EFFICACY OF DIFFERENT REMINERALIZING AGENTS ON ARTIFICIALLY CREATED WHITE SPOT LESIONS :AN IN - VITRO STUDY

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This dissertation is submitted to **THE TAMIL NADU Dr. M.G.R. MEDICALUNIVERSITY** in partial fulfillment for the degree of **Master of Dental Surgery,** in BranchV – Orthodontics and Dentofacial Orthopaedics. It has not been submitted either partially orfully for the award of any other degree or diploma.

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White spot lesions are considered as precursors of dental caries that arises on the teeth as undesirable situation during fixed orthodontic appliance therapy. White spot lesions are defined as the, "subsurface enamel porosity from carious demineralization which can be seen as milky opacity when located on smooth surface"[1]. Dental caries is now being increasingly considered as a dynamic disease process wherein equilibrium exists between demineralization and protective factors causing remineralization.*The carieslesion processis active when demineralization periods are more thanthe periods of remineralization*[2]**.** This new insight into caries provided the scope for remineralizing incipient carious lesion *(WhiteSpot Lesion).*

Demineralization is a reaction of protons (H+) derived from disassociation of plaque acids with tooth enamel causing mineral dissolution[4.] When the critical pH of 5.5 is reached ie below the normal pH of 6.2 to 7.6, the calcium and phosphate ions exit the enamel, weakening it and eventually causing the chalky demineralization known as white spot lesions that may lead to the formation of caries[5.] The white spot lesion can be arrested and even repaired at its early stages without operative intervention by increasing the net mineral gain during the remineralization process.

Remineralization is the natural repair process for non- cavitated lesion and relies on calcium, phosphate ions assisted by fluoride to rebuild a new surface on existing crystal remnants in subsurface lesions remaining after demineralization[3]. When salivary or plaque calcium, phosphate ions and fluoride ions are present in adequate amounts, they can also promote the remineralizing of previously demineralized enamel[2].

Modern dentistry aims to manage white spot lesion non – invasively and effectively to prevent disease progression and improve esthetics, strength and function and other oral conditions. There have been many technologies available forthe

minimal invasive cure of white spot lesions, early diagnosis and early reversal of the initial white spot lesion using non operative techniques. The accurate, reliable detection newer diagnostic aids would enable the dentist to detect and diagnose early such lesions and appropriate preventive measures to promote remineralization.

Individuals with the malocclusions often have many retention sites due to the irregularities of their teeth and bonding attachments to teeth introduces retention sites on surface generally not susceptible to caries. Oral hygiene is thus more important and explain the much stronger relationship between oral hygiene and caries incidence in orthodontic patients than in the non – orthodontic individuals. Therefore it is the orthodontist's responsibility to be aware of the risk of decalcifications and take precautions to avoid or limit the demineralizing process by creating an environment for remineralization by various remineralizing agents[6].

Through remineralizing therapies we can hasten this remineralization procedure. Remineralization is increasingly accepted as a viable non-invasive approach for caries reversal, during the earlier stages of the carious process.

To evaluate demineralization and remineralization, it is important to know how much mineral has been lost or gained, or where the loss or gain occurred. There are different techniques available for direct and indirect mineral quantification including Microhardness, Transverse Micro Radiography, Polarized light microscopy, Scanning electron microscopy, Confocal Laser Scanning Microscopy, Microcomputed tomography, Diagnodent pen, Surface Microhardness ,Conventional radiography,Digital subtraction radiography,laser light methods which includes Digital imaging, Fiber-optic transillumination(DFOTI), Quantitative light induced fluorescence, Scanning Electron Microscopy and EDS (Energy Dispersive

Spectroscopy)are the most commonly used and sensitive techniques for showing mineral changes with respect to demineralization and remineralization.

SEM can provide information on surface topography, crystalline structure and electrical behaviour of the specimen[8]. While EDS detector is to assess the mineral content[4]. Hence, scanning electron microscope and EDS is used in this study to assess the enamel surface demineralization and remineralization.

As individuals who undergo orthodontic treatment are more susceptible to develop high risk of development of white spot lesions, so it is necessary to find the efficacy of the various remineralizing tooth paste that effectively reverses the white spot lesions.

Currently available remineralizing agent, limit the ability to remineralize the enamel, which leads to the research of further agent with the essential elements of enamel remineralization.

Hence this invitro study is undertaken to find out the efficacy of two remineralizing paste Agent I and Agent II to treat the patients effectively.

Aim:

- To evaluate the remineralizing potential of Remineralizing agent I and Remineralizing agent II on demineralized enamel sample using Scanning Electron Microscope (SEM) and Energy Dispersive Spectroscopic (EDS) analysis.

Objectives:

- To compare the efficacy of different remineralizing potential of Remineralizing agent I and Remineralizing agent II on demineralized enamel sample.

The invitro study done was done to assess the effect of concentration of calcium in artificial saliva on the output of fluoride from HEMA/MMA controlled-release devices by Adair SM, Whitford GM et al (1994)[9]. The initial release rates wasdetermined in deionized water, and divided into five groups. One group remained in deionized water throughout the 19-day study while other groups were placed in artificial saliva containing 0, 4.5, 8.0, or 12.0 mg% calcium for 4-13 days. Ten devices of each group were placed in deionized water again on days 14-17. The five devices of each group that were not placed in deionized water on day 14 were inspected for surface crystals.The results indicate that fluoride release from HEMA/MMA devices is reduced in artificial saliva, which is proportional to the concentration of calcium.

The invitro study of treatment of fluorosedand white spot human enamel with Calcium sucrose phosphatewasdone byPamela Den Besten et al (1995)[10]for assessing the relative whiteness of normal, mildly fluorosed, moderately fluorosed and carious white spot lesion extracted teeth quantitatively by light reflectance. Treatment of enamel with a 35% hydrogen peroxide gel resulted in significantly increased whitening, which was not reduced by treatment, removal of enamel surface followed by treatment with 5.25% sodium hypochlorite and artificial saliva was successful for returning white spot lesions to normal enamel colour. SEM imaging of calcium sucrose phosphate treated enamel resulting in a normal light reflectance from the enamel.

The invitro studywas done to evaluate two fluorescence methods DIAGNOdent and QLF (quantitative light-induced fluorescence) to quantify the white spot lesions adjacent to fixed orthodontic appliances; and 2) to determine the inter-observer agreement of the DIAGNOdent and QLF methods for quantification of

incipient enamel lesions adjacent to fixed orthodontic appliances was done by A Aljehani et al (2007)[11]. Samples were forty-one premolar teeth with visually sound smooth surfaces or visually white spot enamel lesions. Orthodontic brackets were fixed adjacent to the lesions. All teeth were measured using both the Diagnodent and QLF methods. Of the 41 teeth, 20 smooth surfaces were analyzed using both Diagnodent and QLF. Results showed that the QLF may be a suitable method for quantifying incipient carious lesions adjacent to fixed orthodontic appliances

The invitro study was done to evaluate the effect of CPP-ACP paste on demineralization by using an FE-SEM by Maki Osiro et al (2007)[14]. A few specimens were stored in 0.1 M lactic acid buffer solution for 10 min and then in artificial saliva (negative control). The remaining specimens were stored in a 10 times-diluted solution of CPP-ACP paste or a placebo paste containing no CPP-ACP for 10 min, followed by 10 min immersion in a demineralizing solution twice a day before storage in artificial saliva. After treatment of the specimens for 3, 7, 21 and 28 days, they were fixed in 2.5% glutaraldehyde in buffer solution. The enamel and dentin specimens were treated with CPP-ACP paste revealed slight changes in their morphological features. Results showed that the CPP-ACP paste might prevent demineralization of the tooth structure.

The invitro study of remineralization of incipient enamel lesions by the topical application of Casein phospho peptide-Amorphous Calcium Phosphate (CPP-ACP) was evaluated by using laser fluorescence and scanning electron microscope by Deepika bhai et al (2008)[19]. Sixty caries free extracted teeth were used in the study. The samples were demineralized and then remineralized by the topical application of CPP-ACP for a period of 14 days. The results of this study showed that a significant number of test samples observed under SEM showed high scores of remineralization.

The effect of nono- hydroxyapatite concentrations on initial enamel lesions under dynamic pH cycling conditions, lesions are prepared in bovine enamel with an acidic buffer was published by S B Huang et al (2009)[15].NaF (positive control), deionized water (negative control) and four different concentration of nano-hydroxyapatite were selected as treatment agents. Surface microhardness measurements were performed before/ after demineralization and after 3,6,9,12 days of application. The specimens were examined by scanning electron microscope. The % of SMHR in nano- hydroxyapatite was greater than that of negative control.When the concentration of nano- HA was under 10%, SMH and %SMHR increased with increasing nano- hydroxyapatite concentrations. It was concluded that 10% concentration of nano- hydroxyapatite had the potential to remineralize initial enamel erosions.

Both invivo and invitro effects of a casein phosphopeptide amorphous calcium phosphate (CPP- ACP) and fluoride containing topical agents in reducing enamel demineralization around orthodontic bracketswas evaluated byT Uysal et al (2010)[16]. The samples include 21 patients and 60 extracted premolars were divided into three groups: two experimental and one control group. Tooth Mousse(CPP- ACP gel) and Fluoridinwere applied to tooth surface around orthodontic brackets in the experimental group. Ininvivo study teeth were extracted after 60 days and samples were cycled through the daily procedure of demineralization. All the teeth were evaluated by superficial microhardness analysiswas made in occlusal – cervical positions. Results showed that in both Invivo and Invitro evaluations indicated that CPP- ACP and fluoride containing agents successfully decreased demineralization around orthodontic brackets.

The effect of casein phosphopeptide–amorphous calcium phosphate tooth mousse on the remineralization of bovine incisor by circularly polarized images was evaluated by Guotao Wuinqiang Liuet al (2010)[18].The samples were divided into Group A -casein phosphopeptide–amorphous calcium phosphate tooth mousse; Group B - fluoride toothpaste; Group C -casein phosphopeptide–amorphous calcium phosphate tooth mousse and fluoride toothpaste; and Group D – no treatment. Circularly polarized images were taken after the specimens were treated for 3, 6, 9, or 12 weeks, and the size of the demineralized area and the mean grey level were measured.Results show that the Casein phosphopeptide–amorphous calcium phosphate tooth mousse can reduce the size of the demineralized areas and promote the remineralization of bovine enamel.

The efficacy of CPP-ACP, and CPP- ACPF on enamel remineralization using Scanning electron Microscope was done by Jayanth Jayarajanet al (2011)[41], in his invitro study.The samples include 90 maxillary premolars were divided into three groups Group A: Artificial saliva, Group B: CPP- ACP, Group C: CPP- ACPF. All the samples were assessed using diagnodent at the baseline and after demineralization and remineralization. Results showed that the CPP- ACP, CPP- ACPF had a significantly higher amount of remineralization than the artificial saliva.

The prevalence, distribution and formation of white spots after orthodontic treatment and about their prevention and management in the post orthodontic treatmentwas viewed by Irfanulla Khan Mahamadet alin (2012)[6], in their article. They occur as a result of improper oral hygiene.The presence of white spot lesions after orthodontic therapy seems to be discouraging whose goal is to improve facial and dental esthetics.

Toevaluate the remineralization potential of casein phosphopeptide-amorphous calcium phosphate paste on enamel subsurface lesions using Scanning Electron microscopy with Energy dispersive X ray analysis was done by Mithra N Hegde and AnuMoany et al (2012)[20], in their invitro study. Ninety enamel specimens were prepared from extracted human molars. The specimens were placed in demineralizing solution for four days to produce artificial carious lesion. The specimens were randomly divided into five study groups and one control group of 15 specimens per group .Except for control group all other group were incubated in the remineralizing paste (CPP-ACP)for 7, 14, 21, 28, 35 days twice daily for 3 minutes. After remineralization, the mineral content of the sample were measured using SEM-EDX. Results show that the CPP- ACP paste could significantly remineralize the artificial enamel subsurface lesions.

The efficacy of remineralizing potential of the specially formulated remineralizing mouth rinse, and the "Gold standard" sodium Fluoride (NaF) rinse was evaluated by using QLF was done by R. Vaderhobliet al (2012)[21].Samples includes 12 subjects and randomly assigned to the control and the test group. Twice daily the subjects rinsed 1 oz of either the test (Calcium and fluoride; 250 ppm F) or the control rinse (Na F; 250 ppm F). Baseline measurements of the progression of the smooth surface caries lesionwere done by QLF at 0, 1, 2, and the end of 3 months.Results showed no significant difference between the two groups in preventing the progression of the caries.

The invitro study to find out the efficacy of CPP- ACP , CPP- ACPF and Tricalcium phosphate fluoride (TCP-F) in remineralizing artificially created caries lesion was assessed by SEM and EDAX by Namrata Patil et al (2013)[23].The changes are analysed using Diagnodent and Scanning electron microscope(SEM) in total of

52 premolars and 24 molars and classified into four groups of 13 premolars and 6 molars each. All the samples were assessed using Diagnodent at the baseline after demineralization and remineralization and for surface evaluation using SEM. The results show that CPP- ACP and TCP- F show more amount of remineralization than the CPP- ACP.

The invitro study was done to assess the white spot lesions treated with NaF plus tricalcium phosphate (TCP) toothpastes using microtomography (micro CT) by Makoto Asaizumik and Arlinsey et al (2013)[24].The samples include the bovine enamel were subjected to demineralization solution to form artificial white spot lesion Following demineralization, the specimens were treated with one of the NaF silica based toothpaste in 10 day pH cycling model. Each day consisted of 2 min treatments, one 4hr acid challenge and immersion in the artificial Saliva between these events. After cycling they were analysed using micro- CT. Micro CT analysis revealed NaF tooth paste containing TCP led to increased WSL densities relative to the fluoride free tooth paste.

The invitro study was done to compare the evaluation of the efficacy of CPP-ACPF and Sodium fluoride with tri- calcium phosphate on enamel remineralization using diagnodent by HetalChapla and Nimisha Shah et al (2013)[25].The samples include 30 extracted premolars were selected and divided into three groups of 10 teeth each Group A: (saliva), Group B:(CPP- ACPF), Group C: (Sodium fluoride with tricalcium phosphate – Clinpro 5000). All the samples were assessed using Diagnodent at the baseline and after demineralization and remineralization after 7 days. Results show that Clinpro 5000 showed marginal more amount of Remineralization than CPP- ACPF.

The invitro study was done to evaluate the remineralizing potential of three different remineralizing agents (GC tooth mousse, Clinpro tooth crème and SHY-NM) on demineralised tooth surfaces using micro CT and microhardness by Arun Balakrishnan et al (2013)[26]. The samples include 45 freshly extracted mandibular premolars. The specimens were then demineralized using Mclnne's demineralizing solution in two cycles. After that remineralization was carried out in two cycles for 30 days using Casein phosphopeptide – Amorphous calcium phosphate (CPP- ACP), 0.21% sodium fluoride- tricalcium phosphate and Calcium sodium phosphosilicate (CSP) containing tooth paste for groups I, II, III respectively. The specimens were evaluated using Micro CT and Vicker's Micro hardness testing at different time periods. The results showed that the CPP- ACP showed the better remineralizing potential.

