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Influence of mode of delivery on cytokine expression in cord blood

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ABSTRACT

The mode of delivery is a known risk factor for immune-related disorders. Normal term vaginal delivery is an inflammatory process and several cytokines are suggested to be involved. The purpose of the study was to evaluate differences in cord blood cytokine expression between modes of delivery in term-born children.

Cord blood was collected from 49 elective Caesarean section (C-section) cases and from 49 normal vaginal term deliveries. Plasma was tested for 17 cytokines with Bio-Plex®-200-system. Mann-Whitney test was used for comparing the groups with Bonferroni correction for multiple testing.

Four cytokines showed significant differences between the modes of delivery. Interleukin-6, Interleukin-8 showed a significantly higher expression in the vaginal delivery group, while Tumor-Necrosis Factor-a, Granulocyte-Colony Stimulating Factor showed a significantly higher level of expression in the C-section cord blood.

Our study shows that there is differential expression of pro-inflammatory cytokines in elective C-section compared with normal term vaginal delivery.

1. Introduction

In recent years, the rate of Caesarean section (C-section) delivery has significantly increased [1,2]. Multiple studies indicate that C-section is a risk factor for immune-related disorders [3–6]. The process of birth itself has gained attention as an immune programming event [7]. Hence the impact of mode of delivery on innate immunity of infants needs to be better understood. Recent literature suggests that the process of normal delivery stimulates the respiratory, metabolic and immunological maturation in infants which prepares them to adapt to the outside world (the exposome) [8]. The mode of delivery is known to influence the innate immune system through altered gut microbiota and hormonal changes during birth like increased catecholamines and cortisol [2], although the role of stress hormones in immune maturation is not clear [7]. A recent study also reported lower monocytes and neutrophils in cord blood of C-section delivered compared to vaginally born infants [9].

Increasing evidence suggests that normal vaginal delivery is a natural inflammatory process which results in increased levels of cytokines like Interleukin-6 (IL-6), Interleukin-8 (IL-8) in maternal and cord blood plasma [10]. Increased levels IL-6 and IL-8 have been reported in the amniotic fluid of subjects who were delivered vaginally compared to those delivered by elective C-section. Existing data suggest that prelabour elective C-section is associated with aberrant short-term innate immune responses such as reduced expression of inflammatory markers in the newborn infant [2], although TNF-a was reported to be increased [11].

The objective of this study was to analyse differences in the cord blood cytokine profile between healthy term infants born vaginally versus those born via elective C-section.

2. Methods

2.1. Patient selection and sample collection

All subjects with uncomplicated pregnancies were recruited from KK Women's and Children's Hospital, Singapore over a period of 8 years. Eligible patients provided informed written consent in accordance with the Declaration of Helsinki. In the initial recruitment, study one, forty-nine pregnant women were enrolled with a further forty-nine pregnant women recruited in study two. Both studies were approved by the institutional board of the hospital, shared the same

