Original Article

DOI: 10.7860/JCDR/2014/8161.4232

Dentistry Section

Short Term Comparative Evaluation of Antimicrobial Efficacy of Tooth Paste Containing Lactoferrin, Lysozyme, Lactoperoxidase in Children with Severe Early Childhood Caries: A Clinical Study

RAVI KUMAR GUDIPANENI[1], VIJAY KUMAR R.[2], JESUDASS G.[3], SURESH PEDDENGATAGARI[4], YESURATNAM DUDDU[5]

ABSTRACT

Background and Objectives: The present study is conducted to compare the anti-microbial efficacy of tooth paste containing lactoferrin, lysozyme, lactoperoxidase (BioXtra ®), a 500ppm fluoride tooth paste, and a non fluoridated tooth paste in children with Severe Early Childhood Caries (S-ECC).

Materials and Methods: Study group included 30 children with S-ECC aged 3-5 years. Subjects were randomly selected & divided into three groups of ten each. Group I: Non–fluoride tooth paste, Group II: 500 ppm fluoride tooth paste, Group III: tooth paste containing lactoferrin, lysozyme & lactoperoxidase. Estimation of salivary *S. mutans* and *L. acidophilus* levels was accomplished by collecting salivary samples and inoculated on Mitis Salivarius Bacitracin agar (MSB) and Rogosa SL media. Colony Forming Units (CFUs) were counted and the results were tabulated and subjected to statistical analysis.

Results: Brushing with tooth paste containing lysozyme, lactoferrin and lactoperoxidase after a week showed highly significant reduction in the CFU counts of both salivary *S. mutans* and *L. acidophilus* ($p<0.001$), whereas 500ppm fluoride tooth paste showed a highly significant reduction in the *S. mutans* levels ($p<0.001$), but a significant reduction in *L. acidophilus* count ($p<0.01$). Non-fluoride tooth paste showed a highly significant reduction in *S. mutans* ($p<0.001$) only, but reduction in the *L. acidophilus* levels was insignificant ($p<0.05$).

Conclusion: The present study revealed that tooth paste containing lactoferrin, lysozyme, and lactoperoxidase was highly significant in reducing the salivary levels of mutans *Streptococci* and *L. acidophilus* in children with S-ECC.

Keywords: Lactoperoxidase, Lysozyme, Lactoferrin, Dentifrices, Saliva, Early childhood caries

INTRODUCTION

Innate human salivary defense proteins such as lysozyme, lactoferrin and peroxidase are known to exert a wide antimicrobial activity against a number of bacterial, viral and fungal pathogens in vitro [1]. One of the leading ideas in adding antimicrobial proteins such as lactoperoxidase, lactoferrin and lysozyme to oral health products has been the purpose to partially restore saliva's own antimicrobial capacity and to add physiological salivary antimicrobial agents into the mouth [2]. Therefore, these proteins alone or in combinations have been incorporated in oral health care products to restore saliva's own antimicrobial capacity [3]. These antimicrobials are used in oral health care products such as dentifrices, mouth-rinses, moisturizing gels and chewing gums have been purified from bovine colostrums [4]. An interesting point is that all these three antimicrobial agents act in an additive way, and even synergistic interactions have been described [5,6]. Therefore, it seems sensible to critically evaluate the clinical efficacy of this kind of preventive approach in children with S-ECC.

Many tooth pastes which contain antimicrobial compounds have been formulated over the years, with the aim of preventing or reducing plaque and dental caries with an attempt to enhance the anticariogenic properties of saliva [7]. A great number of studies with controversial results have been published regarding various individual agents and their possible association to oral health particularly to dental caries [8-11].

The present study was undertaken with the following main aims and objectives:

1. To estimate the salivary levels of *S. mutans* and *L. acidophilus* in children with S-ECC before, immediately after and one week after brushing with a non-fluoridated tooth paste, a tooth paste with 500 ppm of fluoride and tooth paste containing lactoferrin, lysozyme, lactoperoxidase, colostrum-containing growth factors such as IGF-1, TGF-β1 and TGF-β2 (BioXtra ®).
2. To compare their efficacy in reducing the salivary levels of *S. mutans* and *L. acidophilus* in children with S-ECC.
3. To evaluate any additional beneficial effects of lactoferrin, lysozyme & lactoperoxidase in reducing the oral microbial count.