The effects of fluoride varnishes supplemented with sodium trimetaphosphate (TMP) on the remineralization of caries-like lesions in this invitro study was done by M.M Manarelli et al (2014)[27].Bovine enamel discs were selected through surface hardness (SH) and the blocks were divided into 7 experimental groups (n = 24/group): placebo (no fluoride or TMP), 5% TMP, 2.5% NaF, 2.5% NaF/5% TMP, 5% NaF, 5% NaF/5% TMP and (Duraphat™, 5% NaF). Discs were treated with the varnishes and kept in a remineralizing solution for 4 h and a demineralizing solution for 2 h. The remaining discs were submitted to a pH-cycling regimen for 6 days. They concluded that the supplementation of fluoride varnishes with TMP leads to enhanced remineralizing effect of artificial caries lesions invitro.

The invitro study was done to evaluate the efficacy of bioactive glass containing product on remineralization of artificial induced carious enamel lesion by Sai Sathya Narayana, Vinoth Kumar Deepa,et al (2014)[5]. 20 human molar teeth were

subjected to artificial caries lesion formation and verified using high resolution scanning electron microscope. Each demineralized sample was then divided into five test groups .Group A - Bioactive glass (SHY-NM), Group B - Fluoride tooth paste (Amflor), Group C -CPP-ACP (Tooth mousse), Group D - CPP-ACPF (Tooth mousse plus), Group E - control. Following demineralization, the remineralizing agents were applied for 10 min except control. After 10 days period, the entire test groups were evaluated with HRSEM and quantitative assessment by energy dispersive X-ray spectroscopy. Results show that bioactive glass exhibits as effective remineralizing agent.

An invitro study to find the effect of ACP, Tooth mousse, Remin plus remineralizing agents by structural analysis method done by SEM and Elemental analysis procedure by Sathe N et al (2014)[28].Samples include 40 freshly extracted premolars subjected to artificial demineralizing solution and remineralization procedure is carried out for 10 days. They concluded that Tooth mouse and Remin plus show greater effect than APF gel on the enamel resistance to demineralization and future carious lesion.

An invitro study aimed at quantitatively evaluating the enamel remineralization using three different remineralizing agents CPP- ACP, CCP-ACP+F and NaF using surface microhardness analysis was done by Shishir Shetty et al(2014)[29], 50 freshly extracted human molar teeth ,were subjected to create artificial white spot lesion and remineralizing procedure for 28 days, after the process, all the groups of the enamel samples were assessed for surface microhardness using Vicker's hardness test.The results of the study showed that the CPP- ACP effectively remineralizes initial enamel caries compared to CPP- ACPF and NaF. Also, fluoride

to CPP-ACP shows improved remineralization of initial enamel caries when compared with CPP- ACP and NaF.

An invitro study to evaluate the remineralization potential of Novamin on Artificial enamel subsurface lesions around orthodontic brackets using Energy Dispersive X – ray analysis(EDX) by Pritham Mohanty et al (2014)[30]. Samples include 40 extracted teeth were randomly divided into control group and study group. All samples are demineralized and incubated in artificial saliva at 37degree c for 10 days after demineralization, the enamel are treated with the remineralizing agent Novamin for 10 days. It is concluded that Novamin containing remineralizing agent showed significant remineralizing potential in inhibition of artificial enamel subsurface lesion around bracket.

An invitro study to evaluate the assessments of white spot treated with (ICON) and fluoride gel was done Fathimah A et al (2014)[31]. Samples include112 sound premolars were divided into four groups. Group A:Control negative, Group B: NaF, Group C: ICON, Group D: Control positive. Except Group D were demineralized with the buffered demineralized solution for 5 days, following demineralization, the microhardness of the specimens were evaluated using vicker microhardness testing machine and surface roughness using profilometer. The findings of the current study proved the benefits of ICON in enhancing the microhardness and decrease surface roughness of the demineralized enamel more than Na F gel.

The invitro study they examined the effects of fluoridated, casein phosphopepetide- amorphous calcium phosphate complex (CPP- ACP) containing and functionalized b- tricalcium phosphate containing toothpaste on remineralization of white spot lesion by using Quantitative light induced fluorescence (QLF-D) Biluminator by Su- Yeon Jo et al (2014)[32].48 premolars extracted for orthodontic

reasons from 12 patients with artificially induced WSL were randomly and equally assigned to four different treatment groups: Fluoride(1000ppm), CPP-ACP. Ftcp (with sodium fluoride) and control (deionized water) groups Specimens were treated twice daily for 2 weeks and stored in saliva solution. QLF- D Biluminator was used to measure the changes in the fluorescence. Results show that Ftcp and CPP- ACP containing toothpaste seems to be more effective in reducing white spot lesions.

An invitro study to evaluate the effect of pre- conditioning enamel white spot lesions (WSL) surfaces using bioactive glass (BAG) air abrasion prior to remineralization theraphy was conducted by Hussam Milly et al (2015)[33].Ninety human enamel samples with artificial white spot lesions were assigned to three white spot lesion surface pre- conditioning groups(a) air abrasion with BAG polyacrylic acid (PAA- BAG) powder, (b) acid- etching using 37% phosphoric acid gel (positive control), (c) unconditioned (negative control). Structural changes in the lesion were observed using confocal laser scanning microscopy and Scanning electron microscopy- energy dispersive X ray spectrometry. Preconditioning WSL surfaces with PAA – BAG air abrasion reduced subsurface light scattering, increased the knoopmirohardness and the mineral content.

The ability of Novamin to remineralize the artificially created the demineralized lesion was assessed in 120 non carious extracted premolar teeth SEM and EDAX analysis was conducted Saranya Mony, et al (2015)[34].Results showed that the Novamin effectively increases the remineralization by exhibiting the surface characteristics in SEM images and qualitatively by increase in the hardness value and Ca/Po4 ratio.

An invitro study to evaluate the remineralizing potential of four remineralizing agents SHY-NM, GC Tooth Mousse Plus, ReminPro and Colgate strong teeth on

demineralized human teeth by polarized light microscopy was done by Reshma Rajan, Ramesh Krishnan et al (2015)[36]. Samples includes 50 extracted premolars, subjected to demineralization for 48 hours at 37°C. Teeth were grouped into five study groups of 10 teeth in each. Each group was treated with remineralizing agent and sectioned using hard-tissue microtome and visualized under polarized light microscope. Results revealed that SHY-NM has the most remineralizing potential followed by ReminPro, GC Tooth Mousse Plus and fluoridated tooth paste.

The evaluation of the Remineralization potential of fluoride varnish, ACP-CPP-F& TCP-F on artificially created white spot lesions by microhardness was done by Shilpa S Maga et al (2015)[56]. The samples include 30 extracted premolars were subjected to demineralizing solution for a period of 5 days. After artificial enamel lesions are formed they are ready for the measurement of microhardness. A pH cycling regime included demineralization procedure for (3 hrs) and remineralization for (21 hrs) for five consecutive days. Enamel samples are divided into four groups. Group A: Fluoride varnish, Group B: CPP- ACP, Group C: TCP- F, Group D: control group. Results showed the mean hardness of TCP- F group has the highest hardness followed by ACP- CPP-F.

An invitro study was done by Edith Lara Carrilloa,et al (2016)[38],to evaluatethe enamel remineralization of Novamin(Amorphous sodium – calcium phosphosilicate paste) and low- level laser for white spot lesion after orthodontic theraphy in 20 extracted premolars in three phases 1. Placement fixed orthodontic appliances. 2. Demineralizing solution. 3. Remineralization for groups. They concluded that NUPRO sensodyne with Novamin is effective than LLL for treatment for white spot lesions.

An invitro study compared the evaluation of three remineralizing agents ie. Fluoride varnish, CPP-ACP, Functionalized Tricalcium phosphate using Confocal microscopy in 60 permanent central incisors was done by Krunal chokshi,et al (2016)[39]. Artificial white spot lesion is created by immersing all the teeth in the demineralizing solution for a period of 4 days at 4.6 pH. Then the samples are subjected to the remineralizing agents for 2 minutes, for 20 to 40 days and concluded that fluoride varnish remineralizing agent is most effective followed by the CPP- ACP paste and f TCP.

An invitro study of Microcomputed evaluation of white spot lesion remineralization with various procedures was done by EyupBurakKucuket al (2016)[42]. 44 extracted premolars were divided into 4 groups of 11 teeth each. The samples are subjected to demineralizing solution for 96 hrs, for 30 days and micro CT scanning was performed at the pre demineralizing stage at days 0(T1), 15(T2), and 30(T3), after white spot lesions created, remineralizing agent group A: control group, Group B: GC tooth mouse, Group C: Clinpro 5000 group, Group D: 50 ppm sodium fluoride was applied. Results showed that GC tooth mouse and Clinpro 5000 were more effective in remineralization of white spot lesions.

The invivo effects of three different topical agents on Enamel demineralization around orthodontic brackets was done by HammadS and Abdellatif A et al (2016)[43].The samples include first premolar teeth extracted from 28 patients from 13-16 years of age, were divided into four groups three experimental and one control group. Defense, Clinpro fissure sealant and white varnish with TCP were applied to the tooth surfaces around brackets in the experimental group. After one month, two premolars of each patient, were extracted, the samples were stored in refrigerator in flasks with saline. After two months the other 14 premolars from each group were

extracted and treated similarly. They concluded that three protecting agents decrease demineralization around orthodontic brackets.

An invivo study to assess the occurrence of white spot lesions in permanent molars of childrens with and without orthodontic theraphy to evaluate the effect of casein phosphopeptide amorphous calcium phosphate (CPP-ACP) on white spot lesions by Deepti Munjalet al (2016)[44]. The materials include 679 first permanent molars in children aged between 8 to 16 years age. Group I comprised without any orthodontic treatment. Group II comprised who had undergone orthodontic theraphy. Treatment group included 20 post orthodontic patients treated with the remineralizing agent. CPP- ACP cream two times a day for 12 consecutive weeks. CPP- ACP theraphy is highly recommended for the post orthodontic treatment on teeth undergoing fixed orthodontic theraphy.

An invitro study, for the assessment of three fluoride releasing agents on Enamel demineralization around Orthodontic bracketswas done byEsraa S. Jasim et al(2016)[45]. 40 sound human permanent premolars and categorized into four groups, in one group the teeth were bonded with the stainless steel brackets using Resin - modified glass ionomer cement (RMGIC) and other three groups the teeth were bonded with light cured composite Resilience. Group A: Acidulated phosphate fluoride (APF) gel, Group B: Resin modified Glass ionomer cement, used as a bracket adhesive. Group C: Stannous fluoride and Sodium Phosphate fluoride Gel yielding 0.72% fluoride ion was applied daily through experimental study, control group. All the teeth were subjected to 30 days of acid challenge.They concluded that the group B: Resin Modified Glass Ionomer Cement the best caries fighting fluoride measure.

An invivo study with 12 week assessment of treatment of white spot lesions with 10% CPP- ACP paste and /or 5% sodium fluoride varnish on regression of non –

orthodontic white spot lesions was done by Zeynep Ash Guclii et al (2016)[46].The study included 21 children with white spot lesions were randomized into four treatment regimes: weekly clinical application of fluoride varnish for the first month; twice daily applications of CPP- ACP paste; weekly application of fluoride varnish for the first month and twice daily self – applications of CPP- ACP paste (CPP-ACP-FV) and no intervention. The results show that self applications of CPP- ACP paste significantly improved the appearance and remineralization of white spot lesion.

The efficacy of fluoride and casein phoshopeptide - amorphous calcium phosphate for treating white spot lesions during and after treatment with fixed orthodontic appliances was evaluated by Kristina Lopatiene et al (2016)[47], in their invitro study. Use of fluoridated tooth paste had a remineralizing effect on white spot lesions. Fluoride varnish and casein supplements were effective in prevention and early treatment. They conclude that the use of Casein phophopeptide amorphous calcium phosphate can be more beneficial than the fluoride rinse in reduction of the demineralizationspots.

The efficacy of remineralizing agents CPP- ACP containing fluoride varnish by different quantitative methods in 30 bovine samples by SelcukSavas et al (2016)[48]. The test material (MI varnish) was applied on the demineralized area and the treated samples were stored in the artificial saliva. At the 4th week, the enamel surfaces were tested by surface microhardness(SMH), quantitative light induced fluorescence digital (QLF- D),energy dispersive spectroscopy(EDS), and laser fluorescence (LF pen). Results show that CPP- ACP containing fluoride varnish provides remineralization of WSL.

The invitro study was done to compare the evaluation of application of different fluoride varnishes on artificial early enamel lesionby Udita Majithiaet

al(2016)[49] through EDAX. Samples include 80 intact enamel specimens prepared from premolars extracted for orthodontic purposes. Samples were randomly divided into two groups for measurement of baseline surface Vickers microhardness and baseline ca/ Po_4 ratio through EDAX analysis. After demineralization procedure by pH cycling regime,remineralization done for 5 consecutive days for 21 hrs daily Results showed all the remineralizing agents used in this study are capable of treating the early enamel lesions.

The study was done to compare the remineralizing efficacy of novamin and tricalcium phosphate (TCP) was done by Jagga U, Paul U, Padmanabhan V et al (2018)[50].30 premolars samples were taken.Baseline microhardness was measured. Artificial carious lesions were created for all teeth by subjecting them to demineralization process.Then microhardness of demineralized lesion was measured.The samples were equally divided into two groups to treat with remineralization solution for 10 days; group I: novamin and group II: TCP. After 10 days of pH cycling, microhardness was measured. They concluded by saying that both novamin and TCP were effective in remineralizing the carious lesions.

This prospective invitro study was undertaken in Department of Orthodontics, Sri Ramakrishna Dental College, Coimbatore.

Samples which include, 50 extracted maxillary and mandibular non-pathological I^{st} and II^{nd}premolars,(Fig 1) were chosen as they are the most spared teeth in Orthodontic treatment. They were collected from the patients who have undergone Orthodontic extraction from the Department of Oral and Maxillofacial Surgery, Sri Ramakrishna Dental College, Coimbatore.

