International Journal of Nursing Studies 97 (2019) 55–62

Contents lists available at ScienceDirect

International Journal of Nursing Studies

journal homepage: www.elsevier.com/ijns

The relationship between neutrophil-lymphocyte ratio and onset of lactation among postpartum women: A prospective observational cohort study

Shenghong He, Haiou Xia*

School of Nursing, Fudan University, 305 Fenglin Road, Xuhui District, Shanghai, China

ARTICLE INFO

Article history:
Received 14 August 2018
Received in revised form 5 May 2019
Accepted 5 May 2019

Keywords:
Adenosine triphosphate
Breast-feeding
Elective caesarean birth
Lactation
Mitochondrial deoxyribonucleic acid copy number
Neutrophil-lymphocyte ratio
Onset
Vaginal birth

ABSTRACT

Background: Delayed onset of lactation is a key factor in the low rate of exclusive breast-feeding in 6 months after caesarean section. The mechanism of delayed onset of lactation is not clear. Milk production depends largely on mitochondrial adenosine triphosphate synthesis, and the neutrophil-lymphocyte ratio is closely related to mitochondrial deoxyribonucleic acid copy number and adenosine triphosphate production. Presently, it is unclear whether a difference in the neutrophil-lymphocyte ratio exists between those undergoing vaginal delivery and those undergoing caesarean delivery and, if so, whether the difference correlates to the time of onset of lactation.
Objectives: To identify whether the neutrophil-lymphocyte ratio at 24 hours after delivery is different between mothers delivering by caesarean section and those giving birth vaginally and whether the neutrophil-lymphocyte ratio is related to the delayed onset of lactation.
Design: The study adopted a prospective cohort study design.
Settings: Maternity units of an Obstetrics & Gynecology Hospital.
Participants: 327 mother-infant pairs who met inclusion/exclusion criteria and were followed up to the time of onset of lactation.
Methods: Mother-infant pairs were allocated to the vaginal birth group or the caesarean section group according to birth method. The neutrophil-lymphocyte ratio was calculated as the absolute value of neutrophils divided by the absolute value of lymphocytes based on full blood counts. Before delivery, full blood counts were obtained from medical records. After delivery, blood samples were drawn 24 hours postpartum, and blood cells were classified and counted. The onset of lactation was confirmed by the maternal perception of breast fullness. The neutrophil-lymphocyte ratio and its relationship with lactation onset were analyzed by multivariable regression.
Results: The neutrophil-lymphocyte ratios of both groups were elevated after delivery. Based on the covariance analysis, after adjusting for baseline full blood counts before delivery, the neutrophil-lymphocyte ratio in the caesarean group was higher than the ratio in the vaginal group after delivery ($p = .000$). In addition, after adjustment for confounding factors, multivariable regression analyses showed that an increased neutrophil-lymphocyte ratio was correlated with delayed onset of lactation (95% confidence interval 0.285–1.646).
Conclusions: The neutrophil-lymphocyte ratio in the caesarean section group was higher than that in the vaginal delivery group and was related to a delayed onset of lactation. Given the decreased mitochondrial copy number in the elevated neutrophil-lymphocyte ratio and therefore the associated reduction in adenosine triphosphate synthesis, these findings may elucidate the mechanism for delayed onset of lactation in caesarean section births.

What is already known about the topic?

- The onset of maternal lactation in women undergoing caesarean section is usually later than in women giving birth vaginally.
- In early lactation, the mitochondrial deoxyribonucleic acid copy number in peripheral blood is highly related to the mitochondrial

* Corresponding author.
E-mail address: hsxia@fudan.edu.cn (H. Xia).

https://doi.org/10.1016/j.ijnurstu.2019.05.005
0020-7489/

deoxyribonucleic acid copy number in the mammary gland, and the mitochondrial deoxyribonucleic acid copy number in peripheral blood is increased after vaginal delivery.
- When the number of leukocytes of an individual is not significantly changed, a decreased neutrophil-lymphocyte ratio is related to increased mitochondrial deoxyribonucleic acid copy number and adenosine triphosphate production, and vice versa.

What this paper adds

- The neutrophil-lymphocyte ratio after caesarean section was higher than after spontaneous delivery.
- An elevated neutrophil-lymphocyte ratio was related to the delayed onset of lactation.
- Delayed onset of lactation in mothers undergoing caesarean delivery is possibly attributable to elevated neutrophil-lymphocyte ratio and reduced mitochondrial deoxyribonucleic acid copy number and adenosine triphosphate production.

1. Introduction

Although the World Health Organization recommends that infants should be exclusively breast-fed for the first 6 months, the rates of exclusive breast-feeding remain low in China. Two prospective cohort studies have shown the rates to be between 2.6% (Liu et al., 2013) and 21.5% (Chen et al., 2018). Caesarean section is an independent risk factor for the failure of exclusive breast-feeding (Regan et al., 2013; Zhu et al., 2013). Mothers who had a vaginal birth were more likely to practice exclusive breast-feeding than mothers who gave birth via caesarean section (Adugna et al., 2017). The effect of caesarean section on breast-feeding is mainly in the early postoperative period. Early breast-feeding rates are positively related to the exclusive breast-feeding rate and negatively correlated to caesarean section (Guo et al., 2013). The main cause for the lower exclusive breast-feeding rate is the delayed or failed onset of lactation (Brownell et al., 2012). The onset of lactation for mothers with caesarean delivery is more likely to be delayed than for mothers with vaginal delivery (Scott et al., 2007). However, the precise reasons why caesarean section affects the onset of lactation are still unclear. In addition, exploration of the mechanisms underlying the delayed onset of lactation may help to develop appropriate interventions.

