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A Comparative Assessment of Remineralization Potential of Sodium Fluoride (Naf) And Poly Amido Amine (Pamam) on Artificial Caries Like Lesion of Enamel - An In Vitro Study

Varsha Deokar[1]*, Mandakini S Mandale[2], Jayanti G Humbe[2], Vaishali A Nandkhedkar[2], Savita P Wagh[2] and Poonam R Zanwar[2]

[1]Dental Surgeon, Department Oral Pathology and Microbiology, Govt Dental College & Hospital, Aurangabad

[2]Associate Professor, Department Oral Pathology and Microbiology, Govt Dental College & Hospital, Aurangabad

***Corresponding Author:** Varsha Deokar, Dental Surgeon, Department Oral Pathology and Microbiology, Govt Dental College & Hospital, Aurangabad.

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Abstract

Dental caries is a continuous process, involving various cycles of demineralization and remineralization. Initial caries can be prevented or arrested by simple and cost effective interventions like with proper plaque control and remineralization therapy. Hence the study is performed with mineralizing agent like NaF and PAMAM. Aim and objectives - To compare the difference in vicker's hardness number (VHN) of artificial caries like lesion of enamel before and after remineralization with 10% NaF and PAMAM-NH2. Study Design: Experimental randomized analytical- *in vitro* study. Materials and method - Artificial caries-like lesions were prepared on orthodontically extracted premolars and micro hardness was measured. The samples were divided into two group and subjected to 7 days pH cycle with 10% NaF and PAMAM respectively. Micro hardness was recorded & Obtained micro hardness values were analyzed. Results - The mean difference in increase of micro hardness of the two groups was 8.267VHN which was statistically significant with p value 0.020 ($p<0.001$) which indicate that PAMAM pre-treatment has greater potential of than pre-treatment with 10% NaF. Conclusion - PAMAM-NH2 can be used as pre-treatment regimen before remineralization.

Introduction

Dental caries is a biofilm-mediated, diet modulated, multifactorial, non-communicable, dynamic disease resulting in net mineral loss of dental hard tissues [1]. In India the prevalence of dental caries between age group of 3-18 years is 57% and above 18 years it is 77%. In early childhood, it is comparatively low with 29%, in primary dentition 59%, in mixed dentition is 66% and in permanent dentition 43% [2]. As early as 5000 BC cause of dental caries was suggested a "tooth worm" [2]. Further studies confirmed dental caries to be a multifactorial disease which include host, agent, and environmental factors [3].

Clinically caries can be pit and fissure or smooth surface involving proximal, cervical, and root surfaces. According to American Dental Association it is initial, moderate and advanced [4]. Caries histologically can be considered as four porosity-related zones as translucent zone, positively birefringent (dark) zone, body of the lesion and surface zone [5].

Dental caries is a continuous process, involving various cycles of demineralization and remineralization. However currently there are many treatment modalities to intervene this continuing process, to arrest or reverse the progress of the lesion. Initial caries can be prevented or arrested by simple and cost effective interventions like proper plaque control and remineralization therapy [6]. Remineralization is the natural repair process for non-cavitated or incipient carious lesion [7]. These are categorized in two systems as fluoride systems and non- fluoride systems [8].

Fluoride (F) is an efficient measure which is still considered to be the gold standard in the prevention of dental caries and the treatment of early carious lesions. Fluoride form either fluorapatite or fluor-hydroxyapatites which are more acid resistant [9]. But its effect is limited on pit and fissure caries and overexposure to fluoride can lead to adverse effects (e.g., fluorosis) also toxicity of fluoride increases with inadequate nutrition. Though fluoride has had a.

Profound effect on the level of caries prevalence, it is far from a complete cure [10]. Also fluorides are effective in remineralising enamel but do not have the potential to promote formation of organized apatite crystals. Recently, there is an attempt to shift from reparative to regenerative bio mineralization therapies, where in diseased dental tissues are replaced with biologically similar tissues. Enamel regeneration is challenging as mature enamel is acellular and does not resorb or remodel itself unlike bone or dentine. There are various bio mineralizing agents for enamel like P11-4 peptides, Leucine-rich amelogenin, poly amido amine and nanohydroxyapatite [11].