Inclusion criteria:

Morphologically,intact non- carious maxillary and mandibular I^{st} and II^{nd}premolars which are extracted for orthodontic reasons were included in the present study[54]

Exclusion Criteria:

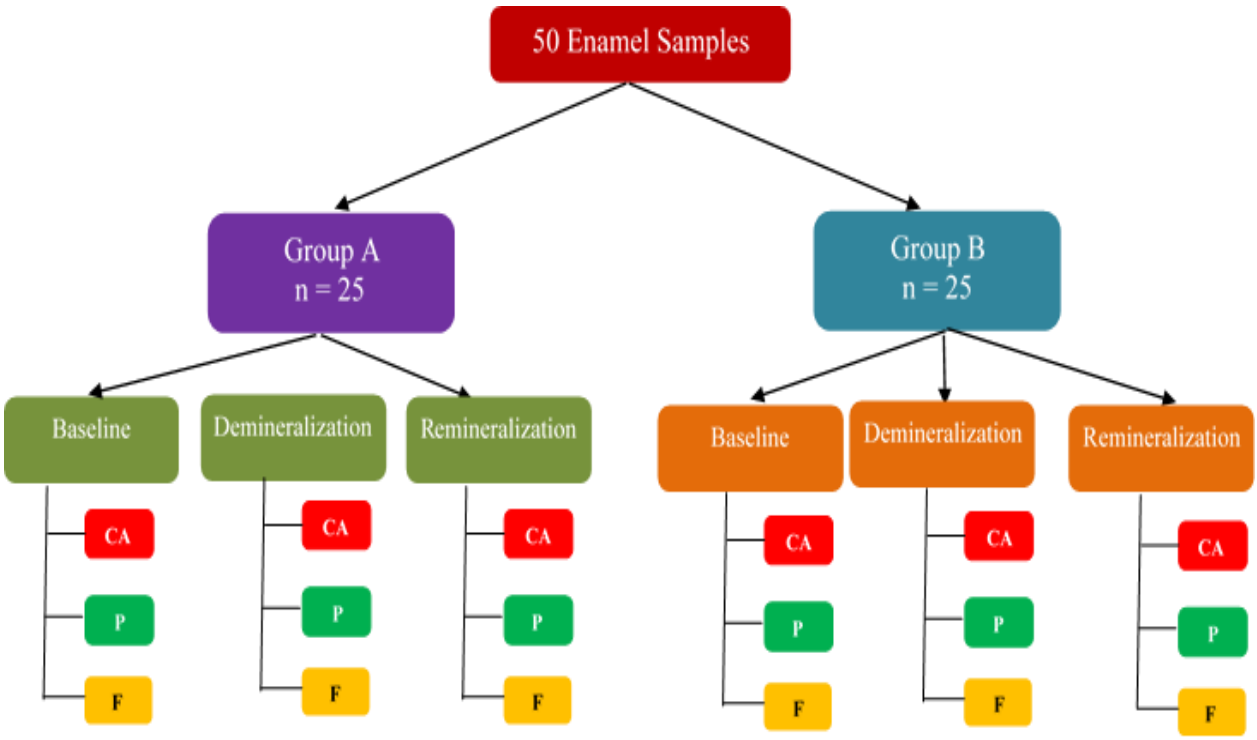
- Decayed, attrited, teeth with visible cracks, developmental anamolies and restored teeth were excluded from the study.[54]

SAMPLE PREPARATION (Fig 2&3):

- The extracted teeth were thoroughly cleaned and washed with normal saline to remove all adherent soft tissues debris and remanants[25,26,27,28,36]
- The teeth were then divided into 2 groups with 25 teeth each.
- A window of 3mm × 3mm of enamel was exposed at the occluso gingival center of the labial surface of the clinical crown, an acid resistant nail varnish(nail lacquer) was applied in a vertical strokes around the exposed enamel surface of all the samples and is the delineated area to be studied[,25,26,36].

- The samples were stored in 10% formalin solution till the experimental procedure at room temperature (20-25 degree celsius)[36]

DIVISION OF SAMPLE:



Group A (n=25): Remineralizing Agent I.

Group B (n =25): Remineralizing Agent II.

Agents I and II were blinded.

SEM evaluation and EDS analysis:

The enamel samples are subjected to gold sputter coating using Sputtering machine(Quorum 150R S) (Fig 13) for high quality image and resolution before SEM (Scanning Electron Microscope) (Quanta 250 FEG) (Fig 16) and EDS (Energy Dispersive Spectrocope) (Quanta 250 FEG) (Fig 17) analysis**.** After sputtering the samples were observed under SEM at 4000x magnification at 10kv**.**

The samples are subjected to baseline SEM (Scanning Electron Microscope) to analyse the surface topography and EDS (Energy Dispersive Spectroscopy) analysis to calculate the mineral content (% weight) at the baseline.

PREPARATION OF DEMINERALIZING SOLUTION:

The demineralization solution was prepared in the Department of Nanoscience and Technology, Bharathiyar University.

500 ml of demineralizing solution was prepared by mixing 2.2 mM Calcium chloride ($CaCl_2$), 2.2 mM Potassium dihydrogen phosphate (KH_2PO_4) and 0.05M Acetic acid. The pH was adjusted at 4.5 by adding 1M Potassium hydroxide (KOH) to the preparedsolution[30,33,34]

DEMINERALIZATION PROCEDURE: (Fig 4&5).

Both the groups of samples were immersed in 250 ml of demineralizing solution in 250ml ofBorosil beaker and kept in an incubator(Universal Scientific Company). (Fig 6) maintained at 37 degree Celsius at a pH of 4.4. The demineralizing solution was changed every day and the procedure was done for 10 days. The Demineralizing procedure was intended to produce a consistent subsurface lesion[30,33,54]

After the demineralization procedure, the samples were subjected to SEM for assessing the surface topographic changes and EDS analysis for mineral content (% weight). (Fig 20&21)

REMINERALIZING PROCEDURE:

Compositions of artificial saliva: (Mc Knight – Hanes, Whitford -1992)[8](Fig 11). The artificial saliva is prepared by the mixingGastric Mucin -2.200 (grams per liter), Na Cl - 0.381 g/ L,$Cacl_2$.2H20 - 0.231 g/ L,

Na_2HPO_4-0.738g / L and Distilled water of 1 litre[30]

The pH of artificial saliva was adjusted to 7 with KOH.

The investigatory part of the study was undertaken in the Department of Nanoscience and Technology,Bharathiyar University, Coimbatore under the guidance of DR. N. Ponpandian, Ph.D. Prof and HOD

Remineralizing agents (Fig 7& 8):

Agent I:100grams - 5% Novamin- 45% SiO2, 24.5 %Na2O, 24.5% CaO and 6% P2O5,glycerine, PEG 400,silica, Calcium sodium phosphosilicate, sodium lauryl sulphate, Titanium Dioxide, carbomer, Potassium Acesulfame

Agent II:40 grams – Hydroxyapatite (calcium and Phosphate), Fluoride (1450ppm) and xylitol, Flavor: strawberry

After 10 days, the process of remineralization was started for the two groups.

In group A, remineralizing agent I was applied over the exposed area of the tooth and left over for 3 minutes with a johnson&johnson applicator bud , while in group B remineralizing agent II was applied over the exposed area of the tooth for 3 minutes , was done twice a day for a period of 10 days. After application the teeth were rinsed with distilled water and stored in artificial saliva.(Fig 12). The artificial saliva was changed every dayand were stored in incubator maintained at a pH of 7 at 37 degree Celsius for 10 days.(Fig 6).

After remineralization procedure the samples are subjected to SEM (Scanning electron microscope) for assessing the surface topographic changes and EDS (Energy Dispersive Spectroscope) analysis for mineral content (% weight)[5,30.,54]

When remineralizing agent comes in contact with saliva or water, first releases sodium ions. This elevates the pH into the range essential for HAP formation (7.5-8.5). The calcium and phosphate are released to supplement the normal levels found in saliva. This increase in ionic concentration, combined with an increase in pH, causes the ions to precipitate onto the tooth surface and form calcium hydroxycarbonate apatite (HCA) to remineralize the defect and to occlude open tubules. Unlike other calcium phosphate technologies, the ions that bioactive glass release from hydroxy carbonate apatite (HCA) directly, without the intermediate amorphous calcium phosphate phase. These particles also attach to the tooth surface and continue to release ions and remineralize the tooth surface after the initial application. Ultimately these particles will completely transform into HCA and result in 80% tubular occludance and desensitization[5,30,33,36,54].

The remineralized enamel sample of SEM image and EDAX is shown in (Fig 22&23)

Remineralizing agent II is a newer remineralizing water-based cream which contains hydroxyapatite, fluoride and xylitol.

The hydroxyapatite contained in Agent II fills the superficial enamel lesions and the irregularities that arise from the enamel surface due to erosion, which adheres to the tooth substances and protects the tooth against demineralization and erosion. The surface is noticeably smoothened, dentinal tubules are superficially sealed. Further, the smooth surface impairs the adhesion of the bacterial plaque.

On tooth surface, fluoride is converted into more stable and more acid resistant fluorapatite through contact with saliva.The fluoride contained in Remin pro strengthens the tooth and thus make it more resistant to acid attacks.

The sugar substitute xylitol is known for its cariostatic properties. Xylitol (unlike saccharose, for example) cannot be converted into harmful lactic acid by cariogenic bacteria.

Remineralizing agent II contains xylitol, thus the harmful effects of these bacteria and the metabolic product lactic acid can be significantly reduced.[36] The remineralized enamel sample of SEM image and EDAX is shown in (Fig24&25).

ARMAMENTARIUM

FIGURE 1 :COLLECTION OF ENAMEL SAMPLE

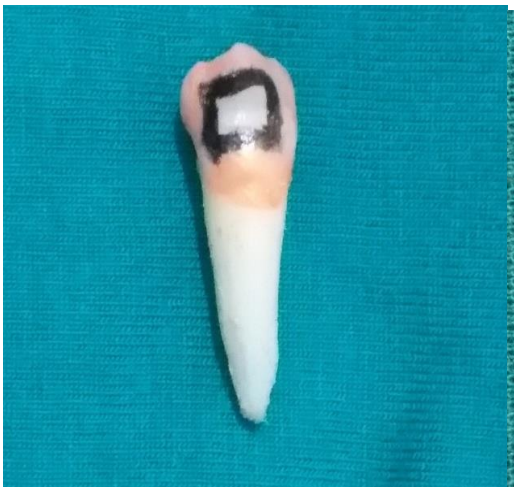


FIGURE 2 :GROUP A

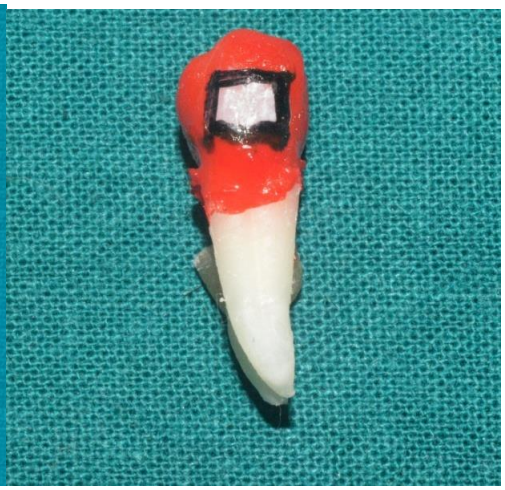


FIGURE 3 : GROUP B

FIGURE 4 :PREPARATION OF ARTIFICIAL DEMINERALIZATION SOLUTION.

FIGURE 5 : DEMINERALIZATION SOLUTION

FIGURE 6 : INCUBATOR(Universal Scientific Company).

FIGURE 7 :REMINERALIZING AGENT I

FIGURE 8 : REMINERALIZING AGENT II

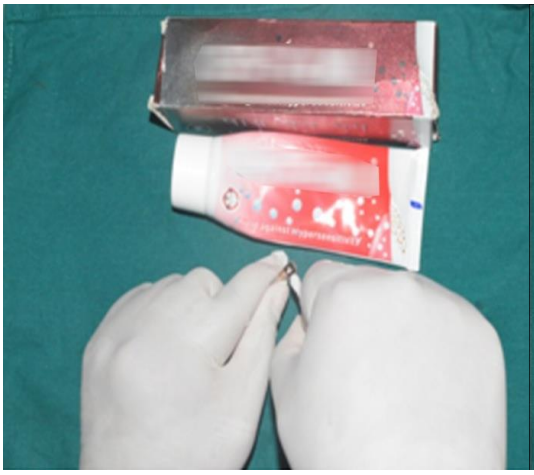


FIGURE 9 :APPLICATION OF AGENT I

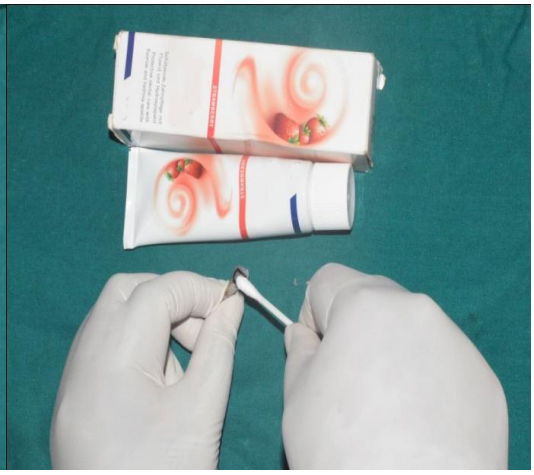


FIGURE 10 : APPLICATION OF AGENT II

FIGURE 11 :PREPARATION OF ARTIFICIAL SALIVA

FIGURE 12 :ENAMEL SAMPLES PLACED IN ARTIFICIAL SALIVA

FIGURE 13 :GOLD SPUTTERING MACHINE (Quorum 150 R S Ion sputter)

FIGURE 14 :ENAMEL SAMPLES BEFORE SUBJECTING TO GOLD SPUTTERING

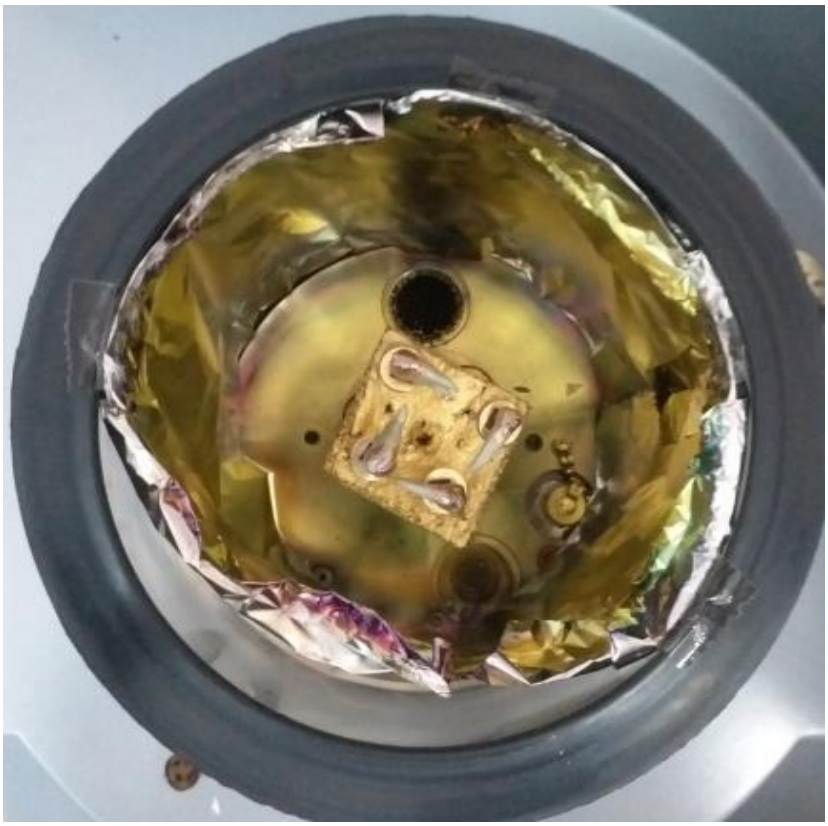


FIGURE 15 :ENAMEL SAMPLES AFTER GOLD SPUTTERING

FIGURE 16 :SEM (SCANNING ELECTRON MICROSCOPE)(Quanta 250 FEG)

