Effect of Lactoperoxidase System Containing Toothpaste on Cariogenic Bacteria in Children with Early Childhood Caries

Sapna Jyoti* / N D Shashikiran** / V V Subba Reddy***

Background and Objectives: *Lactoperoxidase system contains Lactoperoxidase, Hydrogen peroxide and Thiocyanate ions, which have inhibitory action against cariogenic oral microflora. The present study was undertaken to assess the effect of lactoperoxidase system containing toothpaste on cariogenic microflora in children with early childhood caries.* ***Methods:*** *Study group included 30 children with Early Childhood Caries. 15 were considered as test group who used the test product Biotene® toothpaste and other 15 as control group who used Colgate Active® as control product. Salivary samples were analyzed for mutans streptococci (MS) and Lactobacilli, and for the levels of Thiocyanate ions.* ***Results:*** *showed significant increase in the levels of Thiocyanate ion in saliva during experimental period. Compared to the control group test group showed significant increase in the levels of thiocyanate ions during experimental and washout period, whereas the number of colonies of MS and Lactobacilli were significantly reduced in test group during experimental period.* ***Conclusion:*** *The levels of thiocyanate ions can be increased in vivo by supplementing the saliva with natural enzymes like lactoperoxidase. This increased concentration of thiocyanate will reduce the number of cariogenic microflora in children with Early Childhood Caries.*

Keywords: *Lactoperoxidase, Early childhood caries, Thiocyanate ions, Antibacterial.*
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INTRODUCTION

The saliva has a significant role in oral and general health. The antibacterial, antiviral, and antifungal factors of saliva are either non immunoglobulin innate agents (such as lysozyme, lactoferrin, peroxidase systems, agglutinins) or immune acquired agents (IgA, IgG, IgM).[1]

Early childhood is an important period for the colonization of bacteria in the primary dentition, so it is possible that antimicrobial factors in saliva may modify these early events.

Dental caries is an infectious disease that manifests itself by the demineralization of dental tissues. Early Childhood Caries (ECC) is a serious sociobehavioral and dental problem that afflicts infants and toddlers. ECC is associated with prolonged and frequent oral exposure to cariogenic substances. *Mutans streptococci*(MS) are the principal bacteria isolated from children with ECC. *Mutans streptococci* initiate the lesions and *Lactobacilli* progress the dental caries.[2]

The role of dental plaque is the major factor in the etiology of ECC. Supplementation of mechanical brushing with effective antimicrobial agents in tooth paste would promote the control of dental plaque.

However, the addition of antibiotics is not acceptable due to potential hazards of the development of antimicrobial resistance and undesired shifts in the oral ecology. Therefore enhancement of saliva's natural antimicrobial systems could be a useful supplement in maintaining oral health.[3]

Among the host defense mechanism factors in humans, saliva contains an antibacterial system consisting of lactoperoxidase, hydrogen peroxide, and thiocyanate ion. Hydrogen peroxide in the oral cavity is produced by predominant micoorganisms of the oral cavity. Lactoperoxidase catalyzes the oxidation of thiocyanate ions (SCN^-) by hydrogen peroxide to generate hypothiocyanous acid (HOSCN) or hypothiocyanite anion ($OSCN^-$) which is antibacterial. The hypothiocyanite acts as a bacterial inhibitor by interfering with cell metabolism.[4] salivary peroxidase system can be enhanced in-vivo by adding small amount of hydrogen peroxide generating enzymes to toothpaste.

Biotene® toothpaste comprises all the components of the peroxidase system: peroxidase enzyme (lactoperoxidase), SCN^- ions, and Hydrogen peroxide generating enzymes, combined with other prophylactic agents such as sodium

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monofluorophosphate and xylitol.[5]

The aim of the study was to assess the effect of lactoperoxidase system containing toothpaste on cariogenic microflora in children with early childhood caries and to evaluate the effects of lactoperoxidase system containing toothpaste on cariogenic microflora.