It is possible that delayed onset of lactation after caesarean section is related to adenosine triphosphate reduction, which is inferred as follows. Adenosine triphosphate acts as a major co-transmitter substance that directly affects cutaneous vasomotor function and skin temperature (Lang et al., 2017). It has been recognized that caesarean section can lead to a decrease in body temperature, but even active warming with a thermal gown before the intraoperative period cannot absolutely prevent temperature loss during caesarean delivery (Bernardis et al., 2016). Another research study showed that elective limb surgery in normothermic patients resulted in a decrease in adenosine triphosphate plasma levels 24 hours after operation (Seekamp et al., 1999), so caesarean sections may result in the reduction of adenosine triphosphate levels. Caesarean section is a risk factor for delayed onset of lactation (Scott et al., 2007). Meanwhile, most aspects of milk synthesis are dependent on the mitochondrion, which is the key organelle for production of adenosine triphosphate (Huang and Keenan, 1971). Hence, the delayed onset of lactation may be related to the decreased adenosine triphosphate. In addition, previous researchers have reported that the breast temperature in the puerperium of normally lactating women was higher than in women with hypogalactia (Tsubomoto, 1979; Skopichev and Gaidukov, 1990); therefore, the delayed onset of lactation may be related to breast temperature, which is mainly adjusted by adenosine triphosphate.

In light of all the above-mentioned factors, we can reasonably speculate that caesarean section may decrease adenosine triphosphate production and thereby cause delayed onset of lactation. To explore the mechanism hypothesis, we initially wanted to explore the postpartum production of adenosine triphosphate in those delivering via caesarean section as well as those giving birth vaginally. However, since adenosine triphosphate is unstable, it is extremely difficult to measure its levels in a large number of samples collected at different times, necessitating an indirect index to reflect adenosine triphosphate level.

It has been demonstrated that in early lactation, mitochondrial deoxyribonucleic acid copy numbers in peripheral blood in cows were strongly correlated with mitochondrial deoxyribonucleic acid copies in the mammary gland (Laubenthal et al., 2016). In consideration of the research feasibility and ethical requirements, it is proposed that in early-lactating mothers, mitochondrial deoxyribonucleic acid copy numbers in blood might reflect the cellular energy status of the mammary gland. Peripheral blood cells are the cellular components of erythrocytes, leukocytes, and platelets. Because erythrocytes do not contain mitochondria, the peripheral blood mitochondrial deoxyribonucleic acid copy number refers to the leukocyte mitochondrial copy number and platelet mitochondrial copy number.

There are three main types of white blood cells that contain mitochondria, namely, neutrophils, lymphocytes, and monocytes. Neutrophils are the most abundant type of white blood cells, accounting for 50%–70%; the second most abundant type is lymphocytes, accounting for 20%–40%; and the fewest are monocytes, about 3%–8%. Neutrophils contain a very low mitochondrial deoxyribonucleic acid copy number (Maianski et al., 2004), and metabolism in the mitochondria occurs through anaerobic glycolysis rather than oxidative phosphorylation with the production of little adenosine triphosphate (El-Hag and Clark, 1987). Adenosine triphosphate generation via glycolysis is far less efficient (two adenosine triphosphate generated per glucose) compared with oxidative phosphorylation (36 adenosine triphosphate generated per glucose). Given the low mitochondrial copy number within neutrophils coupled with their preferential use of less efficient energy metabolism pathways, a change in total neutrophil counts will not significantly affect whole-body adenosine triphosphate production. The proportion of monocytes in white blood cells is relatively low, and the adenosine triphosphate production mode is mainly anaerobic digestion, which also has little influence on the total mitochondrial deoxyribonucleic acid copy number and adenosine triphosphate level in peripheral blood. Lymphocytes are abundant in mitochondrial deoxyribonucleic acid copy number and produce adenosine triphosphate by aerobic oxidation (Pyle et al., 2010; Kramer et al., 2014). Lymphocytic activity and deoxyribonucleic acid synthesis are inhibited by neutrophils (El-Hag and Clark, 1987). Hence, lymphocyte counts have the ability to significantly affect both mitochondrial copy number and in turn adenosine triphosphate synthesis.

The mitochondrial deoxyribonucleic acid copy number per platelet is much lower than that in a single lymphocyte (Hurtado-Roca et al., 2016). In other words, small changes in platelets do not affect mitochondrial deoxyribonucleic acid copy number quantification and adenosine triphosphate production in peripheral blood. Therefore, the neutrophil-lymphocyte ratio mainly influences mitochondrial deoxyribonucleic acid copy number and adenosine triphosphate production of leukocytes in peripheral blood.

Caesarean section usually causes delayed onset of maternal lactation in comparison with vaginal delivery. Lactation depends on adenosine triphosphate, which may be reduced after caesarean

section. Therefore, we hypothesized that if the neutrophil-lymphocyte ratio after caesarean section was higher than that of vaginal delivery, an elevated neutrophil-lymphocyte ratio may be related to the delayed onset of lactation.

Neutrophil-lymphocyte ratio, as represented by the number of neutrophils divided by the number of lymphocytes, can be easily collected from the data of a routine blood examination. Owing to its convenience and low costs, it may serve as an observational indicator for large-scale prospective cohort studies to help determine whether a decline in adenosine triphosphate after caesarean delivery is related to lactation delay.

2. Methods

2.1. Study design, setting, and participants

We used a prospective cohort study design in this research. We carried out the study in the maternity units of an obstetrics and gynecology hospital, which was a specialized, tertiary (Grade 3, Class A) hospital.

