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REMINERALIZING EFFICACY OF TRI-CALCIUM PHOSPHATE, NANO HYDROXYAPATITE AND RESIN INFILTRATION SYSTEM ON EARLY CARIES LESIONS

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ABSTRACT

Aim : The present study was carried out to compare between non-invasive treatment modalities using (fluoride varnish (FV) and Nano-HAP based reminerlizing agents) and minimal-invasive treatment modalities using (resin infiltrant materials) on surface micro-hardness, surface topography and colour stability of WSLs. **Methods** A total 80 sound human permanent premolars were used in this study. The specimens were divided into four equal groups 20 teeth for each group. According to treatment modality group A_0: resin infiltration material, group A_1 nano hydroxyapatite based remineralizing agent, group A_2 fluoride varnish and group (A_3) artificial saliva as control group. The surface microhardness (SMH) of each specimen was assessed using Vickers microhardness tester. Scanning electron microscope were used to assess surface morphological characters of enamel surface at different stage. Color stability of enamel surface at different stage were assessed by spectrophotometer. **Results**. Both resin infiltration material and Nano hydroxyapatite based remineralizing agents showed the highest statistically significant mean surface microhardness values and highest statistically significant mean of color stability **Conclusion**: Nano hydroxyapatite based remineralizing agent and resin infiltration system are the most effective treatment modalities for incipient carious enamel lesions.

KEYWORDS: Reminalization,Whit spot lesions, resin infiltration, Nano hydroxyapatite

INTRODUCTION

Dental caries is a major public health problem which commonly affecting children in their early childhood. It has a negative impact on children's oral as well as general health. It's caused by an imbalance between demineralization and remineralization through a dynamic and continuous process that occurs throughout the whole life of a tooth.[1,2]

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Enamel carious lesions are characterized by mineral loss in the subsurface body of the lesion, whereas the surface remain highly mineralized. During the progress of sub-surface caries lesions, minerals are dissolved out of the enamel, resulting in increased micro-porosities within the remaining calcified tissues that appear clinically as "white-spot" lesions.[3] White spot lesions (WSLs) are the earliest macroscopic evidence of enamel caries, which appear as opaque white areas with a chalky appearance on smooth tooth surfaces, if left untreated will collapse into non-cavitated lesions, and finally to cavitated lesion.[4,5,6] In the recent years the treatment has been shifted from the large invasive technique to noninvasive or minimal invasive preventive techniques.[1, 7]

Several methods as fluoride therapy, oral hygiene measure and diet control are used to prevent enamel caries but they are not efficient enough to completely prevent tooth decay[,8,9] Fluoride (F) plays important role in the prevention and control of dental caries by being incorporated into demineralized enamel, changing carbonated apatite to a fluoro-apatite-like form which makes the hard tissues more acid resistant[10]

Different forms of calcium phosphate-based delivery systems were developed as alternative remineralizing agents, it can be categorized into one of the three types; crystalline, un-stabilized amorphous, or stabilized amorphous formulations of calcium phosphate which enhance the remineralization process and improve mechanical properties of the demineralized enamel [11,12]. The Nano-hydroxyapatite (Nano-HAP) is considered among the crystalline calcium phosphate remineralizing agents. It is one of the most biocompatible and bioactive materials[13].It contain calcium (Ca) and nanophosphate in the crystalline form of hydroxyapatite (HA) and fluoride withthe concentration of 9000 ppm. Phosphate nanocrystals are smaller than 100nm, which can increase bioactivity and lead to an increase in surface areaand the ability of moisture absorption in hydroxyapatite nanoparticles[11].

