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RESEARCH ARTICLE

COMPARATIVE EVALUATION OF REMINERALIZATION POTENTIAL OF SODIUM FLUORIDE VARNISH AND SODIUM FLUORIDE VARNISH FORTIFIED WITH BIOGLASS -AN IN VITRO STUDY

Dr. Shreya M.S[1], Dr. Barkathunnisa[1], Dr. Anusha, Dr. Krithikashetty[2], Dr. Anandhu Krishnan E.[1], Dr. Kiran Raj[2], Dr. Sowmya B.[2] and Dr. Sarath Kumar[3]

1. Postgraduate Student, Department of Pediatric and Preventive Dentistry AJ Institute of Dental Sciences Karnataka.
2. Professor and Head Professor and Head, Department of Pediatric and Preventive Dentistry, AJ Institute of Dental Sciences Karnataka.
3. Post Graduate Student, Department of Pediatric and Preventive Dentistry, Divya Jyoti College of Dental.

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Abstract

Aim: - The present study is aimed to compare and evaluate the remineralization potential of Sodium fluoride varnish alone and sodium fluoride varnish fortified with bioglass

Material and Method: - caries like lesions were produced and surface microhardness was evaluated. The specimens were then divided into 2 groups with 36 teeth in each group, accordingly remineralization treatment was employed. 36 teeth were treated with sodium fluoride varnish (duraphat) (group 1) and 36 teeth with treated with sodium fluoride fortified with bioglass (Fluro dip bioactive) (group 2). During the next 10 days, the samples were subjected to a pH-cycling system, which simulated the intraoral conditions, surface microhardnesstesting was performed on the 11th day to evaluate remineralization. The values obtained were tabulated and statistically analyzed using one-way ANOVA.

Result: - Significant remineralization was seen with sodium fluoride fortified with bioglass followed by sodium fluoride varnish.

Conclusion: - The results of the study concluded that sodium fluoride varnish fortified with bioglass has better remineralizing potential than Sodium fluoride varnish alone and can be used to reduce caries advancements at the stage of early carious lesions.

Introduction:-

Dental caries are among the most common oral diseases globally. Shafer, Hine, and Levydefine dental caries as a ‘microbial disease of the calcified tissues of the teethcharacterized by demineralization of the inorganic portion and destruction of theorganic substance of tooth’. Despite substantial global public health efforts toward the prevention of this disease epidemiological data shows that dental caries continue to affect a large population in developing countries like India. The recent national oralhealth survey conducted in 2002-2003 stated that the dmft index score for Indian children was around 2% and caries prevalence was increasing with age from 51.9% and 63.1% in the 5-15 years of age group, respectively [1].

Corresponding Author: - Dr. Kiran Raj
Address**: -** Reader, Department of pediatric and preventive dentistry AJ Institute of Dental sciences, Mangalore, Karnataka.

The dynamic caries process consists of rapidly alternating periods of toothdemineralization and remineralization. The process of demineralization involves thedissolution of hydroxyapatite, which is primarily caused by acidic attacks resultingfrom microbial biofilm by fermentation of sugars, resulting in a demineralizedlesion.[2] demineralized lesions can progress from initial demineralization to non cavitated lesions, and finally to cavitated lesions. The initial clinical demineralizationstage is termed a white spot lesion, white spot lesions represent the first visualclinical stage of incipient caries. If left untreated, white spot lesions rapidly progressto visible decay[2].

It is important to balance the pathological and protective factors that influence theinitiation and progression of dental caries, protective factors promote remineralization and lesion arrest. Reconstructing depleted tissues with hydroxyapatite, which is thesame inorganic component as enamel, is the best way to promote remineralization[5].

Our understanding of the interplay between the initiation and progression of cariouslesions and the associated risk and protective factors has improved considerably overthe past few leading to the practice of Non-invasive or minimally invasive interventions.This approach reduces treatment time and expense, and also the fear which can leadto avoidance behavior, particularly in children[6].