MATERIALS AND METHOD

The study was conducted on 30 children between the age group of 3 to 5 years with early childhood caries who have been classified as having mild to moderate caries according to the classification given by Wyne. [6]

At first visit a complete oral examination was performed and saliva samples were collected. This was considered as first baseline period (B1). All the parents of children were informed about the purpose of the study. Among 30 children, 15 children were taken as test group and the other 15 as control group. Test group children used the test toothpaste Biotene® and control group used the control toothpaste Colgate Active®. The test product Biotene® contained sodium monofluoro phosphate (0.14% W/V Fluoride ion), lactoperoxidase, Glucose Oxidase, Lactoferrin, Lysozyme. The control toothpaste Colgate Active® was lacking the enzyme system but had sodium monofluorophosphate.

All the children selected were from the same socio-economic status. Restoration of the carious teeth was done using glass ionomer cement. After 3 months of restoration, saliva samples were collected and this was considered as second baseline period (B2).

After 3 months of the restoration children were instructed to brush with the experimental toothpaste for 1 minute twice a day under parental supervision for 4 weeks. They were instructed to use peanut sized quantity of toothpaste. Children were asked to refrain from using other fluoridated or xylitol-containing products during whole test period. during the washout period, the subjects returned to their normal oral hygiene methods, using any toothpaste except the test ones.

Saliva samples collected at 2 baselines i.e., before treatment of the carious teeth (B1) and 3 months after restoration of carious teeth (B2), after 2nd and 4th weeks' use of the test products, and during wash out period ie., 6th and 8th week. The study procedures took place at the same time of day for each child.

All salivary samples were analyzed for *mutans streptococci* and *lactobacilli,* and for the level of thiocyanate ions. All the examinations were carried out by single investigator.

Collection and treatment of saliva

Whole stimulated saliva was collected by having the children to chew a cotton roll, from which saliva was squeezed into collecting bottle.[7] For microbiological assay a 10 fold dilution of saliva was made with saline and inoculated immediately. The rest of the collected saliva was centrifuged at 18,000 g for 10 min at +4ºC.[8] Portion of centrifuged saliva needed for the analysis of thiocyanate ion levels were stored frozen at -20ºC until analyzed. All the samples were kept on ice during handling.

Chemical assay

The SCN^- content was analyzed from centrifuged saliva and SCN^- ions were quantified by the ferric nitrate method.[9]

Microbiological assay

The saliva samples in 2 baseline periods and after 2 and 4 weeks use of test and control toothpaste were analyzed for *Mutans strptococci*, and *Lactobacilli.* After serial 10-fold dilution the saliva samples were plated as follows: *Mutans strptococci* (MS) on *Mitis Salivarius Bacitracin* agar plates supplemented with 0.5µg/ml and incubated for 3days at 37ºC.

Lactobacilli were cultivated on Rogosa SL agar plates and incubated anaerobically for 3 days at 37ºC.

RESULTS

Comparison of thiocyanate ion level, *Mutans strptococci* level and *Lactobacilli* level was done in different treatment periods and between control and test groups.

There was increase in the levels of thiocyanate ions in saliva on 4th, 6th and 8th week of use of Biotene®. (Fig 1). Also there was significant reduction in MS and *Lactobacilli* during the test period on use of Biotene® (Fig 2and 3). When test group was compared with control group, there was significant increase in the level of thiocyanate ion in the test group during 4th, 6th, and 8th week samples (Table 1). But

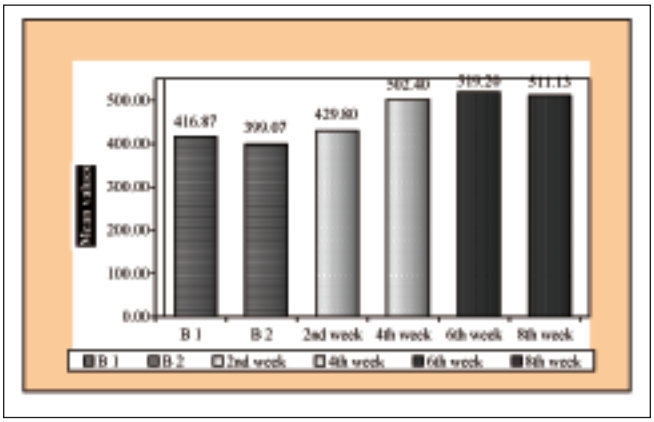


Figure 1. Comparison of Thiocyanate Ion Levels in different treatment periods (Test group)

