White spot lesions appear clinically, which is a first sign of enamel caries. It is a subsurface area with most of the lost mineral beneath the relatively intact enamel surface. It is also known as “initial” or “incipient” lesion. It is softer than the adjacent sound enamel and is much whiter when dried with air, which precedes cavitation. In these lesions, the mineralized layer is lost. This makes the translucent enamel porous and changes its refractive index (RI). Remineralization is one of the noninvasive treatment options for early carious lesions, e.g., through fluoridation, improved oral hygiene, or other processes. A number of methods and agents are available to remineralize early enamel lesions. The mineral-rich concentrates can be topically applied for remineralization of incipient enamel lesions. These provide calcium (Ca$^{2+}$) and phosphate (PO$_4^{3-}$) ions that can easily diffuse through porous enamel in order to remineralize incipient enamel lesion. Milk and milk products were considered to prevent the development of caries due to the existence of casein, calcium, and phosphate contents. Later investigations on the purpose of casein and calcium phosphate concentrations on remineralization concluded that casein phosphopeptide (CPP) complexes stabilized the calcium phosphate and remineralized the incipient caries lesions. The combination of CPP–ACP and fluoride also brings about the remineralization of the incipient caries lesions. A noninvasive technique, application of CPP–ACP with fluoride (CPP–ACP Plus), is considered to be a better alternative to treat early enamel caries lesions. CPP–ACP Plus contains fluoride which helps in the formation of fluorhydroxyapatite. This complex formed is more resistant to undergo remineralization as compared to hydroxyapatite.

A new concept called caries infiltration to treat white spot lesions was introduced. Caries infiltration product ICON-DMG™ was introduced in the year 2009 in Germany; it is a low-viscosity
resin that fills, strengthens, and stabilizes demineralized enamel, without causing any damage to healthy tooth structure. It would also inhibit caries progression in initial carious lesions.

Evaluating the physical property such as hardness of initial enamel caries lesions treated with different techniques and materials will yield a better option to treat these lesions.

Remineralization of early enamel caries was studied extensively in the literature which included fluoride therapy, CPP–ACP, CPP–ACP plus application, but scarce research was focused on the evaluation of physical parameters of initial enamel caries treated with CPP–ACP plus and resin infiltration technique. Hence, the purpose of the present study was to measure the effect of resin infiltration technique and CPP–ACP Plus on the microhardness of initial enamel caries lesions.

**Materials and Methods**

The present study was conducted in the Department of Paediatrics and Preventive Dentistry, Panineeya Institute of Dental Sciences and Hospital, Hyderabad, Telangana, India. A total of 120 maxillary central incisors which were extracted due to diseases of periodontium were collected for the study.

Teeth with decay, infractions, and filled were excluded. These samples were thoroughly cleaned using an ultrasonic scaler, and 0.1% thymol solution was used as storage media to avoid dehydration of the samples until the study was commenced.

**Preparation of Samples**

120 samples were decoronated at cementoenamel junction with the help of a diamond disk, and the crown part of each sample was embedded in a 1.2 × 1 cm thick acrylic resin block. Labial surfaces of each sample were abraded in order to obtain flat enamel surface.

Samples were randomly divided as follows. Computer randomization was carried out. Concealment and allocation was done.

**Control Group I:** All 20 samples were subjected to microhardness test to get the baseline values of untreated sound enamel.

**Creating Window**

On the labial surface of the samples, a 4 × 4 mm window was created using a template made on a sticker paper. This template was attached to the labial surface of the samples to prevent any loss of nail varnish. Template is then removed to expose the window (Fig. 1A).

The demineralization regimen followed was similar to the one conducted by Patil and Anegundi.5 For preparing demineralizing solution, lactic acid of 0.1M, calcium chloride (CaCl₂) of 3 Mm, and potassium dihydrogen phosphate of 1.8 Mm and 40% sodium hydroxide (Na(OH)₂) (SD Fine Chem Limited™) were mixed, and pH was adjusted to 4.5.
The artificial saliva was prepared by mixing 3.90 mM trisodium phosphate, 4.29 mM sodium chloride (NaCl), 17.98 mM potassium chloride (KCl), 1.10 mM calcium chloride (CaCl\(_2\)), 0.08 mM magnesium chloride (MgCl\(_2\)), 0.50 mM sulfuric acid (H\(_2\)SO\(_4\)), 3.27 mM sodium carbonate (Na\(_2\)CO\(_3\)) (SD Fine Chem Limited™), and distilled water, with pH 7.2. The prepared artificial saliva was stored in an amber-colored plastic bottle at room temperature in a cool dark place.