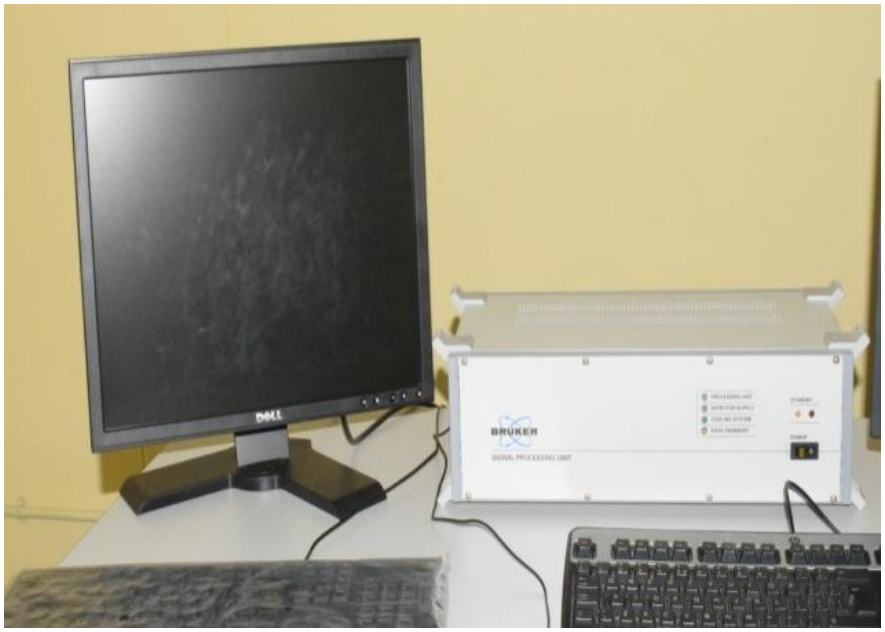


FIGURE 17 :EDS (ENERGY DISPERSIVE SPECTROSCOPY)(Quanta 250 FEG)

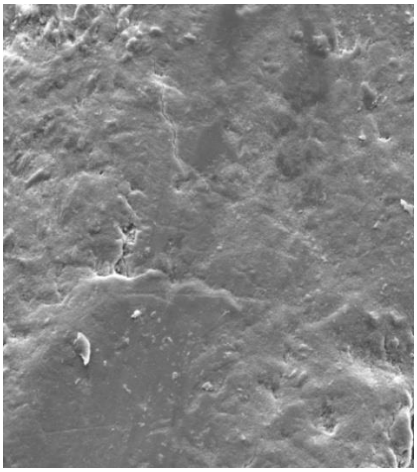


Figure 18 :Normal Enamel sample (SEM) image viewed at 4000 xmagnification

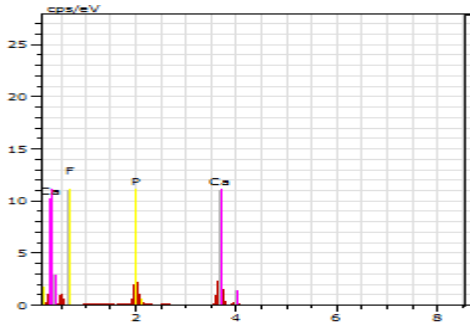


Figure 19 : Normal Enamel (Edax Image)

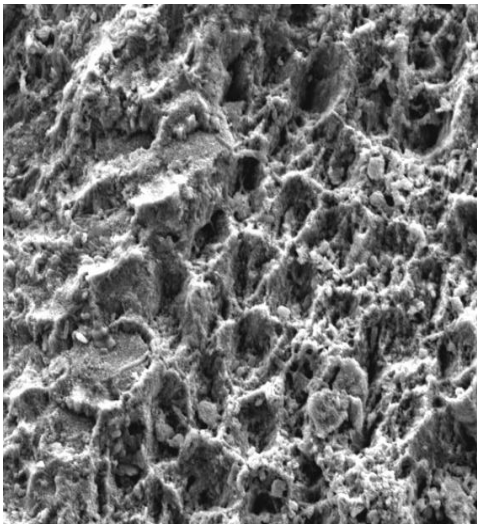


Figure 20 :Demineralised Enamel sample (SEM) image viewed at 4000x magnification

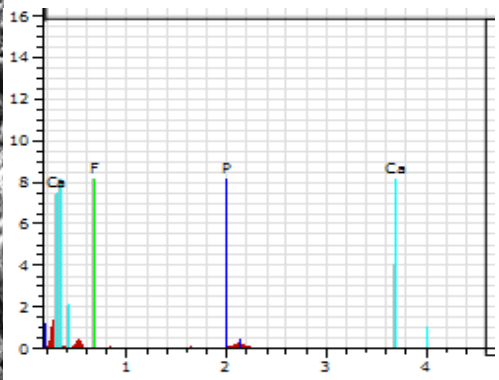


Figure21:Demineralized Enamel (Edax Image)

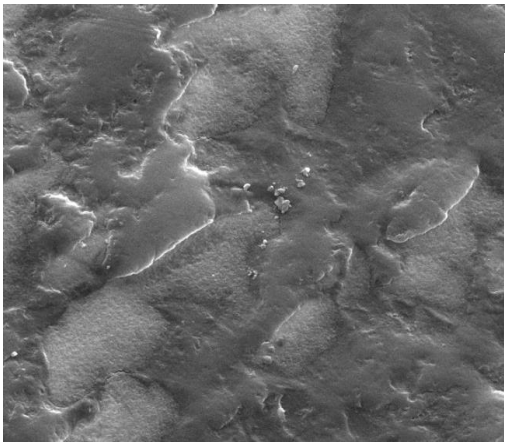


Figure 22 :SEM image of Agent I treated sample viewed at 4000xmagnification

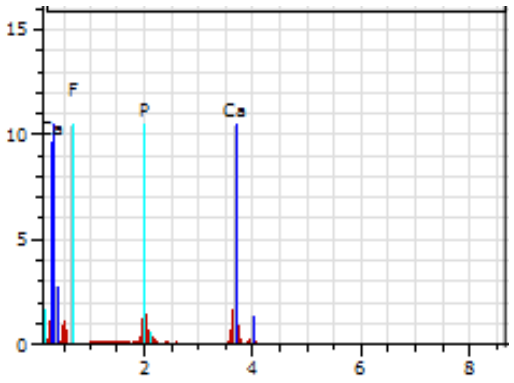


Figure 23 :EDAX image Agent I treatedsample.

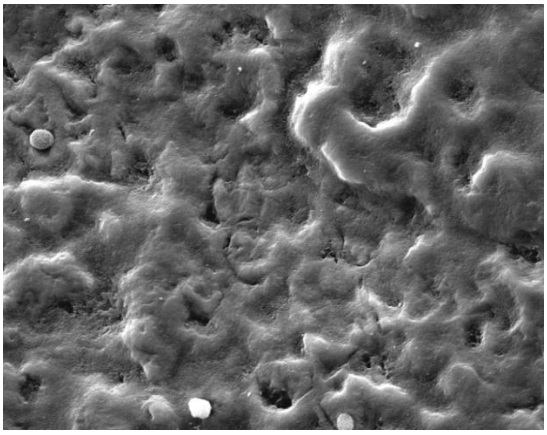


Figure 24 :SEM image of Agent II treated sampleviewed at 4000xmagnification

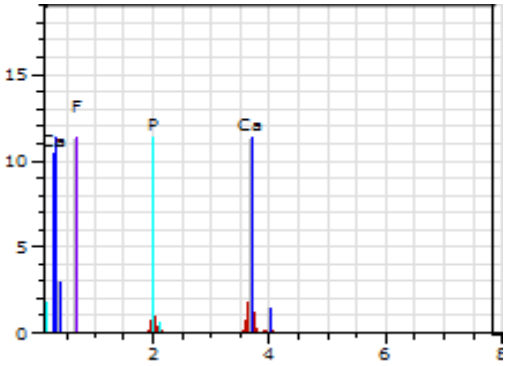


Figure 25 : EDAX image Agent II treated sample.

The aim of the study is to compare the efficacy of different remineralizing agents on artificially created white spot lesions an invitro study.

50 extracted I st and IInd premolars samples were divided into Group A and Group B. Each group included 25 samples. Samples were subjected to artificial white spot lesion formation and they were exposed to two different remineralizing agents. The efficacy of different remineralizing agents were investigated.

INTRA GROUP COMPARISON:

COMPARISON OF Ca BETWEEN BASELINE VS DEMINERALIZATION (GROUP A):

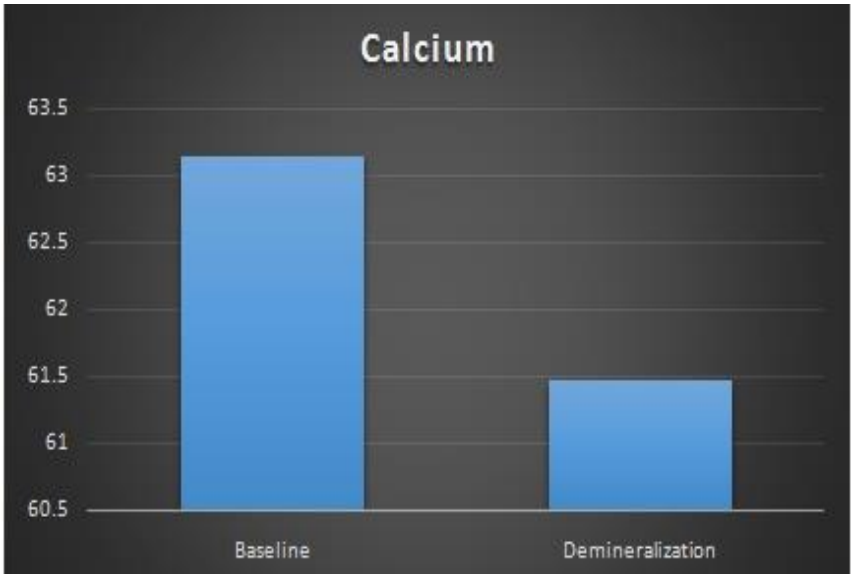
Comparison of Ca percentage between **baseline and demineralization** in Group A is given in Graph 1. The mean, standard deviation,standard error, 95% confidence interval, p value, t value for baseline and demineralization analysed with Paired t test were tabulated in table 1. There is a **statistically significant difference** (P value< 0.05) in **Ca percentage** in baseline and demineralization in Group A, indicating the loss of calcium ions is seen more in demineralization group when compared to baseline group as evident statistically.

TABLE 1:BASELINE Vs DEMINERALIZATION

		Mean	Standard deviation	T value	P Value	Standard error difference	95% Confidence Interval of the Difference	
							Lower	Upper
Baseline	Ca	63.1496	2.05779	9.453	.000	.17768	1.31367	2.04553
Demineralization		61.4700	1.76646					

Pvalue : S < 0.05, NS > 0.05

GRAPH 1



COMPARISON OF F BETWEEN VS DEMINERALIZATION (GROUP A):

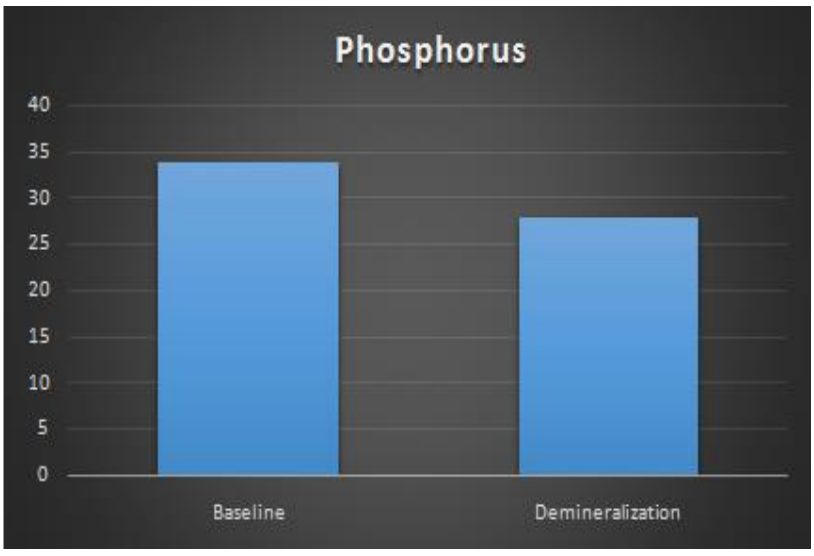
Comparison of P percentage between **baseline and demineralization** in Group A is given in Graph 2. The mean, standard deviation,standard error, 95% confidence interval, p value, t value for baseline and demineralization analysed with Paired t test were tabulated in table 2. There is a **statistically significant difference** (P value < 0.05) in **P percentage** in Baseline and demineralization in Group A, indicating the loss of phosphate ions is seen more in demineralization group when compared to baseline group as evident statistically.

TABLE 2: BASELINE Vs DEMINERALIZATION

		Mean	Standard Deviation	T Value	P Value	Standard Error Difference	95% Confidence Interval of the Difference	
							Lower	Upper
Baseline	P	33.8156	1.76221	13.697	.000	.43633	5.07776	6.87504
Demineralization		27.8392	1.72955					

Pvalue : S < 0.05, NS >0.05.

GRAPH 2



COMPARISON OF F BETWEEN BASELINE VS DEMINERALIZATION (GROUP A):

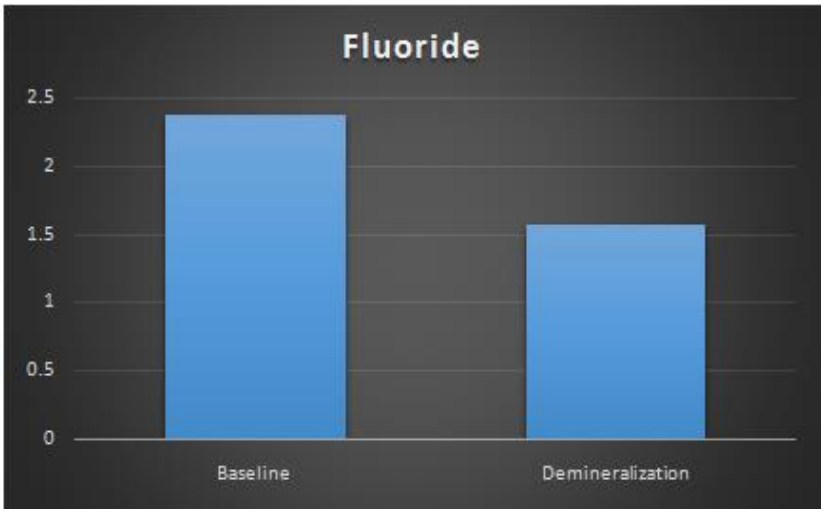
Comparison of F percentage between **baseline and demineralization** in Group A is given in Graph 3. The mean, standard deviation, standard error, 95% confidence interval, p value, t value for baseline and demineralization analysed with Paired t test were tabulated in table 3. There is a **statistically significant difference** (P value < 0.05) in **F percentage** in baseline and demineralization in Group A, indicating the loss of fluoride ions is seen more in demineralization group when compared to baseline group as evident statistically.

TABLE 3 :BASELINE Vs DEMINERALIZATION

		Mean	Standard Deviation	T Value	P Value	Standard Error Difference	95% Confidence Interval of the Difference	
							Lower	Upper
Baseline	F	2.3672	.74730	6.189	.000	.12951	.53486	1.06834
Demineralization		1.5656	.73807					

Pvalue : S < 0.05, NS >0.05.

