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Comparative Evaluation of Antibacterial Efficacy and Effect on Dentin Microhardness of Triple Antibiotic Paste Modified with Nano Zinc Oxide and Nano Titanium Dioxide

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Abstract:-

AIM: The aim of the study is to evaluate the effect of antibacterial efficacy and root dentin microhardness of Triple Antibiotic paste modified with Nano Zinc oxide and Nano Titanium dioxide.

MATERIALS AND METHODS: freshly extracted sixty human mandibular premolar teeth were prepared till Protaper gold F2 and was irrigated with 3% NaOCl and 17% EDTA . The Intracanal medicaments used were Triple Antibiotic Paste modified with Nano Zinc oxide and Nano Titanium dioxide. The antibacterial efficacy was evaluated using Colony Forming Unit analysis and microhardness at middle and apical third after 1 week were measured using Vickers Microhardness Tester.

RESULTS: Within the limitations of this study, TAP with Nano Zinc oxide showed highest antibacterial efficacy and TAP with Nano Titanium dioxide showed least reduction in root dentin microhardness.

Keywords:- *Triple Antibiotic Paste, antibacterial efficacy, nanoparticle, intra-canal, medicaments, root dentin, micro-hardness.*

I. INTRODUCTION

The root canal treatment aims at complete disinfection and elimination of bacteria in periapical region. Complete eradication of bacterial from the root canal with less adverse effect on the microhardness of the dentin is required for the success of endodontic treatment. The most frequently isolated microbial species from the secondary and persistent root canal infections is the E.feacalis[1].

The use of intracanal medicaments is needed to obtain a microbial free environment [2]. Triple Antibiotic Paste combining Metronidazole, Ciprofloxacin and Minocycline is widely used for achieving complete disinfection of the complex root canal morphology [3]. To avoid the discoloration of crown by minocycline, Modified Triple Antibiotic Paste with clindamycin replacing minocycline was most successful in achieving disinfection [4]. The application of intracanal medicaments reduces the root dentin microhardness as they break the linkage between the collagen fibres and hydroxyapatite crystals [5]. Microhardness testing indicates the mineral loss or gain in dental hard tissues [6].

Metal nanoparticles are gaining more attention in dentistry due to their antibacterial properties. They have more surface area and smaller size enhancing the interaction with the cell wall of the bacteria [7]. The Zinc oxide Nanoparticles have selective action against gram-negative and gram-positive bacteria with less effects on human cells [8]. They also have Matrix metalloproteinases inhibiting action [9]. They retain the antibacterial properties against Enterococcus faecalis even after aging for 90 days [10]. The Titanium dioxide Nanoparticles have demonstrated good antiadhesive properties against Streptococcus mutans and free radicals generation rendering antibacterial action.[11]

II. MATERIALS AND METHODS

A. Sample Preparation:

Sixty freshly extracted single-rooted human premolar teeth with straight roots, closed apices, no caries, cracks, fractures, restorations or resorption were included in the study. The single canal was confirmed by taking radiograph. The Phosphate buffered saline was used to store the specimen until use. samples were sectioned at cemento-enamel junction (CEJ) at low speed using diamond disc with water coolant. The Working length was determined by introducing No15 K-file (Dentsply, Switzerland) into the canal and working length was estimated 0.5 mm short of the apex. ProTaper Gold rotary files and Dentsply X-smart plus endomotor (Dentsply Maillefer, Ballaigues, Switzerland) (S1-F2) was used for Cleaning and shaping. RC-Prep (Premier Dental Products, USA) was used as lubricant and 5 - 10 mL of 3% sodium hypochlorite (NaOCl) was used as an irrigant. A final rinse was done with 5.25% NaOCl and 3mL of 17% EDTA. 5 mL saline was used as a final rinse and canals was dried with paper points.

B. Antibacterial Efficacy

For testing antibacterial efficacy, the experiment was conducted at 4°C on BHI Agar. A single isolated colony was taken from the BHIA plate and inoculated in the BHI broth and grown overnight at 37°C. To this sterilised forty samples were added and incubated at 37°C for 7 days and the dentinal shavings were collected using H file for evaluating the antibacterial count of the control group.

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The samples were then divided to 4 treatments groups (n=10)

Group 1: Positive control.
Group 2: Triple Antibiotic Paste group.
Group 3: Triple Antibiotic Paste with Nano Zinc oxide.
Group 4: Triple Antibiotic Paste with Nano Titanium dioxide.

For TAP group Metronidazole, Ciprofloxacin and Clindamycin in 1:1:1 ratio in distilled water (3:1) was used and for TAP with nano titanium group, 2% of Nano Titanium dioxide was added to TAP Paste and for TAP with Nano Zinc oxide group,2% Nano Zinc oxide was added to TAP and applied using lentulospiral. After 7 days the dentinal shavings were collected using 25 H file. The dentinal shavings were resuspended into BHI broth and incubated for 1 hours at 37°C.

