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Original Scientific Paper

COMPARISON OF THE EFFECTIVENESS OF BREAST MILK, DRY-KEEPING, AND USE OF CHLORHEXIDINE IN UMBILICAL CORD CARE

Emine Ela Küçük[1], Dilek Küçük Alemdar[2], Kürşad Yapar[3], Şahin Direkel[4], Nejla Ömerbeyoğlu[5] and Ülker Başak[6]

[1]Giresun University, Faculty of Health Sciences, Nursing Department, Giresun,Turkey;
[2]Ordu University, Faculty of Health Sciences, Nursing Department, Ordu,Turkey;
[3]Giresun University, Faculty of Medicine, Pharmacology Department, Giresun,Turkey;
[4]Malatya Turgut Özal University, Faculty of Medicine, Microbiology Department, Malatya,Turkey;
[5]Giresun Education and Research Hospital, Giresun,Turkey;
[6]Giresun Education and Research Hospital for Obstetrics and Gynecology, Giresun,Turkey

SUMMARY – This research was conducted as a randomized study with the objective of determining the effects of breast milk, dry-keeping, and chlorhexidine methods used in umbilical cord care on the time of umbilical cord separation and on omphalitis and bacterial flora development. The study was carried out in 97 newborns divided into three groups, as follows: breast milk was used for umbilical cord care in group 1 of 32 newborns, dry-keeping was applied in group 2 of 35 newborns, and chlorhexidine was applied in group 3 of 30 newborns. Infants were assigned to three subgroups through randomization performed using a computer program. Swabs for culture were obtained from umbilical cord on postnatal day 2 and day 5. While difference in the results of the care methods applied was not significant on day 2, significant difference in the results was found on day 5. It was determined that there was no bacterial growth in 46.7% of the chlorhexidine group, while maximum bacterial growth was recorded in the dry-keeping group. In the chlorhexidine group, timing of umbilical cord separation was significantly shorter than in other groups. Study results indicated that the most effective method was the use of chlorhexidine. Dry-keeping can be risky since it was inefficient in preventing umbilical colonization, and in cases where the cost of chlorhexidine is an issue, the use of breast milk can be recommended.

Key words: *Bacterial colonization; Umbilical cord care; Cord separation time; Breast milk; Keeping dry; Chlorhexidine*

Introduction

Neonatal infections are among the causes of morbidity and mortality during the neonatal period[1]. Most of them being observed in developing countries, neonatal infections are considered responsible for deaths of 36% of the 4 million newborns who lose their life during the neonatal period[2-4]. Severe bacterial infections resulting from umbilical cord infection, which lead to death of 460,000 newborns, are among these infections[3,5]. In a study conducted in Nepal, where 17,198 newborns were examined, 954 (5.5%) newborns were diagnosed with umbilical cord infection[6]. In a regional study which investigated the causes of death during the neonatal period in Turkey,

Correspondence to: *Professor Dilek Küçük Alemdar, PhD,* Ordu University Health Science Faculty, Nursing Department, Cumhuriyet Mahallesi Altınorudu-Ordu, Turkey
E-mail: dilekkucukalemdar@odu.edu.tr

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it was reported that 15.9% of neonatal deaths were due to infections[7]. In another study conducted in Turkey, where cases of umbilical cord infections between 1991 and 1997 at a university hospital were examined, 44% of the 55 newborns brought to the hospital due to neonatal tetanus had umbilical cord infections and the mortality rate was 40%[8].

The main cause of umbilical cord inflections is the fact that umbilical cord forms an open entrance in the skin during the neonatal period when resistance to infections is very low. After the cord of the newborn is separated (in 7-10 days on average), the remaining area covered with granulation tissue is epithelialized in 2-3 days. Since the risk of developing omphalitis is high especially in this period, the umbilical cord should be cleaned with care[9,10]. The World Health Organization (WHO) gives particular importance to umbilical care, and points out that no other method of umbilical care is superior to keeping the umbilical cord naturally dry[11,12]. Yet, every year 4 million newborn deaths are seen and 1.2 million of them happen in underdeveloped countries such as sub-Saharan African countries where conventional practices are common and births take place in houses and under non-sterile conditions. Umbilical cord infection arising after bacterial colonization is one of the most important causes of newborn morbidity and mortality in these countries[12,13]. Therefore, under the light of related evidence-based studies, the WHO recommends daily umbilical care applications with antiseptic solutions (especially 4% chlorhexidine) during the first week of life, in order to prevent newborn infections in countries where births take place in houses and under non-sterile conditions[12].

