Oropharyngeal colostrum administration in very low birth weight infants: A Randomized Controlled Trial

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ABSTRACT

Objective: Studies have confirmed the safety of oropharyngeal administration of colostrum (OAC) in very low birth weight (VLBW) infants. However, the effect of OAC on immune system is inconclusive. This study aims to evaluate the effect of OAC on secretory Immunoglobulin A (sIgA) and lactoferrin in very low birth weight infants.

Design: Randomized Controlled Trial.

Setting: 40-bedded Neonatal Intensive Care Unit in a university children's hospital in the People's Republic of China.

Patients: VLBW infants were allocated to the study group (n = 32) and control group (n = 32).

Intervention: The intervention was oropharyngeal administration of 0.2 ml of their mother's colostrum every 4 hours during 7 days. The control group received saline solution.

Measurements and Main Results: sIgA and lactoferrin in urine and saliva were measured within 24 hours of life (baseline), at 7 and 21 days. Primary outcomes were changes of sIgA and lactoferrin in urine and saliva between baseline, at 7 and 21 days. Infant's clinical data were also collected during hospitalization.

Change from baseline in lactoferrin in saliva at 7 days (5.18±7.07 vs. -1.74±4.67 µg/ml, $p<0.001$) and 21 days (5.31±9.74vs -1.17±10.38 µg/ml, $p=0.02$) shows statistic difference. No differences were found of lactoferrin in urine and also no differences of sIgA in urine and saliva. There were also no differences between days to full enteral feeding, incidence of clinical sepsis, proven sepsis, and necrotizing enterocolitis.

Conclusions: OAC can increases the level of lactoferrin in saliva in VLBW infants. No effect could be documented of sIgA and lactoferrin in urine. Larger trials are needed to better describe the benefit of OAC, if any, in VLBW infants.

INTRODUCTION

Colostrum is the first milk produced by the mammary when the tight junctions in the mammary epithelium are open (1). It is rich in cytokines (anti-inflammatory cytokines, pro-inflammatory cytokines) and other immune agents such as secretory Immunoglobulin A (sIgA) and lactoferrin (2-7). These immune agents can provide bacteriostatic, bactericidal, antiviral, anti-inflammatory and immunomodulatory protection against infection (8). The tight junctions in the mammary epithelium usually closed after the first day of postnatal for women who give birth at term, much earlier than women who deliver preterm infants [9](9). As a result the immune protective factors are more highly concentrated in the colostrum of mothers delivering premature infants.

Studies confirmed that colostrum contains higher concentrations of immune-protective agents compared with mature human milk, such as lactoferrin, lysozyme, and sIgA (9-11). Therefore, giving colostrum as soon as excreted from their mother can provide more immunological support for immature preterm newborns. However, some VLBW infants can't start enteral feeding because of clinical instability. For these infants, oropharyngeal exposure to protective biofactors from colostrum is delayed. Oropharyngeal administration of colostrum can be given to these infants to eliminate the delay.

Oropharyngeal Administration of Colostrum (OAC) is an intervention placing small amount of colostrum directly onto the oropharyngeal mucosa with a sterile syringe for absorption. In theory, OAC can promote immune response in three ways. First, colostrum is rich in cytokines, which can interact with cells within the oropharyngeal-associated lymphoid tissue (12). For example, Interleukin-6 can stimulate the growth and differentiation of B-lymphocytes to IgA-secreting plasma

cells and secreting sIgA can enhance local mucosal immunity (13). Secondly, colostrum has a high concentration of sIgA and lactoferrin. These factors can be absorbed by mucosal directly and interfere with bacterial colonization (14). Thirdly, during OAC some colostrum can travel to the gastrointestinal tract and interact with gut-associated lymphoid tissues (15).

Although theoretical support for the OAC exists, limited clinical evidence exists to prove the effects of oropharyngeal colostrum administration. Therefore, this study aims to evaluate the effect of OAC on sIgA and lactoferrin in VLBW infants.

MATERIALS AND METHODS

Study Design

A double blind randomized controlled clinical trial was conducted from March 2015 to October 2015 in the Neonatal Intensive Care Unit (NICU) of the stand-alone Children Hospital of Fudan University in Shanghai, China.

