EVALUATION AND COMPARISON OF THE ESTHETIC EFFECT OF REMINERALIZING AGENT [FUNCTIONALIZED TRICALCIUM PHOSPHATE PASTE (*F*TCP)] ON WHITE SPOT LESIONS (WSL) AND ITS EFFECT ON PHYSICO-CHEMICAL, AND MICROBIAL PROPERTIES OF SALIVA.

Dissertation

Submitted to

BABU BANARASI DAS UNIVERSITY

LUCKNOW, UTTAR PRADESH.

In the partial fulfilment of the requirements for the degree

Of

MASTER OF DENTAL SURGERY

In

PEDODONTICS AND PREVENTIVE DENTISTRY

By

Dr. Priyam Chaturvedi

Under the guidance of

Prof. (Dr.) MONIKA RATHORE

Professor

Department of Pedodontics and Preventive Dentistry

BABU BANARASI DAS COLLEGE OF DENTAL SCIENCES, LUCKNOW

(Faculty of Babu Banarasi Das University)

BATCH: 2015-2018

DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation entitled **"EVALUATION AND COMPARISON OF THE ESTHETIC EFFECT OF REMINERALIZING AGENT [FUNCTIONALIZED TRICALCIUM PHOSPHATE PASTE (*F*TCP)] ON WHITE SPOT LESIONS (WSL) AND ITS EFFECT ON PHYSICO-CHEMICAL, AND MICROBIAL PROPERTIES OF SALIVA."** is a bonafide and genuine research work carried out by me under the guidance of **Prof. (Dr.) Monika Rathore**, Professor, Department of Pedodontics and Preventive Dentistry, Babu Banarasi Das College of Dental Sciences, Babu Banarasi Das University, Lucknow, Uttar Pradesh.

Date:

Place:

Dr. Priyam Chaturvedi

CERTIFICATE BY THE GUIDE

This is to certify that the dissertation entitled **"EVALUATION AND COMPARISON OF THE ESTHETIC EFFECT OF REMINERALIZING AGENT [FUNCTIONALIZED TRICALCIUM PHOSPHATE PASTE (*F*TCP)] ON WHITE SPOT LESIONS (WSL) AND ITS EFFECT ON PHYSICO-CHEMICAL, AND MICROBIAL PROPERTIES OF SALIVA."** is a bonafide work done by **Dr. Priyam Chaturvedi,** under my direct supervision and guidance in partial fulfilment of the requirement for the degree of M.D.S. in Pedodontics & Preventive Dentistry.

Date:

Prof. (Dr.)MONIKA RATHORE

Professor

Department of Pedodontics and Preventive Dentistry,

Babu Banarasi Das College of Dental Sciences,

Babu Banarasi Das University,

Lucknow (U.P.)

CERTIFICATE BY THE CO-GUIDE

This is to certify that the dissertation entitled **"EVALUATION AND COMPARISON OF THE ESTHETIC EFFECT OF REMINERALIZING AGENT [FUNCTIONALIZED TRICALCIUM PHOSPHATE PASTE (*F*TCP)] ON WHITE SPOT LESIONS (WSL) AND ITS EFFECT ON PHYSICO-CHEMICAL, AND MICROBIAL PROPERTIES OF SALIVA."** is a bonafide work done by **Dr. Priyam Chaturvedi,** under our direct supervision and guidance in partial fulfilment of the requirement for the degree of M..D.S. in Pedodontics and Preventive Dentistry.

Date:

Dr. PALLAVI PANDEY

Reader

Department of Pedodontics and Preventive Dentistry,

Babu Banarasi Das College of Dental Sciences,

Babu Banarasi Das University,

Lucknow (U.P.)

ENDORSEMENT BY THE HOD / HEAD OF THE INSTITUTION

This is to certify that the dissertation entitled **"EVALUATION AND COMPARISON OF THE ESTHETIC EFFECT OF REMINERALIZING AGENT [FUNCTIONALIZED TRICALCIUM PHOSPHATE PASTE (*F*TCP)] ON WHITE SPOT LESIONS (WSL) AND ITS EFFECT ON PHYSICO-CHEMICAL, AND MICROBIAL PROPERTIES OF SALIVA."** is a bonafide work done by **Dr. Priyam Chaturvedi,** under direct supervision and guidance of **Prof. (Dr.) Monika Rathore,** Professor, Department of Pedodontics & Preventive Dentistry, Babu Banarasi Das College of Dental Sciences, Babu Banarasi Das University, Lucknow, Uttar Pradesh.

Prof. (Dr.) NEERJA SINGH
Professor and Head
Dept. of Pedodontics and Preventive Dentistry
Babu Banarasi Das College of Dental Sciences,
Babu Banarasi Das University,
Lucknow (U.P.)

Prof. (Dr.) B. RAJ KUMAR
Dean, Professor & Head
Dept. of Endodontics and Conservative Dentistry,
Babu Banarasi Das College of Dental Sciences,
Babu Banarasi Das University,
Lucknow (U.P.)

COPYRIGHT DECLARATION BY THE CANDIDATE

I hereby declare that the **Babu Banarasi Das University** shall have the right to preserve, use and disseminate this dissertation in print or electronic format for academic / research purpose.

Date:

Place:

Dr. Priyam Chaturvedi

Acknowledgement

Walking along the journey of life you realize that no one walks alone. So,it is important to thank those that joined you, walked beside you, and helped you along the way.

My journey through this thought provocative, educative, informative enterprising novel work has enriched me with immense knowledge ,most invaluable experiences and sweet memories .Today when my endeavor has successfully reached its culmination, I look back with gratitude to one and all, whose generous help has made this dream come true.

I take this opportunity to express my deep sense of gratitude to my chief guide, ***Prof (Dr.) Monika Rathore****, Professor , Department of Pedodontics, Babu Banarsi Das Institute of Dental and Medical Sciences, Lucknow, and consider myself fortunate enough for getting a privilege to work under her paramount guidance and superb supervision. Her pearls of wisdom coupled with valuable criticism has contributed largely in my way to completion of this work. Her emphasis on punctuality, perseverance and being systematic has helped me in improving myself constantly. Apart from being my mentor and guide she has always been a sire figure for me .Her generosity and motherly attitude kept my enthusiasm alive throughout this study. I stand indebted to her for her constant motivation, unflinching support, persevering encouragement and affection that she has bestowed upon me.*

I am indebted to ***Dr.Pallavi Pandey,*** *Reader, Department of Pedodontics, Babu Banarsi Das Institute of Dental and Medical Sciences, Lucknow, who with her gentle, caring attitude and a scientific bent of mind has guided me in molding this thesis to its present form. Without her inspiring guidance, constant encouragement and valuable insight it would have been impossible for me to accomplish this work.*

I convey my heartfelt gratitude and profound sense of regard to my revered teacher, ***Prof. (Dr.) Neerja Singh,*** *Professor and Head in Department of Pedodontics, Babu Banarsi Das Institute of Dental and Medical Sciences, Lucknow, whose inspiring guidance, constant encouragement and efficient supervision have contributed largely in moulding this thesis to its present form.*

I convey my heartfelt gratitude and profound sense of regard to ***Dr. Shantanu Prakash****, Microbiologist and Virulogist KGMC and Image Diagnostic who with his encouragement,*

constructive criticism, invaluable suggestions and unmatched enthusiasm has always boosted my morale.

I am extremely grateful to my esteemed teachers , ***Dr. Subash Singh , Dr Somya Govil, Dr. Atul Singh*** *and* ***Dr. Madhulika Srivastava*** *for rendering their invaluable help whenever I needed it.*

It is with great privilege that I thank ***Mr. Rajendra Prasad Misra*** *for his able guidance for statistical analysis , unstinted support and generosity .His positive attitude kept my spirits high throughout this study.*

I extend my heartfelt thanks to my colleagues and friends ***Dr. Devyani, Dr. Alok, ,Dr. Monisha*** *&* ***Dr. Neha*** *for their moral support and practical suggestions which were of great help.*

I am extremely thankful to all my juniors ***Dr.Ambreen , Dr. Ankita, Dr. Sana,Dr. Sharlin Dr.Shefali and Dr. Subham*** *extending their help and support.*

Gratitude cannot be seen but only felt, therefore the heartfelt feelings cannot always be described easily. Nevertheless I wish to salute with soul felt esteem and reverence to my loving mother ***Mrs. Neeta Chaturvedi*** *and my respected father* ***Capt. Sunil Chaturvedi*** *who have been a source of inspiration, and have always been by my side with love and affection. It is from them, that I have learnt that hard work, sincerity, honesty and determination are the key words for success in life and in any endeavor. I'm grateful to my mother for extending her help at all those innumerable times when I required it the most.*

I express my humble gratitude to my second family for allowing and helping me to pursue this carrier with their unconditional love and support. I have no words to express my heartfelt appreciation for ***My Father-in Law, Dr. A.K.Chaturvedi , & mother -in law Dr. Meena Chaturvedi ,*** *for their unremitting support and encouragement.*

I thank my sister ***Dr. Oshi Chaturvedi*** *and my Sister in Law* ***Dr. Anumeha Chaturvedi*** *and her Husband* ***Dr. Uday Gajre*** *for guiding me and shaping this thesis to its present form.*

I owe a special thanks to my husband ***Dr. Anshuman Chaturvedi*** *who tolerated my madness and all temper tantrums that I showed during the stressfull days of thesis submission with a smile. He has been a gift , a good companion and a good friend who loved and encouraged me to bring this thesis to its final shape.*

A loving thank to my daughter ***Baby Aadya Chaturvedi*** *for not disturbing me and sleeping quietly during my thesis work.*

I am grateful to ***Mr. Neeraj*** *, Manas printing* ***Centre*** *for their tireless work in processing this manuscript.*

I am obliged to all my patients who provided me the opportunity to conduct this study.

I am also thankful to the non – clinical staff of the department. ***Mr. Nand kumar, Mr.Pradeep, Mr. Indrapal*** *for their help whenever I asked for it.*

Last but not the least, I am full of gratitude to the ***Almighty*** *for his blessings.*

- Priyam Chaturvedi

TABLE OF CONTENT

LIST OF TABLES

LIST OF GRAPHS

LIST OF FIGURES

LIST OF ANNEXURES

Abstract

Background: Incipient lesion is the earliest phase of tooth decay or demineralization. Classical areas of incipient caries are called as "white" spot lesions. These are the localized areas of demineralization caused due to extensive subsurface porosity which gives the lesion a milky appearance.[3] Incipient lesions are capable of being reversed or arrested from progressing to cavitation. Initially the process is reversible and can be treated by deposition of calcium, phosphate and fluoride ions into the crystal voids present in the demineralized enamel[4,5]

The aim of study was to evaluate and compare the esthetic effect of remineralizing agent [functionalized Tricalcium Phosphate Paste (*f*TCP)] on white spot lesion (WSL) and its effect on physico-chemical, and microbial properties of saliva.

Study design :

The study group consisted of 40 children exhibiting at least 1-WSL. Subjects were randomly divided into 2 groups: a test group of using remineralizing agent functionalized tricalcium phosphate (f-TCP) and a control group using regular dentifrices for a period of 3-months. Baseline WSLs were scored using digital imaging CIE L*a*b values and the saliva samples were collected to measure physio-chemical, and microbial properties using real time PCR. After the 3-month period the WSLs were again recorded and the saliva sample collection was repeated for analysis.

Results :

ΔE measurements using CIE L *a*b values were increased by time ($p=0.0001$) in test group and no statistically significant difference ($p<0.5$) was found in control group by the 3-month period. In both the groups, according to PCR reading, bacterial counts

were found to be decreased in 3-months experimental period. S.mutans and S. sanguis count were found to be significantly (p=0.005 & p= 0.006) decreased in the test group. There was significant (p=0.03 & p=0.001) increase in SIgA concentration in both the groups, but higher in test group.

Conclusions: These clinical and laboratory results suggested that remineralizing agent [functionalized Tricalcium Phosphate Paste (fTCP)] had remineralization effect on the WSL in the 3-months evaluation period with anti-cariogenic property. However longer observation is recommended to confirm whether the greater change in WSLs is maintained, or not.

Key words: Remineralization, functionalized Tricalcium Phosphate Paste (fTCP), digital imaging, real time PCR.

LIST OF ABBREVATIONS

1	Percentage	%
2.	Casein phosphopeptide	CPP
3.	Amorphous calcium phosphate	ACP
4.	Casein phosphopeptide- Amorphous calcium phosphate	CPP-ACP
5.	Significance value	P
6.	Functionalized TriCaciumPhosphate	*f*-TCP
7.	Weight by Weight	w/w
8.	Casein phosphopeptide- Amorphous calcium Floride phosphate	CPP-ACFP
9.	Parts per million	ppm
10.	Floride	F
11.	Calcium	Ca
12.	Phosphorus	P
13.	Standard Deviation	SD
14.	Sodium floride	NaF
15.	Acidulated phosphate floride	APF

Dental caries is the name of a disease where an ecologic shift within the dental biofilm environment, driven by frequent access to fermentable dietary carbohydrates, leads to a move from a balanced population of microorganisms of low cariogenicity to a microbial population of high cariogenicity (more aciduric and acidogenic) and to an increased production of organic acids. This promotes net mineral loss of dental hard tissue and results in a carious lesion.[1]The process of caries development takes place within a considerable period of time which varies from months to years. It includes molecular changes in the apatite crystals of the tooth, to a visible white-spot lesion, through dentin involvement and eventually leading to cavitations. Demineralization is the progression through these stages that require a continual imbalance between pathological and protective factors which results in the dissolution of apatite crystals and the net loss of calcium, phosphate, and other ions from the tooth .[2]

Incipient lesion is the earliest phase of tooth decay or demineralization. Classical areas of incipient caries are called as “white” spot lesions. These are the localized areas of demineralization caused due to extensive subsurface porosity which gives the lesion a milky appearance.[3]

Incipient lesions are capable of being reversed or arrested from progressing to cavitation. Initially the process is reversible and can be treated by deposition of calcium, phosphate and fluoride ions into the crystal voids present in the demineralized enamel[4,5]

In the process of remineralization of these white spot lesions exogenous stains may also get incorporated into the lesion which leads to the formation of brown spots causing esthetic problems, hence the treatment of these lesions become necessary and the treatment options for these incipient lesions should aim not only on prevention of caries progression but

should also simultaneously focus on improving the esthetics in relevant zones, by diminishing the opacity.[6]

The white spot lesions can be assessed clinically by the use of International Caries Detection and Assessment System (ICDAS) and by using various range of advanced detection systems, including laser fluorescence, digital imaging fiber-optic transillumination, quantitative light-induced fluorescence and photographic techniques.[7] Quantification of demineralized areas is relatively simple and inexpensive in photographic technique. The CIE L*a*b values and ΔE are calculated in photographic technique to determine the extent of color change in the whiteness of the lesion in pre and post- treatment photographs.