Persistent effect of chlorhexidine in umbilical cord care and its particularly diminishing effect on newborn mortality makes it a preferable antiseptic agent. Chlorhexidine is tied to the stratum corneum of the skin and thereby it maintains its effectiveness for a long time, e.g., 6 hours. Chlorhexidine, which has a broad antimicrobial spectrum, is more effective on gram-positive bacteria, while its effectiveness on gram-negative bacteria and fungi is lower. It shows weak activity against mycobacteria[15-17]. Chlorhexidine is reported to be effective on omphalitis development and to decrease infection development in 26%-56% of cases[18]. In the literature, the effect of chlorhexidine on the time of umbilical cord separation is controversial; some studies report that it shortens the separation while other studies report results indicating that especially when it is used in high doses (more than once), it extends the separation time[18-20]. In a study conducted by Mullany *et al.* on 1923 newborns, it was found that cleaning the umbilical cord with 4% chlorhexidine decreased bacterial colonization, and it was even more effective against some specific bacteria (*Escherichia coli, Klebsiella pneumoniae and Staphylococcus aureus*)[21].

In the guideline published in 2013 by the WHO, entitled WHO Recommendations on Postnatal Care of the Mother and Newborn, especially in countries where neonatal mortality is ≥30 *per* 1000 live births, where births take place in houses and under non-sterile conditions and where conventional harmful practices such as applying fertilizer to the umbilicus are common, it is recommended that daily care of the umbilical cord be performed with chlorhexidine (7.1% chlorhexidine gluconate solution or gel form containing 4% chlorhexidine)[12,22].

In recent years, the effects of the immune compounds and protective enzymes in breast milk on the prevention of bacterial colonization and separation of the umbilical cord are investigated. It is known that the proteins lactoferrin, lactoperoxidase, lysozyme, and N-acetyl-b-D-glucosaminidase that are abundant in breast milk are known to have antimicrobial effects[23]. There are ongoing tests on this topic, considering that the positive effects of alpha and beta growth factors and insulin in breast milk on the stimulation of metabolism and wound healing can contribute to early separation of umbilical cord. Studies using breast milk in umbilical care are reported to shorten the time of umbilical cord separation and can be used as a safe method in the umbilical cord care[24-31].

As the method of natural dry-keeping is a method also recommended by the WHO, in several studies, antiseptic agents were compared with the method of natural dry-keeping. In these studies, the method of clean and natural dry-keeping was found to be a preferred method since it shortened the time of umbilical cord separation and the risk of bacterial colonization was low[32-34]. Again, in the guideline published by the WHO in 2013, in countries where neonatal mortality is low, natural dry-keeping method is recommended for umbilical cord care for those births that take place in hospitals and in healthy conditions[12]. It is suggested that this process be carried out as follows: after cleaning the umbilicus using

a gauze soaked with water, it should be dried using another dry gauze and left open. During this process, it is emphasized that care must be taken to ensure that the hands are clean and the use of separate gauze for each wiping of the umbilical cord and its surrounding area is also emphasized[35].

The present randomized clinical trial was conducted to compare the impact of three therapeutic methods, i.e., breast milk, 4% chlorhexidine, and dry-keeping on bacterial colonization during the time of umbilical cord separation.

Hypotheses

H0 hypothesis: There is no difference among the effects of breast milk, dry-keeping, and chlorhexidine on the formation of colonization, time of umbilical cord separation, and development of omphalitis.

H1 hypothesis: There is a difference among the effects of breast milk, dry-keeping, and chlorhexidine on the formation of colonization, time of umbilical cord separation, and development of omphalitis.