Poly amido amine (PAMAM) is a bimineralizing agent which has multi branched polymers with cascading reactive ends and internal voids. They can be used as similar to amelogenin in bio remineralization of enamel [12]. Among all the PAMAM dendrimers PAMAM NH2 has greatest remineralising potential of artificial caries like lesions of enamel as it is negatively charged and enamel is positively charged [13].

Hence the present vitro study is carried out to compare remineralising potential of most accepted remineralising agent 10% NaF and bio mineralizing agent PAMAM NH2.

Aim and objectives

1. To determine the difference in vicker's hardness number (VHN) of caries like lesions of enamel before and after remineralization with 10 % sodium fluoride (NaF).
2. To determine the difference in vicker's hardness number (VHN) of artificial caries like lesion of enamel before and after remineralisation with PAMAM-NH2.
3. To compare the difference in vicker's hardness number (VHN) of artificial caries like lesion of enamel before and after remineralization with 10% NaF and PAMAM-NH2.

Materials and method

Study design

The present study is a quasi-experimental study.

Study population

Study included premolars extracted for orthodontic treatment. (Consent was taken by orthodontic department) Study population were categorized into two groups.

Group A - To be treated with 10% NaF: 30 samples.

Group B - To be treated with PAMAM-NH2: 30 samples.

Study group

Study included premolars extracted for orthodontic treatment.

Study population were categorized into two groups:
Group A - To be treated with 10% NaF: 30 samples.

Group B - To be treated with PAMAM-NH2: 30 samples.

Inclusion criteria

Extracted premolars removed for orthodontic treatment.

Exclusion criteria

Teeth with caries, abrasion, erosion and cracks.

Teeth with hypoplasia.

Teeth with any other developmental defect.

Equipment's required: (Fig-1)

Straight micromotor handpiece, High speed airotor handpiece, Ultrasonic scaler Mectron, Sectioning diamond disk, Coarse grit diamond points, Applicator tip, Disposable syringe, Rubber bowl, PH meter, Vicker's Microhardness tester, Adhesive tape, Ruler, Marker and Scissor.

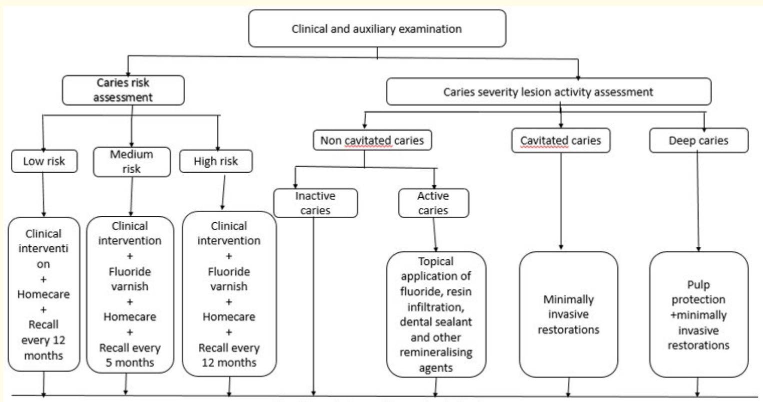

Figure 1: Armamentarium.

List of solutions and reagents (Fig-1)

Distilled water, Acid resistant nail varnish, Self-cure acrylic resin, Pumice powder, 10%formalin, Demineralizing solution {CaCl2 (2.2 mM), NaH2 PO4 (2.2 mM), lactic acid (0.05 M),fluoride (0.2 ppm),adjusted with 50% NaOH to a pH 4.5}.

Remineralizaing solution- (0.2% carbopol, 0.1% lactic acid saturated with calcium phosphate tribasic).

Sodium fluoride-(10%NaF).

Poly amido Amine- (10% PAMAM-NH2).

Procedure

Collection of sample

Thirty premolars extracted for orthodontic treatment and as per exclusion criteria were collected. All teeth were thoroughly cleaned with pumice powder and water to remove soft- tissue debris/calculus. All collected teeth were stored in a 10% formalin solution until further use.

Preparation of samples (Fig-2)

The crown portion of teeth were separated from root. Separated crowns were sectioned longitudinally in a buccolingual direction into two halves (60 sample) with the help of micro motor and diamond disk. All surfaces of each block were coated with two coats of acid-resistant nail varnish except the buccal surface. The teeth were mounted in acrylic resin with enamel surface exposed.

