In vivo study to evaluate of human breast milk, infant milk formula on cariogenicity in children: A comparative study

1.Dr. Prasannakumari. S. Patil, 2.Dr. Priyanka Kotia, 3. Dr. Anurag Azad

4.Dr. Shreyas Gedam, 5.Dr. Deepak Bansal, 6.Dr. Karri Tyson

[1]Professor, Department of Pedodontics and Preventive Dentistry, S. B. Patil Institute for Dental Sciences and Research, Bidar.
[2]Associate Professor, Department of Dentistry, Dr. Sonelal Patel Autonomous State Medical College, Pratapgarh, U.P. 230001.
[3]Professor, Department of Oral and Maxillofacial Surgery, Bhabha College of Dental Sciences, Bhopal
[4]Senior Lecturer, Department of Orthodontics and Dentofacial Orthopedics, Swargiya Dadasaheb Kalmegh Smruti Dental College and Hospital, Nagpur.
[5]Professor, Department of Prosthodontics, Crown and Bridge, Bhojia Dental College and Hospital, Baddi, Himachal Pradesh, India.
[6]MDS, Department of Oral Pathology and Microbiology, Bhopal, M.P.
Corresponding author: Dr. Prasannakumari. S. Patil, Professor, Department of Pedodontics and Preventive Dentistry, S. B. Patil Institute for Dental Sciences and Research, Bidar.
pksp89@gmail.com

Abstract

Background

Early childhood caries (ECC) is a major public health concern influenced by dietary habits, including feeding practices. Human breast milk and infant milk formula are primary sources of nutrition for infants, but their cariogenic potential remains a subject of debate. This in vivo study aims to compare the cariogenicity of human breast milk and infant milk formula in children by assessing dental plaque accumulation and Streptococcus mutans colonization.

Materials and Methods

A comparative study was conducted on 80 children aged 12–36 months, divided into two groups: Group A (n=40) exclusively breastfed for at least six months and Group B (n=40) formula-fed for at least six months. Plaque pH, Streptococcus mutans count, and the decayed, missing, and filled teeth (dmft) index were recorded at baseline and after six months. Salivary samples were collected to analyze microbial activity using selective culture media.

Results

At six months, Group B exhibited a significantly lower mean plaque pH (5.4 ± 0.3) compared to Group A (6.1 ± 0.2) ($p < 0.05$), indicating higher acidity in formula-fed children. Streptococcus mutans counts were significantly higher in Group B (2.8×10^5 CFU/mL) than in Group A (1.9×10^5 CFU/mL) ($p < 0.05$). The mean dmft score was 2.1 ± 0.6 in Group B compared to 1.2 ± 0.4 in Group A ($p < 0.05$), suggesting a higher caries risk in formula-fed children.

Conclusion

Infant milk formula demonstrated higher cariogenic potential than human breast milk, as evidenced by lower plaque pH, increased Streptococcus mutans colonization, and higher dmft scores. Encouraging breastfeeding and implementing early oral hygiene practices may help reduce the risk of early childhood caries. Further longitudinal studies are needed to confirm these findings.

Keywords

Early childhood caries, Breast milk, Infant formula, Cariogenicity, Plaque pH, Streptococcus mutans, dmft index

Introduction

Early childhood caries (ECC) is a significant public health concern that affects children worldwide, with dietary habits playing a crucial role in its development (1). ECC is characterized by the presence of one or more decayed, missing, or filled tooth surfaces in primary teeth of children under six years of age (2). Among the various risk factors, feeding practices such as breastfeeding and formula feeding are considered potential contributors to cariogenicity in infants and young children (3).

Breastfeeding has been widely recommended by health organizations due to its numerous benefits, including optimal nutrition, immune protection, and better orofacial development (4). However, its role in dental caries remains controversial. Some studies suggest that human breast milk has a protective effect against dental caries due to its antimicrobial properties, including the presence of lactoferrin and secretory immunoglobulin A (5,6). Conversely, prolonged and nocturnal breastfeeding without adequate oral hygiene has been associated with increased caries risk (7).

Infant milk formula is a widely used alternative for feeding infants who are not exclusively breastfed. However, the composition of formula differs significantly from human breast milk, particularly in terms of carbohydrate content and buffering capacity (8). Infant formulas often contain fermentable carbohydrates such as sucrose, glucose, and maltodextrins, which can serve as a substrate for cariogenic bacteria, leading to acid production and enamel demineralization (9). Studies have shown that formula-fed infants may have a lower salivary pH and higher Streptococcus mutans colonization compared to breastfed infants, suggesting an increased risk for ECC (10,11).