MATERIALS AND METHODS

The study groups included 30 children who attended the outpatient Department of Pedodontics, Government Dental College and Hospital Kadapa, Andhra Pradesh, India, with S-ECC under the age group of 3–5 years. Subjects were randomly selected & divided into three groups with ten children in each group. Salivary samples were analyzed for mutans *streptococci* and lactobacilli.

Group I: Used Non – fluoride tooth paste.

Group II: Used 500 ppm fluoride tooth paste.

Group III: Used (BioXtra ®).

All the children were examined in adequate day light. A mouth mirror with good reflecting surface and a stainless steel explorer were used to examine the patient.

1. The saliva samples were collected from the children before, immediately after brushing and also on 7th day.
2. The patients were instructed to use the scrub- technique of brushing for a time period of 2-3 minutes twice daily.

Only those children who have not received any antibiotic therapy with in the last three months and children who have not yet received any dental treatment were included into the present study. Informed consent was obtained from the parents.

Collection of Salivary Samples

The subjects were instructed to chew on paraffin wax for 2-3 minutes and expectorate 1-2 ml of saliva directly into the sterile collection bottles. The collected samples were immediately transported to Microbiology department and inoculated on Mitis Salivarius Bacitracin agar (MSB) and Rogosa SL media. The inoculated plates of Mitis Salivarius Bacitracin agar and Rogosa SL medium were incubated at 37 ^{0}C in a candle jar environment for 48 hrs. Colony Forming Units (CFU) were counted and the results were tabulated and subjected to statistical analysis. Confirmation of *S. mutans* and *L. acidophilus* was done by smear examination and biochemical tests.

STATISTICAL ANALYSIS

The comparison of the antimicrobial efficacy of the three toothpastes was analyzed by using two way Anova test and all analyses were performed using SPSS software (SPSS 16.0, SPSS Inc., Chicago, Ill., USA).

RESULTS

Group I demonstrates a reduction in the levels of *L. acidophilus*, immediately after brushing and 7 days after brushing with non fluoridated tooth paste and was not showing any significance ($p<0.05$) as compared to values before brushing [Table/Fig-1]. Group II demonstrates a reduction in the levels of *L. acidophilus*, immediately after brushing and 7 days after the brushing with 500 ppm fluoridated tooth paste and was showing significant reduction ($p<0.01$) compared to values before brushing [Table/Fig-2]. Group III demonstrates a reduction in the levels of *L. acidophilus* and was highly significant ($p<0.001$) with 7 days of brushing than immediately after brushing [Table/Fig-3].

S. No.	Time Points	*S. mutans* Mean ± SD	*L. acidophilus* Mean ± SD
1	Before brushing	110 12.4	122.5 ± 21.39
2	Immediately after brushing	89.5 ± 15.2	109 ± 21.2
3	7 days after brushing	76.2 ± 19.6	99.8 ± 13.4
	F 'ratio	11.4	3.40
	Level of significance	p<0.001	p<0.05

[Table/Fig-1]: Mean ± SD values (CFU) of *S. mutans* and *L. acidophilus* in children with S-ECC used non fluoridated tooth paste (Statistical analysis was done by Anova test)

S.No	Time Points	S.mutans Mean ± SD	*L. acidophilus* Mean ± SD
1	Before brushing	123.1 ± 21.1	122.0 ± 11.0
2	Immediately after brushing	83.2 ± 19.5	106.0 ± 12.2
3	7 days after brushing	33.6 ± 10.3	89.2 ± 9.96
	F 'ratio	64.71	32.9
	Level of significance	p<0.001	p<0.01

[Table/Fig-2]: Mean ± SD values of (CFU) S.mutans and *L. acidophilus* in children with S-ECC used fluoridated tooth paste (Statistical analysis was done by Anova test)

S.No	Time Points	*S.mutans* Mean±SD	*L. acidophilus* Mean±SD
1	Before brushing	112.0 ± 13.3	109.6 ± 14.3
2	Immediately after brushing	55.9 ± 17.56	39.2 ± 18.2
3	7 days after brushing	8.50 ± 5.89	8.60 ± 3.98
	F 'ratio	154.4	146.4
	Level of significance	p<0.001	p<0.001

[Table/Fig-3]: Mean ± SD values of (CFU) *S. mutans* and *L. acidophilus* in children with S-ECC used tooth paste containing lactoferrin, lysozyme and lactoperoxidase (Statistical analysis was done by Anova test)

DISCUSSION

The antimicrobial host proteins most widely used in these products were lysozyme, lactoferrin and lactoperoxidase (BioXtra ®). To make lactoperoxidase enzyme antimicrobial its substrates thiocyanate (SCN-) and hydrogen peroxide (H2O2) were also included in these products. These products also contain colostrum-containing growth factors such as IGF-1, TGF-β1 and TGF-β2 [12]. A large amount of scientific literature exists on how these non-immunoglobulin components work separately and together [3].