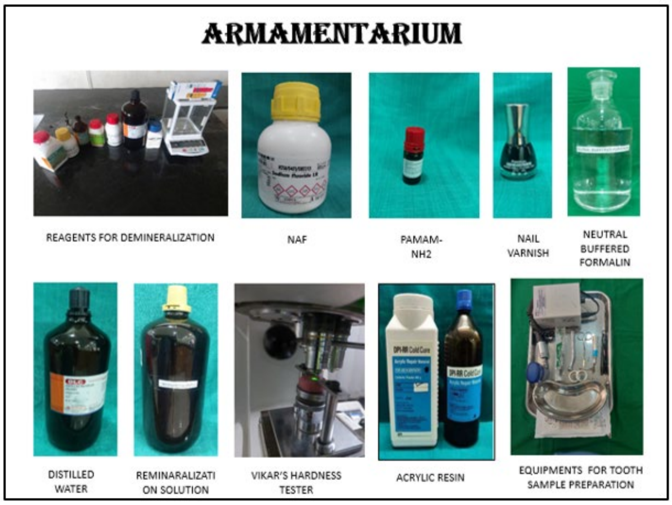

Figure 2: Armamentarium.

Preparation of solutions

Demineralizing solution

Demineralizing solution was prepared with CaCl2 (2.2 mM), NaH2 PO4 (2.2 mM), lactic acid (0.05 M), fluoride (0.2 ppm), adjusted with 50% NaOH to a pH 4.5.

Remineralizing solution

Remineralising solution was prepared with 0.2% carbopol, 0.1% lactic acid saturated with calcium phosphate tribasic.

10% sodium fluoride solution

10 gram of sodium fluoride powder was mixed in 1000 ml of distilled water.

PAMAM-NH2 solution

100mg PAMAM -NH2 (1mg/1ml) was mixed in 1000 ml of distilled water.

Preparation of artificial caries like lesion on enamel (Fig-3)

All the teeth were placed in the demineralizing solution (PH 4.5) for a period of three days to create artificial caries like lesions of enamel [14].

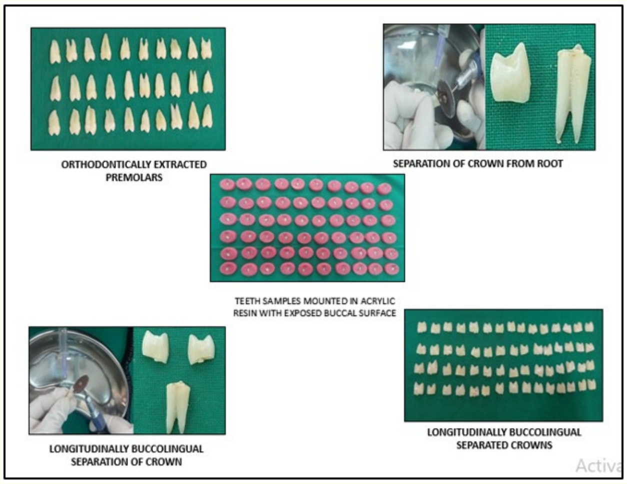

Figure 3: Sample preparation.

After demineralization the Vicker's hardness number were recorded for teeth with Vickers hardness tester under 50gm load with a dwell time of 10 seconds [13].

After demineralization teeth samples were divided into two groups.

1. *Group A* - To be treated with 10% sodium fluoride (30 sample).
2. *Group B* - To be treated with Polyamido Amine (30 sample).

pH cycling regimen for treatment with 10% NaF and PAMAM NH2 (Fig4,5 and 6)

- After preparation of artificial carious lesions Group A and group B were exposed to 10% NaF solution and 10% PAMAM-NH2 solution for 5 min respectively.
- Then both the group were immersed separately in demineralizing solution for three hours.
- One more time Group A and group B were exposed to 10% NaF solution and 10% PAMAM-NH2 solution for 5 min respectively.
- Following both the groups were kept in remineralising solution at PH 7 separately till the next pH cycle was started.
- Again Group A and group B were treated with 10% NaF and PAMAM-NH2 for 5 mins.
- Later both the groups were immersed in demineralizing solution separately for three hours.
- Same PH cycle were repeated for seven days [15].

After completing PH cycle the micro-hardness values for all the samples were recorded with digital Vickers micro-hardness tester at a load of 50 g applied for 10 seconds at room temperature.