On the other hand, resin infiltration (tissue -preserving approach) was introduced to arrest and control carious lesion, this concept aims at occluding the highly porous structures of white spot lesion by means of low-viscosity resins. This approach truly follows the principles of minimally invasive dentistry[14]. Icon infiltration resin is the brand name of noval product which is believed to be minimally invasive. It replaces the lost minerls in the porous enamel with resin compound, using hydrophilic and low viscosity, light-cured resins that can penetrate WSLs subsurface micropores, inhibiting the diffusion pathway to cariogenic bacteria and their products and preventing further lesion progression[15,16]

Thus, the aim of the present study to compare the remineralization potentials between non-invasive treatment modalities using (calcium phosphate and Nano hydroxyapatite based reminerlizing agents) and micro-invasive treatment modalities using (ICON infiltrant materials) on surface micro-hardness of incipient carious enamel lesion,

MATERIALS AND METHODS

Sample size calculation

The sample size was calculated using Epi-info software statistical package created by World Health Organization and center for disease Control and Prevention, Atlanta, Georgia, USA version 2002.

The criteria used for sample size calculation were as follows:

- 95% confidence limit.
- 80% power of the study
- Ratio of 3:1 for treatment and control group
- Expected outcome in treatment group 95% compared to 65% for control group.

The sample size based on the previously mentioned criteria was found at N more than52

Study sample

The study was conducted on 80 sound extracted premolars collected from the Oral Surgery and Maxillofacial Department in Faculty of Dentistry at Tanta University and divided into four equal groups and treated as follow

Group A0: 20 premolars were treated with resin infiltrant system (ICON)

Group A1: 20 premolars were treated with Nano-Hydroxy apatite

Group A2: 20 premolars were treated with fluoride varnish

Group A3: 20 premolars were stored in artificial saliva (control group)

Preparation of the samples

The premolars were sectioned horizontally at 2 mm below the cement-enamel junction to remove the roots using diamond cutting disk (0.3mm Thickness) mounted on straight hand piece Custom made cylindrical plastic molds of dimensions of 10 mm in Diameter and 15 mm in depth were used to contain self-cured acrylic resin which was poured into them. Each specimen was embedded in and mounted horizontally on top of partially set acrylic resin, then allowed to set. So that, the buccal surface was facing Upward and parallel to the base of the mold. A standardized window, (dimension of 5mm mesio-distaland 5 mm inciso-cervical dimensions) was created on the buccal enamel surface of each specimen Figure 1

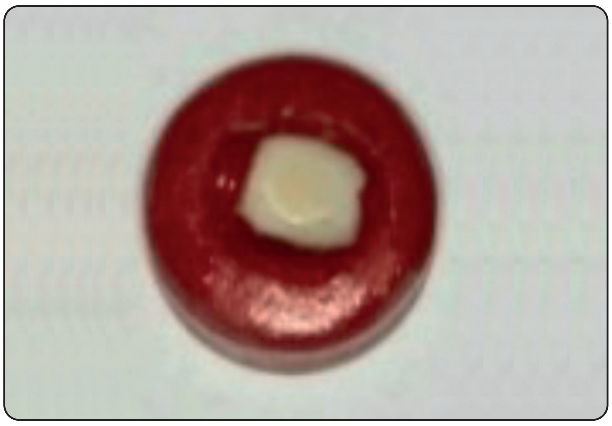

Fig. (1) A standardized window

A standardized window, of 5mm mesio- distal and 5 mm inciso-gingival dimensions, was created on the buccal enamel surface of each Specimen

Grouping of Specimens

As illustrated in table (2)

The eighty premolars were divided randomly into four equal groups, 20 premolars each, according to the surface treatment modality that was done,

- **Group A_0:** Was treated with resin infiltrant system (ICON)
- **Group A_1:** was treated with Nano – hydroxyapatite
- **Group A_2:** was treated with fluoride varnish
- **Group A_3:** was stored in artificial saliva as a control group

In each group, the surface micro hardness, surface topography by scanning electron microscope and colourof each specimen was assessed

- At base line of sound untreated enamel (B0)
- After demineralization (B1)
- After treatment (B2)
- After exposure to acidic challenge (B3).

