Tufts UNIVERSITY | School of Dental Medicine

Radiographic Evaluation of the Effectiveness of Resin Infiltration versus Fluoride Application in Inhibiting Incipient Enamel Proximal Lesion Progression in Primary Molars

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ABSTRACT

Purpose: The goal of this split-mouth, randomized, prospective clinical trial was to evaluate radiographically the effectiveness of resin infiltration as an adjunct to standard-of-care preventative measures (fluoride application, oral hygiene instruction, and diet counseling) compared to standard-of-care preventative measures alone in controlling the progression of non-adjacent, incipient, enamel proximal carious lesions (E1 and E2) in primary molars after six and 12 months of treatment. **Materials and Methods:** A total of 45 healthy children aged 5-8 years old who had been diagnosed radiographically by at least two trained and calibrated examiners to have at least two non-adjacent, incipient, enamel proximal carious lesions in primary molars (total of 90 lesions) were included in the study. The lesions were randomly allocated to either case or control group. Case group lesions were treated using resin infiltration followed by topical fluoride (5% NaF) application versus only topical fluoride (5% NaF) in the control group lesions. All subjects were given oral hygiene instruction, diet counseling and flossing instructions including the proximal areas at the baseline, at the six-month and 12-month follow-ups. Other recorded variables included: (1) Date of birth; (2) gender; (3) race; (4); dmft (decayed, missing, filled primary teeth due to caries) at the treatment day; (5) Caries risk assessment (CAT). To provide standardization, individual bite registration was taken during the initial baseline visit and used at the follow-up appointments. The radiographic evaluation was performed after six and 12 months by two blinded, trained and calibrated examiners using pair-wise reading to determine whether lesions had progressed or not. A p-value of < 0.05 was considered statistically significant. **Results:** After six months of treatment, one of the subjects failed to come to this appointment; thus only 44 lesion pairs could be compared radiographically. Lesions treated with

resin infiltration showed less 5 (11.4%) progression than control group lesions 8 (18.2%), but the difference was not statistically significant (p=0.453). At the 12-month follow-up, six of the subjects (13.3%) failed to come and one subject (2.2%) was excluded due to the exfoliation of control group tooth. Thus, a total of 38 lesion pairs were evaluated radiographically. Six (15.8%) of the lesions treated with resin infiltration showed signs of progression radiographically compared to 13 (34.2%) of the control group lesions; the difference was not statistically significant (p=0.092). **Conclusion:** Resin infiltration as an adjunct to standard-of-care preventative measures (fluoride application, oral hygiene instruction, and diet counseling) was not significantly different from the standard-of-care preventative measures alone in terms of radiographic progression of non-adjacent, incipient, enamel proximal carious lesions in primary molars when evaluated at six and 12 months after treatment.

DEDICATION

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Introduction

Dental Caries

Dental caries is the most common health problem among children and adolescents. It is a complex, multifactorial disease that is influenced by the interaction of several factors, including diet, dental plaque bacteria, saliva composition, and the host's tooth surface [(1)]. Dental caries results from an imbalance between demineralization and remineralization of the dental surface after the intake of fermentable carbohydrates [(2)]. When the microorganisms within the dental plaque biofilm are exposed to fermentable carbohydrates and sugars, they produce organic acids. The acids reduce the plaque's pH to an acidic state that causes the demineralization and dissolution of the calcium and phosphate from the enamel hydroxyapatite crystals, while an increase in pH causes minerals to accumulate. Demineralization continues until equilibrium is achieved between the oral environment and the enamel [(3)].

White spot lesions

Dental caries of the enamel typically is first observed clinically as a non-cavitated subsurface or incipient white spot enamel lesion. White spot lesions are characterized by the loss of minerals within the lesion's body, while the surface of the enamel remains highly mineralized [(4)]. Clinically, early enamel demineralization has a chalky white appearance, particularly when dehydrated by an increase in the surface porosities [(5)].

Histologically, a white spot lesion is composed of four zones: (1) surface zone; (2) body of lesion; (3) dark zone, and (4) translucent zone. The most crucial zone in maintaining the integrity of the tooth structure is the surface zone. The surface zone acts as a barrier to bacterial

invasion and has a low volume of pores (1-5%). It also plays an important role in the remineralization process of the tooth structure; the body zone, having a greater pore volume, constitutes the largest layer of incipient caries [6]. A progressing lesion is called an active lesion. If such a lesion is not arrested, the intact enamel surface will break down and create a cavity. On the other hand, if there is no progression, the lesion is referred to as an arrested/inactive lesion. Inactive enamel lesions may remain so for several years without forming a cavity or they may become arrested permanently and never progress deeper into the dentine [7].

Clinical diagnosis of proximal white spot lesions

Understanding diagnostic test parameters for early carious lesions is crucial in order to carry out the correct treatment [8]. Caries is detected traditionally by a combination of visual examination and tactile pressure (probing). However, impaired visualization makes it difficult to detect and assess the stage and activity of proximal carious lesions. Moreover, proximal surfaces are considered high-risk areas, as they are more difficult to clean, and are subjected less often to the washing benefits of saliva [9]. In 2001, Ratledge reported that direct, visual examination failed to detect up to 83% of cavitated proximal lesions [9]. Therefore, the International Caries Detection and Assessment System (ICDAS) was developed to differentiate between various stages of lesions of the smooth surface (cavitated and non-cavitated) using visual and tactile assessment [10].

Based on ICDAS scores, smooth surface lesions can be divided into: ICADS score 1: no clinical evidence of carious activity; score 2: visual changes in appearance of enamel/white spot lesion; score 3: localized area of enamel breakdown due to caries, with no visible dentin or

underlying shadow: score 4: underlying shadow from dentin, with or without localized enamel breakdown, and score 5: distinct cavity with clinical visible dentin [10] (Figure I).

Radiographic diagnosis of proximal white spot lesions

Due to the difficulty in inspecting the proximal surfaces directly, clinical detection of the stage and activity of proximal carious lesions cannot be assessed properly without temporary tooth separation. Thus, bitewing radiographs are considered the most useful additional method of detection and assessment of proximal lesions [11]. A systematic review carried out by Dove revealed that radiographic examination has greater sensitivity in detecting proximal lesions than does clinical examination. Therefore, the prevalence of proximal lesion typically is underestimated if the clinical examination is not combined with radiographic examination [12, 13]. Furthermore, Dove reported that radiographic examination has greater sensitivity than specificity in facilitating early detection of proximal caries lesions before cavitation [14].

It is well known that the caries process produces a decrease in the mineral content of the enamel and dentin, which decreases the attenuation of the X-ray beam as it passes through the tooth structure. For a carious lesion to be detected radiographically, at least 30 to 40% of the mineral content of the affected area must have been lost [14].

Radiographically, carious lesions may be divided into:

0: No radiolucency.

E1: Radiolucency confined to the outer half of the enamel.

E2: Radiolucency in the inner half of the enamel, including lesions that extend up to, but not beyond the enamel-dentin junction.

D1: Radiolucency in the outer third of the dentin.

D2: Radiolucency in the middle third of the dentin.

D3: Radiolucency in the inner third of the dentin and approaching the pulp [15] (Figure II).

Treatment of proximal white spot lesions

Treatment of incipient proximal carious lesions is limited to noninvasive preventive or invasive restorative treatment. Recently, dental treatment has shifted to early identification of individuals at high risk of developing dental caries [16]. This conservative and minimally invasive approach includes the application of fluoride varnish, together with improved patient oral hygiene and dietary control to inhibit lesion progression and maintain the tooth structure, rather than removing the diseased part of the tooth [9]. However, the success of this approach is dependent upon patient compliance, which is difficult to obtain and maintain, particularly in children, due to their reduced dexterity and visual acuity compared to adults [17, 18].

Conventional treatment of proximal white spot lesions

The conventional restorative approach involves destruction of a large amount of sound enamel and dentin in order to access and remove all of the carious part of the tooth structure, which initiates a cycle of re-intervention due to the limited longevity of dental restorations [19]. Therefore, the goal should be to preserve sound tooth structure, as well as achieve ideal function and aesthetics [20]. Nonetheless, once the lesion is cavitated, plaque removal becomes more difficult for the patient, and leads to gingival inflammation around the area, which makes restorative treatment the best option. Further, at this point, the process of remineralization will fail and the lesion will continue to progress even with preventive measures [9].

Noninvasive treatment for proximal white spot lesions

1- Fluoride varnish

Fluoride varnish (5% NaF) is a highly effective therapeutic agent in controlling and preventing the progression of incipient lesions. It has been applied in many forms, including dentifrices, gel, and solutions. The most common professional fluoride-delivery methods used are gel and varnish. Fluoride varnish was developed in the late 1960s and early 70s to provide site-specific and longer-lasting fluoride delivery, including to the proximal surface of the teeth [(21)]; moreover, it is well tolerated by patients, safe, and can be used quickly and easily, even on infants and very young children [(22)]. Therefore, fluoride varnish was suggested as the first treatment approach for incipient lesions [(23)].

Fluoride reduces the rate of demineralization, enhances remineralization, and alters bacterial metabolism [(24)]. Once the fluoride is applied topically, it works together with the calcium and phosphate ions in the saliva to form a calcium fluoride reservoir layer on the tooth surface [(25)]. The application of sodium fluoride varnish (Duraphat) every three months for three years reduced the progression of proximal lesions in permanent premolars and molars significantly [(26)]. When planning patient treatment, the frequency of topical fluoride application is tailored according to the patients' caries risk assessment, where children who are classified as high-risk patients require more frequent fluoride applications [(16)].

2- Sealing proximal white spot lesions

Caries sealing and resin infiltration are new micro invasive, intermediate approaches introduced to close the gap between conservative, non-operative preventive measures and restorative treatments for non-cavitated, incipient proximal lesions [(27)].

The application of pit and fissure sealant to extracted human teeth with natural, proximal, non-cavitated carious enamel lesions showed the presence of superficial resin tags up to six µm in length [(28)]. These tags act as a physical barrier and reduce the number of bacteria in carious lesions significantly by depriving bacteria of oral fluids and nutrition. Therefore, carious lesions are unlikely to progress if sealed properly [(29)]. Further, the idea of sealing incipient, proximal, non-cavitated carious lesions using either sealant resin [(30-33)] or polyurethane tape [(34)] also has been examined in several clinical studies.

Sealing proximal lesions was found to be statistically significantly more effective in controlling caries progression than was flossing in both permanent [(33)] and primary teeth [(32)]. In one study, a randomized, split-mouth, placebo-controlled clinical trial was conducted to assess the effectiveness of sealing non-cavitated proximal lesions in permanent teeth. Teeth were separated for two days using orthodontic elastic bands, and then the bands were removed, leaving a space of 0.5mm for sealant application. Eighteen months later, 44% of the lesions treated in the sealant group and 84% of those in the flossing group had progressed [(33)]. Another randomized, split-mouth, placebo-controlled clinical trial was conducted to assess the effectiveness of sealing non-cavitated proximal lesions in primary teeth. Most of the children reported no pain or anxiety during the treatment. Sealing was found to be superior to flossing

instructions after one and 2.5 years radiographic follow-ups. During the first year, radiographic follow-up showed that only 27% of sealed lesions progressed compared to 51% of the lesions in the control group. After 2.5 years, radiographic follow-up showed progression in 46% of the sealed lesions and 71% of the control group lesions [32]. Furthermore, after one year [30] and two years radiographic follow-ups [31,34], these studies found a lesser, but not a statistically significant, degree of carious lesion progression in sealed, non-cavitated, proximal carious lesions extending up to half of the dentin thickness [30] compared to non-sealed lesions in permanent teeth.

The main disadvantage of proximal lesions sealing is the need to place separators between the teeth for few days before the treatment. Retention of plague is also one of the main drawbacks with proximal sealing due to the superficial physical barrier [49]. Therefore, sealing proximal lesions has not been widely used for proximal lesions.

3- Resin infiltration for proximal white spot lesions

Resin infiltration is another micro invasive and simple approach for treating incipient non-cavitated enamel lesions. The rationale for the resin infiltration technique is based on using a strong acid and a low viscosity, light-cured resin to penetrate deeply and seal the enamel surface porosities and intercrystalline spaces [35, 36]. Therefore, a diffusion barrier is created inside the enamel lesions, rather than on the enamel surface, as in sealing, which strengthens the enamel structure and prevents further cavitation or breakdown of the tooth [36].

