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IN-VITRO EVALUATION OF FLUORIDE RELEASE AND CARIES INHIBITION OF GLASS IONOMER MODIFIED WITH CHLORHEXIDINE DIACETATE

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Abstract

Background:

Dental caries still remains a major public health problem despite the widespread use of fluoride. Glass ionomer cements aredental restorative materials with an antibacterial effect. This group of restorative materialprovides anti cariogenic effect by fluoride release. Chlorhexidine hasincreased susceptibility of antibacterial andis the most suitable agent in reducing mutans streptococci. Chlorhexidine retains in oral structures for longer duration and is slowly released providing prolonged antibacterial effect than other agents.

Aims and objectives:

1. *To evaluate the effect of Glass ionomer combined with chlorhexidine diacetate on caries mechanism by using polarized microscope.*
2. *To evaluate the amount of fluoride release from Glass ionomer combined with chlorhexidine by using UVspectrophotometry method.*

Materials and Methods:

36 human incisors were selected. Teeth were divided into five groups.

The middle 2 x2 mm of the facial enamel of each tooth was isolated for the purpose of adhesion of restorative material.After bonding the specimens were kept for 4 days in acidic solution to induce artificial lesion formation. All the specimens were mountedin blocks of self cure acrylic and subjected to sectioning using a hard tissue microtome. From each specimen, 150 µm thick sections were taken and fixed on to the slides and observed under polarized light microscope for demineralization.

Fluoride release evaluation:

A total of thirty two disk shaped specimens using two different glass ionomer materials were made and divided into four groups.

The specimens were fabricated by condensing the glass ionomer cement in the metal mold. Specimens were then stored in distilled water at 37°C for 24 hours and 7 days to ensure complete fluoride release. Baseline fluoride measurement of all specimens was made using UV spectrophotometer.

Results:

- *Mean values were compared by one-way analysis of variance (ANOVA)*
- *Among the four groups of fluoride releasing restorative materials, RMGIC+CHX Diacetate showed less area of demineralization followed by conventional GIC+CHX.*
- *The amount of fluoride release was more in the RMGIC+CHX and conventional GIC+CHX.*
- *This combination showed increased fluoride release in first 24hrs which is decreased at 7 days.*

Conclusion:

The cariostatic property is more with RMGIC+CHX and the fluoride release is also more with RMGIC+CHX group over the extended period of time. Though the values are not statistically significant, the choice of restorative material could be RMGIC+CHX due to their advantageous properties like setting on demand, less moisture sensitivity etc. The added CHX can also prevent plaque and calculus formation on the tooth near restoration.

***Key Words:** Chlorhexidine diacetate, UV spectrophotometer, Polarized microscope.*

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INTRODUCTION

Caries disease still remains a major public health problem despite the widespread use of fluoride and the decline in caries prevalence is observed in the majority of highly industrialized countries[1].

The streptococcus mutans is proved primary organism initiatingthedental caries.Various plaque control techniques such as professional tooth cleaning followed by fluoride applications, dental flossing, supervised tooth brushing with fluoride toothpaste or self-administered oral hygiene programs, in different combinationswere developed to eliminate these organisms[2].

The therapeutic procedures do not guarantee the complete removal of microorganisms in the residual tissues, which might lead to residual caries leading to secondary caries. This could be addressed by use of dental materials with bacteriostatic properties.

Due to the high frequency of recurrent caries after restorative treatment, much attention has been paid to the therapeutic effects revealed by direct filling materials.

Remineralization by the release of fluoride is a representative, but the antibacterial effect is another important property because inactivation of bacteria means a direct strategy to eradicate the cause of dental caries [3].

Glass ionomer cements aredental restorative materials with an antibacterial effect by therelease high amounts of fluorides but do not perform as a good restorative material because of their high solubility, poor retention, inadequate physical and esthetic properties.

Chlorhexidine (CHX) hasincreased susceptibilityof antibacterial andis the most suitable agent in reducing mutans streptococci.Chlorhexidine retains in oral structures for longer duration and is slowly released providing prolonged antibacterial effect than other agents.

Recently, researchers have modified restorative materials such as composite resins, acrylic resins, and GIC by incorporation of chlorhexidine andin vitro studies have shown an increased antibacterial effect.

Chlorhexidine has been shown to be the most suitable agent in reducing mutans streptococci due to its increased susceptibility towards this specific group of organisms compared to other groups of microorganisms.

Most of the antibacterial property testing will be done by agar diffusion test etc. Where cariostatic effect is exhibitedinstead of preventing cariogenicity.

So, the present study was designed to evaluate the fluoride release characteristics of glass ionomers and chlorhexidine modified glass ionomers and their ability to reduce enamel demineralization, which is the ultimate clinical requirement.