Dental caries has been considered as a multifactorial disease as it is not only influenced by dietary factors but also by the host factors, such as saliva. The most important caries protective functions of saliva are the flushing and neutralizing effects which are dependent on the flow rate and buffering capacity of saliva.[8] Saliva also contains a number of antibacterial compounds such as lysozyme, lactoperoxidases, lactoferrin, and various immunoglobulins which can control the growth of cariogenic oral microflora.[9] Many studies have shown that the salivary Immunoglobulin A (S-IgA) has a role in the caries resistance shown by certain individuals and decrease in IgA can lead to increase in incidence of acute caries lesions. [10,11] Salivary IgA helps in the antibacterial action of the saliva by neutralizing the bacterial toxins and enzymes, and preventing the adherence of the cariogenic bacteria to the tooth surface by blockage of bacterial adhesions, reduction of hydrophobicity, and agglutination of the bacteria.[10]

Dental caries is also strongly linked to the mutans streptococci, most notably *Streptococcus mutans* and *Streptococcus sobrinus.*[12,13,14,15] *Streptococcus sanguis* is also one of the most predominant species of the indigenous oral biota colonizing saliva and

dental plaque and is frequently present on tooth surfaces free of caries.[14,16] Several investigators using conventional culture methods have deduced from their studies that *S. sanguis* may play an antagonistic role against *S. mutans* colonization.[17,18,19]

The quick and convenient method for detection and quantification of bacteria from patient's saliva samples would simplify diagnosis and treatment.[20] A real-time polymerase chain reaction (PCR) assay is used for the specific quantification of Streptococcus mutans where as a conventional culture serves in qualitative identification of bacterias. Polymerase chain reaction (PCR) is used for simple, rapid and reliable method for identification of *S. mutans*, *S. sanguis*.[21,22] PCR assays are used for detecting *mutans streptococci* which are more specific than conventional culture methods.[23,24]

Effort should be directed to the use of preventive and non invasive measures for the reversal of the lesion. Conventionally treatment of such white spot lesions in esthetic zones, is achieved by various noninvasive procedures which includes long term home care therapies like use of fluorides, Casein phosphopeptide-amorphous calcium phosphate (CPP-ACP), functionalized Tri-Calcium Phosphate(f-TCP) etc. and invasive procedures such as use of composite resins. Non-invasive measures are usually preferred as they remineralize tooth without causing the loss of healthy tooth structure.

Remineralizing agents containing functionalized Tri-calcium phosphate (f-TCP), 950 ppm fluoride, creates a protective barrier around the calcium allowing it to coexist with the fluoride ions. When it comes in contact with saliva during brushing, the barrier breaks down and makes the calcium, phosphate and fluoride readily available to the tooth. The tooth naturally absorbs these components, helping to prevent the initiation and further progression of demineralization and allowing remineralization to occur.[25]

Till now studies have been done to evaluate the efficacy of various fluoridated products, CPP-ACP products, composite resin system and resin Infiltration system. But there are few studies in the literature assessing the clinical performance of these products in enhancing esthetics of teeth and its effect on salivary properties and even though many in vitro studies have been performed, but the amount of interventional studies are still lacking.

Therefore, in the light of above knowledge, the present study was conducted to evaluate the performance of conventional non-invasive treatment like fluoridated dentrifrice with f-TCP, in improving esthetic of teeth and its effect on physico-chemical and microbial properties of saliva.

AIM:

To evaluate and compare the esthetic effect of remineralizing agent [functionalized Tricalcium Phosphate Paste (*f*-TCP)] on white spot lesions (WSL) and its effect on physico-chemical and microbial properties of saliva.

OBJECTIVES:

1. To evaluate and compare the esthetic effect of remineralizing agent [functionalized Tricalcium Phosphate Paste (*f*-TCP)] on white spot lesions.
2. To evaluate and compare the effect of remineralizing agent [functionalized Tricalcium Phosphate Paste (*f*-TCP)] on physico-chemical properties of saliva.
3. To evaluate and compare the effect of remineralizing agent [functionalized Tricalcium Phosphate Paste (*f*-TCP)] on salivary microbes – *Streptococcus mutans* and *Streptococcus sanguis*.

Assessment and Detection of White Spot Lesions

Maria Ângela FF, Dyego Leandro BS, Gilmara CM and Heribert SA(2007)[26] assessed the risk factors involved for active white enamel lesion in 625 school children from age 7 to 12 yrs with the help of various indexes : visible plaque index(VPI), gingival bleeding index (GBI), DMFS-dmfs. Univariate analysis of the result revealed an increased risk of developing active white enamel lesions in individuals with high values in these indexes.

Y iijima (2008)[27] carried out a study for early detection of white spot lesions with the help of digital camera and also to check the degree of remineralization on white spot lesion. After two days of demineralization, the degree of remineralization was assessed at 2^{th}, 6^{th}, and 10^{th} day. They concluded that the combination of in-office bicarbonate and fluoride application followed by oral health care products to promote remineralization would produce additional remineralization effects.

A Caglar, K Yamanel, K Gulsahi, B Bagis and M Özcan(2009)[28] conducted a study to evaluate the colour parameter of composite and ceramic shade guides using colorimeter and digital imaging method with illuminants at different color temperatures. CIE Lab values were obtained using digital imaging and a colorimeter (ShadeEye NCC Dental Chroma Meter, Shofu Inc.).Digital imaging method could be an alternative to the colorimeters when assessing color in clinical dentistry unless the the proper object camera distance, digital camera settings and suitable illumination conditions are supplied.

Tufekcia E ; Julian S.D, J.C. Gunsolley and Steven J.L (2011)[29] conducted a study to check the prevalence of white spot lesions (WSLs) in children undergoing orthodontic treatment for 6 to 12 months using the visual examination method. Most of the patients who

underwent orthodontic treatment had at least one white spot lesion in a mild form, but a few patients presented with moderate or severe demineralization. According to their result prevalence of white spot lesions was 38% in the 6-month group, whereas it was 46% in the 12-month group.

Stafford GL (2011)[30] performed a randomized, parallel-group, controlled clinical trial recruiting 110 participants for six month follow up to see whether fluoride varnish might improve white spot lesions The test group had fluoride varnish applied onto the tooth surfaces with WSLs. Status of the WSLs was assessed using a DIAGNOdent pen. There were statistically significant differences between the mean DIAGNOdent readings of the two groups at the three-month and at the six-month follow-up visits. Thus, it was concluded that topical fluoride varnish application appeared to be a good method to treat WSLs and a routine measure to be used after orthodontic treatment.

N Sagarika, S Suchundran, SC Loganatham and V Gopikrishna(2012)[31] conducted a study to evaluate the prevalence of WSL in section of urban indian population between 12-20 age, undergoing or need of fixed orthodontics therapy for a period of 12 -15 months in 180 patients. The study elicited that Indian patients undergoing fixed orthodontic treatment shows higher prevalence rate of white spot lesions upto 75.6%.

Gugnani N, Pandit IK, Gupta M and Joshan R(2013)[6] conducted a study on treatment of noncavitated lesion by arresting the lesion progression and improving the esthetics by diminishing the opacity. The enamel caries were infiltrated with low viscocity light curing agents called infiltrants . it was concluded that microinvasive technique is designed to bridge the gap between prevention and restoration by filling.

Amely E, Hans J H and Michael K (2015)[32] conducted a study to assess the camouflage effects by concealment of post-orthodontic white-spot lesions (WSLs) to sound adjacent enamel (SAE) achieved over 12 months with resin infiltration in twenty subjects who had received resin infiltration treatment. Color and lightness (CIE-L*a*b*) data for WSLs and SAE was done for 1 year using a spectrophotometer. According to the results, color and lightness characteristics of the resin infiltration and the esthetic camouflage effects achieved by WSL infiltration were not altered significantly or clinically relevant after 12 months. They concluded that the method of resin infiltration can be recommended for an enduring esthetic improvement of postorthodontic WSL.

Korishettar BR(2015)[33] carried out a review to explain the dynamic balance between demineralization and remineralization which can determine the progression of white spot lesion. Recent investigations have primarily focused on various calcium phosphate-based technologies which are designed to supplement and enhance fluoride's ability to restore tooth mineral.

Andrew L, Sercan A, Jeryl D., Tufekci E and Rade P (2016)[34] performed an in-vitro study to analyze the staining and color changes of a resin infiltration system used for management of white spot lesions (WSLs) using a spectrophotometer. These sites were then treated with resin infiltration (RI) while the right halves of the teeth remained as nonresin (NRI) areas. Six groups were formed (n = 8 teeth/group) and were exposed to the following: red wine, coffee, orange juice, combined staining agents, accelerated aging, and distilled water for 1 week. The teeth were then polished with a prophy cup and polishing paste. Color properties were assessed using a spectrophotometer at baseline (T0), after each exposure (T1), and after polishing (T2). The color difference (DE*) was calculated between each time point for both

halves of the teeth (RI and NRI). In their result they concluded that area where resin was infiltrated showed higher staining susceptibility than areas where resin was not infiltrated.

Correlation of Remineralizing Agents with White Spot Lesions

Koch G, Petersson LG, Gleerup A and Löwstedt E.(1982)[35] conducted a study to determine the fluoride kinetics in deciduous enamel after application of fluoride containing varnish (Duraphat). A micro-acid-drop technique was used in 68 clinically intact deciduous upper central incisors in 34 pre-school children 4-5 years of age to determined the fluoride concentration in the enamel.The application of fluoride varnish resulted in an increase in fluoride in surface as well as subsurface enamel from 24 hrs upto six months after treatment. The result indicated that there might be a caries inhibiting effect of fluoride varnish in primary enamel based more upon the kinetics of fluoride rather than a permanent uptake.

Reginald JA, John DP, Joseph K and Steven BK(1983)[36] conducted a clinical trial for three years to evaluate preventive effect of tooth paste containing monofluorophosphate and trimethaphosphate in 1319 children of age group between 11-13yr. The results showed that trimethaphosphate test group had significantly higher caries increments than the monofluorophosphate control group. All groups showed some improvement in oral hygiene and in gingival health.

Ogaard B, Rolla G, Arends J and Ten Cates JM (1988)[37] performed a study to investigate the effect of fluoride on carious lesion development and on lesions established during fixed orthodontic therapy. It was concluded that daily fluoride mouth rinsing with 0.2% solution of sodium fluoride (NaF) retarded lesion development significantly whereas fluoride solution

with low pH inhibited lesion formation completely. Fluoride applied on a plaque covered lesion underneath orthodontic bands retarded lesion progression.

McDonald SP and Sheiham A (1994)[38] conducted a study to compare the three non-traumatic methods of treating dental caries in deciduous teeth over a period of 18 months. The materials used were stannous fluoride (SnF2), silver diamine fluoride (SDF) and composite resin. Caries progressed in only 5 per cent of the SDF/SnF2 group and 11 per cent of the composite resin group. The results indicated that it might be possible to treat carious lesions in a non-traumatic way using minimally prepared cavities and composite resin.

Svante T, Susanna A, Helena D, Anna KH, Carina K, Peter L et al. (2003)[39] conducted a systematic review on existing literature between 1966 to april 2003 on various caries preventive methods with special emphasis on fluoride concentration and supervised v/s non–supervised brushing. The inclusion criteria of randomized or controlled clinical trial was studies with at least 2 years follow-up and caries increment in the permanent (ΔDMFS/T) or primary (Δdmfs/t) dentition as endpoint. . In conclusion, this review reinforced the importance of daily tooth brushing with fluoridated toothpastes for preventing dental caries, although long-term studies in age groups other than children and adolescents are still lacking. They concluded that long term studies on tooth brushing with fluoridated tooth paste for preventing dental caries in age groups other than children and adolescence are still lacking.

A.B Ammari, A. Bloch-Zupan and P.F. Ashley(2003)[40] performed a systematic review on studies with randomized controlled trials comparing low fluoride toothpastes containing 600ppm F or less with toothpaste containg 1000 ppm or more in children or adults. It was

concluded that 250 ppm fluoride dentifrices was not effective in caries prevention in permanent dentition as dentifrices containing 1,000 ppm fluoride or more.

Willmot D. R. (2004)[41] conducted a double-blind prospective randomized clinical controlled trial in twenty-six patients identified as having post-orthodontic demineralized white lesions on removal of their fixed appliance which were then treated with a low fluoride (50 ppm) and a non-fluoride mouth rinse/toothpaste regime**.** Comparison and measurement of the changes in size of these lesions was then done and it was concluded that post-orthodontic demineralized white lesions reduced in size during the 6 months follow up after treatment by approximately half the original size. There was no clinical advantage in using the low fluoride formulation of mouth rinse/toothpaste in this study.

Feng Y, Yin W, Hu D, Zhang YP, Ellwood RP and Pretty IA (2007)[42] conducted a pragmatic cluster-randomized controlled trial to determine the differences in remineralization of early enamel caries on buccal surfaces of anterior teeth using various dentifrices . 296 children with at least 1 visible white-spot lesion were included and were provide with sodium fluoride (NaF; 1,450 ppm F), sodium mono fluoro phosphate (MFP; 1,450 ppm F) dentifrices or a herbal, non-fluoride placebo dentifrices .They examined WSL using QLF at baseline and after 3 and 6 months . According to the results, the groups that received fluoride experienced a more rapid and more substantial remineralization than those in the placebo group.

Anita A, Kerstin SL, Anders H, Lars G.P and Savante T(2007)[43] performed a comparative study on effect of dental cream containing CPP-ACP complexes & Fluoride mouthwashes on WSL regression which were assessed by laser fluorescence, in which they included 26 healthy adolescent with mean age of 14.6 yrs exhibiting 60 teeth with WSL

visible sites on incisors and caries immediately after debonding of fixed orthodontic appliance suggested that both regimens could promote regression of WSL after debonding of fixed orthodontic appliances. The visual evaluation showed an aesthetically more favourable outcome of the amorphous calcium phosphate treatments.

Stecksén-Blicks C, Renfors G, Oscarson ND, Bergstrand F and Twetman S.(2007)[44] conducted a double-blinded randomized placebo-controlled trial in 273 patients with an aim of evaluating the efficacy of topical fluoride varnish(Fluor Protector) applications on white spot lesion (WSL) formation in adolescents during treatment with fixed orthodontic appliances. The results concluded that regular topical fluoride varnish applications during treatment with fixed appliances may reduce the development of WSL adjacent to the bracket base.

Kumar VL, Itthagarun A and King NM (2008)[45] conducted a study to investigate the efficacy of CPP-ACP containing tooth mousse on the remineralization of enamel lesions and compared efficacy to that of fluoride-containing toothpaste. In this study, CPP-ACP showed greater efficiency in remineralization of initial enamel lesions and showed a higher remineralizing potential when applied as a topical coating after the use of topical toothpaste (1100ppm) than when used alone. Additive effects were obtained when CPP-ACP was used in conjunction with fluoride, it was concluded that CPP-ACP could be used as a self-applied topical coating after the teeth have been brushed with fluoridated toothpaste by children who had a high caries risk.

EC Reynolds (2008)[46] performed a study to review the role of various remineralization systems for the treatment of early caries lesions. The study revealed, that there is an evidence

for an anticariogenic efficacy of the enamelon technology for root caries. As well as the Recaldent technology is significantly slowing the progression of coronal caries and promoting the regression of lesions in randomized, controlled clinical trials. The result showed that the calcium phosphate-based remineralization technologies promises as an adjunctive treatments to fluoride therapy for the non-invasive management of early caries lesions.

Cochrane NJ, Saranathan S, Cai F, Cross KJ and Reynolds EC (2008)[47] conducted an in-vitro study to determine the effect of ion composition of CPP-ACP and CPP-ACPF solutions on enamel subsurface lesion remineralization. The mineral deposited in the subsurface lesions was analyzed using transverse microradiography and electron microprobe. The CPP-ACPF solutions produced greater remineralization than the CPP-ACP solutions at pH 5.5 and below. The mineral formed in the subsurface lesions was consistent with hydroxyapatite and fluorapatite for remineralization with CPP-ACP and CPP-ACPF respectively.

E.C. Reynolds, F. Cai, N.J. Cochrane, G.D.Walker and C. Reynolds (2008)[48] conducted an *in situ* randomized, double-blind, cross-over study to determine the ability of CPP-ACP to increase the incorporation of fluoride into plaque and also promotes enamel remineralization .The study involved mouthrinses and dentifrices containing CPP-ACP and fluoride. The addition of 2% CPP-ACP to the 450-ppm-F mouthrinse significantly increased the incorporation of fluoride into plaque. The result showed that dentifrice containing 2% CPP-ACP produced a level of remineralization similar to that achieved with a dentifrice containing 2800 ppm F. The dentifrice containing 2% CPP-ACP plus 1100 ppm F was superior to all other formulations.

