ORIGINAL RESEARCH

A Comparative Evaluation of the Acidogenic Potential of Lactose-based, Soy-based, Protein Hydrolysate-based, and Iron-based Milk Formulas Based on Dental Plaque pH, Salivary pH, and Buffering Capacity: An *In Vivo* Study

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Abstract

Aims and background: Breastfeeding is the gold standard nutrition for infants. However, the inability of infants to latch on, maternal complaints of sore or painful nipples, and insufficient milk supply frequently result in early breastfeeding cessation. Hence, milk has subsequently been replaced by infant formula milks of high quality, which serve as an alternate source of nutrition for infants. There also has been a rising concern among parents and caretakers regarding the cariogenic potential of the various milk formulas available in the market due to the presence of fermentable carbohydrates in them, leading to an increase in nursing caries or early childhood caries. Hence, this study aims to comparatively evaluate the acidogenic potential of four different milk formulas based on dental plaque pH, salivary pH, and buffering capacity.

Materials and methods: This cross-sectional study was carried out to assess the acidogenic potential of various infant milk formulas using three main parameters of measurement of caries: evaluation of salivary pH, plaque pH, and buffering capacity.

Baseline plaque and salivary pH and postconsumption plaque pH and salivary pH were assessed after 5, 10, 15, 20, 30, and 60 minutes in 60 healthy children (15 in each study group) aged 7–12 years after rinsing with group I (lactose-based), group II (soy-based), group III (protein hydrolysate-based), and group IV (iron-based) milk formulas. The pH was assessed using a precalibrated digital pH meter. Plaque pH was also further assessed based on its buffering capacity.

Results: Soy-based and protein hydrolysate-based milk formulas were found to be most cariogenic in both saliva and plaque. Lactose-based formulas were found to be the least cariogenic of all the milk formulas.

Conclusion: Parents and caretakers must be made aware of the various types of milk formulas available, their composition, as well as their relative cariogenic nature. Feeding should always be supervised, and infants should not be left with milk-containing bottles as comforters. Milk feeds at bedtime, when salivary flow is reduced, should be discouraged. Dentists and pediatricians should be aware of the caries-related risk associated with different milk formulas before recommending them to children.

Clinical significance: Knowledge and awareness among parents and caretakers regarding the cariogenicity of various milk formulas available in the market.

Keywords: Caries, Infant formulas, Milk formulas, Nursing caries.

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Introduction

Infant formula has traditionally served as the primary nutritional supplement for feeding babies and young children. It provides all essential nutrients required during the initial 4–6 months of life, or up to 12 months when combined with other complementary infant foods.[1] There exist numerous formulas containing different sources of carbohydrates, including refined and fermentable carbohydrates. Infant formulas are categorized based on their protein levels into three primary groups: formulas containing milk, formulas containing soy, and hydrolyzed protein formulas.[2]

Milk-based formulas are derived from bovine milk and are adjusted to resemble breast milk, primarily incorporating lactose as their main carbohydrate source. Soy-based formulas were developed to meet the needs of children who cannot tolerate bovine milk or lactose due to lactose intolerance or congenital lactase deficiency.[2]

Soy-based formulas are formulated using soy proteins and typically use table sugar and glucose syrup as the primary sugar sources. The inclusion of these carbohydrates may potentially increase the risk of tooth decay compared to lactose-based formulas.[3]

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Protein hydrolysate formulas, also known as allergy-friendly baby formulas, feature proteins that are partially or extensively broken down into smaller units and free amino acids. These formulas are specifically designed for babies with genuine allergies to milk protein. Due to the absence of intact casein, they are considered to have lower cariogenic properties compared to other types of formulas.[4]

Iron-based formulas are recommended for children with low iron intake and bioavailability, especially in developing countries due to the prevalence of iron deficiency anemia during the 1st year of life.[5]

Typically, saliva flow decreases during sleep, and bottle-feeding can limit saliva's contact with teeth under normal circumstances.[6,7] Feeding infants solutions containing sugar appears to be a significant factor contributing to extensive infections of tooth surfaces by *Streptococcus mutans*, which constitutes about 60% of the microbial population found in dental plaque.[8]

