COMPARATIVE EVALUATION OF ROOT CANAL DISINFECTION WITH DIFFERENT TECHNIQUES – AN *IN VIVO* STUDY

DISSERTATION

Submitted to the

BABU BANARASI DAS UNIVERSITY, LUCKNOW, UTTAR PRADESH

In the partial fulfillment of the requirement for the degree

of

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In the subject of

CONSERVATIVE DENTISTRY & ENDODONTICS

Submitted by

DR. RUCHI GUPTA

Under the guidance of

DR. SANDEEP DUBEY

Department of Conservative Dentistry & Endodontics

BABU BANARASI DAS COLLEGE OF DENTAL SCIENCES,

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Dr. B. Rajkumar
Professor & Head
Department of Conservative Dentistry and Endodontics,
Babu Banarasi Das College of Dental Sciences, Lucknow.

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Dr. B. Rajkumar
Principal
Department of Conservative Dentistry and Endodontics,
Babu Banarasi Das College of Dental Sciences, Lucknow.

DEPARTMENT OF

CONSERVATIVE DENTISTRY & ENDODONTICS

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CERTIFICATE BY THE GUIDE/CO-GUIDE

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GUIDE

Dr. SANDEEP DUBEY

READER

Department of Conservative Dentistry and Endodontics,
Babu Banarasi Das College of Dental Sciences, Lucknow.

CO-GUIDE

DR. PALAK SINGH

ASSISTANT PROFESSOR

Department of Conservative Dentistry and Endodontics,
Babu Banarasi Das College of Dental Sciences, Lucknow.

DEPARTMENT OF

CONSERVATIVE DENTISTRY & ENDODONTICS

BABU BANARASI DAS COLLEGE OF DENTAL SCIENCES,LUCKNOW

DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation entitled **"COMPARATIVE EVALUATION OF ROOT CANAL DISINFECTION WITH DIFFERENT TECHNIQUES- AN IN VIVO STUDY"** is a bonafied, & genuine research work carried out by me under the guidance of **Dr. SANDEEP DUBEY ,** READER, Department of Conservative Dentistry & Endodontics, Babu Banarasi Das College of Dental Sciences, Babu Banarasi Das University, Lucknow, Uttar Pradesh.

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"The most important function of education at any level is to develop the personality of the individual and the significance of his life to himself and to others."

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OM NAMAH SHIVAAY

Dr. Ruchi Gupta

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ABBREVIATIONS

S No	Abbreviation	Full Form
1	NaOCl	Sodium Hypochlorite
2	EDTA	Ethylenediamine Tetra Acetic Acid
3	Hz	Hertz
4	W	Watt
5	J	Joule
6	Mm	Mili Meter
7	Ncm	Newton Centimeter
8	Sec	Seconds
9	Fig	Figure
10	P-Value	Probability Number
11	Sd	Standard Deviation
12	%	Percentage

ABSTRACT

AIM

The aim of the study is to compare the root canal disinfection with different techniques.

MATERIALS AND METHOD- 60 patients selected from the Department of Conservative Dentistry and Endodontics, Babu Banarasi Das College of Dental Sciences, Lucknow and divided them into 3 groups who were clinically or/and radiographically diagnosed cases of acute irreversible pulpitis. Percentage of bacterial growth reduction was assessed in blood agar. percentage of bacterial reduction was analyzed with the Mann Whitney U test table.

RESULT- The result showed that there is significant reduction of bacterial count in the group C, sodium hypochlorite along with laser combination than group B and group A.

CONCLUSION- The study showed that sodium hypochlorite and diode laser had antimicrobial effect. However, combined effect of diode laser with NaOCl found to be more significant in bacterial colony count reduction.

