Biomedical & Pharmacology Journal, December 2018. Vol. **11**(4), p. 1781-1787

Effect of CPP-ACP and CPP-ACPF Pastes on the Surface Hardness of Initial Dental Erosion Lesions: An *In Situ* Study

Cibele da Cruz Prates[1], Liege Helena Freitas Fernandes[1], João Baptista da Costa Agra de Melo[2], Catarina Ribeiro Barros de Alencar[1] and Alessandro Leite Cavalcanti[1]

[1]School of Dentistry, State University of Paraíba, Campina Grande, PB, Brazil.
[2]Department of Mechanical Engineering, Federal University of Campina Grande, Campina Grande, PB, Brazil.
*Corresponding author E-mail: dralessandro@ibest.com.br

http://dx.doi.org/10.13005/bpj/1549

(Received: 16 September 2018; accepted: 19 November 2018)

To analyze the effect of intra-oral application of CPP-ACP and CPP-ACPF pastes on the surface hardness of initial dental erosion lesions. Material and Method: **Bovine enamel specimens were randomly assigned into 6 volunteers in 3 treatment groups: GI: CPP-ACP paste, GII: CPP-ACPF paste and GIII (Control): Placebo paste without CPP-ACP and without fluoride. Enamel specimens were selected by surface hardness (SHi), *in vitro* eroded by immersion in hydrochloric acid for 30 seconds (SHdes) and randomized between treatment groups and volunteers, who used the palatal intraoral device for 2 hours, applied the treatment on the specimens and used the palatal intraoral device for an additional 3 hours in 3 crossed phases, interspersed with a 7-day washout period. Subsequently, the surface hardness (SHre) was measured to estimate the re-hardening potential of the softened enamel promoted by treatments. Data were analyzed using the t-test and one-way ANOVA, adopting 5% significance level.** Results: **Mean final and post-erosion hardness values were statistically significant for pastes tested ($p<0.05$), which presented re-hardening effect of the softened enamel, but with no difference between them and placebo ($p>0.05$).** Conclusion: **CPP-ACP and CPP-ACPF pastes did not demonstrate higher efficacy in re-hardening the eroded enamel compared to placebo paste.**

Keywords: Tooth Erosion; Caseins; Toothpastes.

Dental erosion is characterized as a progressive and non-reversible loss of tooth structure caused by chemical processes that do not involve bacterial action[1]. It is a condition that involves the interaction of chemical factors, biological factors and behavioral factors[2-6].

When in contact with the dental surface, acids cause the dissolution of the inorganic hard tissue[7-9], causing decreased mechanical resistance and reduced hardness of the surface layer[10]. In this way, dental erosion can be divided into two phases: Phase 1: Erosion (chemical process), with partial demineralization of the enamel or dentin, causing surface softening; Phase 2: Erosive tooth wear, which characterizes the advanced phase, where there is loss of the previously softened surface through the combined effect of erosion and mechanical wear of the tooth surface[11-13].

The use of casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) is among the strategies to control tooth erosion, a milk protein substance consisting of casein phosphopeptides (CPP) aggregated with calcium phosphate to form agglomerates of amorphous calcium phosphate (ACP)[14,15]. CPP-ACP acts as a supplemental source of calcium and phosphate bioavailable in the oral environment, being proposed both for inhibiting loss of minerals[1] and for intensifying their deposition on demineralized lesions of the dental enamel, such as erosive lesions[16].

Thus, pastes containing CPP-ACP can be considered as potential alternatives for the mineral deposition process [17], being an additional reservoir of calcium and phosphate ions in the oral environment[18]. Furthermore, the addition of fluoride to CPP-ACP, forming CPP-ACPF, has been studied due to its potential to enhance the protective effect promoted by CPP-ACP in the control of dental erosion[19,20]. However, the benefit of this association, to the best of our knowledge, still generates discussions[1], because literature is not consensual regarding its superiority with respect to dental erosion.

Considering the importance of the subject and the scarce literature currently available on the efficacy of CPP-ACP pastes on mineral precipitation of initial erosive lesions, and knowing that *in situ* studies better mimic the clinical reality to obtain scientific information[21], the aim of this study was to analyze the *in situ* effect of a single intrabuccal application of CPP-ACP and CPP-ACPF pastes on the surface hardness of initial erosion lesions in bovine enamel. The null hypothesis tested was that there is no difference in recovery of the enamel hardness by the use of CPP-ACP pastes and placebo.