The 100 samples were immersed for a period of 4 days at 37°C temperature in demineralizing agent to produce white spots on the enamel surface. This is followed by through washing with deionized water and dried. The exposed areas were inspected for chalky white appearance. These were then divided randomly into five experimental groups. The artificial saliva was replenished every 24 hours for 1 month in all the groups.

- **Group Ila:** After demineralization, the samples were placed in artificial saliva at room temperature and tested for microhardness.

- **Group Ilb:** Samples were brushed with a 0.25 g of non-fluoridated dentifrice (Meswak™, Dabur India Limited, New Delhi) using JSB™ Oral Care HF 128 kids manual electronic toothbrush with oscillating bristles for 2 minutes once daily and later stored in artificial saliva.

- **Group Ilc:** Samples were brushed with same 0.25 g of non-fluoridated dentifrice using the similar electronic toothbrush for 2 minutes once daily, and then, the samples were treated by placing 0.5-mm-thick layer of CPP–ACP Plus (GC Tooth Mousse Plus™) on the surface and left without disturbing them for 3 minutes. After this, CPP–ACP Plus was wiped off with cotton and samples were transferred to artificial saliva.

- **Group IId:** Samples were treated with ICON DMG™ resin infiltration technique. For 2 minutes, Icon Etch was applied (Fig. 1B) and then washed with water for 30 seconds. Later, samples were air-dried, and then Icon Dry was applied for 30 seconds (Fig. 1C), which is again rinsed with water and dried. After this, Icon Infiltrant was applied twice—3 minutes for first time and 1 minute for second time (Fig. 1D). Samples were subjected to light cure for 40 seconds and then stored in artificial saliva.

- **Group IIe:** Samples were treated as per the manufacturer’s instructions with resin infiltration technique and brushed with same 0.25 g of non-fluoridated dentifrice for 2 minutes every day using electronic toothbrush and later were stored in artificial saliva.

All the above-mentioned procedures were repeated for 1 month. All 100 samples were rinsed thoroughly in deionized water, blotted dry, and mounted on the indentation table of the microhardness tester (FM-300e) (Fig. 1E). Area of the enamel to be indented was evaluated under a light microscope with camera, which was attached to the microhardness tester. For microhardness evaluation, a load of 100 g was applied for 10 seconds, five indentations at least 100 µm apart were made at center of each sample, and then average was calculated and the values were tabulated, which were subjected to statistical analysis.

### Statistical Analysis

The data thus obtained was entered in an excel data sheet and statistically analyzed with SPSS software version 22. The values were observed to be statistically significant at 95% confidence interval and P value <0.05. The pairwise comparisons between all the groups were carried out using post hoc test.

### Results

On statistical evaluation of microhardness values (VHN), a significant difference was observed between Group I (371.38 ± 39.3 VHN) and Ila (267.84 ± 11.4 VHN) (p < 0.00001*). Similarly, on intergroup comparison of microhardness values (VHN), significant difference was observed between Group Ilb (267.63 ± 10 VHN) and IIC (326.9 ± 16.4 VHN) (p < 0.0001*). On evaluation of microhardness values between Group IId (368.01 ± 8.4 VHN) and IIC (330.3 ± 20.6 VHN), there was significant difference (p < 0.0001*). However, on comparison of overall mean microhardness values, Group I (371.38 ± 39.3 VHN) and IId (368.01 ± 8.4 VHN) showed higher microhardness compared to other groups (Table 1).

The observation showed no statistically significant (p = 0.995) difference in microhardness value between Group IId and I, Group Ila and Ilb, and Group IIC and IIe. The microhardness value of Group IId was highly significant (p < 0.001) when compared to all other experimental groups (Table 2).

### Discussion

Early enamel lesions present an evidently intact outer layer, followed by body of the lesion which is a subsurface porous area. The porosities present in these lesions are filled with either water or air. Surrounding light which falls on the teeth is deflected and scattered. This is due to the difference between the RI of sound enamel (RI:1.62) and that of water (RI:1.33) or that of air (RI:1.0) within the body of the lesion, thus making the initial lesion appear opaque after dessication.

In the present study, maxillary permanent central incisors were included due to their wide surface area of enamel. The enamel thickness was more in the mid-coronal region than in cervical region, and incisal surfaces and flat surfaces were required for microhardness testing. Thus, mid-coronal region was chosen for the window preparation.

Demineralization of the samples was carried out to create the initial enamel carious lesions. The pH was set at 4.5 for demineralizing solution in order to replicate the demineralization processes that occur in vivo below the critical pH. In order to maintain constant pH of the demineralizing solution, it was changed every 12 hours.