Materials and Methods

This research was carried out as a randomized experimental study in order to determine the effects of the methods of using breast milk, dry-keeping, and chlorhexidine for umbilical cord care on the time of umbilical cord separation, and on omphalitis and bacterial flora development.

Setting and samples

The study included newborns who were born healthy at term during the study period in an education and research hospital for obstetrics and gynecology. Based on the study, the sample size was calculated by using G*Power program and taking type I error as 0.05 and type II error as 0.20. At the end of this calculation, it was determined that the sample size should be at least 93. Aiming at an improved impact of the study, this number was increased to 97. The study was carried out on three groups with a total of 97 newborns, where breast milk was used in group 1 of 32 newborns; dry-keeping was applied in group 2 of 35 newborns; and chlorhexidine was applied for umbilical cord care in group 3 of 30 newborns. Infants were assigned to three subgroups through randomization performed using a computer program (www.random.org) (Fig. 1).

Selection criteria:
- infants born between 37th-42nd weeks,
- infants born to mothers who did not develop early membrane rupture,
- infants born to mothers who did not have infections such as genitourinary infection and hepatitis,
- infants born with 5-minute Apgar score >7, and
- infants not born at the end of multiple and risky pregnancies (preeclampsia, eclampsia, ablative placenta, diabetes mellitus).

Exclusion criteria:
- infants born to mothers with chorioamnionitis,
- infants painted with meconium,
- infants taken to the neonatal intensive care unit for any reason, and
- infants who stayed at the hospital for more than four days.

Data collection tool

Personal information form

This questionnaire form, created based on the related literature, comprised questions on descriptive characteristics of the newborns.

Omphalitis follow-up form

This questionnaire form comprised six questions for monitoring the symptoms of omphalitis. The risk of omphalitis was determined by the evaluation of symptoms such as fever, erythema, edema, bad smell, discharge, and bleeding. The symptoms of omphalitis were evaluated in newborns using this form.

Microbiological evaluation of umbilical cord cultures

Samples for bacterial and fungal culture were taken from umbilical cord of the newborns on postnatal day 2 before being discharged from the hospital and on postnatal day 5. Umbilical cord swab samples were taken to transport medium (Amies) and analyzed in a medical microbiology laboratory. The samples were inoculated in the sheep blood agar, eosin methylene blue (EMB) agar and Sabouraud dextrose agar media. Preparations from cultured swabs were prepared and Gram staining was performed. All media were incubated for 24 hours at 37 °C and evaluated for bacterial growth. In order to determine bacterial density in the umbilical cord samples, the colonies of the bacteria growing on the medium were counted. Firstly, Gram staining was applied to different types of colonies and gram-positive bacteria were tested with catalase and coagulase, respectively.