MATERIALS AND METHODS

Experimental Design

A crossover, double blind, *in situ* experimental study was carried out. The factor under study was mineral precipitation in dental erosion lesions according to groups: GI = 10% CPP-ACP paste (MI Paste™, GC America Inc, USA); GII = 10% CPP-ACP paste + 900ppm sodium fluoride NaF (MI Paste Plus™, GC America Inc., USA); and GIII = Paste without CPP-ACP and without sodium fluoride (Dilecta Farmácia de Manipulação & Homeopatia, João Pessoa, Paraíba, Brazil).

Bovine tooth enamel specimens (n = 36) were randomly divided among volunteers (n = 6), who used a palatal device for intraoral paste application. Each group was conducted in a 5-hour phase and the response variable used was surface hardness (hardness recovery percentage).

Preparation of Specimens and Surface Hardness Evaluation

Seventy-one bovine enamel specimens (4x4x3mm) were kept in 0.1% thymol solution, pH 7.0[22], planned and polished with water sands (granulations 600, 1200, 1500, 2000) (3M Company, Minnesota, USA).

The initial surface hardness (SHi) was determined from the mean values of 5 indentations performed in the central region of the specimen equidistant at 100 ìm (Vickers tip, 100g, 15s, Michohardness Tester FM-700, Future-Tech Corp., Fujisaki, Kawasaki-ku, Japan)[22]. Thirty-six specimens with hardness from 266.94 KgF / mm^2 to 375.42 KgF / mm^2 were selected, which were sterilized by exposure to ethylene oxide gas[22].

Erosion lesion was performed *in vitro* by immersing the enamel specimens in 150 ml of 0.01M hydrochloric acid (Vetec Química Fina Ltda., Rio de Janeiro, Brazil) at pH 2.3 with constant stirring for 30 seconds. Then, each specimen was washed with deionized water in order to stop the demineralization process[23].

For the evaluation of surface hardness after erosion (SHdes), the same parameters used to determine the initial surface hardness were adopted. The average hardness (initial hardness of the healthy enamel and after erosion) was used to calculate the hardness loss percentage according to the following formula: [(initial hardness – post-erosion hardness) / (initial hardness)] X 100[24], whose values were used for the stratified random distribution of enamel specimens between volunteers and study groups, so that all the study groups, specimens with lower and higher hardness were allocated. The location of specimens on the right or left side of the device was also randomly determined for each volunteer and each phase of the study[24].

Selection of Volunteers and *in-situ* Experiment

Six volunteers (aged 18-25 years), who met the inclusion criteria (stimulated salivary flow e”1 ml / min, adequate oral health, absence of carious or erosive lesions, and marked tooth wear) and did not violate the exclusion criteria (with systemic diseases, users of orthodontic appliance, pregnancy, breastfeeding, having used fluoride compounds in the 2 months prior to collection, having undergone radiotherapy and chemotherapy, practicing aquatic activities in a chlorinated pool) participated in the study[22].

Palatal intraoral devices were made of acrylic resin from the plaster model of the upper arch of volunteers, being an intraoral device for each phase of the study[22]. Each of them had two 6 x 6 x 3 mm (length x width x depth) cavities, one on the right and one on the left side to fix a specimen in each of them[22]. Specimens were fixed with wax and adapted to the device resin surface level[24] and on them segments of CrNi 0.6mm orthodontic wire were carefully and transversely fixed, without coming in contact with the specimens in order to avoid the contact of the tongue with the surface of specimens[24]. In addition, volunteers were instructed not to touch the specimens with their tongue.

Seven days before and during the experimental period, oral hygiene procedures were standardized[22], and volunteers used the same apparatuses: toothbrush (Colgate Classic Clean Média, Colgate-Palmolive Industrial Ltda., São Paulo, Brazil), fluoride dentifrice (Colgate Total 12®, 1,250 ppm F, Colgate-Palmolive Industrial Ltda., São Paulo, Brazil) and dental floss (Hillo, Aperifio Ind. Com. and Rep. Ltda., Aperibe, Rio de Janeiro, RJ, Brazil).

The three phases of the study were interspersed with a 7-day washout period. At each phase, the volunteer was instructed to wait one hour after oral hygiene procedures and insert the intraoral device containing previously eroded specimens. After 2 hours of use, ensuring the formation of the acquired film, intraoral application of the paste supplied in an amount of the size of a pea grain was made in each specimen. Application followed the manufacturer's recommendations; however, the application was only topical, i.e., without friction, ensuring that the softened surface and the acquired film were not removed.