INTRODUCTION

The most important objective during root canal instrumentation is the removal of vital and necrotic pulp tissue, infected dentin and dentin debris in order to eliminate most of the microorganisms from the root canal system (European society of Endodontology 1994, American Association of Endodontists 1998).[1]

Root canal therapy mainly comprises of disinfecting the root canal space by utilizing a combination of mechanical removal of tissue and chemical decontamination.[2]

The successful endodontic treatment depends on elimination/eradication of microorganism from the root canal system before obturation. The chemical debridement helps in removal of residual tissue and bacterial biofilm, mainly from the non-instrumented areas of root canal system.[3]

The eradication of persisting microorganisms in distant areas of the tubular system is a major challenge in today's treatment regimens and is crucial for the long-term preservation of the endodontically treated tooth.[3]

Approximately 40-60% of microorganisms can survive through chemo-mechanical preparation. This might be due to their inaccessible location in isthmuses, additional canals and apical region.[4]

On average, more than 30% of the canal's surface area remains covered by a smear layer, which protects bacteria in the dentinal tubules against intra-canal disinfection agents. Intra- canal medicament has limited anti-bacterial spectrum and a limited ability to diffuse into the dentinal tubules.[5]

Enterococcus faecalis is one of the most frequent microorganisms that cause post-treatment complication. In the same way some cases have shown that occasionally Candida albicans has also been associated with endodontic failures.[6]

The major cause of endodontic failure is the survival of microorganisms in the apical portion of root canal treated teeth, of which, E.faecalis is considered one of the primary organisms in patients with post treatment endodontic infection.

They are gram positive facultative anaerobic coccoid bacteria which can occur singly, in pairs or as short chains. Enterococci grow at temperatures ranging from 10-45 degree C, at pH 9.6 and in 6.5% (NaOCl) sodium chloride and can survive at 60 degree C for 30 minutes.

E.faecalis has the ability to establish monoinfections in medicated root canals. The organism has the ability to acquire, accumulate and share extra-chromosomal elements, encoding virulence traits, which help to colonize, compete with other bacteria, resist host defense mechanisms and produce pathological changes directly through the production of toxins or indirectly through the induction of inflammation.[7]

Several irrigating solutions have been used to reduce microorganisms, necrotic tissues and residual debries.[8]

Sodium hypochlorite (NaOCl) is known as a strong antibacterial agent and has been used in 0.5-5.25% concentrations in endodontic practices for many years. The effectiveness of NaOCl is well known, although some microorganisms may hide and survive inside tubules orother inaccessible areas.[9]

Bacteria deep in dentinal tubules are apparently protected from instrumentation and irrigation, making their removal or eradication difficult. When 908 nm diode laser was used alone or in conjunction with NaOCl shows a complete elimination of E.faecalis.[10]

QMix 2in1 solution, root canal irrigant contains a chelating agent, an antimicrobial agent and a surfactant in a premixed formulation. This irrigating solution is a single solution used as a final rinse after NaOCl for root canal disinfection and removal of smear layer.[11]

Another endodontic irrigant is CHX. Cationic molecules from CHX bind to microbial cell membranes, which are negatively charged, causing cell lysis. These irrigants must be in direct contact with the microorganisms to be effective, but they have limited penetration into dentinal tubules.[12]

The penetration depth of diode laser is 1000micrometers in root dentin , whereas rinsing solutions reach a depth of approximately 100micrometers . Additionally, curved root canals or side branches also can be obstacles in conventional root canal treatment.[13]

With the introduction of lasers to the field of conservative dentistry and endodontic treatment has been enriched by a multitude of new treatment methods that improved the chances for a successful treatment outcome. The diode laser can achieve an output power of several watts and shown to be highly reliable and effective; the diode laser can be recommended for endodontic treatment because its wavelength of 980nm which is within the infrared range, it also has thin, flexible light-conductor fiber.[14]

Since the development of the ruby laser by Maiman in 1960 and the application of the laser for endodontics by Weichman in 1971, a variety of papers on potential applications for lasers in endodontics have been published.[15]

During laser activation, the formation of vapor bubbles, the collapse of the bubbles, acoustic streaming, and, finally, cavitation processes occur. This cavitation process leads to irrigant activation and subsequent smear layer removal. The threshold for initiation of the cavitation process is more dependent on the output power of laser as evidenced by Hmud Raghad et al.[16]

Apical 1/3rd of root canals was effectively irradiated by inserting the fiber tip 1 mm short of working length and moving outwards slowly at a rate of 1 mm/sec.[17]

The diode laser has radiation range within the visible (mostly 660 nm) and infrared (810 to 980 nm) range of the electromagnetic spectrum.[18]

Diode laser wavelengths (810, 940, and 980 nm) when transmitted show deep penetration in dentine (1000 μm), melanin and hemoglobin and have a similar selective bactericidal effect to that of Nd: YAG 1064 nm laser.[19]

