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Short communication

Different concentration of human cord blood HMGB1 according to delivery and labour: A pilot study

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ABSTRACT

Objective: Oxidative stress is involved in several maternal conditions characterized both by an increase in free radicals synthesis and a parallel decrease in the antioxidant activity. Parturition induces considerable oxidative stress and many inflammatory mediators, among which HMGB1, are involved from the beginning of pregnancy to the birth of the infant. We evaluated serum cord blood HMGB1 levels in a population of neonates to investigate correlation with mode of delivery, as well as the influence of labour.

Setting and patients: The study subjects were 325 neonates delivered at University Hospital "G. Martino" of Messina over an 18-month period. Following cord separation, venous blood sampling was performed on umbelical cords.

Results: In the cord venous blood, we found HMGB1 values significantly more elevated in spontaneous vaginal group when compared to elective or emergency caesarean section group. Regarding labour, umbilical cord venous blood HMGB1 levels were significantly higher in the spontaneous and induced labour group, compared to non-labouring women.

Conclusion: These results could highlight a possible role of HMGB1 during birth time related to mode of delivery and labour.

1. Background

The injury mediated by oxidative stress (OS) is one of the major pathogenic protagonists in the onset of several conditions relating to newborns, which are commonly referred to as "oxygen radical diseases of neonatology" [1,2]. The accumulation of Free Radicals (FR), beyond the capacity of the endogenous antioxidant defence system to scavenge them, results in damage to DNA, proteins and lipids which compromises cellular function, leading to cell death via apoptosis or necrosis. Increasingly emerging evidences from literature reveal that OS-mediated pathways contribute sharply to the preeclampsia, early pregnancy loss, foetal growth restriction and, preterm labour pathogenesis [3]. To date, it is not clear whether also the foetus is subject to OS during the process of labour and birth. Labour is also the result of leukocyte activation which, following uterine invasion, promotes the release of uterotrophins (e.g. cytokines and chemokines) triggering and supporting a myometrial contraction. Whether these events occur early, a preterm labour takes place. Very few literature data are available on changes in OS levels in newborns in relation to delivery mode [3]. When compared to babies born by elective CS, newborns from both spontaneous vaginal delivery (SVD) and emergency caesarean section (CS) show more higher oxidative products levels in the umbilical arterial blood,

Abbreviations: OS, oxidative stress; FRs, free radicals; SVD, spontaneous vaginal delivery; CS, caesarean section; HMGB1, Mobility Group Box 1; RAGE, receptor for advanced glycation end products; TLR2, TLR4, toll-like receptors 2 and 4; IUGR, intrauterine growth restriction; sRAGE, soluble RAGE; ROS, reactive oxygen species

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probably due to delivery-related OS [4]. Also, it could be the expression of a condition of prenatal oxidative status [4].

High Mobility Group Box 1 (HMGB1) is a DNA-binding nonhistone protein (25 kDA) expressed both in intracellular site (nucleus, cytosol, mitochondria, and cell surface membranes) and in extracellular space. Following a specific biochemical stimulus (e.g. antigen presentation, OS, cytokines secretion, tissue damage) HMGB1 can be passively secreted from damaged or necrotic cells [5]. HMGB1 takes part in numerous medical conditions, including pregnancy [6,7], both in early events, primarily embryo implantation, and in later events, including labour and delivery. The release of alarmin, subsequent to tissue damage, aging cell and/or other stress factors, is known to be involved in pathologies of pregnancy, especially in isolated or recurrent abortion, intrauterine growth restriction (IUGR), and preterm labour [8]. It is known that many inflammatory mediators, among which HMGB1, are involved from the beginning of pregnancy to birth of the infant. Changes in amniotic fluid HMGB1 levels both of non-labouring and labouring (term and preterm) pregnant women have highlighted that HMGB1 can be detected into amniotic fluid [9].

In order to further investigate the HMGB1′s role, this study evaluated serum cord blood HMGB1 levels in a population of neonates, to investigate the potential utility of alarmin as a novel marker, and its connection with mode of delivery, in babies born both by SVD and CS (elective or emergency), as well as the influence of labour.

2. Materials and methods

2.1. Subjects

The study subjects included 325 newborns delivered at the Department of Obstetrics, at University Hospital "G. Martino" of Messina, Italy, over an 18-month period. Following cord separation, venous blood sampling was performed on umbelical cords.

Exclusion criteria were: born dead, donation of the umbilical cordon and cord preservation.

Pregnant women admitted for labour were informed about the aims of the research and fully informed about study protocol. Participation in this study was voluntary and enrolment occurred at the same moment of admission for delivery. Prior to start the study, written informed consent was obtained from the pregnant women.

The study was conducted in accordance with the Declaration of Helsinki and was approved by the local Ethics Committee of the University Hospital of Messina (approval number 69/17).

