

Comparative Evaluation of Microhardness and Solubility of Different Combinations of Antibiotic Powders Added to Glass Ionomer Cement: An *In Vitro* Study

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ABSTRACT

Introduction: The imbalance between remineralization and demineralization leads to the formation of secondary caries. Fluoride-releasing ability has been the characteristic property of glass ionomer cement (GIC), but it is uncertain if this property alone will be sufficient for the cessation of the growth of the organisms. Therefore, a restorative material with additional bacteriostatic properties needs to be introduced.

Aim: To evaluate the microhardness and solubility of the conventional GIC after adding different combinations of antibiotic powders.

Materials and methods: In this study, the three groups were conventional GIC (group I), GIC + metronidazole + ciprofloxacin (group II), and GIC + metronidazole + amoxicillin/clavulanic acid (group III). The concentration of the double antibiotic combination was maintained at 1.5% w/w. The antibiotic powders were added to the GIC and evaluated for microhardness and solubility of the specimens.

Results: The mean microhardness level of group II was the highest, which was statistically significant using analysis of variance (ANOVA) with a *p*-value of 0.022. The comparison of each group's solubility in different solutions was not statistically significant.

Conclusion: Along with the conventional properties of GIC, an additional therapeutic gain can also be obtained by incorporating various combinations of antibiotics, thereby arresting the progression of caries at the site of infection itself.

Clinical significance: This new approach shall help in community health programs, where treatment of a large population needs to be done within a short span of time and arrest the progression of caries activity in deep caries.

Keywords: Atraumatic restorative treatment, Double antibiotic, Glass ionomer cement, Microhardness, Solubility.

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INTRODUCTION

Dental caries in children, when left untreated, leads to aching, infection, and interference with eating. Eventually, it badly affects the eruption of the successor teeth. Healthy primary teeth during childhood have an impact on the eruption of healthy successor teeth, healthy nutrition of the individual, and esthetic appearance.¹ Atraumatic restorative treatment (ART) has been proven to have a high rate of success in primary dentition.

The various factors that led to glass ionomer cement (GIC) being a suitable restorative material for ART are its fluoride-releasing action, bonding to both enamel and dentin and biocompatibility with pulp.² Incorporation of antibiotics into GIC was recommended for dealing with carious lesions and aimed to decrease the total number of viable bacteria while preserving dentin and the vitality of the pulp tissue.³ Medicinal benefits can be obtained by incorporation of the antibiotics into GIC.

The conventional double antibiotic combination consists of metronidazole and ciprofloxacin. Amoxicillin has effective antibacterial activity against *Streptococcus*. However, in addition to clavulanic acid, it offers increased antibacterial coverage of microorganisms.

It has been shown that any alteration to the existing combination of dental cement will lead to a reduced survival rate of the restoration and pave the way for secondary caries. Evaluation of microhardness helps prevent fracture of the restoration. Increased solubility of the restoration leads to restoration failure. Therefore, this study aimed to determine the microhardness and solubility of

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conventional GIC after adding different combinations of antibiotic powders.

MATERIALS AND METHODS

The current *in vitro* comparative experimental study was conducted in the Department of Pediatric and Preventive Dentistry in collaboration with the Central Research Cell, Maharishi Markandeshwar College of Dental Sciences and Research, Maharishi Markandeshwar (Deemed to be University), Ambala,

Haryana, India, and the Department of Oral and Maxillofacial Pathology, Maharishi Markandeshwar College of Dental Sciences and Research, Maharishi Markandeshwar (Deemed to be University), Ambala, Haryana, India, following ethical clearance. A simple randomization technique was employed in this study. The minimum requirement was six samples per group, with six control samples. The sample size was estimated according to this formula, as given in Figure 1.

$$n \geq \frac{(Z_{1-\alpha/2} + Z_{1-\beta})^2 \left(\sigma_1^2 + \frac{\sigma_2^2}{r} \right)}{(\mu_1 - \mu_2)^2}$$