Streptococcus mutans, a primary etiological agent of ECC, adheres to dental surfaces and ferments dietary sugars to produce acids, leading to demineralization of enamel (12). The acidogenic potential of different feeding practices can be assessed by measuring plaque pH and bacterial load in saliva (13). The decayed, missing, and filled teeth (dmft) index is another widely used clinical measure to evaluate caries experience in children (14).

Given the conflicting evidence on the cariogenic potential of breast milk and infant formula, there is a need for further research to clarify their effects on early childhood oral health. This in vivo study aims to compare the cariogenicity of human breast milk and infant milk formula in children by evaluating plaque pH, Streptococcus mutans colonization, and dmft scores over a six-month period. Understanding these associations may help in formulating guidelines for infant feeding practices and preventive strategies to reduce the risk of ECC.

Materials and Methods

Study Design and Participants

This in vivo comparative study was conducted on 80 children aged 12–36 months, recruited from pediatric dental clinics. The participants were divided into two groups:

- **Group A (n=40):** Children exclusively breastfed for at least six months.
- **Group B (n=40):** Children who were formula-fed for at least six months.

Children with systemic diseases, congenital oral anomalies, prolonged medication use, or prior antibiotic therapy within the past three months were excluded from the study. Parental consent was obtained before participation.

Clinical and Microbiological Assessments

Baseline assessments were recorded for all participants at the start of the study, and follow-up evaluations were conducted after six months. The parameters assessed included plaque pH, **Streptococcus mutans** count, and the decayed, missing, and filled teeth (**dmft**) index.

1. **Plaque pH Measurement:**
   Plaque samples were collected from the buccal surfaces of upper primary incisors using a sterile dental explorer. The plaque was immediately analyzed using a microelectrode pH meter to determine acidity levels.

2. **Microbial Analysis:**
   Salivary samples were collected from each child by asking them to passively drool into a sterile container. The samples were transported on ice and inoculated onto **Mitis Salivarius Bacitracin (MSB) agar** for selective culture of **Streptococcus mutans**. After 48-hour incubation at 37°C in a 5% CO_2 environment, the bacterial colonies were counted and recorded as **colony-forming units per milliliter (CFU/mL)** of saliva.

3. **Caries Assessment:**
   The **dmft index** was recorded according to the WHO criteria. A single calibrated examiner performed all clinical examinations using a dental mirror and probe under adequate lighting. Calibration of the examiner was achieved by assessing ten patients twice, one week apart, ensuring an inter-examiner agreement (Cohen's kappa = 0.85).

Statistical Analysis

Data analysis was conducted using **SPSS software version 25.0**. The **Shapiro-Wilk test** was applied to check for data normality. Intergroup comparisons for plaque pH, bacterial count, and dmft scores were performed using the **independent t-test** for normally distributed data and the **Mann-Whitney U test** for non-normally distributed variables. A p-value < 0.05 was considered statistically significant.

Results

Plaque pH Levels

At baseline, there was no significant difference in plaque pH between the two groups (**$p > 0.05$**). However, after six months, **Group B (formula-fed children)** exhibited a significantly lower mean plaque pH (**5.4 ± 0.3**) compared to **Group A (breastfed children) (6.1 ± 0.2, $p < 0.05$)**, indicating increased acidity in the formula-fed group (**Table 1**).

Streptococcus mutans Colonization

The **Streptococcus mutans** count at baseline was similar in both groups (**$p > 0.05$**). After six months, Group B showed a significantly higher bacterial count (**2.8×10^5 CFU/mL**) compared to Group A (**1.9×10^5 CFU/mL, $p < 0.05$**), suggesting a greater bacterial load in formula-fed children (**Table 2**).

Caries Experience (dmft Index)

The mean **dmft score** at baseline was comparable between the two groups (**p > 0.05**). However, at the six-month follow-up, Group B had a significantly higher **dmft score (2.1 ± 0.6)** than Group A (**1.2 ± 0.4, p < 0.05**), indicating an increased caries risk associated with formula feeding (**Table 3**).