Resin infiltration was first introduced and explained by Davila et al. in 1975 [37]. The technique of using ultraviolet light-polymerized adhesive after conditioning with 50% phosphoric acid (H3PO4) containing 7% dissolved zinc oxide by weight was called plastification, and was shown to have potential in preventing and arresting incipient, enamel proximal lesions. Subsequently, Robinson et al. conducted preliminary work to find a low viscosity, hydrophilic, and cosmetically acceptable material that could be introduced into the porous enamel and natural carious lesions. The study reported that resorcinol-formaldehyde occluded up to 60% of the internal spaces after the first application. On the second and third application, 80 and 99% of the internal space was occluded, respectively. However, due to the toxicity of resorcinol-formaldehyde, it was not considered appropriate for clinical application [38]. Therefore, single and multiple applications and the infiltration ability of a range of commercially available dental adhesives and bonding agents were compared later with the original resorcinol-formaldehyde formulation [39]. After a single application, all of the materials used showed 50-70% reduction in the pore volume. After a second application, all of the materials, except the cyanoacrylate adhesive, showed further reduction in the pore volume. On the third application, only resorcinol-formaldehyde and Scotchbond Multipurpose showed further significant reductions in pore volume [39].

Several *in vitro* studies investigated the penetration of light-cured resin into artificial [39-44] and natural, non-cavitated enamel lesions [17,43,45] using commercially available adhesives and fissure sealants, and showed that at least partial penetration of the artificial, non-cavitated lesions and natural, non-cavitated caries lesions can be achieved. Moreover, the resin infiltration was

efficacious in preventing further demineralization of artificial enamel lesions under cariogenic conditions *in vitro* [44] and in situ [46].

Penetration of the light-cured resin is controlled primarily by capillary forces; therefore, there is a strong correlation between the penetration coefficient (PC) and the penetration depth of the low viscosity resin (infiltrants). Low viscosity infiltrants with higher PC show superior ability to penetrate completely into artificial [46] and natural enamel lesions *in vitro* [45]. The outer layer of carious enamel is mineralized densely, with lower pore volume compared to the underlying body of the lesion [4, 7]. This may form a barrier and interfere with the resin's ability to penetrate into the carious lesion [41]. Therefore, removing the high mineral and fluoride-rich layer using 36% H3PO4 for five seconds prior to dehydration with ethanol and the application of multiple layers, allows removal of the surface layer and almost complete penetration of artificial lesions by the organic resin [47].

However, due to the alternating cycles in the oral cavity, findings from artificial lesions cannot always be extrapolated to natural lesions. Natural lesions are non-homogeneous, show greater thickness, have higher mineral content, and are contaminated with organic materials, such as protein and carbohydrates, which might interfere with resin penetration [35]. Further, incomplete surface removal and incomplete penetration of the resin into natural enamel caries has been reported after the application of 37% H3PO4 [43]. In natural white spot lesions, acid etching with 15% hydrochloric acid (HCl) gel allowed the resin to penetrate deeper than did 37% H3PO4 gel [35,43]. Recently, Meyer-Lueckel et al. reported that a three-minute application of an

infiltrant following a two-minute etching with 15% HC is sufficient to achieve almost complete penetration of active, non-cavitated enamel caries *in vitro* [48].

An improved infiltrant resin, Icon (DMG America, Englewood, NJ), has been introduced in the United States. The unfilled, light-cured resin is composed of 99% Triethylene Glycol Dimethacrylate (TEGDMA) and Champherquinone. The TEGDMA lowers the viscosity of this material and increases its PC properties (>100 cm/s) [36]. The capability of Icon infiltrants to penetrate deeply into natural lesions has been confirmed (424 μm) in natural, non-cavitated lesions after three minutes application [48]. The combination of both low viscosity resin and etching with HCL technique for 120 seconds achieves almost complete occlusion of natural enamel caries [49]. Icon infiltrant applications require no anesthesia or cavity preparation and can be done in a single visit; a rubber dam is used to protect the adjacent teeth and surrounding soft tissues from the caustic 15% HCL used during etching [50]. Multiple clinical trials have investigated the efficacy of Icon resin infiltration on proximal lesion progression in permanent [51, 52] and primary teeth [53, 54].

Paris et al. investigated the efficacy of resin infiltration on the progression of proximal carious lesions in permanent molars by comparison to non-operative measures (fluoridation, oral hygiene, and dietary instructions). This study evaluated 29 pairs of proximal carious lesions diagnosed radiographically and having radiological extension into the inner half of the enamel or the outer third of the dentin. The lesions identified were assigned randomly to either experimental or control (placebo) groups. The experimental group lesions were infiltrated using Icon, while a placebo treatment was performed in the control group lesions. All participants

received instructions for diet, flossing, and fluoridation. Digital subtraction was used to evaluate the lesions' progression after 18 months. The digital subtraction was performed with Compare software (Dental Health Unit, University of Manchester, UK), which runs as a plug-in to the Image Tool software, version 1.23 (University of Texas Health Science Center at San Antonio, Texas, 2002). Reproducible images and individualized bite registrations were obtained using disposable individual plastic film holders and plastic rings. Eighteen months later, 7% of the lesions treated by resin infiltration showed progression, compared to 37% of the control group lesions [(52)].

The therapeutic effects of infiltration versus sealing in controlling caries progression on the proximal surfaces of permanent teeth after one, two and three years was assessed radiographically in a randomized, split-mouth clinical trial performed by Martignon et al. The study included 39 subjects with three proximal lesions identified radiographically around the enamel-dentin junction and the outer third of the dentin. Lesions were assigned randomly to group A (Infiltration using Icon pre-product; DMG), group B (Sealing), or placebo group. Individualized bite registrations were obtained during the baseline visit to facilitate reproducibility. Digital subtraction radiography using Image Tool (UTHSCSA, San Antonio, TX, USA) showed significant differences in lesion progression between resin infiltration and placebo and between sealing and placebo. Pair-wise radiographic analyses were used to evaluate the therapeutic effects of infiltration versus sealing in controlling caries progression on proximal surfaces of permanent teeth after two, and three years. At three-year follow-up, infiltration and sealing were found to be significantly better than placebo treatment in controlling caries progression of proximal lesions on permanent teeth and a considerably higher percentage of the

placebo group lesions (70%) progressed compared to 32% and 41% of the infiltrated and sealed lesions, respectively. Although no significant difference in controlling the progression of proximal lesions was found between infiltration and sealing, infiltration showed a higher therapeutic effect than did sealing [51].

The clinical and radiographical efficacy of resin infiltration (Icon pre-product; DMG) in controlling the progression of non-cavitated, smooth surface, incipient lesions in permanent teeth after three, six, nine and 12 months of treatment was evaluated by Pancu et al. in 2012. Their study included 19 smooth surface lesions (buccal, lingual, mesial, and distal). One year later, only two lesions (10.52%) showed signs of progression. One lesion progressed from the inner half of the enamel to the outer third of the dentin, and the other progressed from the outer third to the middle third of the dentine [55].

Ekstrand et al. evaluated the clinical and radiographic efficacy of resin infiltration (Icon pre-product; DMG) with fluoride varnish in treating proximal superficial carious lesions in primary molars versus fluoride varnish alone after one year. The clinical examination was performed using the ICDAS visual scoring system. The study included 50 children with at least two proximal carious lesions in the enamel or outer third of the dentin in primary molars. In order to allow reproduction of the X-ray projection geometry, the baseline and follow-up radiographs were taken using a film holder with the central beam pointing to the proximal space between the first and second primary molars. The lesions were assigned randomly to one of two treatments: resin infiltration followed by application of fluoride varnish (2.26% F: case group) versus fluoride varnish only (control group). A year later, all lesions were examined clinically

using the ICDAS visual scoring system and radiography. The clinical examination showed that 67% of the lesions treated by fluoride varnish alone had progressed, compared to 31% of the lesions treated by resin infiltration and varnish, while 23% of the case lesions and 62% of the control lesions showed progression radiographically [53].

Altarabulsi et al. evaluated the clinical safety, quality, and effect of Icon resin infiltration for proximal lesions in primary and permanent teeth after one year. Lesions limited to the enamel and the inner third of the dentin and only five primary teeth were included in the study. One year later, carious lesion progression was assessed by pair-wise visual readings, and 95.3% of the lesions showed no progression. Icon resin infiltration was considered a safe and effective treatment to reduce progression of initial proximal lesions in both primary and permanent teeth [54].

Clinical significance of the study

Combining resin infiltration with caries remineralization program (Oral hygiene, diet counseling, and fluoride application) is a promising therapeutic alternative to conservative non-operative preventive measures and restorative treatments in treating non-cavitated, incipient, enamel proximal carious lesions [27]. There is only on split-mouth randomized clinical trial investigated the efficacy of resin infiltration in treating proximal superficial lesions in primary molars [53]. Therefore, further conclusive randomized clinical studies are required to assess the long–term advantages, indications, and limitations of using the Icon resin infiltration technique.

Aims and objectives

The goals of this study were to evaluate radiographically the effectiveness of Icon resin infiltration as an adjunct to standard-of-care preventative measures (fluoride application, oral hygiene, and diet counseling) compared to standard-of-care preventative measures alone in controlling the progression of non-adjacent, incipient, enamel proximal carious lesions (E1 and E2) in primary molars after six and 12 months of treatment.

Hypothesis

Icon Resin infiltration adjunct to standard-of-care preventive measures (fluoride application, oral hygiene instruction, and diet counseling) is more effective radiographically in inhibiting the progression of non-adjacent, incipient, enamel proximal carious lesions (E1 and E2) in primary molars after six and 12 months of treatment.

Research Design and Methods

Experimental design

This split-mouth, prospective, controlled, randomized clinical trial was conducted at Tufts University School of Dental Medicine in the Department of Pediatric Dentistry between May 2014 and January 2016. The study was approved by the Institutional Review Board at Tufts Medical Center and Tufts University Health Sciences Campus (IRB # 10862).

Screening and Baseline Radiographs

Screening and baseline radiographs were taken during the initial examination or regular follow-up appointments. The objectives of the study were introduced to the parents/legal guardians of patients who met the screening inclusion criteria.

Screening Inclusion Criteria:

1. Children ages five to eight years
2. Healthy children with non-contributory medical history
3. No known allergies and/or sensitivity to hydrochloric acid, pyrogenic silicic acid, ethanol, and methacrylate (components of the Icon products)
4. Willing to return for follow-up visits
5. English speaking

Screening Exclusion Criteria:

1. Subjects with history of any chronic disease (e.g., epilepsy, ectodermal dysplasia, cardiac anomalies)
2. Subjects with abnormalities affecting the tooth structure (e.g., dentinogenesis imperfecta, amelogenesis imperfecta, enamel hypoplasia, fluorosis)
3. Subjects who require sedation and/or general anesthesia for dental treatment

If a parent/guardian agreed to have his/her child participate in the study, she/he signed a screening consent form. To allow standardization, the screening bite-wing radiographs were taken using a Rinn sensor holder to position the sensor in the mouth and align the radiographs so that they were at 90 degrees to the sensor. To duplicate the projection geometry while taking the follow-up radiographs patient specific bite block was utilized using impression material (Futar D (pink), ROYDENT, Johnson City, TN) while the sensor was positioned properly. Each bite

block was the saved in a labeled box. All of the radiographs were taken with the central beam directed between the lower first and second molars using a similar radiographic source (65KV, 7mA, exposure time 0.064 sec) using a MiPACS (Medicor Imaging, Charlotte, NC, USA) digital radiograph. For bite registration, teeth were dried with gauze. The impression material was placed around the teeth after placing the sensor in its proper position. Once the material set (90 seconds), the bite registration was placed in a box and labeled with the subject's number (Figure III).

