Cent. Eur. J. Med. • 5(6) • 2010 • 693-697
DOI: 10.2478/s11536-010-0016-7

VERSITA

Central European Journal of **Medicine**

The relation between delivery type and cord blood levels of chitotriosidase and Troponin T

Research Article

Turan Tunc[1*], Erkan Demirkaya[1], Mustafa Kul[2], Halil Yaman[3], Sinan Karadeniz[4], Tayfun Gungor[5], Faruk Alpay[1], Ismail Kurt[3]

[1] *Department of Pediatrics, Gulhane Military Medical Academy, 06018, Ankara, Turkey*

[2] *Department of Pediatrics, Gulhane Military Medical Academy, 34668, Haydarpasa Military Hospital, Istanbul, Turkey*

[3] *Department of Medical Biochemistry, Gulhane Military Medical Academy, 06018, Ankara, Turkey*

[4] *Departments of Obstetric and Gynecology, Etlik Zubeyde Hanim Maternity Hospital, 06010, Ankara, Turkey*

[5] *Zekai Tahir Burak Maternity and Women's Health Teaching Hospital, 06230, Ankara, Turkey*

Received 14 July 2009; Accepted 16 January 2010

Abstract: The operative deliveries can expose the fetus to acute and systemic hypoxia along with an increase in perinatal morbidity. The aim of this study was to reveal any relationship between delivery type and Chitotriosidase and Troponin T levels in cord blood. Ninety babies born in Ankara Etlik Maternity and Women's Health Teaching Hospital were involved in the study. The babies were divided into three groups; Group 1: Normal vaginal; Group 2: Caesarean section; Group 3: Forceps application. Cord blood samples were drawn from umbilical arteries of the babies soon after the birth. Chitotriosidase enzyme activities in group 3 (141 nmol/ml/h (0–246)) were found higher than groups 1 (100 nmol/ml/h (0–208)) and 2 (91 nmol/ml/h (0–202)) ($p<0.01$ and $p<0.03$ respectively). Although cardiac Troponin T levels were higher in group 3, the difference among groups was not statistically significant ($p=0.79$). Acute or systemic hypoxic exposure of the organism gives rise to a microvascular response characterized by interactions between leukocytes and endothelium. We are hypothesizing that the high levels of chitotriosidase found in the forceps group were due to hypoxia, and that chitotriosidase level can be used as a marker of acute and systemic hypoxia.

Keywords: *Cord blood • Delivery type • Chitotriosidase • Troponin T*

1. Introduction

Labors requiring intervention are accompanied by increased morbidity. In particular, normal vaginal deliveries under epidural anesthesia lengthen the first and second stages of the birth. The most common indication of forceps is elongation of the second stage; midforceps are most often used when there is a risk of fetal asphyxia and neonatal hypoxia [1]. Subdural, cerebral, intraventricular and subarachnoid hemorrhages, facial nerve and brachial plexus injury, convulsion, central nervous system depression and the need for mechanical ventilation are more frequent in babies after births assisted by forceps, vacuum, and caesarean section when compared with spontaneous deliveries [2-4]. Respiratory depression, risks of perinatal asphyxia and hypoxia have been shown to increase in operative deliveries [5,6]. Operative deliveries can expose the fetus to acute and systemic hypoxia along with an increase in perinatal morbidity. Accordingly, the aim of this study was to reveal relationships among delivery type, degree of hypoxia and the levels of two

* *E-mail: drturantunc@yahoo.com*

markers for hypoxia, chitotriosidase (ChT) and troponin (Tn), in cord blood.

An enzyme of the chitinase group, ChT breaks down the chitin sheath, particularly of fungi. Its primary source is activated macrophages [7]. Following an appropriate stimulus, this enzyme can either be secreted by macrophages or by polymorphonuclear leukocytes [8]. It was first defined in patients with Gaucher disease, and was found be increased 1000-fold in macrophages of the tissues of Gaucher patients [9]. Recent studies suggest that ChT levels are related to inflammation [10,11]. Systemic hypoxia causes a microvascular diffuse inflammation in the body, as a result of which leukocyte-endothelial adhesion and vascular permeability increase and leukocytes migrate through the perivascular area [12-15]. Therefore, inflammation resulting from hypoxia can indirectly result in an increase in ChT levels.

