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Research Article

MMP-9 AND MG CHANGES IN CORD BLOOD OF TWO DIFFERENT MODES OF DELIVERY

KS. Abdul-Hamza[1*], MM. Fadil [2] and RB. Sulaiman[3]

[1]Department of Clinical Biochemistry, College of Medicine, Babel University.
[2]Department of Embryology, College of Medicine,
Al-Nahrain University, Baghdad-70036, Iraq.
[3]Department of Chemistry and Biochemistry,
College of Medicine, Al-Nahrain University, Baghdad-70036, Iraq.

ABSTRACT

Objective: to investigate the changes in both serum MMP-9 and Mg levels and their effect on two different modes of delivery, normal vaginal (NVD) and elective cesarean section (C/S). **Method:** a case-control study design has been conducted between January 2015 and May 2016. The current study recruited 64 pregnant women at term gestation (37–41 completed weeks). They were approached from different hospitals in Baghdad. Umbilical cord blood serum magnesium was determined by colorimetric spectrophotometry using colorimetric enzymatic Kit and MMP-9 using Enzyme Liked immune-sorbent assay (ELISA) technique**. Results:** a highly significant increase in MMP-9 concentration was found in umbilical cord blood (UCB) of women sera delivered by C/S *vis.* women delivered normally NVD, while trace element concentration (Mg) was significantly lower in UCB sera of women sera delivered by C/S *vis.* women delivered normally NVD with p values < (0.001 and 0.021 respectively)**. Conclusion:** the decrease of UCB serum magnesium level may effect on UCB serum MMP-9 level especially in women underwent C\S.

Keywords: MMP-9, modes of delivery, term pregnancy, cesarean section.

INTRODUCTION

Labor is a physiological event involving a sequential, integrated set of changes within the myometrium, decidua, and uterine cervix that occur gradually over a period of days to weeks. Biochemical connective tissue changes in the uterine cervix appear to precede uterine contractions and cervical dilation, and all of these events usually occur before rupture of the fetal membranes.[1]

Various types of proteinases are implicated in extracellular matrix (ECM) degradation, but the major enzymes are considered to be matrix metalloproteinases (MMPs), also called matrixins.[2]

Matrixin activities are also regulated by activation of the precursor zymogens and inhibition by endogenous inhibitors, tissue inhibitors of metalloproteinases (TIMPs). Thus, the balance between MMPs and TIMPs are critical for the eventual ECM remodeling in the tissue.[3]

The matrix metalloproteinases-9 (MMP-9) is causing degradation of ECM which is an important feature of development, morphogenesis, tissue repair and remodeling. It is precisely regulated under normal physiological conditions, but when dysregulated it becomes a cause of many diseases such as arthritis, nephritis, cancer, encephalomyelitis, chronic ulcers, fibrosis.[4]

In women with normal pregnancies, elevated levels of MMP-9 have been found in the cervicovaginal fluid and are associated with cervical ripening before labor, but are not a useful predictor for labor induction at term.[5]

Under certain conditions, cervical ripening can be induced by the inflammatory process which involves the catabolism of the cervical ECM by enzymes discharged from infiltrating leukocytes.[6] The MMP-9 has been found to play a role in the events associated with term and preterm labor, comparable changes in MMP-2 protein levels and activity. This may suggest that MMP-2 is expressed continuously

throughout labor, while MMP-9 expression is induced by various factors as previously mentioned.[7]

Almone and his colleagues' findings have implicated magnesium as being an essential element for fetal well-being and supplementation of magnesium may be benefited to fetal outcome.[8]

Magnesium supplementation during pregnancy was associated with significantly fewer maternal hospitalizations, a reduction in preterm delivery, and less frequent referral of the newborn to the neonatal intensive care unit. The results suggest that magnesium supplementation during pregnancy has a significant influence on fetal and maternal morbidity both before and after deliver.[9]

It is known that serum magnesium levels fall during pregnancy with gestational age. This decrease of magnesium plays an important role in the physiology of parturition. Decrease of magnesium in plasma may be responsible for decrease of same in myometrium leading to initiation of uterine contractions and labour.[10]

Since magnesium has an inhibitory role on myometrial contractions, attention has been paid to the role of magnesium deficiency in causing preterm labour. The inhibitory effect of magnesium on preterm labour contractions is attributed to antagonism of calcium mediated uterine contractions.[11]

Hence, hypomagnesaemia leads to neuromuscular hyperexcitability resulting in muscle cramps and uterine hyperactivity. The hyperexcitability of uterine musculature induced by hypomagnesaemia leads to increased cervical dilatation which in turn facilitates approach of vaginal micro-organisms into cervical canal and changes quality and quantity of vaginal discharge while uterine passage is being colonized by pathogenic micro-organisms.[12]

So, this study aimed to investigate the changes in both serum MMP-9 and Mg levels and their effect on two different modes of delivery, normal vaginal (NVD) and elective cesarean section (C/S).