Lactose is the endogenous sugar found in human and bovine milk, and it is also the primary sugar used in the majority of baby formulas.[9] Elevated levels of lactate, the primary acidic by-product in dental plaque exposed to excessive sugars, have been linked to a quicker pH decrease and a lower minimum pH in dental plaque. Birkhed et al. demonstrated that frequent ingestion of milk sugar or milk led to increased acid production in dental plaque in humans.[10]

However, babies who are unable to consume bovine milk or milk sugar do not produce the necessary enzymes to digest milk sugar. Consuming milk sugar under these conditions can result in severe diarrhea. Thus, these infants need an alternative formula, such as soy-based formula, which is free from lactose.[10] A study by Bhatia et al. stated that the American Academy of Pediatrics recommends soy-based formulas for children who cannot consume animal protein, as well as for infants diagnosed with galactosemia or congenital lactase deficiency.[3] Studies have previously demonstrated that milk, especially its casein component in infant formulas, could protect the enamel from dissolution in acids. The absence of casein in soy formulas may result in a lower ability to prevent tooth decay compared to other types of infant milk.[9]

Hydrolyzed protein formulas include nonmilk extrinsic sugars like table sugar and glucose syrup as sources of carbohydrates, akin to soy-based formulas.[11] In one study conducted by Moynihan et al., glucose polymers were observed to cause a smaller decrease in plaque pH compared to 10% table sugar.[9]

Iron-fortified formulas include iron to prevent iron deficiency anemia in newborns. A study conducted by Ribeiro et al.[12] found that iron treatment reduced the number of bacteria in *S. mutans* biofilm and also decreased enamel demineralization. Additionally, Pecharki et al. demonstrated *in situ* that dental biofilm formed in humans exposed to iron exhibited a reduced count of *S. mutans*.[13]

Baby formulas containing milk and their manufacturing processes are continually evolving to resemble human milk more closely. However, to achieve high caloric content, these formulas often contain significant amounts of simple carbohydrates such as table sugar, glucose syrup, and glucose polymer, which can increase their cariogenic potential.[14,15]

There are many different host factors that influence the acidogenic potential, such as buffering capacity of saliva and plaque, among others. Assessing the pH response after consuming milk formulas is among several methods used to gauge the acid-producing potential of foods. The acidogenic potential of dietary items is well-documented, with ample evidence indicating that saliva plays a crucial role in regulating plaque pH. Furthermore, foods that stimulate saliva production are pivotal in determining their acidogenic impact.[16]

On thorough literature search of scientific papers, very few documented studies were found worldwide, and no such comparative study has been conducted in India so far to evaluate the acidogenicity of different types of milk formulas mentioned here. Therefore, the objective of this study is to compare and assess the acid-producing potential of lactose-based, soy-based, iron-based, and protein-hydrolysate infant formulas on dental plaque pH, salivary pH, and buffering capacity.

Materials and Methods

Materials Used

- Lactodex (lactose-based formula).
- Isomil (soy-based formula).
- Nutramigen (protein hydrolysate formula).
- Enfamil (iron formula).
- A digital pH meter calibrated using buffer solutions of pH 4.0 and 7.0.

Study Design

This study was conducted in the Department of Pediatric and Preventive Dentistry using a single-point-in-time study design. Sixty healthy children aged 7–12 years, with decayed, missing, and filled teeth [decayed, missing, and filled teeth for primary teeth (dmft) ≤ 3, decayed, missing, and filled teeth for permanent teeth (DMFT) ≤ 3] according to Moller's index (1966), were randomly chosen from the outpatient department. The parents were then asked to sign an informed consent form prior to the conduction of the study.

Inclusion Criteria

- Healthy, cooperative children aged between 7 and 12 years were included.
- Children who had restoration-free labial, buccal, palatal, and lingual surfaces.
- The DMFT index score was observed to be 3 or lower.

Exclusion Criteria

- Children who experienced xerostomia or were using medications that affected salivary flow within the last 2 weeks prior to the study.
- Children with a general allergy to milk.
- Children who wore orthodontic appliances.
- Children with physical challenges such as severe orofacial defects like cleft lip and cleft palate.
- Children with medical conditions.
- Children undergoing radiotherapy or chemotherapy, or on antibiotic therapy for the past 1 month.

Ethical Clearance

Approval from the Institutional Ethics Committee of Sardar Patel Post Graduate Institute of Dental and Medical Sciences, Lucknow was obtained prior to the commencement of the study (PEDO/02/522021/IEC).

