Relationship between Severe Early Childhood Caries and breast milk`s lactose among 12-24 month old children.

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Research

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Abstract

**Aim:** The study aimed to investigate the relationship between the occurrence of Severe Early Childhood Caries (S-ECC) and breast milk lactose, in infants aged 12 to 24 months.

**Material and Methods:** This cross-sectional descriptive-analytical study was carried out in April to July 2020 on 30 children aged 12 to 24 months with or without S-ECC who were solely breastfed. Also, the mothers answered questions about their breastfeeding. After the child’s dental visit, the mother was asked to express 10 to 20 ml of her milk as a sample and give it to the researchers. The samples were immediately kept at -4 ° C and then they were tested to lactose measurement. Finally, the test results were analyzed by SPSS 21 software.

**Results:** The average amount of lactose in the breast milk of cases with S-ECC infant was 5.74g/100 ml, and the average amount of lactose in the breast milk of cases without S-ECC infant was 4.64g/100 ml. There was no significant difference in lactose concentration between the two groups (p=0.64). The average number of breastfeeding times in cases with S-ECC infants was 7.87 per day while in the healthy cases this was 7.33 per day. There was no significant difference between the numbers of breastfeeding times per day in the two groups.

**Conclusion:** According to this study, the average level of lactose in the breast milk samples was lower than the average level of lactose in breast milk in other countries, and the amount of lactose in breast milk of children with S-ECC was slightly higher than the amount of lactose in breast milk in children with healthy teeth.

**Introduction**

Early Childhood Caries (ECC) is a non-communicable but transmissible infectious disease (1). Caries usually progresses to the pulp and causes pain, eventually leading to the formation of dental abscesses that adversely affect the child’s health [5]. ECC means the presence of one or more decayed tooth surfaces in each tooth of a child aged 71 months or less. Severe Early Childhood Caries (S-ECC) is a severe type of ECC that is characterized by the age of less than three years (2). The cause of this disease is demineralization of tooth enamel and dentin by organic acids of microbial origin. Various factors affect the onset and progression of the disease, such as food substrate and acid-producing bacteria, which form a biofilm that adheres to the tooth surface and over time the substrate acts as a bacterial nutrient and the acid is produced by bacteria (3). There are many predisposing factors for this disease such as breastfeeding at night, diet with high sugar content, poor parental awareness of the child’s oral health, low socioeconomic level of the family and lack of access to oral care centers. Also, lack of sufficient fluoride in drinking water and/or in foods can be other predisposing factors for this disease (4, 5). If performed in an inappropriate way, breast-feeding can be a predisposing factor for S-ECC (6). Factors such as the amount of carbohydrates in breast milk or formula, and the duration of milk contact with the erupted teeth, determine the risk of tooth decay (7-9). Breast milk is beneficial for both mother
and baby (1). Unlike formula, breast milk contains casein, which prevents the growth and adhesion of cariogenic bacteria, especially streptococci, to the enamel (10). Breast milk has the highest amount of lactose compared to other species (7 grams per 100 ml). Lactose is converted to glucose and galactose before intestinal absorption (11). Mohebbi et al. evaluated the eating habits of children and its effect on early childhood caries in communities that were normally breastfed for a long time. According to their findings, breast milk was the cause of early childhood caries due to its high lactose content (1). Branger et al. reviewed articles that assessed the association between breastfeeding and S-ECC.

**Methods**

According to the study, breastfeeding until the age of one is not associated with an increased risk of tooth decay and may even be protective compared to formula. In contrast, infants who were breastfed after the age of one year showed an increase in S-ECC levels (12). Prevalence of ECC has been reported up to 71% in the world. In Iran, the prevalence of S-ECC in different regions has been reported 10% to 45% (13-15). Therefore, this study was conducted due to the high prevalence of S-ECC in Iran and many countries, and a likely relationship between prevalence and severity of S-ECC with the amount of breast milk lactose and lack of related studies.

This study was approved by the ethics committee of Kerman University of Medical Sciences with the code IR.KMU.REC.1398.549. The cross-sectional descriptive-analytical study was performed on 30 children of 12 to 24 months with or without S-ECC. The mothers who did not consent to participate in the study were excluded. The study population was selected from mothers who brought their children to public health centers in Kerman for vaccination as well as to the pediatric dentistry clinics. The mothers were interviewed by third author and they were selected from low socio-economic families because ECC is more prevalent in these families. The children were examined for maxillary anterior teeth in a knee-to-knee position. Clinical examination of children's teeth was performed by disposable oral mirror after drying the surface of maxillary incisors. The presence of any signs of caries on the labial surfaces of the four maxillary incisors was assessed for S-ECC diagnosis, and the presence or absence of caries was noted in the relevant checklist. Inclusion criteria for the mothers are the following:

1- Age at delivery between 18 to 45 years

2. Pregnancy period of 37 weeks and more.

Exclusion criteria:

1- Diabetes 2- Tobacco and/or alcohol consumption

3. Feeding by formula

Mothers were asked to answer the checklist questions while reading and signing the informed consent form to participate in the study. Finally, they extracted 10 to 20 ml of milk by hand or with a special pump to a collection tube.
To ensure blindness, all collection tubes were coded as recorded in the consent form and checklist. The samples were immediately placed at -4 °C and kept at this temperature until the experiment was performed. On the day of the experiment, the samples were transferred to the laboratory.