Preparation of Specimen

Metal band molds of 10 mm in diameter and 6 mm in thickness were made with the help of a spot welder and were used for disk preparation. The disks were divided into group I containing GIC (3M Ketac Molar), group II containing GIC + metronidazole + ciprofloxacin, and group III containing GIC + metronidazole + amoxicillin/clavulanic acid. Group I served as the control group, and groups II and III served as the experimental groups. The powder and liquid were mixed according to the manufacturer’s guidelines for all groups. To obtain the powdered form of antibiotics, the enteric coating of the tablets was scraped off using a sharp blade and crushed with a sterilized mortar and pestle. The pulverized form of each antibiotic was individually weighed using a digital precision analytical balance, and the addition of antibiotic powders to GIC was at 1.5%. The disks were allowed to set for 30 minutes before retrieval from the molds. Until further use, disks were immersed in deionized water.

For Microhardness Evaluation

A total of 18 disks were mounted within acrylic, where the acrylic was poured into a rectangular stainless steel split mold measuring 5 mm in height, 2 mm in length, and 1 mm in breadth.

The indenter of 200 gf load was applied for a dwell time of 10 seconds, and Vickers hardness value was recorded. From each sample, the mean value was derived from 3 readings, and the mean Vickers hardness value for each group was recorded.

For Solubility Evaluation

A total of 18 samples were divided according to various solutions—deionized water, acid artificial saliva, and neutral artificial saliva. With the help of a pH meter, the artificial saliva was adjusted to acidic (pH 5.5) and neutral (pH 7) by adding hydrochloric acid (6 M) or sodium hydroxide (5 N) dropwise, respectively. The weight of these samples was recorded on a precision analytical balance. Subsequently, they were placed in a heater at 37°C and 100% humidity to reach a constant mass (m), with a maximum weight variation of +0.005 gm.

The samples were immersed in different solutions and weighed after 24 hours, 7, 14, and 21 days post-immersion (m2). Each sample received 5 mL of the solution, which was changed daily at the same time. Throughout the experiment, the solutions containing the samples were maintained in a heater at 37°C (Fig. 2). After 21 days, the samples were removed from the solutions and placed in a desiccator with dehydrated silica gel to reach a constant weight (m3). The diameter of each disk was measured at four points, and the thickness was measured with a caliper rule to calculate the volume (mm³). The equation for assessing solubility is (m1 – m3)/V (µg/mm³).

Statistical Analysis

Results of the study were tabulated, and statistical analysis was performed using Statistical Package for the Social Sciences (SPSS) software (version 17). Scores obtained were evaluated using analysis of variance (ANOVA) test for comparisons between the groups, and intergroup comparisons were conducted using *post hoc* tests. A significance level of *p* ≤ 0.05 was considered statistically significant.

RESULTS

Comparison of Microhardness Levels between the Groups

This table shows the comparison of microhardness levels between the groups (Table 1 and Fig. 3A). The mean microhardness level of group I was 114.50 ± 28.808, group II was 119.83 ± 30.571, and group III was 60.5 ± 14.95. The difference was statistically significant using ANOVA with a *p*-value of 0.022.