All the teeth were placed in the demineralizing solution (PH 4.5) for a period of three days to create artificial caries like lesions of enamel [14].

After demineralization the Vicker's hardness number were recorded for teeth with Vickers hardness tester under 50gm load with a dwell time of 10 seconds [13].

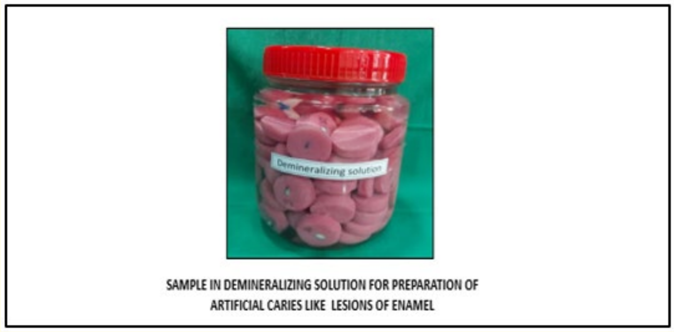

Figure 4: Preparation of artificial caries like lesions on enamel.

Figure 5: Pretreatment with Group-A and Group-B.

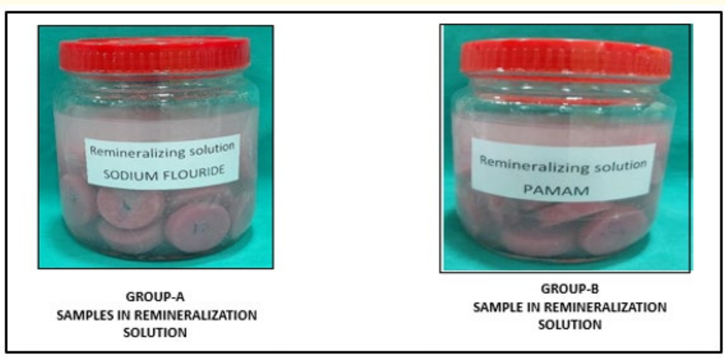

Figure 6: Group-A and Group-B in remineralization solution.

After demineralization teeth samples were divided into two groups.

1. Group A - To be treated with 10% sodium fluoride (30 sample).
2. Group B- To be treated with Polyamido Amine (30 sample).

Evaluation technique of micro hardness

Micro-hardness values for the samples were obtained before remineralization with digital Vickers micro-hardness tester at a load of 50 g applied for 10 seconds at room temperature. Micro-hardness values for the samples were obtained after remineralization with digital Vickers micro-hardness tester at a load of 50 g applied for 10 seconds at room temperature. Micro-hardness values obtained for samples were analysed by using SPSS version 23. Test for normality, Descriptive statistics, Paired t test and independent t test were done for intragroup and intergroup comparison respectively.

Results

Artificial caries-like lesions were prepared on orthodontically extracted premolars and micro hardness was measured. The samples were divided into two group and subjected to 7 days pH cycle with 10% NaF and PAMAM respectively. Micro hardness was recorded & Obtained micro hardness values were analyzed.

The observed values are tabulated in table - 1.

Comparison of Micro hardness values of artificial caries like lesion of enamel among the 10% NaF Group (Group A) before and after remineralization. There is statistically significant difference present in micro hardness of artificial caries like lesion of enamel among the 10% NaF Group (Group A) before and after remineralization. (Table 1, 2 and fig- 1)

Comparison of Micro hardness of artificial caries like lesion of enamel among the PAMAM Group (Group B) before and after remineralization. There is statistically significant difference present in micro hardness of artificial caries like lesion of enamel among the PAMAM Group (Group B) before and after remineralization. (Table1, 3and fig- 2)

Comparison of Micro hardness of artificial caries like lesion of enamel between 10% NaF Group (Group A) and PAMAM Group (Group B) before and after pre-treatment with 10%NaF & PAMAM and remineralization. There is statistically significant difference present in mean micro hardness when Group A was compared with Group B after remineralization ($p<0.001$). (table1,4 and fig-3)

Discussion

Dental caries is an irreversible microbial disease of the calcified tissues of the teeth, characterized by demineralization of the inorganic part and destruction of the organic substance of the tooth, which often leads to cavitation [16]. According to a recent survey by the Global Oral Health Data Bank, dental caries is a widespread disease with prevalence ranging from 49% - 83%. In India the incident rate of dental caries is approximately 60-65 % which is rising day by day. Continuous efforts have been made to reduce its prevalence, it is still prevalent, especially in the lower socioeconomic groups [17]. The etiological factors that directly contribute to the progression of dental caries include the diet, dental plaque, susceptible dental hard tissue, and time [18].