Elmar H, Markus A, Thomas A, Adrian L and Wolfgang B (2009)[49] carried out an in-vitro study to evaluate the remineralization of initial carious lesions in deciduous enamel. This was done after application of dentrifices of different fluoride concentration with the help of an intra oral appliance for the period of 4 weeks. In their study it was concluded that it is possible to remineralize initial carious lesions in deciduous enamel in a similar way as it has for enamel of permanent teeth.

Karlinsey RL, Mackey AC, Stookey GK and Pfarrer AM (2009)[50] performed a in vitro study to determine the fluoride dose response of experimental NaF dentifrices containing a prospective calcium phosphate technology, along with the corresponding relative enamel and dentin abrasion values. Four test dentifrices (A, B, C, D) with three of the four (A, B, C) containing a promising calcium phosphate ingredient, Crest Cavity Protection, MI Paste Plus, and PreviDent Booster 5000. The groups were cycled in a lesion reversal pH cycling model. Results showed that the fluoride dose response was observed for the test dentifrices after 10 and 20 days of pH cycling, with test dentifrice C promoting the highest remineralization among the groups while both the MI Paste Plus and PreviDent systems provide the least remineralization. With respect to enamel fluoride uptake, the group facilitating the highest incorporation of fluoride into the enamel lesion was test dentifrice C, while the least effective NaF system was the MI Paste. Concluded that, the developmental test dentifrices demonstrates a fluoride dose response and shows great promise in remineralizing white-spot enamel lesions relative to MI Paste Plus and PreviDent.

Karlinsey RL, Mackey AC and Stookey GK (2009)[51] conducted a in vitro study to determine the in vitro remineralization potential of a new calcium phosphate technology in a

1000 ppm F system. Results showed a difference in results between the distilled water and fluoride-containing groups. Among the fluoride-containing groups, Group B demonstrated statistically low levels of enamel fluoride deposition and change in Vickers hardness number, while Group E statistically outperformed Group D. so can be concluded that the combination of a new calcium phosphate technology plus 1000 ppm F, produced significantly greater remineralization relative to both the 1000 ppm F test dentifrice and MI Paste Plus, and was statistically equivalent to Theramed SOS.

Karlinsey RL and Mackey AC (2009)[52] conducted a study To determine the feasibility of creating functionalized β-TCP (fTCP) using solid-state mechanochemical ball milling and to evaluate whether fTCP and fluoride provide better remineralization of weakened enamel than fluoride alone. In the study TCP-sodium lauryl sulfate (SLS) material was created via milling. Bovine enamel specimens with white-spot lesions were divided into five treatment groups distilled water, 500 parts per million (ppm) fluoride, 1,100 ppm fluoride, 500 ppm fluoride plus 0.025 percent TCP SLS and 1,100 ppm fluoride plus 0.05 percent TCP SLS. Specimens cycled for 5 days between four 2-minute treatments and one four-hour acid challenge. Results showed that milling β-TCP with SLS created fTCP and protected calcium from prematurely interacting with ionic fluoride while coexisting in solution. The fTCP combined with fluoride significantly boosted remineralization efficacy compared with only fluoride.

Karlinsey RL, Mackey AC, Walker ER and Frederick KE (2009)[53] evaluated the remineralization effects of seeding native β-TCP, milled β-TCP (mTCP) and β-TCP milled with sodium lauryl sulfate (fTCP) into weakened dental enamel. Enamel specimens from bovine molars were immersed in an acid solution (pH=5.0) at 37°C for 26 hours to produce

caries-like lesions. Specimens were then subjected to a 30-minute seeding period in solutions or suspensions containing sodium lauryl sulfate (SLS), native β-TCP, mTCP or fTCP. Results show that fTCP provided significant mineralization potential compared with native β-TCP and mTCP. While native β-TCP and mTCP produced no dose response, fTCP produced a dose response dependent on SLS content.

Mackey AC, Karlinsey RL,Gidley J and Stookey G (2009)[54] evaluated the in vitro occlusion of dentin tubules from fluoride- and fluoride-free hypersensitivity dentifrices, including two prototype sodium fluoride (NaF) dentifrices containing a functionalized TCP (fTCP) technology, using a remineralization/demineralization model. In the study Bovine dentin specimens were demineralized and divided into six treatment groups: distilled water; a conventional 1,100 ppm fluoride dentifrice, 1,100 parts per million (ppm) fluoride; a dentifrice prototype, 1,100 ppm fluoride with functionalized TCP (fTCP); a dentifrice prototype, 5,000 ppm fluoride with fTCP; a calcium sodium phosphosilicate dentifrice; and a paste with CPP-ACP and 900 ppm fluoride. Groups were remineralized for 7 days and demineralized for 3 days. Results shows that The two dentifrices containing the fTCP technology showed complete tubule occlusion with mineral layer formation, calcium sodium phosphosilicate dentifrice and paste with CPP-ACP and 900 ppm fluoride showed considerable occlusion and distilled water and conventional 1,100 ppm fluoride dentifrice showed virtually no occlusion .

Karlinsey RL, Mackey AC, Walker ER and Frederick KE (2009)[55] investigated the efficacy of TCP-sodium lauryl sulphate (SLS) plus 5,000 parts per million (ppm) fluoride relative to 5,000 ppm fluoride alone in remineralizing weakened enamel emulating early caries formation. In this study Bovine enamel specimens cycled between two 2- minute

treatments in either distilled water, 5,000 ppm fluoride solution or 5,000 fluoride solution plus 800 ppm TCP-SLS, one 4-hour acid challenge and two more 2-minute treatments per day for 5 days, interspersed with immersion in artificial saliva. Results shows that TCP-SLS plus 5,000 ppm fluoride significantly boosted remineralization of subsurface enamel lesions, with microhardness values increasing up to 30 percent greater than fluoride alone.

Bailey DL, Adams GG, Tsao CE, Escobar K, Manton DJ, Morgan MV et al. (2009)[56] conducted a clinical trial to test whether, in a post-orthodontic population that used fluoride toothpastes and received supervised fluoride mouth rinses, more lesions would regress in participants using a remineralizing cream containing casein phosphopeptide- amorphous calcium phosphate. 408 white-spot lesions were recruited in forty-five participants (aged 12–18 yrs) with 23 participants randomized to the remineralizing cream and 22 to the placebo. Product was applied twice daily after fluoride toothpaste use for 12 weeks. In their result they concluded that significantly more post-orthodontic white-spot lesions regressed with the remineralizing cream compared with a placebo over 12 weeks.

Karlinsey RL, Mackey AC ,Walker ER, Amaechi BT, Karthikeyan R and Najibfard K (2010)[57] conducted study to determine the anti-caries potential of two dentifrices, one with fast dispersion for improved enamel fluoride uptake and one containing an innovative TCP system for enhanced remineralization, in an in vitro pH cycling model. After white-spot lesion formation, enamel specimens were divided into three treatment groups: a fluoride-free dentifrice, a 5,000 ppm fluoride dentifrice, liquid gel and a 5,000 ppm fluoride dentifrice with TCP. The groups cycled between four 1-minute treatment periods and one 4-hour acid challenge per day for 10 days, interspersed with immersion in artificial saliva. Results showed that the dentifrice containing fTCP imparted superior remineralization at both the

enamel surface and within the subsurface lesion and may provide more significant anti-caries benefits than fluoride-only and fluoride-free dentifrices.

Karlinsey RL, Mackey AC, Walker TJ, Frederick KE, Blanken D, Flaig SM, et al. (2011)[58] performed a study to evaluate the in vitro remineralization effects of four dentifrice systems using microhardness and fluoride uptake analyses. The study involved the following NaF silica-based dentifrices: 1) placebo (0 ppm F), 2) 500 ppm F, 3) 1150 ppm F, and 4) 500 ppm F plus functionalized tricalcium phosphate (fTCP) and analysis for surface microhardness (SMH), enamel fluoride uptake (EFU), and cross-sectional microhardness (CSM) was then done. The results revealed Statistical significant differences among the four groups, with the placebo and 500 ppm F dentifrices providing significantly less remineralization relative to the 1150 ppm F and 500 ppm F plus fTCP dentifrices.

Bröchner A, Christensen C, Kristensen B, Tranæus S, Twetman S, **Karlsson L et al. (2011)**[59] performed a randomised controlled trial to investigate the effect of topical applications of 10% CPP-ACP on white spot lesions (WSL) detected using QLF after treatment with fixed orthodontic appliances. Sixty healthy adolescents with ≥1 clinically visible WSL at debonding were recruited and were instructed to use CPP-ACP -containing agent (Tooth Mousse, GC Europe) and standard fluoride toothpaste. In this study, they concluded that topical treatment of white spot lesions after debonding of orthodontic appliances with a CPP-ACP agent resulted in significantly reduced fluorescence and a reduced area of the lesions after 4 weeks as assessed by QLF. The improvement was however not so superior to the "natural" regression following daily use of fluoride toothpaste.

Jayarajan J, Janardhanam P, Jayakumar P and Deepika. (2011) [60] performed an in vitro study to evaluate the efficacy of casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) and casein phosphopeptide-amorphous calcium phosphate fluoride (CPP-ACPF) in remineralizing enamel surface on which artificial caries lesion had been created. The changes were analyzed using DIAGNOdent® (KaVo) and scanning electron microscope (SEM). Ninety maxillary premolars were selected and divided into three groups of 30 teeth each: A (artificial saliva), B (CPP-ACP), and C (CPP-ACPF). It was concluded that because of the added benefit of fluoride (NaF 0.2%), CPP-ACPF (Tooth Mousse-Plus) showed marginally better remineralization than CPP-ACP (Tooth Mousse).

Hegde MN, Devadiga D and Jemsily PA(2012)[61] conducted a study to evaluate the effect of cola based beverages on the calcium loss of enamel surface pre-treated with fluoride enriched casein phosphopeptide amorphous calcium phosphate (CPP-ACPF) and Beta-tricalcium phosphate using energy dispersive X-ray analysis (EDX). In this study, 24 enamel specimens were prepared from the buccal and palatal surfaces of extracted intact human premolars and were randomly assigned to study groups and control group. According to the result, it was concluded that both the remineralizing agents tested were found to be effective in inhibiting the demineralization caused by cola based beverage. Among the remineralizing agents tested, TCP was found to be more effective than CPP-ACPF.

Sharma E, Vishwanathamurthy RA, Nadella A, Savitha AN, Gundannavar G and HussainMA (2012)[62] conducted a randomized clinical trial on 50 patients to estimate the pH of saliva, concentration of calcium and inorganic phosphate, and calculus formation before and after usage of Recaldent, functionalized Tricalcium Phosphate and standard dentifrice. Clinical parameters were assessed using Plaque index, Gingival index, and Calculus index.

The result showed that the usage of remineralizing dentifrices led to an increase in the salivary calcium, phosphate, and pH. However, it did not reach the level of super saturation of the ions caused by elevated pH which could lead to calculus formation.

Aularawat WS, Nakornchai S, Thaweboon S and Korsuwannawong S (2012)[63] conducted an in-vitro study in 20 extracted third molar to evaluate the remineralization potential of tricalcium phosphate (TCP), casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) and sodium fluoride (NaF) products. The demineralized area of each section was examined by a polarized light microscope. The result showed that the area changes were significantly different among groups. It was concluded that all products can decrease demineralization on advanced enamel lesions. Among all the groups, 1.1% NaF gel showed the most effective in reduction of demineralization followed by 0.21% NaF paste with TCP, CPP-ACP tooth mousse with 0.2% NaF, CPP-ACP toothmousse and 0.22% NaF toothpaste.

Patil N, Choudhari S, Kulkarni S and Joshi SR (2013)[64] conducted a study to evaluate the efficacy of casein phosphopeptide-amorphous calcium phosphate (CPP-ACP), casein phosphopeptide-amorphous calcium phosphate fluoride (CPP-ACPF), and tricalcium phosphate fluoride (TCP-f) in remineralizing the artificial caries lesion on enamel surface. The changes were analyzed using DIAGNOdent and scanning electron microscope (SEM). A total number of 52 premolars and 24 molars were included in the study. The results showed that all the experimental groups had a significantly higher amount of remineralization except for group using regular dentifrice. Remineralization efficacy was TCP-F>CPP-ACPF>CPP-ACP.

Prabhakar AR, Manojkumar AJ and Basappa N (2013)[65] performed an in-vitro study with Tooth Mousse and Clinpro tooth crème on remineralization and tubule occluding ability with 5000ppm fluoride-containing toothpaste. Thirty third molar teeth were placed in demineralized and were treated with Group I, 5000ppm sodium fluoride; Group II, GC MI paste plus and Group III, Clinpro tooth crème. On evaluating results it showed that Group I showed a significantly greater percentage of remineralization than Group III and Group II.

He Yuan, Jiyao Li, Liang Chen, Lei Cheng, Richard D. C and Li Mei (2013)[66] performed a study to compare the esthetic improvement of WSL treated by fluoride, CPP-ACP, or resin infiltration by spectrophotometer, QLF. WSLs were created on human enamel and randomly assigned to four groups: NaF (500 ppm), CPP-ACP, resin infiltration (Icon), or distilled deionized water (DDW; control group). The color change (DE) of each specimen was measured with a Crystaleye spectrophotometer, and fluorescence loss (DQ) was measured by quantitative light-induced fluorescence (QLF), at different time points after treatment: baseline (0 weeks), 2 weeks, 4 weeks, and 6 weeks. According to the results, it was concluded that resin infiltration is more effective than NaF or CPP-ACP in providing esthetic improvement of wsls.

Hetal C, Nimisha S, Vaishali P, Ruchi R and J. R. Patel (2013)[67] conducted a study to compare the efficacy of CPP-ACPF and Sodium fluoride with tri-calcium phosphate on enamel remineralisation using the Diagnodent. Thirty extracted human premolars were selected and divided into three groups of 10 teeth each: A(artificial saliva), B (CPP-ACPF), C (Sodium fluoride with tri-calcium phosphate-Clinpro 5000). The result showed that group B (CPP-ACPF) and group C (Clinpro 5000) had significantly higher amount of remineralisation than group A (artificial saliva) concluding that all the three groups showed a statistically

significant amount of remineralization. Clinpro 5000 showed marginally more amount of remineralisation than CPP-ACPF.

Ruchi V, Rajamani I, Ramachandra S, Anil K and Manali R S (2013)[68] conducted a study on 60 subjects exhibiting atleat 1 WSL to evaluate remineralizing effect on CPP-ACP on WSL using diagnodent and its inhibitory effect on S. mutans. Their result suggested that CPP-ACP containing cream had a slight remineralization effect on WSL.

Vanichvatana S and Auychai P (2013)[69] **c**onducted a double-blind crossover in situ study to test the efficacy of two calcium phosphate pastes compared to that of fluoride toothpaste on remineralizing artificial caries. Nine healthy subjects participated in the study . Group A, brushing with 1.0 g of Colgate Regular Flavor, followed by applying 0.25 g of Tooth Mousse Plus; group B, brushing with 0.25 g of Clinpro Tooth Cream; and group C, brushing with 1.0 g of Colgate Regular Flavor. On analysing all the three groups which were remineralized, indicated sensitivity to fluoride. The differences in usage amounts and treated regimens, Clinpro Tooth Cream provided similar benefit to the fluoride toothpaste; however, no additional benefit of Tooth Mousse Plus was observed when used in conjunction with the fluoride toothpaste.

A A Yetkiner, N Kara, M Ates, N Ersin and F Ertugrul (2014)[70] conducted a study to evaluate the remineralizing effect of CPP-ACP on WSL using diagnodent and its inhibitory effect on S. mutans. The study consisted of 60 children exhibiting at-least one WSL which were divided into two groups. Group A using CPP-ACP and group B using regular fluoride dentifrice. It was concluded that mutans count were decreased in three months of period in both groups and CPP-ACP has slight remineralizing effect on WSL.