After 3 minutes of application, intraoral devices were washed with mineral water (Crystal Águas do Nordeste Ltda., Fluorides 0.04 mg / L, pH 6.21, Maceió, AL, Brazil) and reinserted in the mouth only after 3 hours of use (paste substantivity time) [25], being removed, wrapped in gauze soaked in mineral water (Crystal Águas do Nordeste Ltda., Fluorides 0.04 mg / L, pH 6.2, Maceió, AL, Brazil) and returned to the researcher.

For the evaluation of surface hardness after remineralization (SHre), the same microdurometer with the same specifications was used, at 100 ìm of post-erosion indentations. The average hardness (post-erosion hardness and post-remineralization hardness) was used to evaluate the hardness recovery percentage (% SHR) according to the following formula: [(final hardness – post-erosion hardness) / (initial hardness – post-erosion hardness)] X 100.

Table 1. Mean of surface hardness after erosion (SHdes), after treatment (SHre) and hardness recovery percentage (% SHL) according to the experimental phases

Groups	SH*des* - KgF/mm²	SH*re* - KgF/mm²	p-value*
G1 Placebo (n=10)	306.40 (±37.44)	316.98 (±41.34)	0.006
G2 CPP-ACP (n=12)	299. 24 (±20.03)	336.55 (±22.03)	0.003
G3 CPP-ACPF (n=12)	295.19 (±50.55)	314.29 (±45.96)	0.007
p-value**	0.79	0.307	

*T test for paired samples; **One-way ANOVA.

Table 2. Average and hardness recovery percentage after treatment, according to experimental groups

Groups	Hardness Recovery	Percentage
G1 Placebo (n=10)	10.58 (±9.42)	3.19 (±2.72)
G2 CPP-ACP (n=12)	37.31 (±33.53)	10.64 (±9.12)
G3 CPP-ACPF (n=12)	19.10 (±19.90)	6.29 (±7.20)
p-value*	0.036	0.06

*One-way ANOVA.

Statistical Analysis

Data normality was checked by means of the Shapiro-Wilk test, also using the t-test and One-way ANOVA. The significance level adopted was 5% and the SPSS software version 20.0was used (SPSS Inc., Chicago, Illinois, USA).

Ethical Aspects

This *in-situ* study was approved by the local Institutional Ethics Research Committee (Protocol No. 57770916.4.0000.5187) and followed the guidelines of the Declaration of Helsinki.

RESULTS

Final and post-erosion hardness averages were statistically significant for the three pastes tested (Table 1), but with no difference between them (Table 2).

DISCUSSION

Dental caries[26-33] and erosion[7,12,13,34] are two major dental problems in children and adolescents. Among the various types of treatment for dental erosion, phosphopeptide casein nanocomplex stabilized by amorphous calcium phosphate was postulated as a remineralizing agent of dental structure due to its proven ability to increase salivary levels of calcium and phosphate[35].

CPP-ACP can be made available in the oral environment by means of several formulations, and the use of pastes based on this nanocomplex has shown to be promising in the control of dental erosion[36]. In this perspective, the present study evaluated the use of pastes containing CPP-ACP (supplemented or not by fluoride) compared to placebo paste. Using the *in situ* protocol, it was possible to accept the initially formulated null hypothesis, since the use of CPP-ACP and CPP-ACPF pastes provided hardness recovery equivalent to placebo paste.

The short-term *in situ* treatment protocol adopted to evaluate the effects of products in the initial phase of erosion also has the advantage of favoring the adherence of volunteers in the precise conduction of the proposed treatment protocol[37]. The first development stage of the erosive lesion is characterized by the reduction of the surface layer hardness[10,38], so surface hardness variation was used in this study to measure the effect of the application of CPP-ACP pastes on the recovery of the eroded enamel hardness.

Hydrochloric acid, which in addition to simulating the acid present in the gastric juice, was used by other authors [39] to prepare the erosion lesion. In addition, initial indentations were located after the erosive challenge and after the *in situ* step in order to guarantee the absence of erosive dental wear, that is, that the surface evaluated at the initial phase and at the end of the study were similar[22].