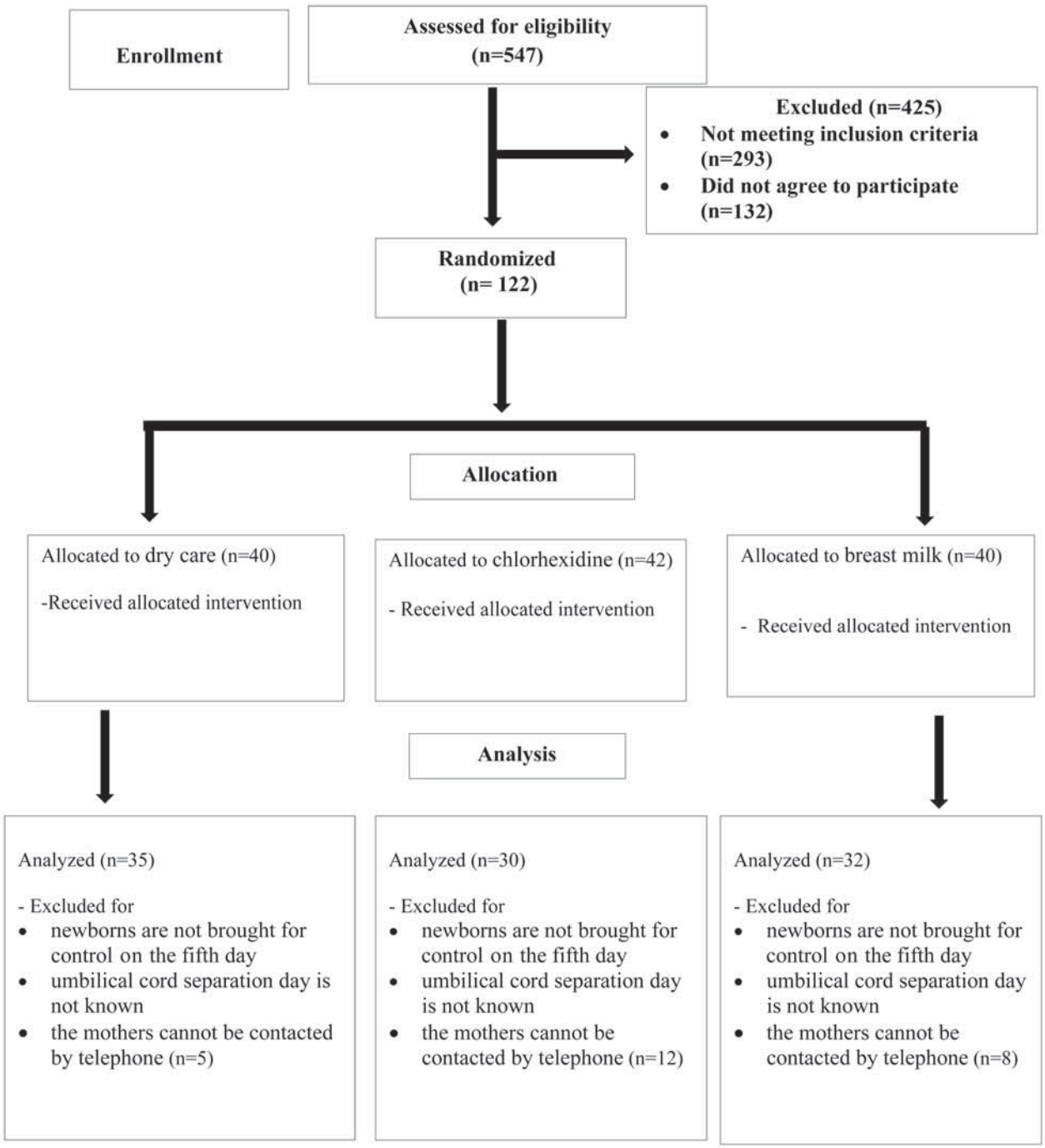

Fig. 1. Study enrollment, randomization, and procedures.

If the result of Gram staining was negative, the bacteria were identified by classic methods. If no growth of bacteria was observed on the media after 24 hours of incubation, the medium was reevaluated at the end of 48 hours. In the case of two umbilical cord samples taken at different times from newborns, the microorganism species and their density were compared.

Procedure

After delivery, the mothers were informed about the study by the training nurse and the mothers filled out the informed consent form. Next, the training nurse trained the mothers about the topic of umbilical cord care using a booklet on umbilical cord care prepared for the study. Before the newborns were discharged from the hospital, the Newborn Information Form and Omphalitis Follow-up Form were filled out by the infection nurse, and umbilical swabs were obtained from umbilical regions of the newborns and evaluated with aerobic culture method. During the newborn stay at the hospital, the type of umbilical care used in the respective subgroup (breast milk, dry-keeping, or 0.4% chlorhexidine) was applied once in 24 hours by the infection nurse. The mothers having normal vaginal delivery were discharged within 1-2 days on average when the mother and the newborn were in good health, and the infants born by cesarean delivery were discharged in 2-3 days on average.