GRAPH 3



COMPARISON OFCa BETWEEN DEMINERALIZATION VS REMINERALIZATION (GROUP A):

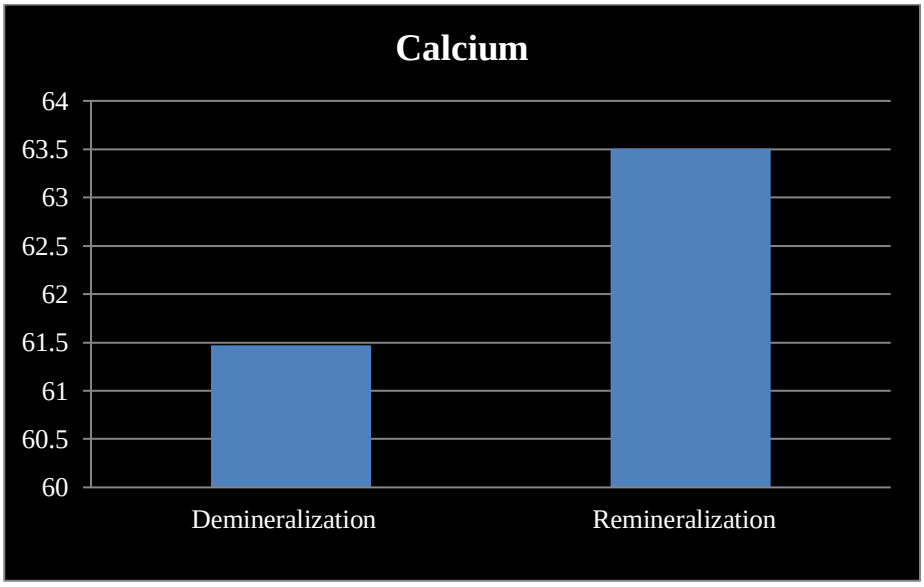
Comparison of Ca percentage between **demineralization and remineralization** in Group A is given in Graph 4. The mean, standard deviation, standard error, 95% confidence interval, p value, t value for demineralization and remineralization analysed with Paired t test were tabulated in table 4. There is **statistically significant difference** (P value < 0.05) in **Ca percentage** in demineralization and remineralization in Group A, indicating that there is more increase in calcium ions in remineralizing group when compared to demineralization group as evident statistically.

TABLE 4: DEMINERALIZATION Vs REMINERALIZATION

		Mean	Standard Deviation	T Value	P Value	Standard Error Difference	95% Confidence Interval of the Difference	
							Lower	Upper
Demineralization	Ca	61.4700	1.76646	-8.285	.000	.24599	-2.54464	-1.53136
Remineralization		63.5080	1.51479					

Pvalue : S < 0.05, NS > 0.05

GRAPH 4



COMPARISON OF P BETWEEN DEMINERALIZATION VS REMINERALIZATION (GROUP A):

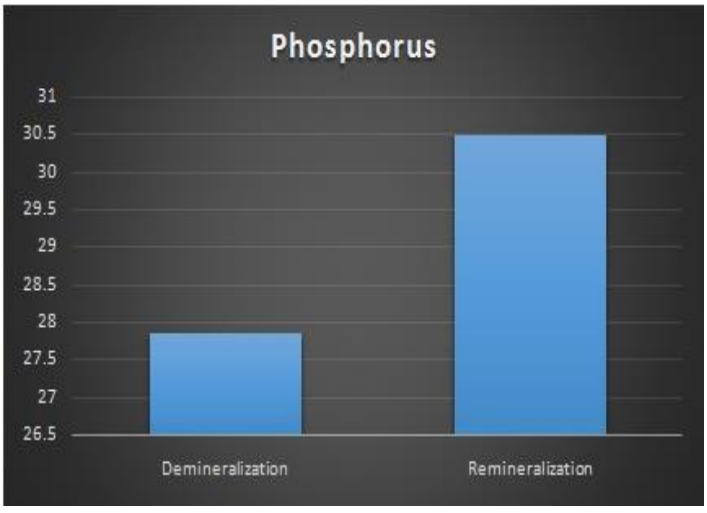
Comparison of P percentage between **demineralization and remineralization** in Group A is given in Graph 5. The mean, standard deviation,standard error , 95% confidence interval, p value, t value for demineralization and remineralization analysed with Paired t test were tabulated in table 5 .There is **statistically significant difference** (P value < 0.05) in **P percentage** in demineralization and remineralization in Group A, indicating that there is more increase in phosphororus ions in remineralization group when compared to demineralization group as evident statistically.

TABLE 5: BETWEEN DEMINERALIZATION Vs REMINERALIZATION

		Mean	Standard Deviation	T Value	P Value	Standard Error Difference	95% Confidence Interval of the Difference	
							Lower	Upper
Demineralization	P	27.8392	1.72955	-8.028	.000	.43033	-3.58429	-1.81171
Remineralization		30.4868	1.99719					

Pvalue : S < 0.05, NS > 0.05

GRAPH 5



COMPARISON OF F BETWEEN DEMINERALIZATION VS REMINERALIZATION (GROUP A):

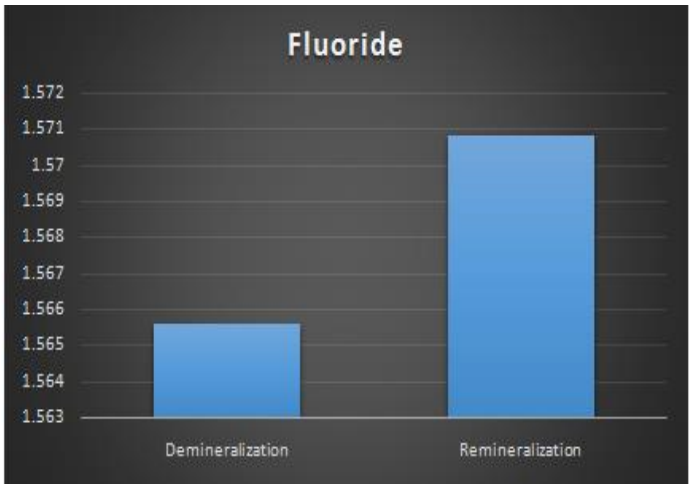
Comparison of F percentage between **demineralization and remineralization** in Group A is given in Graph 6. The mean, standard deviation,standard error, 95% confidence interval,p value, t value for demineralization and remineralization analysed with Paired t test were tabulated in table 6. There is **no statistically significant difference** (P value > 0.05) in **F percentage** in demineralization and remineralization in Group A, indicating that there is less increase in fluoride ions in remineralization group when compared to demineralization group as evident statistically

TABLE 6: BETWEEN DEMINERALIZATION VS REMINERALIZATION

		Mean	Standard Deviation	T Value	P Value	Standard Error Difference	95% Confidence Interval of the Difference	
							Lower	Upper
Demineralization	F	1.5656	.73807	-034	.973	.15074	-.31566	.30526
Remineralization		1.5708	.61282					

Pvalue : S < 0.05, NS > 0.05

GRAPH 6



COMPARISON OF Ca BETWEEN BASELINE VS REMINERALIZATION (GROUP A):

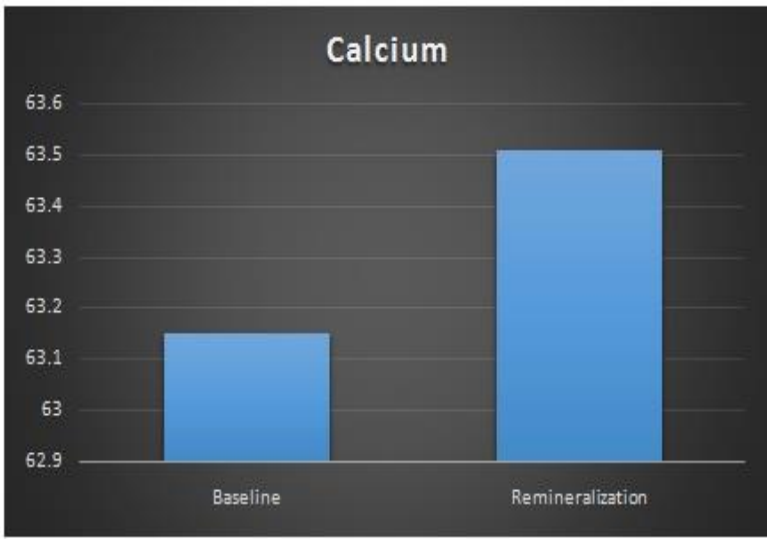
Comparison of Ca percentage between **baseline and remineralization** in Group A is given in Graph 7. The mean, standard deviation, standard error , 95% confidence interval , p value, t value for baseline and remineralization analysed with Paired t test were tabulated in table 7 .There is **no statistically significant difference** (Pvalue > 0.05) in **Ca percentage** in baseline and remineralization in Group A, indicating that there is increase in calcium ions in remineralization group to the same level as that of baseline group as evident statistically

TABLE 7: BASELINE Vs REMINERALIZATION

		Mean	Standard Deviation	T Value	P Value	Standard Error Difference	95% Confidence Interval of the Difference	
Baseline	Ca	63.1496	2.05779	-1.390	.177	.25790	Lower	Upper
Remineralization		63.5080	1.51479				-.88955	.17275

Pvalue : S < 0.05, NS > 0.05

GRAPH 7



COMPARISON OF P BETWEEN BASELINE VS REMINERALIZATION (GROUPA):

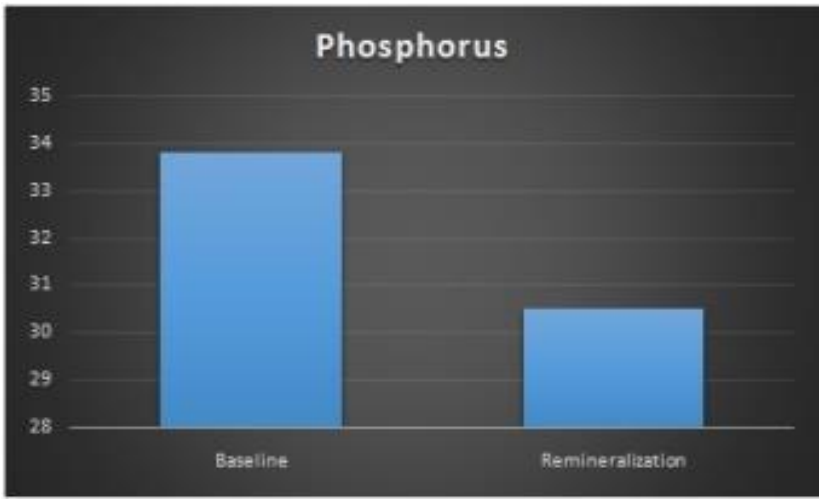
Comparison of P percentage between **baseline and remineralization** in Group A is given in Graph 8. The mean, standard deviation, standard error, 95% confidence interval, p value, t value for baseline and remineralization analysed with Paired t test were tabulated in table 8. There is a **statistically significant difference** (P value < 0.05) in **P percentage** in baseline and remineralization in Group A, indicating that there is less decrease in phosphate ions in remineralization group when compared to baseline group as evident statistically

TABLE 8: BASELINE Vs REMINERALIZATION

		Mean	Standard Deviation	T Value	P Value	Standard Error Difference	95% Confidence Interval of the Difference	
							Lower	Upper
Baseline	P	33.8156	1.76221	6.650	.000	.50059	2.29781	4.35979
Remineralization		30.4868	1.99719					

Pvalue : S < 0.05, NS > 0.05

GRAPH 8



COMPARISON OF F BETWEEN BASELINE VS REMINERALIZATION (GROUP A):

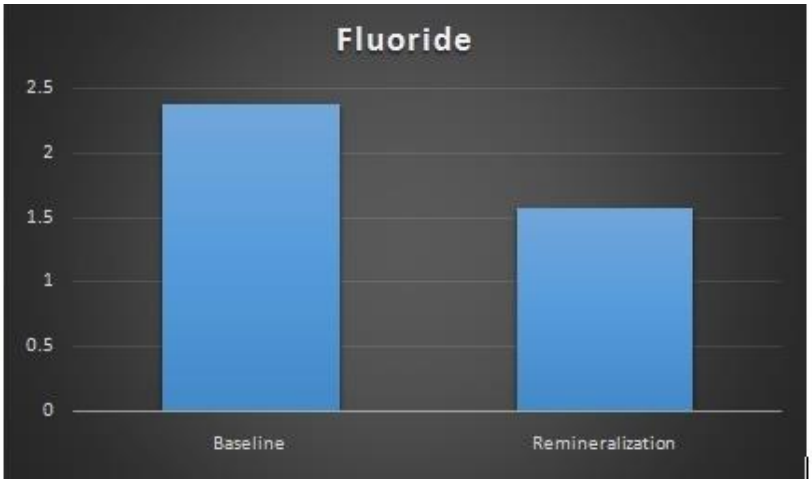
Comparison of F percentage between **baseline and remineralization** in Group A is given in Graph 9. The mean, standard deviation, standard error, 95% confidence interval, p value, t value for baseline and remineralization analysed with Paired t test were tabulated in table 9. There is a **statistically significant difference** (P value < 0.05) in **F percentage** in baseline and remineralization in Group A, indicating that there is more decrease in fluoride ions in remineralization group when compared to baseline group as evident statistically

TABLE 9: BASELINE Vs REMINERALIZATION

		Mean	Standard Deviation	T Value	P Value	Standard Error Difference	95% Confidence Interval of the Difference	
Baseline	F	2.3672	.74730	5.031	.000	.15829	Lower	Upper
Remineralization		1.5708	.61282				.47039	1.12241

P value : S < 0.05, NS > 0.05

GRAPH 9



COMPARISON OF Ca BETWEEN BASELINE VS DEMINERALIZATION (GROUP B):

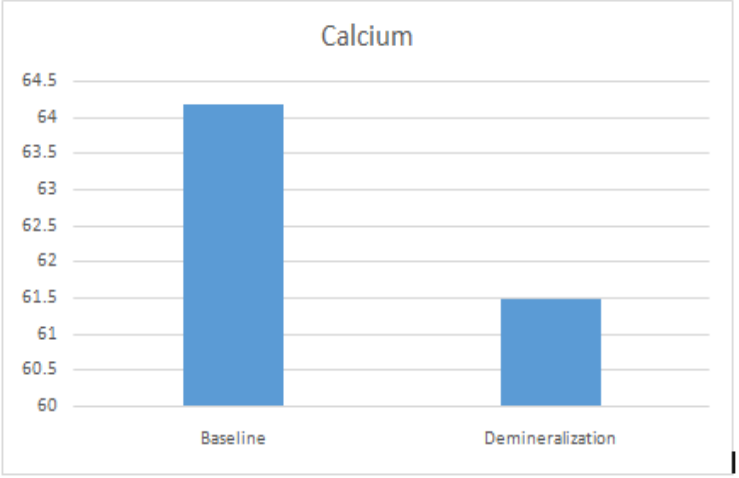
Comparison of Ca percentage between **baseline and demineralization** in Group B is given in Graph 10. The mean, standard deviation, standarderror, 95% confidence interval p value, t value for baseline and demineralization analysed with Paired t test were tabulated in table 10. There is **statistically significant difference** (P value < 0.05) in **Ca percentage** in baseline and demineralization in Group B, indicating that there is more decrease in calcium ions in demineralization group when compared to baseline group as evident statistically

TABLE 10:BASELINE Vs DEMINERALIZATION

		Mean	Standard Deviation	T Value	P Value	Standard Error Difference	95% Confidence Interval of the Difference	
							Lower	Upper
Baseline	Ca	64.1764	1.09220	11.616	.000	.23164	2.21372	3.16788
Demineralization		61.4856	1.29813					

Pvalue : S < 0.05, NS > 0.05

GRAPH 10



COMPARISON OF P BETWEEN BASELINE VS DEMINERALIZATION (GROUP B):

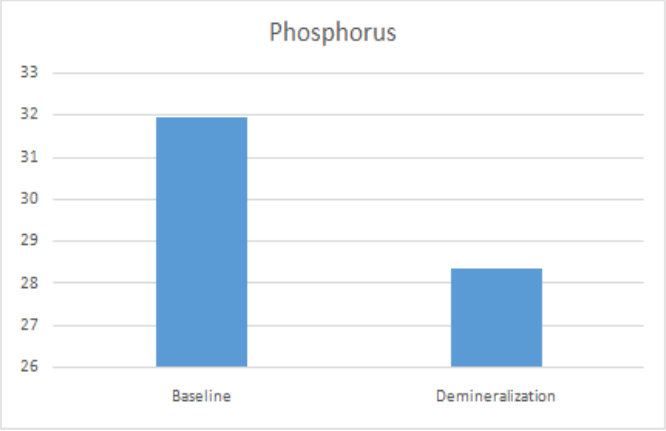
Comparison of P percentage between **baseline and demineralization** in Group B is given in Graph 11. The mean, standard deviation, standard error, 95% confidence interval, p value, t value for baseline and demineralization analysed with Paired t test were tabulated in table 11. There is **statistically significant difference**(P value < 0.05) in **P percentage** in baseline and demineralization in Group B, indicating that there is more decrease in phosphate ions in demineralization group when compared to baseline group as evident statistically

TABLE 11:BASELINE Vs DEMINERALIZATION

		Mean	Standard Deviation	T Value	P Value	Standard Error Difference	95% Confidence Interval of the Difference	
							Lower	Upper
Baseline	P	31.9464	1.23170	9.356	.000	.38699	2.82379	4.41781
Demineralization		28.3256	2.03763					

Pvalue : S < 0.05, NS > 0.05.