Methodology

The sample size calculation was conducted using G*Power software (version 3.0), specifically for F-tests—analysis of variance (ANOVA): fixed effects, omnibus, one-way design.

The 60 children were then randomly assigned into the four study groups (15 each): group I (lactose-based formula), group II (soy-based formula), group III (iron-based formula), and group IV (protein hydrolysate formula) (Fig. 1).

The evaluation of buffering capacity was conducted in collaboration with CytoGene (Research and Development-Biotechnology Company, Lucknow), which is well-equipped with the facilities.

Presampling Preparation

The children were instructed to abstain from oral hygiene procedures for at least 48 hours to allow for adequate dental plaque accumulation, and to avoid eating or drinking anything for at least 2 hours before each examination procedure, except for plain water. The study was conducted in the early morning hours, specifically between 8 and 11 AM, to minimize the influence of food on plaque pH and to reduce the influence of circadian rhythms on salivary flow rate and buffering capacity.

Evaluation of Preconsumption Salivary pH

In accordance with the saliva spitting method by Navazesh,[17] subjects were asked to spit saliva into a fresh container once every minute for up to 10 minutes on the day of the study. Baseline, unstimulated saliva was collected into 10 mL sterile plastic containers and subsequently mixed with distilled water to measure the baseline/resting salivary pH using the saliva spitting method.

Evaluation of Preconsumption Plaque pH

Likewise, following the plaque pooling/plaque harvesting method by Frostell (1980) and Jensen (1982),[18] baseline unstimulated dental plaque was collected from all reachable surfaces of both maxillary and mandibular teeth using a sharp-edged spoon excavator and dissolved into 10 mL sterile plastic containers containing 50 µL of deionized water.

The resting/baseline/prerinse salivary pH and the resting/baseline/prerinse plaque pH readings were taken using a digital pH meter that had been calibrated beforehand with buffer solutions at pH 4.01 and 7.0.

Evaluation of Postconsumption Plaque pH

After the baseline pH values were obtained, the subjects were asked to rinse/swallow 100 mL of one test formula from a particular group for 1 minute to maintain homogeneity (Figs 2A and B). Following consumption of the milk formula, dental plaque samples were obtained from all easily reachable surfaces, including the maxillary central incisors, buccal surfaces of maxillary first and second molars (if present), premolars, as well as the lingual surfaces of mandibular incisors and molars, and placed in 10 mL dry, millimetric, sterile plastic containers with 50 µL of deionized water (Fig. 3). The

Figs. 2A and B: Consumption of/rinsing with the milk formula used in the study

Fig. 3: Postconsumptional collection of plaque samples

Fig. 1: The various infant milk formulas used in the study

plaque samples were collected at time intervals of 5, 10, 15, 20, 30, and 60 minutes, and their postconsumption pH values were measured using the digital pH meter (Fig. 4).

Evaluation of Postconsumption Salivary pH

Similarly, postconsumption salivary samples were collected in 10 mL sterile plastic containers with 2 mL of deionized water added. Samples were collected at intervals of 5, 10, 15, 20, 30, and 60 minutes. The pH values of these samples after consumption were measured using a digital pH meter at each time interval (Figs 5 and 6).

Evaluation of Buffering Capacity

After determination of the salivary and plaque pH, the samples were then transferred to CytoGene (Research and Biotechnology Company) for determination of buffering capacity (Fig. 7). The buffering capacity of each sample was calculated using the buffering intensity formula described by Van Slyke.[19]

$$B\ value = d(x)/d(pH) \times 100$$

Statistical Analysis

The values obtained from the samples were then subjected to statistical analysis, and the results were tabulated and evaluated.

Results

Descriptive analysis was conducted to express salivary pH, plaque pH, and buffering capacity levels in each study group, using mean and standard deviation. A one-way ANOVA test followed by Tukey's *post hoc* test was employed to compare the mean levels of salivary pH, buffering capacity, and plaque pH among the four groups.

A paired Student's *t*-test was conducted to compare the mean plaque pH levels between prerinse and the end of the 1-hour period within each study group. The significance level was set at $p < 0.05$.

Acidogenic Potential

Group I (lactose-based milk formula) was the least acidogenic and cariogenic among all four different milk formulas tested (Table 1).