For the measurement of lactose, phenol-sulfuric acid method, which is one of the most common methods for measuring carbohydrates in solution, was adopted. Initially, to draw a standard curve for lactose, specific concentrations of lactose were prepared (concentrations of 400-200-100-50 μg / ml). The milk samples were then centrifuged in Falcon tubes to separate lactose at 10,000 rpm for 7-8 minutes. Samples were taken from the supernatant and added to the numbered test tubes. To each tube was added 10 μl of milk sample with 1990 μl of water. Then, the steps were taken to draw a standard curve, which included the addition of 50 μl of 80% phenol and 5 ml of sulfuric acid immediately, and mixing on the device to completely mix the acid with the solution. Then, the tubes were kept fixed for 10 minutes. After that, the tubes were shaken again for 2 seconds and placed in a 30-degree oven for 20 minutes. The spectrophotometer [Shimadzu Co., Japan] was again zeroed with the blank solution and then the wavelength of the samples was read at 490 nm. The wavelengths obtained from each sample were recorded. Finally, the absorptions obtained from the milk samples were placed in the line equation of the standard lactose diagram, and the equivalent concentration was obtained. The number obtained was multiplied by 200 (because the milk sample was diluted 200 times) and lactose concentration was calculated in mg / L. To report values in percentage, (g of lactose in 100 ml of milk) the numbers were divided by 10,000 and the concentrations obtained from the sample were recorded.

The obtained data were statistically analyzed by a statistical expert using SPSS 21 software. Independent t-test was used to evaluate the relationship between the amount of the milk lactose and suffering from ECC.

Results

In this study, the amount of lactose in 30 mother milk samples was measured. The children of 15 mothers did not have S-ECC. Their characteristics are given in Table 1, and characteristics of 15 children with S-ECC are given in Table 2. Table 3 shows the comparison of the two groups.

Discussion

Thirty infants aged 12 to 24 months (mean age 16.4) of both genders who were exclusively breastfed were included in the study with their mothers. Fifteen children had S-ECC. The maximum amount of lactose was 10.24 g per 100ml of milk (belong to the S-ECC group) and the minimum amount was 1.54g/100ml (belong to the non-S-ECC group). (Tables 1 and 2). The average of lactose among 30 mothers was 5.19g/100ml (Table 3). A study by Sadaf Khan et al. reported that the average lactose level in the milk samples of 15 mothers was 6.8g/100ml (16), and the study by Aumeister et al. has shown 6.53g/100ml of milk (6). In both studies, the amount of lactose in breast milk was higher than the amount of lactose in samples in the present study. These differences can be due to different quality and quantity of nutrition in
the mothers in different countries. There was no study on the level of lactose in mother breast milk in relation to the occurrence of S-ECC in their children's teeth. In the present study, the amount of lactose in S-ECC group was slightly more than the group of without S-ECC. Although the difference was not significant (Table 3), the difference can be a reason for higher occurrence of S-ECC. It means higher level of lactose in the breast milk increases the risk of occurrence of S-ECC in their children's teeth.

Sadaf Khan et al. found that more breast sucking and breastfeeding cause an increase in the amount of lactose in the milk. In the present study, the average of lactose was slightly higher in the breast milk of mothers who breastfed their baby more frequently than the average of lactose in the milk of mothers who breastfed their baby less frequently (Tables 1,2) (16). In that study, the average breastfeeding of children was 11 times in 24 hours whereas in the present study, the average number of times of breastfeeding was 7.6 per 24 hours. In both studies, spectrophotometric method was employed to determine the concentration of lactose (16). In Sadaf Khan Study, milk samples were taken from both breasts, but no significant difference was observed in the amount of lactose in the right and left breasts. In our study, the milk samples were taken from either the right or left breast. In the present study, we did not assess the amount of fat and protein in the milk samples, but in the Sadaf Khan study, it was carried out and resulted lack of enough of protein in breast milk prevents the baby from getting full. Consequently this increase in the number of breastfeeding times can eventually cause more caries (16). Table 4 shows studies related to breast feeding and dental caries.

**Suggestions for further research:** Future studies can focus on simultaneous evaluation of the amount of fat and protein with lactose. Also, mothers’ nutrition can be considered as a factor in the relationship between breast milk’s lactose and S-ECC.

Limitations: Small sample size was a limitation for the present study. This limitation had two reasons: 1. Fewer mothers visited clinics due to the spread of COVID-19; and 2. Some mothers were reluctant to give their milk samples.

**Conclusion**

According to the results of this study, the average level of lactose in the breast milk samples was lower than the average level of lactose in breast milk in other countries, and the amount of lactose in breast milk of children with S-ECC was slightly higher than the amount of lactose in breast milk in children with healthy teeth.