C. Enumeration Of Colony Forming Units

The whole procedure was carried out in a Laminar Air Flow hood. 20µL from each sample were applied to respective Petri plates and were swabbed on to BHI Agar. The plates were incubated at 37°C for 24 hours in a microbiological incubator. The CFUs were counted using a Digital Colony counter and were expressed as CFUs/mL.

D. Microhardness Evaluation

For evaluating microhardness value, twenty teeth were sectioned in a bucco-lingual direction longitudinally using a diamond disk at low speed, passing through the canal. The root segments were mounted horizontally with auto polymerizing acrylic resin with the canals exposed. The dentin surface was polished with carbide abrasive papers under distilled water to remove surface scratches to obtain smooth glossy surface.

The samples were then divided to 4 treatments groups (n=10)

Group 1: Positive control.
Group 2: Triple Antibiotic Paste group.
Group 3: Triple Antibiotic Paste with Nano Zinc oxide.
Group 4: Triple Antibiotic Paste with Nano Titanium dioxide.

The TAP was prepared by mixing Metronidazole, Ciprofloxacin and Clindamycin in 1:1:1 ratio in distilled water (3:1) and 2% Nano Zinc oxide,2%Nano Titanium dioxide was added to TAP for respective groups.

Baseline microhardness value of the specimens was evaluated at a 400X magnification using Vickers Microhardness Tester at 25 gf load,for 14 s. The microhardness values were evaluated at middle and apical third. Three indentations were marked for each section. Intracanal medicaments were then applied to each specimen. The specimens were stored in an incubator at 37°C for 1 week. After 1-week saline irrigation was used to remove the medicament activated with endoactivator, then microhardness was tested and was compared with the baseline values.

III. RESULTS

Table 1: Antibacterial efficacy of samples after 1 week inoculation

SAMPLE	MEAN NO OF COLONY COUNTED	MEAN VALUE CFU/mL
Group I: positive Control	354	177 x10^2
Group II: TAP	7	3.5 x 10^2
Group III: TAP ZnO	1	0.5 x 10^2
Group IV: TAP Ti02	4	2 x 10^2