Remineralization is defined as the process by which calcium and phosphate ions areprovided from an external source, it promotes ion deposition into decalcified enamelcrystal voids, which helps in net mineral gain[7]. The term "void" is used to define the accessible space within a crystal caused by ion loss from the demineralizationprocess. Therefore, this definition of remineralization includes crystal repair toprovide a net subsurface mineralization effect of enamel lesions[11]. However, salivahelps in remineralization, net remineralization produced by saliva is a slow processand small, due to the low ion concentration gradient from saliva into the lesion (Silverstone, 1972)[8].

As known, the role of fluoride in caries prevention has been widely accepted since the 1930s.[7] since the 1970s, a considerable amount of published studies have documentedthis significant finding; further, these varnishes are capable of remineralizingincipient caries in the form of white spot lesion, thus slowing the progression orpreventing disease. However, the effectiveness of fluoride alone with an inadequatesource of calcium and phosphate could be limited[4]. It has been reported thatproviding both calcium and phosphate together with fluoride had a superior impact inpreventing caries when compared to fluoride alone. Different formulations of calcium phosphate have been incorporated into fluoride products, including caseinphosphopeptide–amorphous calcium phosphate (CPP-ACP) and bioactive glass. Bioactive glass has the ability to release calcium, sodium, and phosphate ions into anaqueous solution. Thus providing an adequate source of calcium and phosphate. Recently Fluoridated-bio glass toothpaste has proven to have better remineralizationpotential when compared to fluoridated toothpaste. This is possibly due to itssustained release along with other useful ions such as calcium and phosphate[3], leading to the formation of fluorapatite and a consequent increase in surface microhardness. Also, fluoride along with bioglass has better penetration as reported in theprevious studies[10].

Fluoride bioglass toothpaste has shown better remineralization when compared tofluoride toothpaste alone, but varnish studies of this combination are scarce in the literature. Varnish is advantageous as retention on the tooth surface is an importantfactor in delivering effective factors for remineralization of hard tissue. Longerretention of varnish might enable a decrease in the frequency of application andmaximize the remineralizing effect with a single application in clinics. Thus thepresent study aims to compare and evaluate the remineralization potential of sodiumfluoride varnish and sodium fluoride varnish fortified with bioglass.

Methodology:-

This study was conducted on 72 selected teeth which were stored and surfaced as per the Center for Disease Control Global Health protocol. The teeth were stored in 0.1% thymol solution at ambient temperature. The crowns and the roots were separatedfrom 2 mm below the Cemento-Enamel Junction (CEJ). They were mounted withepoxy resin so that their buccal surfaces will be exposed. In order to enclose thesurface to an exposed area of 4 × 4 mm, two layers of nail varnish were applied to thesurrounding area. As a next step, the Surface Microhardness (SMH) of the samples was tested by a Vickers microhardness testing machine (model No.402 MVD), whichis appropriate for determining the hardness of brittle materials, such as tooth structure.

In this test, a diamond-shaped square-based pyramid of 136^{o} was pressedinto the surface of the specimen, and placed on the machine podium under a specific loadof 50 g with a Dwell time of 15 s. The surface of each sample received 3 indentations. The dimensions of the indent were noted and entered. The calculation of themicrohardness at each

point was done based on the equation below (Avner, 2006). Hardness testing was conducted in V&G Laboratories Pvt. Ltd Baikampady, Mangalore, Karnataka.

$HV = 1.854\ L / d^2$
Where,
L = Denotes the applied load on indenter in kg.
d = for, mean diagonal of indentation, in mm.
HV= Vicker'smicrohardness degree (kg/mm^2)