Caries management includes two aspects—controlling caries risk factors and different measures for managing individual lesions [19]. (fig-1)

The caries management philosophy has shifted from the traditional surgical manners to minimal intervention dentistry. Minimal intervention dentistry aims to extend the longevity of natural teeth. It places the non-restorative approaches as a priority.

Sample number	*Micro hardness number before 10% NaF treatment and Remineralizationn (VHN).*	*Micro hardness number after10% NaF treatment and Remineralizationn (VHN).*	*Sample number*	*Micro hardness number before PAMAM Treatment and remineralizationn (VHN).*	*Micro hardness number after PAMAM Treatment and remineralizationn (VHN).*
1	413	440	31	382	404
2	376	431	32	368	486
3	369	442	33	410	476
4	379	422	34	429	487
5	391	421	35	415	463
6	454	464	36	381	447
7	423	453	37	383	449
8	387	428	38	388	449
9	426	471	39	423	489
10	376	427	40	387	446
11	374	442	41	385	474
12	379	420	42	412	447
13	391	430	43	446	501
14	395	455	44	491	524
15	378	443	45	392	479
16	363	408	46	429	505
17	385	462	47	407	432
18	420	466	48	443	468
19	401	422	49	415	456
20	414	475	50	379	435
21	435	454	51	424	473
22	451	499	52	422	461
23	401	476	53	426	463
24	439	468	54	417	501
25	432	447	55	444	471
26	408	437	56	458	479
27	446	488	57	398	460
28	392	474	58	391	425
29	394	463	59	408	481
30	393	462	60	374	419

Table 1

Group	Parameter	Sample size	Minimum/ Maximummicro hardness (VHN)	Mean (Standard Deviation/standard error)	Mean	t value	P value
Group A-10%NaF treatment before reminerali zation	micro hardness for Group A Before pre-treatment with 10% NaF and remineralization	30	363/454	25.899/4.7290	46.833	10.807	<0.001**
	micro hardness for Group A After pre-treatment with 10% NaF and remineralization	30	408/499	22.55/4.117			
	Mean difference of micro hardness for Group A before and after pre-treatment with 10% NaF and remineralization	30	10/82	19.85			

**-highly significant (p<0.001).

Table 2

Group	Parameter	Sample size	Minimum/ maximum	Standard Deviation/standard error	Mean difference	t value	P value
PAMAM Group-B	Micro hardness of articifial caries like lesions of enamel before PAMAM remineralization.	30	368/492	28.06/5.122	54.10	23.07	<0.001**
	Micro hardness ofartificial caries likelesion of enamel after PAMAM remineralization.	30	404/524	27.04/4.938			
	Difference between Micro hardness of artificial caries like lesion of enamel before and after PAMAM remineralization.	30	21.00	118.00			

**-highly significant (p<0.001).

Table 3

	NaF		PAMAM		Mean	P value
	Mean	SD	Mean	SD		
Micro hardness of artificial caries like lesionof enamel after remineralization	449.67	22.55	465.00	27.04	8.267	0.020*

*-Significant (p<0.05).

Table 4

After the onset of the COVID-19 pandemic, a focus was placed on the reduction of aerosol- generating procedures aimed to minimise the risk of cross-infection amongst oral health care workers and patients and emphasis is laid more on remineralization of early lesions. After the onset of the COVID-19 pandemic, a focus was placed on the reduction of aerosol-generating procedures aimed to minimise the risk of cross-infection amongst oral health care workers and patients and emphasis is laid more on remineralization of early lesions. To achieve this, minimally invasive strategies for the management of caries lesions are preferred. These interventions include use of tooth paste, varnishes, and sealant containing remineralising agents like fluoride, ACP, CPP/ACP and CPP/ACFP, Nano-hydroxyapatite resin [20]. Amongst all these the researches in the literature states that fluoride treatment remains the best remineralising method for early enamel caries [21]. If fluoride molecule (F) is present in the vicinity of a demineralising HAP crystals, during remineralization fluorapatite crystals are formed which are more resistant for subsequent demineralization [22]. If fluoride molecule (F) is present in the vicinity of a demineralising HAP crystals, during remineralization fluorapatite crystals are formed which are more resistant for subsequent demineralization [23]. So much safer, biomimetic remineralising agent with good remineralising potential are needed for remineralising dental tissue for examples bioactive glass, Pchi-ACP, Amelogenin - Chitosan system, oligopeptide and PAMAM. Chris Ying Cao et al (2015) showed that PAMAM could induce the formation of HAP crystals on demineralised enamel and can induce nanorod-like HAP remineralisation on acid-etched enamel [24].