Artificial saliva was changed every 24 hours throughout the period of remineralization, to guarantee ionic balance and maintain pH.

Studies have shown that remineralization of small lesions with low-dose fluoride therapy was more efficient. Usage of low fluoride concentration (0.2% or 900 ppm of sodium fluoride (NaF)) leads to complex localization of free Ca\(^{10}\), (PO\(_4\))^\(^{2-}\), and fluoride ions, which help in maintaining a supersaturated state by repressing demineralization.

ICON-DMG™ resin infiltrant used in this study consists of three components, namely, Icon Etch containing 15% hydrochloric acid (HCl), Icon Dry containing 99% ethanol, and Icon infiltrant. The itching of enamel surface with 15% HCl causes deep surface porosities, leading to deeper penetration of the resin. Acid conditioning with 15% HCl enhanced the penetration of resin compared with 37% phosphoric acid gel.

ICON Dry, which contains 99% of ethanol, was administered for 30 seconds before the application of infiltrant, thus facilitating...
In the present study, digital display Vickers hardness test was used at a load of 100 g along with a diamond indenter for 10 seconds. The lowest load of 100 g for enamel was selected because it created Vickers diagonals longer than 20 m, which is because of the gaps filled by the material that enhanced the strength of the tooth structure.\textsuperscript{11} In a study conducted by Paris et al.,\textsuperscript{12} it was concluded that ethanol was linked with greater penetration coefficient of the resin by decreasing the viscosity and contact angle so it can be helpful in rapid penetration of resin. Higher penetration coefficient and satisfactory hardening were also noticed and proved to be present in mixtures containing large amounts of triethylene glycol dimethacrylate, 2-hydroxyethyl methacrylate, and ethanol.

Infiltrating resin in ICON-DMG\textsuperscript{TM}, containing triethylene glycol dimethacrylate, is light cured and has low viscosity. This property aids in creeping the material deep into the active lesion with the aid of capillary forces and forms a diffusion barrier. Hence, this technique is proved to be efficient.\textsuperscript{10}

In the present study, digital display Vickers hardness test was used at a load of 100 g along with a diamond indenter for 10 seconds. The lowest load of 100 g for enamel was selected because it created Vickers diagonals longer than 20 m, which was recommended to prevent errors in optical measurement. The duration of indentation was fixed to 10 seconds, because this was sufficient for indentation on the tooth surface permanently.\textsuperscript{13}

Group Ila (demineralized enamel) showed lower mean microhardness value (267.84 VHN), compared with the Group I (sound enamel 371.38 VHN), and there was highly significant (<0.00001) difference between Group I and Group Ila. Crombie et al. concluded that the polycrystalline structure of hypomineralized enamel was extremely porous and unorganized than normal enamel with a report of 28% reduction in mineral content, with elevations in protein content up to 3–15-fold, and also with 80% more carbonated apatite; thus, the hypomineralized enamel hardness was significantly lower than sound enamel.\textsuperscript{14}

The mean microhardness value of Group IId (resin infiltration technique, 368.01 VHN) was similar to that of Group I (sound enamel, 371.38 VHN). This proves that resin infiltrated enamel microhardness is almost equal to the sound enamel.

Resin infiltrant resulted in high score of mean microhardness, which is because of the gaps filled by the material that enhanced the strength of the tooth structure.\textsuperscript{11}