Null hypothesis tested was that no difference could be detected in degree of demineralization and amount of fluoride release by the Glass ionomer cement modified with chlorhexidine diacetate.

AIM:

Aim of the study was to evaluate the fluoride release and to evaluate the effect of Glass ionomer combined with chlorhexidine diacetate on caries mechanism.

OBJECTIVES:

1. By UVspectrophotometry method the amount of fluoride release, was assessed.
2. The caries inhibition of Glass ionomer mixed with chlorhexidine diacetate on demineralized tooth surface was assessed using polarized light microscope

MATERIALS & METHODS

The study involved two parts:

Part I: Quantitative measurement of the areas of demineralization and lesion depth adjacent to the restorative material.

Part II: Evaluation of the fluoride release from the restorative materials.

Specimen Preparation: To measure the areas of demineralization and lesion depth

A total of 36 human incisors free of fracture, caries, calculus stored in saline were chosen for the study and were randomly divided into five groups. The teeth were sectioned horizontally at the CEJ using a diamond saw so that the crowns of the teeth could be obtained for the study.

CHX diacetate (SIGMA, India) which is commercially available as solid substance was added to GC Fuji II (GC Corporation, Tokyo, Japan) and GC Fuji IILC (GC Corporation, Tokyo, Japan) in order to obtain 2.5% concentrations of CHX in the GIC formulation.

To obtain 2.5% diacetate formulations, 0.44 gm CHX diacetate was mixed with each 15 gm of GC Fuji II and GC Fuji IILC respectively. GIC-CHX mixture and GIC liquid was manipulated according to the manufacturer’s instructions at room temperature on a mixing pad with a plastic spatula.

The middle 2 x2 mm of the facial enamel of each tooth was isolated for the purpose of adhesion of restorative material used in the study. The following test materials were used in the study which was grouped as follows:

Group I: - Positive control group (Intact Teeth)

Group II: - Conventional Glass ionomer Cement

Group III: - Conventional Glass Ionomer Mixed with Chlorhexidine Diacetate

Group IV: - Resin modified Glass Ionomer Cement

Group V: - Resin Modified Glass Ionomer Mixed with Chlorhexidine Diacetate

Transparent nail varnish was applied at a distance of 1 mm from the test materials and also was applied onto the top surface of restored materials so that the material should not come in contact with the artificial caries formation solution, leaving the edges exposed.

Each of these specimens were then suspended in 500 ml of unstirred acidic buffer solution which consisted of 50 mM acetic acid, 1.5 mm calcium nitrate tetrahydrate and 0.9 mM potassium dihydrogen orthophosphate buffered to pH of 4.7 by using pH meter and by adding 0.1 M sodium hydroxide.

The specimens were kept for 4 days to induce artificial lesion formation. Further were subjected to sectioning using a hard tissue microtome.

From each specimen, a 150 µm thick section were taken by sectioning parallel to the longitudinal axis of the tooth.

The sections were fixed on to the slides and observed under polarized light microscope, projected at a magnification of 200×. Areas and depth of demineralization adjacent to the test material were measured.

SPECIMEN PREPARATION: FOR EVALUATION OF FLUORIDE RELEASE

A total of thirty two disk shaped specimens using two different glass ionomer materials measuring 10 mm in diameter and 2 mm in thickness were prepared using a steel mold. Samples were divided into four groups.

Group I - Conventional glassionomer

Group II - Conventional glass ionomer mixed with 2.5% chlorhexidine diacetate

Group III- Resin modified glass ionomer

Group IV-Resin modified glass ionomer mixed with 2.5% chlorhexidine diacetate

The specimens were fabricated by condensing the glass ionomer cement in the metal mold having a circular shaped hole (10 x 2 mm).

Specimens were then stored in distilled water at 37°C for 24 hours and 7 days to ensure complete fluoride release. Baseline fluoride measurement of all specimens was made using UV spectrophotometer.

RESULTS

TABLE 1: Mean comparison for demineralization (µm)

GROUPS	MEAN	SD	MIN	MAX
Group I	453.23	143.62	348.90	663.10
Group II	178.88	54.41	91.03	244.30
Group III	135.69	104.06	76.76	379.00
Group IV	195.02	60.49	114.10	293.10
Group V	122.17	87.04	30.37	246.50

GRAPH 1: Mean comparison for demineralization(µm)