At least two of the calibrated examiners evaluated the screening radiographs. Each examiner scored each lesion in a dark room on a 19-inch screen. No magnification was used. If the innermost border of the lesion was located before the enamel-dentin junction, the lesion was considered to be within the enamel. If the innermost border of the lesion was located before the midline of the enamel, the lesion was considered to be in the outer half of the enamel (E1). On the other hand, if the lesion extended beyond the midline of the enamel, but did not cross the enamel-dentin junction, the lesion was considered to be in the inner half of the enamel (E2). The depth of each lesion was determined based on the two examiners' agreed score. If the examiners disagreed, a third calibrated examiner evaluated the lesion's depth.

Children diagnosed radiographically by at least by two of the calibrated and trained examiners to have at least two, non-adjacent, incipient, enamel proximal carious lesions in primary molars (E1 and E2 lesions) were qualified for the study.

Phone calls were made to the subject's parent/legal guardian to inform them whether or not the subject qualified for the study. If the subject qualified, a treatment appointment was scheduled. Other recorded variables included: (1) Date of birth; (2) gender; (3) race; (4); dmft (decayed, missing, filled primary tooth due to caries) at the treatment day; (5) Caries risk assessment (CAT).

Treatments

The case group lesions were treated by infiltration with Icon resin infiltration (Icon, DMG America, and Englewood, NJ). First, topical anesthesia was applied to reduce the discomfort caused by the placement of the rubber dam. Then, the proximal surfaces were flossed and cleaned, and a dental wedge was emplaced. Following the manufacturer's instructions, 15% HCL was placed on the lesion for two minutes (Icon Etch). The surface was then rinsed and dried for 30 seconds each with oil free and water free air. Thereafter, Icon Dry was applied for 30 seconds and then dried for 30 seconds with oil free and water free air. Finally, after removing excess material with a gentle air blow, thus leaving no overhang, the first layer of infiltrating Icon resin was applied for three minutes and polymerized for 40 seconds with blue light (Figure IV).

For the control and case group lesions, fluoride varnish was applied (5% NaF). Subjects and their parents/guardians were given oral hygiene instruction, diet counseling, and instructions on how to floss proximal areas, including the infiltrated surface daily.

Follow-up appointments

The subjects' medical history was reviewed during a regularly scheduled follow-up appointment. To provide standardization, the individual bite registration taken during their initial baseline visit was used.

Subjects and their parents/guardians were reminded to floss the proximal areas daily, including the infiltrated surface. Oral hygiene instruction and diet counseling was also provided at the six and 12 month follow-ups.

Radiographic evaluation

The radiographs were evaluated by the conventional pair-wise visual reading. Two of the calibrated, blind examiners made an independent visual assessment of the depth of each lesion selected to determine whether it had progressed (increased in size) or not.

At the six-month follow-up, the baseline and follow-up radiographs of each lesion were paired with the dates that they were taken, and imported into MiPACS (Medicor Imaging, Charlotte, NC, USA). Each set of radiographs was then assigned a random number. The radiograph on the right side of the set was the baseline, while the six month follow-up radiograph was placed on the left side of the set (Figure V). The radiographs were evaluated in a dark room on a 19-inch screen. No magnification was used. The examiners were blind to case and control group lesions. At the 12-month follow-up, the baseline and 12-month follow-up radiographs were paired.

If the first two examiners disagreed, a third calibrated blind examiner evaluated the lesion depth and determined whether or not the lesion had progressed. If two of the examiners agreed that there was no caries progression, the lesion was considered arrested (no progression). If they agreed that there was caries progression, the lesion was considered to have progressed.

At the six or 12-month follow-ups, when signs indicated that a lesion had progressed from an outer enamel (E1) to an inner enamel lesion (E2) or from an inner enamel (E2) to a dentin lesion (D1) the lesion was considered to have progressed and was restored conventionally.

Sample size calculation

A power calculation was performed using nQuery Advisor (Version 7.0). Assuming that the percentage with progression would differ by 38.4% between groups and that 43.6% of subjects would progress on exactly one side [53] a sample size of n=27 subjects (two lesions per subject) was adequate to obtain a Type I error rate of 5% and a power over 80%. To account for possible dropout and exfoliation, n=45 subjects per group (90 lesions) were recruited. In addition, 75% screening failure was expected. Therefore, a total of 180 subjects were screened to identify 45 subjects that met the inclusion criteria.

Statistical analysis

The statistical significance of the effectiveness of resin infiltration as an adjunct to standard-of-care preventive measures (fluoride application, oral hygiene instruction, and diet counseling) versus standard-of-care preventive measures (fluoride application, oral hygiene instruction, and diet counseling) only in inhibiting the progression of non-adjacent, incipient,

enamel proximal carious lesions was assessed via McNemar's test. SPSS version 21 software was used, and a p value < 0.05 was considered statistically significant.

Randomization

Each subject had at least two identified non-adjacent, incipient, enamel proximal carious lesions. A maximum number of two lesions per subject were included in the study. A randomization scheme was generated using R (Version 2.13.1); randomization was performed at the level of the lesion and occurred at the beginning of visit two. Each lesion was selected randomly to be in either the case or the control group. The remaining non-adjacent, incipient, enamel proximal carious lesions (E1 and E2) were treated with standard-of-care preventive measures (fluoride application, oral hygiene instruction, and diet counseling). Other lesions (D1, D2, and D3 lesions) were not included in the study and were treated by conventional restorative methods.

Blinding

Due to the nature of the study, the investigator who performed the treatment was not blind with respect to which group a lesion belonged. However, the investigators who evaluated the radiographs were blind with respect to which group the lesion they were observing belonged.

Results

Screening

A total of 90 subjects were screened between May and December 2014 during their initial or recall appointments. The screening was performed after the screening consent and assent

forms were signed. The majority of the participants were Asians, 49 (54.4%); followed by African Americans, 17 (18.9%); and White-non-Hispanic, 12 (13.3%). The mean (SD) age of the subjects on their screening day was 6.82 (1.09). The analysis with the Cardiogram program (CAT) disclosed that the majority of the subjects screened, 55 (61.1%), were characterized as being at high risk. Only 27 (30%) of the participants had a primary dmft score of zero. The mean (SD) primary dmft score for the subjects was 4.02 (4.05) (Table I).

After the evaluation of the radiographs by two calibrated examiners, only 61 (67.8%) were eligible to participate in the study. Upon contacting them, nine (14.8%) of the eligible subjects refused to participate in the study. Another seven (11.5%) failed to come to the treatment appointment. Thus, a total of 45 (73.8%) of the eligible subjects participated in the study. The flow chart of the study is presented in Figure VI.

The inter-examiner reliability (kappa) values of the three examiners ranged from 0.59 to 0.80, while the intra-examiner reliability values ranged from 0.64 to 0.87.

Baseline

At baseline, the plurality of participants were Asian 21 (46.7%) followed by White-non Hispanic 9 (20%) and African American, 9 (20%). The mean (SD) age of the participants at their treatment day was 7.29 (1.09). The percentage of females, 24 (53.3%), was slightly higher than males, 21 (46.7%) participants. The analysis with the Cardiogram program (CAT) disclosed that the majority of the participants, 28 (62.2%), were characterized as being at high risk. Only 10

(22%) of the participants had a primary dmft score of zero. The mean (SD) primary dmft score for the participants was 4.60 (3.78) (Table II).

Ninety randomly selected proximal lesions were included in the study (45 cases and 45 control group lesions). The plurality of the case group lesions were located on the mesial surface of the lower right second primary molar 8 (17.8%), while the plurality of the control group lesions were located on the mesial surface of the upper right second primary molar 10 (22.2%). With respect to the contact area between the primary molars, most of the case and control group lesions were located on the mesial surface of the second molars and the distal surface of the first molars, 34 (75.6%) and 37 (82.2%), respectively (p=0.453). The distribution of the case and control group lesions according to tooth surface and initial radiographic depth is shown in Figures VII and VIII. The majority of the lesions were E2 lesions, 29 (64.4%) and 32 (71.1%), in the case and control groups, respectively (p=0.629). The distribution of the case and control group lesions according to each lesion's initial radiographic depth is illustrated in Figure IX.

Six-month follow-up

At the six-month follow-up appointment, the mean age (SD) of the participants was 7.74 (1.10). The mean (SD) time between the treatment and the six-month follow-up appointment was 180.42 days (24.59). One of the subjects failed to come to this appointment; thus only 44 lesion pairs could be compared radiographically. None of the participants reported any side effects.

Radiographically, the majority of the case and control group lesions were E2 lesions, 31 (70.5%) and 31 (70.5%), respectively. One (2.3%) lesion in the case group and four (9.1%) of the control group lesions reached the dentine radiographically at the six-month follow-up. A total of three (6.8%) participants showed progression in both lesions, 34 (77.3%) participants showed no lesion progression in the case or control lesions, two (4.5%) showed progression in case group lesions only, and five (11.4%) had progression in the control group lesions only. Thus, a total of 39 (88.6%) case group lesions and 36 (81.8%) control group lesions showed no sign of progression radiographically at the six-month follow-up. McNemar's test showed no statistically significant difference between the groups (p=0.453). Figure X shows information about lesions' progression radiographically among the participants at the six-month follow-up. Five lesions (11.4%) of the case group showed signs of progression and a higher number of lesions in the control group, 8 (18.2%), showed signs of progression compared to the case group lesions (Figure XI).

Twelve-month follow-up

At the 12-month follow-up appointment, the mean age (SD) of the participants was 8.19 (1.07). The mean (SD) time between the treatment and the 12-month follow-up appointment was 367.25 days (31.46) and between the six-month and 12-month follow-up was 181.91 days (48.40). Six of the subjects (13.3%) failed to come for the 12-month follow-up appointment. One subject (2.2%) was excluded due to the exfoliation of control group tooth. Thus, 38 lesion pairs could be compared radiographically at the 12-month follow-up. Total dropout at this follow-up was 15.6%. None of the participants reported any side effects.

Radiographically, 26 of the case group lesions (68.4%) and 19 of the control group lesions (50.0%) were E2 lesions at the 12-month follow-up. Two (5.3%) lesions in the case group and 10 (26.3%) of the control group lesions reached the dentine radiographically at the 12-month follow-up. The radiographic depths of the case and control group lesions at the baseline, six-month, and 12-month follow-up are illustrated in Figures XII and XIII. A total of three (7.9%) participants showed progression in both lesions, 22 (57.9%) participants showed no lesion progression in case or control lesions, three (7.9%) showed progression in case group lesions only, and 10 (26.3%) had progression in the control group lesions only. Thus, a total of 32 (84.2%) of case group lesions and 25 (65.8%) of control group lesions showed no sign of progression radiographically at the twelve-month follow-up. McNemar's test did not exhibit a significant difference between the groups (p=0.092). Figure XIV shows information about lesion progression radiographically among the participants at the 12-month follow-up. Six lesions (15.8%) of the case group showed signs of progression compared to 13 (34.2%) of the control group lesions (Figure XV).

Discussion

The goals of this study were to evaluate radiographically the effectiveness of Icon resin infiltration as an adjunct to standard-of-care preventative measures (fluoride application, oral hygiene instruction, and diet counseling) compared to standard-of-care preventative measures alone in controlling the progression of non-adjacent, incipient, enamel proximal carious lesions (E1 and E2) in primary molars after six and 12 months. The primary outcome was to measure whether there was lesion progression radiographically after six and 12 months of treatment.

Lesions either progressed from E1 to E2 or from E2 into the dentine. Once signs of progression were reported, the lesion was considered a failure and was excluded from the study.

During the initial screening, only 61 subjects (67.8%) were found to be eligible to participate in the study, in addition to the 16 subjects who refused or failed to come for the treatment. Thus, screening failure was 50%, which is lower than what was expected during sample size calculation. During the six-month follow-up, only one (2.2%) subject failed to come back. Unfortunately, at the 12-month, follow-up appointment, a total of seven (15.6%) were lost, mainly due to their failure to come for the follow-up appointment or due to the exfoliation of the primary molars. This is lower than what was estimated in the sample size calculation and to what was reported by Ekstrand et al. [53] and Martignon et al [32]. Ekstrand et al. reported that 19% of the children who received resin infiltration for their proximal surface lesions were lost at the one-year follow-up [53]. Similarly; Martignon et al. reported that 20% of the participating children were lost after one year of treatment [32].

