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RESEARCH ARTICLE

COMPARATIVE EVALUATION OF COMPRESSIVE STRENGTH OF VARIOUS GLASS IONOMER CEMENTS MODIFIED WITH *CHITOSAN*: AN IN VITRO STUDY.

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Abstract

Recent studies have reported the incorporation of modifiers with conventional Glass Ionomer Cement (GIC) to enhance its physical properties. One such modification being the addition of Chitosan (CH). The present study was done to investigate the effect of modifying the liquid phase of two types of conventional GIC with 10% and 50% v/v CH in comparison to conventional GIC. The liquid of commercially available restorative GIC, GC Fuji IX and ketac molar was modified with 10% and 50% v/v CH solution (Everest Biotech, Bengaluru). GIC powders were mixed with the unmodified and the CH-modified liquids at the desired powder/liquid ratio. Universal testing machine was used to determine the difference in compressive strength between the conventional and CH modified GIC liquid with GIC powder. Shiprowilk test, one way anova test and post-hoc bonferroni was used to compare the compressive strength between CH modified GIC and unmodified GIC. Results showed that the modification with 10% v/v CH solution improved the physical property of both GICs but found insignificant in case of modification with 50% v/v CH solution. Compressive strength strength of CH modified GIC compared to conventional GIC was found to be statistically significant. Modifying the liquid phase of a conventional GIC with 10% v/v CH significantly improves the physical property of GIC.

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Introduction:-

Dental caries is an age-old disease which has been the bane of affliction in the oral cavity. It is commonly seen affecting the pediatric population due to high ingestion of refined carbohydrates and lack of awareness. In order to preserve primary teeth in the arch until its normal exfoliation, restoring such carious lesion at an early age is of utmost importance in order to preserve the primary teeth until its normal anticipated exfoliation. This helps to assist in the maintenance of a healthy oral environment and arch length as well as to preserve the function of mastication and speech.¹

However, even a simple restorative treatment plan is likely to evoke anxiety in a young patient and may prove to be a challenge to the clinician. Hence when choice of the restorative material is made, simplicity of clinical application of the material should be considered along with other properties of the restorative material. The interest in the

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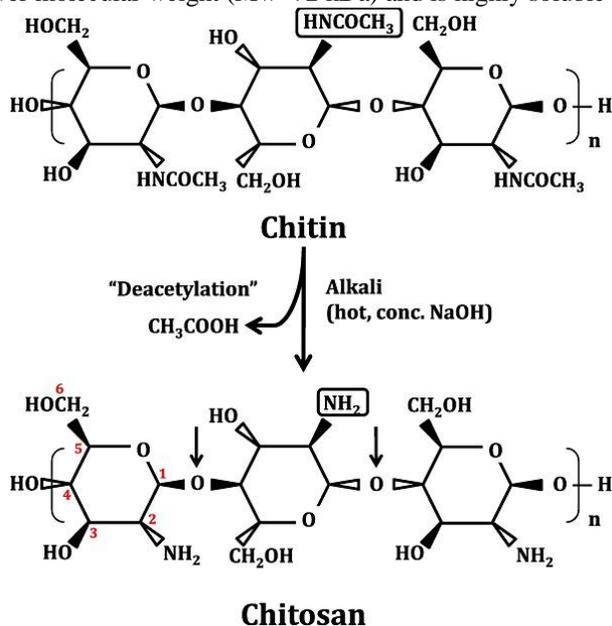
clinical use of glass ionomer cements (GIC) arose mainly from their particular advantage of requirement of a short time to fill the cavity which is a desirable property while treating children and even adults.²

Glass ionomer cement (GIC) for dental restorative applications are formed by an acid-base reaction between calcium fluoro-alumino-silicate glass and polyacrylic acid. Since their introduction in 1972 by Wilson and Kent, to the dental field, they have been widely used as restorative materials, sealant, luting cement, and cavity base materials. Glass ionomers have certain exceptional properties such as chemical adhesion to dental hard tissues, anticariogenic/antibacterial properties from the release of fluoride, good thermal compatibility with tooth structure, and acceptable biocompatibility. However, GICs have some disadvantages or limitations including early moisture sensitivity, brittleness, and inferior mechanical strength when compared to resin-based restorative materials.³ Condensable or high-viscosity glass ionomer cements, developed early in the 1990s, as filling materials in the atraumatic restorative therapy technique, were desirable due to their advantageous properties like faster setting, adequate strength and polishability in a single visit. However, the risk of fracture exists for large restorations. High-viscosity glass ionomers are still inferior to resin-based restorative materials when it comes to fracture toughness. Hence, there has been a constant quest for further improvement in the properties of the material while retaining its multitude of clinical advantages.⁴

Thus, there was a need for an alternative biocompatible additive material which has a potential for enhancing the physical properties of GIC, however, addition of any agent into a material to improve the properties, should not jeopardize any other desirable property of the parent material.