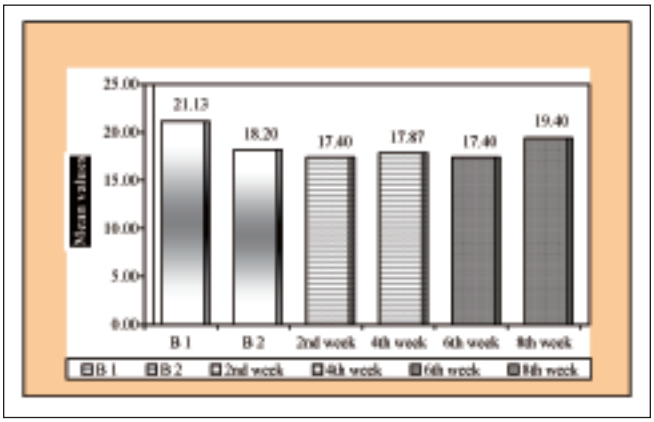


Figure 2. Comparison of S. Mutans (x10^5CFU/ml of saliva) in different treatment periods (Test group)

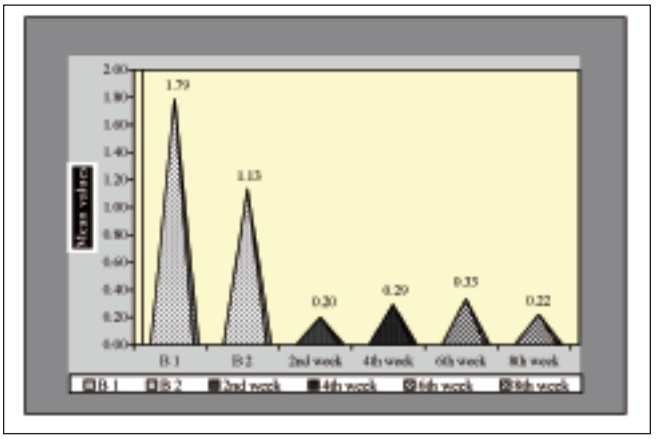


Figure 3. Comparison of Lactobacilli (x10^{5}CFU/ ml of saliva) in different treatment periods (Test group)

no significant reduction was seen in MS and *Lactobacilli* when both the products were compared (Table 2 and 3).

Table 1. Comparison of Thiocyanate ion levels between control and test groups

Treatment period	Control		Test		Unpaired t-value	p-value	Significance
	Mean(μM)	S.D.	Mean(μM)	S.D.			
B 1	420.27	59.86	416.87	47.03	0.17	0.86	NS
B 2	419.87	23.36	399.07	31.80	2.04	0.05	NS
2nd week	422.20	46.88	429.80	33.54	-0.51	0.61	NS
4th week	423.53	38.42	502.40	15.93	-7.34	0.00	S
6th week	419.73	48.01	519.20	20.10	-7.40	0.00	S
8th week	422.33	46.95	511.13	41.17	-5.51	0.00	S

Table 2. Comparison of mutans streptococci levels (x10^5 CFU/ml of saliva) between control and test groups