One (2.27%) of the participating children was excluded at the 12-month follow-up due to the exfoliation of the primary molars, and a slightly higher number of children were excluded by Ekstrand et al. due to exfoliation of primary molars, three (6.25%) [53]. On the other hand, Martignon et al. [32] did not report any loss of the participating children due to exfoliation, and this can be explained by the fact that the mean (SD) age of the participants at their treatment day was 7.27 (1.09), which is similar to the mean age of the subjects included in the study published by Ekstrand et al. at their treatment day, 7.18 (0.68) [53] and higher than mean age 5.3 (0.70) of the children who received superficial lesion sealing as reported by Martignon et al. [32].

A split-mouth design was used in the study to eliminate the subject's related factors and increase the precision of the treatment effects, thus enhancing the study's power to determine if one treatment is more effective than another if the carry-across effects were under control ([56]. As both lesions received fluoride application, oral hygiene instruction, and diet counseling at the time of treatment, after six and 12 months, we believe that the carry-across effect was under control. On other hand, due to the nature of the study, both the parents and the subject were not blinded as to which group a lesion was in. Thus, we can expect that the subjects or parents might have paid extra attention to one of the lesions to an extent that could influence the final results.

In the study two non-adjacent incipient proximal lesions per subject were randomly allocated to either case or control group. This left the possibility that case group lesions could differ from the control group lesions in the lesion depths at the baseline and tooth surface locations. The analysis showed that the plurality of the case group lesions were located on the mesial surface of the lower right second primary molar, 8 (17.8%), while the plurality of the control group lesions were located on the mesial surface of the upper-right second primary molar, 10 (22.2%). With respect to the contact area between the primary molars, most of the case and control group lesions were located on the mesial surface of the second molars and the distal surface of the first molars, 34 (75.6%) and 37 (82.2%), respectively. This can be related to the size of the contact area between the primary molars [(57)]. The large contact area between the primary first and second molars contributes to a high prevalence of proximal dental caries at those surfaces [(58)]. Further analysis using McNemar's test showed that the initial depths and the locations of the lesions did not differ significantly between the case and control, and therefore we

can be confident that the difference in the outcome among the two groups is not attributable to their initial depth or the lesions' location.

The radiographic evaluation at the six and 12-month follow-ups was performed by at least two calibrated and trained examiners to evaluate the lesions' depths and determine whether the lesions had progressed. Therefore, before the study was initiated, training sessions were carried out in which all the examiners were trained and calibrated, and the inter- and intra-examiner reproducibility agreement using kappa value was obtained using randomly selected bitewing radiographs for primary molars with proximal lesions of various depths. Each one of the three examiners was asked to evaluate the radiographs at two times one month apart. The inter-examiner reliability values of the three examiners ranged from 0.59 to 0.80 (kappa), while the intra-examiners values ranged from to 0.64 to 0.87 (kappa). The first two examiners were asked to evaluate all the lesions and evaluate whether or not the lesion had progressed and if they disagreed, the third examiner evaluated the lesions. Therefore, the third examiner always evaluated the lesions that the first two examiners disagreed on; this explains the low value of the inter-examiners kappa value. Since infiltrated lesions cannot be distinguished from untreated lesions radiographically, the calibrated examiners were blinded to whether the lesions they were examining were case or control group lesions.

Recently dental treatments have shifted into more conservative approaches including the application of fluoride varnish together with improving the patient's oral hygiene and diet control to inhibit lesion progression and maintain the tooth structure rather than removing the diseased part of the tooth [9]. Both caries sealing [30-33] and resin infiltration [51-55] are new micro-

invasive intermediate approaches and their effectiveness in controlling the progression of proximal lesions in primary and permanent teeth has been investigated by many authors.

Resin infiltration is based on using a strong acid and a low-viscosity, light-cured resin to penetrate deeply and seal the enamel surface porosities and inter-crystalline spaces [35,36]. Therefore, a diffusion barrier is created inside the enamel lesions rather than on the enamel surface, as in sealing, which strengthens the enamel structure and prevents further cavitation or breakdown of the tooth [36]. The superficial sealing can lead to plaque retention around the sealed proximal lesion [34]. In a study done by Martignon et al. a one-year radiographic follow-up of sealed non-cavitated proximal lesions in primary teeth showed that 27% of the sealed lesions progressed after one year of treatment [32]. This is higher compared to resin infiltration as reported by both Ekstrand et al. [53] 23% and our study 15.8% after one year.

Another advantage of resin infiltration over sealing is that sealing does not require teeth separation for few days prior to treatment [32]. Also, resin infiltration eliminates the need of local anesthesia administration and cavity preparation [50]. Considering local anesthesia administration is one of the most painful procedures for children [59], eliminating the need of anesthesia application and cavity preparation can be a major advantage when it comes to treating fearful children and allows postponing more invasive operative approaches.

To the best of our knowledge, there are only two published clinical trials that evaluated the therapeutic effect of resin infiltration on proximal superficial carious lesions on the primary molar teeth [53, 54]. Only one used a split-mouth design and reported that 23% of the case group

lesions and 62% of the control lesions progressed [53] radiographically compared to six (15.8%) of case lesions and 13 (34.2%) of the control group lesions after one year in our study. In the previous study, most of the lesions included in the case (66.7 %) and control (53.8%) group lesions were in the outer half of the dentine radiographically. In our study, only lesions within the enamel were included and it is well-known that the progression of caries is faster in the dentine than enamel [60].

The lower progression rate in our study can also be explained by the remineralization effect of topical fluoride. The effect of topical fluoride in both inhibiting the demineralization of dental hard tissue and enhancing its remineralization are well established, particularly for initial enamel carious lesions [61]. Since all the patients received 5% NaF at the baseline, six and 12 month follow-up appointments and only enamel lesion were included in the study less progression rate was expected at the follow-ups compared to the study done by Ektracnd et al. [53]

Water fluoridation,[62] the use of fluoridated tooth paste,[63] the use of fluoridated fluoridate mouth wash [64] have been shown to be effective in reducing caries progression in children but the subjects' additional fluoride exposure prior to and during the study period was not evaluated in this study.

The other study reported by Altarabulsi et al. evaluated the clinical safety, quality, and effect of resin infiltration for proximal lesions in both primary and permanent molars in children, with the majority of the infiltrated lesions located on permanent molars and premolars. They reported that only two (4.7%) of the lesions showed signs of progression after one year from E2

into the dentine [54]. No specific data were given regarding which teeth showed the signs of progression (primary or permanent), and therefore their results cannot be compared with ours.

When compared to the effectiveness of resin infiltration on proximal lesions in permanent teeth 18 months after treatment, significantly lower percentage (7%) of the lesions treated by resin infiltration showed sign of progression radiographically [52]. Which is lower to what was reported by both Ekstrand et al. [53] (23%) and. and our study (15.8%) after one year of treatment for primary molars. This result can be due to the fact that the primary teeth have thinner enamel [65] and the progression of proximal carious lesion in primary teeth is faster than in permanent teeth [66]. It takes an average of 12 months for a lesion to progress through the outer half of the enamel and, on average, 10–12 months for a lesion to progress through the inner half of the enamel in primary teeth [67] compared to three to four years for a lesion to progress through enamel into dentine in permanent teeth [68].

Another explanation of the higher failure rate of resin infiltration in primary teeth compared to permanent teeth is that the Icon resin infiltration kit provides a universal applicator for permanent and primary teeth and does not consider the anatomical differences between these teeth. Primary teeth have thinner enamel and dentine and demonstrate board and flattened contact area, while the permanent teeth have a small distinct circular contact point [65].

When the inhibition effect of resin infiltration alone was compared to resin infiltration and fluoride varnish on artificial enamel lesions, resin infiltration in combination with fluoride varnish was better in arresting the initial lesions in primary and permanent teeth than resin

infiltration alone [69]. Therefore, in the present study, we used a combination of resin infiltration and standard of care (fluoride application, oral hygiene instruction, and diet counseling) to provide better protection in case the resin infiltration failed to infiltrate the entire lesion.

The study was conducted on a small sample with most of them being a high-risk group, and the study period was only 12 months. Therefore, its value in terms of external validity might be limited. However, if resin infiltration in addition to the standard of care showed fewer lesion progressions than standard of care preventive measures (fluoride application, oral hygiene instruction, and diet counseling) after six and 12 months, it will probably also work in subjects with lower caries risks over longer time periods.

Limitations of the study

The poor compliance with instructions to floss regularly, especially by children [33], emphasizes the need for motivation and improvements of other preventive strategies. All children and parents were given oral hygiene instruction, diet counseling and flossing instructions including the proximal areas at the baseline at the six-month and 12-month follow-ups, but their compliance during the study period was not evaluated in our study.

Resin infiltration using 15% hydrochloric acid was found to penetrate most parts of the demineralized enamel lesions in vitro when examined under the microscope, while it failed to fill up cavitated lesions. Therefore, the efficacy of resin infiltration is impaired significantly when the lesions treated are cavitated [66]. The lesion cavitation was evaluated clinically prior to treatment by both previous studies done on primary teeth [32, 53, 54] and only non-cavitated lesions

were included. In our study, the lesions were only evaluated radiographically and the level of cavitation was not evaluated clinically prior to the treatment. Therefore, some doubts concerning proper diagnosis may remain and it is questionable whether the failure of resin infiltration in some of the lesions was due to the lesion's cavitation or due to the actual failure of the treatment.

Conclusions

Within the limitations of the study, it can be concluded that Resin infiltration as an adjunct to standard-of-care preventative measures (fluoride application, oral hygiene instruction, and diet counseling) was not significantly different from the standard-of-care preventative measures alone in terms of radiographic progression of non-adjacent, incipient, enamel proximal carious lesions in primary molars when evaluated at six and 12 months after treatment. Data will be collected at 18 and 24 months after treatment for further statistical analysis to compare the effectiveness of these two preventative measures.

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Appendix A: Tables

Table I: Characteristics of the screened subjects (N=90).

Variables	**Mean**	**SD**
Age (Yrs)	6.82	1.09
Primary teeth dmft	4.02	4.05
	Number	**Percentage**
Gender		
Male	48	53.3
Female	42	46.7

Race		
Asian	49	54.4
White-non Hispanic	12	13.3
African American	17	18.9
Hispanic	11	12.2
Others	1	1.1
CAT		
Low	28	31.1
Moderate	7	7.8
High	55	61.1

Table II: Characteristics of participating subjects at baseline (N=45).

Variables	**Mean**	**SD**
Age (Yrs)	7.29	1.09
Primary teeth dmft	4.60	3.78
	Number	**Percentage**
Gender		
Female	24	53.3
Male	21	46.7
Race		
Asian	21	46.7
White-non Hispanic	9	20.0
African American	9	20.0
Hispanic	5	11.1
Others	1	2.2
CAT		
Low	14	31.1
Moderate	3	6.7
High	28	62.2

Appendix B: Figures

Figure I: Smooth surface ICDAS stage [49].

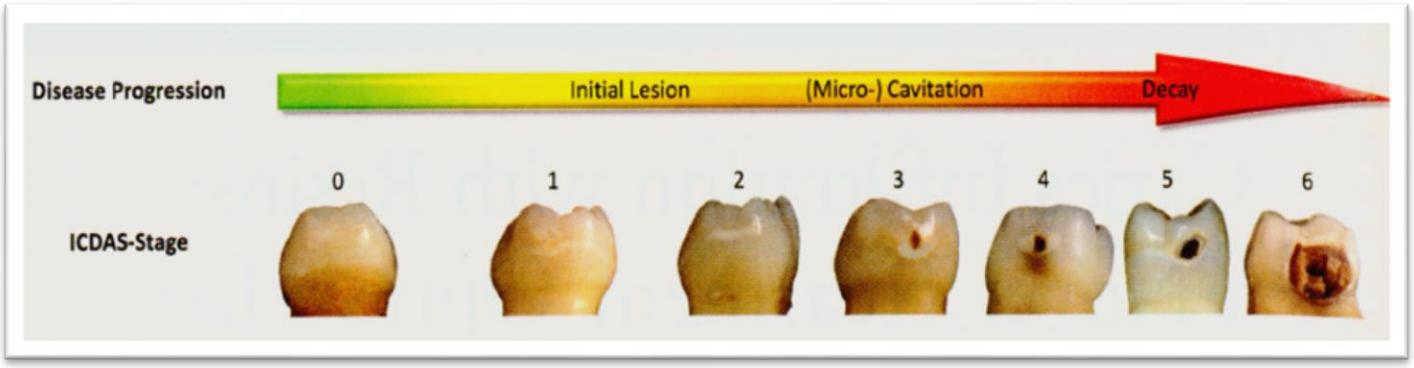


Figure II: Radiographic stages of proximal lesion [15].