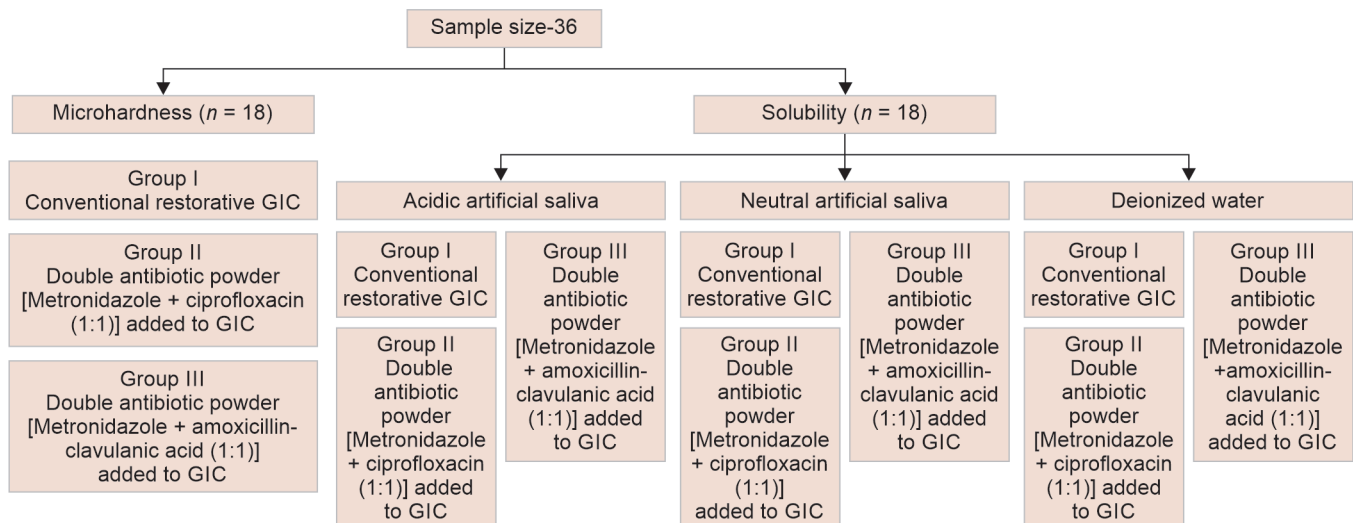


Fig. 1: Allocation of the samples into groups

Intergroup Comparison of Microhardness Levels

This table shows the intergroup comparison of microhardness levels (Table 2). The difference between groups I and II was not statistically significant with a *p*-value of 1.00. However, the differences between groups I and III, and between groups II and III were statistically significant with *p*-values of 0.007 and 0.004, respectively, using *post hoc* tests.

Comparison of Solubility Levels between the Groups

This table presents the comparison of solubility in different solutions—acidic saliva, neutral saliva, and deionized water among the different groups (Table 3 and Fig. 3B).

For the acidic saliva solution, the solubility levels were as follows—group I, 14.00 ± 9.899; group II, 28.50 ± 21.920; and group III, 29.00 ± 25.456. The difference was not statistically significant using ANOVA with a *p*-value of 0.727.

For the neutral saliva solution, the solubility levels were as follows—group I, 18.00 ± 12.728; group II, 24.50 ± 16.263; and group III, 27.00 ± 8.485. The difference was not statistically significant using ANOVA with a *p*-value of 0.787.

For the deionized water solution, the solubility levels were as follows—group I, 15.00 ± 15.556; group II, 28.00 ± 24.042; and group III, -13.00 ± 86.267. The difference was not statistically significant using ANOVA with a *p*-value of 0.749.

Intergroups Comparison of Solubility Levels

This table shows the intergroup comparison between solubility levels of different solutions (Table 4).

For acidic saliva, the differences between groups I and II, groups I and III, and groups II and III were found to be not statistically significant using *post hoc* tests with a *p*-value of 1.000.

For neutral saliva, the differences between groups I and II, groups I and III, and groups II and III were found to be not statistically significant using *post hoc* tests with a *p*-value of 1.000.

For deionized water, the differences between groups I and II, groups I and III, and groups II and III were found to be not statistically significant using *post hoc* tests with a *p*-value of 1.000.

DISCUSSION

Dental caries is the most common chronic disease associated with oral health in school-aged children. Caries primarily develops due to the interaction among the host, microorganisms, and food substrates. The cycle of remineralization and demineralization leads to caries formation based on the imbalances that occur.

The various restorative materials used for the restoration of caries include amalgam, GIC, composite, giomer, etc. Bönecker et al. stated that ART has shown remnants of caries-producing bacteria near the cavity floor.⁴ Therefore, it is uncertain whether the characteristic fluoride-releasing capability of GIC will be sufficient to prevent the recurrence of caries around the restoration.

Secondary caries, also known as recurrent caries, are usually associated with defective restoration or inadequate removal of caries. Gaps between the restoration and the tooth have also been found to be a major cause of secondary caries development.