Cynthia CS, Juliana LM, Thereza CL, Eduardo M and Monica AT(2014)[71] performed a study to evaluate the effect of different application frequencies of dentrifrices containing CPP-ACP and fluoride on enamel demineralization inhibition using a pH cycling model. It was found that there was similar effect of all, except fluoridated dentifrices which has more effect than MI plus. The enamel samples treated with CPP-ACP topical paste showed smoother surfaces than that of control and fluoride dentrifrice.

Pritam M, Sridevi P and Arun BC(2014)[72] carried out a study to evaluate and compare the Calcium phosphorus ratio of enamel samples around the orthodontic brackets. 48 samples were demineralized and incubated for a period of 10 days after demineralization. Then, they were treated with remineralizing paste (Nupro-Nu-solution containing Novamin). At the end of incubation period the Calcium phosphorus ration was analysed, which concluded that novamin containing remineralizing toothpate showed significant remineralizing potential in inhibition of artificial enamel sub-surface lesion around bracket after 10 days of remineralization phase. EDX element analysis was found to be efficient method to quantify the changes in mineral content of a sample during in vitro caries studies.

Al-Kawari HM and Al-Jobair AM (2014)[73] performed a study to evaluate and compare the effects of different preventive agents namely Casein phosphopeptide-amorphous-calcium-phosphate(CPP-ACP), fluoride-containing-CPP-ACP (CPP-ACPF) and 5% sodium fluoride (5% NaF).They were applied on acid etched enamel of extracted human teeth to check the bracket shear bond strength (SBS) . It was concluded that brackets SBS was significantly increased when fluoride-containing-CPP-ACP was applied after acid-etching.

Patricia L, Micaelle TG, Fabricio ED and Andre L F(2016)[74] performed a systemic review which included clinical trials that investigated the effectiveness of materials containing fluorides to lute brackets or cover the bonding interface in order to inhibit the development and progression of white spot lesions. The result suggested that fluoride releasing materials can reduce the risk of white spot lesions around bracket but there was no effect of fluoride releasing material upon lesion extent.

Sombir S, Satinder P S, Ashima G, Ashok K U and Ashok K J(2016)[75] carried out a randomized clinical trial on 45 subject with atleast one post orthodontic WSL to check the effect of various remineralizing agents, like fluoride varnish and CPP-ACP plus creme on the outcome of post-orthodontic white spot lesion. In their result, they conclude that the use of varnish and CPP-ACP plus crème in addition to twice daily use of fluoride toothpaste had no additional benefit in the remineralization of post-orthodontic white spot lesions.

Correlation between white spot lesion and salivary properties and microbial analysis

Reynolds and Wong (1983)[76] conducted a study to show the inhibition of adherence of Streptococcus mutans to apatite discs following treatment with casein fraction. It was confirmed that CPP incorporation into the salivary pellicle not only increases its remineralizing potential but also inhibits the incorporation of the cariogenic *streptococci* by affecting their adherence.

Lundstorm F.(1987)[77] carried out a study to evaluate the effect of chlorhexidine treatment on *Streptococcus mutans* and *Lactobacilli* in orthodontic patients. It was found that the number of micro-organisms did not differ from that given for a general population of

corresponding ages. After insertion of fixed orthodontic appliances, the levels of both species were found to have increased, despite a pre-treatment oral hygiene education and training. Chlorhexidine treatment significantly reduced the number of *Streptococcus mutans*, both prior to and during the orthodontic treatment period. No effect on numbers of lactobacilli could be demonstrated.

E.C. Moreno and H.C. Margolis(1988)[78] performed a study to determine the composition of pooled resting plaque fluid in 50 individual aged 18-22yrs, who abstained from oral hygiene for 36 hours and did not eat or drink for at least one hour prior to plaque collection. They concluded that higher levels of calcium and phosphate ions may result in a higher degree of saturation in the enamel leading to an increase in the level of remineralization with a simultaneous decrease in the level of demineralization.

Winter, Holt and William (1989)[79] performed a study to compare the effectiveness of 550ppm and 1055 ppm fluoride dentifrices. In their study they concluded, that the caries increment was slightly higher (10%) in the low- fluoride dentifrices group, but the difference was not statistically significant.

Nesser JR, Golliard M, Woltz A, Rouvet M, Dillmann ML and Guggenheim B (1994)[80] conducted a study which demonstrated that cariogenic diet containing micellar casein or CPP significantly reduced the numbers of *Streptococcus sobrinus* colonizing the teeth of experimental rats. In their result, they also suggested that reduction in cariogenic *Streptococci* was at least partly responsible for the substantial reduction in caries obtained with the CPP.

P. Schüpbach, J.R. Neeser, M. Golliard, M. Rouvet and B. Guggenheim (1996)[81] conducted an *in vitro* and animal study to demonstrate the protective effects of milk and milk products against dental caries. Incorporation of CGMP and/or CPP into salivary pellicles reduced the adherence of both *S. sobrinus* and *S. mutans* significantly. It is suggested that the calcium-and phosphate-rich micellar casein or caseinopeptides are incorporated into the pellicle resulting in ecological shifts, together with the increased remineralization potential of this biofilm, explain its modified cariogenic potential.

Featherstone JDB (1999)[82] carried out a study, which highlighted that dental caries was a bacterial based disease that progresses when acid is produced by bacterial action on dietary fermentable carbohydrates diffused into the tooth and dissolved the mineral, that is, demineralization. The level of fluoride incorporated into dental mineral by systemic ingestion was insufficient to play a significant role in caries prevention. The effect of systemically ingested fluoride on caries was minimal. Fluoride "supplements" could be best used as a topical delivery system by sucking or chewing tablets or lozenges prior to ingestion.

Rose RK (2000)[83] conducted a study to measure the effect of CPP–ACP on calcium diffusion in plaque. Using Dibdin's effusion system, calcium diffusion was measured in streptococcal model plaques. This demonstrated that by providing a large number of possible binding sites for calcium, 0.1% CPP–ACP reduces the calcium diffusion coefficient by about 65% at pH 7 and 35% at pH 5. In their study , it was concluded that the CPP-ACP nanocomplexes attached to the *Streptococcus mutans* and the plaque to produce a reservoir of calcium ions which will restrict the caries process.

Akira Y, Noboru K, Hirohisa I , Toshikazu Y and Nobuhiro H(2002)[84] performed a study for quantification of *Streptococcus mutans*. A real-time polymerase chain reaction (PCR) assay was developed for the quantification of *Streptococcus mutans*. Primers targeting gtf genes of *S. mutans* were designed and tested for their specificity using 28 oral streptococcal strains, three other bacterial strains, and human DNA. The results of the real-time PCR assay corresponded well to those of conventional culture assays for *S. mutans* in saliva samples. A realtime PCR assay for *Streptococcus sobrinus* and *Streptococcus downei* was also established and produced results that corresponded well to those from conventional culture assays for *S. sobrinus* in saliva samples. These assays will be useful as a new means to assess one of the important risk factors for caries.

Cristiane Y, Clélia A, Ivan B and Antonio O (2004)[85] conducted a study to analyse the correlation among mutans streptococci counts, dental caries, and IgA to *Streptococcus mutans* in saliva in 240 individuals which were divided into five groups as follows: caries-free children, children with caries, children with rampant caries, young adults with and without caries. Whole stimulated saliva was collected and quantitative analysis of the total aerobic flora and mutans streptococci in saliva was performed and the level of salivary anti-*S. mutans* IgA was determined by ELISA. After analyzing, the highest total counts of microorganisms were found in the group of children with caries. No correlation between *mutans streptococci* counts and anti-Streptococcus mutans IgA levels was observed in the studied groups. A correlation between increased anti-Streptococcus mutans IgA levels and caries-free status was observed among young adults but not among children.

Magnusson K, Petersson LG and Birkhed D (2007)[86] conducted a study on 59 subjects to evaluate the effect of daily use of fluoride dentifrices containing various antimicrobial agents

on mutans streptococci (MS) in saliva and approximal dental plaque in 12-14 year aged children, undergoing orthodontic treatment with fixed appliances. They were provided with zinc lactate (group I), amine fluoride-stannous fluoride (group II), triclosan (group III), and no antimicrobial agent (grioup IV) for 6 months. Changes of MS scores were determined using the Dentocult SM Strip mutans test. In their result they showed that In saliva and in the 1- and 3-month plaque samples, no changes of MS were detected in any of the four groups and concluded that dentifrices with various antimicrobial agents only result in small or no changes of the MS scores in saliva.

Shifa S , Muthu M , Amarlal D and Rathna P(2008)[87] conducted a study to determine the IgA levels in the unstimulated whole saliva of caries-free and caries-active children aged 3–6 years and to correlate its role in protection of the tooth against dental caries. It was concluded that there was an increase in the mean IgA value of the caries-resistant group, the results were not statistically significant.

Y. Ge , P.W. Caufield , G.S. Fisch and Y. Li (2008)[88] conducted a study to examine the colonization of Streptococcus mutans and Streptococcus sanguinis in the oral cavity and the association with severe early childhood caries (S-ECC). Saliva and plaque samples were collected from 14 S-ECC children and 8 caries-free (CF) children. All S-ECC children were S. mutans positive; 100% of CF children and 93% of S-ECC children were S. sanguinis positive. On analysis, it showed that the interaction of S. sanguinis with S. mutans was a significant factor associated with the caries status in children, suggesting that the relative levels of these two microorganisms in the oral cavity play an important role in caries development.

Chawda JG, Chaduvula N, Patel HR, Jain SS and Lala AK (2011)[89] performed a case-control study to determine the protective role of salivary secretory immunoglobulin A (S-IgA) levels in the unstimulated whole saliva of dental caries active (Group I and II) and caries free children (Group III). Thirty children aged 4-8 years were selected. Their DMFT (Decayed Missing Filled teeth for permanent teeth) and/or df-t (decayed, filled teeth for deciduous teeth) scores were determined and the salivary S-IgA levels were measured using Immunoturbidometry. The obtained result of the study showed that the S-IgA levels of the whole unstimulated saliva has some role in protection against dental caries , but regarding the severity of diseas, it does not show any significant results.

E. Ranadheer, U Anand Nayak , N. V Reddy and V. Arun (2011)[90] conducted a study to find the relationship between salivary IgA (S-IgA) levels and dental caries in 40 children (8-12 yrs). The whole unstimulated S-IgA levels were estimated using ELISA method. It was concluded that there was an increase in S-IgA levels in caries-active mouth to give protection mechanism against dental caries and the Streptococcus mutans which are active in caries-active mouth. The S-IgA antibodies can play an important role in control of dental caries.

Sepideh S, Lars O, Kristina H, Peter C, Dan E and Lennart L (2013)[91] conducted a study to compare a new means of quantifying bacteria using FTATM Elute cards and Real-Time Polymerase Chain Reaction to a conventional culture-based assay using oral *S. mutans* as a model sample. The results show a significant negative correlation between the two methods, with a correlation coefficient of −0.577 (Spearman's Correlation) and $p < 0.01$. The method demonstrated a high sensitivity, specificity and reli- able quantitative results, covering a large range of bacterial concentrations.

A Bagherian and Gholamreza A(2013)[92] conducted a cross-sectional study to compare resting salivary pH, buffering capacity, and secretory immunoglobulin A (S-IgA), calcium, and phosphate concentrations between children with and without ECC. Samples of unstimulated saliva of 90 children (45 in ECC group and 45 in caries-free group) were taken with Scully method. The pH and buffering capacity were determined by pH meter. S-IgA, calcium, and phosphate concentrations were quantitated with ELISA, CPC photometric, and phosphomolybdate/ UV methods. According to the results, it was concluded that the higher mean resting salivary pH and better buffering capacity found in children without ECC are probably the contributing factors that protect against caries development; but further studies are needed to understand the effects of saliva and its characteristics and components on ECC.

Dwitha A, Venkata T R, Pranitha V, Sunil B K, Swetha A and Noorjahan M (2014)[93] conducted a study to evaluate the pH, buffering capacity, viscosity and flow rate of saliva in caries free, minimal caries and nursing caries children and to evaluate the relationship of these on the caries activity of children. Saliva samples were collected from 75 subjects (4-12 yrs) and were estimated for flow rate, pH, buffering capacity and viscosity. The result showed that there was a significant decrease in the mean salivary flow rate, salivary ph and salivary buffer capacity and a significant increase in the salivary viscosity among caries-free subjects, subjects with minimal caries and subjects with nursing caries. It was concluded that the physicochemical properties of saliva, such as salivary flow rate, pH, buffering capacity and viscosity, has a relation with caries activity in children and act as markers of caries activity.

Pallavi P, N. Venugopal R, V. Arun P. R, Aditya S and C. P. Chaudhary (2015)[94] performed a study to evaluate the salivary flow rate, pH, buffering capacity, calcium,

total protein content and total antioxidant capacity in relation to dental caries, age and gender. Included 120 healthy children aged 7–15 years. According to the result is was concluded that total protein and total antioxidants in saliva were increased with caries activity. Calcium content of saliva was found to be more in caries-free group and increased with age.

The present study was conducted in the Department of Pedodontics and Preventive Dentistry, BabuBanarsi Das College of Dental Science, Lucknow, with an aim to compare and evaluate the esthetic effect of remineralizing agent [functionalized Tricalcium Phosphate Paste (*f*TCP)] on white spot lesion(WSL) and its effect on physio-chemical properties and microbial analysis of saliva, after a thorough review and approval by Institutional Research Committee of BabuBanarasi Das University, Lucknow (**Annexure- I**).

Study design:

The subjects were selected from outpatient Department of Pedodontics and PreventiveDentistry, BabuBanarsi Das College of Dental Science, Lucknow. A total of 40 subjects aged between 6-12 years of both the genders were included in the study. The entire procedure was explained to the parents and a prior written informed consent was obtained from the parents. (Annexure II)

The subjects were randomly divided into 2 groups:

- Group I - comprised of 20 children using fluoridated dentifrice for a period of 3 months,
- Group II - comprised of 20 children using regular dentifrice.

The study was carried out in three phases:

A: Assessment of esthetic effect on White Spot Lesion by digital imaging analysis using CIEL*a*b* values at baseline and after 3 months of interventional period.

B: Chair side assessment of physio-chemical properties of saliva, i.e., flow, pH, buffering capacity using Saliva-check buffer testing mat and SIgA using ELISA at baseline and after 3 months of interventional period.

C: Microbial Analysis of Saliva using Real time PCR for Streptococcus mutans count and Streptococcus sanguis count at baseline and after 3 months of interventional period with the help of Image Diagnostic Lab.

At baseline, both control group (group I) and test group (group II) were instructed to brush twice a day, his/her teeth with a standardized amount (approx. 1 g) of products given, respectively for a period of 3 months.

The results were then compared and statistically analyzed.

Sample size :

40 participants were included in the study.

N = {8 * (psdˆ2) / (mdˆ2)} + 1 = 14.76 +1~ 16 formula was used

Add 20% contingency, sample size becomes 20 per group.

Where, md is mean difference and psd is pooled standard deviation.

Inclusion criteria:

- Children exhibiting at least one white spot lesion in maxillary and mandibular anterior teeth.
- Children with score of 1 or 2 according to International Caries Detection and Assessment System II (ICDAS II) index.

Exclusion criteria:

- Children who had taken antibiotics within the last 3 months.
- Children with systemic disease.

- Children with deep dental caries.