The mothers in the breast milk group were told to drop milk (5-6 drops) directly from the breast to the umbilical cord and its surroundings once a day in the morning and not to apply any other type of care. Chlorhexidine solutions in the form of 50 mL spray were provided to the mothers in the chlorhexidine group. They were taught how to apply chlorhexidine spray; they were told to apply it to the umbilical cord and its surroundings once a day but not to use any other type of care. The mothers in the dry-keeping group were told to keep the umbilical cord clean and dry and not to apply any other method of care.

The mothers were instructed to observe the umbilical cord when changing diapers, and if the umbilical cord was contaminated with feces and urine, to reapply the umbilical care. Telephone numbers were obtained from the mothers for later contact, and they were contacted daily. The existence of omphalitis symptoms in the umbilical cord were evaluated by asking questions by the infection nurse in the Omphalitis Monitoring Form. In the cases where omphalitis was suspected, the newborns were invited to the hospital and their physical examination was performed by a pediatrician. On postnatal day 5, swabs for second cultures were obtained by the infection nurse from the umbilical cords of the newborns who were brought to the pediatric clinic for follow-up, and their omphalitis findings were evaluated.

Ethical considerations

In order to carry out the study, legal permission was obtained from the hospital and approval was obtained from the institutional Ethics Committee (No: 2016/106.01). The aim of the study was explained to the families of the children who met the research group criteria, their questions were answered and their written approval was obtained. Since the use of human phenomena necessitates protection of individual rights, the related ethical conditions of the Informed Consent Principle, Voluntarism Principle, and Privacy Protection Principle were fulfilled.

Statistical analysis

The data collected were evaluated in Statistical Package for Social Sciences (SPSS) program using convenient statistical analyses. The data were hence statistically analyzed using percentage distributions, mean, standard deviation, Fisher exact test, variance analysis for intergroup comparisons, and χ^2-test.

Results

There was no significant difference in descriptive characteristics (i.e., they were similar) of the newborns among the breast milk, dry-keeping, and chlorhexidine groups ($p>0.05$) (Table 1). Table 2 shows colonization results obtained from culture samples collected on day 2 and day 5 in the breast milk group. Culture results of 12 newborns revealed that colonization was observed both on postnatal day 2 and day 5. Table 3 shows colonization results obtained from culture samples collected on day 2 and day 5 in the dry-keeping group. Culture results of 14 newborns revealed that colonization was observed both on postnatal day 2 and day 5. Table 4 shows colonization results obtained from culture samples collected on day 2 and day 5 in the chlorhexidine group. Culture results of 6 newborns revealed that colonization was observed both on postnatal day 2 and day 5.

Table 5 shows comparison of colonization results on postnatal day 2 and day 5 among the breast milk, dry-keeping, and chlorhexidine groups. The results of Fisher exact test yielded a significant difference in colonization among the groups ($p<0.05$).

Comparison of the time of umbilical cord separation among the breast milk, dry-keeping, and chlorhexidine groups is shown in Table 6. The time of umbilical cord separation was 6.62±0.84 days in the breast milk group, 7.15±0.67 days in the dry-keeping group, and 6.43±0.77 days in the chlorhexidine group. Further analysis revealed the time of umbilical cord separation to be shortest in the chlorhexidine group newborns. Table 6 also presents comparison of omphalitis results on postnatal day 2 and day 5 in the breast milk, dry-keeping, and chlorhexidine groups. According to the evaluations performed on day 2, the highest number of newborns with omphalitis development was observed in the breast milk group. According to the evaluations performed on day 5, the highest number of omphalitis development was recorded in the dry-keeping group. Fisher exact test showed that there was no significant difference in the omphalitis development rates among the groups ($p>0.05$).