Participants

Inclusion criteria were infants with birth weight ≤1500g, transferred to our hospital within 24 hours after birth and parents agree to provide colostrum. Exclusion criteria were infants suffering from life-threatening conditions, such as severe heart disease, whose life time is expected less than 30 days; infants suffering from any kind of disease influencing enteral feeding, including gastrointestinal malformations and necrotizing enterocolitis (NEC) or infants whose mother's milk was contraindicated (mothers with a history of drug abuse or HIV infection).

All infants were randomized into a study group or a control group from a 1:1 ratio random number table generated by computer. Allocation was hidden from nurses, doctors and parents. Only the principal investigator (PI) knew the allocation and

prepared the colostrum and normal saline in blinded syringes. The infants started their enteral feeding with nasogastric or orogastric tube. The attending physicians decided the feeding status based on the infants' individual factors. In both groups infants were fed with preterm formula first followed by their mothers' human milk.

Sample size

On the basis of results from pilot data, we supposed that a mean difference of the changes of sIgA at 7 days from baseline in saliva was 580 ng/ml between the two groups; the SD was 537 ng/ml in study group and 509 ng/ml in control group. With α=0.05 and β=0.1, we calculated 26 infants for each group. Assuming a 20% dropout rate, we estimated that 64 infants were needed.

Intervention

1. Collection of Colostrum

Within 24 hours after delivery the infants were transferred to our NICU. The PI met with each father whose infant was enrolled in the study to educate the standardized protocol for milk removal utilizing an electric breast pump and hand expression of colostrum. Only fathers were contacted because mothers were at home or in the maternity hospital following the Chinese tradition of 'zuo yuezi' ('sitting the month'). This tradition is a 30-day recovery of new mothers staying at home to protect their body against cold. Fathers were encouraged to help mothers pumping every 2-3 hours for a total of 8 times per 24-hour period; pour the colostrum separately into breast milk storage bags labeled with the infant's information including the medical record number, the infant's name, the date and time of colostrum pump and deliver the colostrum specimens to the NICU for immediate refrigeration.

2. OAC Protocol

Once received the colostrum, the PI and another nurse checked the infant's information against the label on the breast milk storage bags. In the control group we put the storage bags in -18^{o}C freezer for cryopreservation until the infants start enteral feeding. In the study group we collected the storage bag with nearest pumping time, extracted 0.2ml of colostrum with 1ml sterile syringe and placed in room temperature for 5 minutes to bring the colostrum to room temperature for the OAC. Then put the bag in the refrigerator cold storage with temperature of 0-4 ^{o}C for the following intervention and the colostrum was stored for 24h. All the other storage bags were placed in -18^{o}C freezer for cryopreservation until the infants start enteral feeding.

The nurses followed an oropharyngeal administration protocol to administer the drops: Removed the needle and then put the syringe into the infant's mouth along one side of the cheek gently. Adjust the syringe tip pointing the infant's oropharyngeal. Administered 0.1 ml of the colostrum with constant speed over a period of at least 20 seconds, then moved the syringe to the opposite side of the cheek and administered the remaining 0.1 mL of the colostrum in the same way. Control group infants also started with the oropharyngeal administration protocol using normal saline instead of colostrum. This protocol was carried out every 4 hours for 7 days.

Monitoring and Recording

The infants' heart rate, respiration, pulse, blood pressure and oxygen saturation were monitored and recorded during the intervention. The intervention would be stopped and continue later when any of the following cases happened: the infant shows significant agitation, the infant's basic vital signs changes (heart rate> 200 beats / min or <80 beats / min; periodic breathing or apnea; oxygen saturation less than 88%).