The surface microhardness was assessed at baseline. A demineralizing solution was prepared (2.2 mM calcium chloride, 2.2 mM monopotassium phosphate, 0.05 mMacetic acid having pH adjusted to 4.4 and 1M potassium hydroxide) and all thesamples were immersed in this demineralizing solution for 4 days to create artificialdemineralization, then the specimens were washed with distilled water for 20 seconds, surface microhardness testing was performed. Specimens were then stored inartificial saliva. Artificial saliva was according to the formulation of Gohring et al.and consisted of hydrogen carbonate (22.1 mmol/L), potassium (16.1 mmol/L), sodium (14.5 mmol/L), hydrogen phosphate (2.6 mmol/L), boric acid (0.8 mmol/L), calcium (0.7 mmol/L), thiocyanate (0.2 mmol/L), and magnesium (0.2 mmol/L). ThepH was maintained between 7.4 and 7.8. The specimens were then divided into 2 groups with 36 teeth in each group, accordingly remineralization treatment wasemployed. 36 teeth were treated with sodium fluoride varnish (duraphat) (group 1) and 36 teeth with treated with sodium fluoride fortified with bioglass (Fluro dipbioactive) (group 2). The specimens were air-dried and a thin, uniform layer ofvarnish was applied in a single stroke painting motion and left in contact, accordingto the manufacturer's specifications. During the next 10 days, the samples were subjected to a pH-cycling system, which simulated the intraoral conditions, SMH testing was performed on the 11th day. The values obtained were tabulated and statistically analyzed using one-way ANOVA

Result:-

At baseline, group 1 showed a mean microhardness score of 335.16±6.19, and group 2 showed 335.05±7.16 (Table 1). The mean difference between the groups was notfound to be statistically significant. After initial demineralization, group 1 showed a mean score of 168.80±3.47, and group 2 gave a mean score of 168.55±4.09 (Table 1). The mean difference between the groups was not found to be statistically significant. After remineralization, Group 1 showed a mean score of 220.44±5.68, and group 2 provided a mean score of 255.83±7.26 (Table 2). The mean difference between bothgroups was found to be highly statistically significant (<0.01**). The comparison between demineralization and remineralization within the groups was found to behighly statistically significant (<00.1**).

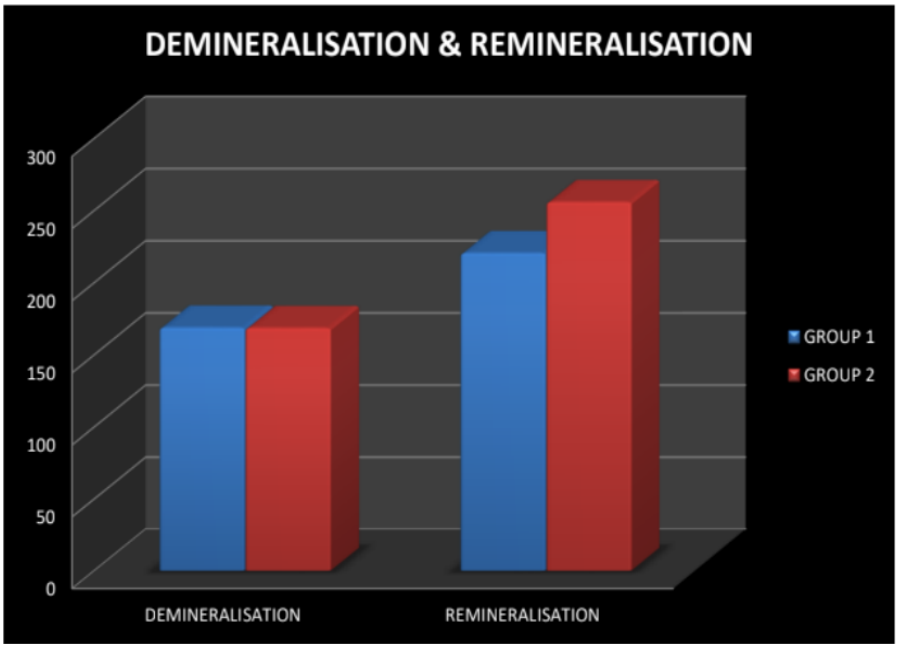
Table 1:- Vicker's micro hardness (kg/mm2) of two groups in.