A few examples of natural biomaterials include collagen, fibrin, natural silk, and Chitosan. Chitosan is a natural biomaterial that is purified mainly from Chitin. Chitin is a natural polysaccharide from crustacean shells, insect cuticles, and on fungal cell walls is the second most abundant polymerized carbon found in nature. The chitosan (Figure 1) is obtained from the alkaline deacetylation of chitin. This copolymer obtained is biodegradable, consisting of D-glucosamine units containing a free amino group.⁵

During the process of deacetylation (Figure 1), the water-insoluble chitin ($M_w > 1000$ kDa) changes to chitosan ($M_w > 100$ kDa) that is poorly soluble in water. Further enzymatic hydrolyzation transforms chitosan to chitosan oligosaccharide that has a lower molecular weight ($M_w < 2$ kDa) and is highly soluble in water.



Chitosan and its derivatives have excellent biocompatibility, non-toxicity to human beings, biodegradability, reactivity of the deacetylated amino groups, selective permeability, polyelectrolyte action, antimicrobial activity, ability to form gel, film and sponge, absorptive capacity, anti-inflammatory and wound healing. One of the most important properties of chitosan is high bioactivity, that makes this material very interesting to develop new biomaterials for application in dental restorations.^{5,6}

Not much of the studies has been carried out to check the improved mechanical properties of chitosan enhanced GIC. Thus, the aim of this study is to check and compare the compressive strength of commonly used GIC's enhanced with chitosan in various concentration and thus, to investigate the potential of Chitosan, based on promising properties and reliable biological functionalities emphasizing the dental restoration procedure. Look for new methods and applications due to their excellent biocompatibility.

Materials And Methods:-

The present study was carried out at Daswani dental collage and research centre in Department of Pedodontics and Preventive Dentistry between the academic years 2016 to 2019. The materials used in study were, GC Fuji Type IX gold label (GC corporation, Tokyo, Japan) glass ionomer and KetacMolar (3M ESPE, Deutschland GmbH, Germany) was selected in the study since these glass ionomer cements are most commonly used restorative material for posterior teeth in because of its various advantageous properties like fluoride release, chemical adhesion and type IX is high strength GIC most commonly used for ART technique.

The experimental cement was formulated from the same batch; by incorporation of 10% v/v, 25% v/v, 50% v/v Chitosan into liquid component of glass ionomer cement, after dissolving it in 1% acetic acid.

Modification of Glass Ionomer with Chitosan:

Almost 1.8 ml of acetic acid was made up with 100 ml of distilled water to obtain 0.3 N acetic acid. 20 mg of CH (Everest Biotech, bangalore) was dissolved in 0.3 N acetic acid and made up to 100 ml to get 0.2 mg / ml Chitosan solution.⁷ Then, 0.1 ml of this 0.2 mg/ml of CH solution was added to 0.9 ml of both GIC liquid to get 10% v/v CH modified glass ionomer solution, and 0.5 ml of 0.2 mg/ml of CH solution was added to 0.9 ml of both GIC liquid to get 50% v/v CH modified glass ionomer solution.

1. Group I- GC Type IX GIC
2. Group II- KetacMolar GIC
3. Group III- GC Type IX GIC modified with 10% Chitosan
4. Group IV- KetacMolar GIC modified with 10% Chitosan
5. Group V- GC Type IX GIC modified with 50% Chitosan
6. Group VI- KetacMolar GIC modified with 50% Chitosan

Here, Group I and Group II are conventional forms of both GIC's acting as a control group. Group III and Group IV are has 10% of modification by adding Chitosan to both conventional GIC cements while Group V and Group VI has 50% of modification by adding chitosan to both conventional GIC cements.

30 specimens were prepared using both GIC and CH-GIC. Five specimens were prepared for each group, to evaluate the compressive strength. 30 non-carious extracted mandibular molars were taken and placed in hydrogen peroxide for 24 hours. An extensive cavity was then prepared using clinical airtor (KavoKerr, Danaher corporation, United States) with Round and Inverted cone burs (Mani, Mani inc, japan). The cement was then mixed according to the manufacturer's instruction, placed and condensed tightly into the cavities with the help of cement condenser, allowed to set for 10 minutes at room temperature. A thin layer of Petroleum jelly was applied to all the fillings as to avoid excessive dryness. These tooth samples were then embedded on an acrylic base that will give the tooth proper vertical height required for testing and stability. Acrylic powder and liquid was mixed according to manufacturers instructions and poured into a round cylindrical Teflon mould of 25mm diameter and 35mm height. After the setting, the base were removed from the moulds and excess was trimmed using acrylic trimming bur, the specimens were stored at temperature of 37⁰ for 24 hours.

