RESEARCH ARTICLE

Assessment of Cariogenicity by pH-value Decrement of Plaque Solution with Four Infant Milk Formulas: An *In Vitro* Study

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Abstract

Introduction: People all over the world use a wide variety of infant formulas to nourish the infants. Recent studies demonstrated the high caries-inducing potential of infant formulas. This indicates a need for awareness toward the possible role of the infant formulas in the etiology of early childhood caries (ECC).

Aim: The present study was undertaken to evaluate and comparatively assess the change in plaque pH solutions after fermenting four commonly used infant milk formulas.

Materials and methods: This simple randomized study was carried out on 40 healthy children aged 4–6 years old. The children received full-mouth prophylaxis before examination. They were asked not to implicate oral hygiene for 24 hours and not to have anything at least 2 hours prior the study. Supragingival plaque from the buccal surface of posterior teeth was collected using a Hu-Friedy's curette. Four commonly used infant milk formulas were prepared and given to children for rinsing. The pH of plaque samples were measured at 30 and 60 minutes at 37°C.

Results: The present study showed that all of the four infant milk formulas decreased the mean pH values in plaque solutions significantly after 30 and 60 minutes of preparation.

Conclusion: Our results showed that the plaque pH varied in response to the oral rinsing with the various infant formulas and most of infant formulas were able to reduce the pH significantly below the pH before the rinse. Based upon this study further evaluation of the cariogenicity of infant formulas is recommended.

Keywords: Early childhood caries, Infant formulas, Plaque pH.

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Introduction

Early childhood caries is an ever-increasing global problem and particularly predominant in developing and underdeveloped nations.[1] Early childhood caries primarily presents in initial 3 years of life as a consequence to a diet high in sugar. A submanifestation of this condition, baby bottle decay, occurs due to frequent and prolonged periods of bottle feeding.[2] In contrast to the global decline of adulthood caries incidence, ECC has not shown any signs of downswing.[3]

As per the American Academy of Pediatrics, infants are to be maintained on an exclusive diet of breast milk for the first 6 months of life. Albeit this recommendation, several factors such as altered lifestyles, difficulty in suckling, soreness and pain in nipples, and deficient breast milk production are leading to an increased dependency on formula milk. Milk formulas constitute fermentable carbohydrates such as sucrose, lactose, and glucose as primary ingredients which may serve as sources of nutrition for cariogenic bacteria.[4]

Infant formulas are given exclusively during the first 6 months and until 12 months in conjunction with other infant foods and are a leading cause for ECC during this period. It cannot be emphasized enough that to reach a goal of caries-free child population a comprehensive research framework with weightage on preventive care is the need of the hour. This process must include identification of high-risk populations and implementation of rigorous preventive programs for them.[5,6]

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Limited data are available in the literature on the impact of infant formulas on incidence of ECC. Our aim, through this study, is to explore the cariogenic potential of commonly available infant milk formulas in aspect to their ability to alter the plaque pH.

Materials and Methods

Sample Selection

The study included 40 healthy preschool children 4–6 years old, who reported to outpatient department and were selected randomly and divided into four groups. Informed consent was obtained from parent or guardian before the procedure.

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Ethical clearance was obtained from Institutional Ethics Committee, Rungta College of Dental Sciences and Research, Bhilai, Chhattisgarh.

Inclusion Criteria

- Age between 4 and 6 years.
- Systemically healthy.
- Caries-free.

Exclusion Criteria

- Aversion to infant milk formulas.
- Antibiotic therapy in the past 6 months.
- Allergy to milk or soy products.
- Drugs affecting salivary flow.
- Xerostomia.

Materials Used for the Study

The infant milk formulas for the study were selected based on the local market survey and its availability in major counters and more frequently dispensed: Lactodex 2 (Raptakos Brett & Company Ltd., India), Lactogen 1 (Nestle India Ltd.), NAN PRO 2 (Nestle India Ltd.), and Similac 1 advance (Abbott Pharma India Ltd.). Hu-Friedey's Gracey curette (#3–4) was used to collect plaque sample whereas glass beakers of 100 mL capacity and digital pH meter (waterproof HI 98,128 pH tester by HANNA) were used to record pH of sample.