Salivary pH and Plaque pH

Group I (lactose-based formula) and group III (iron-based formula) resulted in higher mean salivary as well as plaque pH compared to group II (soy-based formula) and group IV (protein hydrolysate formula) (Figs 8 and 9).

Mean Drop in Salivary pH and Plaque pH

Group I (lactose-based formula) and group III (iron-based formula) also resulted in a lower mean drop in salivary as

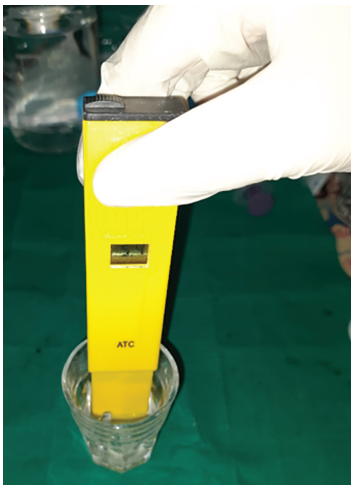

Fig. 4: Measurement of postconsumptional plaque pH

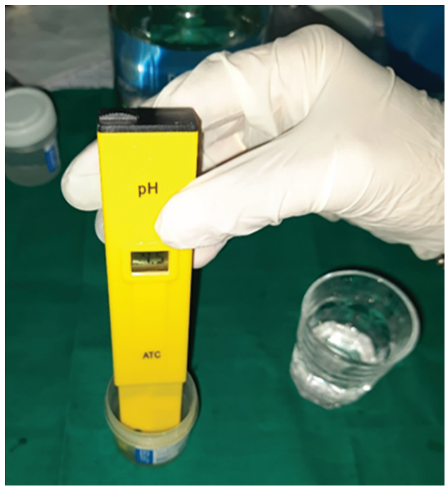

Fig. 6: Measurement of postconsumptional salivary pH

Fig. 5: Postconsumption collection of salivary samples

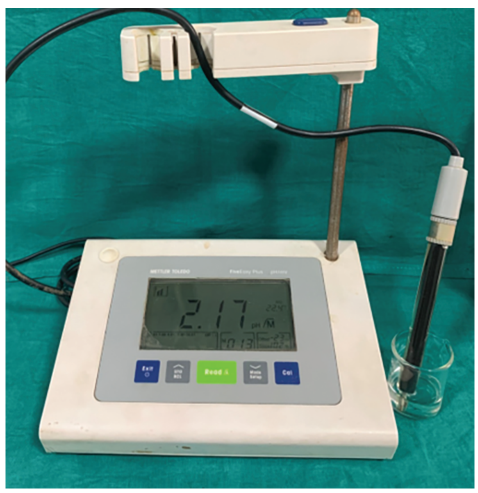

Fig. 7: Evaluation of buffering capacity

Table 1: Descriptive of mean values of different parameters at various time intervals in different study groups

		Group I (lactose-based)		*Group II (soy-based)*		*Group III (iron-based)*		*Group IV (protein hydrolysate-based)*	
Parameter	*Time*	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*
Salivary pH level	Baseline	6.69	0.17	6.55	0.19	6.64	0.17	6.57	0.17
	5 minutes	6.39	0.14	5.77	0.56	6.36	0.13	5.72	0.22
	10 minutes	6.15	0.2	5.44	0.41	6.06	0.21	5.41	0.13
	15 minutes	5.85	0.22	5.23	0.42	5.67	0.21	5.14	0.17
	20 minutes	5.66	0.25	4.99	0.46	5.43	0.16	4.75	0.22
	30 minutes	5.84	0.3	4.8	0.36	5.2	0.17	4.53	0.15
	60 minutes	6.09	0.29	4.94	0.36	5.16	0.16	4.68	0.3
Buffering capacity		6.44	0.25	5.48	0.39	5.91	0.35	5.68	0.12
Plaque pH	Prerinse	6.38	0.15	6.26	0.32	6.37	0.14	6.32	0.19
	After 1 hour	5.99	0.11	5.29	0.44	5.8	0.15	5.19	0.17