CPP-ACP can be made available in different types of vehicles, notably chewing gum[22] and topical application paste[1]. However, other forms are also studied, as in solution[40] and added to beverage compositions[41]. However, the use of paste as a bioavailability vehicle for CPP-ACP seems to be the best form, since its concentration is higher in this formulation (7.5% in the mousse of CPP-ACP to 10% and the mousse of 10% fluorinated CPP-ACP + 900 ppm fluoride), and the direct contact allowed by topical application probably favors its action.

Most studies on the use of CPP-ACP pastes in the control of dental erosion have been conducted in strictly laboratory approaches[1,17,18,25], where saliva and acquired film, which are crucial elements in erosive dynamics, are not present[42]. Some studies have shown promising results with the use of CPP-ACP-based pastes[16].

In the present study, the three pastes used resulted in significant optimization in the re-hardening process, compared to the initial period. However, no difference was observed between CPP-ACP, CPP-ACPF and control pastes. Possibly, this finding is a result of the salivary effect, which, as already established in literature, plays an important role in the reduction of tooth wear in the face of an erosive / abrasive attack[43]. In addition to being able to protect the dental structure from erosion through its buffering capacity, washing and demineralization inhibition, saliva also has the potential to re-harden the eroded enamel, providing calcium, phosphate and fluoride necessary for mineral precipitation [44].

It should be emphasized that this study followed recommendations present in literature, resembling natural clinical situation, and, therefore, provides information pertinent to the reality of the oral environment. However, for being a short-duration test, it cannot be inferred about the effectiveness of the tested groups regarding more advanced erosion stages. Therefore, it is suggested that the action of CPP-ACP and CPP-ACPF pastes on erosive wear should be evaluated in erosive cycling studies.

CONCLUSION

CPP-ACP pastes, supplemented or not by fluoride, did not demonstrate higher efficacy in re-hardening the eroded enamel compared to placebo paste.

ACKNOWLEDGMENTS

The authors would like to thank the Multidisciplinary Laboratory of Materials and Active Structures and the Metallography Laboratory of the Federal University of Campina Grande for the use of their equipment; to company Acecil Central de Esterelização Comércio e Indústria Ltda for the sterilization of specimens, the Pro-Rector's Office for Graduate Studies and Research of the State University of Paraíba (EDITAL 2015 - PROPESQ/UEPB), the Coordination for the Improvement of Higher Education Personnel (CAPES) and the National Council for Scientific Development and Technological (CNPq) - Fellowship of Research Productivity (Process 302850/2016-3) - for financial support.