All mother-infant pairs in this study were based on the following inclusion criteria: (a) healthy single birth and full-term primiparas aged 20–35 years (because the neutrophil-lymphocyte ratio in periparturient primiparous and multiparous dairy cows is quite different before and after calving (Bühler et al., 2016)), (b) willing to breast-feed babies, (c) blood cell counts within 1 week before the delivery (since the neutrophil-lymphocyte ratio is changed less in the first 3–7 days before birth but changed greatly in the prenatal 7–14 days (Bühler et al., 2016)), and (d) normal temperature when venous blood samples were drawn and during labor (because fever affects the neutrophil-lymphocyte ratio). The exclusion criteria were (a) breast-feeding contraindications, (b) smoking during pregnancy, (c) a pregestational body mass index $\geq$28 kg/m^2 (obesity), (d) complications related to pregnancy or childbirth, (e) dystocia, or use of midwifery forceps during labor, (f) a fever (temperature $\geq$38.0 °C) within 24 hours postpartum, (g) chronic medical and psychiatric problems, (h) night delivery, (i) using cortisol drugs, (j) emergency caesarean section (after onset of labour), or (k) other disease such as asthma. Any patient with factors affecting the neutrophil-lymphocyte ratio, mitochondrial deoxyribonucleic acid copy number, or adenosine triphosphate production, such as age (Chistiakov et al., 2014), obesity (Hastie and Lappas, 2014), complications (Crovetto et al., 2013), or smoking (Garrabou et al., 2016), was excluded.

2.2. Ethical considerations

The ethics committee of the Obstetrics & Gynecology Hospital of Fudan University approved the study protocol (No. 2016-61), and the study was registered in the Chinese Clinical Trial Registration (No. ChiCTR-ROC-16010102) and conducted from December 2016 to June 2017. Before the study, we provided all potential participants with both verbal and written information about the purpose and procedures of the study, informed them of the right to withdraw from the study at any time and without any reason, and obtained the written informed consent from each participant.

2.3. Measurement

2.3.1. Blood cell counts

Blood cell counts included the numbers of leukocytes, neutrophils, lymphocytes, monocytes, and platelets of all pregnant women enrolled in the study. Prenatal blood cell counts were obtained from the medical records of the pregnant women, whose blood was drawn within 1 week before delivery and whose body temperature were normal. Postpartum blood cell counts were obtained 24 hours after delivery.

2.3.2. Onset of lactation

From 24 hours after delivery to the onset of lactation, mothers were asked twice a day if and when they noticed a sudden feeling of fullness in their breasts. All mothers' reported time of lactation onset was recorded. This is a traditional method used to measure the time of the onset of lactation and has been shown to correlate with the actual time (Parker et al., 2011; Chapman and Perez-Escamilla, 2000).

2.3.3. Prenatal, delivery, and postpartum data

We investigated a wide range of variables as potential confounding factors of the onset of lactation. Via one-to-one interviews, we collected a series of demographic data including maternal age, education level, household income per capita, marital status, employment status, residential address, race, and breast-feeding or not. Based on the maternal health manual, a type of medical record for pregnant women, we obtained the prepregnancy height and weight and calculated the prepregnancy body mass index using the following formula: mass index = weight/height^2. We also estimated gestational weight gain, which was the difference between maternal weight at the time of the first prenatal care visit and maternal weight before labor.

In addition, we collected the following data via medical records: health problems during pregnancy, attendance of antenatal care, and delivery-related factors, including delivery mode, anesthesia and labor analgesia, and newborn birth weight, gender, and gestational age.

Finally, we examined postpartum variables such as time to first breast-feeding, duration of first breast-feeding, breast-feeding frequency and duration over 24 hours, supplementation of formula within 24 hours, onset of lactation, and maternal psychological factors. We gathered the feeding data from the feeding records of parents or caregivers, who were trained in the data collection methods by us at the inception of the study.

We measured the confounding factors of pain (Daoudia et al., 2015), fatigue (Chae et al., 2018), and depression (Kim et al., 2011) that affected the neutrophil-lymphocyte ratio, mitochondrial deoxyribonucleic acid copy number, and adenosine triphosphate levels. We measured the mother's stress level using the Chinese version of the Edinburgh Postnatal Depression Scale (Lee et al., 1998), a self-evaluation tool, within 24 hours after delivery. The Chinese version of the Edinburgh Postnatal Depression Scale with a cutoff score of 9/10 is recommended for screening depressive illness in a general postnatal population. We used a questionnaire from the Fatigue Scale-14 to measure mother's degree of fatigue (Wang et al., 2010; Chalder et al., 1993). Mothers were asked to score pain at 24 hours postpartum on a verbal numeric rating scale ranging from 0 to 10, with 0 indicating *no pain* and 10 indicating *worst pain imaginable* (Holdgate et al., 2003).

2.4. Statistical analysis

The sample size was determined as follows based on preliminary experiments. A two-sided significance level of 0.05 and power level of 0.9 were defined. According to the data of 57 dyads in preliminary experiments, the time of onset of lactation was 54.95 hours for the vaginal birth group and 63.28 hours for the caesarean group; the standard deviation (SD) was 21.00 hours. Factoring in dropouts, the sample size was increased by 30%. A total of 386 subjects were required in the two groups.

The data were imported into Epidata 3.1 and analyzed using SPSS Statistics, version 22.0. Continuous variables with normal distribution were expressed with mean and standard deviation

and analyzed with two sample *t*-test. Allowing for the imbalanced baseline of levels of blood cells and subtype counts, we conducted covariance analysis for the adjusted blood cell counts before delivery. Data that did not meet the condition of covariance analysis were first transformed by coding rank method and then conducting covariance analysis. Composition ratio data were counted by using a chi-square test, while ranked data were analyzed by rank sum test. Multivariate regression was adopted for analyzing the relationship between the neutrophil-lymphocyte ratio and the onset of lactation.

3. Results

The flow from eligibility to data analysis of participants is shown in Fig. 1. A total of 367 mothers of singleton live births were eligible, of whom 7 were excluded because of the lack of blood cell tests at 24 hours postpartum (5 in the vaginal group and 2 in the caesarean group), 22 were excluded because of dropout (18 in the vaginal group and 8 in caesarean group), and 7 were excluded for a temperature ≥38 °C within 24 hours postpartum (5 in the vaginal group and 2 in the caesarean section group). The statistical analysis covered a total of 327 dyads' effective data. Among them, the numbers of women in the caesarean group and vaginal group were 146 (44.6%) and 181 (55.4%), respectively. Of the 181 women in the vaginal group, 48 did not receive labor analgesia, while the remaining women received anesthesia in labor for pain relief. As for the caesarean group, combined spinal-epidural anesthesia was employed in all elective caesarean sections. All but three mothers of the elective caesarean group used in patient-controlled intravenous analgesia.