Armamentarium

A) Intra Oral Examination

- Mouth mirror
- Probe
- Tweezer
- Gloves
- Kidney tray
- Nikon D80 digital camera (Nikon Corp., Tokyo, Japan)
- 105-mm micro Nikon (Nikon) lens

B) Saliva Collection and analysis

- Saliva-Check Buffer Testing Mat (GC Dental Products Corp., Kasugai City, Aichi, Japan)
- Sterile vial
- Ice box

C) Microbial Analysis

- ELISA kit (Abelisa Elisa kit)
- Opticon real-time machine (Monitor-2, MJ Research Inc., Alameda, CA)

D) Dentifrices

- Remineralizing agent –Clinpro Tooth Creme (0.21% w/w, 950 ppm fluoride ion, 3M ESPE)
- Regular dentifrice –Colgate (650 ppm fluoride ion, Colgate –Palmolive Company)

Methodology :

Sample selection

The children were introduced with the environment and were made comfortably seated in dental chair and examined under dental chair light. Prior to clinical examination, a brief explanation about the procedure was made to them. A cheek retractor was placed into the mouth and surfaces of teeth were wiped with cotton and dried with three-way air syringe. A ball end explorer was used to aid in examination in order to detect white spot lesions using ICDAS II criteria to check severity of lesion.

The labial surfaces of the incisors, canine were examined for the presence of white spot lesions according to ICDAS criteria.[95] Codes for detection and classification of carious lesions on the smooth surfaces according to ICDAS criteria **(Annexure VI)** are as follows:

Code 0: Sound tooth surface

Code 1: First visual change in enamel

Code 2: Distinct visual change in enamel when viewed wet

Code 3: Localized enamel breakdown due to caries with no visible dentin

Code 4: Underlying dark shadow from dentin with or without localized enamel breakdown

After examination, subject with ICDAS criteria, code 1 or 2 were included in the study.

A: Assessment Of White Spot Lesions

1) Recording of White spot lesions

- The image (color) of each lesions was captured by a digital camera with a fixed distance of 25.0 cm from the tooth.
- Exposure data were as follows: exposure index, ISO 100; aperture, f/2.8; shutter speed, 1/100 second; focus, manual.
- Preadjustedtwo flashlights (Nikon SB 200) at half-power with position of at 3 and 9 o'clock in relation to the objective and were inclined 45º to the long axis of the lens, which was positioned perpendicular to the labial surface of the teeth.
- The captured images were standardized with respect to time and place, so there was no influence of indirect external light.
- The same procedure was performed at the end of experimental period.

2) White spot lesions Assessment

- The digital images were analyzed using Adobe Photoshop 7.0. The CIEL*a*b* color space system was selected through the tool Lab Color system and the values of each color parameter were obtained. The L* represents the values lightness(+100) or darkness(0/zero).
- The a* value is a measure of redness (+127 a*) or greenness (-127 a*), while the b* value is a measure of yellowness (+127 b*) or blueness (-127 b*). **(Annexure VII)**
- Whitening occurs mainly by increasing the lightness (higher L*) and reducing the yellowness (lower b*) and, to a lesser extent, by a redness reduction (lower a*).

- The extent of color change suggesting obvious change in the whiteness of the lesion in pre- and post-treatment photographs wasdetermined byΔ E, where $\Delta E = [(a_f-a_b)^2+ (b_f-b_b)^2+(L_f-L_b)^2]^{1/2}$.Any value > 6 has been shown to correlate with the obvious changes in the color differences when two different photographs are compared.[96]

B: Assesment Of Physico-Chemical Properties Of Saliva

1) Collection of saliva

10ml ofparaffin wax stimulated whole saliva was collected for the study. Sample collection was carried out in the day time, 2 h after breakfast. The children were made to sit comfortably in a ventilated and well-illuminated room, and were instructed to chew paraffin wax to stimulate salivary flow. After 30 seconds patient expectorate saliva and continued chewing for further 5 minutes. Saliva was collected in a pre-weighed graduated cylinder andthe values were noted .

2) Chair-side analysis of saliva for flow, pH, and buffering capacity:

Collected samples of saliva were estimated for flow rate, pH, buffering capacity and viscosity. The salivary flow rate was obtained from the volume of saliva collected in the initial 5 min of saliva collection. The “Saliva-Check Buffer Testing Mat” was used to estimate the pH and buffering capacity of saliva.[97]

For pH evaluation a pH test strip was immersed in the stimulated saliva for 10 seconds and compared for colour change with a testing chart. **(Annexure VIII)**

The buffer strip was removed from the foil package and placed onto an absorbent tissue with the test side up. Using pipette, sufficient saliva was drawn from the collection cup and dispense one drop onto

each of 3 test pads. Immediately the strip was turned around 90 degree to soak up excess saliva on absorbent tissue. (This is to prevent the excess saliva from swelling on the test pad and possibly affect the accuracy of the result). The test pads started changing the color immediately and after 2 minutes the final results can be calculated by adding the points according to the final colour of each pad.

The remaining saliva was collected in sterile vial and were immediately stored in ice box. The samples were then transported to laboratory where it was stored as aliquots at -80◦until use.

3) Immunoglobulin A quantification in saliva:

Portion of the stored saliva was used for the detection of sIgA which was done by using sandwich ELISA. In these assays, polystyrene Maxisorb F96 microtitre plates were coated overnight at 4ºC with 0.2 µg/well of affinity purified rabbit anti-IgA antibodies with alpha chain-specificity in 0.05 M $NaHCO_3$, pH: 9.5. Blocking was performed by use of phosphate buffer containing 0.5% bovine serum albumin (BSA) at room temperature for 90 min. One hundred microliters of saliva samples (in duplicate) and standard samples (in duplicate) were pipetted into the microtitre wells. The plates were incubated for 90 min at 37ºC. The wells were washed 5 times with washing solution. Then, 100 µl of goat anti-human IgA conjugated with hours radish peroxidase (HRP) were pipetted into each well, and the plates were incubated for 30 min at 37ºC. The wells were washed 5 times with washing solution and tapped dry. A fresh substrate solution, tetramethylbenzidine (100 µl), was added, and the plates were incubated for 15 min at room temperature. The enzyme reaction was stopped with 100 µl of 1 N HCl. Salivary IgA levels were detected by use of a standard curve. The percent coefficient of variation (%CV) for this ELISA was 3.8%.[98]

Salivary IgA levels were quantitated by using appropriate dilution of a standard IgA sample with a known concentration of IgA, provided by the manufacturer and expressed as mg/dL.

C : Analysis of *Streptococcus mutans & Streptococcus sanguis* in Saliva

1) Microbial analysis (PCR and real-time quantitative PCR assays)

The remaining saliva sample was then evaluated for microbial assay. The species specificity of each newly developed primer set was evaluated initially against the 7 S. mutans and 48 non-S.mutans reference strains, and further validated using the randomly selected purified S. mutans DNA of clinical isolates and the mixed bacterial samples described above. The limit of detection of the primers was evaluated using PCR against a set of 10-fold serially diluted concentrations of UA159 genomic DNA samples and further validated using the mixed S. mutans DNA samples containing known concentrations of Streptococcus sobrinus or Streptococcus sanguinis DNA.

PCR assays were performed using a standardized protocol in a thermal cycler (GeneAmp PCR system 9700, Applied Biosystems, Foster City, CA). Each reaction mixture (25 µl total volume) contained 1X PCR buffer (10 mMTris-HCl, 50 mMKCl, pH 8.3), 1.5 µl of 2.5 mMdNTP mixture, 1 mM MgCl2, 10 pmoles each of forward and reverse primers, 1.5 U of Taq DNA polymerase, and 10 ng of template DNA. The reaction was conducted as follows: 95°C for 2 min, followed by 40 cycles of 95°C for 30 s, 60±5 °C for 30 s, and 72°C for 1 min, then finally 5 min at 72°C for extension. The PCR amplicons were evaluated in a 1.5% agarose gel in TBE (Tris-borate-EDTA) buffer and stained with ethidiumbromide solution (1 µg/ml). The final images of the gels were captured by a digital camera (AlphaImager 3300 System, Alpha Innotech Corp., San Leandro, CA).

The species specificity and limit of detection of the primers in identifying S. mutans colonization in children was determined using real-time quantitative PCR (real-time qPCR). Briefly, real-time qPCR was performed using an Opticon real-time machine (Monitor-2, MJ Research Inc., Alameda, CA) with low-profile 96-well polypropylene microplates. Tenfold serially diluted, known DNA concentrations of

S. mutans UA159 were used as an external standard for absolute quantification. Each tube contained 25 µl of reaction mixture, including 1X PCR Master Mix (QuantiTect SYBR Green, Qiagen Inc.), 10 to 100 ng of the mixed bacterial DNA samples obtained from MM10 culture plates and 0.4 µM of each primer. The cycling conditions were 15 min at 95°C for activation of HotStarTaq DNA polymerase, 45 cycles of 15 s at 94°C for denaturation, 30 s at 56°C for annealing and 30 s at 72°C for extension, followed by a melting curve analysis of the PCR product. All reactions were carried out in duplicate and the final analysis was based on the mean of the two reactions. Furthermore, the PCR products were reconfirmed for correct size by electrophoresis in a 1.5% agarose gel alongside molecular size standards. The real-time qPCR results were compared with the results previously obtained using conventional culture methods.[99]

2) DNA sequencing analysis

To further confirm the species specificity of the primers, 50% of the PCR products of the S. mutans reference strains, the clinical isolates, and the mixed bacterial DNA samples were randomly selected, purified, and sequenced from both directions (ABI Prism cycle sequencing kit, BigDye Terminator chemistries with AmpliTaq DNA polymerase FS; Perkin-Elmer, Chen et al. Page 3 FEMS MicrobiolLett. Author manuscript; available in PMC 2009 September 3.NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript Wellesley, MA). A sequence similarity search of the nonredundantGenBank database was performed using the standard nucleotide-nucleotide BLAST (BLASTn) algorithm, and sequences were aligned using ClustalW (Chenna et al., 2003).[99]

Statistical analysis

The results are presented in mean±SD. Unpaired t-test/Mann-Whitney U test was used to compare the study parameters between the groups before and after intervention. Paired t-test was used to compare

mean change from before to after intervention within the groups. The p-value<0.05 was considered significant. All the analysis was carried out on SPSS 16.0 version (Chicago, Inc., USA).

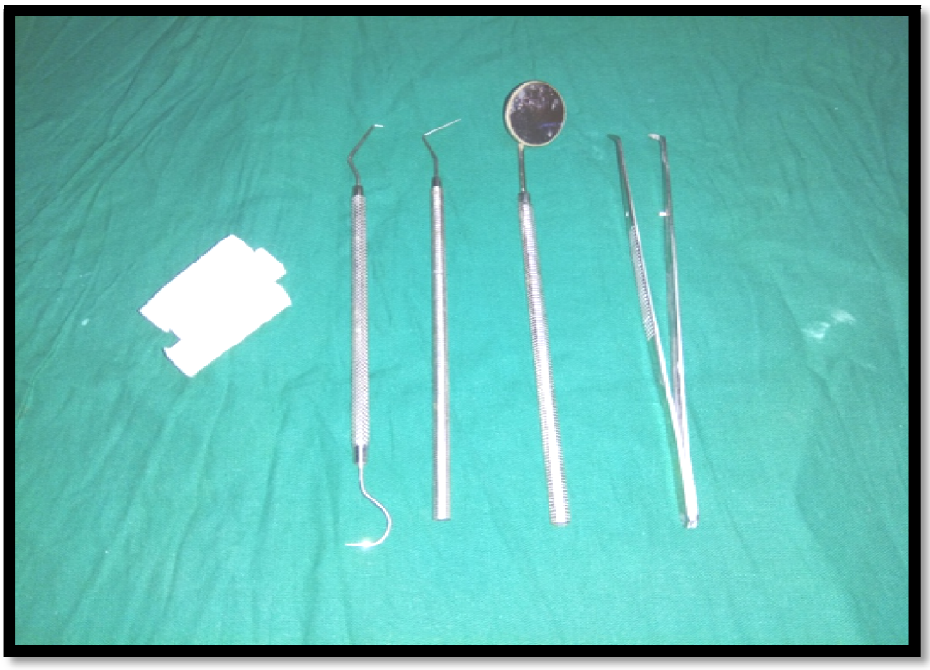


Figure 1 : Armamentarium used for screening

Figure 2 : Nkon d80 digital camera

Figure 3 : Dentifrice containing Remineralizing agent (fTCP) and Regular dentifrice