Working of the laser and its effect on biological tissue is determined by interaction of laser radiation parameters, such as: wavelength, physical characteristics of the illuminated tissue, energy radiation, continuous or pulsed mode, diameter of the laser beam, and the exposure time.[20]

Recently the 940 nm wavelength came to the attention, as it was demonstrated that a limited form of cavitation could be generated in aqueous fluids around the top of the fibre tip.[21]

The thin flexible fiber of 200 μm tip provides better access to the apex. It also works efficiently at lower power with less heat production with power output ranging from 0.5 W to7 W.[22]

Moritz and his colleagues (2000) found that Gram-negative organism showed immediate structural injury, whereas the Gram-positive organism (E. faecalis) required repeated application of laser irradiation.[23]

Laser energy can eliminate microorganisms existing in main canal, lateral canals and dentinal tubules which may cause pulp and peri-apical infection.[24]

Clinically a fine 200µm diameter endodontic tip is positioned 1mm short of the apical terminus and using a helical movement the tip is retracted coronally at a steady pace in 5 to10 seconds. The lasing procedure is done in canal filled with an irrigant such as EDTA or NaOCl to avoid undesirable thermal effects.[25]

Klinke et al. achieved an elimination rate of 92.9 % with an Nd:YAG laser adjusted to 1.5 W and 15 Hz, and with a slice thickness of 300 µm. Gutknecht et al., a bacterial reduction on an average of 99.91% was able to be achieved by means of an Nd:YAG laser and an output of 1.5 W at 15 Hz— this in vitro work, though, was performed on roots of extracted human teeth. The fiber, therefore, was in direct contact with the bacteria.

Gutknecht et al., the 810-nm diode laser was checked for its bactericidal effect. With a distal output of 0.6 W in c.w. operation, an average bacterial reduction of 88.38% was able to be achieved with a dentin slice of 300 µm in thickness.[26]

The reason behind the better performance of diode laser is its mode of delivery, which is 200 µm fiber optic tip, with the length of 14 mm. Penetration of endodontic irrigant is limited due to the presence of smear layer in root dentin.[27]

Diode laser causes partial to complete obliteration of the dentinal tubules in the intra-canal system, leading to a sterile environment and reducing the chances of re-infection thus increasing the success rate of the root canal treatment if used as an adjunct to the conventional root canal therapy.[28]

Diode lasers are mainly used as soft-tissue lasers, but are proved to be particularly effective in disinfection of the root canal and do not alter the shape of the canal as hard tissue lasers such as carbon dioxide lasers and erbium lasers do.[29]

Application of semiconductor laser in root canal therapy effectively removes the smear layer within the root canal. It also improves root canal closure through melting and remineralization of hard tissue and closed dentinal tubules.[30]

Field of antibacterial chemotherapy is a constant challenge. The current problem of bacterial drug resistance perhaps best illustrates the continuing requirement both for new agents and new approaches to eliminate infection from root canal system.

Ng et al. suggested that it would be superior to develop adjunctive antibacterial therapeutic strategies to chemo-mechanical methods to target residual microorganisms and thus enhance the healing rates of teeth with infected root canals.[31]

AIM AND OBJECTIVES

AIM-

The aim of the study is to compare the root canal disinfection with different techniques.

OBJECTIVES-

1. To compare the root canal disinfection potential of Diode laser along with saline, diode laser along with 5.25% sodium hypochlorite and 5.25% sodium hypochlorite alone.
2. To evaluate the root canal disinfection potential of Diode laser along with saline, diode laser along with 5.25% sodium hypochlorite and 5.25% sodium hypochlorite alone.

REVIEW OF LITERATURE

Eliana Barbosa de Souza, MS Silvana Cai (2008)[15] evaluated the disinfection degree of dentine caused by the use of diode laser after biomechanical procedures. Diode laser irradiation provided increased disinfection of the deep radicular dentin in the parameters and samples tested.

Gutknecht N (2008)[55] assessed that laser treatments may effectively replace conventional techniques. Especially its improved disinfection efficacy, more effective root canal cleaning, reduction of permeability, reduction of micro-leakage, and elimination of the need to use toxic solvents represent the main advantages for patients and dentists.