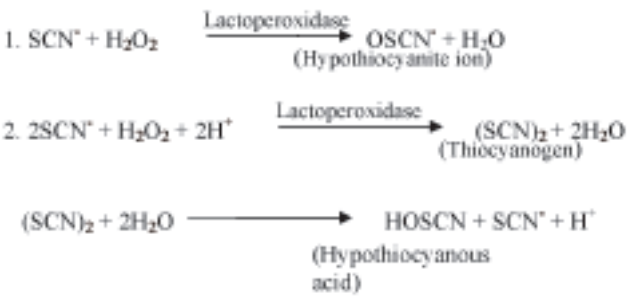
Treatment period	Control		Test		Unpaired t-value	p-value	Significance
	Mean(x10^5)	S.D.	Mean(x10^5)	S.D.			
B 1	20.67	5.21	21.13	3.14	-0.30	0.77	NS
B 2	18.40	6.03	18.20	5.32	0.10	0.92	NS
2nd week	19.13	3.62	17.40	3.33	1.36	0.18	NS
4th week	20.67	6.77	17.87	4.24	1.36	0.19	NS
6th week	21.13	4.32	17.40	5.38	2.09	0.05	S
8th week	22.40	5.62	19.40	3.68	1.73	0.09	NS

Table 3. Comparison of Lactobacilli levels (x10^5 CFU/ml of saliva) between control and test groups

Treatment period	Control		Test		Unpaired t-value	p-value	Significance
	Mean(x10^5)	S.D.	Mean(x10^5)	S.D.			
B 1	1.03	1.35	1.79	1.25	-1.59	0.12	NS
B 2	0.75	0.82	1.13	1.07	-1.11	0.28	NS
2nd week	0.34	0.47	0.20	0.46	0.82	0.42	NS
4th week	0.34	0.56	0.29	0.53	0.24	0.82	NS
6th week	0.67	0.97	0.33	0.44	1.23	0.23	NS
8th week	1.29	1.13	0.22	0.55	3.30	0.00	S

DISCUSSION

Human saliva not only lubricates oral tissues, but also protects oral tissues, teeth, and mucosal surfaces in many ways. *In vitro* studies have indicated that most of the salivary factors are able to limit the growth of MS. These salivary factors inhibit sugar transport, agglutinate the bacteria and even kill MS through different mechanisms,[10] Salivary peroxidase system is one among them and is an innate defense factor in saliva. It consists of the Peroxidase Enzyme, the Thiocyanate ion (SCN^-) and Hydrogen Peroxide (H_2O_2).[11] Peroxidase enzyme and thiocyanate ions are the normal components of saliva whereas, hydrogen peroxide comes from oral microorganisms in the oral environment.[12] Lactoperoxidase is the bovine homologue of human salivary peroxidase.[13] The presence of lactoperoxidase in both milk and saliva was shown by Mosimann & Suner and also showed that they have an inhibitory effect on bacterial growth. Lactoperoxidase catalyzes the oxidation of thiocyanate ions (SCN^-) by hydrogen peroxide to generate hypothiocyanous acid (HOSCN) or hypothiocyanite anion ($OSCN^-$) which is antibacterial. The oxidation of SCN^- may yield $OSCN^-$ directly or may yield thiocyanogen, $(SCN)_2$, which hydrolyzes rapidly to yield hypothiocyanous acid(HOSCN). HOSCN and OSCN have the antimicrobial property.[14]



SCN^- concentrations which lie above the physiological salivary concentrations have a positive effect on gingival health and also have an antiplaque effect. So SCN^- ion is the main component among the lactoperoxidase system which is responsible for the antimicrobial effect on the microbial flora.[2]

The effect of lactoperoxidase system could be assessed by analyzing increase in the levels of SCN^- ions and decrease in the number of cariogenic bacteria in saliva during the use of toothpaste and during washout period.

In this study the test product (Biotene®) containing Lactoperoxidase system was compared with the control toothpaste (Colgate Active®) which lacks this system. Here two baseline periods have been recorded i.e., first baseline (B1) at the first visit, and second baseline (B2); 3 months after restoration of the carious lesions. Berg *et al*[15] showed that the cariogenic microorganisms stabilized at 3 months post restoration. Saliva samples were collected at these two baseline periods (B1 and B2), and at the end of 2nd and 4th weeks' use of experimental toothpastes (Biotene®/ Colgate Active®), and during washout period i.e., 6th and 8th week.

The first baseline sample (B1) was compared with the second baseline period (B2), and 2nd, 4th, 6th, and 8th weeks' samples in both control and test groups.