GRAPH 11



COMPARISON OF F BETWEEN BASELINE VS DEMINERALIZATION (GROUP B):

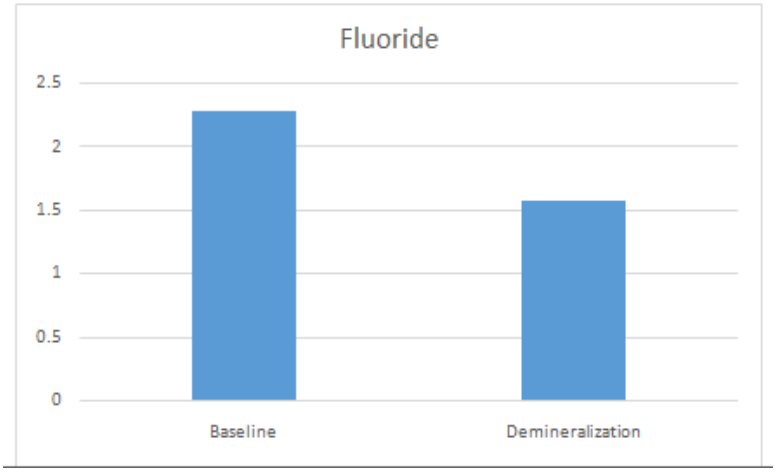
Comparison of F percentage between **baseline and demineralization** in Group B is given in Graph 12. The mean, standard deviation, standard error, 95% confidence interval, p value, t value for baseline and demineralization analysed with Paired t test were tabulated in table 12. There is **statistically significant difference** (P value < 0.05) in **F percentage** in baseline and demineralization in Group B, indicating that there is more decrease in fluoride ions in demineralization group when compared to baseline group as evident statistically

TABLE 12:BASELINE Vs DEMINERALIZATION

		Mean	Standard Deviation	T Value	P Value	Standard Error Difference	95% Confidence Interval of the Difference	
							Lower	Upper
Baseline	F	2.2768	.86735	5.850	.000	.12184	.46187	.96373
Demineralization		1.5640	.82209					

P value : S < 0.05, NS > 0.05.

GRAPH 12



COMPARISON OF Ca BETWEEN DEMINERALIZATION VS REMINERALIZATION (GROUP B):

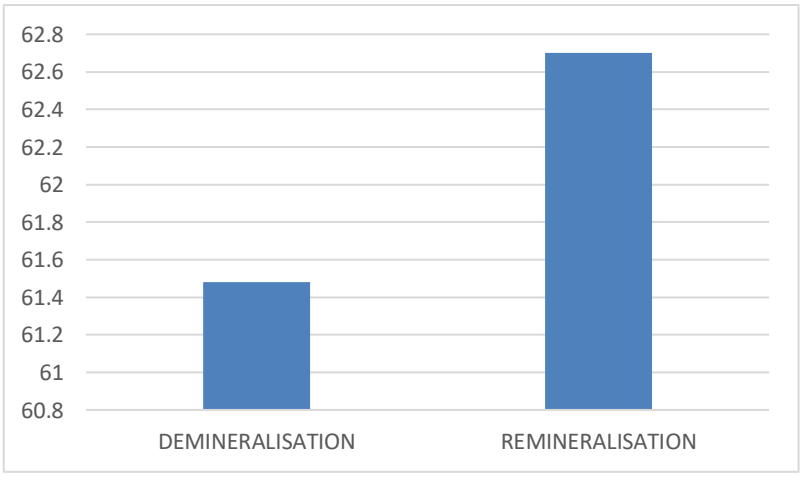
Comparison of Ca percentage between **demineralization and remineralization** in Group B is given in Graph 13. The mean, standard deviation, standard error, 95% confidence interval, p value, t value for demineralization and remineralization analysed with Paired t test were tabulated in table 13. There is **statistically significant difference** (P value <0.05) in **Ca percentage**indemineralization and remineralization in Group B, indicating that there is less increase in calcium ions in remineralization group when compared to demineralization group as evident statistically.

TABLE 13: DEMINERALIZATION Vs REMINERALIZATION

		Mean	Standard Deviation	T Value	P Value	Standard Error Difference	95% Confidence Interval of the Difference	
							Lower	Upper
Demineralization	Ca	61.4856	1.29813	-10.473	.000	.31327	-3.92599	-2.63561
Remineralization		62.7664	1.23579					

Pvalue : S < 0.05, NS > 0.05.

GRAPH 13



COMPARISON OF P BETWEEN DEMINERALIZATION VS REMINERALIZATION (GROUP B):

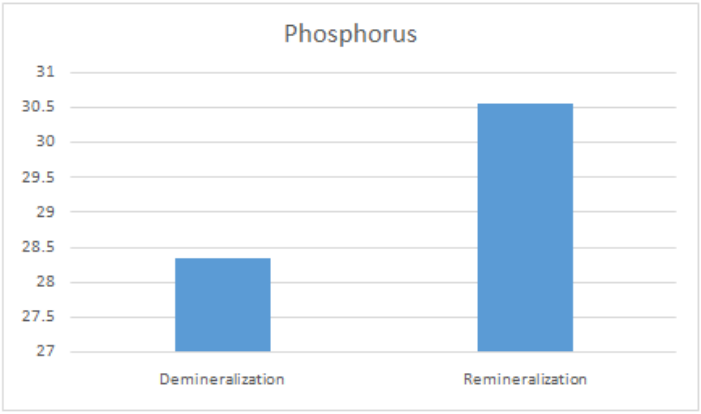
Comparison of P percentage between **demineralization and remineralization** in Group B is given in Graph 14. The mean, standard deviation, standard error, 95% confidence interval, p value, t value for demineralization and remineralization analysed with Paired t test were tabulated in table 14. There is **statistically significant difference** (P value < 0.05) in **P percentage** in demineralization and remineralization in Group B, indicating that there is more increase in phosphate ions in remineralization group when compared to demineralization group as evident statistically

TABLE 14:DEMINERALIZATION Vs REMINERALIZATION

		Mean	Standard Deviation	T Value	P Value	Standard Error Difference	95% Confidence Interval of the Difference	
							Lower	Upper
Demineralization	P	28.3256	2.03763	-6.103	.000	.36236	-2.95790	-1.46530
Remineralization		30.5372	1.12905					

Pvalue ; S < 0.05, NS > 0.05.

GRAPH 14



COMPARISON OF F BETWEEN DEMINERALIZATION VS REMINERALIZATION (GROUP B):

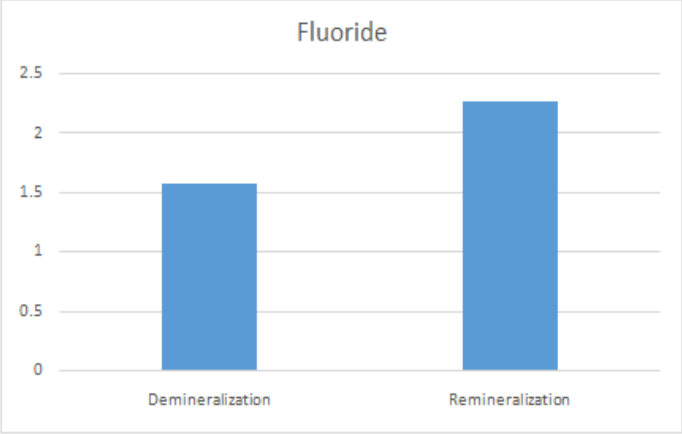
Comparison of F percentage between **demineralization and remineralization** in Group B is given in Graph 15. The mean, standard deviation,standard error, 95% confidence interval, p value, t value for demineralization and remineralization analysed with Paired t test were tabulated in table 15. There is **statistically significant difference** (P value < 0.05) in **F percentage** in demineralization and remineralization in Group B, indicating that there is more increase in fluoride ions in remineralization group when compared to demineralization group as evident statistically.

TABLE 15: DEMINERALIZATION Vs REMINERALIZATION

		Mean	Standard Deviation	T Value	P Value	Standard Error Difference	95% Confidence Interval of the Difference	
							Lower	Upper
Demineralization	F	1.5640	.82209	-4.267	.000	.16321	-1.03255	-.36025
Remineralization		2.2604	.75185					

P value : S < 0.05, NS > 0.05

GRAPH 15



COMPARISON OF Ca BETWEEN BASELINE VS REMINERALIZATION (GROUP B):

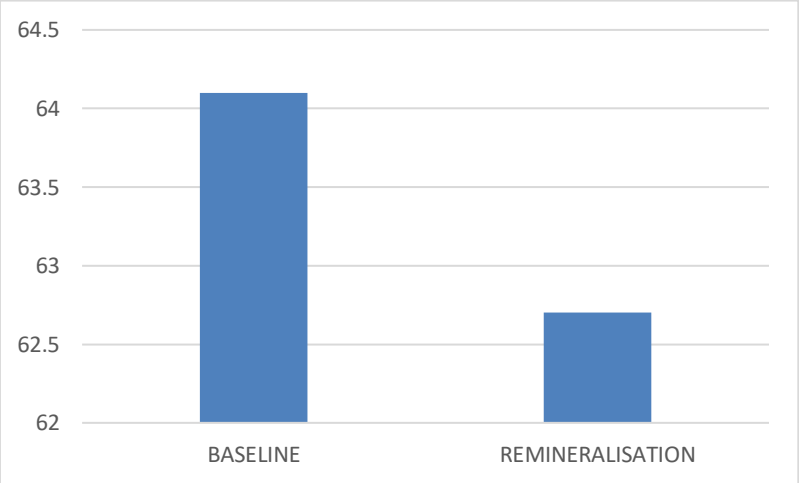
Comparison of Ca percentage between **baseline and remineralization** in Group B is given in Graph 16. The mean, standard deviation, standard error, 95% confidence interval, p value, t value for baseline and remineralization analysed with Paired t test were tabulated in table 16. There is **no statistically significant difference** (P value > 0.05) in **Ca percentage** in baseline and remineralization in Group B, indicating that there is less decrease in calcium ions in remineralization group when compared to baseline group as evident statistically

TABLE 16:BASELINE Vs REMINERALIZATION

		Mean	Standard Deviation	T Value	P Value	Standard Error Difference	95% Confidence Interval of the Difference	
Baseline	Ca	64.1764	1.09220	-1.887	0.71	.31266	Lower	Upper
Remineralization		62.7664	1.23579				-1.23393	.05393

Pvalue : S < 0.05, NS > 0.05

GRAPH 16



COMPARISON OF P BETWEEN BASELINE VS REMINERALIZATION (GROUP B):

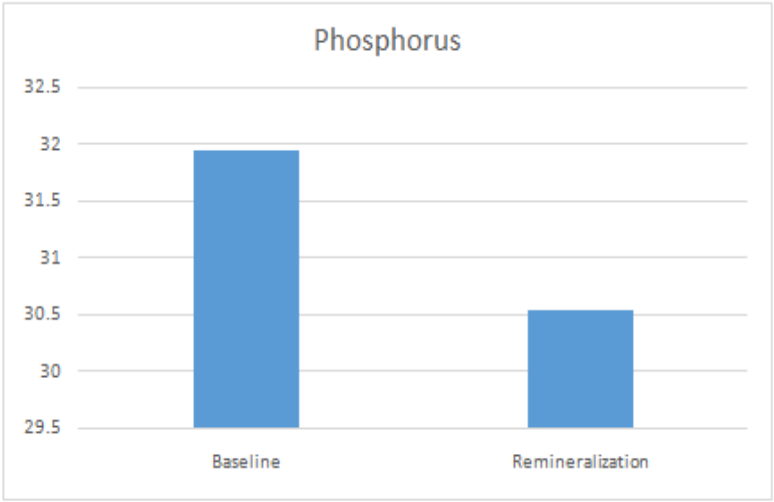
Comparison of P percentage between **baseline and remineralization** in Group B is given in Graph 17. The mean, standard deviation,standard error, 95% confidence interval, p value, t value for baseline and remineralization analysed with Paired t test were tabulated in table 14. There is **statistically significant difference** (P value < 0.05) in **P percentage** in baseline and remineralization in Group B, indicating that there is less decrease in phosphate ions in remineralization group when compared to baseline group as evident statistically.