Assessment of Compressive strength

Conventional glass ionomer cement (GC Fuji IX gold label and KetacMolar) served as control (Group I & II) and rest as experimental group (Group III, IV, V & VI). The compressive strength of each specimen was measured by using a Universal testing machine (AG-50kNG) with a tip size of 1mm. A compressive load along long axis was applied using cross head speed of 0.5mm/min. The maximum force required to fracture the specimen was recorded in kg/cm².

Statistical Analysis

The results were tabulated and statistically analysed with intragroup comparisons. **Normality test** and **Shapiro-Wilk test** was first done to find normal distribution. **One-way ANOVA test** was done to evaluate and compare mean

force value of each group. The inter-group comparison of mean force value was done using the **post-hoc bonferroni test**.

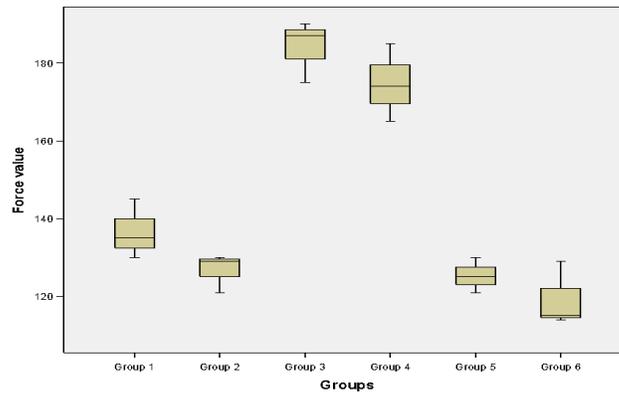
Results:-

Normality test was done to see the distribution of data followed the normal distribution or not. The Shapiro-Wilk test showed that all groups followed the normal distribution. So, parametric tests were used.

Groups	Shapiro-Wilk		
	Statistic	df	p-value
Group 1	0.964	5	0.637
Group 2	0.832	5	0.194
Group 3	0.893	5	0.363
Group 4	0.997	5	0.890
Group 5	0.996	5	0.878
Group 6	0.800	5	0.114

Table 1:-showing normal distribution of data through Shapiro-Wilk test.

Graph 1:-graphical representation of distribution of data.



One-way ANOVA test

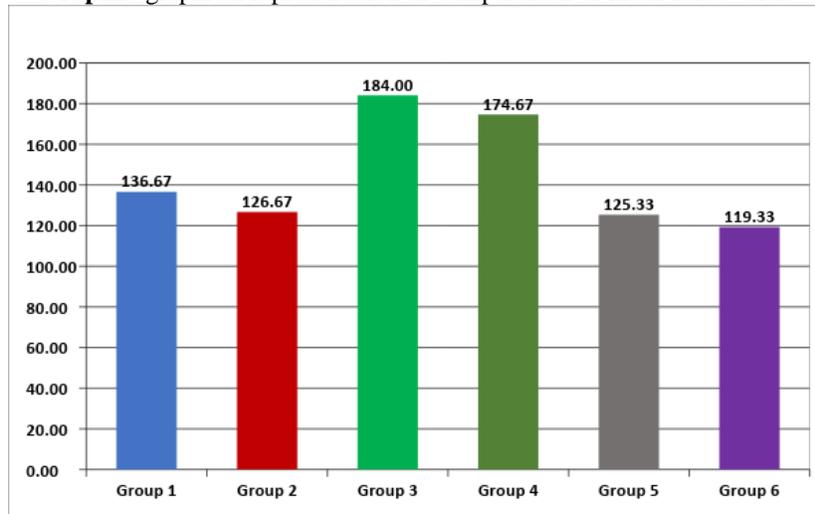
Significant difference

The comparison of mean force value was done between groups 1, 2, 3, 4, 5 and 6 **One-way ANOVA test**. There was a significant difference in mean force value between groups 1, 2, 3, 4, 5 and 6.

	Mean	Std. Deviation	F-value	p-value
Group 1	136.67	7.64	41.172	<0.001*
Group 2	126.67	4.93		
Group 3	184.00	7.94		
Group 4	174.67	10.02		
Group 5	125.33	4.51		
Group 6	119.33	8.39		

Table2:-The comparison of mean force value was done between groups 1, 2, 3, 4, 5 and 6 **One-way ANOVA test**. There was a significant difference in mean force value between groups 1, 2, 3, 4, 5 and 6.