### Table 1: Comparison of microhardness values (VHN) between groups

| S. No. | Group I | Group Ila | Group Iib | Group Iic | Group IId | Group Ile |
|--------|---------|-----------|-----------|-----------|-----------|-----------|
| 1      | 439.3   | 267.4     | 253.4     | 350.2     | 368.9     | 300.0     |
| 2      | 315.3   | 288.4     | 283.7     | 299.9     | 372.6     | 359.3     |
| 3      | 371.2   | 252.2     | 262.4     | 315.6     | 369.6     | 320.1     |
| 4      | 350.1   | 260.1     | 270.2     | 325.6     | 350.5     | 309.5     |
| 5      | 383.6   | 271.3     | 268.3     | 342.8     | 379.3     | 360.2     |
| 6      | 430.2   | 263.4     | 252.4     | 349.3     | 365.2     | 302.1     |
| 7      | 310.1   | 285.4     | 280.2     | 302.2     | 375.2     | 357.3     |
| 8      | 372.2   | 258.2     | 265.5     | 319.6     | 367.6     | 320.4     |
| 9      | 353.3   | 262.2     | 271.3     | 323.4     | 355.4     | 310.2     |
| 10     | 386.2   | 270.1     | 269.4     | 345.1     | 378.2     | 359.2     |
| 11     | 374.4   | 267.2     | 281.6     | 321.4     | 366.2     | 358.5     |
| 12     | 325.2   | 275.1     | 264.4     | 347.2     | 374.5     | 325.6     |
| 13     | 345.6   | 255.2     | 267.3     | 310.2     | 370.4     | 315.7     |
| 14     | 385.4   | 286.2     | 271.2     | 321.4     | 351.4     | 352.4     |
| 15     | 425.2   | 256.2     | 251.3     | 342.7     | 376.2     | 309.4     |
| 16     | 436.3   | 280.4     | 266.4     | 342.3     | 357.2     | 324.5     |
| 17     | 318.3   | 275.4     | 280.6     | 337.5     | 369.2     | 336.2     |
| 18     | 365.2   | 248.2     | 268.1     | 317.2     | 367.2     | 324.2     |
| 19     | 356.1   | 264.1     | 274.4     | 306.2     | 369.2     | 330.2     |
| 20     | 383.6   | 270.2     | 250.5     | 318.2     | 376.2     | 331.1     |
| Mean ± SD | 371.38 ± 393 | 267.84 ± 11.4 | 267.63 ± 10 | 326.9 ± 16.4 | 368.01 ± 8.4 | 330.3 ± 20.6 |
| p-value   | <0.00001* | <0.0001* | <0.0001* | <0.00001* | <0.0001* | <0.0001* |

*very significant

### Table 2: Pairwise comparison of microhardness values among groups

| Group | Group I | Group Ila | Group Iib | Group Iic | Group IId | Group Ile |
|-------|---------|-----------|-----------|-----------|-----------|-----------|
| Group I | 0       | <0.001*  | <0.001*  | <0.001*  | 0.996 NS  | <0.001  |
| Group Ila | <0.001* | 0        | 1.00 NS   | <0.001*  | <0.001*  | <0.001*  |
| Group Iib | <0.001* | 1.00 NS  | 0         | <0.001*  | <0.001*  | <0.001*  |
| Group Iic | <0.001* | <0.001*  | <0.001*  | 0        | <0.001*  | 0.995 NS  |
| Group IId | 0.995 NS | <0.001*  | <0.001*  | <0.001*  | 0        | <0.001*  |
| Group Ile | <0.001* | <0.001*  | <0.001*  | 0.995 NS  | <0.001*  | 0        |

NS, not significant; *very significant
Paris et al. stated that twice application of infiltrant was shown to enhance microhardness of lesions infiltrated with resin. It is therefore possible that twice application of resin might fill porosities and crevices within the infiltrated lesion body and compensate polymerization shrinkage, thus increasing hardness and decreasing mineral loss when challenged again by demineralization.

Group Ile showed lesser microhardness value compared to Group IId. This could be attributed to the slight but significant mineral loss during brushing with non-fluoridated dentifrice. When Group Ilc and Group IId were compared, the samples treated with resin infiltration showed significantly (P < 0.0001) higher microhardness. This might be due to the fact that low-viscosity resin could reach the deeper areas of the initial enamel caries lesion immediately after the treatment.

Shimaa et al. concluded in the in vitro study conducted that the resin infiltration had the highest depth of penetration with Icor that resulted in higher microhardness, which is statistically significant compared with CPP–ACP Plus. The results obtained in this study were in agreement with the present study.

The present study suggested that resin infiltration can be observed as microinvasive treatment option for initial enamel caries lesions as it showed maximum microhardness and depth of penetration. This microinvasive technique for the treatment of initial enamel caries not only provides therapeutic benefits but also significantly reduces both long-term restorative needs and costs, thus encouraging the concept of minimum intervention dentistry. However, the present study acknowledges certain limitation such as chemical makeup of dental resins that favors absorbing liquids. Therefore, discoloration of resin may be inevitable over time.

**Conclusion**

Based on the results of present study, it could be concluded that:

- The microhardness of resin-infiltrated carious enamel was almost the same as that of microhardness of sound enamel.
- Resin infiltration technique enhanced the microhardness of initial enamel caries lesions compared with CPP–ACP Plus application.
- The decrease in microhardness of initial enamel caries lesions treated with resin infiltration technique followed by brushing may be because of significant mineral loss due to brushing.

**Clinical Significance**

Resin infiltration technique is a noninvasive or a microinvasive approach for increasing the microhardness of hypoplastic enamel. This technique may decrease the need of laminates or other full coverage restorations for such teeth.

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