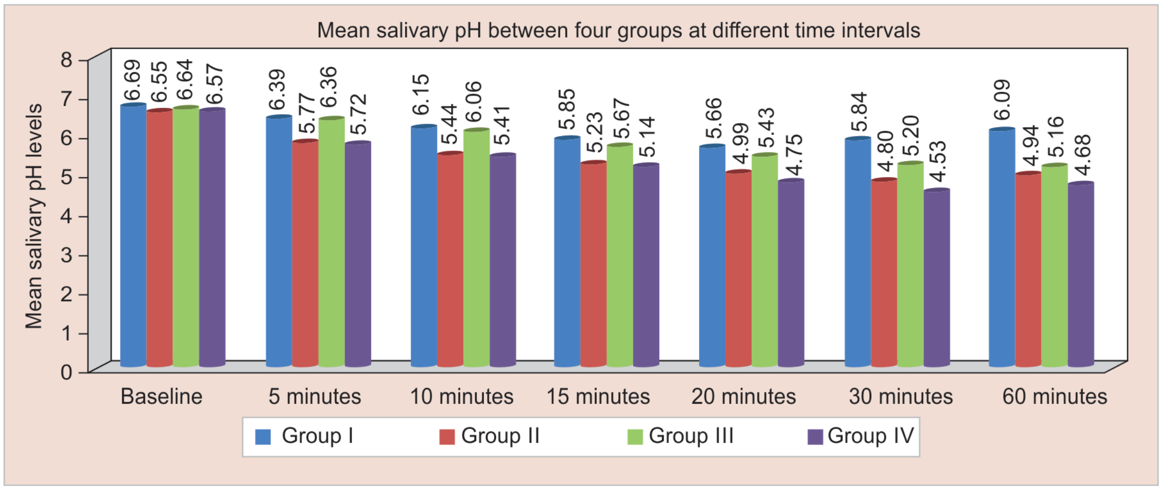

Fig. 8: Mean salivary pH between four groups at different time intervals

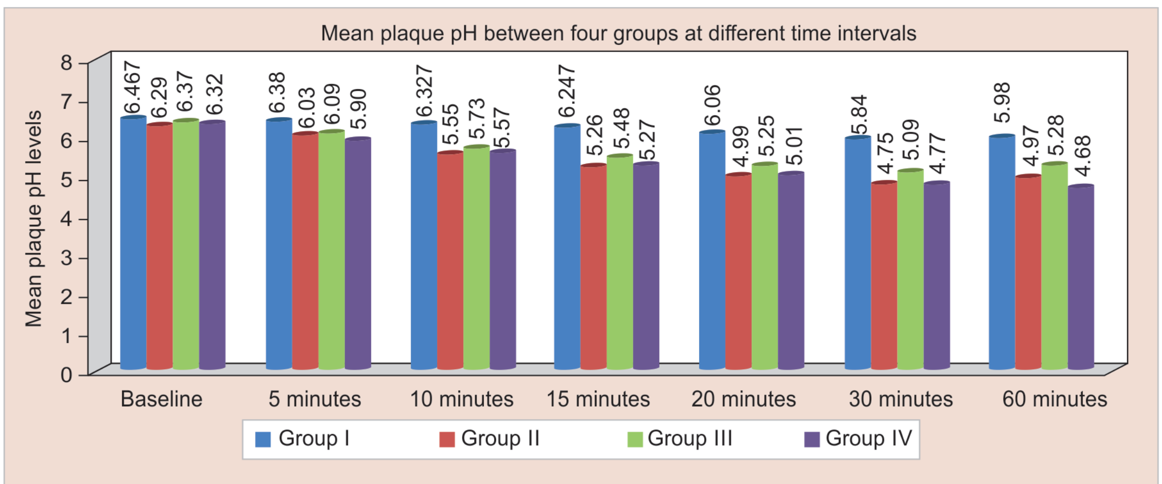

Fig. 9: Mean plaque pH between four groups at different time intervals

well as plaque pH values when compared at various evenly spaced post-consumption time intervals of 5 minutes (from 5 to 60 minutes) assessed from the baseline pH values (Figs 10 and 11).

Buffering Capacity

Group I (lactose-based formula) also showed the highest buffering capacity among all four milk formulas (Fig. 12).