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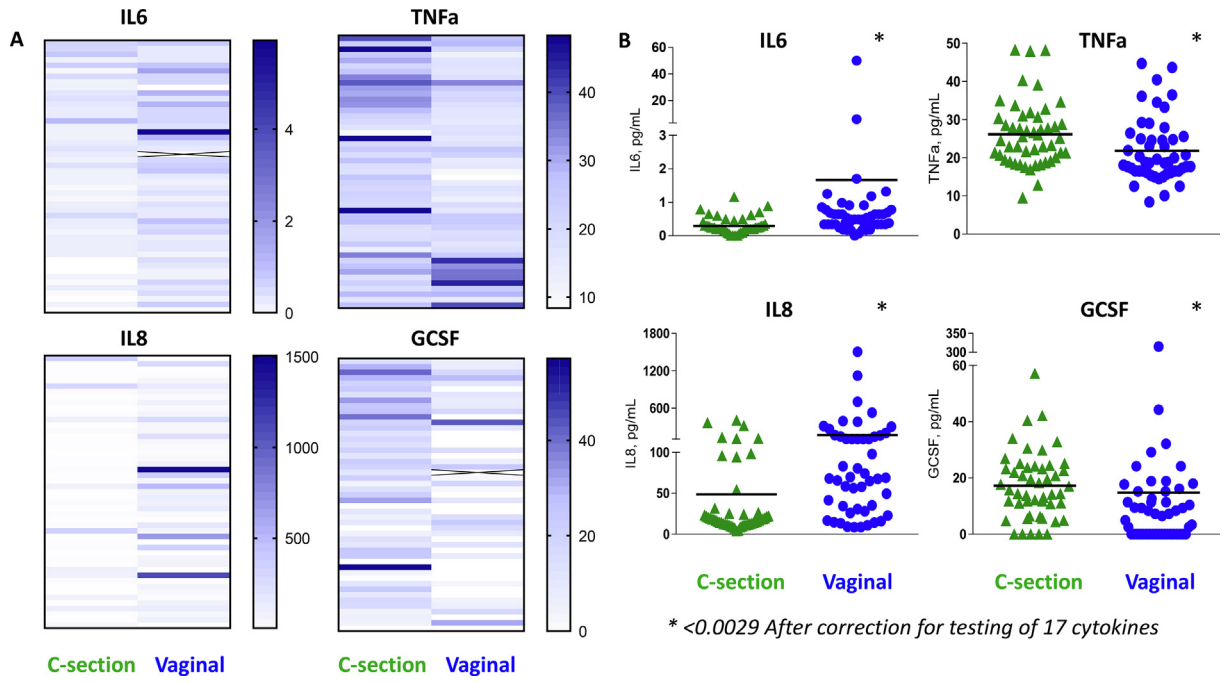

Fig. 1. Expression of IL-6, IL-8, TNF-a, G-CSF in C-section and vaginal cord blood plasma. A heatmap of IL-6, IL-8, TNF-a, G-CSF in C-section and vaginal cord blood plasma. Outliers are removed and represented by cross on white background. B dot blots of IL-6, IL-8, TNF-a, G-CSF expression in C-section and vaginal cord blood plasma. Each dot represents the level of expression of an individual sample, a line represents a median expression in each group. All P values were compared with 0.0029 after correction for comparison of 17 cytokines.

study design for the collection of cord blood and were conducted by the same research team. The venous umbilical cord blood was collected into heparinized tubes (BD Vacutainer Tubes, ThermoFischer). The collected samples were stored at room temperature during transportation to the lab. The blood was processed as soon as possible within a maximum of 10 h from collection, plasma was aliquoted and stored at −80 °C until analysis. The two studies are registered at https://clinicaltrials.gov (NCT03134768) and www.trialregister.nl (registration number 2838).

2.2. Cytokine assays

Plasma was tested for seventeen cytokines using Bio-Plex® 200 system, Luminex (Bio-Rad Laboratories, Singapore). Each final cytokine concentration was corrected for background concentration by subtracting the concentration of the negative control samples. The following cytokines were measured: Interleukins (IL) - IL-1b, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12(p70), IL-13, IL-17, Granulocyte-colony stimulating Factor (G-CSF), Granulocyte-macrophage-colony stimulating Factor (GM-CSF), Interferon-gamma (IFN-g), Monocyte chemoattractant protein-1 (MCP-1), Macrophage Inflammatory Protein-1b (MIP-1b), Tumour-Necrosis Factor-alpha (TNF-a). All samples were analysed with the same Bio Rad Kit (Lot number 64093502) within one week.

2.3. Data analysis

Data analysis was performed using GraphPad software. Online portal http://scistatcalc.blogspot.sg/2013/11/home.html was consulted. Non-normal distribution was assumed using visual examination of histograms and Shapiro-Wilk test. Thus, non-parametric methods were used for comparison between the modes of delivery, Mann-Whitney test was used to compare between groups, Spearman correlation and multiple regression were performed. Bonferroni correction was performed for multiple testing.

3. Results

3.1. Study cohort and characteristics

Obstetric and newborn characteristics of the two groups are described in Supplementary Table 1. Mean birthweight of the 98 infants was 3150 g (range: 2235–4245 g), 49% of the infants were male. All characteristics were similar except for maternal age, neonatal birth length and head circumference.

All mothers who had elective C-section were given standard prophylactic doses of antibiotics before the surgery. Forty-six subjects were given spinal anesthesia and four subjects were given general anesthesia. All subjects who had vaginal delivery were induced with vaginal prostaglandin E2 (Prostin E2) suppository.