Table 1. Comparison of newborns in the breast milk, dry-keeping and chlorhexidine groups based on their descriptive characteristics

Characteristic	Group			Test and p
	Breast milk (n=32)	Dry-keeping (n=35)	Chlorhexidine (n=30)	
	n (%)	n (%)	n (%)	
Gender				
Girl	16 (50.0)	18 (51.4)	14 (46.7)	χ^2=0.188
Boy	16 (50.0)	17 (48.6)	16 (53.3)	p=0.936
Birth type				
Normal	9 (28.1)	6 (17.1)	7 (23.3)	χ^2=0.616
Cesarean	23 (30.7)	29 (82.9)	23 (76.7)	p=0.568
	M±SD	M±SD	M±SD	
Gestational age (weeks)	38.84±1.13	38.68±1.15	39.00±0.87	F=0.015 p=0.500
Birth weight (g)	3246.93±548.20	3293.93±405.27	3300.73±347.20	F=0.858 p=0.870
Birth length (cm)	50.65±0.82	50.91±0.95	50.86±0.73	F=0.858 p=0.427

Table 2. Bacterial growth results in the breast milk group on postnatal day 2 and day 5

Culture bacterial growth	Breast milk			
	A (n=5) M±SD	B (n=6) M±SD	C (n=5) M±SD	D (n=12) M±SD
None	0	0	5	0
CNS	5	5	0	8
AHS	0	1	0	2
SA	0	0	0	2

A = bacterial growth existed on day 2 but did not exist on day 5;
B = bacterial growth did not exist on day 2 but did exist on day 5;
C = bacterial growth did not exist either on day 2 or on day 5;
D = bacterial growth existed on day 2 and on day 5;

CNS = coagulase negative *Staphylococcus;* SA = *Staphylococcus aureus;* AHS = alpha hemolytic *Streptococcus*

Table 3. Bacterial growth results in the dry-keeping group on postnatal day 2 and day 5

Culture bacterial growth	Dry-keeping			
	A (n=7) M±SD	B (n=10) M±SD	C (n=4) M±SD	D (n=14) M±SD
None	0	0	4	0
CNS	4	8	0	9
AHS	1	1	0	2
SA	2	1	0	2
AHS+CNS	1	0	0	0
Klebsiella	0	0	0	1

A = bacterial growth existed on day 2 but did not exist on day 5;
B = bacterial growth did not exist on day 2 but did exist on day 5;
C = bacterial growth did not exist either on day 2 or on day 5;
D = bacterial growth existed on day 2 and on day 5;

CNS = coagulase negative *Staphylococcus;* SA = *Staphylococcus aureus;* AHS = alpha hemolytic *Streptococcus*

Table 4. Bacterial growth results in the chlorhexidine group on the postnatal day 2 and day 5

Culture bacterial growth	Chlorhexidine			
	A (n=5) M±SD	B (n=6) M±SD	C (n=14) M±SD	D (n=6) M±SD
None	0	0	14	0
CNS	3	3	0	3
AHS	0	0	0	2
SA	1	1	0	1
AHS+CNS	1	1	0	0
Pseudomanas	0	1	0	0

A = bacterial growth existed on day 2 but did not exist on day 5;
B = bacterial growth did not exist on day 2 but did exist on day 5;
C = bacterial growth did not exist either on day 2 or on day 5;
D = bacterial growth existed on day 2 and on day 5;

CNS = coagulase negative *Staphylococcus;* SA = *Staphylococcus aureus;* AHS = alpha hemolytic *Streptococcus*

Table 5. Comparison of bacterial growth results on postnatal day 2 and day 5 in the breast milk, dry-keeping, and chlorhexidine groups

Culture bacterial growth	Group				Test and p
	A (n=18) n (%)	B (n=21) n (%)	C (n=23) n (%)	D (n=35) n (%)	
Breast milk	6 (18.8)	6 (18.8)	5 (15.6)	15 (46.9)	χ^2=13.240 **p=0.036**
Dry-keeping	8 (22.9)	9 (25.7)	4 (11.4)	14 (40.0)	
Chlorhexidine	4 (13.3)	6 (20.0)	14 (46.7)	6 (20.0)	

A = bacterial growth existed on day 2 but did not exist on day 5;
B = bacterial growth did not exist on day 2 but did exist on day 5;
C = bacterial growth did not exist either on day 2 or on day 5;
D = bacterial growth existed on day 2 and on day 5.