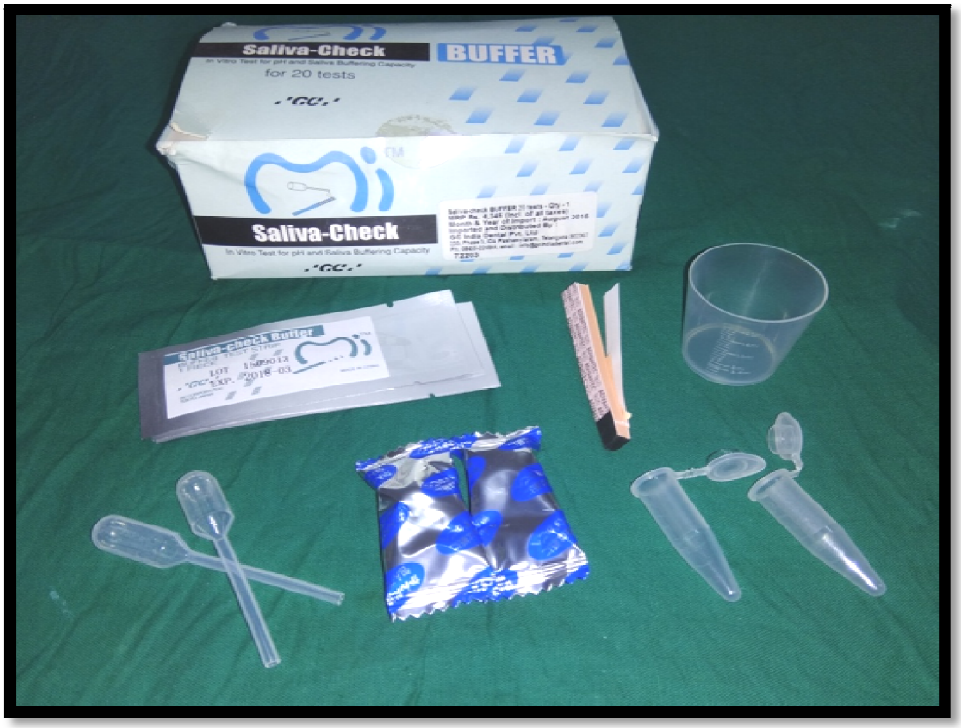


Figure 4 : GC Saliva-check buffer kit

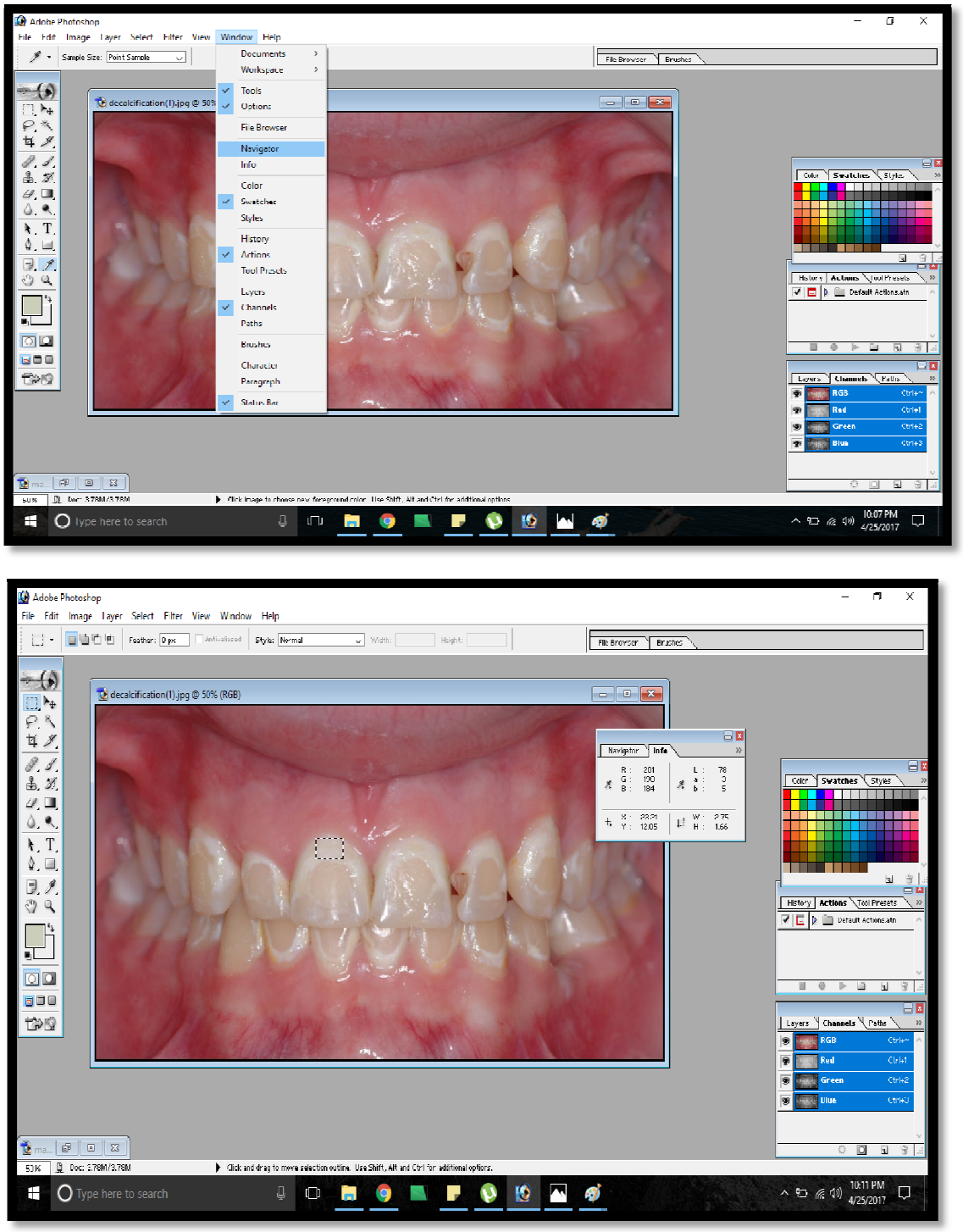
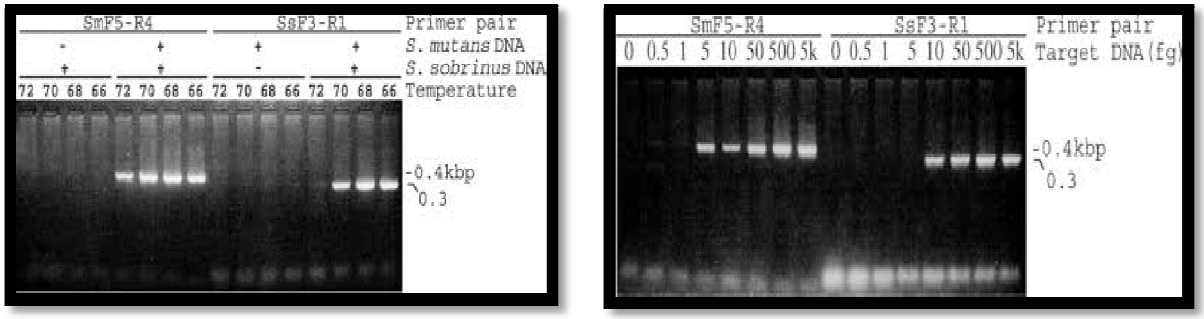


Figure 7 : CIE L a b values, using adobe photoshop CS2 software

Figure 8: Real time PCR machine

Figure 9 : Elisa kit used for S-IgA



S. MUTANS **S. SANGUIS**

Figure 10 : Qiagen bacterial dna extraction for S. mutans & S. sanguis

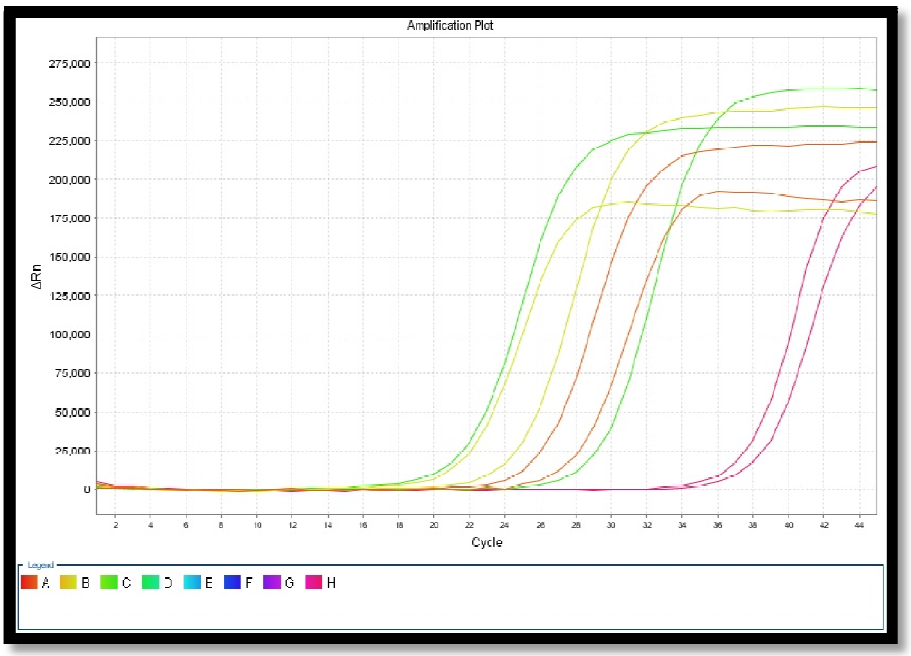


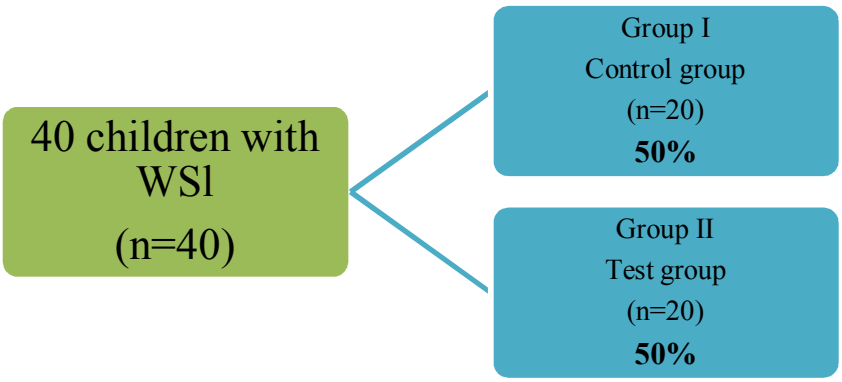
Figure 11 : Real time PCR graph of S. mutans and S. sanguis

RESULTS

The present study was conducted in the Department of Pedodontics and Preventive Dentistry, Babu Banarasi Das College of Dental sciences, Lucknow with an aim to evaluate and compare the esthetic effect of remineralizing agents [functionalized Tricalcium Phosphate Paste (*f*TCP)] on white spot lesion(WSL) and its effect on physico-chemical and microbial properties of saliva.

40 children, aged 6-12 years were included in the present interventional study. The group wise distribution of children is shown in **Table- 1.**

Children were randomly divided into two groups of 20 participants each (50%).



Group I participants used regular dentifrices and Group II participants used remineralizing agent [functionalized Tricalcium Phosphate Paste (*f*TCP)]. **[Graph-1]**

The mean age of the participants ranged from 9.1 ± 0.17 years in Group I i.e Control group and 8.95 ± 0.65 years in Group II i.e Test group. The mean age of the participants in Group I was observed to be higher as compared to that in Group II. **[Table -2 & Graph -2]**

In Group I i.e Control group, proportion of males was lower (40%) compared to Group II i.e Test group (55?%). **[Table-3 & Graph-3]**

In the first part of the study, assessment of esthetic effect of remineralizing agents on white spot lesion was done, using digital imaging. Evaluation of CIE L*a*b values and ΔE value of images was done.

Table 4 and Graph 4 shows the comparison of L, a, b values between the groups at baseline. L(p=0.02) & a(p=0.001) values were significantly higher in Group II.

Table 5 and Graph 5 shows the comparison of L, a, b values between the groups after intervention. L(p=0.001) & b(p=0.001) and the values were highly significant in Group I.

Table 6 & Graph 6 depicts pre and post intervention L, a and b values and comparison between the groups . Before intervention, L was significantly (p=0.02) higher in Group II than Group I. However, L became significantly lower in Group II after 3 months of intervention.

Table-7& Graph. 7 shows the pre & post intervention comparison of mean change in L, a and b values. There was significant ($p<0.01$) mean change in L, a and b values after intervention in Group II. The mean change in L, a and b was higher in Group II compared

to Group I. There was significant ($p<0.05$) difference in the mean change in L, a and b between the groups.

Table 8 and Graph 8 shows the comparison of delta E values which depicts the change in color of lesion. delta E value between the groups after intervention was significantly higher in Group II (P=0.0001).

In the second part of the study, the effect of dentifrice containing remineralizing agent on salivary characteristics pH, salivary flow, buffering capacity and Salivary IgA were analysed.

Table 9 & Graph 9 shows the value of salivary characteristics at baseline for both groups. There was statistically no significant ($p>0.05$) difference between both the groups, in salivary flow, pH, buffering capacity. S-IgA was significantly (p=0.006) lower in Group II than in Group I at baseline.

Table 10 & graph 10 shows the value of salivary properties after intervention for both groups. There was statistically no significant ($p>0.05$) difference between both the groups in salivary flow, pH, buffering capacity,S-IgA at baseline.

Table 11 & graph 11 shows the pre and post intervention comparison of mean change in salivary flow, pH, buffering capacity, and S-IgA in both groups.

There was significant ($p<0.05$) mean change in flow rate from before to after intervention in both the groups. The mean change in flow rate was higher in Group II compared to Group I. There was no significant ($p>0.05$) difference in the mean change in flow rate between the groups.

There was insignificant ($p>0.05$) mean change in pH from pre and post intervention in both the groups. The mean change in pH was higher in Group II compared to Group I. There was no significant ($p>0.05$) difference in the mean change in pH between the groups.

There was insignificant ($p>0.05$) mean change in buffer in both Group I and Group II after intervention.

There was significant ($p<0.05$) mean change in S-lgA after intervention in both the groups. The mean change in S-lgA was higher in Group II compared to Group I. There was significant ($p=0.001$) difference in the mean change in S-lgA between the groups.

In the third part of the study comparison of the effect of remineralizing agent on salivary microbes including S. mutans and S. sanguis count (real time PCR) was done.

Table 12 & graph12 shows the comparison of S.mutans & S.sanguis (copies/ml) in both groups at baseline. There was no significant ($p>0.05$) difference in S.mutans between the groups at baseline. S.sanguis was significantly ($p=0.001$) higher in Group II than Group I after intervention.

Table 13 & graph 13 shows the comparison of S.mutans & S.sanguis (copies/ml) in both groups after intervention. There was no significant ($p>0.05$) difference in S.mutans and S.sanguis between the groups after intervention.

Table-14 & graph 14 shows the comparison of mean change between pre & post intervention in Streptococcus mutans and Streptococcus sanguis counts. There was significant ($p=0.005$) mean change in Streptococcus mutans counts after 3 months of intervention in Group II. The mean change in Streptococcus mutans counts was higher in Group II compared to Group I. There was no significant ($p>0.05$) difference in the mean change in Streptococcus mutans counts between the groups.

There was significant ($p=0.007$) mean change in Streptococcus sanguis counts after intervention in Group II. The mean change in Streptococcus sanguis counts was higher in Group II compared to Group I. There was significant ($p=0.004$) difference in the mean change in Streptococcus sanguis counts between the groups.

Table -1 Group wise distribution of particants (n=40)

S.NO.	GROUP	DESCRIPTION	NO. OF PARTICIPANTS	PERCENTAGE (%)
1.	**I**-Control group (CG)	Children using regular dentifrices	20	50
2.	**II**-Test group (TG)	Children using remineralizing agent [functionalized Tricalcium Phosphate Paste (*f*TCP)]	20	50

Table -2 Age wise distribution of participants

	GROUP I (CONTROL GROUP) n=20		GROUP II (TEST GROUP) n=20	
	Mean	**SD**	**Mean**	**SD**
AGE	9.1	0.17	8.95	0.67

Table -3 Gender profile of participants in both the groups

	GROUP I (n=20)	GROUP II (n=20)
Gender		
Male	8	11
Female	12	9

Table 4: L.a.b. values at baseline in both the groups

Parameter	GROUP I (n=20)	GROUP II (n=20)	P-Value
L	83.40 ± 5.20	87.10 ± 6.65	0.02
a	-2.25 ± 3.33	-6.00 ± 3.06	0.001
b	5.55 ± 3.92	2.80 ± 4.61	0.05

TABLE 5: L. a .b. Values after intervention in both the groups

Parameter	GROUP I (n=20)	GROUP II (n=20)	P-Value
L	84.85 ± 7.84	70.75 ± 5.60	0.001
a	-0.90 ± 3.50	-2.40 ± 1.98	0.21
b	2.25 ± 3.49	0.25 ± 2.75	0.001

Table-6: Pre and post intervention comparison of L,a,b values in both the groups

Time period	GROUP I (n=20)	GROUP II (n=20)	p-value[1]
L			
Pre Intervention	83.40±5.20	87.10±6.65	0.02*
Post Intervention	84.85±7.84	70.75±5.60	0.001*
A			
Pre Intervention	-2.25±3.33	-6.00±3.06	0.001*
Post Intervention	-0.90±3.50	-2.40±1.98	0.21
B			
Pre Intervention	5.55±3.92	2.80±4.61	0.05
Post Intervention	2.25±3.49	0.25±2.75	0.001*

[1]Mann-Whitney U test, *Significant

Table 7: Pre and post intervention comparison of mean change in L, a, b values in both the groups

	L		A		b	
Groups	**Mean change**	**p-value[1]**	**Mean change**	**p-value[1]**	**Mean change**	**p-value[1]**
GROUP I	1.45±5.97	0.29	1.35±2.30	0.01*	0.30±0.84	0.69
GROUP II	16.35±7.56	0.001*	3.60±2.81	0.001*	2.55±3.79	0.007*
p-value[2]	0.001*		0.009*		0.04*	

[1]Paired t-test, [2]Unpaired t-test, *Significant

Table 8-: Comparison of delta E value in both the groups

Groups	**Delta value**
GROUP I	5.16±5.03
GROUP II	17.80±6.91
p-value	0.0001*

Table 9 : Salivary properties at baseline in both the groups

Parameter	**GROUP I (n=20)**	**GROUP II (n=20)**	**P-Value**
Salivary Flow	5.70 ± 1.94	6.15 ± 2.23	0.05
pH	7.10 ± 0.34	7.02 ± 0.23	0.39
Buffering capacity	10.55 ± 1.70	10.30 ± 1.62	0.63
SIgA	199.45 ± 53.82	155.15 ± 42.56	0.006

Table 10 : Salivary properties after intervention in both the groups

Parameter	GROUP I (n=20)	GROUP II (n=20)	P-Value
Salivary Flow	7.40 ± 1.69	6.45 ± 1.39	0.06
pH	7.12 ± 0.25	7.12 ± 0.34	1.00
Buffering capacity	10.95 ± 1.70	10.95 ± 1.19	0.15
SIgA	207.10 ± 43.7	197.55 ± 16.04	0.36

Table-11: Pre and post intervention comparison of mean change in salivary properties in both the groups