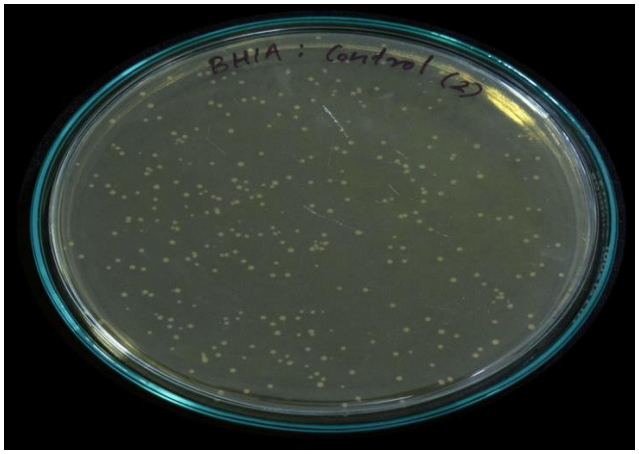

Fig. 1: CFU/ml of Positive control

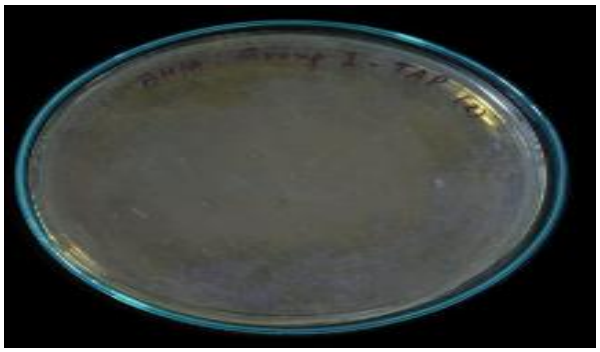

Fig. 2: CFU/ml of Triple Antibiotic Paste

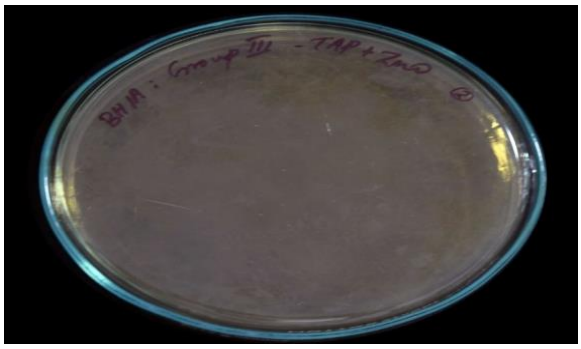

Fig. 3: CFU/ml of Triple Antibiotic Paste with nano Zincoxid

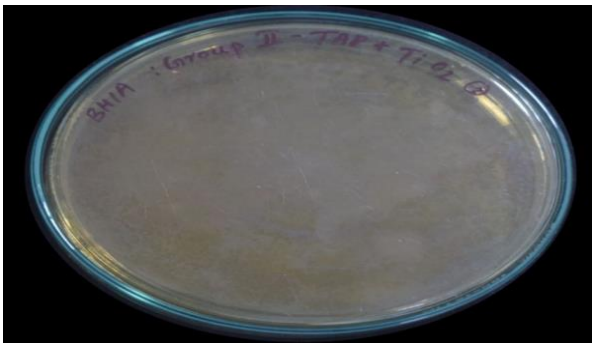

Fig. 4: CFU/ml of Triple Antibiotic Paste with nano Titanium dioxide

Table 2: Microhardness value (VHN) at middle and apical third.

SAMPLE		MEAN	SD
GROUP 1: BASELINE	middle	43.78	4.80
	apical	33.14	4.89
GROUP 2: TAP	middle	37.56	7.29
	apical	35.26	6.94
GROUP 3: TAP+ nZnO	Middle	54.38	8.06
	Apical	41.84	7.91
GROUP 4: TAP+ nTiO2	Middle	56.84	6.54
	apical	80.4	20.88

Fig. 5: Vickers Microhardness Tester

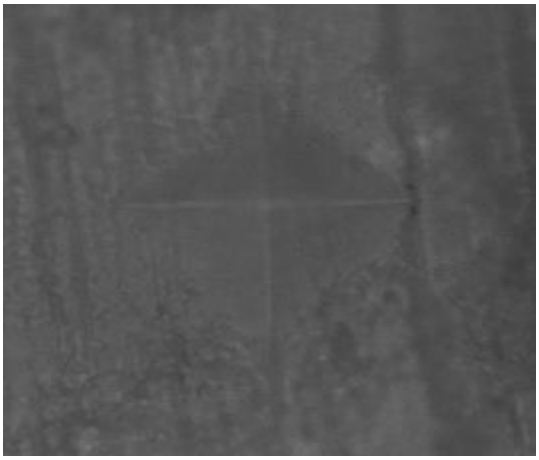

Fig. 6: Indentation formed by Vickers Microhardness Tester

IV. STATISTICAL ANALYSIS

Data was analysed using the statistical package SPSS 26.0 (SPSS Inc., Chicago, IL) and level of significance was set at $p<0.05$. Descriptive statistics was performed to assess the mean and standard deviation of the respective groups. Normality of the data was assessed using Shapiro Wilkinson test. Inferential statistics to find out the difference between the group was done using Kruskal Wallis test followed by Bonferroni test and between group analysis was done by Mann Whitney U test to find out the difference between groups.

V. DISCUSSION

As resistance to single antibiotic is most common and hence required multiple antibiotics to overcome the resistance and disinfection with all the polymicrobial. Enterococcus faecalis, though makes a small proportion of the microbial flora in untreated canals, plays a vital role in the aetiology of persistent infections and are resistant to traditional intracanal medicaments [12]. The most effective combined drug to overcome the resistance of bacterial strains is the Triple antibiotic Paste. It has clinical activity against anaerobic gram-negative cocci and anaerobic gram-positive bacilli. The TAP and MTAP currently used in regenerative endodontics caused significant reduction in the microhardness of dentin, due to the demineralizing effect of this antibiotic mixture on dentin [13]. Hence, in this study the effect of addition of Zinc oxide and Titanium dioxide Nanoparticles on root dentin microhardness and antibacterial efficacy was evaluated.

Antimicrobial efficacy of Triple antibiotic paste with nano titanium dioxide and nano zinc oxide were evaluated after 7 days incubation. Among the treatment groups, TAP group showed highest CFU /ml compared TAP with Nano Zinc oxide and TAP with Nano Titanium dioxide. TAP with Nano Zinc oxide showed better reduction in CFU when compared with TAP with Nano Titanium dioxide.

Vickers microhardness test was used as it helps to evaluate surface changes of deeper dental hard tissues. Other hardness tests such as Knoop hardness are only restricted to evaluate the microhardness of only the superficial dentin at 0.1 mm and not advocated for deep dentin. The results of this study showed increased bacterial reduction and statistically significant difference in TAP with Nano Zinc oxide (Group III). TAP group showed highest reduction in root dentin microhardness when compared with TAP with Nano Zinc oxide and TAP with Nano Titanium dioxide. TAP with Nano Titanium dioxide showed least reduction in root dentin microhardness.

VI. CONCLUSION

The Triple Antibiotic Paste modified with Nano Zinc oxide showed more antibacterial action as compared to Triple Antibiotic Paste and Triple Antibiotic Paste modified with Nano Titanium dioxide the combination of Triple Antibiotic Paste modified with Nano Titanium dioxide particles showed least effect on root dentin microhardness compared to Triple Antibiotic Paste and Triple Antibiotic Paste modified with Nano Zinc oxide groups.

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