So the present study was a small step towards finding safer remineralising agent with better remineralising potential. This study was attempted to compare remineralising potential of PAMAM a biomimetic agent with old and efficient remineralising agent that is Sodium Fluoride.

Pre-Treatment With 10% Sodium Fluoride and Remineralization. (TABLE-1, 2 AND Figure-8)

The micro hardness values of artificial caries like lesions induced by demineralization were 363VHN (minimum) and 454 VHN (maximum) with mean 402.83 VHN. The micro hardness value after treatment with 10% NaF and remineralization were tabulated. They were as 408 VHN (minimum) and 499 VHN (maximum) with mean 449.67VHN. Calculated mean difference in micro hardness between pre and post treatment with10 % NaF and remineralization was 46.84 VHN which was statistically significant with t value 10.80.

Figure 7: Group-A and Group-B in Demineralization solution.

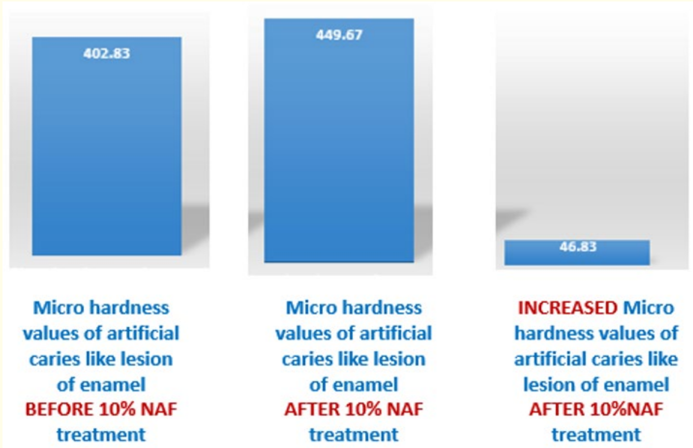

Figure 8: Comparison of Micro hardness of artificial caries like lesion of enamel among the 10% NaF (Group-A) BEFORE and AFTER remineralization.

This increase in micro hardness values of artificial caries like lesion after pre-treatment with 10% NaF and remineralization was due to absorbtion of fluoride into the enamel and formation of fluor hydroxyapatite crystals which are harder than HA crystals These fluor hydroxyapatite crystals are more resistant to the acidic environment and prevent further progression of caries [25].

In Farooq I et al (2021) study the micro hardness values of pre and post treatment with sodium fluoride and remineralization showed mean difference of 61.13VHN which in accordance with our study [26]. According to Bandekar S et al (2019) [27] and Basir L et al (2020) mean difference of micro hardness values of pre and post treatment with sodium fluoride and remineralization of enamel caries like lesion were 53.7VHN and 53VHN [28] respectively which are in favour of our study.

In Jabbarifar SE et al (2011) study and, Baothman A et al (2017), study micro hardness of artificial caries like lesions of enamel pre and post treatment of sodium fluoride treatment and remineralisation showed mean difference of 45.40 VHN and 45.1VHN respectively which is in accordance to our study [29, 30].

S. Lata et al (2010) in their vitro study on enamel block, used fluoride varnish containing 0.77% fluoride and found that Fluoride had remineralising potential of enamel caries like lesions with mean difference in micro hardness as 40.35 VHN, this is in favour of our study. In the present study mean micro hardness value after treatment with 10%NaF and remineralization was more which might be due concentration of fluoride we used was more as compared to their study [31].

Similar results were seen in studies done by Puangpanboot N. et al (in 2018), with mean difference 17.73VHN [32] Farhad F et al (2021), with mean difference 23.25VHN [33] Alkhalifah TS et al (2022), with mean difference 20.1VHN [34] Rafiee A et al (2022) in with mean difference in micro hardness value as 22.95VHN [35] Remi RV et al (2023), with mean difference 26.51VHN [36].