MATERIALS AND METHODS

A case-control study design has been conducted between January 2015 and May 2016. The current study recruited 64 pregnant women at term gestation (37–41 completed weeks). They were approached from different hospitals in Baghdad. All pregnant women were at reproductive age.

Women were divided into two groups (33 women delivered normally (NVD) represents the first group) with mean age (26.44±1.1), while the second group contain 31 women with mean age (29.1 ±1.2) delivered by cesarean section (C/S). It should be mentioned that the primigrvida cases were excluded from the study.

Pregnant women with any serious systemic illnesses or medical disorder (*e.g.* diabetes or hypertension, Thyroid disease, Preeclampsia, Renal disease), and smoking were excluded from the study. This exclusion was done by a gynecologist and all women participate in this study were with no type of infection for at least one month before their delivery. Because all conditions above could effect on study results by abnormally increasing the oxidative stress status which may overestimate its effect on pregnancy duration. Written informed consent was obtained from pregnant women before delivery. This study received ethical clearance from the Research Board of the Institution at Al-Nahrain College of Medicine in Baghdad. Medical and obstetrics data were collected using a pretested questionnaire.

Five mls of umbilical cord blood (UCB) was taken and allowed to clot, centrifuged at 2500 rpm for 15 min at room temperature to obtain serum which was stored at −20 °C till the time of analysis. Magnesium was determined by colorimetric spectrophotometry using colorimetric enzymatic Kit and MMP-9 using Enzyme Liked immune-sorbent assay (ELISA) technique (Biomaghreb, France kits).

STATISTICAL METHODS

The data were encoded and entered into SPSS statistical package (v. 22). Data cleaning and management was then conducted to ensure that no mistakes were made during the data entry phase. Data analysis was then conducted. Descriptive data analysis was performed to describe the characteristics of study sample. The mean and Standard Error (S.E.) was used to describe the continuous variables while frequencies and percentages were used to describe categorical variables. Bivariate analysis was performed. P value less than 0.05 was considered significant. Moreover, the Receiver Operating Characteristics (ROC) curve was calculated to assess the sensitivity and specificity of the used kits in classifying the true positive versus false positive cases in this study. The ROC curve plots were presented as well.

RESULTS

Sixty-four women were involved in this study. Thirty one women were delivered by cesarean section (C/S) while the rest 33 women were all deliver normally (NVD). Women's age was nearly comparable as well as their gestational age with no significance differences (p>0.05) as shown in table-1.

A highly significant increase in MMP-9 concentration was found in umbilical cord blood (UCB) of women sera delivered by C/S *vis.* women delivered normally NVD, while trace element concentration (Mg) was significantly lower in UCB sera of women sera delivered by C/S *vis.* women delivered normally NVD with p values < (0.001 and 0.021 respectively)(table-1).

Also, the area under the corresponding ROC curve for serum Mg concentration was compared between women with C/S and NVD for the prediction of type of delivery. ROC analysis revealed that serum Mg concentration had a significant area under the curve (AUC) 0.657, indicating that a threshold of 2 mg/dl gave sensitivity of 90% and specificity 73%. Less of serum Mg concentration was associated with increased rate of C/S (table-2).

Regarding the inverse results shown in table-1, no significant correlation was found between UCB serum MMP-9 and Mg as shown in figure-1 in both delivery groups.