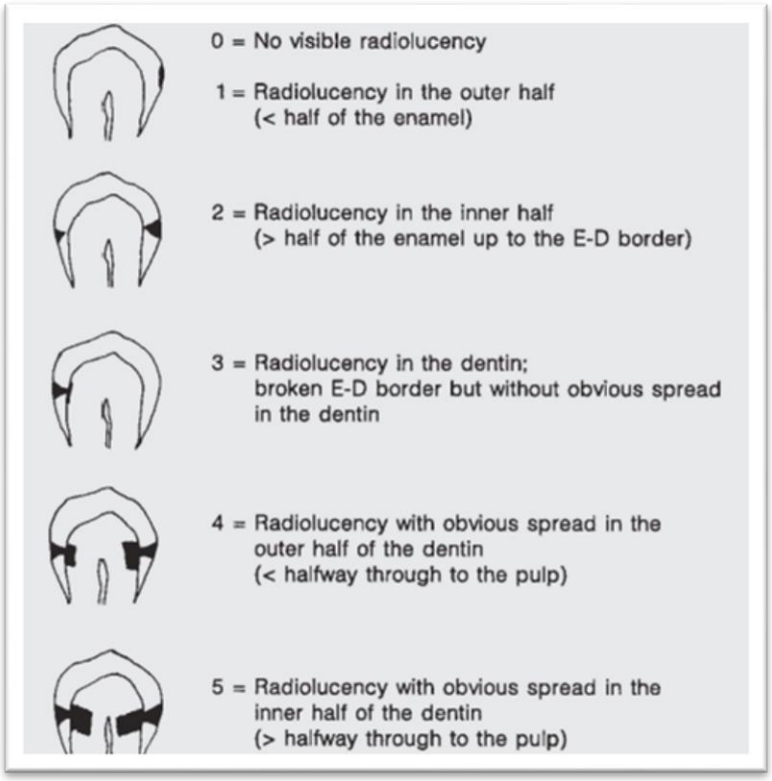


Figure III: Taking a standardized bite-wing radiograph. A: Using a Rinn sensor holder to position the sensor in the mouth and align the radiographs so that they were at 90 degrees to the sensor. B: Bite registration using impression material (Futar D (pink), ROYDENT, Johnson City, TN) was recorded for each subject while the sensor was positioned properly**.**

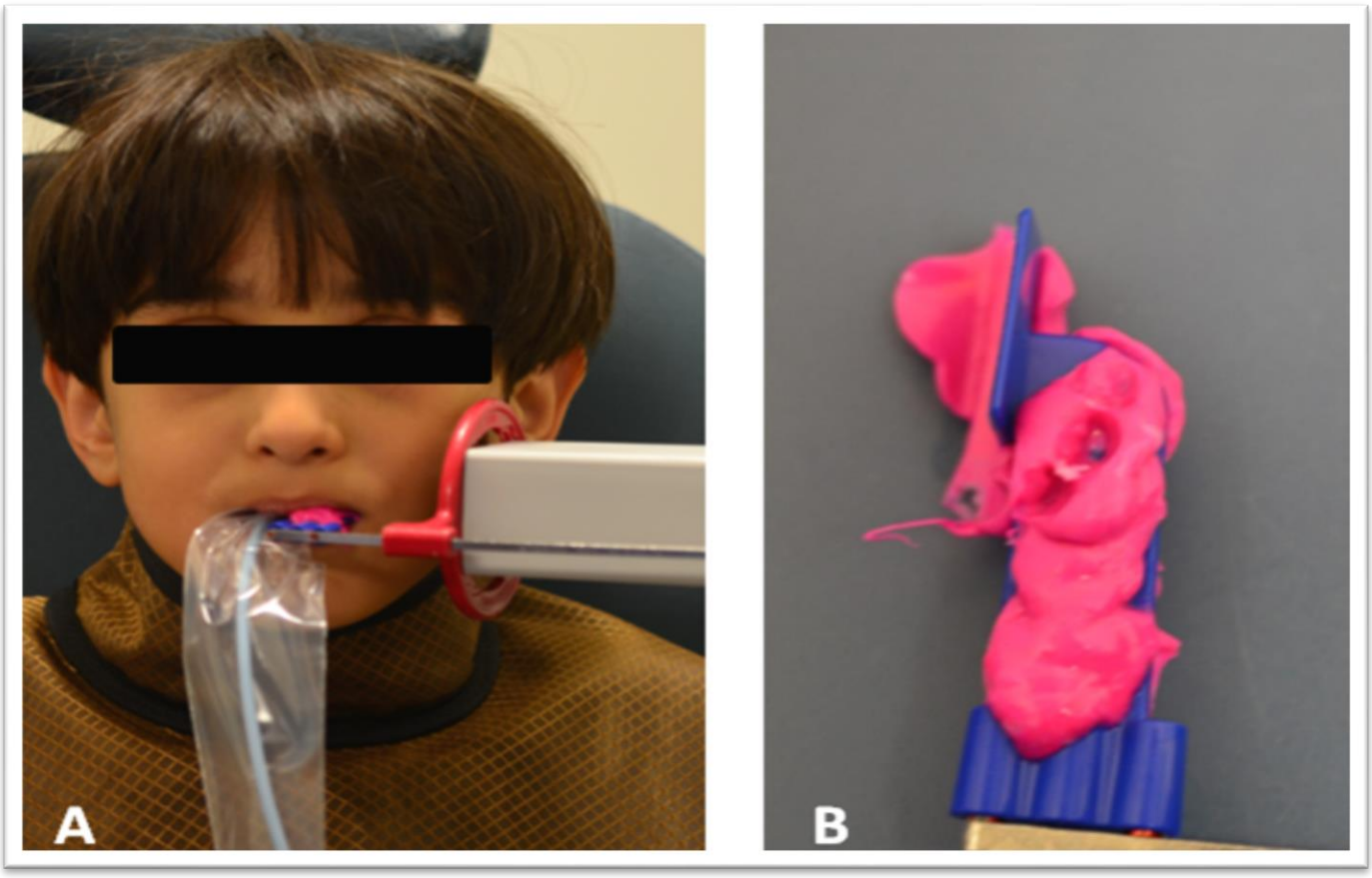


Figure IV: Resin infiltration of the distal surface of the upper-left first primary molar. A: Rubber dam Isolation. B: Flossing and cleaning of the proximal surface. C: Wedge placement. D: 15% HCL for two minutes (Icon Etch). E: Rinsing for 30 seconds and drying for 30 seconds. F: Icon Dry application for 30 seconds. G: Infiltrating resin (Icon) application for three minutes. H: removal of the excessive material before polymerization.

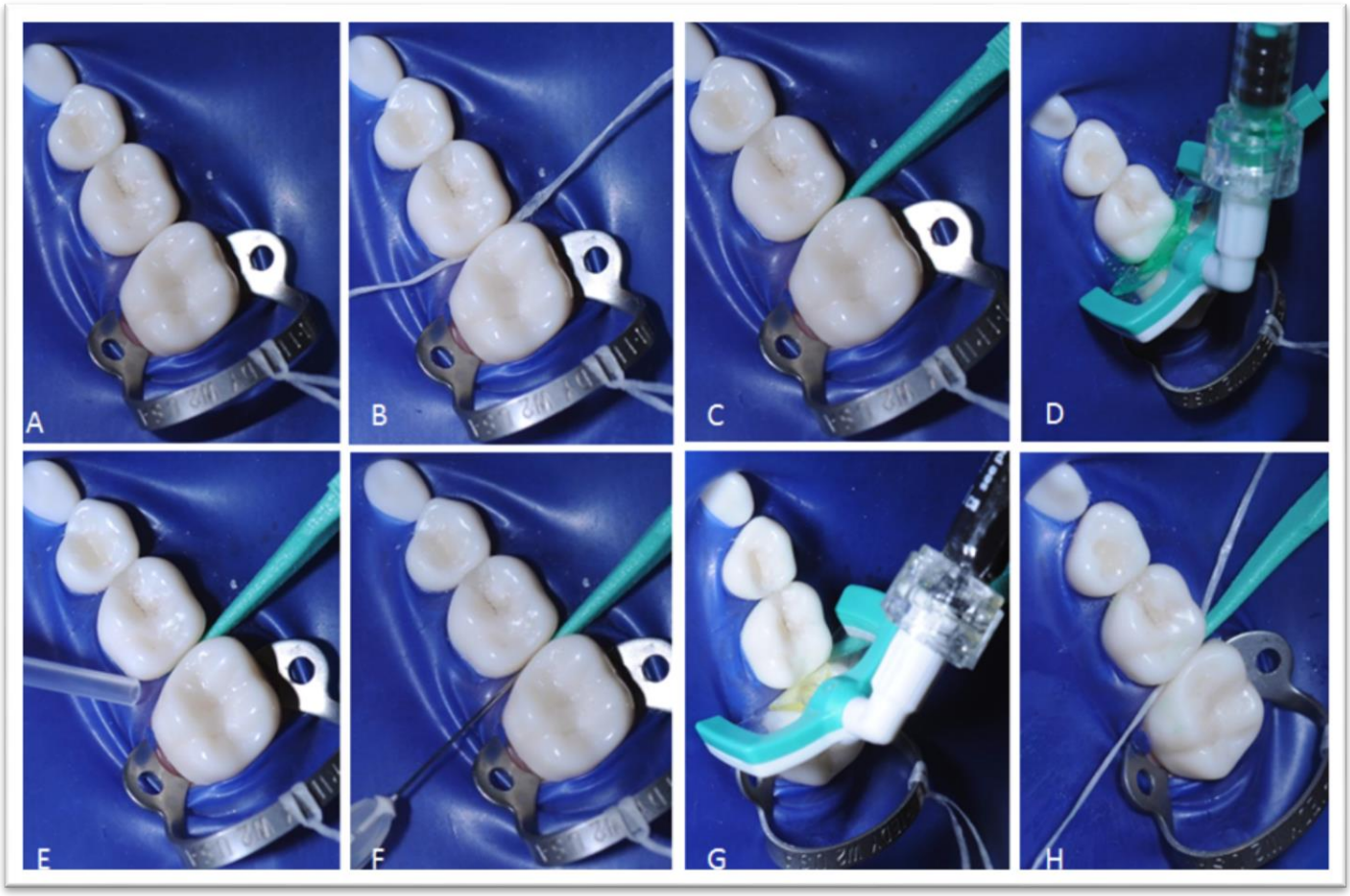


Figure V: Baseline and the follow-up radiographs of each lesion were paired. A: Represent the initial and 12-month follow-up radiographs for progressed lesion on the mesial surface of the lower left second primary molar. B: Represent the initial and 12-month follow-up radiographs for non-progressed lesion on the mesial surface of the lower left second primary molar.