Caries-like lesions induction

All specimens were immersed in a demineralizing solution. The solution was composed of $CaCl_2$ = 2.2mM, NaH_2 PO_4 = 2.2 mM, Lactic acid = 0.05 M, Fluoride = 0. 2ppm, Solution was adjusted with 50% NaOH to a pH 4.5[2]. The specimens were soaked in a proportion of 2 ml solution/mm2 of exposed enamel individually in tightly sealed glass containers[9, 10] Thus, the total amount of demineralizing solution was 50 ml for each specimen. The pH of the solution was checked and verified using a pH meter and re-adjusted if any change has occurred before adding it to the specimens. The specimens were stored for 72 hours, in an incubator at a temperature of 35 degree[11]. The demineralizing solution was replaced every 24 hours by a freshly prepared solution

Application of Treatments

The specimens were removed from the demineralizing solution, washed with distilled water for 10 seconds and dried on absorbent paper. All treatments were applied following the manufacturers' instructions

Artificial Saliva

After application of the tested materials, all specimens were immersed in artificial saliva [2], and the pH was set at a level of 7. 2. The specimens were soaked in a proportion of 50 ml of artificial saliva for each specimen. in tightly sealed glass containers. The pH of the solution was checked using a pH meter and re-adjusted if any change has occurred before adding it to the specimens. The specimens were stored in artificial saliva for four weeks at a temperature of

PH CYCLE

All specimens were subjected to pH cycling model over a period of 60 day by immersing in demineralizing solution for 3 hours and in artificial saliva for 21 hours, PH of demineralizing solution checked daily and readjusted using PH meter

1-Assessment of Surface Micro-hardness (SMH)

The surface micro hardness (SMH) was assessed at baseline of sound un treated enamel (SMH/Baseline), after demineralization (SMH/ Demineralization), after treatment for four weeks (SMH/Treatment) and finally, after being subjected to acidic (SMH/Acidic Challenge). Assessment of Surface micro-hardness was performed using a Vickers micro hardness tester with a Vickers diamond indenter degree figure 2

The surface micro hardness calculation was obtained using the following equation [18]

Fig. (2): Vickers Microhardness tester with diamond indenter.

HV= 1:8544 P/d2

Where, HV is Vickers hardness in Kgf/mm2; P is the load in Kg force; d is the average length of the diagonal left by the indenter in mm2.

Also, percentage of surface microhardness recovery (%SMHR) was calculated

After treatment [4,19,20] using the following formula:

%SMHR= ((SMHT-SMHD) / (SMHB-SMHD)) ×100

Where …….

***-SMHT** is the surface microhardness after treatment*

***- SMHD** is the surface microhardness after demineralization*

***- SMHB** is the surface microhardness at baseline.*

In addition,

Percentage of surface microhardness change (**%SMHC**) was calculated [14,21] using the following formula:

%SMHC= ((SMHF-SMHI) / (SMHI)) ×100

Where…….

***SMHF** is the Final surface micrhardness;*

***SMHI** is the Initial Surface microhardness.*

Fig. (3) Scanning electron microscope, surface topography

2-Scanning electron microscope exmaination

Exmination was carried out at the Armed Research Center,Ministery of Defense.

For SEM examination two specimen in each group were randomly selected and evaluated for surface changes. The scanning electron microscope was used to determine and compare the morphological varation between the different treated specimen, for comparison, the surface of enamel were examined at baseline, after deminerlizing soloutin, after application of material, and after reminerlizing, deminerlizing cycle.images were obtained at 500X magnification **figure 3**

3- Assessment of colour stabilty

Exmination was carried out at calibration laboratory, GizaThe CARY 5000 spectrophotometer will be used to assess the colour of white spot lesions and the adjacent sound enamel(23.24),using the Munsell system: CIE colour parameters (ΔE): L, a and b.

$$\Delta E = (\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2})^{(1/2)}$$

L refers to the lightness coordinate, and its value ranges from 0 for perfect black to 100 for perfect white. a* andb* are the chromaticity coordinates in the red-green axis and yellow-blue axis, respectively*(22,25)

RESULTS

At base line B0, there were no statically significance difference between mean surface microhardness of all treatment modalities 387.71, 388.51, 386.17 and 388.21 respectively. P <.05.