Presampling Measures

Before commencement of the study, complete oral prophylaxis of selected children was performed to attain a minimal and uniform baseline plaque score, determined by applying a disclosing solution (Basic Fuchsin—0.075%). The caretakers of study subjects were instructed not to perform any method of oral hygiene procedures for 48 hours to enable natural plaque deposition.

Plaque Collection Procedure

On the 3rd day, the children were recalled on an empty stomach. The tooth surfaces were dabbed with cotton blobs to absorb saliva so as to avoid contamination. Supragingival plaque samples were collected from buccal surfaces of maxillary teeth using curettes, mixed into 50 mL of deionized water, and the initial pH was recorded within 20–30 seconds of sampling.[7] The infant milk formulas were prepared according to the manufacturer's instructions and after recording the resting prerinse plaque pH, each child was given 15 mL of the prepared test solution and asked to swish it around the mouth for 60 seconds before spitting it.[8] Fresh samples of the plaque were taken after 30 and 60 minutes of consuming the test substances, and pH changes were recorded.

Prerinse and postrinse pH values (recorded at varying intervals) were used to compute the maximum pH drop, i.e., the difference between initial prerinse plaque pH and the minimum plaque pH obtained. The difference between the prerinse plaque pH and the postrinse plaque pH recorded at 60 minutes past the time of initial plaque sampling was calculated to account for alterations in plaque acidogenicity caused by the sampling process.[2,9]

The results were tabulated and subjected for statistical analysis (SPSS software version 20).

Results

In the present study, it was observed that all the test samples produced significantly lower plaque pH values after 30 and 60 minutes of preparation in comparison to pH before rinsing with infant formula. The amount of different ingredients in the four infant milk formulas (per 100 gm powder) has been tabulated in Table 1. Lactogen 1 has the highest carbohydrate content whereas Similac 1 contains the highest fat content.

The results of the present study showed that all of the infant milk formulas decreased the mean pH values significantly after 30 and 60 minutes of preparation (Table 2). The pH decrement was nearly same for Lactogen 1, Similac 1, and NAN PRO 2, and slightly higher for Lactodex 2 (0.35 ± 0.07) after 30 minutes of preparation. Similarly pH decrement after 60 minutes was nearly same for Similac 1 and NAN PRO 2, slightly higher for Lactogen 1, and highest for Lactodex 2 (0.71 ± 0.09). The difference in pH decrement after 30 and 60 minutes with initial pH was statistically significant for all the groups except negative control ($p = 0.001$*) (Fig.1).

Discussion

Early childhood caries was historically considered as a consequence to inappropriate and prolonged bottle use; hence, older terms such as "baby bottle tooth decay" and "nursing caries" were used to describe this condition. Recent studies show that ECC is a multifactorial disease with prevalence rates as high as 70%, particularly in developing countries. The risk factors for ECC point to various parenting practices with the unfortunate consequence of high treatment costs.[10]

Although a wide range of methods are utilized to test the cariogenic nature of foods, plaque pH measurement methods have been widely used and accepted as.[11] Sampling and testing of undisturbed plaque is a reliable and proven method for a wide range of oral investigations. Although sufficient studies report on the multiple aspects of plaque acidogenesis, its pH measurements have been doubted for their reliability. However, measurement of pH enables examination of plaque as a metabolic unit and a significant indicator for caries activity.[12]

Originally proposed by Frostell, plaque sampling was mandated to be performed in 5-minute cycles after exposing the oral environment to the cariogenic substrate. Jensen modified this protocol to include both pre and postrinse sampling.[13] In our study, plaque samples were collected prerinse and after 30 and 60 minutes of postrinse with infant formula.