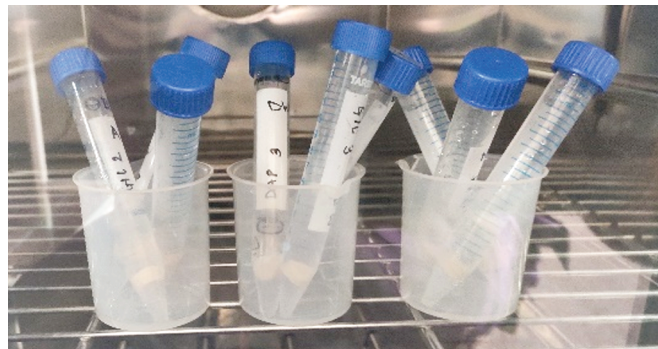


Fig. 2: Samples receiving 5 mL of solution every day and being placed in the heater

Table 1: Comparison of microhardness levels between the groups

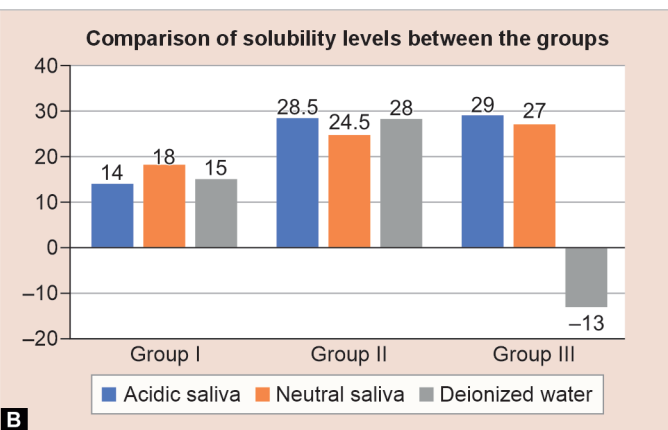
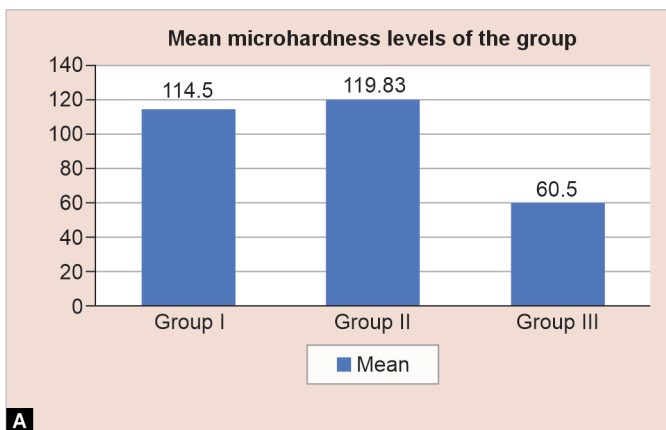
Group	N	Mean	Standard		p-value
			deviation	Minimum Maximum	
I	6	114.50	28.808	68 149	0.022*
II	6	119.83	30.571	82 162	
III	6	60.50	14.950	42 79	

*, Denotes statistically significant using ANOVA

Table 2: Intergroup comparison of microhardness levels

Group vs	Mean difference	Significance
I vs II	-5.333	1.000
I vs III	54.000*	0.007*
II vs III	59.333*	0.004*

*, Denotes statistically significant using *post hoc* test



Figs 3A and B: Mean microhardness levels of the group and comparison of solubility levels between the groups

These gaps may result from flawed initial placement of the restoration or inadequate light curing of the material.⁵

In order to overcome this shortcoming, a dental material with bacteriostatic properties should be used.⁶ A dental material alone has been shown to have limited bacteriostatic properties. Therefore, there is a need for a restorative material incorporating newer antibiotics to overcome secondary caries.

In dentistry, the history dates back to 1951, when antibiotics were first used as an intracanal medicament by Grossman. Ciprofloxacin is a potent fluoroquinolone that is active against a wide range of bacteria, especially aerobic gram-negative bacilli. It is mainly effective against species of Enterobacteriaceae and *Neisseria*, as well as *Escherichia coli*. Metronidazole acts against anaerobic bacteria and has been found helpful in preventing the growth of obligate anaerobes. It is effective against species of *Fusobacterium*, *Clostridium*, *Prevotella*, and *Veillonella*. Minocycline has been found to be active against both gram-positive and gram-negative bacteria.⁷ The conventional triple antibiotic combination consists of ciprofloxacin, metronidazole, and minocycline. However, the addition of minocycline has resulted in various problems such as tooth structure discoloration, antiangiogenic effects, and radicular dentin chelation, which can weaken the root structure.⁸ Therefore, this led to the introduction of double antibiotics over triple antibiotics. In our study, we used amoxicillin/clavulanic acid to replace ciprofloxacin in the conventional double antibiotic combination. Amoxicillin/clavulanic acid is a combination of amoxicillin and clavulanic acid. With the incorporation of clavulanic acid, the spectrum of action expands to include β -lactamase-producing strains of *Enterococcus* species, *Streptococcus* species, etc.⁹

In this study, a double antibiotic powder combination was chosen over a triple antibiotic powder combination to prevent resistance against microbes.