Mothers in the caesarean group were older ($p = .000$), less frequently received antenatal care ($p = .001$), had higher gestational mass index gains ($p = .009$), had a younger gestational age at births ($p = .000$), and had babies with a heavier birth weight ($p = .006$) than those in the vaginal group (Table 1). As shown in Table 2, mothers in the vaginal group took less time to begin their first breast-feeding ($p = .001$), had a greater breast-feeding frequency ($p = .001$), and had a longer duration of first breast-feeding ($p = .029$) and sucking duration ($p = .007$) than those in the caesarean group. Also, formula feeding frequency ($p = .000$) and formula feeding volume ($p = .000$) were greater in the caesarean group. Pain ($p = .000$) and fatigue ($p = .002$) were worse in the caesarean group (Table 2). The onset of lactation ($t = -3.071$; $p = .002$) was earlier in the vaginal group (56.58 ± 15.23 h) than that in the caesarean group (62.25 ± 18.16h).

Compared with the prenatal period, total leukocytes ($p = .000$), neutrophils ($p = .000$) and their percentage ($p = .000$), monocytes ($p = .000$), and the neutrophil-lymphocyte ratio ($p = .000$) were increased after delivery. The percentage of lymphocytes ($p = .000$) and monocytes ($p = .000$) and the number of platelets ($p = .000$ in the vaginal group, $p = .010$ in the caesarean group) were decreased after deliveries. The absolute value of lymphocytes was increased in the vaginal group ($p = .000$) but decreased in the caesarean group ($p = .006$). After adjusting the baseline blood cell values of the prenatal and postnatal intergroup covariance analysis, total leukocytes ($p = .000$), neutrophils ($p = .000$), lymphocytes ($p = .000$), and monocytes ($p = .000$) of the caesarean group were decreased drastically; platelets ($p = .003$) and the neutrophil-lymphocyte ratio ($p = .000$) of the caesarean group were increased more significantly than those of the vaginal group (Table 3).

Candidate variables (delivery modes, $p = .002$; formula frequency, $p = .001$; formula feeding volume, $p = .001$; postnatal absolute number of lymphocytes, $p = .015$; postnatal lymphocyte percentage, $p = .021$) with a p value ≤.05 on univariate analysis were included in the multivariable model, and variables that were considered clinically relevant (time to first breast-feeding, $p = .898$; duration of first breast-feeding, $p = .830$; breast-feeding frequency, $p = .450$; sucking duration, $p = .356$) were entered into the multivariate regression model. Variables for inclusion were carefully chosen, given the number of events available, to ensure parsimony of the final model. Multivariate regression analysis with a stepwise method showed that the neutrophil-lymphocyte ratio was correlated with the onset of lactation after adjusting for these confounders (Table 4). That is, the greater the formula intake within 24 hours after births, the higher the neutrophil-lymphocyte ratio and the later the onset of lactation.

4. Discussion

Based on the study, the absolute number of lymphocytes was decreased drastically after caesarean delivery, while the maternal white blood cell count and its subtype counts, except for lymphocytes, were increased after delivery, regardless of whether it is a caesarean section or spontaneous delivery. Hence, the neutrophil-lymphocyte ratios of the two groups were increased after delivery, but the extent of the increase in the neutrophil-lymphocyte ratio in the caesarean section group was much higher than that in the spontaneous delivery group.

Intriguingly, it has been reported that following spontaneous delivery, there is a remarkable increase in total maternal white blood cell count and neutrophil count, whereas the percentage and absolute number of lymphocytes are decreased drastically (Delgado et al., 1994). Following elective caesarean delivery in our study, no leukocytosis was found. However, the percentage and absolute number of lymphocytes was also decreased, though not as pronounced as after spontaneous delivery (Delgado et al., 1994). Presumably, the results suggest that although the neutrophil-lymphocyte ratio of both groups was increased, the extent of the increase in the spontaneous delivery group was higher than that in the caesarean section group. One of the possible factors that might reconcile the different results between the two studies could be the time of blood draw. The time of blood draw in Delgado's study

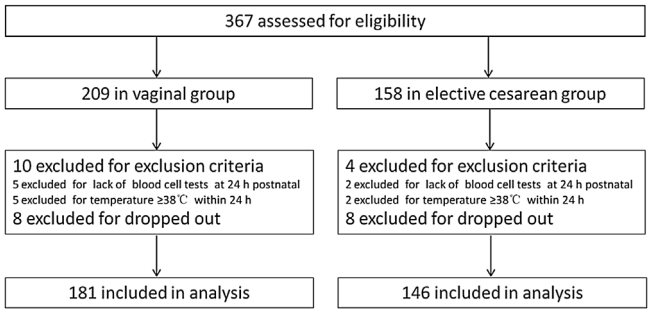

Fig. 1. Flow diagram of this study.

Table 1
Demographic Information for Mother–Infant Dyads*.