The inhibitor protein complex Tn is found in the actin filaments of striated muscles. It comprises three subtypes: troponin T (TnT), troponin C (TnC) and troponin I (TnI). Cardiac TnT levels increase in hypoxic myocardial injuries [16-18]. Cardiac TnT is expressed in fetal cardiac myocytes, and it is found at increased levels in cord blood as a marker of cardiac injury [19]. This phenomenon arises from low perfusion of the heart in hypoxic fetuses. A high TnT value in cord blood is assumed to be a marker of serious chronic hypoxia in abnormal umbilical venous return. This occurs in fetuses that have retrograde blood flow or a loss of diastolic blood flow in the umbilical artery, and in fetuses with severe placental insufficiency [20,21]. TnT levels are thought to increase with fetal asphyxia and neonatal hypoxia, influencing the myocardium in operative deliveries.

2. Material and Methods

2.1. Subjects

Ninety babies born in Division of Maternal-Fetal Medicine of Ankara Etlik Maternity and Women's Health Teaching Hospital were involved in the study. Only full-term babies were included in the study. Mothers with risk factors including hypertension, diabetes mellitus, preeclampsia and smoking, as well as resuscitated and/or congenitally malformed babies, were excluded. Since ChT is a marker of infection, patients with preterm premature rupture of membranes were also excluded. Maternal hypertension was defined according to the Working Group (2000) criteria as high blood pressure ≥ 140/90 mmHg after the 20th week of gestation [22]. Gestational diabetes mellitus was defined as any degree of glucose intolerance with onset or first recognition during pregnancy [23].

The study was approved by the local ethical committee of Gulhane Military Academy of Medicine. Informed consent was obtained from all subjects.

2.2. Study groups

The babies were divided into three groups according to delivery type: Group 1: Normal vaginal delivery (NVD); Group 2: Caesarean section (C/S); Group 3: Forceps application. In Group 2; 12 patients were underwent C/S for fetal distress, whereas C/S was elective in the others. Cord blood samples were drawn from umbilical arteries of the babies soon after the birth, and collected into tubes without any additives. Supernatants were taken after the centrifugation at 4000 rpm for 10 min, and kept at -80°C until measurement.

2.3. Activity of Chitotriosidase

ChT enzyme activities were analyzed according to the method described by Guo et al. [24]. Briefly, 25 µl of milk supernatant was incubated with 100 µl of 22 µmol/L 4-methylumbelliferyl-β-D-N,N′,N″-triacetylchitotriose in McIlvain's phospate-citrate buffer, pH=5.2, for 1 h at 37°C. The reaction was terminated by adding 120 µl 0.5 mol/L Na_2CO_3–$NaHCO_3$ buffer, pH 10.7, and the fluorescence of 4-methylumbelliferone was read in a Microfluor 2® fluorimeter (Bio-Tek Instruments, Neufahrn—Germany; excitation 355 nm, emission 460 nm). The ChT activity was expressed as nanomoles of substrate hydrolyzed per milliliter per hour (nmol/ml/h).

2.4. Troponin T determination

Cord blood TnT levels were assessed by Elecsys Troponin-T STAT Immunoassay, Elecsys® 1010/2010 systems. The electrochemiluminescence immunoassay was performed using an Elecsys® 2010 immunoassay analyzer (Roche® Diagnostics GmbH, Mannheim, Germany) with a lower limit of 0.010 ng/ml. Any value below this limit was considered to be zero. The upper limit of the test was 25.00 ng/ml; the normal range was 0.010–0.100 ng/ml.

2.5. Statistical analysis

Data were analyzed using SPSS® for Windows® 10.0. Descriptive statistics were given as mean ± standard deviation and median (min-max) values. Multiple group comparisons were done by the Kruskal-Wallis test and post hoc Bonferroni adjusted Mann-Whitney U tests. Statistical significance was set at $p<0.05$.

Table 1. Clinical characteristics of the subjects.