Graph2:-graphical representation of comparision of mean force value

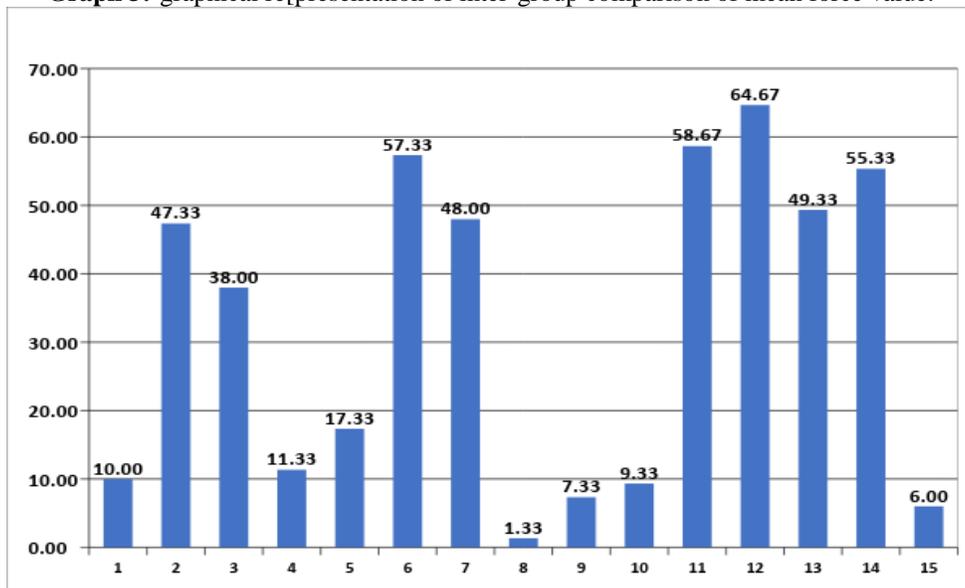


Post-hoc bonferroni test * Significant difference

The inter-group comparison of mean force value was done using the **post-hoc bonferroni test**. The mean force value was significantly more among group 3 and 4 than groups 1, 2, 3 and 5.

		Mean Difference	p-value
Group 1	Group 2	10.00	1.000
Group 1	Group 3	-47.33	<0.001*
Group 1	Group 4	-38.00	0.001*
Group 1	Group 5	11.33	1.000
Group 1	Group 6	17.33	0.226
Group 2	Group 3	-57.33	<0.001*
Group 2	Group 4	-48.00	<0.001*
Group 2	Group 5	1.33	1.000
Group 2	Group 6	7.33	1.000
Group 3	Group 4	9.33	1.000
Group 3	Group 5	58.67	<0.001*
Group 3	Group 6	64.67	<0.001*
Group 4	Group 5	49.33	<0.001*
Group 4	Group 6	55.33	<0.001*
Group 5	Group 6	6.00	1.000

Table 3:-The inter-group comparison of mean force value was done using the **post-hoc bonferroni test**. The mean force value was significantly more among group 3 and 4 than groups 1, 2, 3 and 5.

Graph 3:-graphical representation of inter-group comparison of mean force value.**Discussion:-**

Dental caries is ubiquitous and is one of the most prevalent infectious diseases of man. Glass Ionomer Cement is used in dentistry for restorative and preventive applications because of its unique properties. They have received intensive investigation as restorative materials for deciduous teeth.⁸ These applications motivated various modifications in conventional GICs so as to enhance the antibacterial and/or physical properties without adversely affecting the chemical adhesion to enamel and dentin.³

Rapid development of civilization and technology involve a number of advantages and also many consequences. More often we are turn to the nature in search for materials and solutions that will be friendly to us and our environment. Chitin and chitosan are one of those products.

The discovery of chitosan was in 1859 by Rouget when the chitin was subjected to a treatment with hot potassium hydroxide solution. In the period of 1894, Gilson confirmed the presence of glucosamine in chitin and in the same period was named chitosan by Hopper-Seyler . Since then several researches with interests in the applications of chitin and aiming to broaden the knowledge about the structural relations and properties of this polysaccharide and its derivatives.⁹

Chitosan ,derived from Chitin, is a natural polysaccharide from crustacean shells, insect cuticles, and on fungal cell walls is the second most abundant polymerized carbon found in nature. The chitosan is obtained from the alkaline deacetylation of chitin. This copolymer obtained is biodegradable, consisting of D-glucosamine units containing a free amino group. Chitosan can be used in a large number of industrial applications, among which the following stand out: biocompatibility, biodegradability, antibactericidal, emulsifying and chelating properties. Due to these biological characteristics, several applications have been found for this biomaterial, among them: in agriculture, in the food industry and, recently, in the medical and dental field.^{6,10}