A number of studies have been tried to find a clinically significant association between selected salivary agents and caries prevalence or incidence but the results were controversial. This may be explained by several reasons, e.g., various salivary agents interact with each other in a complicated way and also inadequate immune defence (usually IgA deficiency) can be compensated by an enhanced non-immunoglobulin activity or by compensatory IgM response [8,10].

This approach is more physiological, safe and suitable for daily use as no harmful side-effects of host proteins have been described to human oral cell lines [3]. Although solely bovine milk derived proteins have been used so far in commercial applications, no short (e.g., allergy) or long-term (e.g., auto-immunity) adverse effects have been observed [13]. Clinical trials both in rats and human beings have suggested that long term use of peroxidase containing tooth paste resulted in significant reduction in caries incidence [7,14,15].

There are a number of studies which describe the antimicrobial mechanisms of lysozyme, lactoferrin and HOSCN/OSCN. Most of these studies are done in vitro by using *Streptococcus* mutans as a target bacterium; also, the effects against periodontal pathogens have been published. In most conditions which imitate the in vivo situation in oral fluids, the effects are bacteriostatic, but the spectrum against various microbial pathogens is large [6,11]. The antimicrobial proteins in these toothpastes may also inhibit the appearance of aphthous ulcers may be explained by the absence of ulcer-provoking sodium lauryl sulphate in all these antimicrobial tooth-pastes [16]. A rough estimate is that in human trials the reduction in plaque accumulation and gingivitis is 10-20%, but as high as 50-90% reduction in the occurrence of aphthous ulcers [17].

The present study revealed that tooth paste containing lactoferrin, lysozyme, lactoperoxidase was highly significant in reducing the salivary levels of mutans *Streptococci* and *L. acidophilus* in children with S-ECC ($p<0.001$). Non-fluoridated tooth paste and 500 ppm fluoride tooth paste produced highly significant reduction ($p<0.001$) of *S.mutans* only, whereas the reduction of *L. acidophilus* count was not showing much significance thus indicating that the reduction of *L. acidophilus* was mainly due to tooth paste containing lactoferrin, lysozyme and lactoperoxidase.

CONCLUSION

As most of the areas in India are in fluoride belt, already the risk of systemic fluorosis is already severe. As, it is debatable whether to prescribe the fluoride tooth paste in fluorosis areas, it can be

proposed that use of tooth paste containing lysozyme, lactoferrin and lactoperoxidase may be used as a substitute to fluoride tooth paste to reduce the salivary bacterial activity and caries progression in children with S-ECC. In addition, further clinical evaluations particularly longitudinal studies are needed to know their significant role and extensive clinical trials and long-term in-vivo studies are required to support this perspective.

REFERENCES

[1] Tenovuo J. Antimicrobial function of human saliva how important is it for oral health? *Acta Odontol Scand.* 1998; 56: 250-6.

[2] Gil-Montoya JA, Guardia-López I, González-Moles MA. Evaluation of the clinical efficacy of a mouthwash and oral gel containing the antimicrobial proteins lactoperoxidase, lysozyme and lactoferrin in elderly patients with dry mouth--a pilot study. *Gerodontology.* 2008; 25(1):3-9.

[3] Tenovuo J. Clinical applications of antimicrobial host proteins lactoperoxidase, lysozyme and lactoferrin in xerostomia. Oral Diseases. 2002; 8:23-9.

[4] Güneri P, Alpöz E, Epstein JB, Çankaya H, AteşMIn vitro antimicrobial effects of commercially available mouth-wetting agents. *Spec Care Dentist.* 2011; 31(4): 123-8.

[5] Soukka T, Lumikari M, Tenovuo J. Combined inhibitory effect of lactoferrin and lactoperoxidase system on the viability of Streptococcus mutans, serotype c. *Scand J Dent Res.* 1991; 99: 390-6.