Test product (Biotene®) showed significant increase in the levels of thiocyanate ions in different periods of use of toothpaste. The levels of thiocyanate ion increased from B1 to 6th week in the test group, and at the end of washout period i.e., 8th week there was slight decrease in the level when compared to 6th week. Control group showed no significant increase in SCN^- ion levels. A study on xerostomic patients by Kirstila *et al,*[4] showed increase in the levels of thiocyanate ions in washout period but concentration did not increase during the experimental period.

In our study during experimental period (2nd & 4th week), the use of test product supplemented the saliva by Lactoperoxidase, SCN^-, and H_2O_2 generating enzymes. So the H_2O_2 produced by microorganisms was utilized in the oxidation of SCN^- to produce $OSCN^-$ (antibacterial agent) which in turn reduced the number of microorganisms. Reduction in number of microorganisms further reduced the production of H_2O_2. During washout period (6th & 8th week), due to reduced amount of H_2O_2, the SCN^- which was already present in the saliva would not be oxidized to produce $OSCN^-$. This resulted in increased levels of SCN^- ions during washout period.

Simultaneously there was reduction in the number of colony forming units by MS per ml. of saliva during the second baseline, 2nd wk, 4th wk, and 6th wk, of use of test product. Similarly, there was significant reduction in the number of *Lactobacilli* CFU/ml. in 2nd,4th, 6th, and 8th week of use of test product, but no significant results seen in the control group($p>0.05$). This shows that increase in the level of SCN^- ion concentration reduced the number of colonies of MS and *Lactobacilli*. In a study by Kirstila *et al,*[17] use of Biotene® toothpaste relieved the oral symptoms in xerostomic patients but there was no significant reduction in cariogenic microflora. Mulligan *et al*,[16] found a significant decrease in salivary total anaerobes after the use of Biotene® mouth rinse.[17] whereas Kirstila *et al,*[17] found no significant decrease in the cariogenic bacteria in healthy adults after 2–weeks use of toothpaste. On the other hand, lactoperoxidase system seems to be a well designed as an inhibitor of bacterial metabolism and growth in the oral environment. Lumikari *et al*, found that the amount of antimicrobial agents generated by use of Biotene® toothpaste were high enough to exert an antibacterial effect on the metabolic activities of MS.[7]

When comparison was made between control and test group, there was significant increase in the level of thiocyanate ions during experimental period i.e., 4th week and during washout period i.e., 6th and 8th week in the test group. At baseline (B1) the mean value of thiocyanate ion level was 420.27 in control group and 416.87 in test group and at the end of experimental period i.e., 4th week; mean value was 423.53 in control group and 502.40 in the test group. During washout period mean value was 422.33 in control group and 511.13 in the test group. This shows there was gradual increase in the level of thiocyanate ions from baseline to washout period by use of Biotene® toothpaste.

When comparison of levels MS was made between control and test groups, significant reduction was found in the test group only during 6th week (washout period). Similarly when comparison of levels of *Lactobacilli* was made between control and test group, significant reduction was found in the test group only during 8th week. Both MS and *Lactobacilli* showed significant reduction during washout period in test group compared to control group. Control group showed reduction in number of MS and *Lactobacilli* only during experimental period. The fluoride content of control toothpaste may be the reason for reduction in number of microorganisms during experimental period. But once the use of control toothpaste was stopped, its antibacterial effect was reduced, whereas Biotene® had its antibacterial effect even during washout period and thus reduced the number of cariogenic microflora.

Our results show that it is possible to increase significantly the levels of thiocyanate ion which is one of the main components of lactoperoxidase system in saliva of children with ECC by means of Biotene® toothpaste. This increase in thiocyanate ion will result in increased level of antimicrobial agents which in turn reduce the number of cariogenic microflora. So far, very little evidence exists that this system really helps to protect oral tissues from harmful microbes. There was definite increase in the levels of thiocyanate ions after the use of Biotene® toothpaste. This increase had antimicrobial effect on cariogenic microflora. As this study was of short term duration, long term efficacy of this Biotene® can be studied on large number of subjects in future.

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