Discussion

Early childhood caries is highly prevalent as a chronic disease among children. Severe early childhood caries is defined as the presence of any signs of smooth surface caries in children under 3 years old. According to research by Kaste and Gift, almost 20% of children aged 6 months to 5 years have been put to sleep with a bottle containing liquids other than water.[20]

Hinds et al. observed in their study that many parents opt not to breastfeed and instead rely solely on infant formula for nutrition.[21] As humanity has evolved, there has been a significant shift in dietary fermentable carbohydrates, which has been linked to an increased prevalence of dental caries. Furthermore, the prolonged presence of milk formulas in the mouth during nighttime, exacerbated by reduced salivary flow and the suckle-sleep-suckle cycle, may contribute to the enzymatic breakdown of protective proteins like casein.[15]

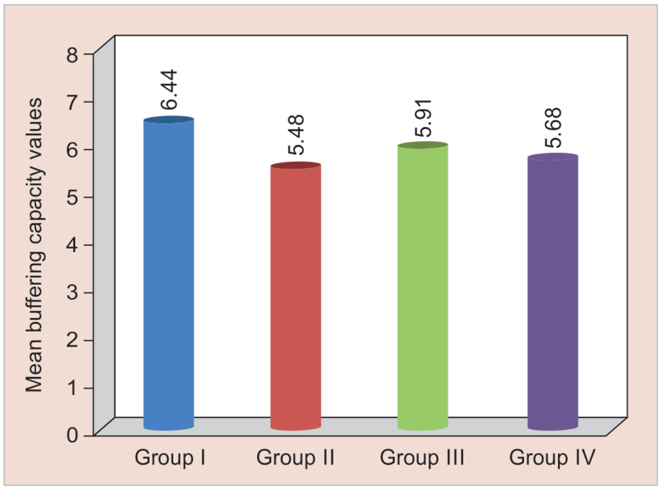

Fig. 12: Comparison of mean buffering capacity between four groups of milk formulas

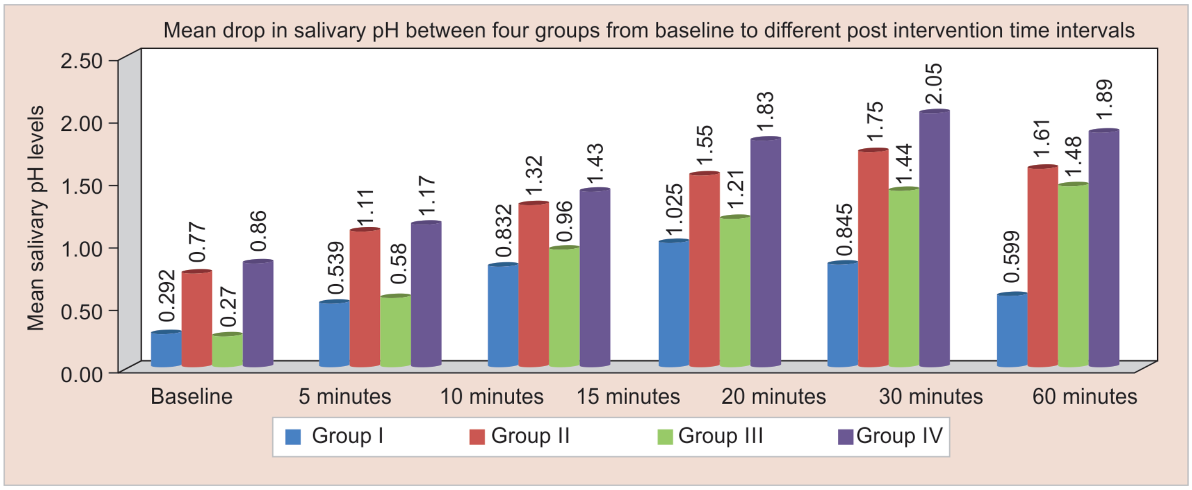

Fig. 10: Mean drop in salivary pH in the four different groups at various time intervals