	Group 1 (n=30)	Group 2 (n=30)	Group 3 (n=30)	P value
Maternal age (year)	28.87±5.32	29.28±4.89	30.77±5.21	>0.05
Maternal weight (kg)	77.13±8.91	81.72±7.86	79.72±11.14	>0.05
Gestational age (week)	38.68±1.49	37.85±2.88	38.31±1.79	>0.05
Birth weight (g)	3120 (2610–3925)	3030 (2520–3780)	3220 (2710–3890)	>0.05
Birth length (cm)	49.2±2.6	50.1±3.1	50.05±4.10	>0.05
Sex (Male/Female)	13/17	14/16	17/13	>0.05
Apgar scores				
1 min	8.7 (7–10)	8.5 (7–10)	8.2 (6–9)	>0.05
5 min	9.5 (8–10)	9.8 (9–10)	9.3 (6–10)	

Table 2. Chitotriosidase and Troponin T levels in cord blood.

	NVD (n=30)	C/S (n=30)	Forceps (n=30)	P value*
Chitotriosidase (nmol/mL/h)	100 (0–208)	91 (0–202)	141 (0–246)	=0.02
Troponin T (ng/mL)	0.018 (0.010–0.074)	0.020 (0.00–0.182)	0.021 (0.010–0.390)	=0.79

**P<0.05 was accepted as significant*

3. Results

Clinical characteristics of the babies and mothers are shown in Table 1. ChT enzyme activities in Group 3 (141 nmol/ml/h (0–246)) were found higher than Group 1 (100 nmol/ml/h (0–208)) and Group 2 (91 nmol/ml/h (0–202)) (p=0.01 and p=0.03 respectively). There was no significant difference between Groups 1 and 2 (p=0.55). Although cardiac TnT levels were higher in Group 3, the difference among groups was not statistically significant (p=0.79) (Table 2).

4. Discussion

In this study, we aimed to discover whether ChT and TnT levels were related to delivery type. No significant difference was seen in ChT levels between the NVD and C/S groups, whereas the forceps group had significantly increased ChT levels; the groups were similar regarding cardiac TnT levels.

Despite the extensive technological improvements in modern medicine in recent years, parturition asphyxia can progress in the form of hypoxic ischemic encephalopathy. Sometimes that clinical condition can result in permanent neurological injuries [6]. Several studies have shown that there is a positive relationship between operative deliveries, particularly forceps application, and asphyxia at birth [5,6]. Acute or systemic hypoxic exposure of the organism gives rise to a microvascular response characterized by interactions between leukocytes and endothelium [15]. Leukocyte migration to the affected area occurs with increased vascular permeability [14,25]. Recent data suggest that the balance between reactive oxygen species (ROS) and nitric oxide (NO) plays a role in microvascular response to hypoxia. A study by Steiner et al. [28] showed that ROS formation increased in the mesenteric microcirculation during hypoxia. ROS formation, as well as leukocyte adherence and migration, was shown to decrease with an antioxidant pretreatment before the hypoxia. Another study by Wood et al. [15] revealed that increased NO within tissues diminished the leukocyte-endothelium interaction.

Another potential result of the oxidative stress that results from the imbalance between ROS and NO is an increase in local inflammatory mediators, which are readily formed from arachidonic acid; these substances play a role in the microvascular rapid response to hypoxia [27,28]. Circulatory levels of cytokines, such as leukotriene B4 (LTB4), have been shown to increase during hypoxia [29]. LTB4 is a potent proinflammatory mediator that not only leads to adhesion and migration of the leukocytes, but also increases vascular permeability. Moreover, in parallel with the response to hypoxia, LTB4 was shown to also increase ROS formation in polymorphonuclear leukocytes [30,31]. However, it must be mentioned that findings to the contrary have also been reported [32].

ChT is an antifungal functioning member of chitinase enzyme system, and its main origin is the macrophages.