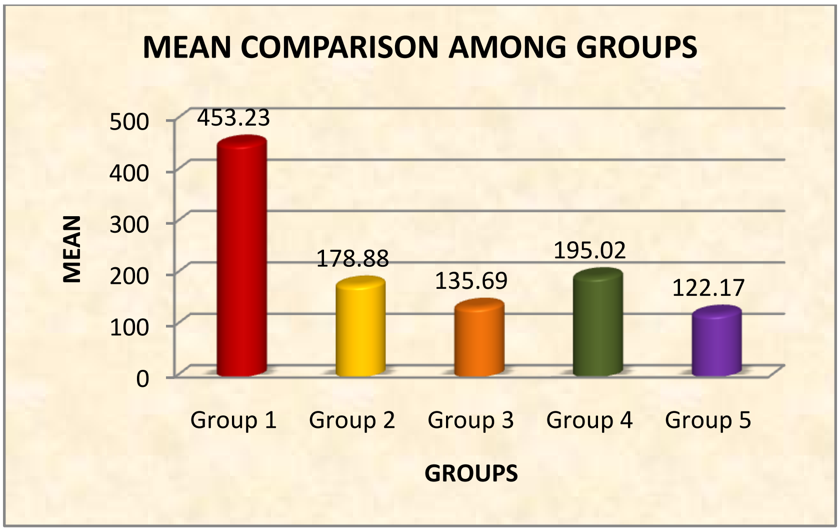
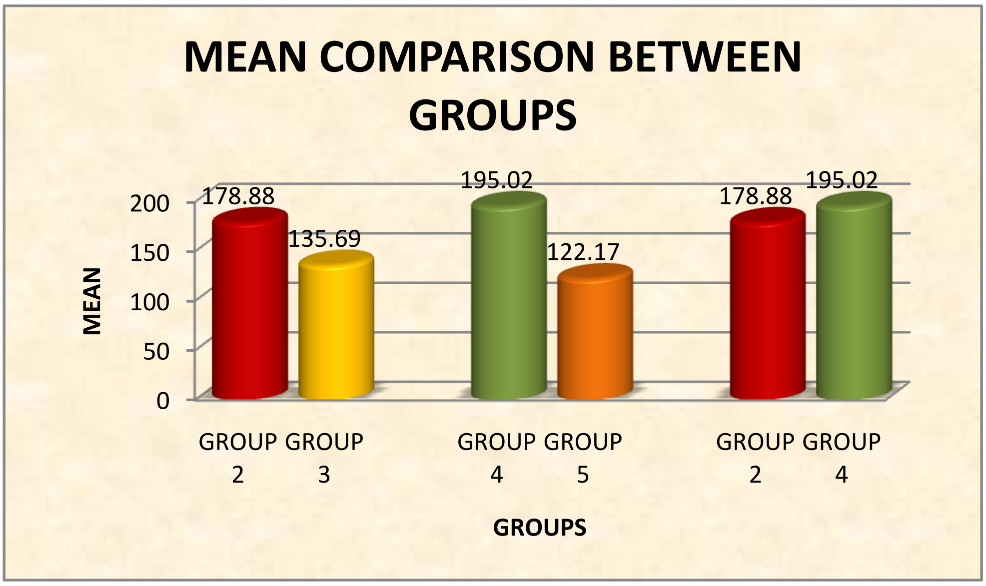


TABLE 2: Mean comparison of demineralization between groups

COMPARISON BETWEEN	MEAN	SD	DIFFERENCE	P VALUE
GROUP II	178.88	54.41	43.19±49.65	0.316 NS
GROUP III	135.69	104.06		
GROUP IV	195.02	60.49	72.85±26.55	0.072 NS
GROUP V	122.17	87.04		
GROUP II	178.88	54.41	16.14±6.08	0.584 NS
GROUP IV	195.02	60.49		

GRAPH 2: Mean comparison of demineralisation between groups



Conventional glass ionomer showed 178.88µm of demineralisation, where as conventional glass ionomer mixed with chlorhexidine diacetate showed decreased demineralisation i.e., 135.69µm

Resin modified glass ionomer showed 195.02µm of demineralisation, where as resin modified glass ionomer mixed with chlorhexidine diacetate showed decreased demineralisation i.e., 122.17µm.

Among the four groups of fluoride releasing restorative materials, RMGIC+CHX Diacetate shows less area of demineralisation

TABLE 3 Shows fluoride release values in µg/ml

Groups	Day 1(fluoride release in µg/ml)	Day 7(fluoride release in µg/ml)
GIC	13.2328	3.0992
GIC+CHX	13.2328	6.9732
RMGIC	15.1984	5.1030
RMGIC+CHX	13.8626	7.2404

On day 1 conventional glass ionomer showed 13.2328µg/ml of fluoride release where as conventional glass ionomer mixed with chlorhexidine diacetate showed 13.2328µg/ml.

RMGIC showed 15.1984µg/ml of fluoride release where as RMGIC+CHX showed 13.8626µg/ml.

On day 7 GIC showed 3.0992µg/ml of fluoride release where as GIC+CHX showed 6.9732µg/ml .

RMGIC showed 5.1030µg/ml of fluoride release where as RMGIC+CHX showed 7.2404µg/ml.