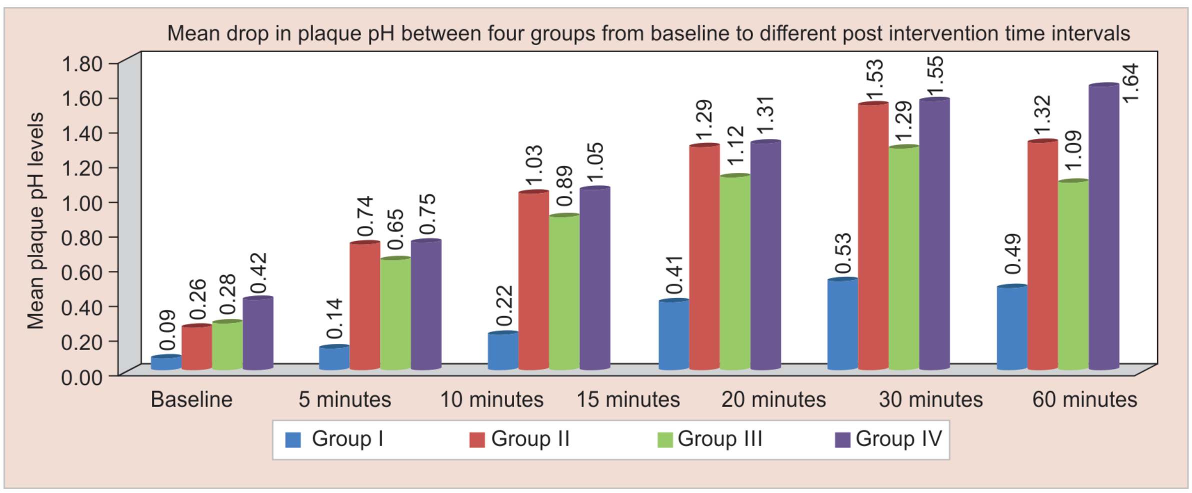

Fig. 11: Mean drop in plaque pH in the four different groups at various time intervals

Factors such as the buffering capacity of saliva and plaque have been reported to greatly influence the acidogenic potential of milk formulas. Saliva plays a crucial role in maintaining oral health, and there is reasonable evidence that salivary buffering capacity protects the tooth from dental caries.[22] Bhat et al. concluded from their study that saliva has the capacity to neutralize acids and sustain pH levels above critical thresholds, thus safeguarding teeth against demineralization.[16] Tayab et al. [23] emphasized the role of saliva in regulating plaque pH.

The formation of dental caries is driven by the dynamic processes of demineralization and remineralization at the enamel-plaque interface.[24] Research indicates that acidic plaque, which harbors acid-producing microorganisms, promotes demineralization.

There is an inverse relationship between the pH of plaque and the content of carbohydrate in saliva, as shown by Bibby et al.[25] Furthermore, studies by Alamoudi et al.[26] have provided sufficient evidence that acids may be neutralized by the buffering nature of saliva.

According to the recommendations by World Health Organization (WHO), Oral Health Survey (1997), and Deshpande et al. (2011, 2016), children in the primary and early mixed dentition stages tend to have a high caries risk and higher susceptibility to dental caries since they have lower salivary calcium levels. Additionally, there is an inverse relationship between dental caries and salivary calcium concentration.[27] Hence, children aged 7–12 years were included in our study. According to Masih et al.,[28] children in the current study were instructed to abstain from oral hygiene procedures for 48 hours to ensure adequate accumulation of dental plaque.

In our study, significantly lower post-rinse plaque and salivary pH values were observed compared to their pre-rinse/baseline values, consistent with findings reported by Sheikh and Erickson,[14] Munshi et al.,[29] Masih et al.,[28] and Chaudhary et al.[30] This clearly highlighted the cariogenic nature and subsequent acidogenic potential of the milk formulas.

On intragroup comparisons regarding the salivary and plaque pH values, the results observed a shallow decline in the pH from baseline to 5, 10, 15, and 20 minutes postconsumption of the milk formulas, after which the pH then increased toward the baseline value at 60 minutes. This is in accordance with Stephan's curve, which indicates that the caries process can only be active if there is a repeated renewal of carbohydrate. This finding was observed previously in studies by Lingström et al.[31]

On intergroup comparison of the mean salivary pH and plaque pH values, it was observed that the lactose-based formula (group I) showed the highest mean pH value, followed by the iron-based formula (group III), protein-hydrolysate-based formula (group IV), and then the soy-based formula (group II). This aligns with earlier studies conducted by Sheikh et al. and Koulourides et al.,[32] where the mean minimum pH values following rinsing with soy-based or protein hydrolysate formulas were significantly lower compared to lactose-based formulas. Additionally, in a study by Moynihan et al., it was noted that casein, a phospho-protein found in milk, may act as a buffer against pH reduction in plaque.[9]

It was also observed that soy-based formulas (group II) showed the least mean pH value. This is supported by the findings of Sheikh et al., where formulas containing sucrose and corn syrup were more readily fermented by oral bacteria, resulting in the lowest mean plaque pH.[14] Furthermore, Moi et al.[33] and Aarthi et al.[34] also observed that among all fermentable sugars, sucrose is considered to be the carbohydrate with the most cariogenic potential.