Similarly, after demineralizing solution B1, the mean surface microhardness of group A0 was 263.22, for group A1 was 258.20, for groupA2 was 256.97 and for group A3 was 261.06 respectively and P <.05. and the difference among them didn't reach the level of statistically significant difference.

Regardly, immediately after treatment B2, the mean surface microhardness of group A0 and group A1 367.41, 363.77 respectively P>.05.It was significantly higher than the corresponding values of group A2 and group A3 334.20, 273.46 respectively P>.05

TABLE (1) Mean and Standard Deviation (SD) of surface Microhardness ofdifferent treatment modalities within each stage

Treatments	Group A_0	Group A_1	Group A_2	Group A_3	p-value
	Mean ±S.D	Mean ±S.D	Mean ±S.D	Mean ±S.D	
B_0	387.71±6.41	388.51±9.59	386.17±8.01	388.21±5.53	0.999 NS
B_1	263.22±5.80	258.20±4.47	256.97±7.05	261.06±6.29	0.981 NS
B_2	367.41^a±6.26	363.77^a±7.81	334.20^b±5.87	273.46^c±8.93	≤0.001**
B_3	336.45^a±6.51	332.32^a±4.74	309.09^b±8.93	233.07^c±5.73	≤0.001**

TABLE (2): Mean and Standard Deviation (SD) of percentage of change in surface Microhardness for different treatment modalities at different stages.

Treatments	SMHC %		
	SMHC% after demineralization (B_1)	SMHC% after treatment (B_2)	SMHC% after acid challenge (B_3)
	Mean ±S.D	Mean ±S.D	Mean ±S.D
Group A_0	-32.59±6.31	41.63^a±13.35	-13.43^a ±4.09
Group A_1	-33.87±4.69	40.69^a±9.41	-14.64^a±2.18
Group A_2	-33.79±5.08	31.04^b±12.22	-20.28^b±6.69
Group A_3	-33.31±4.89	5.25^c±9.88	-40.49^c±7.43
p-value	0.862 NS	≤0.001**	≤0.001**

There is a significant difference at p-value <0.05*, highly significant difference at p-value <0.001**.

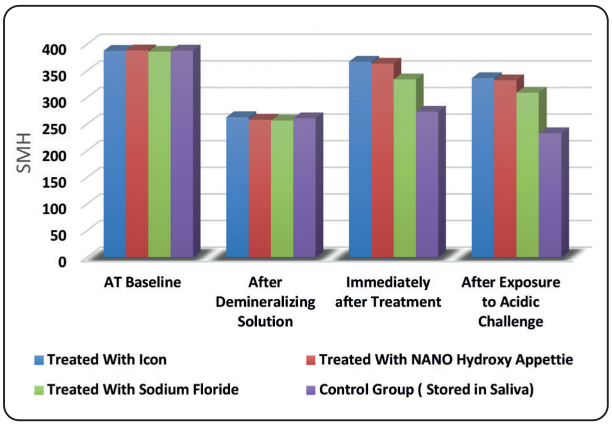

Fig. (4): bar graph showing mean surface Microhardness of different treatment modalities within each stage.

Percentage of change in surface Microhardness

Superscripts with different letters within each column indicate statistically significant difference.

The results showed that there was no statistically significant difference between percentages of change in Mean of surface micro hardness for all groups after demineralization compared to baseline.

The Mean of percentage of change in surface micro hardness for different treatment modalities after treatment compared to after demineralization revealed that group A_0 showed the highest Mean of percentage of change in micro hardness 41.63, followed by group A_1 40.69, with no statistically significant difference between them.

Additionally, the corresponding value of them were statistically higher than mean percentage of recovery of group A_2 31.04. Moreover, all treatment groups were statistically significantly higher than group A_3 5.25.

Using Tukey test revealed a significant difference in SMHC% when comparing group A_0 or group A_1 versus group A_2 or group A_3 and group A_2 & group A_3. On the other hand, the difference not significant when comparing group A_0 & group A_1.