Characteristic	Vaginal Group, n = 181	Caesarean Group, n = 146	t/χ^2/Z	*p*
Maternal age (years), mean ± SD	28.20 ± 2.65	29.22 ± 2.48	−3.565	0.000
Race, n (%)			0.000	1.000
Han	179 (98.90)	145 (99.32)		
Minority	2 (1.10)	1 (0.68)		
Per capita household income (yuan/month), n (%)			−1.110	0.267
≤6000	20 (11.05)	14 (9.59)		
6000–7999	39 (21.55)	25 (17.12)		
8000–9999	47 (25.97)	39 (26.71)		
≥10000	75 (41.43)	68 (46.58)		
Maternal education (years), n (%)			−1.456	0.145
≤ 12	11 (6.08)	15 (10.27)		
12-15	35 (19.34)	31 (21.23)		
16-17	108 (59.67)	83 (56.85)		
≥18	27 (14.91)	17 (11.65)		
Employment status, n (%) unemployed	41 (22.65)	35 (23.97)	0.079	0.779
Number of antenatal care interviews, mean ± SD	13.45 ± 2.11	12.68 ± 1.99	3.336	0.001
Pregestational mass index (kg/m^2), mean ± SD	20.48 ± 2.21	20.35 ± 2.34	0.556	0.578
Gestational mass index gain (kg/m^2), mean ± SD	5.65 ± 1.70	6.14 ± 1.65	−2.638	0.009
Residential address, n (%), city	174 (96.13)	134 (91.78)	2.796	0.094
Accepted breast-feeding education, n (%), yes	44 (24.31)	33 (22.60)	0.131	0.718
Breast-feeding or not, n (%), yes	138 (76.24)	100 (68.49)	2.450	0.118
Gestational age at birth (weeks), mean ± SD	39.84 ± 1.01	39.36 ± 0.74	4.729	0.000
Birth weight (g), mean ± SD	3331.16 ± 343.47	3447.53 ± 410.96	−2.789	0.006
Newborn gender, n (%), male	96 (53.04)	79 (54.11)	0.037	0.847

* Continuous data were tested by independent-sample *t* test, dichotomous data by chi-square test, and rank data by rank sum test.

Table 2
Breast-Feeding Characteristics Within 24 hours After Birth*.

Variable	Vaginal Group, n = 181	Caesarean Group, n = 146	t/χ^2	*p*
Early breast-feeding				
Time to first breast-feeding (hours), mean ± SD	5.32 ± 3.25	7.34 ± 7.16	−3.398	0.001
Duration of first breast-feeding (minutes), mean ± SD	18.78 ± 12.51	15.95 ± 10.31	2.194	0.029
Breast-feeding frequency, number of sessions, mean ± SD	5.98 ± 3.08	4.95 ± 2.56	3.224	0.001
Sucking duration, (minutes), mean ± SD	131.66 ± 89.49	106.60 ± 73.05	2.730	0.007
Formula intake				
Formula frequency, number of feedings, mean ± SD	3.82 ± 2.48	5.05 ± 2.17	−4.746	0.000
Formula volume (ml), mean ± SD	47.62 ± 36.08	64.49 ± 37.42	−4.134	0.000
Potential related factors				
Edinburgh Postnatal Depression Scale, score, n (%)			0.000	0.982
≤9	166 (91.71)	134 (91.78)		
≥10	15 (8.29)	12 (8.22)		
Pain, score, mean ± SD	2.91 ± 1.75	4.67 ± 2.19	−8.063	0.000
Fatigue, score, mean ± SD	4.90 ± 2.50	5.77 ± 2.59	−3.091	0.002

* Continuous variables were analyzed with independent-sample *t* test, and categorical variables were compared using chi-square test.

was 30 minutes postpartum, whereas it was 24 hours postpartum in our study. The blood counts may vary depending on the time of blood drawing.

It is well known that a rise in cortisol induces lymphocyte apoptosis (Smith and Cidlowski, 2010; Planey and Litwack, 2000) and attenuates neutrophil apoptosis (Cox, 1995). The maternal cortisol level increased in both the vaginal and caesarean groups from 2 hours after delivery and lasted for 2–3 days postpartum (Stjernholm et al., 2016; Zanardo et al., 2012; Campbell et al., 1987). Given that the cortisol levels were higher in the spontaneous delivery group than in the caesarean section group (Stjernholm et al., 2016; Zanardo et al., 2012), an increase in the neutrophil-lymphocyte ratio in women delivering vaginally may occur more quickly than in women with caesarean delivery.

Compared with vaginal deliveries, caesarean deliveries can bring surgical trauma. The influence of operations in women undergoing caesarean deliveries is an important factor that determines the elevated neutrophil-lymphocyte ratio. It has been shown that the number of leukocytes and neutrophils increases greatly whereas the number of lymphocytes drops significantly in patients after cardiac surgery (Franke et al., 2006). In patients who underwent surgical resection for colorectal cancer, the white blood cell count increased greatly and the lymphocyte count dropped drastically on the first day after the operation (Girardot et al., 2017). Hence, we propose a similar mechanism underlying the changes in white blood cell count and its subtypes' counts for people undergoing surgical operations.

In this study, postpartum leukocyte counts of women in the spontaneous delivery group were significantly higher than among those of the caesarean section group after childbirth, while the neutrophil-lymphocyte ratio of the spontaneous delivery group was significantly lower than that of the caesarean section group. Since the increased neutrophil-lymphocyte ratio is associated with decreases in mitochondrial deoxyribonucleic acid copy number and adenosine triphosphate production, we speculate that the mitochondrial deoxyribonucleic acid copy number and adenosine

Table 3
Comparison of Maternal Blood Cell Counts Before and After Deliveries and the Differences in Maternal Blood Cell Counts Postpartum Between the Two Delivery Groups.