[6] Welk A, Meller Ch, Schubert R, Schwahn Ch, Kramer A, Below H. Effect of lactoperoxidase on the antimicrobial effectiveness of the thiocyanate hydrogen peroxide combination in a quantitative suspension test. *BMC microbiology.* 2009; 9: 134.

[7] Lenander-Lumikari M, Loimaranta V. Saliva and dental caries. *Adv Dent Res.* 2000; 14: 40-7.

[8] Roger V, Tenovuo J, Lenander-Lumikari M, Söderling E Vilja P. Lysozyme and Lactoperoxidase Inhibit the Adherence of Streptococcus mutans NCTC 10449 (Serotype c) to Saliva-Treated Hydroxyapatite in vitro. *Caries Res.* 1994; 28: 421–8.

[9] Jyoti S, Shashikiran ND, Reddy VV. Effect of lactoperoxidase system containing toothpaste on cariogenic bacteria in children with early childhood caries. *J Clin Pediatr Dent.* 2009; 33(4): 299-303.

[10] Lee JY, Kim YY, Chang JY, Park MS, Kho HS The effects of peroxidase on the enzymatic and candidacidal activities of lysozyme. *Archives of oral biology.* 2010; 5(9): 607-12.

[11] Lenander-Lumikari M, Tenovuo J. Mikola H. Effects of a Lactoperoxidase System-Containing Toothpaste on Levels of Hypothiocyanite and Bacteria in Saliva. *Caries Res.* 1993; 27(4): 285–91.

[12] Wei H, Loimaranta V, Tenovuo J, et al. Stability and activity of specific antibodies against Streptococcus mutans and Streptococcus sobrinus in bovine milk fermented with Lactobacillus rhamnosus strain GG or treated at ultra-high temperature. *Oral Microbiol Immunol.* 2002; 17:9-15.

[13] Hettinga K, van Valenberg H, de Vries S, Boeren S, van Hooijdonk T, van Arendonk J, Vervoort J. The host defense proteome of human and bovine milk. *PLoS One*. 2011; 6(4): e19433.

[14] Hatti S, Ravindra S, Satpathy A, Kulkarni RD, Parande MV. Biofilm inhibition and antimicrobial activity of a dentifrice containing salivary substitutes. *Int J Dent Hyg.* 2007; 5(4): 218-24.

[15] Welk A, Rudolph P, Kreth J, Schwahn Ch, Kramer A, Below H. Microbicidal efficacy of thiocyanate hydrogen peroxide after adding lactoperoxidase under saliva loading in the quantitative suspension test. *Arch Oral Biol.* 2011; 56(12): 1576-82.

[16] Brokstad-Herlofson B, Barkvoll P. The effect of two toothpaste detergents on the frequency of recurrent aphthous ulcers. *Acta Odontol Scand.* 1996; 54: 150-3.

[17] Herlofson BB, Barkvoll P. Sodium lauryl sulfate and recurrent aphthous ulcers. A preliminary study. *Acta Odontol Scand.* 1994; 52: 257-9.

PARTICULARS OF CONTRIBUTORS:

1. Assistant Professor, Department of Pedodontics and Preventive Dentistry, Government Dental College and Hospital, Kadapa, Andhra Pradesh, India.
2. Senior Lecturer, Department of Periodontics, Government Dental College and Hospital, Kadapa, Andhra Pradesh, India.
3. Associate Professor, Department of Pedodontics and Preventive Dentistry, Government Dental College and Hospital, Kadapa, Andhra Pradesh, India.
4. Associate Professor, Department of Periodontics, Government Dental College and Hospital, Kadapa, Andhra Pradesh, India.
5. Assistant Professor, Department of Oral and Maxillofacial Surgery, Government Dental College and Hospital, Kadapa, Andhra Pradesh, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:

Dr. Ravi Kumar Gudipaneni,
Assistant Professor, Department of Pedodontics and Preventive Dentistry,
Government Dental College and Hospital, Kadapa-516002, Andhra Pradesh, India.
Phone: 9247278135, E-mail: drravimds@gmail.com

FINANCIAL OR OTHER COMPETING INTERESTS: None.

Date of Submission: **Nov 29, 2013**
Date of Peer Review: **Jan 20, 2014**
Date of Acceptance: **Feb 12, 2014**
Date of Publishing: **Apr 15, 2014**