocoiled and hypercoiled cord is produced by different mechanisms. During hypercoiling, mechanical compression plays the major role, while during hypocoiling, the increased extracellular matrix hayluronat in addition to accumulation of collagen and some other proteins in Wharton jelly produce massive increment of the connective tissues surrounding the umbilical vessels of hypocoiled cords resulting in their forceful compression.

The (MMP-9) immunohistochemical reactivity around the perivascular mesenchymal tissue was shown in this study to be significantly higher in hypercoiled and hypocoiled cords compared to normal cords. This result is supported by the establishment that MMP-9 is an enzyme synthesized by blood cells and blood vascular cells; it degradation ECM

components [25,26]. The MMP-9 breaks the ECM component around the umbilical vessels to relieve the compression of these vessels in both types of abnormal coiled cords. The limited effect of MMP-9 could secure satisfactory space around the umbilical vessels to a certain point beyond which obstruction of the umbilical circulation take place.

The increased MMP-9 immunohistochemical reactivity associated with abnormal cord coiling documented in this study indicates the high degradation of ECM components in the subamniotic mesenchyme. The increased MMP-9 activity reduces the subamnion mesenchyme to minimize the strain of the overlying amnion surrounding the cord. The marked increased subamnion MMP-9 reactivity in hypocoiled cords is a predictable outcome to minimize the massively increased mesenchyme resulting from the dual effect of increased hyalunate and collagen accumulation in hypocoiled cord.

Therefore, the perivascular immunohistochemical reactivity could indicate that the MMP-9 localization has a role degradation of the extra-tissue collagen and proteins to maintain a harmless perivascular pressure that preserve the normal vascular blood flow.

The results of this study do not contradict with the suggestion that reduced activity of collagen-degrading enzymes is a possible factor which enhances the accumulation of collagen and some other proteins in the pre-eclamptic umbilical cord tissues resulting in hypocoiling [27]. The pattern of MMP-9 immunohistochemical reactivity found in this study is a protective physiological response that preserves the healthy outcome of pregnancy resulting in a normal newborn with a normal Apgar score.

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 contribution

Abdul Sattar: M.Sc. candidate performing the laboratory research work. Dr. Mobarak: The advisor of the M.Sc. Research performing the production and 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. Kashanian M, Akbarian A, Kouhpayehzadeh J. The umbilical coiling index and adverse perinatal outcome. Int J Obstetetric Gynecol. 2006; 95(1): 8-13. doi: 10.1016/j.ijgo.2006.05.029.
2. Chitra T, Sushanth YS, 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.
3. de Laat MW, Franx A, Bots ML, et al. Umbilical Coiling Index in Normal and Complicated Pregnancies. Obstet. Gynecol., 2006; 107(5): 1049-55. doi: 10.1097/01.AOG.0000209197.84185.15.
4. Tahmasebi M. and Alighanbari R. Evaluation of umbilical cord thickness, cross-sectional area, and coiling index as predictors of pregnancy outcome. Indian J Radiol Imaging. 2011; 21(3): 195-8. doi: 10.4103/0971-3026.85367.
5. Ezimokhai M, Rizk DE, 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.
6. Ezimokhai M, Rizk DE, Thomas L. Maternal risk factors for abnormal vascular coiling of the umbilical cord. Am J Perinatol. 2000; 17(8): 441-6. doi: 10.1055/s-2000-13452.
7. Van Lint P, Libert C. chemokine and cytokine processing by matrix metalloproteinases and its effect on leukocyte migration and inflammation. J Leukoc Biol. 2007; 82(6): 1375-81. doi: 10.1189/jlb.0607338.
8. Woessner JF. Matrix metalloproteinases and their inhibitors in connective tissue remodeling. FASEB J. 1991; 5(8): 2145-54.
9. Galewska Z, Romanowicz L, Jaworski S. Matrix metalloproteinase-7 of the umbilical cord in preeclampsia. Arch Perinat Med. 2008; 14(2): 41-4.