Specimen Collection

To measure the concentrations of sIgA and lactoferrin we collected saliva and urine specimens at three times: first time when parents agreed to participate in this study (T0); at 7 (T1) and 21 (T2) days of life. Sterile urine bags were used to collect urine specimens and weak suction to collect saliva in a sterile container. All specimens were centrifuged and frozen at -70^{o}C until transported to the laboratory for biochemical analysis. The concentrations of sIgA and lactoferrin were measured using enzyme-linked immunosorbent assay kits. The sIgA kit (Jianglai Biological Technology, Shanghai, China), limit of detection was 1.5 ug/ml -48ug/ml. The minimum detectable dose is typically less than 0.1ug/ml. The lactoferrin kit (Jianglai Biological Technology, Shanghai, China), limit of detection was 125ug/ml-4000ug/ml. The minimum detectable dose is typically less than 10ug/ml. Both of the kit coefficient of variations between kits were less than 10%, within kit coefficient of variations were less than 10%.

Outcome measures

To evaluate the local and systemic immune effect of OAC we measured the concentration of sIgA and lactoferrin in saliva and urine. The primary outcomes are the changes from baseline in sIgA and lactoferrin at the finish of OAC and two weeks later. We also collect the incidence of sepsis (clinical or proven) and NEC, time to reach full enteral feeding (140 ml/kg/day), time to start oral feeding (start bottle feeding by mouth >5ml/once) as secondary outcomes.

According to the international sepsis definitions we defined clinical sepsis as clinical signs of infection with antibiotic treatment more than three days (16). Sepsis was confirmed if bacterial growth occurred in at least one blood culture and symptoms of clinical sepsis. We defined NEC grade ≥ II according to the modified Bell' staging classification (17). Vital signs were assessed during the study period and

adverse events were recorded. Clinical data from each infant were collected until hospital discharge.

Statistical Analysis

Statistical analyses were performed using SPSS, version 21.0. Fisher's exact tests and t-test were used to analyze clinical data. Changes from baseline in lactoferrin and sIgA between two groups were calculated by the t-test.

Ethics

The local institutional review board approved this study. The PI explained the purpose, process, voluntary participation and confidentiality of this study to the parents. A consent form was required to be signed before the study. The study was registered at the US National Institutes of Health (ClinicalTrials.gov) with number NCT02389478.

RESULTS

In total, 64 VLBW infants were included and randomly assigned to the control or study group. Four infants were excluded from the study group and five infants were excluded from the control group (Figure 1). In total, 55 infants (28 in the study group and 27 in the control group) completed the study protocol receiving totally 42 times of treatment during a period of 7 consecutive days. The median gestational age of the population was 30^{+2}weeks (range: 25^{+2}~33^{+4} weeks), and the median birth weight was 1245g (650~1490g).

No significant differences were found in the baseline characteristics between both groups (Table 1).

Figure 1: presents the level of sIgA and lactoferrin in saliva and urine (μg/ml). Data are presented as mean and standard deviation (SD). The saliva level of sIgA at T1 was 31.92$\pm$8.69 vs. 27.78$\pm$5.26 (p=0.04). Changes of sIgA and lactoferrin in

saliva and urine were measured at different time-points. The only statistically significant differences between the two groups were observed in lactoferrin in saliva between baseline and T1, 5.18+7.07 vs. -1.74+4.67 (p<0.001) and T2 5.31+9.74 vs. -1.17+10.38 (p=0.02) (Figure 2).

No significant differences were observed in the secondary outcome measures between both groups at time of discharge (Table 2). However, the control group had a higher incidence of NEC, sepsis, and needed more days before reaching full enteral feeding.

DISCUSSION

Since the first study in 2010 reported the safety and feasibility of OAC to extremely low birth weight infants, more researchers and clinicians have paid attention to OAC (18). Oropharyngeal administration of own mother's colostrum is feasible and safe to VLBW infants. However, only a few studies have investigated the beneficial effects of OAC. One of these studies reported that the OAC may reduce the days reaching full enteral feeding (19). Another study reported the clinical advantages of OAC related to the immune system (20).

In our double blind, randomized, placebo-controlled trial we were able to document significant differences on the changes of lactoferrin at 7 days from baseline in saliva. Also, difference in sIgA levels in saliva between both groups at 7 days of life was significant. However, the results did not confirm the differences on sIgA in the change from baseline to 7 and 21 days. Our results suggest that OAC may increase the concentration of local lactoferrin and might implicate a beneficial effect on the immunologic system of VLWB infants.