Unlike chitin, chitosan is soluble in dilute acid medium forming a cationic polymer that confers special properties differentiated with respect to the vegetal fibers . Chitosan is soluble in dilute acids, such as acetic acid, formic acid, lactic acid, as well as inorganic acids, after prolonged agitation. However, the solubility is dependent on several parameters, such as the degree of deacetylation, molar mass, concentration of acid and biopolymer and ionic strength.^{6,11}

Chitosan in the present study was used with acetic acid to modify the GIC liquid and the pH values of the modified liquids were kept in the acidic range. CH solubility in the acidic environment is explicated by the protonation of the free amino groups (NH₂) to NH₃⁺. This is because CH can be considered a strong base as it possesses primary

amino groups with a pKa value of 6.3. The presence of the amino groups indicates that pH substantially alters the charged state and properties of chitosan. At low pH, these amines get protonated and become positively charged and that makes chitosan a water-soluble cationic polyelectrolyte.⁷

Bonifacio et al. carried out a study comparing the mechanical properties of different types of glass ionomer cement and the data suggested that Fuji IX (FIX), and Ketac Molar Easymix (KME) presented the best in vitro performance in terms of flexural strength, Knoop hardness and compressive strength.¹²

The aim of the study was to investigate the effect of modification of GIC liquid to Chitosan on the compressive strength of the commonly used GIC's in various concentration (10%,50%). It is important that any modification of GIC should not affect both mechanical and chemical properties of parent material. Previous studies has shown no declined status in any of the mechanical and chemical properties of Chitosan modified GIC and Conventional GIC'S.¹³

Study conducted by Petri D et al.(2007) determined the setting reaction between the CH modified and conventional GIC. The study revealed that the reaction had taken place between amino (-NH₂) group of CH and the functional group (OH group and C=O group) of GIC. The final matrix showed no additional peaks or downs representing that the CH was thoroughly mixed in the GIC matrix. Since CH possess hydroxyl and acetamide groups, they bind to a hydroxyl group of powder particles and carboxylic groups of polyacrylic acid by hydrogen bonding. This interaction reduces interfacial tension among GIC components, thereby improving mechanical performance.¹⁴

Ibrahim et al. 2015 investigated the effect of modifying the liquid phase of a conventional glass ionomer restorative material with different chitosan volume contents on the antibacterial properties and adhesion to dentin. The liquid of GIC was modified with chitosan (CH) solutions at different volume contents (5%, 10%, 25%, and 50%). Modification with 25% and 50% CH adversely affected the micro-tensile bond strength. However, no significant difference was found between the control and the 5% and 10% CH-modified groups.¹³

On a contrary,Chitosan chains carry many hydroxyl groups and acetamide groups which are able to bind to hydroxyl groups of the GIC particles and to polyacrylic acid (PAA) carboxyl groups by hydrogen bonding. The network formed by CH and PAA around the inorganic GIC particles might reduce the interfacial tension among the GIC components, improving the mechanical performance. However, with the increase in chitosan content, the mechanical performance was adversely affected due to segregation of CH chains which interact with each other, and no longer with PAA and/or the GIC particles surfaces resulting in poor mechanical performance.¹³ Likewise in our present study, this adverse effect of high CH content on the reinforcement of GIC could be the reason of the failure observed with 50% CH-modified GIC specimens.

Limitation Of The Study

The present study considered only 10% and 50% concentration of Chitosan mixed with GIC,effect of different concentration can be considered.

The study included 2 types of GIC's , more brands of GIC's can be included for their effect on Chitosan.

Only Compressive strength was included in study since the influence of this cement on other mechanical properties can be studied.

Conclusion:-

Based on the result of the current study and other related studies, modification of GIC with CH could be potential clinical significance in preventive dentistry owing to its proven mechanical reinforcements effects. Therefore , CH being biocompatible ,a low cost additive could easily be incorporated into traditional GIC's and the modified cement is being recommended as a restorative material of choice.

Within the limitations of the present *in vitro* study design, real-time longevity of GICs for restorative procedures could not be certainly determined because of certain factors such as saliva, pH changes, food, liquids, and masticatory functions in the oral environment. Since the oral cavity being a dynamic field where initiation and progression of dental caries becomes a long and continuous process with variations in microbial load in response to changes in oral environment. Thus, more clinical and longitudinal studies employing the use of CH incorporated in GIC should be undertaken to establish its use for restorative procedures in the field of preventive dentistry in coming years.

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