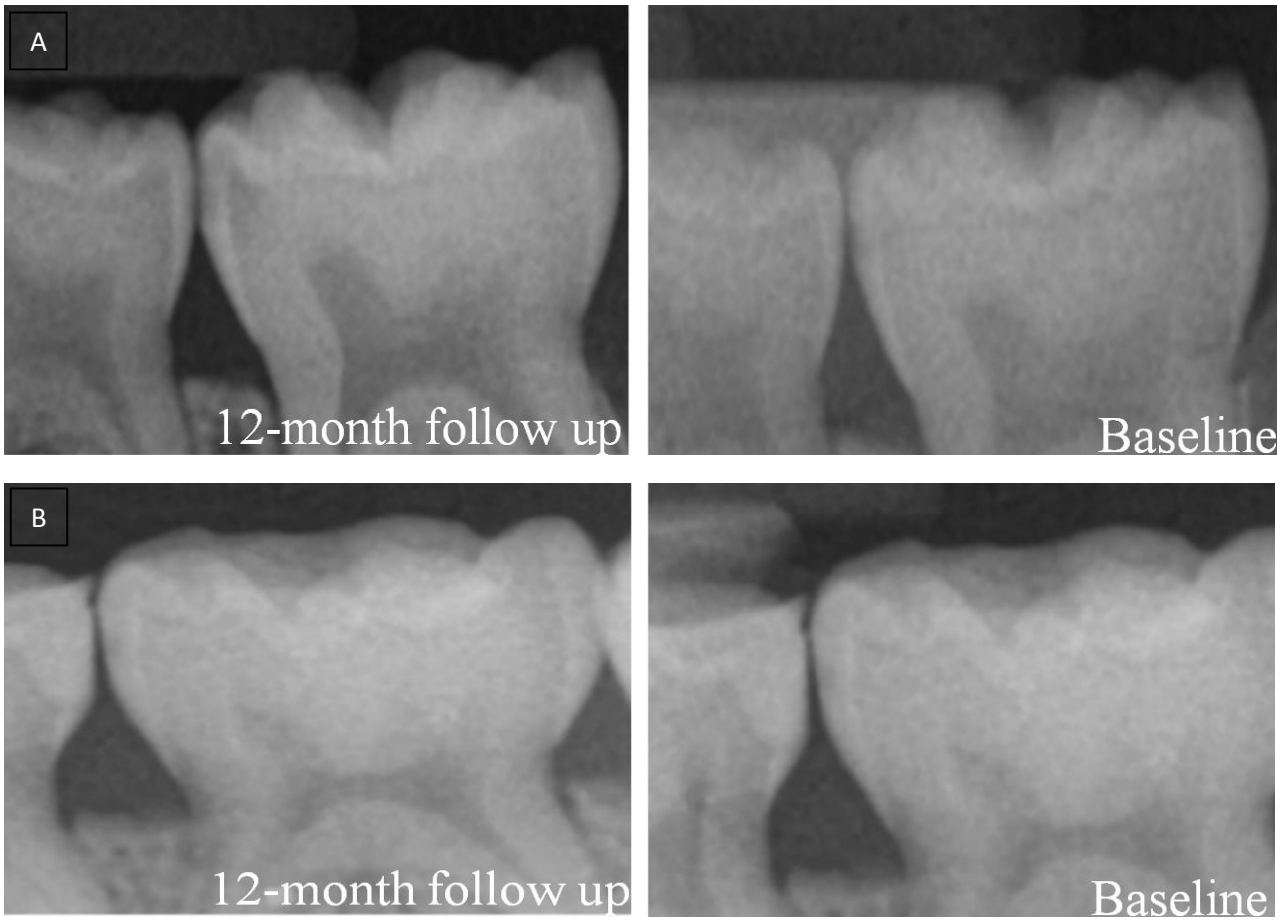


Figure VI: The study flow chart.

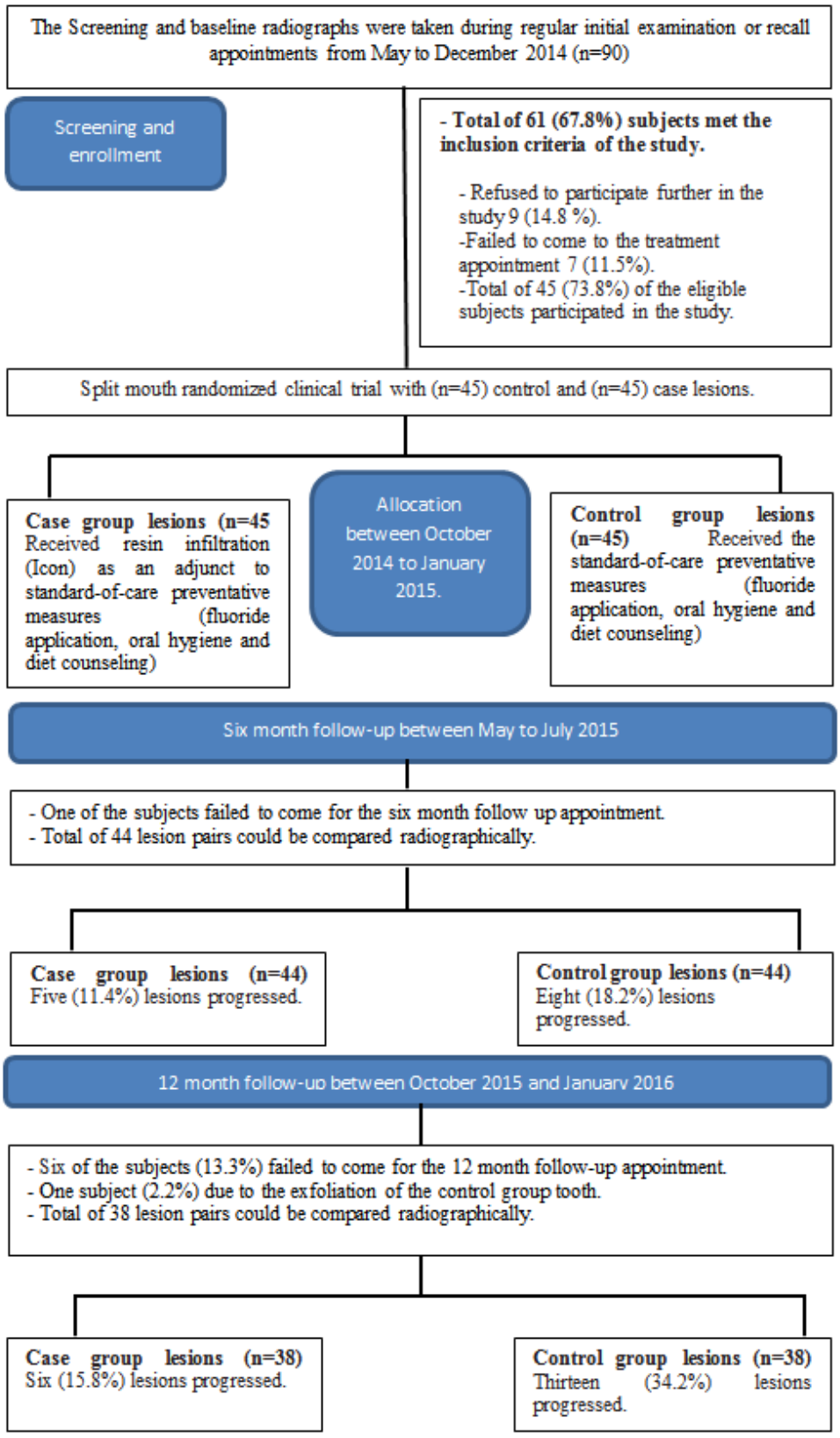


Figure VII: The distribution of case group lesions according to tooth surface and initial radiographic depth.

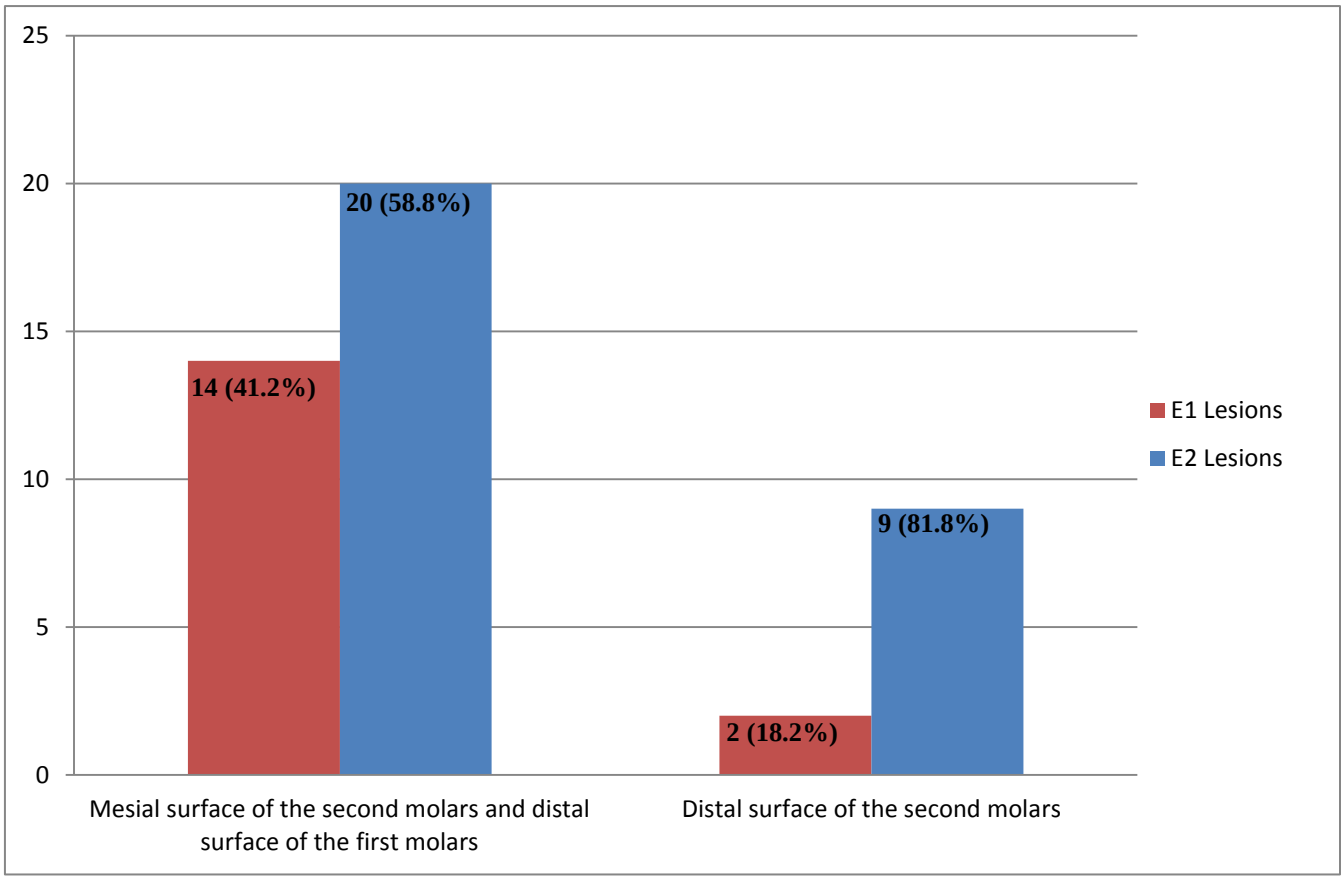


Figure VIII: The distribution of control group lesions according to the location and initial radiographic depth.