**Declarations**

**Ethics Approval and consent to participate**

Title of study: Evaluation of relationship between Sever Early Childhood Caries and breast milk`s Lactose among 12-24-month-old Children.
Ethical code of Study: IR.KMU.REC.1398.549.

I ....... (Mother name), agree to participate in the mentioned research and to examine my child's teeth. Iacknow ≤ d ≥ twi ≤ therestofthisstudymay be helpful in preventing of Early Childhood Caries (ECC) in other children in future.

Mother`s name:
Signature

Consent for publication

Authors of manuscript titled “Evaluation of relationship between Sever Early Childhood Caries and breast milk’s Lactose among 12-24-month-old Children” agree to publish the article in INTERNATIONAL BREASTFEEDING JOURNAL. We also announce that the article has not been previously published in another journal and has not been submitted to any journal for publication.

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Availability of data and material

Not applicable

Competing interests

There is no conflict of interests in this study.

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Authors contributions

Writing of the study proposal: Hamidreza Poureslami, Maryam Sharifi.

Selection of the mothers & Preparation of the breastmilk samples: Mahla Vahedi, Parnian Poureslami.

Examination of the children teeth: Nima Hatami, Perisa Jafari.

Performing of the test for measuring of lactose: Salehe Sabour, Naghmeh Satarzadeh, Mahla Vahedi.
Writing of the article: Hamidreza Poureslami, Maryam Sharifi.

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Tables

Table 1. Distribution of frequency of variables in the group without S-ECC.

| Sample Code | Child age in month | Child gender | Amount of lactose in breast milk | Number of feedings in 24 hours |
|-------------|--------------------|--------------|----------------------------------|-------------------------------|
| 1           | 16                 | girl         | 5.66                             | 7                             |
| 2           | 14                 | boy          | 4.30                             | 6                             |
| 4           | 12                 | boy          | 7.01                             | 8                             |
| 5           | 19                 | girl         | 1.54                             | 10                            |
| 6           | 14                 | girl         | 5.70                             | 7                             |
| 8           | 18                 | boy          | 4.50                             | 7                             |
| 9           | 16                 | girl         | 1.81                             | 4                             |
| 10          | 14                 | girl         | 3.10                             | 8                             |
| 11          | 16                 | boy          | 7.07                             | 10                            |
| 12          | 23                 | girl         | 4.77                             | 8                             |
| 14          | 15                 | boy          | 4.40                             | 2                             |
| 16          | 18                 | girl         | 4.57                             | 10                            |
| 17          | 13                 | boy          | 3.58                             | 9                             |
| 18          | 12                 | girl         | 5.34                             | 6                             |
| 23          | 14                 | boy          | 6.27                             | 8                             |

Table 2. Distribution of frequency of variables in the group with S-ECC.
| Sample Code | Child age in month | Child gender | Amount of lactose in breast milk | Number of feedings in 24 hours |
|-------------|--------------------|--------------|---------------------------------|-------------------------------|
| 3           | 23                 | girl         | 5.35                            | 10                            |
| 7           | 16                 | boy          | 7.86                            | 9                             |
| 13          | 13                 | girl         | 10.24                           | 13                            |
| 15          | 13                 | girl         | 4.84                            | 12                            |
| 19          | 19                 | boy          | 8.80                            | 6                             |
| 20          | 13                 | boy          | 4.87                            | 6                             |
| 21          | 14                 | boy          | 4.72                            | 15                            |
| 22          | 22                 | girl         | 6.60                            | 2                             |
| 24          | 16                 | boy          | 7.20                            | 7                             |
| 25          | 23                 | boy          | 7.46                            | 5                             |
| 26          | 22                 | boy          | 5.75                            | 3                             |
| 27          | 14                 | boy          | 4.34                            | 9                             |
| 28          | 16                 | girl         | 3.07                            | 7                             |
| 29          | 15                 | boy          | 2.92                            | 6                             |
| 30          | 18                 | boy          | 2.10                            | 8                             |

Table 3. Amount of Lactose in the breast milk samples in the two groups.

| Suffering from S-ECC | Number of children | Average lactose content (SD) | P value |
|----------------------|--------------------|------------------------------|---------|
| Yes                  | 15                 | 5.74 (2.28)                  |         |
| No                   | 15                 | 4.64 (1.65)                  | 0.14    |
| Total                | 30                 | 5.19 (1.96)                  |         |

Table 4. The studies related to breast feeding and dental caries.

| Researchers          | year | Sample size |
|----------------------|------|-------------|
| Aumeister et al.     | 2017 | 28          |
| Khan et al.          | 2013 | 15          |
| Mohebbi et al.       | 2008 | 504         |
| Feldens et al.       | 2018 | 345         |
| Hong et al.          | 2014 | 509         |
| Soto et al.          | 2014 | 160         |
| Sugito et al.        | 2010 | 565         |
| Branger et al.       | 2019 | 35          |