	Salivary flow		pH		Buffering capacity		SIgA	
Groups	**Mean change**	**p-value**	**Mean change**	**p-value**	**Mean change**	**p-value**	**Mean change**	**p-value**
GROUP I	0.75±1.20	0.01*	0.02±0.18	0.62	0.40±0.99	0.08	7.65±14.72	0.03*
GROUP II	1.25±1.16	0.001*	0.10±0.31	0.17	0.90±0.98	0.09	42.40±42.02	0.001*
p-value[2]	0.19		0.33		0.54		0.001*	

Table 12: Streptococcus mutans and Streptococcus sanguis counts at baseline in both the groups

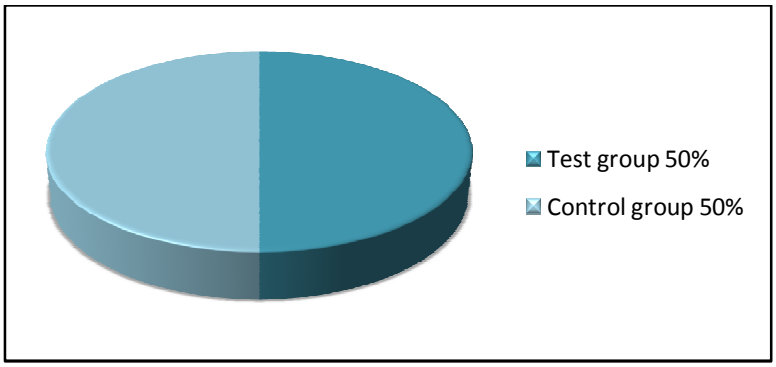
Parameter	GROUP I (n=20)	GROUP II (n=20)	P-Value
S. mutans (copies/ml)	38064.22 ± 79114.64	29196.23 ± 39337.29	0.55
S. sanguis (copies/ml)	20698.50 ± 17733.29	260365.70 ± 336861.40	0.001*

Table 13: Streptococcus mutans and streptococcus sanguis counts after intervention in both the groups

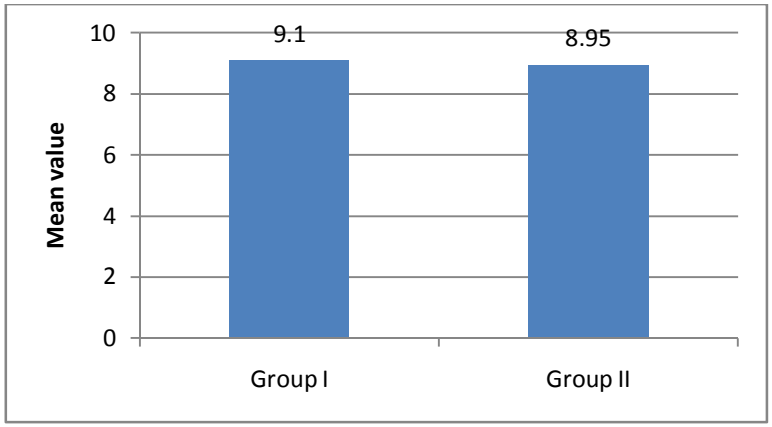
Parameter	GROUP I (n=20)	GROUP II (n=20)	P-Value
S. mutans (copies/ml)	1453.00 ± 1866.17	1901.11 ± 2110.58	0.25
S. sanguis (copies/ml)	19433.65± 33990.19	26033.50 ± 20041.99	0.32

Table-14: Pre and post intervention comparison of mean change in Streptococcus mutans & Streptococcus sanguis counts in both the groups

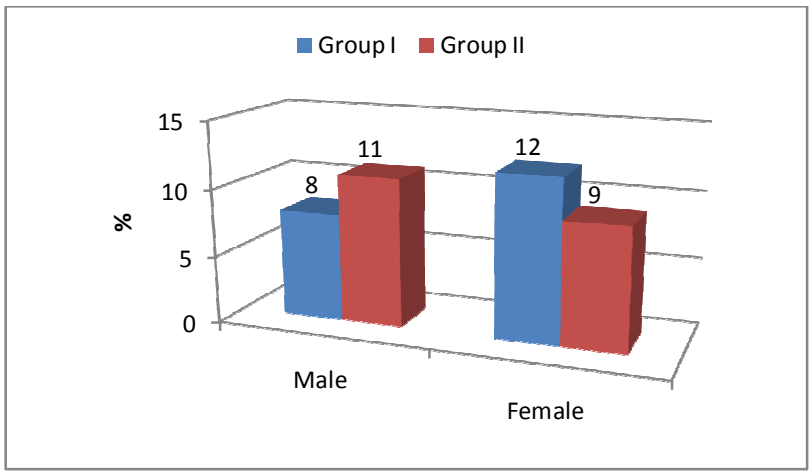
	S.mutans (copies/ml)		S.sanguis (copies/ml)	
Groups	Mean change	p- value	Mean change	p- value
GROUP I	24357.00±63744.04	0.13	1264.85±28787.98	0.84
GROUP II	27743.22±38612.26	0.005*	234332.20±345306.90	0.006*
p-value[2]	0.84		0.004*	



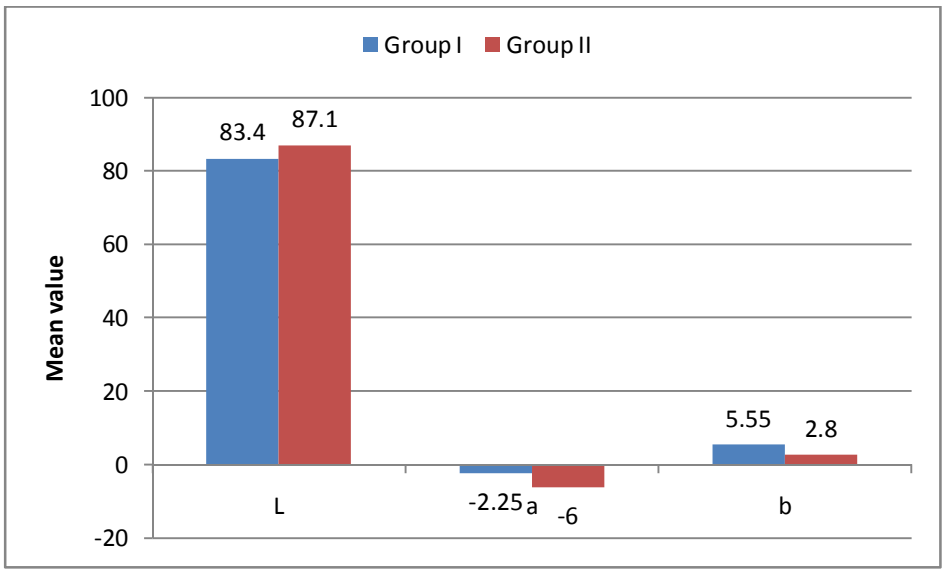
Graph-1: Group wise distribution of participants (n=40)



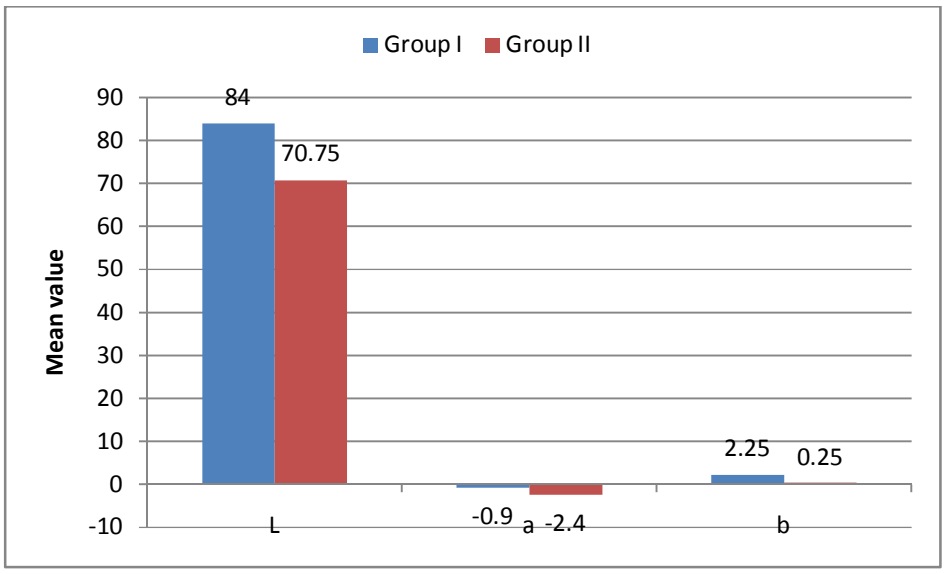
Graph -2: Age comparison of participants in both the groups



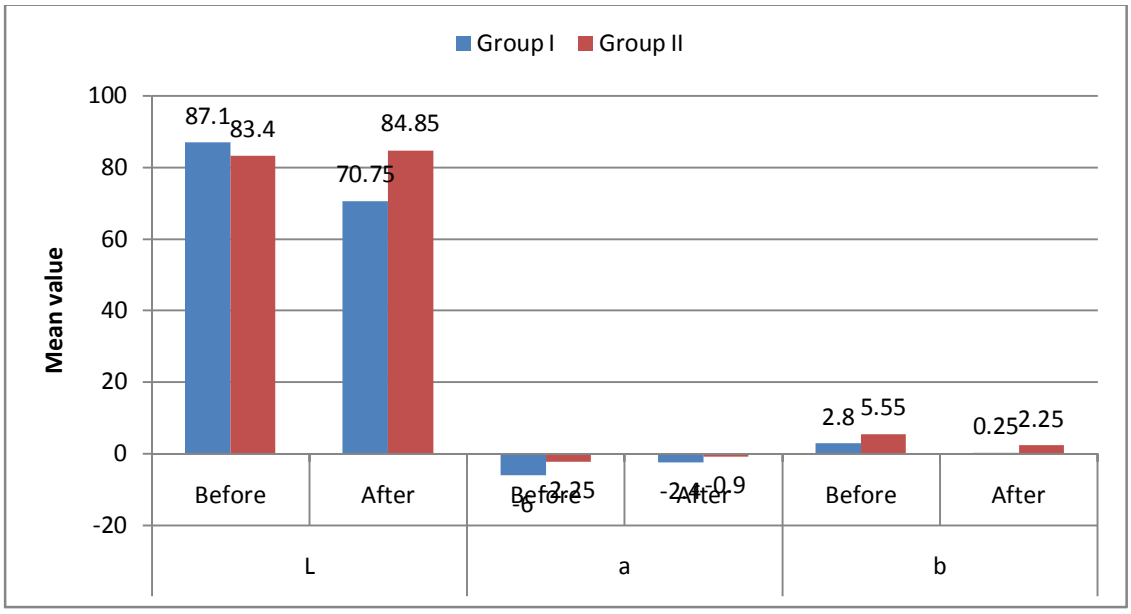
Graph-3: Gender profile of participants in both the groups



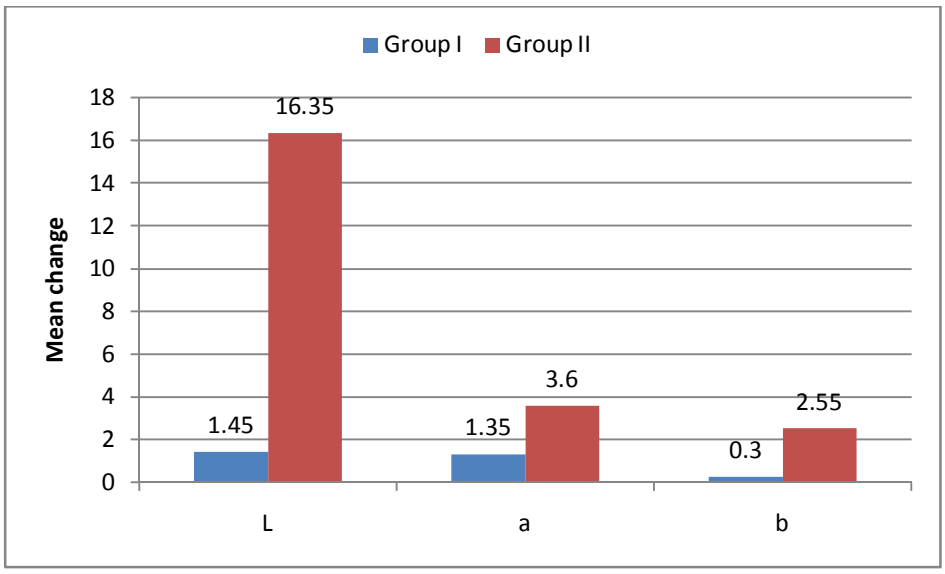
Graph 4: L.a.b. values at baseline in both the groups



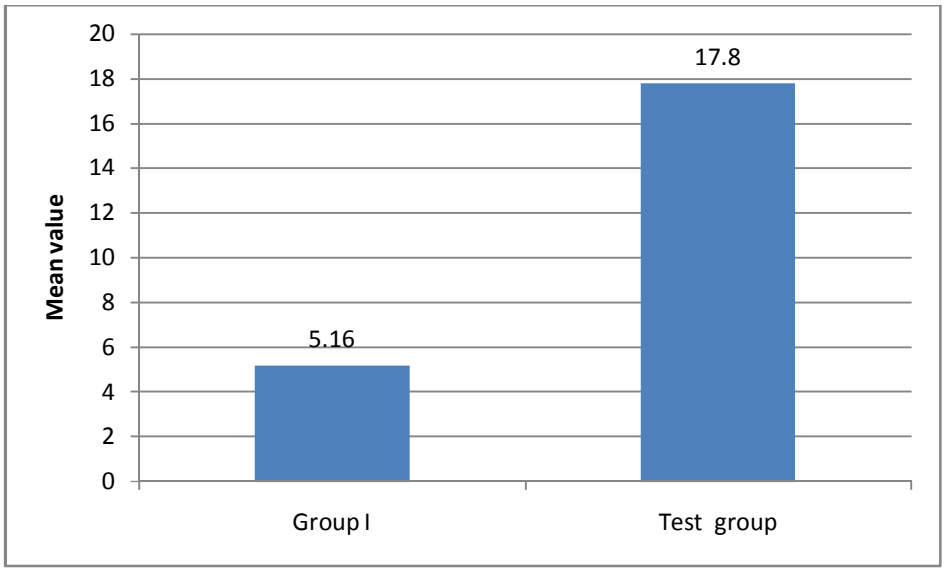
Graph 5: L.a.b. values after 3 months intervention in both the groups



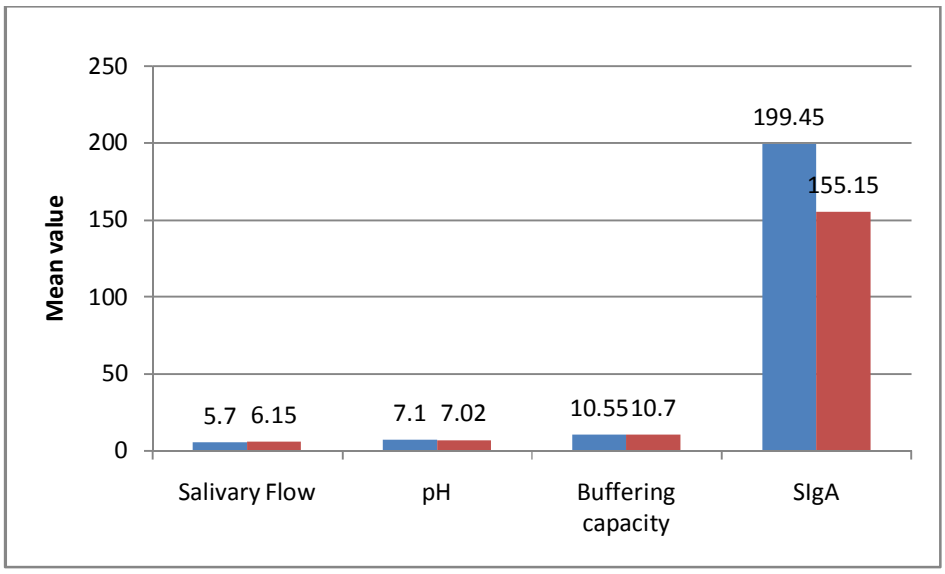
Graph 6: Pre and post intervention comparison of L,a,b values in both the groups