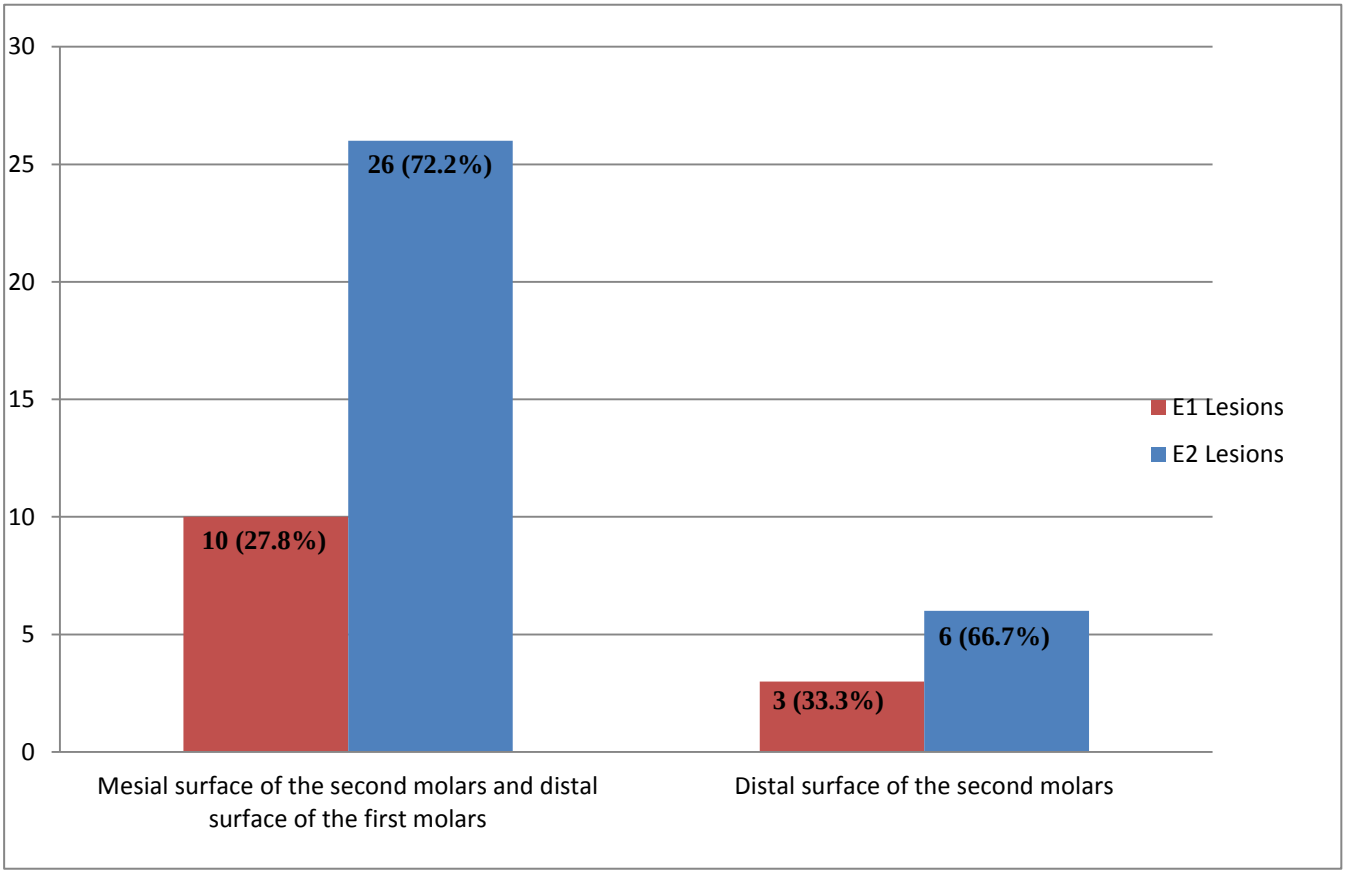


Figure IX: The distribution of case and control group lesions according to each lesion's initial radiographic depth.

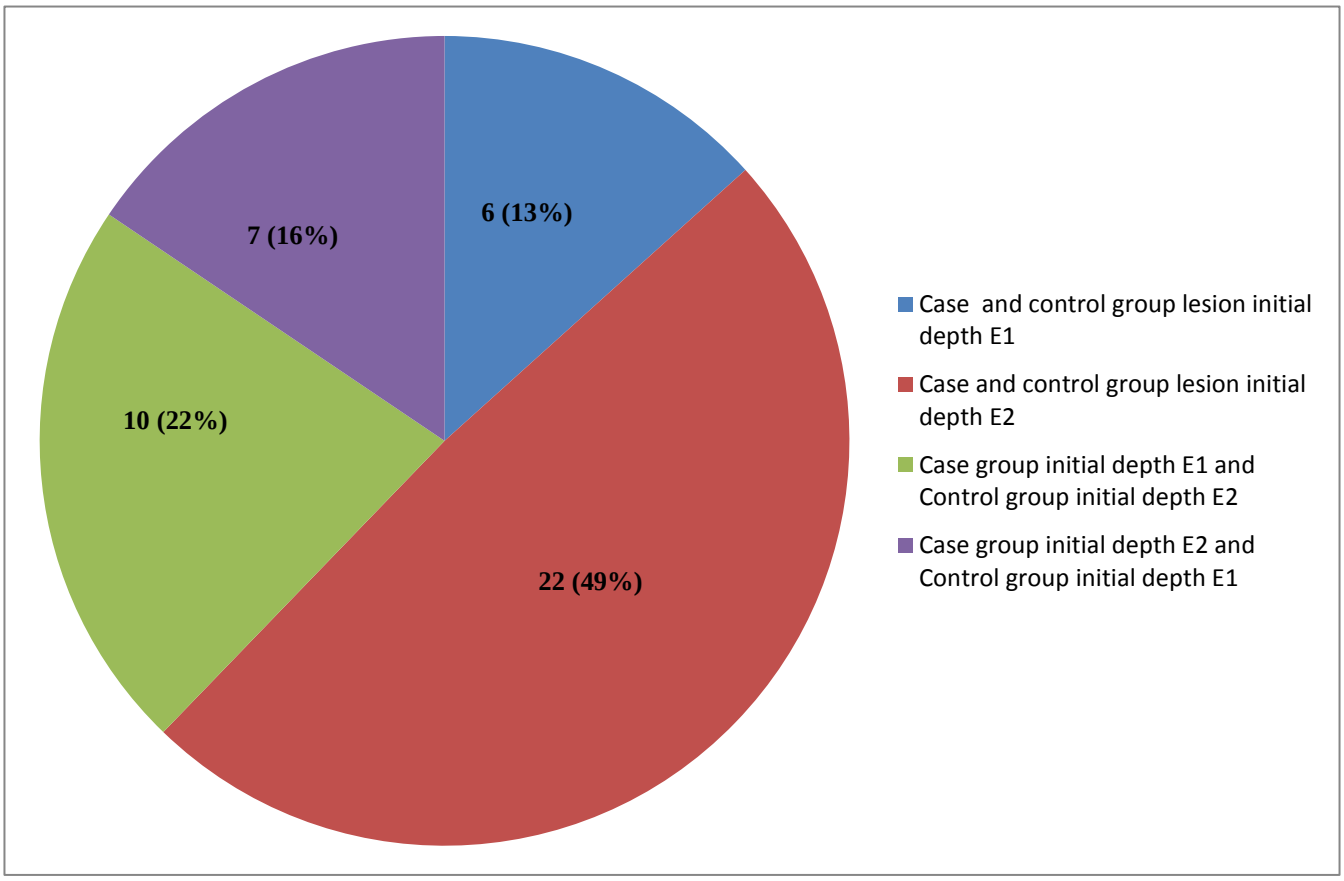
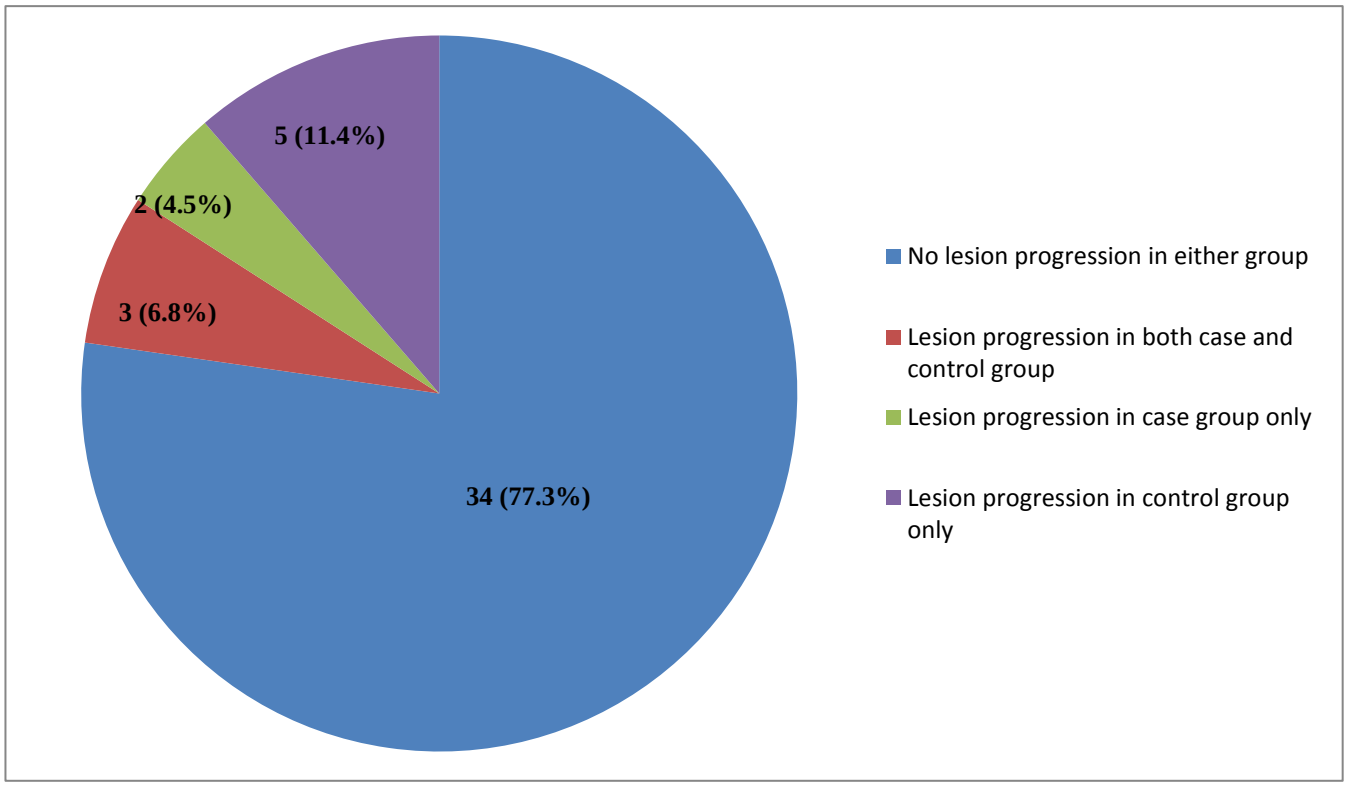


Figure X: Lesion progression radiographically among the participants at six-month follow-up (N=44).



* McNemar's test showed no statistically significant difference between the case and control groups (*P*=0.453).

Figure XI: The behavior of the case and control group lesions at six-month follow-up (N=44).