Ferreira et al. disclosed the improved antibacterial performance of GIC due to the addition of antibiotics. Incorporating antibiotics in GIC has been shown to reduce or completely inactivate microorganisms in dentin.¹⁰ Studies done by Botelho,¹¹ Yesliyurt et al.,¹² and Prabhakar et al.¹³ have shown that conventional GIC had no antibacterial action against *S. mutans*, *Lactocaseibacillus casei*. Therefore, with the innovation of incorporation of antibiotics into GIC, we can use antibacterial GIC as a liner/base/restorative material as it shall decrease the danger of pulp exposure during the necessity of remaining dentin repair.

Rahman et al. evaluated the antibacterial effect of GIC with individual antibiotics (amoxicillin, minocycline, ciprofloxacin, and metronidazole) and a combination of all of them against *S. mutans*. It was found that amoxicillin showed the smallest zone of inhibition, despite its potent antibacterial action against *Streptococcus*.¹⁴ Therefore, amoxicillin/clavulanic acid was included in this study. The double antibiotic combinations used in the current study were metronidazole/ciprofloxacin and metronidazole/amoxicillin/clavulanic acid. Ciprofloxacin, being a narrow-spectrum antibiotic, was replaced with amoxicillin/clavulanic acid to broaden the spectrum of its antibacterial action.

Prabhakar et al. compared the physical parameters of adding double antibiotics (metronidazole and ciprofloxacin) at 1 and 2% w/w and concluded that adding 1% w/w of the antibiotic mixture provided antimicrobial activity to GIC without compromising the physical parameters.¹³ However, Yesliyurt et al. stated that incorporating triple antibiotic powder (metronidazole, ciprofloxacin, and minocycline) at 1.5% maintained suitable physical characteristics.¹² Chaudhari et al. found a considerable amount of zone of inhibition and microhardness of GIC with metronidazole and ciprofloxacin at 1.5%.

Based on evidence from Chaudhari et al., who used 1.5% w/w of double antibiotic powder and triple antibiotic powder, this concentration was chosen as the required concentration for the current study.¹⁵ Amoxicillin/clavulanic acid has a broad-spectrum antibiotic range. Hence, the concentration of the modified double antibiotic combination was kept low at 1.5%.

Table 4: Intergroup comparison of solubility levels

Dependent variable			Mean difference (I-J)	Significance
Acidic saliva	1	2	-14.500	1.000
		3	-15.000	1.000
	2	3	-0.500	1.000
Neutral saliva	1	2	-6.500	1.000
		3	-9.000	1.000
	2	3	-2.500	1.000
Deionized water	1	2	-13.000	1.000
		3	28.000	1.000
	2	3	41.000	1.000

Table 3: Comparison of solubility levels between the groups

		N	Mean	Standard deviation	Minimum	Maximum	p-value
Acidic saliva	1	2	14.00	9.899	7	21	0.727 [#]
	2	2	28.50	21.920	13	44	
	3	2	29.00	25.456	11	47	
Neutral saliva	1	2	18.00	12.728	9	27	0.787 [#]
	2	2	24.50	16.263	13	36	
	3	2	27.00	8.485	21	33	
Deionized water	1	2	15.00	15.556	4	26	0.749 [#]
	2	2	28.00	24.042	11	45	
	3	2	-13.00	86.267	-74	48	

[#], Denotes statistically not significant using ANOVA

Therefore, in our study, we added the antibiotic mixture at a constant 1.5% concentration for both groups. In group II, GIC was combined with metronidazole and ciprofloxacin, as ciprofloxacin alone has limited activity against microorganisms. This combination of double antibiotic powder has been used as a conventional combination for intracanal medicaments. Group III consisted of GIC with metronidazole and amoxicillin/clavulanic acid, as both cover a wide range of microorganisms. To date, no research has been done on the efficacy of metronidazole and amoxicillin/clavulanic acid, even though amoxicillin offers effective antibacterial action against *Streptococcus*.