The Mean of percentage of change in surface Microhardness for different treatment modalities after exposure to acidic challenge compared to base line revealed that group A_0 showed the lowest mean of percentage of change in microhardness -13.43, followed by group A_1 -14.64, the two groups were statistically significant lower than the group A_2 -20.28

Moreover, all treatment groups were statistically significantly lower than group A_3 -40.49.

Using Tukey test revealed that the difference in SMHC% not significant when comparing group A_0 & group A_1. On the other hand there were a significant difference in SMHC% when comparing group A_0 or group A_1 versus group A_2 or group A_3 and group A_2 & group A_3.

Scanning electron microscop morphological characters

All tested groups showed increased of density of hydroxyapatite crystals. **Resin infiltration system group** the enamel surface exhibited thickening of the inter rod substance with decreased fish scale pattern and revealed smooth surface topographic features of normal unetched enamel **fig. 5A**. **Nano hydroxyapatite group** the enamel surface began to heal by deposition of calcium and phosphate on the prism peripheries in regular and uniform pattern over the enamel surface **fig. 5B. Also, sodium fluoride group** the enamel surface appeared undisturbed and showed irregular in discriminate erosion of enamel prism so there was decreased in fish scale pattern

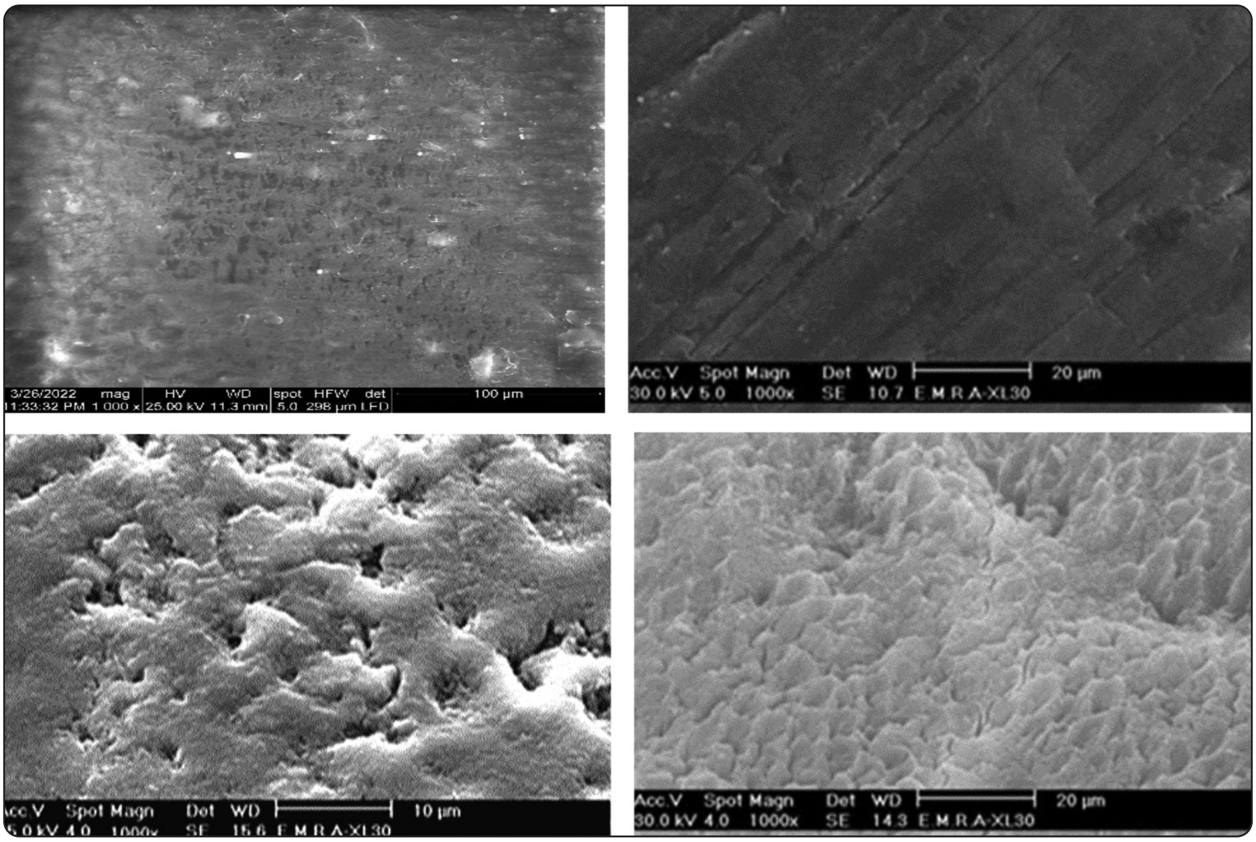

Fig. (5) Show morphological characters after treatment with different materials: A) resin infiltration system, B) Nano hydroxyapatite, C) sodium fluoride, D) control group.

TABLE (3) Mean and Standard Deviation (SD) of colour change of different treatment modalities within each stage

Treatments	GROUP (A_0)	GROUP (A_1)	GROUP (A_2)	Group (A_3)	p-value
	Mean ±S.D	Mean ±S.D	Mean ±S.D	Mean ±S.D	
B_0	9.02±.61	8.96±.35	8.72±.6	8.41±.47	0.9834 ns
B_1	5.21±.34	5.07±.33	4.63±.72	4.01±,45	0.8947 ns
B_2	8.56^{a}±.14	8.17^{a}±.46	7.19^{b}±1.3	$4,32^{c}$±.59	≤0.001*
B_3	7.03^{a}±.51	6.71^{a}±.5	4.21^{b}±.5	3.12^{c}±.5	≤0.001*

There is a significant difference at p-value <0.05*, highly significant difference at p-value <0.001**. Superscripts with different letters within each column indicate statistically significant difference.

fig. 5C. **control group** showed increased in density but more prominent fish scale pattern than fluoride varnish group **fig. 5D.**

Assessment of colour stability

As shown in table 13, at base line and after demineralization the mean color change of all treatment modalities showed no statistically significant differences among them.