Table 6. Comparison of the time of umbilical cord separation and omphalitis results in the breast milk, dry-keeping, and chlorhexidine groups

	Group			Test and p
	Breast milk (n=32) M±SD	Dry-keeping (n=35) M±SD	Chlorhexidine (n=30) M±SD	
Time of umbilical cord separation (days)	6.62±0.84	7.15±0.67	6.43±0.77**	F=13.240 **p=0.036**
	n (%)	n (%)	n (%)	
Omphalitis, day 2*	4 (50.0)	3 (37.5)	1 (12.5)	χ^2=1.677 p=0.476
Omphalitis, day 5**	2 (14.3)	7 (50.0)	5 (35.7)	χ^2=2.809 p=0.243

*Bonferroni test; **Omphalitis monitoring result (erythema, fever, edema, bleeding, discharge, bad smell)

Discussion

In this study, the effects of using breast milk, dry-keeping, and chlorhexidine (0.4%) during umbilical care on umbilical cord colonization, time of umbilical cord separation, and omphalitis development were investigated. As a result of the study, it was found that there was no colonization in 46.7% of the chlorhexidine group, while the highest percentage of colonization existed in the dry-keeping group. In a multi-center study performed in south Nepal, the effects of using 4% chlorhexidine, water-soap, and dry-care during umbilical cord care on mortality and omphalitis development were assessed. The incidence of omphalitis was found to be significantly lower in the chlorhexidine group. This points to the importance of umbilical cord care with an antiseptic solution. Growth of coagulase negative staphylococcus was observed in 13 newborns from the group where breast milk was used and 16 newborns from the dry-keeping group. It was determined that the newborn mortality was by 34% lower in the group where chlorhexidine was applied as compared to the dry-care group[36]. Chlorhexidine was found to be effective on omphalitis development and decreased development of infection at a percentage of 26% to 56%[14]. The results of our study also support these findings.

According to literature data, the effect of chlorhexidine on the time of umbilical cord separation is debatable. Some studies argue that it shortens the time of cord separation, whereas other studies report results indicating that it extends the time of cord separation, particularly if used in high doses (more than once)[18-20]. Yet, its effect on reducing newborn mortality is quite important[20,37,38].

In our study, the time of umbilical cord separation was found to be significantly shorter in the chlorhexidine group than the time of separation in the other two groups. In a related study, the time of umbilical cord separation was shorter in the group of newborns who received umbilical cord care with breast milk as compared to the group where dry-keeping was applied[27]. In the study by Vural and Kısa, it was also found that the mean time of umbilical cord separation was 7.7 days in the newborns who received umbilical cord care with breast milk *versus* 9.9 days in the group of newborns who received umbilical cord care with povidone-iodine, and this difference was statistically significant[31]. Umbilical cord care with breast milk is recommended since it shortens the time of umbilical cord separation, decreases bacterial colonization, and is cheaper[28]. The cost of chlorhexidine is high.

Therefore, when costs are taken into consideration and chlorhexidine cannot be supplied, it is concluded that the use of breast milk is more effective than the method of dry-keeping.

Farahani *et al.* compared the methods of dry cord care and application of breast milk to the cord in 118 healthy newborns with respect to the time of umbilical cord separation and percentage of colonization. The authors found that the time of umbilical cord separation was significantly shorter in the breast milk group. Omphalitis and sepsis were not observed in any of the cases[28]. In another study, the effects of umbilical cord care methods of breast milk, 96% ethyl alcohol, silver sulfadiazine, and dry care on the time of umbilical cord separation and infection development were investigated. The time of cord separation in the breast milk group was found to be significantly shorter when compared with the other groups. As a result of the study, it was pointed out that dry cord care could be risky in underdeveloped countries. It was emphasized that breast milk could be used in cord care due to its easy availability and anti-infectivity; yet, it was also pointed out that large-scale and well-planned further studies were required to assess its effect on reducing infections and time of separation[26].

For umbilical cord care, WHO recommends dry care in developed countries and the use of antiseptics, especially chlorhexidine, in underdeveloped and developing countries in particular[12]. In a study conducted in Turkey, it was indicated that umbilical cord care with sterile gauze was sufficient for healthy newborns who were born on term[13]. In the meta-analysis, Sener Toplak and Bayat recommended antisepsis with chlorhexidine in developing countries[40].