3.2. Cytokine measurements in cord blood

Among the seventeen cytokines measured in cord blood, IL-4, IL-7, IL-13 and GM-CSF were below detection limits in both groups. Two heatmaps were built for cytokine expression: one with a high range of expression (0–1550 pg/ml) and a second with a low range (0–15 pg/ml) (Supplementary Fig. 1). No clear overall pattern was observed. However, some individual cytokines showed a visual difference between modes of delivery. Subsequently, individual differences in cytokine expression were analysed.

There were no significant differences in the expression of IL-1b, IL-10, IL-17, IL-12p70, IFN-g and MIP-1b between the two groups. The levels of IL-6, IL-8, IL-5 and IL-2 were increased in the vaginal group but after Bonferroni correction for multiple testing of 17 cytokines only IL-6 and IL-8 showed a consistent significantly higher expression in the vaginal group when compared to C-section (Fig. 1). Similarly, G-CSF, TNF-a and MCP-1 levels were significantly increased in the C-section group. But after the Bonferroni correction, only G-CSF and TNF-a remained significant (Fig. 1).

3.3. Relationship between cytokines and obstetric, newborn characteristics

The four cytokines that demonstrated significant difference between modes of delivery (IL-6, IL-8, TNF-a and G-CSF) were analyzed for correlation with obstetric and newborn characteristics for both C-section and vaginal deliveries. No correlation was observed between any of the factors. Multiple regression for each cytokine and maternal, newborn parameters also did not reveal any significant relationship (data not shown).

No significant correlation of IL-6, IL-8, TNF-a and G-SCF expression to each other have been observed. (data not shown).

4. Discussion

This study identified important differences in the immune responses between elective C-section and vaginal deliveries.

The findings are consistent with previous studies which showed an increase in IL-6 levels in cord blood plasma of vaginal deliveries compared to elective C-section [12,13]. As for IL-8 expression, previous studies reported inconsistent results with no significant difference in two studies and increase in IL-8 in relation to labour events in another study [12–14]. IL-8 is known to have neutrophil migration ability, which helps in enhanced chemotaxis [15]. Lower neutrophils in C-section cord blood have been observed [9]. The activation of cells through increased IL-8 during labour could be a biological adaptation to prepare the newborn to face challenges ex-utero [15].

In contrast to high IL-6, IL-8 levels in vaginally delivered cord blood plasma, a statistically significant increase in TNF-a in C-section cord blood plasma was observed. These results are not in line with previous studies which reported either no difference between C-section and vaginally delivered cord blood [16] or high levels of TNF-a in vaginally delivered cord blood [13]. However, our study supports the results of Zanardo et al. [11] where the TNF-a levels are increased in C-section plasma levels.

A significant new finding in our study is an increase in G-CSF in C-section compared to vaginally delivered cord blood plasma which has never been reported to date. One previous study has reported no difference between modes of delivery in the levels of G-CSF in cord blood, probably due to the study's small sample size [17].

It is important to note that some cord blood samples in vaginal and C-section deliveries show very low cytokine expression. However, more vaginal cord blood samples showed increased expression of IL-6 and IL-8 whereas more C-section cord blood samples show increased TNF-a and G-CSF expression. These differences between samples within each mode of delivery could be due to multiple factors like genetic differences, duration of labour, order of birth and labour intensity [18–20]. Here, Bonferroni correction was used for multiple testing. IL-5, IL-2 and MCP1- initially showed differences but did not survive the correction for multiple testing. It is possible that the differences in the expression of these cytokines in the cord blood exist and could be demonstrated with a higher sample size.

As indicated, many studies have been published demonstrating differences in cytokine expression in the cord blood plasma of vaginal and C-section births which may reflect the stresses that the newborn experiences during the process of birth [8,12,13]. But the results are not always consistent. These inconsistencies could be due to the differences in the methods of cord blood collection, cytokine analysis, or maternal confounders such as anthropometric differences between studies. Most studies collect cord blood from the umbilical vein or mix cord blood from both artery and vein [10,12]. The study by Duncombe et al. [16] demonstrated differences in the levels of IL-6 and TNF-a in cord blood collected from the umbilical artery, umbilical vein and from the placenta, showing that blood from the umbilical vein shows the lowest level of reported cytokines. In our study, the cord blood was collected from the umbilical vein and despite that, significant differences between the four cytokines were observed.