TABLE 17: BASELINE Vs REMINERALIZATION

		Mean	Standard Deviation	T Value	P Value	Standard Error Difference	95% Confidence Interval of the Difference	
Baseline	P	31.9464	1.23170	7.405	.000	.19031	Lower	Upper
Remineralization		30.5372	1.12905				1.01724	1.80116

Pvalue : S < 0.05, NS > 0.05

GRAPH 17



COMPARISON OF F BETWEEN BASELINE VS REMINERALIZATION (GROUP B):

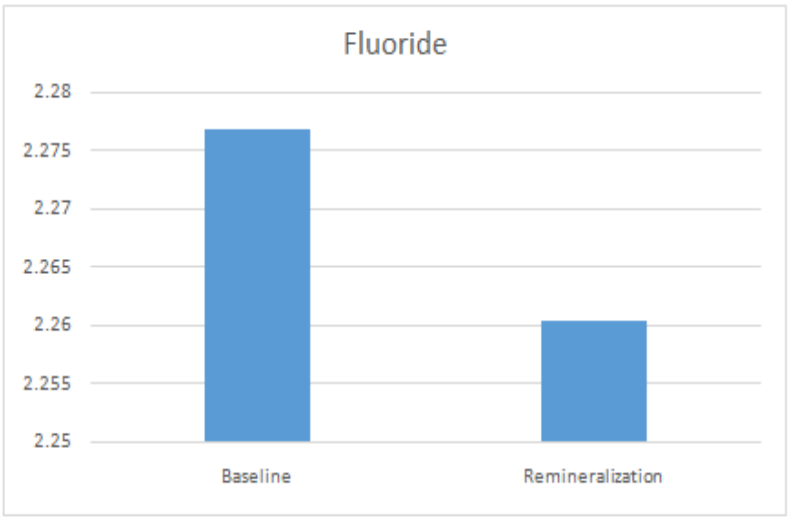
Comparison of F percentage between **baseline and remineralization** in Group B is given in Graph 18. The mean, standard deviation,standard error, 95% confidence interval, p value, t value for baseline and remineralization analysed with Paired t test were tabulated in table 18. There is **no statistically significant difference** (P value> 0.05) in **F percentage** in baseline and remineralization in Group B, indicating that there is less decrease in fluoride ions in remineralization group when compared to baseline group as evident statistically

TABLE 18:BASELINE Vs REMINERALIZATION

		Mean	Standard Deviation	T Value	P Value	Standard Error Difference	95% Confidence Interval of the Difference	
							Lower	Upper
Baseline	F	2.2768	.86735	.154	.879	.10623	-.20239	23519
Remineralization		2.2604	.75185					

Pvalue : S < 0.05, NS > 0.05

GRAPH 18



INTERGROUP COMPARISON BETWEEN GROUP A AND GROUP B AT BASELINE:

BASELINE VS BASELINE:

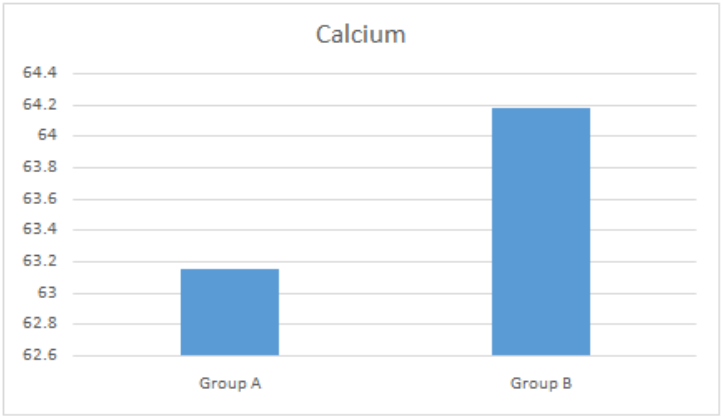
The mean, standard deviation, standard error, 95% confidence interval for inter group findings are calculated and tabulated with Independent sample Test (Table 19). There were **no statisticallysignificant difference** in **Capercentage**in baselinein Group A and B. (P value >0.05).The mean value of Ca in Group A&B in Baseline was given Graph 19,indicating that there is increase in ca ions in group A when compared to group B in baseline group as evident statistically.

TABLE 19

		Group	Mean	S. D.	T	p value	Std. Error Difference	95% Confidence Interval of the Difference		N
								Lower	Upper	
Baseline	**ca**	**Group A**	63.1496	2.10023	-2.15	.086	.47554	-1.98294	-.07066	25
		Group B	62.1764	1.11472						

Pvalue : S < 0.05, NS > 0.05.

GRAPH 19



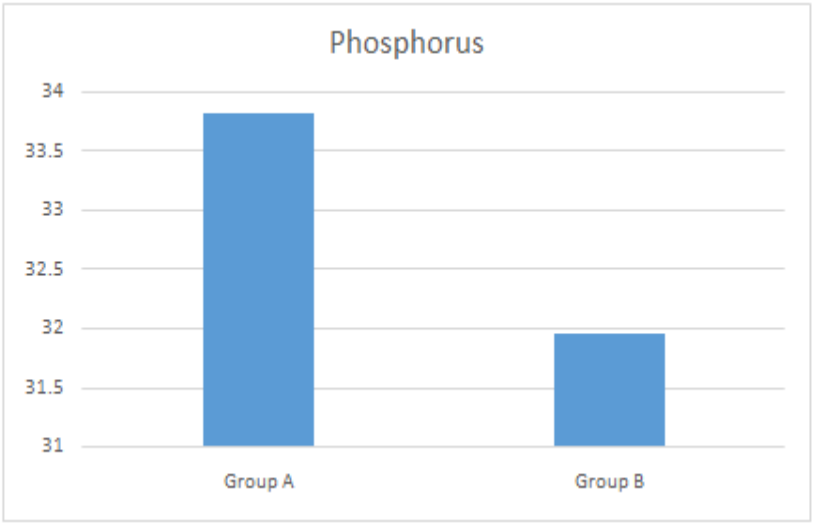
The mean, standard deviation, standard error, 95% confidence interval for inter group findings are calculated and tabulated with Independent sample Test (Table 20). There were**no statisticallysignificant difference in P percentage in baseline** in Group A and B. (P value >0.05). The mean value of P in Group A&B in Baseline was given Graph 20,indicating that there is increase in P ions in group A when compared to group B in baseline group as evident statistically.

TABLE 20

		Group	Mean	S. D.	T	p value	Std. Error Difference	95% Confidence Interval of the Difference		N
								Lower	Upper	
Baseline	**P**	**Group A**	33.8156	1.79855	4.259	.060	.43886	.98680	2.75160	25
		Group B	31.9464	1.25710						

P value :S < 0.05, NS > 0.05.

GRAPH 20



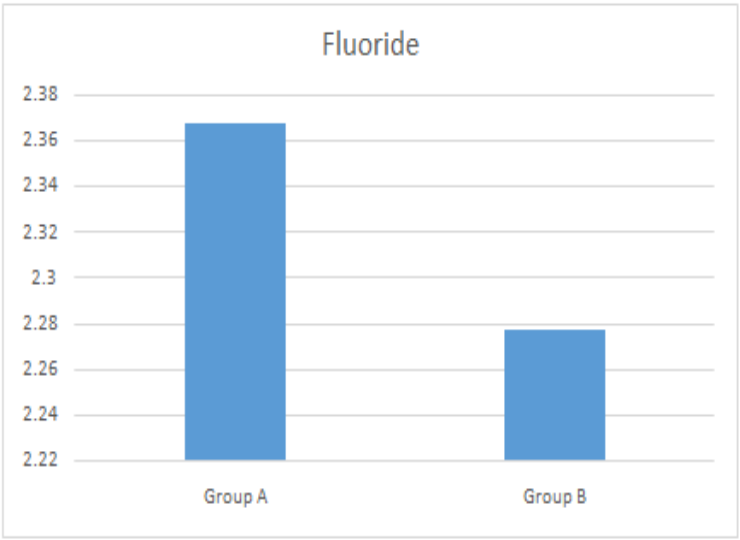
The mean, standard deviation, standard error, 95% confidence interval for inter group findings are calculated and tabulated with Independent sample Test (Table 21). There were **no statisticallysignificant difference in F percentage in baseline** in Group A and B. (P value >0.05).The mean value of F in Group A&B in baseline was given Graph 21,indicating that there is increase in F ions in group A when compared to group B in baseline group as evident statistically.

TABLE 21

		Group	Mean	S. D.	T	p value	Std. Error Difference	95% Confidence Interval of the Difference		N
								Lower	Upper	
Baseline	**F**	**Group A**	2.3672	.76271	.387	.701	.23370	-3.7948	.56028	25
		Group B	2.2768	.88524						

P value : S < 0.05, NS >0.05

GRAPH 21



INTERGROUP COMPARISON BETWEEN GROUP A AND GROUP B IN DEMINERALIZATION:

DEMINERALIZATION VS DEMINERALIZATION

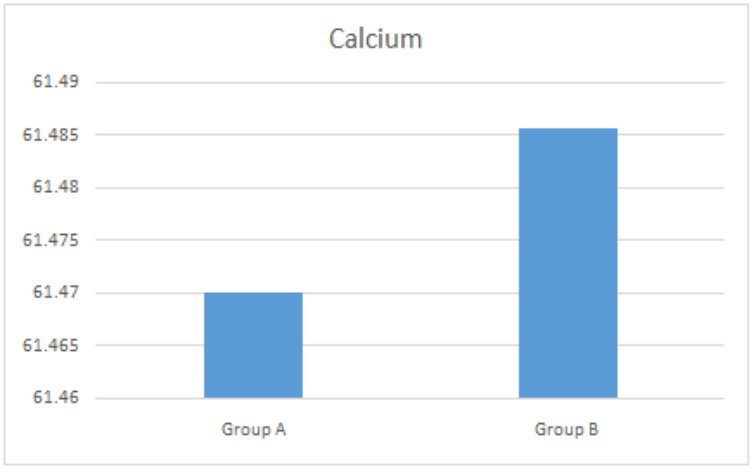
The mean, standard deviation, standard error, 95% confidence interval for inter group findings are calculated and tabulated with Independent sample Test (Table 22). There were **no statisticallysignificant difference in Ca percentagein demineralization** group in Group A and B. (P value >0.05).The mean value of Ca in Group A&B in Demineralization was given Graph 22, indicating that there is decrease in Ca in both group A and B in Demineralization group as evident statistically.

TABLE 22

		Group	Mean	S. D.	T	p value	Std. Error Difference	95% Confidence Interval of the Difference		N
								Lower	Upper	
Demineralization	**Ca**	**Group A**	61.4700	1.802	-.035	.972	.44747	-.91530	.88410	25
		Group B	61.4856	1.324						

Pvalue :S < 0.05, NS > 0.05.

GRAPH 22



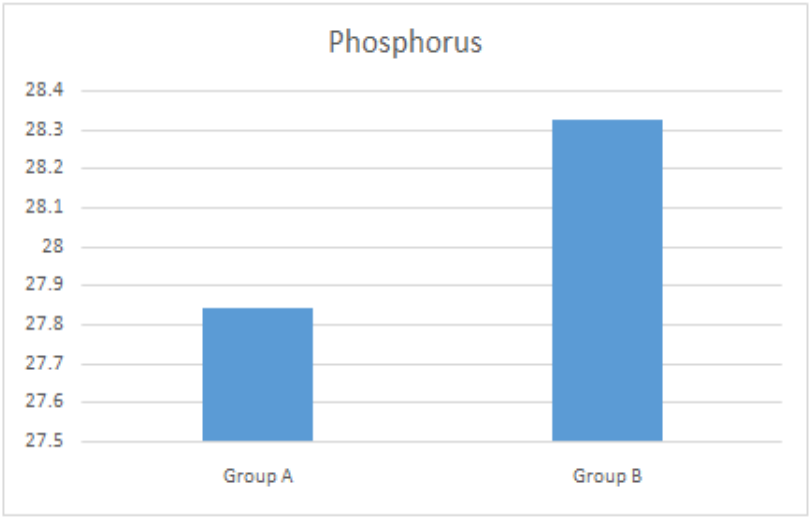
The mean, standard deviation, standard error, 95% confidence interval for inter group findings are calculated and tabulated by Independent sample Test (Table 23).There were **no statisticallysignificant difference in P percentage**in **demineralization** group in Group A and B. (P value >0.05).The mean value of P in Group A&B in Demineralization was given Graph 23, indicating that there is more decrease in P ions in group A when compared to group B in Demineralization group as evident statistically.

TABLE 23

		Group	Mean	S. D.	T	p value	Std. Error Difference	95% Confidence Interval of the Difference		N
								Lower	Upper	
Demineralization	**P**	**Group A**	27.83	1.765	-8.92	.377	54556	-1.58332	.61052	25
		Group B	28.32	2.07						

Pvalue : S < 0.05, NS > 0.05.

GRAPH 23



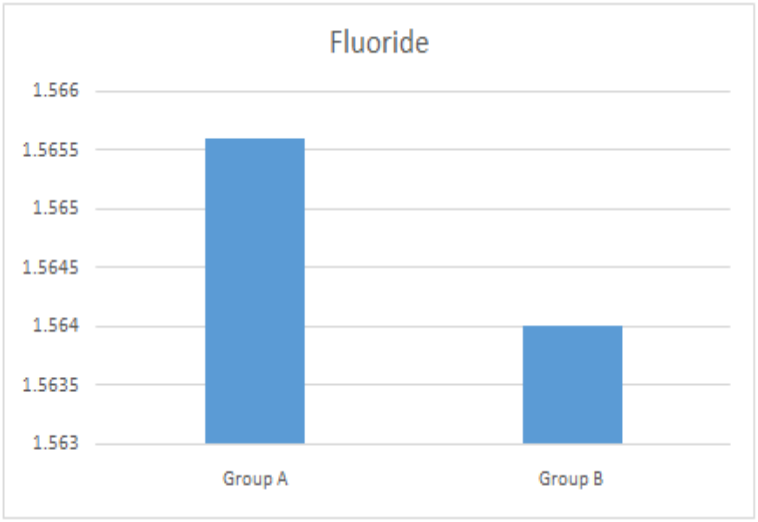
The mean, standard deviation, standard error, 95% confidence interval for inter group findings are calculated and tabulated with Independent sample Test (Table 24).There were **no statisticallysignificant difference in F percentage in demineralization** group in Group A and B. (P value >0.05).The mean value of F in Group A&B in Demineralization was given Graph 24, indicating that there is less decrease in F ions in group B when compared to group A in Demineralization group as evident statistically.

TABLE 24

		Group	Mean	S. D.	T	P value	Std. Error Difference	95% Confidence Interval of the Difference		N
								Lower	Upper	
Demineralization	**F**	**Group A**	1.565	.753	.007	.994	.2252	-.45183	.45606	25
		Group B	1.564	.839						

P value: S < 0.05, NS > 0.05.

GRAPH 24



INTERGROUP COMPARISON BETWEEN GROUP A AND GROUP B IN REMINERALIZATION:

REMINERALIZATION Vs REMINERALIZATION

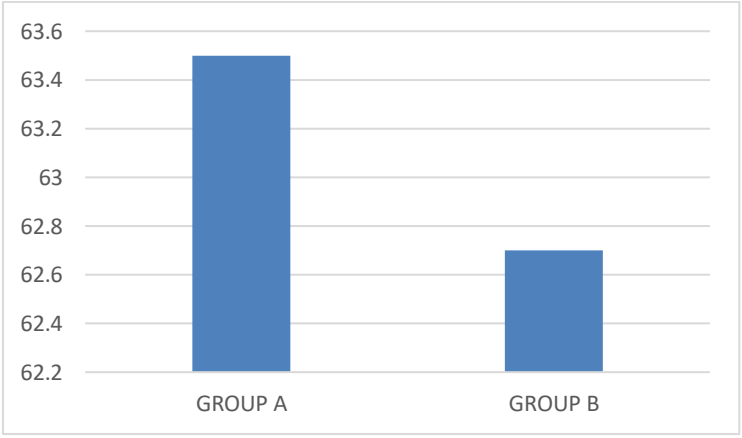
Comparison of Ca percentage between Group A & B in Remineralization group is given in Graph 25. The mean, standard deviation, standard error, 95% confidence interval , p value, t value for Group A & B is analysed with Paired t test were tabulated in table 25. There is **no statistically significant difference(P value > 0.05) in Ca percentage** in **remineralization** group in Group A & B ,indicating that there is less increase in calcium ions is seen in group A when compared to group B in remineralization group as evident statistically.