DISCUSSION

Findings of present study suggest that the mode of delivery may be one of the factors affecting the umbilical cord level of MMP-9. Also the increases of MMP-9 level in cord blood of women underwent cesarean section more than in cord blood of women with normal vaginal delivery may be due to the significantly decrease in the level of magnesium in cord blood of woman underwent cesarean section compared with cord blood of woman underwent normal vaginal delivery (table-1). An inverse relationship between magnesium and MMP-9 was found. Therefore, it's possible that magnesium affected MMP-9 production in the Vascular Smooth Muscle Cells (VSMCs) through calcium mediated signal transduction. Mean serum level of magnesium concentration was significantly higher in woman underwent normal vaginal delivery compared with woman underwent cesarean section delivery as shown in table-1. In agreement with this result, Dobrowski et al have found that magnesium is the most effectively reduced plasma MMP-9 concentration and they suggested that magnesium may effect on TIMP activity. Although their findings were agreed with present results; still this relation has not been documented.[13] Regarding correlation between Mg and MMP-9, Lee 2004 found a negative correlation between magnesium and MMP-9. It may result from N-methyl-D-aspirate (NMDA) receptor activity because magnesium is a non-competitive NMDA receptor antagonist.[14] Glutamate activation of the NMDA receptor leads to increased cellular calcium, which activates a complex biochemical cascade that includes protein kinase activation, calpain induced cytoskeletal breakdown, DNA fragmentation and reactive oxygen species accumulation.[15] After Prolonged activation, neurons become damaged and subsequently die. The activation of NMDA receptors is reversibly modulated by MMP-9. However, the mechanism underlying MMP-9's regulation of the NMDA receptor is not clear. Some investigators have postulated that MMP-9 modulation of NMDA receptors requires proteolytic activity of MMP-9 and is not dependent on protein–protein interactions.[16,17] Therefore, it assumes that changes in serum umbilical cord magnesium concentration content can affect MMP-9 level; nevertheless, the interaction between Mg remains to be elucidated.[15] In this study, Mg levels were increased in NVD group compared with CSD group. This result did not agreed with a study done by Kleopatra *et al* . They found Mg concentration in serum of umbilical cord was remarkably decreased in NVD group compared with CSD group and they suggested that the uterus and skeletal muscles participated in this natural process. Their findings were similar to those found in athletes post-exercise (exercise provides an excellent model of tissue aseptic inflammation) .They attributed the decreases of their levels may be due to excretion in urine and sweet.[18]

CONCLUSION

From all the above, it can be concluded that the decrease of UCB serum magnesium level may effect on UCB serum MMP-9 level especially in women underwent C\S.

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Table 1: Mean ± standard error for anthropometric and biochemical parameters in study groups

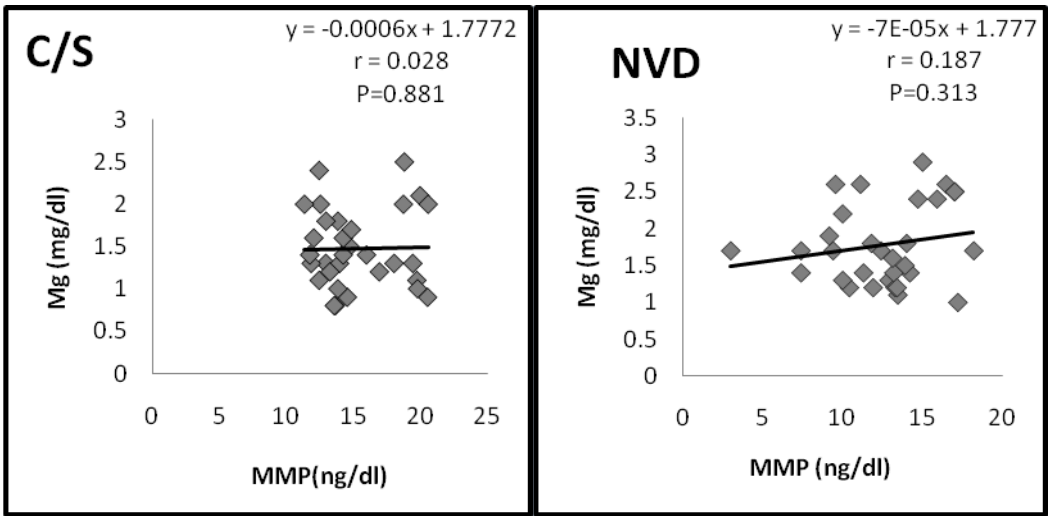
Anthropometric parameters	C/S (n= 31)	NVD (n= 33)	*P* value
Age (years)	29.1±1.21	26.44±1.1	*NS*
Gestational age (wks)	37.26±0.1	37.42±0.1	*NS*
Biochemical parameters			
Serum MMP-9 (ng/dl)	15.24±0.54	12.51±0.60	**<0.001**
Serum Mg (mg/dl)	1.47±0.08	1.77±0.09	**0.021**

C/S= cesarean section, NVD= normal vaginal delivery, n= number, wks=weeks, MMP-9= matrix metalloproteinase-9, Mg= magnesium, mg= milligram, ng= nanogram and NS=not significant

Table 2: Estimated area under curve and predictive cut-off values for serum MMP-9 and Mg concentrations in cesarean section and normal vaginal delivery women

Parameters	Area	Cutoff point value	Sensitivity	Specificity	*P* value	95%CI	
						Lower Bound	Upper Bound
MMP-9(ng/dl)	0.708	13.5	68%	65%	**0.005**	0.580	0.835
Mg (mg/dl)	0.657	2.0	90%	73%	**0.031**	0.524	0.791

MMP-9= matrix metalloproteinase-9, Mg= magnesium, ng= nanopictogram, mg= milligram and CI= confidence interval.