On intergroup comparison, it was observed that iron-based formulas (group III) had higher mean salivary pH and plaque pH compared to protein hydrolysate formulas (group IV). This can be supported by findings from previous studies by Sheikh and Erickson, wherein iron-based formulas were considered to have cariostatic properties.[14] Furthermore, Hopkins et al. also observed a positive relationship between iron status and protein intake, suggesting that the phospho-protein casein plays a role in reducing the pH drop.[5]

Previous studies conducted by More et al.[35] also observed that lactose is less readily fermented by *S. mutans* compared to sucrose. In yet another study by Murray et al. (2003), it was observed that milk formulas made up of soy and protein showed higher cariogenic potential than lactose. Bowen and Lawrence (2005)[36] and Peres et al. (2009) also observed that sucrose initiated smooth-surface carious lesions.

On intergroup comparison of the mean drop in salivary pH and plaque pH values, Lactose-based (group I) and Iron-based milk formulas (group III) showed the lowest mean drop in pH compared to Soy-based (group II) and Protein hydrolysate-based milk formulas (group IV). This finding can be supported by the results of studies by Danchaivijitri et al.[37] and Sheikh and Erickson,[14] where it was demonstrated that protein hydrolysate formulas caused a more significant maximum plaque pH drop compared to lactose-based and soy-based formulas. Earlier research conducted by Al-Ahmari and Adenubi (2003) similarly found that lactose-based formulas containing the highest amount of casein content resulted in the smallest plaque pH drop. Consequently, the absence of casein in soy formulas (group II) may contribute to a lower pH drop.[2]

According to Upreti et al.,[38] it was noted that saliva and milk buffers play a role in neutralizing acid produced during acid production. This further explains the findings observed in our present study, wherein lactose-based milk formula (group I) showed the highest mean buffering capacity, followed by iron-based (group III), protein hydrolysate-based (group IV), and then soy-based milk formulas (group II). Hedge et al. (2007) also observed that the greater the buffer capacity, the greater the resistance of the solution to alterations in pH.[14]

Outcome

The current study aims to investigate the potential cariogenic effects of different commercially available infant milk formulas. Our study used the three commonly used parameters for the measurement of acidogenic potential, which helped in providing a broader understanding of the potential for milk formulas to cause tooth decay. The results of this investigation also emphasized that infant milk formulas can be as cariogenic as sucrose. As a result, an instructional program on early childhood caries aimed at prospective or new parents is urgently needed for advising and educating them regarding the high carbohydrate concentration of the various milk formulas.

Limitations of the Study

- This study was conducted among 7-12-year-old children. Following the recommendations by WHO, further studies with younger age groups can be carried out.
- This study employs a cross-sectional design with a smaller sample size. In the future, more carefully planned longitudinal studies with a larger number of participants could be conducted.

These studies could provide guidance to pediatricians and pediatric dentists on advising parents about the contents of different commercially available milk formulas and the potential risks associated with diluting them. Such research is crucial in understanding their significant impact on the development of early childhood caries.

Conclusion

Therefore, it is evident that the method of consuming infant formulas significantly influences their cariogenic potential. Establishing guidelines for feeding practices to reduce dental caries is crucial for both parents and healthcare providers. Moreover, educating parents and caregivers about oral healthcare for children, including milk consumption practices and oral hygiene, particularly for infants using high-caries risk formulas, is imperative.

Clinical Significance

- An instructional program on nursing caries aimed at prospective or new parents is urgently needed for advising and educating them about the various formula milks that can be given.
- Parents need to be informed about the connection between persistent bottle or breastfeeding, the practice of nighttime nursing, and the use of a sweetened pacifier with the prevalence of dental decay. It is best to discourage these behaviors.
- Along with various nutritional factors, a comprehensive approach targeted at the prevention of dental caries among preschool children and adolescents must be made available, including the maintenance of proper oral hygiene, appropriate and timely fluoride use, and access to preventive and restorative dental care.

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