TABLE 25:

		Mean	Standard Deviation	T Value	P Value	Standard Error Difference	95% Confidence Interval of The Difference	
							Lower	Upper
Group A	Ca	63.5080	1.54602	-3.154	.063	.39905	-2.06074	-.45606
Group B		62.7664	1.2612					

Pvalue : S < 0.05, NS > 0.05.

GRAPH 25



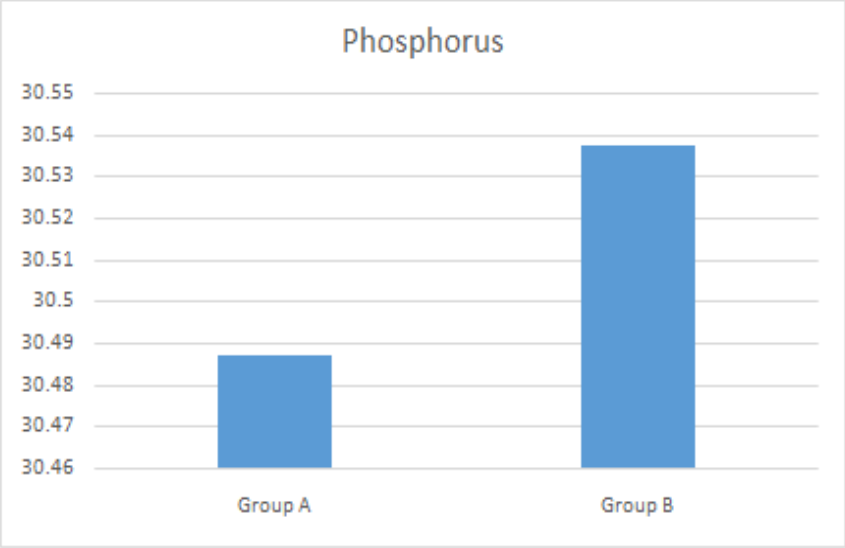
Comparison of P percentage between Group A & B in Remineralization is given in Graph 26. The mean, standard deviation, standard error, 95% confidence interval , p value, t value for Group A & B is analysed with Paired t test were tabulated in table 26. There is **no statistically significant difference** (P value > 0.05) **in P percentage** in **remineralization**group in Group A & B, indicating that there is less increase in phosphorous ions seen in group B when compared to group A in remineralization group as evident statistically.

TABLE 26

		Mean	Standard Deviation	T Value	P Value	Standard Error Difference	95% Confidence Interval of the Difference	
							Lower	Upper
Group A	P	30.4868	2.038	-.108	.915	.46831	-.99200	.89120
Group B		30.5372	1.152					

Pvalue : S < 0.05, NS > 0.05.

GRAPH 26



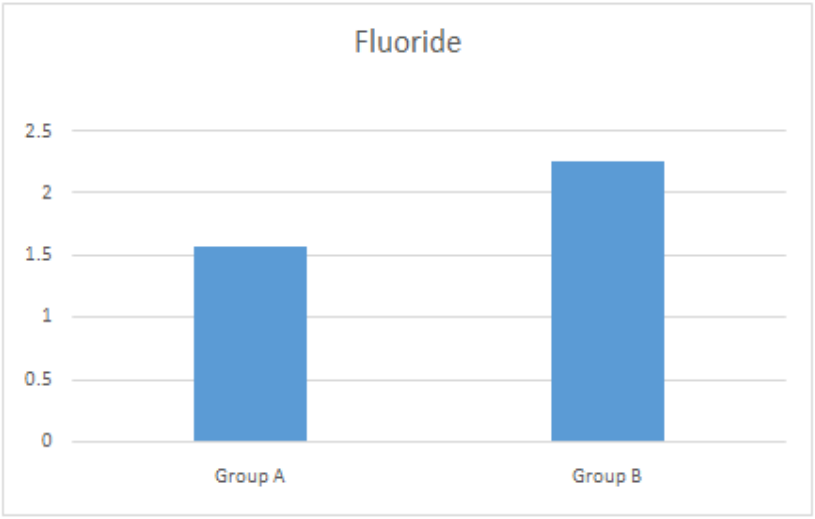
Comparison of F percentage between Group A & B in Remineralization is given in Graph 27. The mean, standard deviation, standard error, 95% confidence interval , p value, t value for Group A & B is analysed with Paired t test were tabulated in table 27. There is **statistically significant difference** (P value < 0.05) in **F percentage in remineralization**group in Group A &B, indicating that there is more increase in fluoride ions seen in group B when compared to group A in remineralization group as evident statistically.

TABLE: 27

		Mean	Standard deviation	T value	P value	Standard error difference	95% confidence interval of the difference	
Group A	F	1.5708	.62545	-3.483	.001	.19799	Lower	Upper
Group B		2.2604	.76736				-1.08769	-.29151

Pvalue : S < 0.05, NS > 0.05.

GRAPH 27



White spot lesions and enamel demineralization can occur during and after the orthodontic treatment. Enamel demineralization become a clinical problem ever since directly bonded orthodontic brackets were introduced. The prevalence of white spot lesion varies from 4.9% to 84% [52] .Preexisting white spot lesions may be present in orthodontic patients, not all white spot lesions are carious demineralization-related lesions. The prevalence of orthodontically related lesions was significantly greater in treated orthodontic arches when compared with untreated control arches in the same patients. **Derrick willmot et al** stated that the prevalence of demineralized white spot lesions is disturbingly high after orthodontic treatment[52].

The demineralization process can be stopped by creating an environment that permit remineralization by various remineralizing agents.[5.] It has been proven that oral hygiene and topical fluoride regimens during treatment and after treatment can reduce the occurrence of white spot lesions. Variety of methods are used to assess and detect the presence of demineralization of enamel lesions. The postorthodontic demineralized white spot lesions when subjected to intervention with a proper oral hygiene /toothpaste regimen or formulated low fluoride mouthrinse (50 ppm) test combination were able to remineralize early enamel lesion[53].Remineralization of white-spot lesions will be possible with a variety of currently available agents containing fluoride, bioavailable calcium and phosphate, casein phosphopeptide - amorphous calcium phosphate, Casein phosphopeptide amorphous calcium phosphate along with the fluoride, self-assembling peptide, Calcium sodium phosphosilicate, tricalcium phosphate, Amorphous Calcium phosphate, Dicalcium phosphate dehydrate, Reminpro (Hydroxyapatite + Fluoride + Xylitol) and tricalcium phosphate[5].

The mechanism of action of these remineralizing agents varies based on the composition. In the present study, remineralizing agent I & IIwere chosen to find the efficacy of their Remineralizing potential.

The mechanism of action of remineralizing agent I is as follows,"when calcium sodium phosphosilicate comes in contact with saliva or water, it first releases sodium ions, which increases the pH into the range suitable for HAP (Hydroxyapatite) formation as a result the calcium and phosphate are released to supplement the normal levels found in saliva"[2]. It contains 45 wt% SiO2, 24.5 wt% Na2O and CaO and 6 wt% P2O5[1],[29,36].The increase in ionic concentration, combined with an increase in pH, causes the ions to precipitate onto the tooth surface and form calcium hydroxycarbonate apatite (HCA) to remineralize the defect and to occlude open tubules and reduces the possibility of reopening of dentinal tubule[37]

The mechanism of action of remineralizing agent II is as follows, when it comes in contact with saliva, fluoride is converted into fluorapatite on the tooth surface. Once the fluorapatite layer is formed, the tooth surface becomes more resistant to acid attack[2]. The hydroxyapatite contained in the agent II fills the superficial enamel lesions and the smallest irregularities that arise from the erosion. It adheres to the tooth substances and protects the tooth against demineralization and erosion. The surface is smoothened,dentinal tubules are superficially sealed. Further, the smooth surface prevents the adhesion of the bacterial plaque. Natural remineralization is simultaneously promoted and the tooth thereby reinforced. Cariostatic properties of Xylitol prevents formation of harmful lactic acid by cariogenic bacteria thus allowing the mouth to naturally remineralize damaged teeth with less interruption[2,24,36]..

In this study, the intra-group findings for both, group A and group B tooth samples, for **base line to demineralization** indicate, a significant loss of Ca, P, and F ions, during the demineralizing procedure. Similar to this study, **Pritham Mohanty et al**[30]also confirmed of significant loss of Ca , P and F ions after demineralizing procedure when compared to baseline samples.The reason for similarity of ion loss during demineralization procedure could be attributed to the attack of acetic acid from demineralizing solution causing, chemical dissolution of both organic and inorganic components[3.]

The intra group findings of **demineralization to remineralization** for Group A samples for Ca and P ions were statistically significant, which could be due to the presence of calcium ions present in the remineralizing agent I.The intragroup finding for fluoride is statistically insignificant due to less increase in fluoride ion in remineralization group when compared to demineralization as the remineralizing agent I is a non –fluoridated remineralizing agent [30,34]**.** Similar to my study, **Prithammohanty et al** [30]**and AnuMohny etal**[34] also found, Ca and P ion during the remineralization procedure, to be statistically significant. The reason could be attributed to, the calcium sodium phosphosilicate present in the remineralizing agent I ,when it comes in contact with saliva or water which releases calcium and sodium ions, causing an increase in the pH into the range essential for the formation of HAP (Hydroxyapatite) on the enamel surface which replaces the lost calcium and phosphate ions[2,30,37]

The intra group findings of **baseline to remineralization** for Group A samples for Ca ions were stastically insignificant, indicating a net replacement of Ca ions to the level of baseline samples. But,the intragroup findings for P and F ions were statistically significant, indicating that, less remineralization of phosphate ions,

when compared to baseline sample. The reason for fluoride ions to be statistically less, when compared to baseline samples, is, because remineralizing agent I is a non-fluoridated remineralizing agent[2,5,30,34].Similar to my study **Zaheer et al [55]**found that, Ca and P ions were statistically significant, indicating less remineralization, of the ions, when compared to baseline samples.

The intra group findings for Group B for **demineralization to remineralization,** for Ca, P and F were statistically significant due to the increase of Ca, P and F ions in remineralizing group, which could be due to the presence of hyroxyapatite and fluoride is present in the Group B remineralizing paste. The hydroxyapatite contained in the remineralizing agent I fills the superficial enamel lesions and the smallest irregularities that occurs due to erosion. The surface is noticeably smoothened,dentinal tubules are superficially sealed and protects the tooth against demineralization and erosion , which replaces the lost calcium, phosphate and fluoride ions in demineralizing group **[2,24,25,36]**.Similar to my study, **Kamath P et al[55]**found that, F ion was found to be statistically significant which could be due to the presence of fluoride present in the remineralizing agent II.

The intra group finding for Group B for **baseline to remineralization**, for Ca, and F were statistically insignificant due to increase of Ca and F ions in remineralizing group to the level of baseline is due to the conversion of Hydroxyapatite crystals present in the remineralizing agent II into calcium ions which replaces the lost calcium ions in remineralization group and the fluoride ions, which is present in the remineralizing agent II is converted into fluorapatite crystals on the enamel surface, which is more resistant to acid attack that replaces the lost fluoride ions to remineralization group **[2,24,25,36]**.The intragroup finding for P is

statistically insignificant which is due to less increase of phosphate ions in remineralization group.

The intergroup comparison of baseline samples of group A and group B for Ca, P and F ions were statistically found to be insignificant, which could be due to proper standardization in collection of extracted tooth samples. The findings of this study for Ca, P and F ions,is similar to the findings of the study done by **Pritam Mohanty et al [30] and Anumaony et al [20]**

The intergroup findings for demineralization of group A and B samples were also found to be statistically insignificant, which could be due to proper demineralization standardization procedure, followed in methodology for both the groups A and B in this study. Similar to the findings of my study was observed for intergroup demineralization studies done by **Kamath P et al [55]**, **Pritam Mohanty et al[30] and Anu maony et al [20].**

The intergroup findings, in this study , for remineralization for Group A and Group B samples, for Ca and P ions were found to be **statistically insignificant**, which could be due to the efficacy of remineralizing capacity of both the samples used, were found to be good. There is a statistically significant difference for fluoride ions during the remineralizing procedure, which could be due to the presence of fluoride ions in remineralizing agent II as opposed to **non- fluoridated group A** sample. Similar to my study **Narayana et al [5],** in his study compared the efficacy of remineraizng agent I with CPP-ACP, Amflorfluoridated tooth paste and CPP-ACPF they concluded that remineralizing agent I showed better remineralizing capacity when compared to the above remineralizing agent.

In this study, the **intergroup comparison** for remineralizing efficacy, of remineralizing agent I &II was done and both were found to have good remineralizing potential. Many authors have compared the remineralizing agent I which have been used in this study, with other materials like Casein phosphopeptide- amorphous calcium phosphate(CPP-ACP) with and without fluoride, fluoridated tooth paste and resin infiltrate Icon remineralizing agents, for Ca, P and F ions at different time intervals ie. (7^{th}day,10^{th}day,14^{th}day,21^{st}day & 28^{th}day)[5,30,34,54]**.** Contradictory results were confirmed in some studies[34,54]**.** Most of the studies confirmed, remineralizing agent Iwas found to have better remineralizing efficacy of Ca and P ions than other remineralizing agents. Comparison of remineralizing agent II, which have used in this study,was also compared to other materials like Casein Phosphopeptide-Amorphous Calcium phosphate with fluoride, fluoridated dentrifice, tooth crème containing Tricalcium phosphate and sodium fluoride.No statistically significant difference was found in the remineralizing efficacy among the above group of materials [55]**.**The mechanism of action of the above used material have good remineralizing potential for replacing Ca, P and F ions, when compared to the Group B material in this study.

Remineralization efficacy of Group A agent revealed as SHY-NM(Calcium sodium phosphosilicate) was found to be better when compared to Group B remineralizing agent revealed as Remin pro(Hydroxyapatite+fluoride+Xylitol).

SUMMARY:

An invitro study was done in 50 extracted premolars samples to find the efficacy of SHY-NM (Calcium sodium phosphosilicate) and Remin pro (Hydroxyapatite+Fluoride+xylitol) remineralizing agents using SEM and EDAX analysis. The samples were divided into two groups each group has 25 samples. Remineralizing agent I is SHY-NM(Calcium Sodium Phosphosilicate) and remineralizing agent II is Remin pro(Hydroxyapatite+ fluoride+ Xylitol). All the samples were subjected to Demineralizing solution to produce artificial white spot lesions for a period of 10 days. After Demineralizing procedure, they were subjected to respective remineralizing agents for the period of 10 days and analysed to find the efficacy of the remineralizing agents.After demineralization and remineralization SEM and EDAX was done. Statistical analysis was performed using paired t test and independent sample test for both intragroup and intergroup comparison.

The statistical analysis for intergroup comparison reveals that there is no statistical significant difference between Ca and P ions in remineralizing agents I & II and statistical difference is seen in F ions in remineralizing agents I&II. Both group exhibited statistically higher remineralizing potential, while remineralizing agent I showed statistical significant remineralizing potentialwhen compared to remineralizing agent II.

CONCLUSION:

White spot lesions arise as an undesirable side effects during and after orthodontic treatment. Various Remineralizing agents are available to prevent and reverses the white spot lesions. In this study, the evaluation and comparison of the efficacy of different remineralizing agent SHY-NM (Calcium Sodium Phosphosilicate) and Remin pro (Hydroxyapatite + Fluoride + Xylitol) is done. Group A remineralizing agent (SHY-NM) was considered to bean effective remineralizing agent when compared to Group B remineralizing agent (Remin pro).

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