Pre-Treatment with PAMAM and Remineralization. (TABLE-1, 2 AND Figure -9)

The micro hardness values of artificial caries like lesions before treatment with PAMAM were tabulated. Minimum value of micro hardness was 368 and maximum was 492 VHN with mean value of micro hardness was 410.90 VHN.The micro hardness values after treatment with PAMAM and remineralization were tabulated. Minimum value of micro hardness was 404 and maximum micro hardness value was 524 VHN with mean micro hardness value 465VHN. The mean difference between pre and post treatment with PAMAM micro hard ness was 54.10 VHN with t value 12.84.

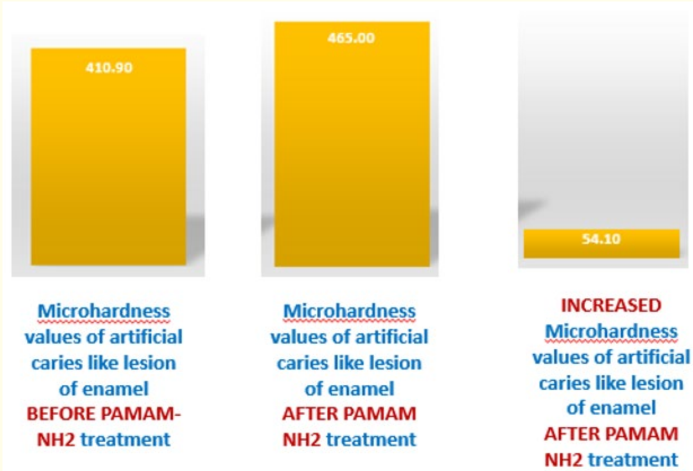

Figure 9: Comparison of Micro hardness of artificial caries like lesion of enamel among the PAMAM (Group-B) BEFORE and AFTER remineralization.

It showed statistically significant increase in micro hardness of artificial caries like lesion of enamel among the PAMAM (Group B) before and after remineralization. The micro hardness of caries like lesion of enamel of PAMAM (Group B) increased because when the samples were pre-treated with PAMAM which gets adsorbed onto the artificial like caries like lesions of enamel surface and provides nucleation sites and act as mineralisation template for HAP. It also regulates the growth of crystals and induces nanorod-like HAP with high uniformity on Demineralized enamel [24]. This proves that PAMAM has remineralising potential of artificial caries like lesions of enamel which is in favour of previous studies.

Similar in vitro studies were done by Fan M. et al (2020) who showed that the PAMAM dendrimer with different terminal groups have remineralising potential on artificial enamel caries like lesion. The mean difference between pre and post treatment PAMAM micro hardness values was 76.42 which is in accordance with our study [13].

Chen M et al (2014) carried out Scanning electron microscopic and X-ray diffraction (XRD) study for the evaluation of remineralization potential of PAMAM done on acid-etched tooth enamel of human and animal teeth. On XRD they observed the type and orientation of the newly formed crystals of the tooth enamel which were similar to normal enamel. On SEM they found that the gaps between the prism-like structures of the eroded enamel surfaces had disappeared after remineralization. This was because new crystals grew on and between the prisms [37]. Wu D et al used Knoop hardness tester for evaluation of remineralising potential of PAMAM and found that PAMAM regulate the remineralization process to form ordered new crystals and produce an enamel prism-like structure that is similar to that of natural tooth enamel [38].

Scanning electron microscopic study of Liang Chen et al in (2015) [39] and Bapat RA et al (2019) [40] - showed that in the presence of PAMAM, a large number of new crystals were formed on the etched enamel. On the transverse section, the gaps between enamel prisms were restored by new-grown crystals. Also PAMAM induces regeneration of HA crystals which has shown to increase the hardness of acid etched enamel which was similar to the hardness of natural tooth enamel.

Comparison of increase in micro hardness of artificial caries like lesion of enamel between 10% naf & pamam treated lesions after remineralisation (Table-3 and 4 and Figure- 10)

Calculated mean difference in micro hardness between pre and post treatment with10 % NaF and remineralization was 46.84 VHN which was statistically significant with t value 10.80 and the mean difference between pre and post treatment with PAMAM micro hard ness was 54.10 VHN with t value 12.84. The increase in VHN was higher in PAMAM pretreated and remineralized samples as compared to 10 % NaF pretreated and remineralized samples.