10. Wang Y, Luo W, Reiser G. Trypsin and trypsin-like proteases in the brain: proteolysis and cellular functions. Cell Mol Life Sci. 2008; 65(2):237-52. doi: 10.1007/s00018-007-7288-3.
11. Mannello F, Tonti G, Papa S. Are matrix metalloproteinases the missing link. ISJ. 2005; 2(69): 69-74.
12. Nagase H, Woessner JF Jr. Matrix metalloproteinases. J Biol Chem. 1999; 274(31): 21491-4.
13. Ikechebelu J, Eleje G, Ofojebe C. True umbilical cord knot leading to fetal demise. Ann Med Health Sci Res. 2014; 4(Suppl 2): S155-8. doi: 10.4103/2141-9248.138044.
14. Baergen RN. Cord abnormalities, structural lesions, and cord accidents. Semin Diagn Pathol. 2007; 24(1): 23-32.
doi: http://dx.doi.org/10.1053/j.semdp.2007.02.004.
15. Collins JH. Umbilical Cord Accidents. BMC Pregnancy and Childbirth. 2012; 12(1): 1. doi: 10.1186/1471-2393-12-S1-A7.
16. Parast MM, Crum CP, Boyd TK. Placental histologic criteria for umbilical blood flow restriction in unexplained stillbirth. Hum Pathol. 2008; 39(6): 948-53. doi: 10.1016/j.humpath.2007.10.032.
17. Peng HQ, Levitin-Smith M, Rochelson B, et al. Umbilical cord stricture and overcoiling are common causes of fetal demise. Pediatr Dev Pathol. 2006; 9: 14-9. doi:10.2350/05-05-0051.1.
18. French AE, Gregg VH, Newberry Y, et al. Umbilical cord stricture: a cause of recurrent fetal death. Obstet Gynecol. 2005; 105: 1235-9. doi: 10.1097/01.AOG.0000159041.55845.
19. Phithakwatchara N, Phatihattakorn C. Specificity of hypocoiled umbilical cord in prediction of fetal trisomy 21 in the second trimester. Siriraj Med J. 2010; 62(4): 170-2.
20. Mauro AM, Buscemi M, Leone A, et al. Role of matrix metalloproteinases 2, 9 (MMP− 2, − 9), orphanin FQ and others peptides in vascular tone of human umbilical cordo at term. Dent Med Probl. 2008; 45(2): 111-20.
21. Sobolewski K, Bankowski E, Chyczewski L, et al. Collagen and glycosaminoglycans of Wharton's jelly. Biol Neonate. 1997; 71(1): 11-21. doi: 10.1097/01.AOG.0000159041.55845.
22. Wadhwa PD, Buss C, Entringer S, et al. Developmental origins of health and disease: brief history of the approach and current focus on epigenetic mechanisms. Sem Reproduct Med. 2009; 27(5): 358-68. doi: 10.1055/s-0029-1237424.
23. Bańkowski E, Pawlicka E, Jaworski S. Collagen biosynthesis in the wall of the umbilical cord artery and its alteration in EPH-gestosis. Pathophysiology. 1997; 4(1): 9-13. doi: http://dx.doi.org/10.1016/S0928-4680(96)00141-1.
24. Cox TR, Erler JT. Remodeling and homeostasis of the extracellular matrix: implications for fibrotic diseases and cancer. Dis Models Mech. 2011; 4(2): 165-78. doi: 10.1242/dmm.004077.
25. Davis GE, Senger DR. Endothelial extracellular matrix biosynthesis, remodeling, and functions during vascular morphogenesis and neovessel stabilization. Circulation Res. 2005; 97(11): 1093-107. doi: 10.1161/01.RES.0000191547.64391.e3.
26. Daniels JT, Schultz GS, Blalock TD, et al. Mediation of transforming growth factor-β1-stimulated matrix contraction by fibroblasts: a role for connective tissue growth factor in contractile scarring. Am J Pathol. 2003; 163: 2043-52.
27. Romanowicz L, Galewska Z. Extracellular matrix remodeling of the umbilical cord in pre-eclampsia as a risk factor for fetal hypertension. J Pregnancy. 2011; 69(4): 212-8. doi: 10.1155/2011/542695.

Correspondence to Hiba A. Abdul Sattar
E-mail: hibaahmad455@gmail.com
Received Sep. 29th 2016
Accepted Feb. 12th 2017