We observed that the urinary concentration of lactoferrin and sIgA was not significantly increased in both of the groups, which was different from the study of Lee and colleagues (20). Three reasons might explain these differences. First, the inclusion criteria of our study were defined in terms of birth weight than gestation age. Therefore, the average gestation age at baseline in our study was 30 weeks and thus more mature than reported in the study of Lee and colleagues, 26^{+5} weeks. Previous studies have confirmed that immuno-protective factors in the colostrum are related to gestation age — more highly concentrated of mothers who deliver extremely preterm infants (6-8). Secondly, sIgA and lactoferrin are absorbed from breast milk via neonatal gut mucosa with subsequent excretion of their intact maternal forms in the urine (7,19,21,22). This exceptional mucosal absorption might be expected to occur only in preterm infants, particularly before "gut closure". Thirdly, the half-lives of sIgA and lactoferrin are 3 to 6 days (21,23), shorter than the time of specimens' collection. Therefore, future studies need to pay attention on extremely preterm infants and consider collecting specimens every day after OAC to observe trends of changes.

In saliva concentration of lactoferrin we observed statistical differences on change from baseline, 7 days, and 21 days. Although we didn't observe any other immunologic effect in our study, previous studies has shown that lactoferrin plays an important role in the establishment of immune system. It can decrease the incidence of late-onset sepsis and NEC grade $\geq$ II in preterm infants (24-26). It suggests that OAC may increase the concentration of lactoferrin in saliva and then enhance local mucosal immunity. In our study the average time to start the OAC was 69.45±34.34 hours after the infants' birth. Previous study have confirm the concentration of lactoferrin in colostrum from mothers delivered before 33 weeks of gestation

appeared significantly higher than in colostrum from mothers delivered full-term baby (10), and it decrease slowly and maintain rather constant values (9). Thus, OAC has effect on lactoferrin even if the start time is nearly three days later after birth.

We observed differences in sIgA levels in saliva between both groups after 7 days of OAC. No differences were found on changes from baseline, 7 and 21 days in urine and saliva. Because the changes play a more important role, we believe OAC did not have an impact on the concentration of sIgA in saliva in this study. This might be because the average time to start OAC was 69.45±34.34 hours after the infants' births. This was a delay of more than one day compared to the study of Montgomery et al., 40±28 hours (22). Evidence confirmed that the levels of sIgA in colostrum were significantly more elevated on the first day after delivery when compared with other days (27). On the fourth day, the concentration of sIgA was almost a tenth of the first day (27). Thus, when we started the OAC the concentrations of sIgA were decreased, and the impact on immunologic effect may also have decreased. The delayed start of OAC was because of the delayed acquired of colostrum. Our study was conducted in a NICU in a stand-alone children's hospital where mothers and infants were separated. We were only able to provide education about breast feeding to the infants' father or other family members instead of to their mother directly. We also needed to rely on other family members to transport the colostrum to the hospital. The delayed first time to administer the colostrum resulted in a delayed start of the OAC intervention. Future studies need to consider the cooperation with maternity hospitals to ensure that mothers can obtain relevant knowledge after postpartum, start pumping earlier, shorten the time to obtain the initial colostrum, and shorten the time to start OAC.

The difference of days in reaching full enteral feeding (140ml/kg.d^{-1}) between two groups has no statistical significance. However, we observed an 8-days-delayed

to reach full enteral feeding in the control group. Previous studies pointed that the impact of human milk on prematurity-related morbidities has emerged as a dose-response relation (28,29), and infants with a delayed achievement of full feedings are at risk of NEC [30](30). It is interesting to note that although there were no differences about the incidence of NEC between the two groups, we notices that five of the 28 infants in the control group developed NEC while only one infant in the study group. Evidence suggests that oral lactoferrin prophylaxis can decreases the incidence of NEC stage II or greater in preterm infants without adverse effects (30). Colostrum contains high concentration of lactoferrin and other immunomodulatory nutrients which have a potential protection to prevent the incidence of NEC (31-34).

Limitations

Our study was conducted in a children's hospital without a maternity ward and the NICU has limited rooms for parents to stay. Therefore, maternal separation causes delay in delivering the colostrum to the NICU. The sample size included in this study could not effectively test the difference in secondary outcomes between two groups. Therefore, future studies need to consider expanding the sample size based on other power variables.