DISCUSSION

Enamel demineralization is the earliest step in caries formation and prevention or reduction, and enamel remineralization is the key to long-term caries control [4].

The ability of dental materials to inhibit recurrent caries formation is an important clinical property. GICs have been used for more than 30 years, and it is well known that their major advantage is their potential to inhibit caries because of fluoride release and their clinical adhesion to dental hard tissues.

The methods that have been employed to estimate the amount of fluoride release include spectrophotometry, ion chromatography, capillary electrophoresis and fluoride ion selective electrodes with an ionanalyzer[13.]

Dental literature reveals that chlorhexidine has been incorporated into GIC and those invitro studies have shown an increased antibacterial effect, which was done by agar diffusion inhibitory test [2 5 10]. However in the clinical situation this property can be proved if the material can inhibit the demineralization of tooth in caries process.

Among the four groups of fluoride releasing restorative materials, RMGIC+CHX Diacetate shows less area of demineralisation.

RMGIC+CHX<GIC+CHX<GIC<RMGIC.

The amount of demineralization was less in the RMGIC+CHX and Conventional GIC+CHX because inclusion of antibacterial compound chlorhexidine.

Chlorhexidine is a bis-biguanide& is strongly basic, containing 2 positive charge surfaces, which is therefore described as dicationic and having affinity for negatively charged bacterial cell walls, extracellular polysaccharides of bacterial origin [38].

It eliminates the recurrence of decay around the margins of restoration, inhibit the plaque formation on and near the restored surface and reduce the number of microorganisms in salivary fluids & oral cavity.

According to a study wherein antibacterial activity for Streptococcus mutans, Streptococcus sobrinus, Lactobacilluscasei using agar diffusion method found that GIC mixed with chlorhexidine showed better antibacterial property [2].

Conventional GIC showed significantly lesser amount of demineralisation when compare to that of RMGIC because this material is classified as a water based material that hardens following an acid base reaction between flour alumina silicate glass powder and an aqueous solution of poly acid.

The increased level of fluoride in the conventional GIC is due to the erosive leaching of glass particles in the bulk of cement and diffusion of the leached fluoride through the porous cement matrix [4].

This fluoride gets incorporated within the adjacent tooth structure, forming flour apatite or hydroxyl flour apatite. However, HEMA present in resin modified glass ionomers slowly absorbs water to allow for the diffusion of fluoride ions [33].

Since, there have been no studies evaluating the cariostatic effect of chlorhexidine modified with GIC, results could not be compared.

FLUORIDE RELEASE

Fluorides have been incorporated into restorative materials for their unique property of formation of fluorapatite crystals and thus making the enamel more resistant to acid breakdown and demineralization[4].As early as 1977, it was suggested that GICs could offer particular advantages as restorative materials in the primary dentition because of their ability to release fluoride and to adhere to dental hard tissues.

The initial fluoride release from the glass ionomer is due to an acid base reaction, with the amount of fluoride release proportional to the concentration of fluoride in the material. This is responsible for the phenomenon of "burst effect", wherein high amount of fluoride are released during the first two days [27].

Any advances in material sciences of Glass ionomer should not compromise on the property of fluoride release. Hence, fluoride release was also assessed in the test group by incorporating Chlorhexidine.

In the present study on day 1 fluoride release was same in both conventional GIC and GIC+CHX groups. Whereas on day 7 fluoride release was less in conventional GIC groupwhen compare to theGIC+CHX group. This is in accordance with the study. [15] This combination showed increased release of fluoride in first 24hrs which is decreased at 7 days [15].

The test and control groups of resin modified GIC showed increased fluoride release in the first 24 hours and decreased fluoride release at 7 days. The lesser fluoride release from the RMGIC can be attributed to its setting reaction. The setting reaction of RMGIC is "dual setting", in which both polymerization and acid base reaction take place[4].

The fluoride release was found to be decreasing from day 1 to day 7.

Null hypothesis was rejected as there is difference in degree of demineralization and amount of fluoride release by the Glass ionomer cement modified with chlorhexidine diacetate.

The cariostatic efficiency of chlorhexidine modified glass ionomer is evident in this study. This is also supported by increased fluoride levels by chlorhexidine modified GIC. Hence the addition of CHX improves the anticariogenic activity along with fluoride release levels can give rise to a advantageous clinical property.

CONCLUSION

1. The amount of demineralisation was less in the RMGIC+CHX and conventional GIC+CHX.
2. Among the control groups conventional GIC shows less area of demineralisation compare to the RMGIC.
3. The amount of fluoride release was more in GIC mixed with chlorhexidine both in conventional and RMGIC.
4. Resin modified glass ionomers were mostly found to have a potential for releasing fluoride in equivalent amount as conventional glass ionomers cements.

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