After treatment the mean of color stability of group A_0 and group A_1 8.56, 8.17 respectively, P<0.05 were significantly higher than corresponding value of group A_2 and group A3 7.19,4,32 respectively, P<0.05.

Using Tukey test revealed a highly significant difference of color stability after treatment when comparing group A_0 or group A_1 versus group A_2 or group $A_{3,}$ and when comparing group A_2 & group A_3. On the other hand, the difference not significant when comparing group A_0 & group A_1.

After exposure to acid the mean color stability of group A_2 and group A_3 4.21, 3.12 respectively, P<0.05 were significantly lower than corresponding value of group A_0 and group A_1 7.03, 6.71 respectively, P<0.05.

Then using Tukey test revealed non-significant differences on colour stability after acid challenge when comparing group A_0 & group $A_{1.}$ On the other hand the difference were highly significant when comparing group A_0 or group A_1 versus group A_2 or group $A_{3,}$ and when comparing group A_2 & group A_3.

DISCUSSION

Treatment of white spot lesions can be introduced by using either fluoride remineralizing agents in the form of dentifrice, mouth rinses, foams, gels, varnishes and bonding agents or non-fluoride remineralizing agents which could be accomplished through crystalline, un-stabilized amorphousca phosphate systems or stabilized amorphous formulations of calcium phosphate such as Nano-hydroxyapatite[17,18]. these remineralizing agentscapable to arrest, reharden and revert enamel caries to healthy enamel condition through a remineralization process involving the diffusion of minerals into the defective tooth structure [19]. In addition. Resin infiltration was introduced for treatment of white spot lesions by occluding highly porous structures of WSLs with low viscosity resins

The selected premolars were incubated at 35°C throughout all the steps of the study to mimic optimum temperature for storage this is in agreement with study conducted by Arslan et al[20]. Artificial saliva were selected in this study as storage media to partially simulate clinical condition and prepared according to the composition specified by lata et al[21], Schipper et al[22] and was daily replaced to minimize bacterial growth. In this study, demineralization was induced on enamel surfaces to mimic enamel condition of white spot lesions (wsls). Each specimen was placed in amount of demineralizing solution of PH 4.5. Tramontino et al88 suggested a proportion of 2 ml solution for each mm2 to avoid the production of under-demineralized or over-demineralized enamel lesions. so 50ml is sufficient enough for induce WSLs in a standardized window (5mm *5mm).