CONCLUSION

Our results suggest that OAC can increase the concentration of lactoferrin in saliva but the effect on other immune-protective agents was not confirmed. We recommend OAC for VLBW infants especially infants who cannot be fed in early times of life.

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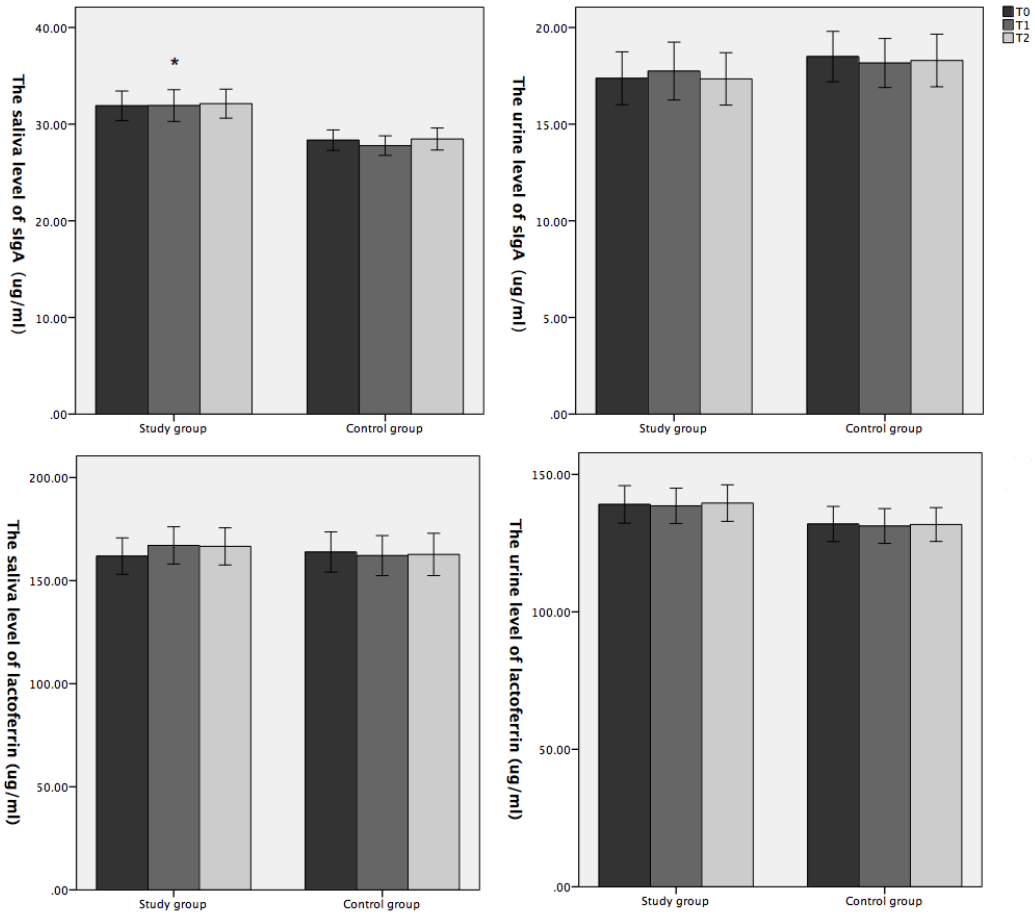

Figure 1. Level of sIgA and lactoferrin in saliva and urine

Each error bar represents 1 SE. T tests were used for group comparisons.