	GROUP 1	GROUP 2	P VALUE
Baseline	335.16 ±6.19	335.05 ±7.16	0.94
Demineralization	168.80 ±3.47	168.55 ±4.09	0.78
Remineralization	220.44±5.68	255.83±7.26	<0.01**

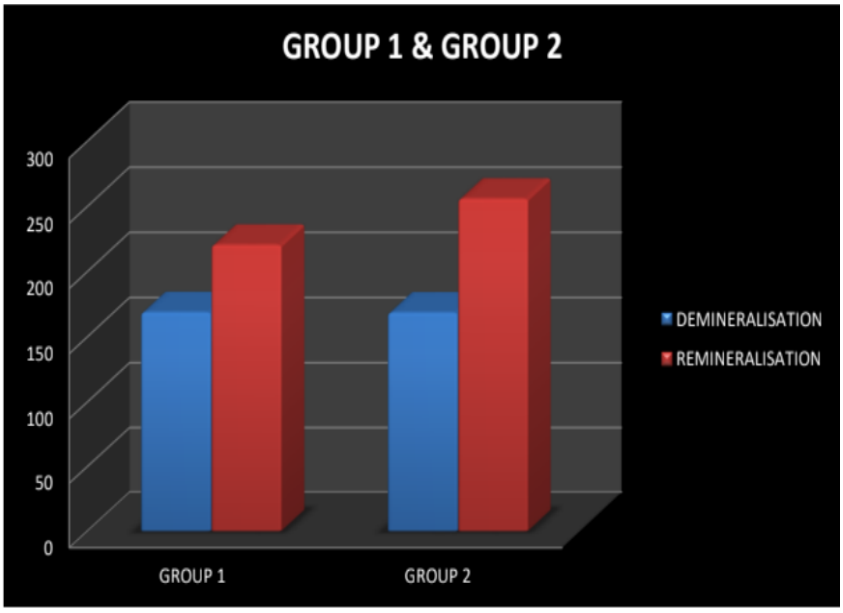
Table 2:- Vicker's micro hardness (kg/mm2) before and after remineralization in both the groups.

	Demineralization	Remineralization	P Value
GROUP 1	160.80±3.47	220.44±5.68	<0.01**
GROUP 2	168.55±4.09	255.83±7.26	<0.01**

Graph1:- comparison of microhardness before and after remineralization.



Graph2:- comparison of microhardness before and after demineralization and remineralization between group 1 and group 2.



Discussion:-

Remineralization of early carious lesions is an essential cornerstone of caries controlwhich is naturally achieved by salivary ions and can be accelerated by adjunctiveremineralizing agents[15]. Among them, sodium fluoride varnish is the most widelyused and has the highest level of supporting evidence [10]. However, inadequate sources of calcium and phosphate can limit the effectiveness offluoride alone. It has been reported that administration of both calcium and phosphatewith fluoride has a better effect on caries prevention compared to fluoride alone. Various calcium phosphate formulations such as casein phosphopeptide amorphouscalcium phosphate (CPP-ACP) and bioglass have been incorporated into fluorideproducts by researchers. These formulations have recently been introduced as part ofthe fluoride varnish and have long-term adherence to the tooth surface, which mayserve as a sustained release fluoride reservoir[10]. However, CPP-ACP does not adhereto the enamel surface well. Unlike bioactive glass, it adheres to teeth due to itsamorphous nature[12].