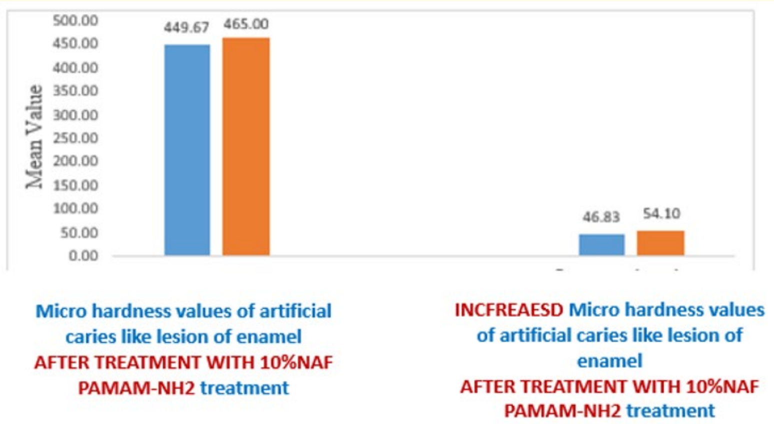

Figure 10: Comparison of Micro hardness of artificial caries like lesion of enamel BETWEEN the 10% NaF (Group-A) and PAMAM (Group- B) BEFORE and AFTER pretreatment with 10%NaF and PAMAM and remineralization.

The mean difference in increase of micro hardness of the two groups was 8.267VHN which was statistically significant with p value 0.020 ($p<0.001$). Which indicates that the enamel pre- treatment with 10% NaF and PAMAM followed by remineralization significantly increased the Vickers micro hardness number of artificial caries like lesion of enamel. It also shows that PAMAM pre-treatment has greater potential of remineralisation as compared with pre- treatment with 10% NaF. The reasons for greater remineralisation potential of PAMAM than 10% NaF might be

- PAMAM dendrimers are highly branched polymers. These dendrimers of PAMAM grasps calcium and phosphate ions and induces novel mineral crystal regeneration in demineralised enamel [41].
- PAMAM-NH2 has greater remineralising potential of artificial caries like lesion of enamel as it is positively charged it get easily adsorbed on negatively charged enamel through electrostatic forces [18].
- PAMAM could induce the formation of HAP crystals on demineralised enamel and can induce nanorod-like HAP remineralisation on acid-etched enamel [49].
- The new crystals created by the PAMAM organic templates had the same structure, orientation, and mineral phase of the intact enamel, with the HAP nanorods closely paralleling the original prisms, so the micro hardness increased by PAMAM is greater as compared to 10 % sodium fluoride [48].

In the present study, observed that pre-treatment with 10% NaF and PAMAM increases the micro hardness value of artificial caries like lesions of enamel. Pre-treatment with PAMAM &remineralisation has greater remineralising potential than the pre-treatment with 10% sodium fluoride & remineralisation with statistical significance.

Conclusion

- Dental caries is one of the most prevalent and ubiquitous non-communicable diseases worldwide [42]. Tooth demineralization can be arrested or reversed when remineralization agents are applied to incipient carious or non-cavitated carious lesions. Fluoride has been widely recommended as a remineralization agent for preventing early enamel carious lesion. Because of adverse effect of fluoride, non- fluoridated products has been developed to enhance enamel remineralization [43]. So the present study was performed to evaluate and compare remineralising potential of PAMAM and 10% sodium fluoride and concluded that.

- There was increase in micro hardness of artificial caries like lesion of enamel in 10% NaF and PAMAM pre-treated group after remineralization was statistically significant.
- There was increase in micro hardness of artificial caries like lesion of enamel in PAMAM pre-treated group after remineralization was statistically significant as compared with 10% NaF pre-treated group after remineralisation with mean difference 8.267 VHN & p value 0.020 ($p<0.001$).

This is the first study to compare the micro hardness of artificial caries like lesion of enamel between 10% NaF & PAMAM treated lesions after remineralisation. Therefore PAMAM can be used as pre-treatment regime before remineralization treatment for better and regenerative and reparative remineralisation of enamel caries. Further vivo studies need to be performed to clinically establish the effect of PAMAM pre-treatment on remineralization with variable concentration and application.

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