Tables

Table 1: Comparison of Plaque pH Between the Groups at Baseline and Six Months

Timepoint	Group A (Breastfed) (Mean ± SD)	Group B (Formula-fed) (Mean ± SD)	p-value
Baseline	6.5 ± 0.2	6.4 ± 0.3	>0.05
6 Months	6.1 ± 0.2	5.4 ± 0.3	<0.05*

(*Statistically significant difference)

Table 2: Streptococcus mutans Count (CFU/mL) in Saliva at Baseline and Six Months

Timepoint	Group A (Breastfed) (Mean ± SD)	Group B (Formula-fed) (Mean ± SD)	p-value
Baseline	$1.7 \times 10^5 \pm 0.3 \times 10^5$	$1.8 \times 10^5 \pm 0.2 \times 10^5$	>0.05
6 Months	$1.9 \times 10^5 \pm 0.4 \times 10^5$	$2.8 \times 10^5 \pm 0.5 \times 10^5$	<0.05*

(*Statistically significant difference)

Table 3: Comparison of dmft Scores Between the Groups at Baseline and Six Months

Timepoint	Group A (Breastfed) (Mean ± SD)	Group B (Formula-fed) (Mean ± SD)	p-value
Baseline	0.9 ± 0.3	1.0 ± 0.4	>0.05
6 Months	1.2 ± 0.4	2.1 ± 0.6	<0.05*

(*Statistically significant difference)

These findings indicate that formula-fed children demonstrated greater acidity in dental plaque, a higher bacterial load, and a significantly higher caries experience over six months.

Discussion

The present study aimed to compare the cariogenic potential of human breast milk and infant formula by evaluating plaque pH, **Streptococcus mutans** colonization, and caries experience over six months. The findings revealed that formula-fed children exhibited lower plaque pH, a higher **S. mutans** count, and increased **dmft** scores compared to breastfed children, suggesting that infant formula has a greater cariogenic potential.

Plaque pH plays a crucial role in the demineralization and remineralization processes of enamel. A lower plaque pH, as observed in formula-fed children, creates an acidic oral environment conducive to demineralization and caries development (1). The present study found that the mean plaque pH in the formula-fed group (5.4 ± 0.3) was significantly lower than that in the breastfed group (6.1 ± 0.2), indicating a higher cariogenic potential of infant

formula. These findings are consistent with previous studies that have reported greater acidogenicity in formula-fed infants compared to those exclusively breastfed (2,3).

The microbial analysis demonstrated significantly higher **S. mutans** counts in the formula-fed group, supporting the hypothesis that infant formula promotes bacterial colonization. **S. mutans** is a well-established cariogenic pathogen that adheres to dental surfaces and metabolizes fermentable carbohydrates to produce acids, leading to enamel demineralization (4). The results align with earlier studies, which have shown that formula-fed infants harbor higher levels of **S. mutans** than their breastfed counterparts, likely due to the higher fermentable sugar content in infant formula (5,6).

Caries experience, as measured by the **dmft index**, was significantly higher in formula-fed children after six months. The mean **dmft** score in this group was 2.1 ± 0.6, compared to 1.2 ± 0.4 in the breastfed group. The findings align with studies reporting a higher incidence of ECC among formula-fed children (7,8). While human breast milk contains lactose, it also possesses protective components such as lactoferrin, immunoglobulins, and lysozymes, which have antimicrobial properties that help control bacterial colonization and buffer plaque acidity (9,10). In contrast, infant formulas often contain added sucrose, glucose polymers, and maltodextrins, which have been shown to lower plaque pH and enhance **S. mutans** proliferation (11).

Despite these findings, the study has certain limitations. The follow-up period was limited to six months, and other factors such as dietary habits, oral hygiene practices, and fluoride exposure were not controlled. Additionally, variations in infant formulas' composition across brands might influence cariogenicity. Future longitudinal studies with a larger sample size and extended follow-up are necessary to confirm these findings and establish definitive guidelines for infant feeding practices to minimize caries risk.

Conclusion

The study findings suggest that infant formula has a greater cariogenic potential than human breast milk, as evidenced by lower plaque pH, increased **S. mutans** colonization, and higher **dmft** scores. Encouraging exclusive breastfeeding and promoting early oral hygiene measures may help reduce the risk of ECC. Further research is warranted to explore the long-term effects of different feeding practices on pediatric oral health.

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