In the course of an appropriate stimulus, this enzyme is secreted from the granules of macrophages and polymorphonuclear leukocytes [33-36]. Having assessed the ChT levels in cord blood, our study showed no significant difference between the NVD and C/S groups. However, ChT levels increased in the forceps group compared with C/S and NVD. Risk of fetal asphyxia can increase, particularly in prolonged deliveries, and there may be an indication for forceps intervention. High levels of ChT in the forceps group may be an outcome of fetal exposure to acute hypoxia, and to increased levels of mononuclear and polymorphonuclear leukocytes secreting leukotrienes such as LTB4. Both the increased number of inflammatory cells in circulation and the secretion of the enzymes stored in their granules may give rise to high ChT levels.

A second finding was that TnT levels in cord blood were not related with the parturition type. Since TnT shows myocardial cellular injury, our results suggest that the subjects were not exposed to significant hypoxia that would induce cardiac injury.

We hypothesize that the high levels of ChT found in the forceps group resulted from hypoxia, and that the ChT level can be used as a marker of acute and systemic hypoxia. Long-term studies must be done to evaluate the degree of that hypoxic effect. The clinicians should bear in mind that C/S is an alternative approach in these conditions to avoid the risk of a hypoxic effect.

References

[1] Neuberg M. The use of forceps delivery in own analysis. Ginekol Pol 2000;71:258-262

[2] Towner D, Castro MA, Eby-Wilkens E, Gilbert WM. Effect of mode of delivery in nulliparous women on neonatal intracranial injury. N Engl J Med 1999;341:1709-1714

[3] Rijhsinghani A, Belsare TJ. Neonatal intracerebellar hemorrhage after forceps delivery: report of a case without neurologic damage. J Reprod Med 1997;42:127-130

[4] Johanson RB, Rice C, Doyle M, et al. A randomised prospective study comparing the new vacuum extractor policy with forceps delivery. Br J Obstet Gynaecol 1993;100:524-530

[5] Baskett TF, Allen VM, O'Connell CM, Allen AC. Predictors of respiratory depression at birth in the term infant. BJOG 2006;113:769-774

[6] Palsdottir K, Dagbjartsson A, Thorkelsson T, Hardardottir H. Birth asphyxia and hypoxic ischemic encephalopathy, incidence and obstetric risk factors. Laeknabladid. 2007;93:595-601

[7] Van Eijk M, van Roomen CP, Renkema GH, at al. Characterization of human phagocyte-derived chitotriosidase, a component of innate immunity. Int Immunol 2005;17:1505-1512

[8] Bouzas L, Carlos Guinarte J, Carlos Tutor J. Chitotriosidase activity in plasma and mononuclear and polymorphonuclear leukocyte populations. J Clin Lab Anal 2003;17: 271-275

[9] Hollak CE, van Weely S, van Oers MH, Aerts JM. Marked elevation of plasma chitotriosidase activity. A novel hallmark of Gaucher disease. J Clin Invest 1994;93:1288-1292

[10] Artieda M, Cenarro A, Ganan A, at al. Serum chitotriosidase activity is increased in subjects with atherosclerosis disease. Arterioscler Thromb Vasc Biol 2003;23:1645-1652

[11] Di Rosa M, Dell'Ombra N, Zambito AM, Malaguarnera M, Nicoletti F, Malaguarnera L. Chitotriosidase and inflammatory mediator levels in Alzheimer's disease and cerebrovascular dementia. Eur J Neurosci 2006;23:2648-2656

[12] Steiner DR, Gonzalez NC, Wood JG. Mast cells mediate the microvascular inflammatory response to systemic hypoxia. J Appl Physiol 2003;94:325-334

[13] Gonzalez NC, Wood JG. Leukocyte-endothelial interactions in environmental hypoxia. Adv Exp Med Biol 2001;502:39–60

[14] Wood JG, Johnson JS, Mattioli LF, Gonzalez NC. Systemic hypoxia increases leukocyte emigration and vascular permeability in conscious rats. J Appl Physiol 2000;89:1561–1567

[15] Wood JG, Mattioli LF, Gonzalez NC. Hypoxia causes leukocyte adherence to mesenteric venules in non-acclimatized, but not acclimatized rats. J Appl Physiol 1999;87:873–881

[16] Gunes T, Ozturk MA, Koklu SM, Narin N, Koklu E. Troponin-T levels in perinatally asphyxiated infants during the first 15 days of life. Acta Paediatr 2005;94:1638-1643

[17] Matuszczak-Wleklak M, Szymankiewicz M. Perinatal asphyxia, known topic, new diagnostic prospects-advisability of troponin T in perinatal medicine. Ginekol Pol 2003;74:487-493

[18] Szymankiewicz M, Matuszczak-Wleklak M, Hodgman JE, Gadzinowski J. Usefulness of cardiac troponin T and echocardiography in the diagnosis of hypoxic myocardial injury of full-term neonates.