Table 1: The amount of different ingredients in four infant milk formulas (per 100 gm milk powder)

Ingredient/100 gm powder	*Lactodex 2*	*Lactogen 1*	*Similac 1*	*NAN PRO 2*
Carbohydrate (gm)	59.5	60.4	49.8	59.1
Protein (gm)	10.5	10.2	14.5	14.3
Fat (gm)	23	23.7	28.2	20
Calcium (mg)	300	270	369	420
Phosphate (mg)	150	145	230	270
Iron (mg)	5	6	9.2	6.8

Table 2: The mean of pH values and pH decrements of samples at 30 and 60 minutes after preparation

Samples	*pH at the beginning*	*pH after 30 minutes*	*pH after 60 minutes*	*pH decrement after 30 minutes*	*pH decrement after 60 minutes*	*p-value*
Lactodex 2	6.77 ± 0.14	6.42 ± 0.15	6.06 ± 0.12	0.35 ± 0.07	0.71 ± 0.09	0.001*
Lactogen 1	6.69 ± 0.14	6.35 ± 0.14	6.0 ± 0.06	0.33 ± 0.16	0.69 ± 0.13	0.001*
Similac 1	6.89 ± 0.08	6.62 ± 0.12	6.43 ± 0.10	0.33 ± 0.05	0.46 ± 0.04	0.001*
NAN PRO 2	6.66 ± 0.15	6.32 ± 0.14	6.22 ± 0.12	0.32 ± 0.05	0.42 ± 0.07	0.043*

* Signifies statistically significant difference

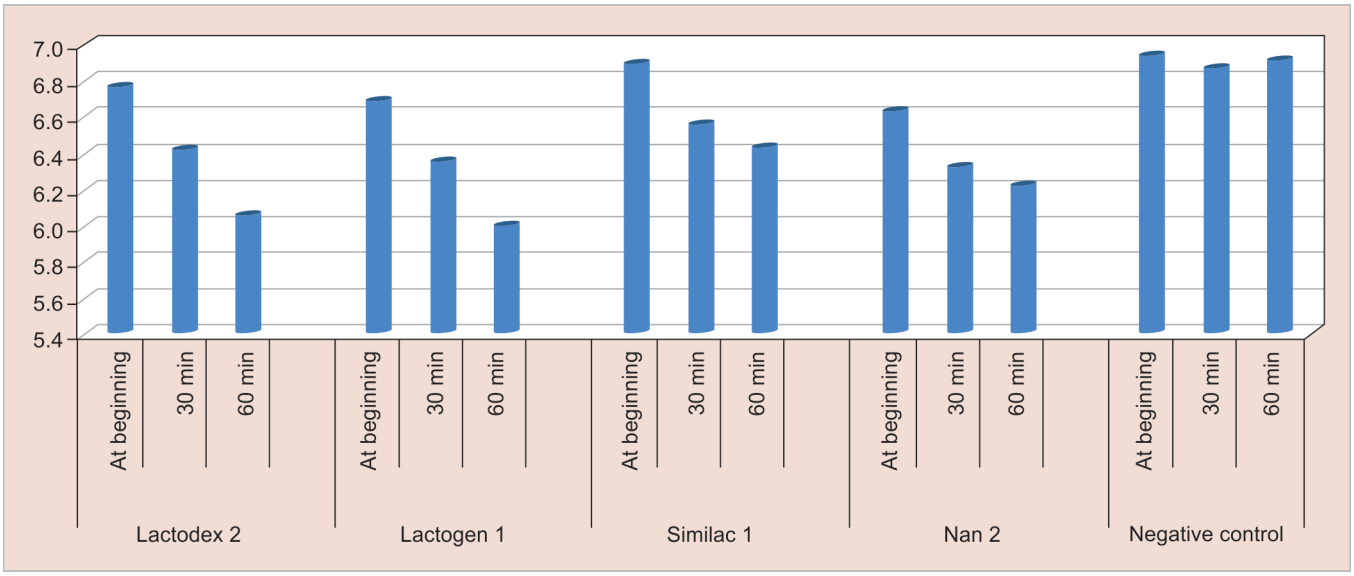


Fig. 1: Graphical representation of pH changes at different time intervals

The transition phase from exclusive breast milk feeding to including a variety of foods post 6 months of age marks a crucial juncture for molding eating habits in children. Repetitive training by parents and peer influence in preschool environments tend to shape the diet patterns at this age. Infants who are given sugars early in life favor products with higher sugar levels when they are toddlers. In a study, the rate of dental caries incidence in 3-year-old children having high sugar intake during infancy was significantly greater than in children who had less exposure to sugar. Dissuading intake of sugary foods during this phase could markedly alter the caries incidence.[14]