Conclusion

The results of the study showed that in the swabs obtained on postnatal day 5, there was no colonization in 46.7% of the newborns in the chlorhexidine group, whereas the highest percentage of bacterial growth was observed in the dry-keeping group, predominated by coagulase negative staphylococcus. When colonization results on postnatal day 2 and day 5 were compared, the difference among the methods of umbilical cord care employed was statistically significant. When the time of umbilical cord separation was compared among the three methods, differences were statistically significant and the order of the time of separation was as follows: chlorhexidine, breast milk, and dry-keeping groups. When the methods were compared according to the rate of omphalitis development, differences among the methods were not statistically significant. According to these results, caution is required when using dry-keeping in underdeveloped and developing countries since it is insufficient to prevent umbilical colonization, while leading to a longer time of cord separation. As the use of breast milk is more effective than dry-keeping and it implies no additional cost, it can be used in underdeveloped and developing countries. Institutions should develop protocols of umbilical cord care and ensure their sustainability. Performing similar studies with a larger number of cases is recommended.

Limitations

The home environment factor that is not under our control but has an important role in omphalitis development should not be ignored. Large varieties exist related to this factor. These varieties include whether or not mothers detect the symptoms of omphalitis correctly, and if they do, whether they inform us correctly or not, and whether they apply the treatment that is described at the hospital appropriately or not. In our study, the symptoms of omphalitis in the umbilical cord on postnatal day 2 and day 5 were evaluated by the researchers through observation. The evaluation until the umbilical cord separation were left to the families.

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Sažetak

USPOREDBA UČINKOVITOSTI MAJČINA MLIJEKA, ODRŽAVANJA SUHOM I PRIMJENE KLORHEKSIDINA U NJEZI PUPKOVINE

E. E. Küçük, D. Küçük Alemdar, K. Yapar, Ş. Direkel, N. Ömerbeyoğlu i *Ü. Başak*

Cilj ove randomizirane studije bio je utvrditi učinke majčina mlijeka, održavanja suhom i primjene klorheksidina u njezi pupkovine,na vrijeme odvajanja pupkovine te na razvoj omfalitisa i bakterijske flore. Istraživanje je provedeno na ukupno 97 novorođenčadi podijeljene u tri skupine. Kod prve skupine od 32 novorođenčadi primijenjeno je majčino mlijeko, u drugoj skupini od 35 novorođenčadi primijenjena je metoda održavanja područja suhim, a u trećoj skupini od 30 novorođenčadi primijenjen je klorheksidin za njegu pupkovine. Novorođenčad je podijeljena u tri skupine pomoću računalnog programa randomizacije. Uzorci za kulturu uzeti su iz pupkovine drugog i petog dana nakon rođenja. Razlika u rezultatima kolonizacije među primijenjenim metodama njege pupkovine nije bila značajna drugog dana nakon rođenja, dok je petog dana utvrđena značajna razlika u tim rezultatima. U skupini na klorheksidinu nije bilo kolonizacije u 46,7% slučajeva, dok je najveća kolonizacija utvrđena u skupini gdje je primijenjena metoda održavanja područja suhim. Vrijeme do odvajanja pupkovine bilo je značajno kraće u skupini na klorheksidinu nego u drugim dvjema skupinama. Rezultati istraživanja pokazuju da je primjena klorheksidina najučinkovitija metoda njege pupkovine. Održavanje područja suhim može biti udruženo s određenim rizikom, jer se ova metoda pokazala neučinkovitom u sprječavanju kolonizacije pupkovine, a tamo gdje cijena klorheksidina predstavlja problem preporuča se primijeniti metodu majčina mlijeka.

Ključne riječi: *Bakterijska kolonizacija; Njega pupkovine; Vrijeme odvajanja pupkovine; Majčino mlijeko; Održavanje pupkovine suhom; Klorheksidin*