REFERENCES

1. Wang CP, Huang SB, Liu Y, Li JY, Yu HU. The CPP-ACP relieved enamel erosion from a carbonated soft beverage: An in vitro afm and xrd study. *Arch Oral Biol;* **59**(3):277-82 (2014).
2. Lussi A, Jaeggi T. Erosion-diagnosis and risk factors. *Clin Oral Investig;* **12**(1):5-13 (2008).
3. Lazzaris M, Farias MMAG, Araújo SM, Schmitt BHE, Silveira EG. Erosive potential of commercially available candies. *Braz Res Pediatr Dent Integr Clin*; **15**(1):7-12 (2015).
4. Oliveira AF, Sampaio FC, Meira IA, Bezerra MGPG, Fernandes NL, Paiva VM. Erosive potential of industrialized teas: An in vitro study. *Pesq Bras Odontoped Clin Integr*; **17**(1):e3165 (2017).
5. Vieira BR, Firmino BO, Santos RL, Guenês GMT, Carvalho FG. In vitro effect of erosive challenge on surface microhardness of orthodontic composites. *Braz Res Pediatr Dent Integr Clin;* **16**(1):43-50 (2016).
6. Castro RD, Costa GA, Carvalho FG, Peixoto LR, Brandt LMT, Abílio GMF, Cavalcanti AL. Cariogenic and erosive potential of industrialized cashew juice. *Braz Res Pediatr Dent Integr Clin;* **16**(1):123-8 (2016).
7. Shitsuka C, Corrêa MSNP, Duarte DA, Leite MF. Quantification of dental biofilm in children with dental erosion. *Braz Res Pediatr Dent Integr Clin;* **15**(1):95-101 (2015).
8. Almenara OCPL, Rebouças AG, Cavalli AM, Durlacher MM, Oliveira AMG, Florio FM, Zanin L. Influence of soft drink intake on the salivary pH of schoolchildren. *Braz Res Pediatr Dent Integr Clin*; **16**(1):249-55 (2016).
9. Emmi DT, Cabral CP, Rocha MPC. Evaluation of the erosive potential of different amazonian fruits on bovine enamel. *Braz Res Pediatr Dent Integr Clin;* **16**(1):359-367 (2016).
10. Kanzow P, Wegehaupt FJ, Attin T. Wiegand A. Etiology and pathogenesis of dental erosion. *Quintessence Int*; **47**(4):275-8 (2016).
11. Huysmans MC, Young A, Ganss C. The role of fluoride in erosion therapy. Monogr Oral Sci 2014; 25:230-43.
12. Diniz MB, Lussi A. Dental erosion in pediatric dentistry: What is the clinical relevance. *Pesq Bras Odontoped Clin Integr;* **17**(1):1-2 (2017).
13. Mendonça FL, Ionta FQ, Alencar CRB, Oliveira GC, Gonçalves PSP, Oliveira TM, Honório HM, Rios D. Impact of saliva and intraoral appliance on erosion lesions rehardening ability - A pilot study. *Braz Res Pediatr Dent Integr Clin;* **16**(1): 51-8 (2016).
14. Reynolds EC. Casein phosphopeptide-amorphous calcium phosphate: The scientific evidence. *Adv Dent Res*; **21**(1):25-9 (2009).
15. Pereira JV, Maciel RP, Monteiro MJF, Conde NCO, Vieira JMR, Rebelo MAB. Effect of chewing gum containing CPP-ACP on salivary flow and buffer capacity: An in vivo study. *Braz Res Pediatr Dent Integr Clin;* **16**(1):425-31 (2016).
16. Srinivasan N, Kavitha M, Loganathan SC. Comparison of the remineralization potential of cpp-acp and cpp-acp with 900 ppm fluoride on eroded human enamel: An in situ study. *Arch Oral Biol;* **55**(7):541-4 (2010).
17. Turssi CP, Maeda FA, Messias DC, Neto FC, Serra MC, Galafassi D. Effect of potential remineralizing agents on acid softened enamel. *Am J Dent;* **24**(3):165-8 (2011).
18. Tantbirojn D, Huang A, Ericson MD, Poolthong S. Change in surface hardness of enamel by a cola drink and a CPP-ACP paste. *J Dent;* **36**(1):74-9

(2008).