It is generally accepted that the nutrients with more acid production after fermentation by plaque microorganisms have more cariogenicity. Our results showed that all infant formula categories produced significantly lower plaque pH values than as compared to prerinse plaque samples. These results are in conjunction with the results observed by Munshi et al.,[2] Chaudhary et al.,[6] Sheikh and Erickson,[13] and Masih et al.[15] The average resting plaque pH of all the children was 6.76 ± 0.20. This average resting plaque pH is comparable to the resting plaque pH seen in studies conducted by Anderson et al.,[16] Saigal and Tewari,[17] and Jensen and Schatele.[18] This is an important finding as it has been found that the children having less cariogenic potential had high resting pH approx 6.8 when compared to the children with active carious lesions.[19,20]

The results of four infant milk formulas tested in our study showed that Lactodex 2 was highly acidogenic and Similac 1 was the least acidogenic. All of the four milk formulas used in the current study were milk-based formulas. Carbohydrate content in all these four milk formulas was more than 50 gm per 100 mL, whereas protein was about 10 gm in Lactdex 2 and Lactogen 1 and 14 gm in Similac 1 and Nan Pro 2. Iron and calcium content in Similac 1 and NAN PRO 2 was much higher than two other formulas, calcium being highest in NAN PRO 2 while iron in Similac 1.

The mineral content of formula feeds may exert an influence on their cariogenicity. Few studies have reported a probable cariostatic action of iron although it does not seem to have a direct effect on plaque pH. Masih et al., who studied on three different milk formulas, stated that infant formula with higher iron content was least cariogenic.[15] Bowen et al. conducted a study to investigate relationship between caries experience and iron concentration in rats and concluded that cariostatic action of iron and fluoride are compatible with each other and have additive effect.[21]

Casein constitutes a major portion of protein in milk. Reynolds and Del Rio investigated the role of casein in a rat model and found that 2% casein in drinking water reduced the extent of fissure and smooth surface caries.[22] In our study, Lactodex 2 and Lactogen 1 decreased the mean plaque pH value more than Similac 1 and NAN PRO 2 in all of the samples. It is evident than Similac 1 and NAN PRO 2 have higher protein content than two other samples. Peres et al. found in a study that the infant formula milk was as cariogenic as sucrose, whereas cows' milk has negligible cariogenicity with human milk showing moderate caries tendency.[23]

Conclusion

The present study has attempted to elucidate the possible cariogenic potential of infant milk formulas. It underlines the need for the parent as well as the clinician to be fully aware of these affects of infant milk formulas and their possible role in

ECC. It can be conjectured that changes in plaque pH after rinsing with infant milk formulas when assessed as risk factors associated with caries implicate infant milk formulas in promoting caries in children. Continued studies on the cariogenicity of infant milk formulas are important to assess the risks associated with their consumption.

Along with nutritional factors, a comprehensive approach for prevention of dental caries in preschool children must include good oral hygiene, appropriate use of fluorides, and access to preventive and restorative dental care.

Clinical Significance

- Parents should be educated from time to time about the importance of diet in the development of caries (with regard to the type of carbohydrate).
- Parents should be discouraged from the frequent use of bottles for feeding, especially for prolonged periods at night.
- As a preventive measure, the dental professionals must thrive hard to make appropriate feeding of infants and children a primary objective, supported by all health professionals. The parents and the pedodontist should work as partners in providing oral health care for children with ECC.

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