Absolute cell numbers and percentage	Vaginal Group, n = 181 mean ± SD	Caesarean Group, n = 146 mean ± SD	Postnatal intergroup		Pre-post in Vaginal Group		Pre-post in Caesarean Group	
			F/Z	P^{*}	t	P^{**}	t	P^{**}
Antenatal leukocytes, 10^9/L	8.80 ± 1.97	8.60 ± 1.91	16.835	0.000	−17.625	0.000	−13.554	0.000
Postnatal leukocytes, 10^9/L	13.18 ± 2.70	12.05 ± 2.41						
Antenatal neutrophils, 10^9/L	6.58 ± 1.77	6.44 ± 1.62	3.636	0.057	−17.313	0.000	−15.483	0.000
Postnatal neutrophils, 10^9/L	10.43 ± 2.41	9.91 ± 2.17						
Antenatal neutrophils, %	74.09 ± 6.24	74.62 ± 5.10	0.017	0.895	−5.295	0.000	−13.587	0.000
Postnatal neutrophils, %	78.18 ± 8.30	82.21 ± 4.44						
Antenatal lymphocytes, 10^9/L	1.59 ± 0.45	1.51 ± 0.41	70.059	0.000	−6.172	0.000	2.756	0.006
Postnatal lymphocytes, 10^9/L	1.91 ± 0.51	1.37 ± 0.43						
Antenatal lymphocytes, %	18.69 ± 5.34	17.88 ± 4.62	11.039	0.001	6.110	0.000	12.731	0.000
Postnatal lymphocytes, %	14.75 ± 3.79	11.60 ± 3.64						
Antenatal monocytes, 10^9/L	0.56 ± 0.17	0.58 ± 0.19	10.957	0.001	−8.958	0.000	−5.215	0.000
Postnatal monocytes, 10^9/L	0.77 ± 0.27	0.71 ± 0.25						
Antenatal monocytes, %	6.53 ± 1.66	6.73 ± 1.68	0.082	0.775	6.110	0.000	4.322	0.000
Postnatal monocytes, %	5.86 ± 1.76	5.91 ± 1.54						
Antenatal platelet, 10^9/L	205.59 ± 46.29	213.83 ± 48.41	8.905	0.003	9.595	0.000	2.581	0.010
Postnatal platelet, 10^9/L	192.02 ± 42.86	203.28 ± 48.88						
Antenatal neutrophil-lymphocyte ratio	4.42 ± 1.73	4.50 ± 1.34	23.797	0.000	−6.946	0.000	−13.132	0.000
Postnatal neutrophil-lymphocyte ratio	5.83 ± 2.10	7.89 ± 2.82						

Note: The postnatal comparisons between the two groups were made by conducting a covariance analysis with the baseline intention as the covariate. The pre-post changes of the two groups were assessed using the paired *t*-test.
* Covariance analysis.
** Paired *t*-test.

Table 4
Relationship between the neutrophil-lymphocyte ratio and onset of lactation*.

Measure	B	Standard error	Beta	t	*p*	95% Confidence Interval	
Formula intake within 24 hours after birth	0.075	0.024	0.169	3.097	0.002	0.028	0.123
Postnatal neutrophil-lymphocyte ratio	0.966	0.346	0.152	2.792	0.006	0.285	1.646

* Multivariate regression.

triphosphate level would be lower after childbirth in women with caesarean section than in women delivering vaginally.

To provide further proof, we focused on the changes in platelets and differential counts of leukocytes in peripheral blood to explore the changes in mitochondrial deoxyribonucleic acid copy number and adenosine triphosphate after vaginal and caesarean deliveries. From more than 3000 samples, the platelets and leukocytes collected were 231.16×10^9/L and 7.34×10^9/L, respectively. In whole blood and leukocytes, mitochondrial deoxyribonucleic acid copy numbers were 68.25 and 31.86, respectively (Hurtado-Roca et al., 2016). It was calculated that the mitochondrial deoxyribonucleic acid copy number was 0.158 of per 1×10^9/L platelets. The differences in platelet counts between the two groups or between predelivery and postdelivery were all about 10×10^9/L, so the change in mitochondrial deoxyribonucleic acid copy number was negligible for the total mitochondrial deoxyribonucleic acid copy number in peripheral blood.

Based on the data of the differential counts of white blood cells, monocyte numbers were increased in both groups, but the extent of the increase of monocytes in the spontaneous delivery group was higher than that in the caesarean section group. The postpartum mitochondrial deoxyribonucleic acid copy number of monocytes in the spontaneous delivery group was higher than that in the caesarean section group. Neutrophils contain very small amounts of mitochondria that can be ignored. Lymphocytes were decreased in the caesarean section group after delivery but increased in the spontaneous delivery group. The postpartum mitochondrial deoxyribonucleic acid copy number of lymphocytes in the spontaneous delivery group may be higher than that in the caesarean section group.

The total mitochondrial deoxyribonucleic acid copy number and adenosine triphosphate in peripheral blood can be determined mainly by lymphocytes. Therefore, postpartum mitochondrial deoxyribonucleic acid copy number and adenosine triphosphate in the spontaneous delivery group may be increased after childbirth. Of note, this notion is in agreement with previous studies showing elevated mitochondrial deoxyribonucleic acid copy number and adenosine triphosphate level in the peripheral blood of cows with early lactation (Hadsell et al., 2011; Laubenthal et al., 2016), whereas in our study, postpartum mitochondrial deoxyribonucleic acid copy number and adenosine triphosphate in the caesarean section group was decreased after childbirth, consistent with elective limb surgery (Seekamp et al., 1999).

Taken together, we propose that the delayed onset of lactation in women undergoing caesarean section may be due to decreased mitochondrial deoxyribonucleic acid copy number and adenosine triphosphate level after childbirth.

Dyads in the caesarean group experienced a delay in first breast-feeding compared with those in the vaginal birth group. However, time to first breast-feeding did not influence the onset of lactation after controlling for other confounding factors. In the present study, formula intake within 24 hours after birth was related to the delayed onset of lactation. Further study is needed to determine the mechanism for this relationship.

4.1. Implications

Our findings that birth by caesarean section increased the neutrophil-lymphocyte ratio in mothers and that the elevated neutrophil-lymphocyte ratio was related to the onset of lactation expand our existing knowledge about the causes of delayed onset of lactation. These results provide evidence of the physiological mechanisms that contribute to the delayed onset of lactation among mothers who experience caesarean birth. Future studies

should further confirm its biological mechanisms and explore the method to promote the onset of lactation in mothers undergoing caesarean section.

4.2. Limitations

The drawback of this study may be that mothers in the vaginal delivery group may have had symptoms of irregular contractions and premature rupture of membranes, which might have affected the results of prenatal blood cell counts. In addition, the study did not include mothers who had emergency caesarean section.