In our study, we have investigated the effectiveness of sodium fluoride varnish (dycal group 1) and sodium fluoride varnish fortified with bioglass (FluoroDip Bioactive group 2). Demineralized enamel samples have been used in this study and can beregarded as a suitable substitute for white spot lesion[9.] Surface hardness testing using Vickers indentations was used in our study to evaluate the hardness of enamelsurfaces. This method has proven to be suitable for measuring surface hardness withhigh accuracy[2]. The main advantage of this technical evaluation includes the abilityto make repeatable measurements with the application of different loads in a limitedtime. The average microhardness value for normal enamel is in the range of 250-360 Vickershardness number (VHN)[5]. In this study, the baseline microhardness valuewith an overall average of 335.16VHN, which was within the standard range. When the baseline microhardness values of the various treatment groups werecompared, the difference was found to be statistically nonsignificant which impliesthat all the pre-treatment enamel samples were similar[9].After subjecting the enamel to demineralization the microhardness values of all the enamel specimens were found to decrease considerably. When the demineralizationmicrohardness values were compared with the baseline values, the difference wasfound to be statistically significant. In the comparison of post demineralization valuesbetween group 1 and group 2, the difference was found to be statisticallynonsignificant average being 168.80±3.47VHN which indicates that all the enamelspecimens were demineralized to the same extent. Furthermore, it has to be considered that the storage of samples in artificial saliva is not able to adequately imitate the intraoral mineralizing processes [White, 1995][51].Remineralization in vitro is likely to be greater than in vivo as proteins. Natural human saliva binds calcium and thus inhibits calcium phosphateprecipitation [Shellis et al., 2011][6]. Other modifying factors such as pellicleformation, bacteria, and fluoride in saliva and plaque fluid are not regarded in thisstudy. Furthermore, it has to be considered that compared to a pH-cycling model, thestudy design does not adequately reflect the dynamic process of caries progressionwith alternating phases of de and remineralization[6, 10]. Thus, this study can provide information about the remineralizing potential of the used products, in a net remineralizing in vitro model, this study has to be regarded as an initial investigation.After varnish application and ph cycling for 10 days, the microhardness values of both the groups were found to increase considerably indicating mineral gain aftervarnish application. During the course of the study, the hardness number of thedemineralized teeth had increased in both groups after the application of the studymaterials. However, this increase did not reach the same level as that of the teeth atbaseline (before demineralization). This could be due to the in vitro nature of thepresent study as in an in vivo study factors such as diet, salivary flow, and oralhygiene habits like tooth brushing using fluoride-containing toothpaste would result ina higher increase in remineralization, also samples were evaluated after 10 days ofvarnish application. This may be related to the low frequency of applying varnishover the short period of this study (10 days).

The comparison was found to be highly statistically significant, with an increasedmicrohardness in group 2, even though 50mg of sodium fluoride in 1ml of varnish, equivalent to 22.6 mg of fluoride was used in both groups. This could be due to theadequate availability of calcium and phosphate from bioglass. Also, bioglass in thisvarnish leads to its sustained release of calcium and phosphate leading to theformation of fluorapatite crystals.Increased microhardness was seen in group 2 (sodium fluoride fortified with bioglass) when compared to group1 (sodium fluoride varnish) is suggestive of betterremineralization potential in group 2, this is in accordance with studies conducted by Al-Hussain et al, Srivastava et al and Mohapatra et al, using toothpaste, whichcompared fluoride toothpaste and fluoride bioglass toothpaste and showed betterremineralization with fluoride bioglass tooth paste[13].Further studies are warranted to assess the effect of long-term use of remineralizingagents on the prevention and regression of WSLs in children. The use ofsupplementary instruments to measure the degree of improvement of incipient carieslesions is also helpful. Although the re-mineralizing effects of sodium fluoridefortified with bioglassare better in this study, further studies with a larger sample sizeand a longer period of treatment are required to ensure the lack of significantdifference in the performance of these remineralizing agents. One of the limitations

of the present study is that remineralization was physically characterized solely by means of the microhardness test; however, the effect of saidremineralization should be determined by chemical characterization.

Although there are no studies, in which sodium fluoride varnish fortified withbioglass has been used to study the remineralization potential, the results noted in thisstudy are comparable to the previous studies using regular methods, in which aftervarnish application, the Vickers microhardness value was found to increase which isindicative of the remineralizing capability ofthis material. Further in vivo studies with a longer period of treatment is required to ensure the performance of theseremineralizing agents.

Conclusion:-

The results of the study concluded that sodium fluoride varnish fortified with bioglasshas better remineralizing potential than Sodium fluoride varnish alone and can be used to reduce caries advancements at the stage ofearly carious lesions.

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