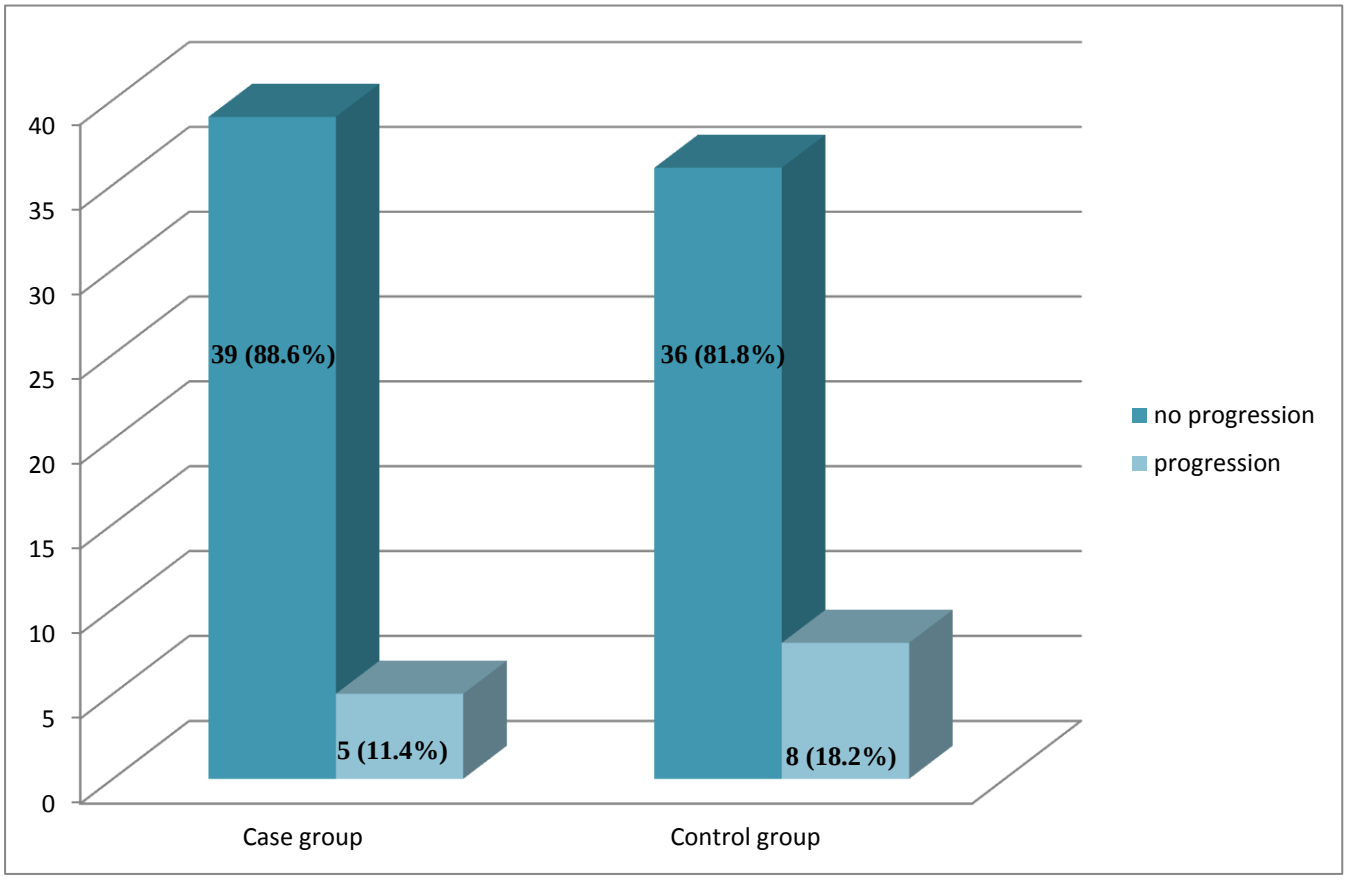


Figure XII: The radiographic depth of the case group lesions at the baseline, six-month and 12- month follow-ups.

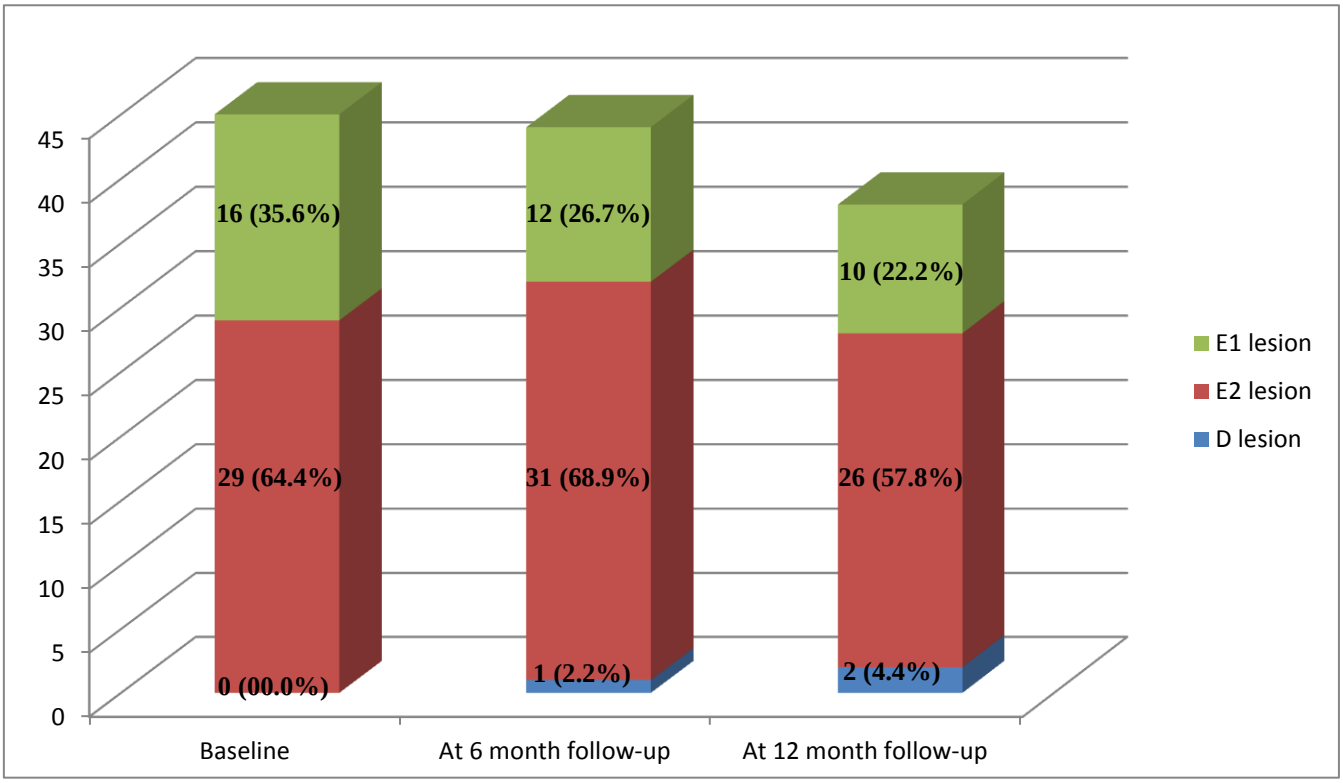


Figure XIII: The radiographic depth of the control group lesions at the baseline, six-month and 12-month follow-ups.

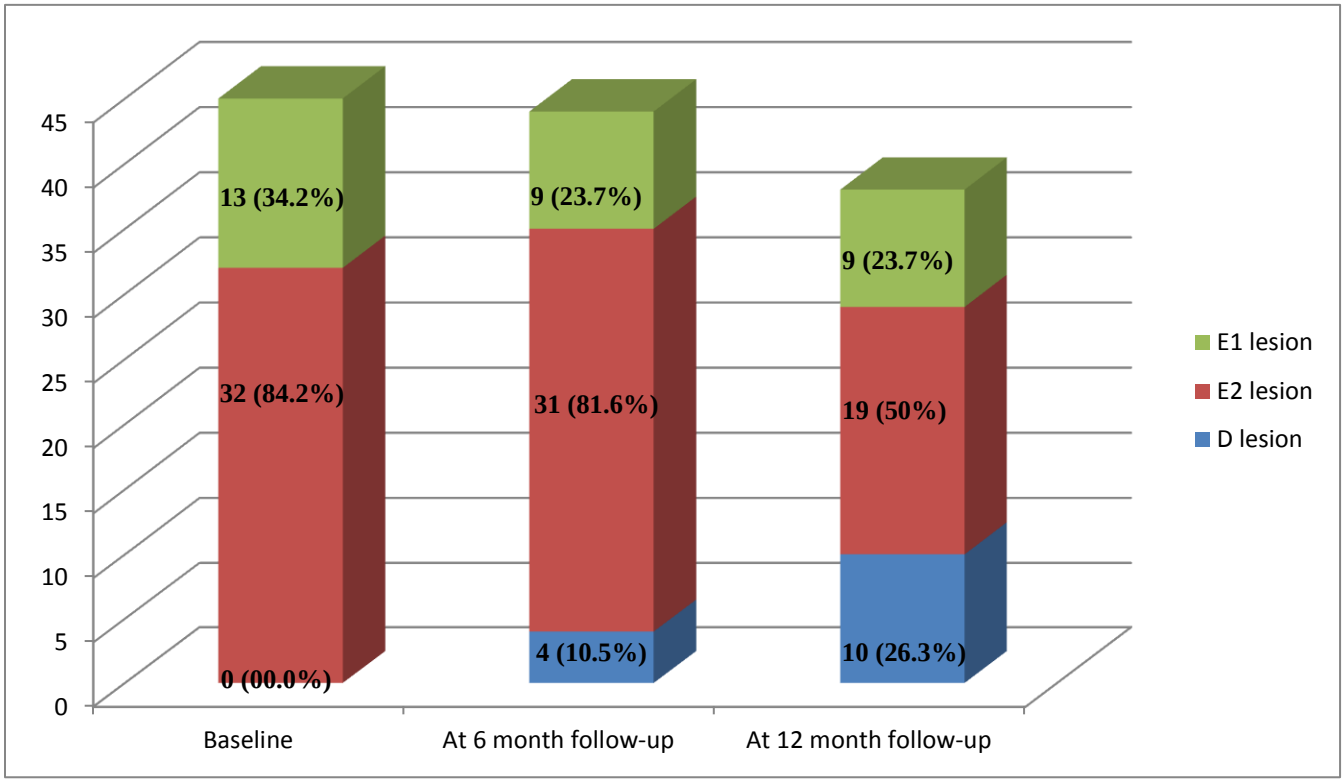
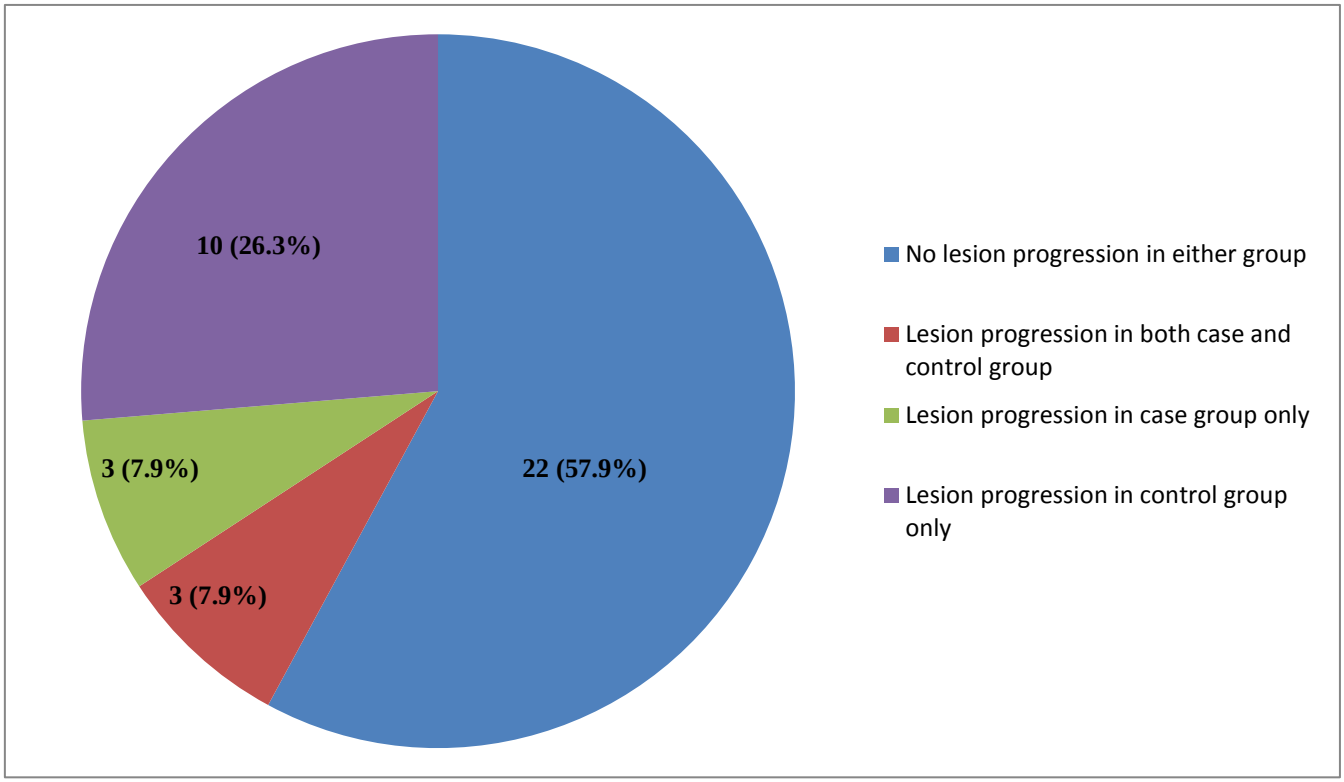


Figure XIV: Lesion progression radiographically among the participants at 12-month follow-up.



* McNemar's test showed no statistically significant difference between the case and control groups (*P*=0.092).

Figure XV: The behavior of the case and control group lesions at 12-month follow-up (N=38).