The resistance of a dental material to indentation or penetration is known as hardness, which also predicts the material's ability to abrade or be abraded by the opposing tooth. Vickers hardness test was used in this study to measure the hardness of GIC. This test utilizes a diamond pyramid-shaped indenter with a square base and an angle of 136° between the faces. The depth of the indentation is approximately 1/7 of the diagonal length of the impression produced. Samples were prepared in cylindrical molds, and the mean hardness value was determined from three measurements.¹⁶ The mean microhardness level of group II (GIC + metronidazole + ciprofloxacin) was higher compared to the other two groups. Specifically, group I had a mean microhardness of 114.50 ± 28.808 , group II had 119.83 ± 30.571 , and group III had 60.5 ± 14.95 . The difference was statistically significant using ANOVA with a *p*-value of 0.022. The higher microhardness level observed in group II compared to group I (GIC alone) suggests that the addition of double antibiotics to GIC may confer advantages in terms of microhardness. This improvement in microhardness in group II could be attributed to synergistic interactions between the antibiotics and GIC. Enhanced microhardness ultimately contributes to increased longevity of the restoration and helps prevent fracture under high masticatory loads.

Solubility is a crucial parameter to evaluate the durability of dental cements. During solubilization, the material loses mass, making the cement susceptible to marginal microleakage, which can lead to restoration failure. Artificial saliva serves as the storage medium to simulate conditions in the oral cavity.¹⁷ In our study, we prepared artificial saliva in both acidic and neutral forms to simulate the respective conditions of the oral cavity, with deionized water used as a control group.

In both acidic and neutral saliva solutions, it was observed that the solubility increased when altering the composition of conventional GIC. For acidic saliva solution, the solubility levels were as follows—group I, 14.00 ± 9.899 ; group II, 28.50 ± 21.920 ; and group III, 29.00 ± 25.456 . For neutral saliva solution, the solubility levels were group I, 18.00 ± 12.728 ; group II, 24.50 ± 16.263 ; and group III, 27.00 ± 8.485 . However, these differences were not statistically significant. Lima et al. found that Ketac Molar EasyMix GIC had the lowest solubility values compared to other brands of GIC on the market. This can be attributed to the presence of a large number of carboxylic acid groups in the liquid component of such cements. Additionally, numerous crosslinking interactions between polymer chains reduce available spaces, thereby decreasing water infiltration into the cement.¹⁷ Subramaniam et al. modified GIC by adding propolis into it and found that the solubility of the GIC increased on modification with propolis.¹⁸ Chitosan-modified GIC had shown to be more effective in reducing solubility.¹⁹

Both microhardness and solubility contribute to the longevity of a restoration. Therefore, it was necessary to evaluate whether

the addition of various combinations of double antibiotic powder would affect the physical properties of glass ionomer cement.

The main limitation of this study was the small sample size. Further studies are needed to assess various other physical parameters and the antibacterial efficacy of the antibiotic combinations used in this study.

CONCLUSION

Based on the results drawn from the study, it has been concluded that:

- The intergroup comparison of microhardness levels showed no statistically significant difference between groups I and II, but there was a significant difference between groups I and III, and between groups II and III.
- There was no statistically significant difference in the comparison of solubility in different solutions—acidic saliva, neutral saliva, and deionized water between the different groups.

Hence, in addition to the conventional properties of glass ionomer cement, incorporating various combinations of antibiotics can provide an additional therapeutic benefit by arresting the progression of caries at the site of infection itself.

Clinical Significance

In dentistry, oral formulations of antibiotics do not completely eradicate microorganisms. By incorporating antibiotics into the cement, this material can be used as a base or restorative material in both primary and permanent teeth. This approach holds promise for ART. It could be particularly valuable in community health programs requiring rapid treatment of large populations and halting the progression of caries activity in deep lesions.

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