(a) **(b)**

Fig. 1: Correlation between MMP-9 and Mg concentrations in umbilical cord blood of (a): cesarean section delivery and (b): normal vaginal delivery

REFERENCES

1. Nathanielsz PW, Giussani DA and Wu WX. Stimulation of the switch in myometrial activity from contractures to contractions in the pregnant sheep and nonhuman primate. Equine Vet J Suppl. 1997;83.
2. Visse R and Nagase H. Matrix metalloproteinases and tissue inhibitors of metalloproteinases: structure, function, and biochemistry. Circ Res. 2003;92:827–839.
3. Nagase H, Visse R and Murphy G. Structure and function of matrix metalloproteinases and TIMPs.; Pub Med, Cardiovasc Res. 2006;69(3):562-73.
4. Newby AC. Dual role of matrixmetalloproteinases (matrixins) in intimal thickening and atherosclerotic plaque rupture. Physiol Rev. 2005;85:1–31.
5. Xu P, Alfaidy N and Challis JR. Expression of matrix metalloproteinase MMP-2 and MMP-9 in human placenta and fetal

membranes in relation to preterm and term labor. J Clin Endocrinol Metab. 2002;87:1353–1361.
6. Watari, Watari H, DiSanto ME, Chacko S, Shi GP and Strauss III JF. Pro-inflammatory cytokines induce expression of matrix-metabolizing enzymes in human cervical smooth muscle cells. American Journal of Pathology, 1999;154(6):1755–1762,
7. Olgun NS and Reznik SE. The matrix metalloproteases and endothelin-1 in infection-associated preterm birth.:PubMed. ObstetGynecol Int. 2010.
8. Almonte RA, Heath DL, Whitehall J, Russell MJ, Patole S and VinkR. Gestational magnesium deficiency is deleterious to fetal outcome. Biol Neonate. 1999;76:26-32.
9. Parizadeh SM, Mohammadzadeh A, Farhat A, Valaee L, Khajedaluee M and Faal G. Maternal serum magnesium level and low birth weight neonate. Int J Prev Med. 1999;4:1476-9.
10. Kamal S, Sharan A, Kumar U and Shahi SK. Serum magnesium level in preterm labour. Indian J PatholMicrobiol. 2003;46(2):271-3.
11. Swain R, Kaplan and Machlis B. Magnesium for the next millennium.South Med J. 1999;92: 1040-7.
12. Hantoushzadeh S, Jafarabadi M and Khazardoust S. Serum magnesium levels, muscle cramps and preterm labour. Int J Gynae Obst. 2007;98(2):153-4.
13. Dabrowski W, Rzecki Z, Czajkowski M and Pilat J. Magnesium reduces matrix metalloproteinase-9, but not glial fibrillary acidic protein, in cardiac surgery patients; Future Neurol. 2012;7(3):349–359.
14. Lee JS, Han YM, Yoo DS, Choi BH, Kim YH, Huh PW, Ko YJ, Rha HK, Cho KS and Kim DS. A molecular basis for the efficacy of magnesium treatment following traumatic brain injury in rats. J Neurotrauma. 2004;21:549-61.
15. Dabrowski W, Rzecki Z, Czajkowski M, JacekPilat J, Biernacka J, Kotlinska E, Pasternak K, Stążka K and Sztanke M. Plasma matrix metalloproteinase 9 correlates with disorders of brain magnesium homeostasis in patients undergoing coronary artery bypass surgery; Magnesium Research. 2010;23 (4):169-79.
16. Gorkiewicz T, Szczuraszek K, Wyrembek P, Michaluk P, Kaczmarek L and Mozrzymas JW. Matrix metalloproteinases– 9 reversibly affects the Time course of NMDA–induced currents in cultured rat hippocampal neurons. Hippocampus 2010 (in press).
17. Michaluk P, Mikasova L, Groc L, Frischknecht R, Choquet D and Kaczmarek L. Matrix metalloproteinase – 9 controls NMDA receptor surface diffusion through integrin beta 1 signaling. J Neurosci. 2009;29:6007-12.
18. Kleopatra H Schulpis, Theodore karakonstantakis, George D Vlachos , Alexios-Fotios a. Mentis, George A Karikas, DespoinaAfordakou, Maria Papastamataki, ArisAntsaklis and Ioannis Papassotiriou. Maternal-eonatal magnesium and zinc serum concentrations after vaginal delivery. Scandinavian Journal of Clinical & Laboratory Investigation. 2010;70:465–469.