Gerek M, S. Asci & D.I. Yaylali (2010)[17] According to the data evaluated, although EDTA, Nd:YAG, and Diode lasers were effective as a bactericidal agent in contaminated root canals, NaOCl had a significantly higher antibacterial effect. The Nd:YAG and Diode lasers showed more antimicrobial effect than EDTA in the E. faecalis group while the Nd:YAG and Diode lasers and EDTA showed the same level of antimicrobial action in the C. albicans group.

Verma SK, Maheshwari S et al (2012)[26] concluded that Use of the laser proved to be an effective tool to increase efficiency, specificity, ease, and cost and comfort of the dental treatment.

Castelo-Baz P ,Martin-Biedma B et al (2012)[29] evaluated combined Sodium Hypochlorite and 940 v nm Diode Laser Treatment Against Mature E. Faecalis Biofilms and concluded that the combination of sodium hypochlorite and diode laser light (940 nm) has a synergistic effect, intensifying the bactericidal action.

Ashofteh K, Sohrabi K, Iranparvar K et al (2013)[17] done an In vitro comparison of the antibacterial effect of three intracanal irrigants and diode laser on root canals infected with Enterococcus faecalis, result showed that sodium hypochlorite was the most effective agent against Enterococcus faecalis.

Bhatia S, Kohli S (2013) [16] stated that the laser is an effective tool as it has the ability to kill the bacteria, remove debris and smear layer from the root canal walls following biomechanical instrumentation by the use of energy and wavelength characteristics. This article review goes on to explain, the effects of laser on tissue, bacteria, types of laser, delivery systems ,emission modes and about the use of lasers in root canal sterilization.

Kaiwar A, usha HL (2013)[5] done study on the efficiency of root canal disinfection using a diode laser and concluded that the 980 nm diode laser can eliminate bacteria that has immigrated into dentine,thus being able to increase the success rate in endodontic therapy.

Asnaashari M ,Safavi N (2013)[24] done study on disinfection of contaminated canals by different laser wavelengths, while performing root canal therapy and concluded that maximum effect is obtained when laser light in combination with sodium hypochlorite irrigating solution with appropriate concentration, is used in canals. Therefore use of laser energy can increase the success rate of teeth root treatments.

Njwan F. Shehab ,Alshamaa Z A et al (2013)[13] Evaluated the antibacterial efficacy of elexxion diode laser 810nm on the infected root canals and concluded that diode laser at a wave length 810nm has antibacterial effect against Enterococcus faecalis.

Shetty K R , Hegde M N et al (2013)[14] done comparative evaluation of bactericidal effects on Enterococcus faecalis using Diode Laser irradiation, Sodium Hypochlorite and Chlorhexidine Gluconate Irrigation and concluded that 3% NaOCl was the most effective irritant comparatively and combination of 2% CHX and laser was as effective as 3% NaOCl and hence can be used as an alternative for 3% NaOCl.

Mashalkar S, Mansing G Pawar et al (2014)[9] done comparative evaluation of root canal disinfection by conventional method and laser and concluded that conventional method by using sodium hypochlorite and hydrogen peroxide as irrigating solutions is highly effective in disinfecting the root canal. Lasers when used can also reduce the bacterial load of the infected root canal.

De Moor, Meire M (2014)[2] done a survey of the use of high-power lasers for root canal cleaning and disinfection. There are two approaches: the first using a fiber in a dry root canal and exposing the root canal wall to the laser light with a spiral motion, and the other using the fiber in irrigant in the root canal or at the orifice. The laser-target interaction is different: a direct exposure of the substrate to laser light is the aim of the spiral motion, whereas the aim of the second technique is activation and agitation of the irrigant (laser activated irrigation / LAI).

Darmiani S, Salmani F (2019)[8] Compared the antibacterial effect of endo activator and diode laser on root canals infected with entrococcus faecalis an in vitro study and concluded that although 5.25% NaOCl seems to reduce E.faecalis more effectively, EA also reduced the

bacterial count. Therefore EA could be considered as a complementary disinfection method in root canal treatment (RCT).

Kumar S, kailasam S K et al (2014) [10] done comparative evaluation of antimicrobial efficiency of diode laser, sodium hypochlorite and their synergistic effect againstenterococccus faecalis contaminated root canals-an in vitro study and result showed thatdiode laser alone and diode laser with sodium hypochlorite shows complete elimination of E.faecalis from the root canal.