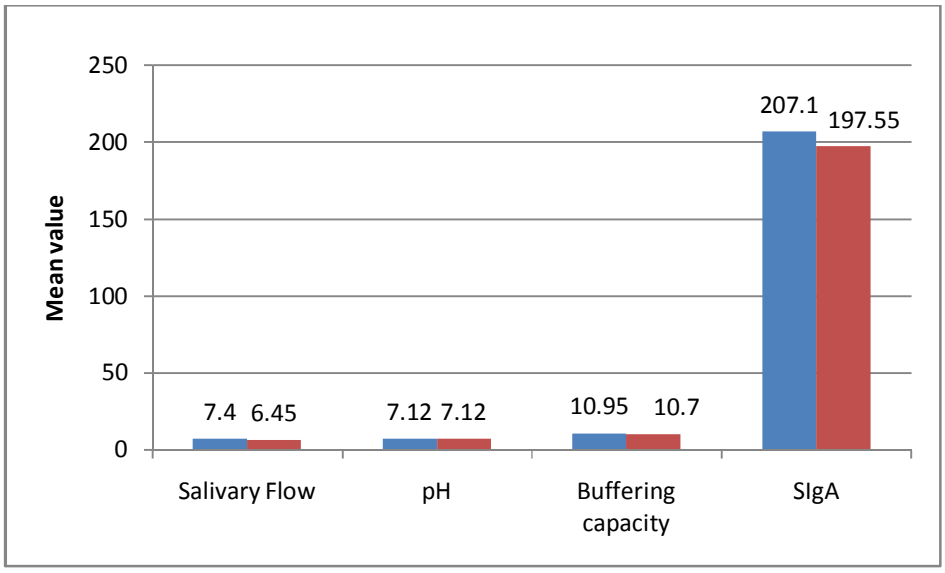
Graph 7 : Pre and post intervention comparison of mean change in L, a, b values in both the groups



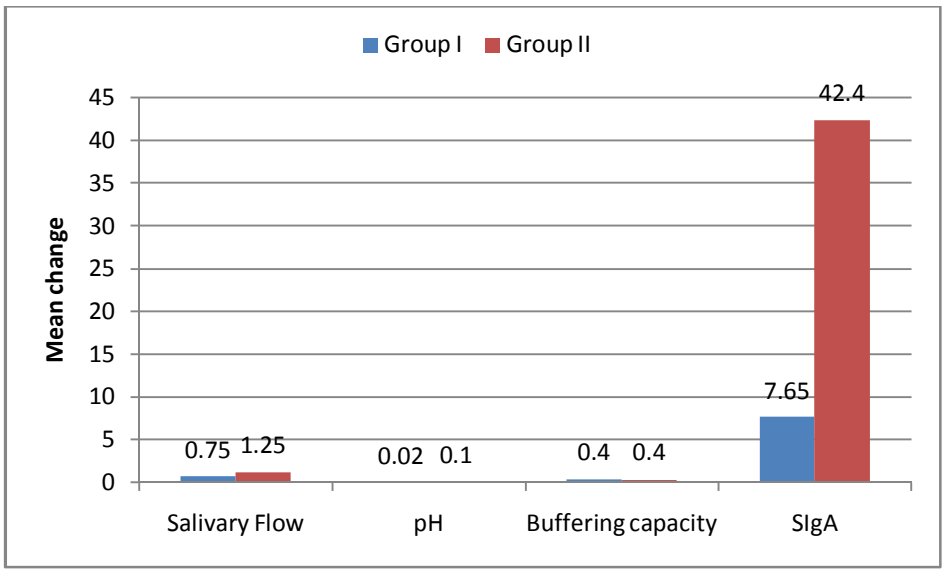
Graph 8: Comparison of delta E value in both the groups



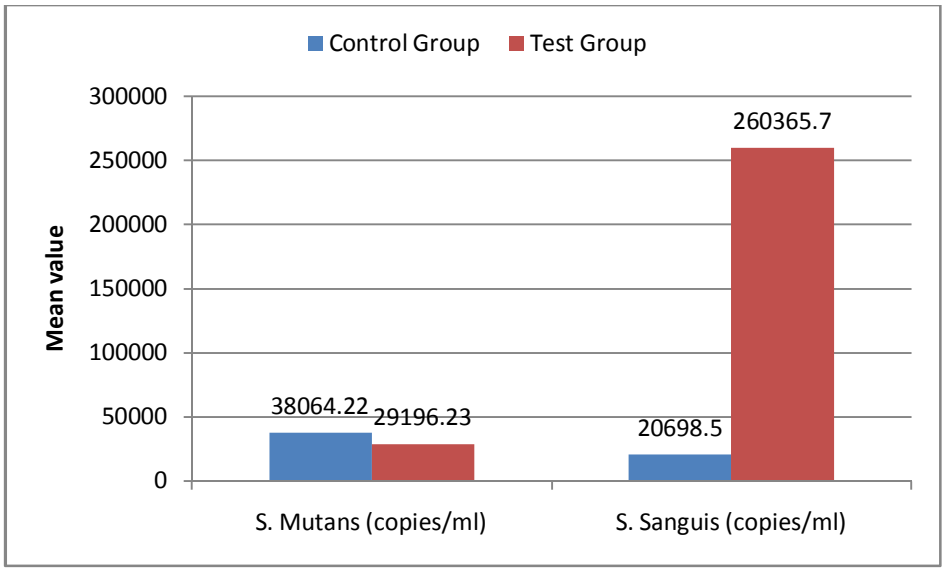
Graph 9: Salivary properties at baseline in both the groups



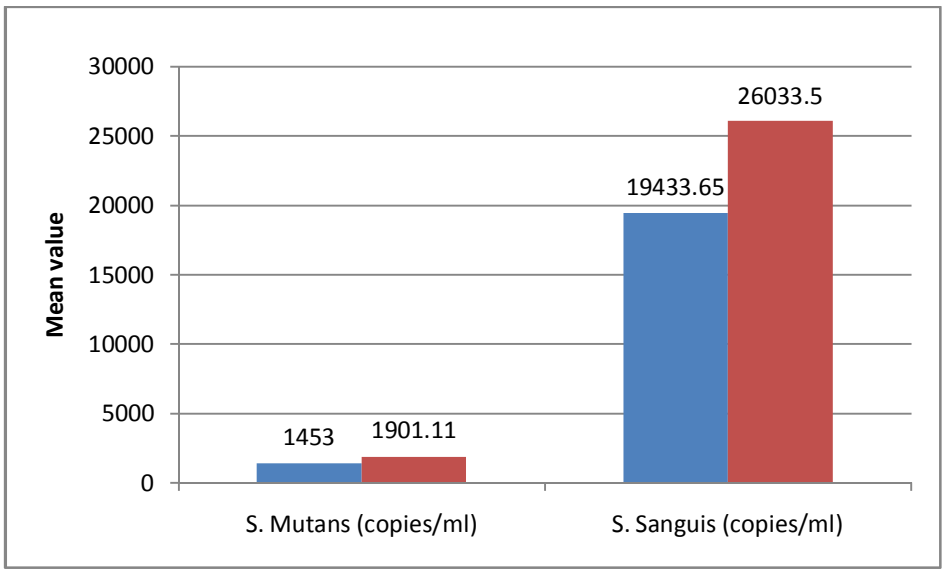
Graph 10 : Salivary properties after intervention in both the groups



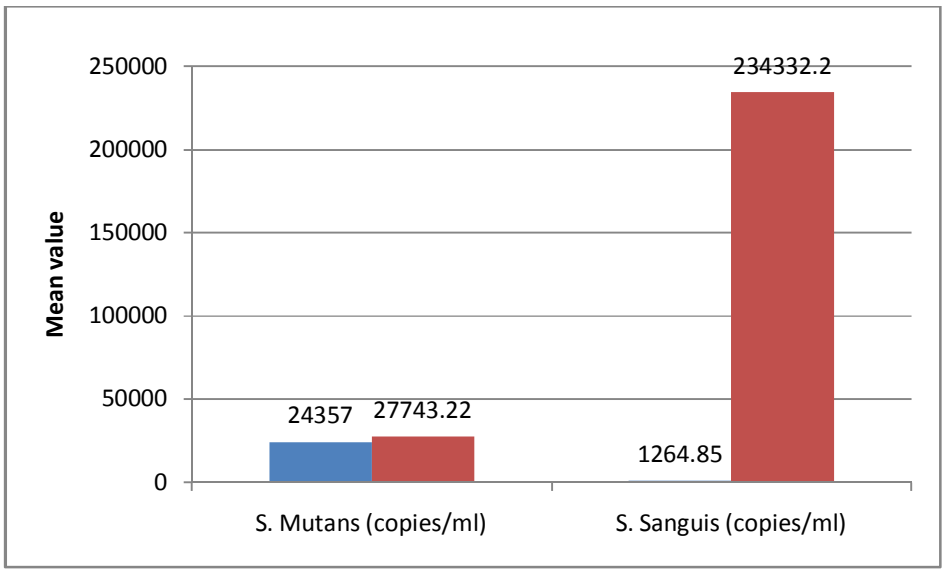
Graph 11: Pre and post intervention comparison of mean change in salivary properties in both the groups



Graph 12: Streptococcus mutans and Streptococcus sanguis count at baseline in both the groups



Graph 13: Streptococcus mutans and streptococcus sanguis count after intervention in both the groups



Graph 14: Pre and post intervention comparison of mean change in Streptococcus mutans & Streptococcus sanguis count in both the groups

The present study was carried out in Department of Pedodontics and Preventive Dentistry, Babu Banarsi Das College of Dental Science, Lucknow. The study was done with an aim of esthetic evaluation of remineralizing agent [functionalized Tricalcium Phosphate Paste (fTCP)] on white spot lesion and its effect on physico- chemical and microbial properties of saliva in children of age 6-12 years.

On the basis of results in the study, the following conclusion have been drawn:

1. The higher mean change($p=0.0001$) in the CIE L*a*b and ΔE value in Group II depicts positive effect of remineralizing agent [functionalized Tricalcium Phosphate Paste (fTCP)] on white spot lesion.

2. In group II , there was significant ($p=0.001$) increase in SIgA level in saliva, although salivary flow rate, pH and buffering capacity were found to be unchanged.

3. There was significant decrease in streptococcus mutans and streptococcus sanguis count in both the groups ($p=0.004$). However, the mean change in both streptococcus mutans and streptococcus sanguis count was higher in Group II.

Tricalcium phosphate (f-TCP) containing remineralizing paste improved the esthetic appearance of white spot lesions after 3 months of intervention, suggestive of bioactive, biocompatible and esthetic efficiency of the material. But in order to extrapolate the findings of present study, studies involving a larger ample size are required.

Summary

Dental caries is the name of a disease where an ecologic shift within the dental biofilm environment, driven by a frequent access to fermentable dietary carbohydrates, which leads to a move from a balanced population of microorganisms of low cariogenicity to a microbial population of high cariogenicity (more aciduric and acidogenic) and to an increased production of organic acids. This promotes net mineral loss of dental hard tissue and results in a carious lesion.[1]

Incipient lesion is the earliest phase of tooth decay or demineralization. Classical areas of incipient caries are called as "white" spot lesions. These are the localized areas of demineralization caused due to extensive subsurface porosity which gives the lesion a milky appearance.[3]

Dental caries has been considered as a multifactorial disease as it is not only influenced by dietary factors but also by the host factors, such as saliva. The most important caries protective functions of saliva are the flushing and neutralizing effects which are dependent on the flow rate and buffering capacity of saliva.[8] Salivary IgA helps in the antibacterial action of the saliva by neutralizing the bacterial toxins and enzymes, and preventing the adherence of the cariogenic bacteria to the tooth surface by blockage of bacterial adhesions, reduction of hydrophobicity, and agglutination of the bacteria.[10]

Dental caries is also strongly linked to the mutans streptococci, most notably *Streptococcus mutans* and *Streptococcus sobrinus*.[12,14,14,15] *Streptococcus sanguis also* one of the most predominant species of the indigenous oral biota colonizing saliva and dental plaque and is frequently present on tooth surfaces free of caries.[14,16]

The present study was conducted in the Department of Pedodontics and Preventive Dentistry, Babu Banarsi Das College of Dental Science, Lucknow, with an aim to evaluate and compare the esthetic effect of remineralizing agent [functionalized Tricalcium Phosphate Paste (*f*TCP)] on white spot lesion (WSL) and its effect on physio-chemical properties and microbial properties of saliva.

The subjects were selected from outpatient Department of Pedodontics and Preventive Dentistry, Babu Banarasi Das College of Dental Science, Lucknow. A total number of 40 subjects aged between 6-12 years of both male and females were included in the study. A total of 20 subjects in each groups, group I (control group) received regular dentifrices whereas, group II (test group) received remineralizing agents containing functionalized tri-calcium phosphate (f-TCP) and sodium fluoride with regular dentifrices as an interventional agents for 3 months period.

The study was carried out in three phases:

A: Assessment of esthetic effect on White Spot Lesion by digital imaging analysis using CIEL*a*b* values at baseline and after 3 months of interventional period.

B: Chair side assessment of physio-chemical properties of saliva, i.e., flow, pH, buffering capacity using Saliva-check buffer testing mat and S-IgA using ELISA at baseline and after 3 months of interventional period.

C: Microbial Analysis of Saliva using Real time PCR for Streptococcus mutans count and Streptococcus sanguis count at baseline and after 3 months of interventional period with the help of Image Diagnostic Lab.

Results in the present study, showed that on assessing CIE L a* b values ,there was significant ($p<0.01$) mean change in L, a and b values in Group II. The mean change in L, a and b was higher in Group II compared to Group I. delta E value between the groups after intervention was significantly higher in Group II ($P=0.0001$) which depicts the change in color of lesion.

On assessment of flow rate, pH, buffering capacity and S-IgA, there was significant ($p<0.05$) mean change in flow rate from before to after intervention in both the groups. The mean change in flow rate was higher in Group II compared to Group I. Though, there was no significant ($p>0.05$) difference in the mean change in flow rate between the groups. There was insignificant ($p>0.05$) mean change in pH and buffering capacity in both the groups. There was significant ($p<0.05$) mean change in S-lgA in both the groups. The mean change in S-lgA was higher in Group II compared to Group I. There was significant ($p=0.001$) difference in the mean change in S-lgA between the groups.

On assessment of S. mutans and S. sanguis count using real time PCR ,there was significant mean change in Streptococcus mutans($p=0.005$) and Streptococcus sanguis ($p=0.007$) in Group II. The mean change in Streptococcus mutans and Streptococcus sanguis was higher in Group II compared to Group I.

On the basis of results & observation, and within the limitations in this study , the following conclusions have been drawn:

1. The higher mean change($p=0.0001$) in the CIE L*a*b and ΔE value in Group II depicts positive effect of remineralizing agent [functionalized Tricalcium Phosphate Paste (fTCP)] on white spot lesion.

2. In group II, there was significant (p=0.001) increase in SIgA level in saliva, although salivary flow rate, pH and buffering capacity were found to be unchanged.

3. There was significant decrease in streptococcus mutans and streptococcus sanguis count in both the groups (p=0.004). However, the mean change in both streptococcus mutans and streptococcus sanguis count was higher in Group II.

Tricalcium phosphate (f-TCP) containing remineralizing paste improved the esthetic appearance of white spot lesions after 3 months of intervention. suggestive of bioactive, biocompatible and esthetic efficiency of the material. However, large observation with more sample size is recommended to confirm whether the greater change in WSL is maintained.