Moreover, demineralizing solution cycle was 72 hours sufficient for storing specimens, and this was confirmed by study conducted by Lata et al 14, Majithia et al [23] to create subsurface demineralization of approximately150 microns width, with an intact surface, thus simulating an early enamel lesion. Remineralization was used to evaluate the effects of the different treatment modalities as it offers the opportunity to effectively monitor caries-prevention on dental hard tissues on a short-time basis, thus simulating a best treatment option this is in consistence with study conducted by

Tschoppe etal[24].While the second demineralization simulated the acidic challenge was performed by using the same demineralizing protocol used for inducing caries-like lesions to evaluate the stability of the treated lesions this is in agreement with Torres et al4, Elkasass et al [19]

The results of the present study revealed that the mean surface microhardness and surface microhardness recovery of Resin infiltrant agents was the highest significant values compared to other groups. This result is in agreement with previous studies conducted by Arslan et al [20], Liuet al [25], Attia [26], Ahmed [27] and AbdEllahy et al [28] they concluded that Icon resin infiltration is significantly superior to fluoride varnish treatment. Resin infiltration is dependent on the occlusion and impregnation of the pores within the body of the caries lesion by replacing lost minerals with low viscosity light curing resin as reported in study by Paris,[29] so the infiltrant is soaked into the lesion body and occludes diffusion pathways for acids and dissolved minerals. The result wasn't coincide with study conducted by Montasser et al, who found that no significant difference between ICON resin infiltration and Fluoride varnish. This contradiction could be attributed to the difference in the formulas used to induce enamel demineralization, difference in sample size and demineralization protocol.

Concerning Nano-HAP in this study, it show highest statistically significant surface micro hardness values and the highest statistically significant surface micro hardness recovery parallel with resin infiltration system with no significant difference between two groups. This results is coincided with previous study conducted by Reddy et al [30], Grewal et al [31] and Kooshki et al [32], they concluded that found that Nano-Hydroxyapatite had highest remineralization potential and mineral gain than fluoride varnish. On the other hand, the result is in contrary to the results obtained by, Comar et al [33], Nozari et al [34] they concluded that there were no significant differences between Nano-hydroxyapatite and fluoride varnish. This contradiction could be attributed to the difference in nature of examined specimens, the difference in form,composition and properties of the used materials might have been reflected by differences in the results.

Concerning to color stability RI system showed the highest color stability when compared to other treatment modalities this result is in agreement with study conducted by Prasada et al [35], Abdel Hafez et al [36] who concluded that icon can improve esthetic characters of WSLs and lowes tcolor change at all time of measurement. This result was confirmed by the explanation of Ajami et al who reported that RI system provide more colorstability over eight week duration. On the other hand, the results of this study doesn't coincided with the results obtained by Borges et al [37], Cohen et al [38] evaluated color stability ofresin infiltration compared to Fluoride varnish and they reported that RI hasmore stain suscbtabilty than fluoride varnish. Difference in result could be attributed to difference in nature of examined specimens and the difference in the methodologies which may lead to difference in results.

Nano-HAP show high color stability parallel to resin infiltration more than other groups andthis result is coincided with the result obtained by Khater et al [39] who concluded that Nano-HAP has least color change when compared to remineralizing agent. On the other hand this results wasn't coincided with findings reported by, Hammad et al [40] they concluded that Nano-hydroxyapatitecant restore natural appearance or improve color of White spot lesions when compared with resin infiltration. Difference may be due to difference incomposition, mechanism of action and properties of the used materials.

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