19. Wang X, Megert B, Hellwig E, Neuhaus KW, Lussi A. Preventing erosion with novel agents. *J Dent;* **39**(2):163-70 (2011).
20. Somani R, Jaidka S, Singh DJ, Arora V. Remineralizing potential of various agents on dental erosion. *J Oral Biol Craniofac Res;* **4**(2):104-8 (2014).
21. West NX, Davies M, Amaechi BT. In vitro an in situ erosion models for evaluating tooth substance loss. *Caries Res;* **45**(1):43-52 (2011).
22. Alencar CRB, Magalhães AC, Machado MAAM, Oliveira TM, Honório HM, Rios D. In situ effect of a commercial CPP-ACP chewing gum on the human enamel initial erosion. *J Dent;* **42**(11):1502-7 (2014).
23. Tereza GP, Oliveira GC, Machado MAAM, Oliveira TM, Silva TC, Rios D. Influence of removing excess of resin-based materials applied to eroded enamel on the resistance to erosive challenge. *J Dent;* **47**(1):49-54 (2016).
24. Jordão MC, Alencar CR, Mesquita IM, Buzalaf MA, Magalhães AC, Machado MA, et al. In situ effect of chewing gum with and without CPP-ACP on enamel surface hardness subsequent to ex vivo acid challenge. *Caries Res*; **50**(3):325-30 (2016).
25. Poggio C, Lombardini M, Vigorelli P, Ceci M. Analysis of dentin / enamel remineralization by a CPP-ACP paste: AFM and SEM study. *Scanning*; **35**(6):366-74 (2013).
26. Souza ESS, Bezerra ACB, Amorim RFSG, Azevedo TDPL. Caries diagnosis in the mixed dentition using ICDAS II. *Braz Res Pediatr Dent Integr Clin;* **15**(1):13-21 (2015).
27. Queiroz BM, Alencar NA, Requejo MEP, Antonio AG, Maia LC. Risk factors, perception of caregivers and impact of early childhood caries on quality of life related to oral health of preschool children and their families. *Braz Res Pediatr Dent Integr Clin;* **15**(1):85-94 (2015).
28. Viana SVC, Piovesan C, Imparato JCP, Raggio DP, Almeida ARS, Bonini GC. Prevalence of dental caries in preschool children by ICDAS diagnostic methodology. *Braz Res Pediatr Dent Integr Clin;* **15**(1):291-300 (2015).
29. Hartwig AD, Ferreira LM, Costa FS, Cademartori MG, Goettems ML, Schardosim LR. Experience of dental caries and use of continuous medication in children with neuropsychomotor disorders. *Braz Res Pediatr Dent Integr Clin*; **16**(1):59-67 (2016).
30. Moura LFAD, Sousa HCS, Lima CCB, Moura MS, Lopes TSP, Lima MDM. Oral health of children and adolescents victims of maltreatment housed in foster care: A case-control study. *Braz Res Pediatr Dent Integr Clin;* **16**(1): 279-87 (2016).
31. Martins LGT, Pereira KCR, Costa SXS, Traebert E, Lunardelli SE, Lunardelli AN, Traebert J. Impact of dental caries on quality of life of school children. *Braz Res Pediatr Dent Integr Clin;* **16**(1):307-12 (2016).
32. Aragão AS, Fernandes LFH, Brandt LMT, Auad SM, Cavalcanti AL. Association between nutritional status and dental caries in Brazilian teenagers with and without risk for eating disorders. *Braz Res Pediatr Dent Integr Clin*; **16**(1): 479-89 (2016).
33. Castinho LS, Abreu MHNG, Paula LF, Souza e Silva ME, Resende VLS. Oral health status among girls with developmental disabilities: A cluster analysis. *Pesq Bras Odontoped Clin Integr;* **17**(1):e3403 (2017).
34. Oliveira PAD, Paiva SM, Costa MLG, Abreu MHNG, Carvalho SD, Auad SM. Dental erosion in Brazilian children with gastroesophageal reflux disease. *Braz Res Pediatr Dent Integr Clin;* **15**(1):227-34 (2015).
35. Reynolds EC, Cai F, Shen P, Walker GD. Retention in plaque and remineralization of enamel lesions by various forms of calcium in a mouthrinse or sugar-free chewinggum. *J Dent Res;* **82**(3):206-11 (2003).
36. Fernandes LHF, Moura EFF, Alencar CRB, Cavalcanti AL. Use of pastes containing casein-amosphorus calcium phosphate with and without fluoride in the prevention of dental erosion: A literature review. *Arch Health Invest*; **6**(11):513-8 (2017).
37. Alencar CRB, Mendonça FL, Guerrini LB, Jordão MC, Oliveira GC, Honório HM, et al. Effect of different salivary exposure times on the rehardening of acid-softened enamel. *Braz Oral Res;* **30**(1):1-7 (2016).
38. Mathew S, Luke AM, Walia T, Masri AG, Jamal H, Pawar AM. Effect of fruit juices and other beverages on loss of tooth structure. *Pesq Bras Odontoped Clin Integr;* **18**(1):e3888 (2018).
39. Kensche A, Pötschke S, Hannig C, Richter G, Hoth-Hannig W, Hannig M. Influence of calcium phosphate and apatite containing products on enamel erosion. *Scientific World Journal*; 2016(7959273):1-12 (2016).
40. Zheng L, Zheng J, Zhang YF, Qian LM, Zhou ZR. Effect of CPP-ACP on the remineralization of acid-eroded human tooth enamel: Nanomechanical properties and microtribological behaviour study. *J Phys D Appl Phys*; **46**(40):1-9 (2013).
41. Ferrazzano GF, Coda M, Cantile T, Sangianantoni G, Ingenito A. SEM investigation on casein phosphopeptides capability in contrasting cola

drinks enamel erosion: An in vitro preliminary study. *Eur J Paediatr Dent;* **13**(4): 285-8 (2012).

42. Lussi A, Carvalho TS. Erosive tooth wear: A multifactorial condition of growing concern and increasing knowledge. *Monogr Oral Sci;* **25**:1-15 (2014).

43. Amaechi BT, Higham SM. Dental erosion: possible approaches to prevention and control. *J Dent;* **33**(3):243-52 (2001).

44. Baumann T, Kozik J, Lussi A, Carvalho TS. Erosion protection conferred by whole human saliva, dialysed saliva, and artificial saliva. *Sci Rep;* **6**(34760):1-8 (2016).