The methods used for cytokine analysis, such as ELISA or multiplex systems could also influence the concentrations of cytokines analysed. It is therefore crucial to use the same lot of the multiplex analysis kit to make such a comparison [21]. In our study, we ensured that the analysis was performed at the same time by the same lot of the multiplex kit.

Previous studies looking at the difference between the modes of delivery have focused mainly on analysis of two to three cytokines [11,22]. We analysed seventeen cytokines and identified four cytokines that showed significant differences even after the stringent Bonferroni correction for multiple testing.

No correlation with maternal age, duration of labour and birthweight for both C-section and vaginal deliveries was observed in our cohort. This is not surprising as only healthy uncomplicated pregnancies were included. Interestingly, we also did not observe a correlation between the four differentially expressed cytokines which indicated that the mechanisms responsible for the induction of these cytokines were different and not related to each other.

The limitation of this study is that the clinical protocols did not allow to assess the potential effect of medication on cytokine expression. All C-section delivered subjects received antibiotic prophylaxis and all mothers who delivered vaginally received prostaglandin E2. Prostaglandins are known to increase IL-8 [23], and it's possible that the observed increase in IL-8 expression in our study is due to the use of prostaglandins. Morikawa et al. [24] showed a modulatory effect of antibiotics on cytokine production in vitro. Previous report has observed the influence of anesthesia on immune responses in newborns with maternal epidural anesthesia having less of an impact than general anesthesia [25]. Thus, we cannot exclude that some of the differences observed could be due to medication use and not due to the process of labour itself.

The current study also did not address the topic of oxidative stress and alarmins, such as High Mobility Group Box-1 (HMGB1), which are known to accompany normal vaginal delivery [26–29]. Thus, the relationship between oxidative stress, alarmins and cytokines could not be evaluated.

In conclusion, our study demonstrates clear differential expression of IL-6, IL-8, TNF-a and G-CSF in the cord blood of term children born vaginally and by elective C-section.

Evidence is accumulating that a few cytokines, chemokines, catecholamines and stress hormones are involved in the event of labour. A comprehensive study on cytokines, chemokines, stress hormones, oxidative stress markers, alarmins and catecholamines in cord blood in relation to maternal and infant blood along with an analysis of the gut microbiome of infants would help to build a better understanding of their effects during labour and the roles in immune development. Pinpointing the relevance of these immunological changes might help to develop intervention strategies to reduce the risk of immune disorders in later life.

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Conflicts of interest

Leon Knippels, Johan Garssen, Bindu Nandanan, Dinesh Kumar and Elena Sandalova are employees of Danone Nutricia research.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.humimm.2019.03.018.