The recruited patients were divided into 3 groups related to mode of delivery: spontaneous vaginal delivery (SVD) (group A), elective CS (group B), emergency CS (group C).

Regarding labour, subjects were divided into 3 groups: spontaneous labour (Group S), induced labour (Group I), absent labour (Group O).

Within 5 min after birth, blood samples from the umbilical vein were collected. Blood was left to clot for 5 min at room temperature. Then, blood samples were centrifugated and serum aliquots were stored at 80 °C until use. Serum HMGB1 was assessed using ELISA Kit following recommended protocol (Phoenix Pharmaceutical; Belmont, CA. ST51011 HMGB1 ELISA 96). Absorbance was measured using a microplate reader (Bio-Rad, Milan, Italy), and standard curves were constructed by the Bio-Rad Microplate Manager program V.5.1.

Other aliquots of the collected cord blood samples obtained in heparin-washed syringes were used to perform blood gas analysis.

Birth body weight, gestational age, and all maternal characteristics were recorded.

2.2. Statistical analysis

Numerical data are expressed as median and range (minimum and maximum). The non-parametric approach was used since the numerical variables were not normally distributed, as verified by the Kolmogorov Smirnov test. The Kruskall Wallis test was applied to compare HMGB1 among the 3 different kinds of delivery; since it was highly significant, we performed pair wise comparisons between groups using the Mann Whitney test. The same analysis was performed to compare the different kinds of labour. For these multiple comparisons, we had to apply Bonferroni's correction, for which the significance alpha level 0.050 has to be divided by the number of the possible comparisons; thus, the new "adjusted" significance level for this analysis is equal to 0.050/6 = 0.008. The non-parametric Spearman correlation test was applied in order to assess the existence of any significant interdependence between HMGB1 and numerical parameters. Statistical analyses were performed using SPSS 17.0 for Window package.

$P < 0.05$ two sides was considered to be statistically significant.

3. Results

From among the enrolled 325 subjects, only 295 were included in this analysis. Thirty neonates with evident hemolysis in umbilical cord blood were excluded from the study.

All neonates were born healthy, as estimated by Apgar scores at 1 and 5 min and pH values of blood gas performed at birth. The mean (± SD) gestational age was 38.6 ± 1.8 weeks, mean birth weight 3100 ± 500 g. Characteristics of infants and their mothers are summarized in Table 1.

Statistical analysis performed to identify any potential significant interdependence between umbilical vein blood HMGB1 levels and obstetric history (e.g. personal history of acute and chronic disease or infectious diseases; taking drugs during pregnancy; smoking during pregnancy; weight gain during pregnancy; abortion threats; premature birth threats; presence and length of labour), clinical characteristics (gestational age ($p = 0.213$); Apgar Index at 1′ and 5′; birth weight ($p = 0.612$); need of aspiration of airways, oxygen supplementation, positive pressure ventilation, emergency tracheal intubation, chest compressions, umbilical vein catheterization, and drugs administration), and laboratory findings (arterial blood gas test) of enrolled population failed. Correlations between HMGB1 levels, clinical features, and laboratory findings are summarized in Table 1, but no significant differences were observed.

Subjects were divided into the following 3 groups related to mode of delivery: SVD (group A, n = 196), elective CS (group B, n = 49), emergency CS (group C, n = 42). Serum HMGB1 levels significantly and directly were correlated with mode of delivery. In the cord venous blood, we found HMGB1 values significantly more elevated in spontaneous vaginal group when compared to elective or emergency caesarean section group (p = 0.004). While there was no significant HMGB1 difference between groups of neonates born by caesarean delivery, both elective or emergency (Group B and C) (p = 0.046).

Regarding labour, subjects were divided into 3 groups: spontaneous labour (Group S, n = 131), induced labour (Group I, n = 99), absent labour (Group O, n = 55). In the cord venous blood, we found HMGB1 values significantly more elevated in the two groups characterised by presence of labour, both spontaneous and induced (Group S and I), with concentrations significantly higher than the group without labour (Group O) (p = 0.010).

Over the years, research on HMGB1 has been quite thorough and other properties have been revealed. HMGB1 has resulted to be a leading mediator in both acute and chronic inflammation, and plays a crucial role in several medical conditions, including autoimmune diseases, cancer, hepatitis, malaria, myocardial ischemia, infection-elicited inflammatory diseases [10]. It is known how inflammation is essential in maintaining uterine homeostasis, for successful embryo implantation and delivery. Even though inflammation is needed for a successful reproduction, early and uncontrolled activation of inflammatory proceedings can cause important adverse effects on childbearing outcomes, including preterm birth [11,12]. Recent findings by Romero et al. have highlighted the importance of HMGB1 in amniotic fluid sterile