* P <0.05 versus placebo group

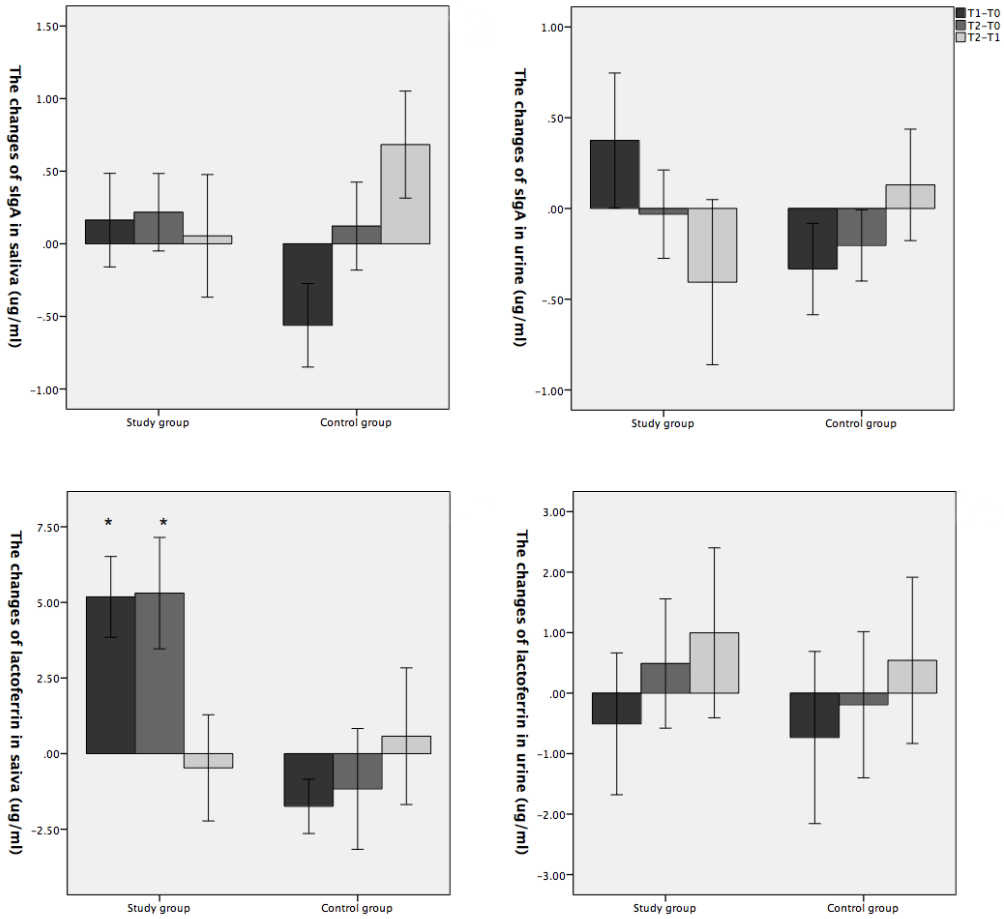

Figure 2. Changes of sIgA and lactoferrin in saliva and urine

Each error bar represents 1 SE. T tests were used for group comparisons.

* P <0.05 versus placebo group

Table 1. Patient Demographics

	Study group (n = 32)	Control group (n = 32)
Gestational age in weeks: mean, (SD)	29.86 (2.02)	30.46 (2.50)
Birth weight in grams: mean, (SD)	1241 (275)	1248 (233)
Gender: Male (n)	18	17
Apgar score at 1 min: mean, (min- max)	7 (3~10)	8 (3~10)
Apgar score at 5 min: mean, (min- max)	8 (4~10)	8 (1~10)
Multiple gestation: Yes (n)	13	12
Vaginal delivery: Yes (n)	8	9
Receiving colostrum in hrs: mean, (SD)	67.3 (36.1)	69.4 (34.4)
Start enteral feeding in days: mean, (SD)	2.0 (0.5)	2.3 (1.5)

SD, Standard Deviation; hrs, hours.

Table 2. Clinical outcomes between two groups at the time of discharge.

	Study group (n = 27)	Control group (n = 28)	P-value
NEC, stage≥2; (n)	1	5	0.10[a]
Clinical sepsis; (n)	4	8	0.61[a]
Proven sepsis; (n)	3	6	0.17[a]
Days starting oral feeding, >5ml; mean (SD)	15.38 (10.74)	16.29 (10.13)	0.75[b]
Days reaching full enteral feeding; mean (SD)	24.71 (11.23)	32.72 (20.11)	0.09[b]

[a] Fisher's exact test; [b] t-test; NEC= necrotizing enterocolitis