Moreover, maternal blood draw occurred 24 hours after delivery, whether caesarean section or vaginal delivery, and this is a bit far too long the onset time of lactation. In fact, 48 hours after birth is a better time. However, most mothers delivering vaginally are discharged from the hospital 24 hours after delivery. This limiting factor needs to be addressed in future research.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was supported by the Funding of Nursing Scientific Research at Fudan University (Program no. FNF201607).

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Acknowledgements

This work would not have been possible without the support and help from doctors and nurses in maternity units of the Obstetrics & Gynecology Hospital. We would also like to thank Yan Ding for her various assistance. In particular, we are indebted to Jiejing Sheng and Feifei Zhou, who supported us with their work in breast-feeding. Last but not least, we are very grateful to Hui Peng for his guidance in statistics and to Wanling He for her English language assistance.

References

Adugna, B., Tadele, H., Reta, F., Berhan, Y., 2017. Determinants of exclusive breastfeeding in infants less than six months of age in Hawassa, an urban setting, Ethiopia. Int. Breastfeed. 12, 45.

Bernardis, R.C., Siaulys, M.M., Vieira, J.E., Mathias, L.A., 2016. Perioperative warming with a thermal gown prevents maternal temperature loss during elective cesarean section. A randomized clinical trial. Rev. Bras. Anestesiol. 66, 451–455.

Brownell, E., Howard, C.R., Lawrence, R.A., Dozier, A.M., 2012. Delayed onset lactogenesis II predicts the cessation of any or exclusive breastfeeding. J. Pediatr. 161, 608–614.

Bühler, S., Frahm, J., Tienken, R., Kersten, S., Meyer, U., Huber, K., Dänicke, S., 2016. Influence of energy level and nicotinic acid supplementation on apoptosis of blood leukocytes of periparturient dairy cows. Vet. Immunol. Immunop. 179, 36–45.

Campbell, E.A., Linton, E.A., Wolfe, C.D., Scraggs, P.R., Jones, M.T., Lowry, P.J., 1987. Plasma corticotropin-releasing hormone concentrations during pregnancy and parturition. J. Clin. Endocrinol. Metab. 64, 1054–1059.

Chae, J., Chua, P.S., Ng, T., Yeo, A.H.L., Shwe, M., Gan, Y.X., Dorajoo, S., Foo, K.M., Loh, K. W., Koo, S., Chay, W.Y., Tan, T.J.Y., Beh, S.Y., Lim, E.H., Lee, G.E., Dent, R., Yap, Y.S., Ng, R., Ho, H.K., Chan, A., 2018. Association of mitochondrial DNA content in peripheral blood with cancer-related fatigue and chemotherapy-related cognitive impairment in early-stage breast cancer patients: a prospective cohort study. Breast Cancer Res. Treat. 168, 713–721.

Chalder, T., Berelowitz, G., Pawlikowska, T., Watts, L., Wessely, S., Wright, D., Wallace, E. P., 1993. Development of a fatigue scale. J. Psychosom. Res. 37, 147–153.

Chapman, D., Perez-Escamilla, R., 2000. Maternal perception of the onset of lactation is a valid, public health indicator of lactogenesis stage II. J. Nutr. 130, 2972–2980.

Chen, C., Yan, Y., Gao, X., Xiang, S., He, Q., Zeng, G., Liu, S., Sha, T., Li, L., 2018. Influences of cesarean delivery on breastfeeding practices and duration: a prospective cohort study. J. Hum. Lact. 34, 526–534.

Chistiakov, D.A., Sobenin, I.A., Revin, V.V., Orekhov, A.N., Bobryshev, Y.V., 2014. Mitochondrial aging and age-related dysfunction of mitochondria. Biomed Res. Int. 2014, 238–463.

Cox, G., 1995. Glucocorticoid treatment inhibits apoptosis in human neutrophils. Separation of survival and activation outcomes. J. Immunol. 154, 4719–4725.

Crovetto, F., Lattuada, D., Rossi, G., Mangano, S., Somigliana, E., Bolis, G., Fedele, L., 2013. A role for mitochondria in gestational diabetes mellitus? Gynecol. Endocrinol. 29, 259–262.

Daoudia, M., Decruynaere, C., Le Polain, D.W.B., Thonnard, J.L., Plaghki, L., Forget, P., 2015. Biological inflammatory markers mediate the effect of preoperative pain-related behaviours on postoperative analgesics requirements. BMC Anesthesiol. 15, 183.

Delgado, I., Neubert, R., Dudenhausen, J.W., 1994. Changes in white blood cells during parturition in mothers and newborn. Gynecol. Obstet. Invest. 38, 227–235.

El-Hag, A., Clark, R.A., 1987. Immunosuppression by activated human neutrophils. Dependence on the myeloperoxidase system. J. Immunol. 139, 2406–2413.

Franke, A., Lante, W., Kurig, E., Zoller, L.G., Weinhold, C., Markewitz, A., 2006. Hyporesponsiveness of T cell subsets after cardiac surgery: a product of altered cell function or merely a result of absolute cell count changes in peripheral blood? Eur. J. Cardiothorac. Surg. 30, 64–71.

Garrabou, G., Hernandez, A.S., Catalan, G.M., Moren, C., Tobias, E., Cordoba, S., Lopez, M., Figueras, F., Grau, J.M., Cardellach, F., 2016. Molecular basis of reduced birth weight in smoking pregnant women: mitochondrial dysfunction and apoptosis. Addic. Biol. 21, 159–170.

Girardot, T., Rimmele, T., Venet, F., Monneret, G., 2017. Apoptosis-induced lymphopenia in sepsis and other severe injuries. Apoptosis 22, 295–305.

Guo, S., Fu, X., Scherpbier, R.W., Wang, Y., Zhou, H., Wang, X., Hipgrave, D.B., 2013. Breastfeeding rates in central and western China in 2010: implications for child and population health. Bull. World Health Organ. 91, 322–331.