References

[1] A.P. Betran, et al., The increasing trend in caesarean section rates: global, regional and national estimates: 1990–2014, PLoS One 11 (2) (2016) e0148343.
[2] C.E. Cho, M. Norman, Cesarean section and development of the immune system in the offspring, Am. J. Obstet. Gynecol. 208 (4) (2013) 249–254.
[3] A. Sevelsted, et al., Cesarean section and chronic immune disorders, Pediatrics 135 (1) (2015) e92–e98.
[4] P. Bager, J. Wohlfahrt, T. Westergaard, Caesarean delivery and risk of atopy and allergic disease: meta-analyses, Clin. Exp. Allergy 38 (4) (2008) 634–642.
[5] N. Mitselou, et al., Cesarean delivery, preterm birth, and risk of food allergy: nationwide Swedish cohort study of more than 1 million children, J. Allergy Clin. Immunol. (2018).
[6] N.P. Ly, et al., Mode of delivery and cord blood cytokines: a birth cohort study, Clin. Mol. Allergy 4 (2006) 13.
[7] M.J. Hyde, et al., The health implications of birth by Caesarean section, Biol. Rev. Camb. Philos. Soc. 87 (1) (2012) 229–243.
[8] S.L. Liao, et al., Caesarean Section is associated with reduced perinatal cytokine response, increased risk of bacterial colonization in the airway, and infantile wheezing, Sci. Rep. 7 (1) (2017) 9053.
[9] G. Dunsmore, et al., Mode of delivery by an ulcerative colitis mother in a case of twins: immunological differences in cord blood and placenta, World J. Gastroenterol. 24 (42) (2018) 4787–4797.
[10] C.J. Chan, et al., Cytokines in umbilical cord blood and the impact of labor events in low-risk term pregnancies, Early Hum. Dev. 89 (12) (2013) 1005–1010.
[11] V. Zanardo, G. Solda, D. Trevisanuto, Elective cesarean section and fetal immune-endocrine response, Int. J. Gynaecol. Obstet. 95 (1) (2006) 52–53.
[12] N. Takahashi, et al., Cytokine profiles of seventeen cytokines, growth factors and chemokines in cord blood and its relation to perinatal clinical findings, Cytokine 49 (3) (2010) 331–337.
[13] A. Malamitsi-Puchner, et al., The influence of the mode of delivery on circulating cytokine concentrations in the perinatal period, Early Hum. Dev. 81 (4) (2005) 387–392.
[14] J. Dembinski, et al., Cell-associated interleukin-8 in cord blood of term and preterm infants, Clin. Diagn. Lab. Immunol. 9 (2) (2002) 320–323.
[15] E. Yektaei-Karin, et al., The stress of birth enhances in vitro spontaneous and IL-8-induced neutrophil chemotaxis in the human newborn, Pediatr. Allergy Immunol. 18 (8) (2007) 643–651.
[16] G. Duncombe, et al., IL-6 and TNFalpha across the umbilical circulation in term pregnancies: relationship with labour events, Early Hum. Dev. 86 (2) (2010) 113–117.
[17] P. Gessler, C. Dahinden, Increased respiratory burst and increased expression of complement receptor-3 (CD11b/CD18) and of IL-8 receptor-A in neutrophil granulocytes from newborns after vaginal delivery, Biol. Neonate 83 (2) (2003) 107–112.
[18] M. Kragh, et al., Divergent response profile in activated cord blood T cells from first-born child implies birth-order-associated in utero immune programming, Allergy 71 (3) (2016) 323–332.
[19] M. Schirmer, et al., The causes and consequences of variation in human cytokine production in health, Curr. Opin. Immunol. 54 (2018) 50–58.
[20] C.A. Logan, et al., Delivery Mode, duration of labor, and cord blood adiponectin, leptin, and C-reactive protein: results of the population-based ulm birth cohort studies, PLoS One 11 (2) (2016) e0149918.
[21] E.C. Breen, et al., Multisite comparison of high-sensitivity multiplex cytokine assays, Clin. Vaccine Immunol. 18 (8) (2011) 1229–1242.
[22] M. Haghshenas Mojaveri, et al., The comparison of serum interleukin-6 of mothers in vaginal and elective cesarean delivery, Caspian J. Intern. Med. 5 (4) (2014) 223–226.
[23] R. Bakker, S. Pierce, D. Myers, The role of prostaglandins E1 and E2, dinoprostone, and misoprostol in cervical ripening and the induction of labor: a mechanistic approach, Arch. Gynecol. Obstet. 296 (2) (2017) 167–179.
[24] K. Morikawa, et al., Modulatory effect of antibiotics on cytokine production by human monocytes in vitro, Antimicrob. Agents Chemother. 40 (6) (1996) 1366–1370.
[25] A. Gasparoni, et al., Effect of general and epidural anaesthesia on thyroid hormones and immunity in neonates, Paediatr. Anaesth. 12 (1) (2002) 59–64.
[26] G. D'Angelo, et al., HMGB1 and markers of oxidative stress in human cord blood, Pediatr. Int. (2019).
[27] J.M. Salas-Pacheco, et al., Oxidative stress equilibrium during obstetric event in normal pregnancy, J. Matern. Fetal. Neonatal. Med. 30 (15) (2017) 1836–1840.
[28] G. D'Angelo, et al., Different concentration of human cord blood HMGB1 according to delivery and labour: a pilot study, Cytokine 108 (2018) 53–56.
[29] G. Carlomagno, et al., From implantation to birth: insight into molecular melatonin functions, Int. J. Mol. Sci. 19 (9) (2018).