Biol Neonate 2005;88:19-23
[19] Clark SJ, Newland P, Yoxall CW, Subhedar NV. Cardiac troponin T in cord blood. Arch Dis Child Fetal Neonatal Ed 2001;84:34-37
[20] Makikallio K, Vuolteenaho O, Jouppila P, Rasanen J. Association of severe placental insufficiency and systemic venous pressure rise in the fetus with increased neonatal cardiac troponin T levels. Am J Obstet Gynecol 2000;183:726-731
[21] Kahila H, Stefanovic V, Loukovaara M, Alfthan H, Hämäläinen E, Halmesmäki E. Prenatal buprenorphine exposure: effects on biochemical markers of hypoxia and early neonatal outcome. Acta Obstet Gynecol Scand 2008;87:1213-1219
[22] Report of the National High Blood Pressure Education Program Working Group on High Blood Pressure in Pregnancy. Am J Obstet Gynecol 2000;183:1-22
[23] American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care 2007;l1:42-47
[24] Guo Y, He W, Boer AM, Wevers RA, de Bruijn AM, Groener JE, Hollak CE, Aerts JM, Galjaard H, van Diggelen OP. Elevated plasma chitotriosidase activity in various lysosomal storage disorders. J Inherit Metab Dis 1995;18:717-722
[25] Steiner DR, Gonzalez NC, Wood JG. Leukotriene B(4) promotes reactive oxidant generation and leukocyte adherence during acute hypoxia. J Appl Physiol 2001;91:1160-1167
[26] Wood JG, Johnson JS, Mattioli LF, Gonzalez NC. Systemic hypoxia promotes leukocyte-endothelial adherence via reactive oxidant generation. J Appl Physiol 1999;87: 1734–1740
[27] Lefer AM. Significance of lipid mediators in shock states. Circ Shock 1989;27:3–12
[28] Sullivan GW, Sarembock IJ, Linden J. The role of inflammation in vascular diseases. J Leukoc Biol 2000;67:591–602
[29] Richalet JP, Hornych A, Rathat C, Aumont J, Larmignat P, Remy P. Plasma prostaglandins, leukotrienes and thromboxane in acute high altitude hypoxia. Respir Physiol 1991;85:205–215
[30] Granger DN, Grisham MB, Kvietys PR. Mechanisms of microvascular injury. In: Physiology of the Gastrointestinal Tract. 3rd ed. New York: Johnson LR; 1994:1693–1722
[31] Kurose I, Argenbright LW, Wolf R, Lianxi L, Granger DN. Ischemia/reperfusion-induced microvascular dysfunction: role of oxidants and lipid mediators. Am J Physiol Heart Circ Physiol 1997;272:2976–2982
[32] Neumann M, Kownatzki E. The effect of adherence on the generation of ROS by human granulocytes. Agents Actions 1989;26:183–185
[33] Renkema GH, Boot RG, Strijla-d A, at al. Synthesis, sorting, and processing into distinct isoforms of human monocyte-derived macrophage chitotriosidase. Eur J Biochem 1997;244:279–285
[34] Tunc T, Kul M, Yaman H, at al. Chitotriosidase activity in human milk from mothers of premature and full-term infants during the first month of lactation. Clin Biochem 2008;41:693-696
[35] Bouzas L, Carlos Guinarte J, Carlos Tutor J. Chitotriosidase activity in plasma and mononuclear and polymorphonuclear leukocyte populations. J Clin Lab Anal 2003;17: 271-275
[36] Musumeci M, Malaguarnera L, Simpore J, Barone R, Whalen M, Musumeci S. Chitotriosidase activity in colostrum from African and Caucasian women. Clin Chem Lab Med 2005;43:198-201