Hadsell, D.L., Olea, W., Wei, J., Fiorotto, M.L., Matsunami, R.K., Engler, D.A., Collier, R. J., 2011. Developmental regulation of mitochondrial biogenesis and function in the mouse mammary gland during a prolonged lactation cycle. Physiol. Genomics 43, 271–285.

Hastie, R., Lappas, M., 2014. The effect of pre-existing maternal obesity and diabetes on placental mitochondrial content and electron transport chain activity. Placenta 35, 673–683.

Holdgate, A., Asha, S., Craig, J., Thompson, J., 2003. Comparison of a verbal numeric rating scale with the visual analogue scale for the measurement of acute pain. Emerg. Med. (Fremantle) 15, 441–446.

Huang, C.M., Keenan, T.W., 1971. Membranes of mammary gland. I. Bovine mammary mitochondria. J. Dairy Sci. 54, 1395–1405.

Hurtado-Roca, Y., Ledesma, M., Gonzalez-Lazaro, M., Moreno-Loshuertos, R., Fernandez-Silva, P., Enriquez, J.A., Laclaustra, M., 2016. Adjusting MtDNA quantification in whole blood for peripheral blood platelet and leukocyte counts. PLoS One 11, e163770.

Kim, M., Lee, J., Kang, H., Kim, E., Lee, D., 2011. Leukocyte mitochondrial DNA (mtDNA) content is associated with depression in old women. Arch. Gerontol. Geriatr. Suppl. 53, e218–e221.

Kramer, P.A., Ravi, S., Chacko, B., Johnson, M.S., Darley-Usmar, V.M., 2014. A review of the mitochondrial and glycolytic metabolism in human platelets and leukocytes: implications for their use as bioenergetic biomarkers. Redox Biol. 2, 206–210.

Lang, J.A., Krajek, A.C., Smaller, K.A., 2017. Evidence for a functional vasoconstrictor role for ATP in the human cutaneous microvasculature. Exp. Physiol. 102, 684–693.

Laubenthal, L., Hoelker, M., Frahm, J., Danicke, S., Gerlach, K., Sudekum, K.H., Sauerwein, H., Haussler, S., 2016. Mitochondrial DNA copy number and biogenesis in different tissues of early- and late-lactating dairy cows. J. Dairy Sci. 99, 1571–1583.

Lee, D.T., Yip, S.K., Chiu, H.F., Leung, T.Y., Chan, K.P., Chau, I.O., Leung, H.C., Chung, T. K., 1998. Detecting postnatal depression in Chinese women. Validation of the Chinese version of the Edinburgh Postnatal Depression Scale. Br. J. Psychiatry 172, 433–437.

Liu, P., Qiao, L., Xu, F., Zhang, M., Wang, Y., Binns, C.W., 2013. Factors associated with breastfeeding duration: a 30-month cohort study in northwest China. J. Hum. Lact. 29, 253–259.

Maianski, N.A., Geissler, J., Srinivasula, S.M., Alnemri, E.S., Roos, D., Kuijpers, T.W., 2004. Functional characterization of mitochondria in neutrophils: a role restricted to apoptosis. Cell Death Differ. 11, 143–153.

Parker, L.A., Sullivan, S., Krueger, C., Kelechi, T., Mueller, M., 2011. Effect of early breast milk expression on milk volume and timing of lactogenesis stage II among mothers of very low birth weight infants: a pilot study. J. Perinatol. 32, 205–209.

Planey, S.L., Litwack, G., 2000. Glucocorticoid-induced apoptosis in lymphocytes. Biochem. Biophys. Res. Commun. 279, 307–312.

Pyle, A., Burn, D.J., Gordon, C., Swan, C., Chinnery, P.F., Baudouin, S.V., 2010. Fall in circulating mononuclear cell mitochondrial DNA content in human sepsis. Intensive Care Med. Exp. 36, 956–962.

Regan, J., Thompson, A., DeFranco, E., 2013. The influence of mode of delivery on breastfeeding initiation in women with a prior cesarean delivery: a population-based study. Breastfeed. Med. 8, 181–186.

Scott, J.A., Binns, C.W., Oddy, W.H., 2007. Predictors of delayed onset of lactation. Matern. Child Nutr. 3, 186–193.

Seekamp, A., van Griensven, M., Hildebrandt, F., Wahlers, T., Tscherne, H., 1999. Adenosine-triphosphate in trauma-related and elective hypothermia. J. Trauma 47, 673–683.

Skopichev, V.G., Gaidukov, S.N., 1990. The reactions of the vascular bed of the breast in normally lactating women and in hypogalactia. Fiziol Zh SSSR Im I M Sechenova 76, 891–896.
Smith, L.K., Cidlowski, J.A., 2010. Glucocorticoid-induced apoptosis of healthy and malignant lymphocytes. Prog. Brain Res. 182, 1–30.
Stjernholm, Y.V., Nyberg, A., Cardell, M., Höybye, C., 2016. Circulating maternal cortisol levels during vaginal delivery and elective cesarean section. Arch. Gynecol. Obstet. 2, 267–271.
Tsubomoto, S., 1979. Treatment and diagnosis of Hypogalactia by measurement of breast temperature. Adv. Obstet. Gynecol. 31, 607–617.
Wang, W., Wang, P., Zhou, X., Liu, Y., Zhang, H., 2010. Correlation analysis between fatigue and job stressors of nurses. Wei Sheng Yan Jiu 39, 76–78.
Zanardo, V., Savona, V., Cavallin, F., D'Antona, D., Giustardi, A., Trevisanuto, D., 2012. Impaired lactation performance following elective delivery at term: role of maternal levels of cortisol and prolactin. J. Matern. Fetal. Neonatal. Med. 25, 1595–1598.
Zhu, P., Hao, J., Jiang, X., Huang, K., Tao, F., 2013. New insight into onset of lactation: mediating the negative effect of multiple perinatal biopsychosocial stress on breastfeeding duration. Breastfeed. Med. 8, 151–158.