Xhevdet A , Stubljar D et al (2014)[6] compared the disinfecting efficacy of root canals with laser photodynamic therapy concluded that PUI still remains the most effective method for disinfection of infected root canals in endodontics compared to hand instrumentation for both microorganisms. SEM analysis only confirmed the results. Other results ex vivo suggested that prolonging the time from 1 to 5 minutes of PDT increased the number of killed microorganisms significantly, therefore longer times of photodynamic therapy wererecommended. Irrigation with 2.5% NaOCl showed similar results to 5 min irradiation.

Naghavi N,Rouhani A et al (2014)[6] done study on diode laser and calcium hydroxide for elimination of enterococcus faecalis in root canal and result showed that combination therapy with naocl irrigation and diode laser irradiation can be recommended as an effective treatment option for elimination of E. faecalis from the root canal system.

Mathew A ,Lajevardin M et al (2015) [3]done an in vivo study on comparison of disinfection of root canal with chemical disinfectants and disinfectant-diode laser-photodynamictreatment combined system concluded that In both of the microbiological study by Blood Agar and Mitis Salivarius media, it showed that application of either Diode laser or NaOCl alone will not bring considerable reduction in the bacterial colony. It was observed that the synergic effect of Diode laser combined with NaOCl was found to be very effective.

Hegde M N ,Bhat R et al (2015)[1] evaluated efficiency of a semiconductor diode laser in disinfection of the root canal system in endodontics. Laser irradiation resulted in significantly higher antimicrobial effects compared with the Endovac and Stropko irrigation groups when in conjunction with sodium hypochlorite.

Markovic D, Rakasevic D et al (2015)[20] done study on application of high-power diode laser and photodynamic therapy in endodontic treatment and stated that a precise protocol for a PDT or high-power diode laser therapy does not exist. It has not been determined how

many sessions or repetitions of therapy are needed to create completely sterile conditions. A research is necessary to define a precise protocol for a PDT and highpower laser in therapy of the root canal. Looking to the future, it is expected that specific laser technologies will become essential components of contemporary dental practice over the next decade.

Jhingan P,Sandhu M et al (2015)[28] done an in-vitro evaluation of the effect of 980 nm diode laser irradiation on intra-canal dentin surface and dentinal tubule openings after biomechanical preparation scanning electron microscopic study and concluded that 980 nm diode laser (2 watt power, and 200 µm fiber size) causes melting of intra-canal dentin and partial to complete obliteration of the dentinal tubule openings if the intra-canal dentin is irradiated for four cycles in helical movements at the speed of 1 mm/s. Thus to increase the success rate of the root canal treatment and prevent reinfection, use of diode lasers can be recommended as an adjunct to conventional root canal treatment as it would limit the re-entryof microflora into the root canal system.

Agrawal AA, Kolhe S et al (2016)[7] done study on root canal disinfection potential of 5.25% sodium hypochlorite, 2% chlorhexidine and 810nm diode laser-a comparative In vitro antimicrobial study and concluded that 5.25% sodium hypochlorite or 2% chlorhexidine can be efficiently used as an adjuvant to mechanical root canal cleaning.

Purayil T P,Chakravarthy A et al (2016)[25] concluded that with the advancements in laser properties, it has been possible to invade the obscure apical zone of the root canal system thus providing synergistic gains to conventional endodontic therapy. The use of photoinduced disinfection techniques and contemporary tip designs have paved channels to eliminate pathogens and expedite periapical tissue repair, hence creating promising multifaceted applicability.

Kumari A,Loomba K et al (2017)[11] studied effect of diode laser when used alone or in combination with various irrigants on root canal microbes-an in vivo study and assessment showed that diode laser in combination with saline was comparable to a multimodal modality of naocl, 2%chx and saline combination.

Dandan Su , Xingxue Hu et al (2017)[26] evaluated the effect of semiconductor laser irradiation on root canal sealing after routine root canal therapy and concluded that the application of semiconductor laser prior to root canal obturation increases the apical sealing of the roots treated.

Iselinni C, Meidyawati R et al (2017)[12] evaluated the effects of a 980-nm diode laser's activation of 2.5% NaOCl and 2% chlorhexidine antifungal irrigation solutions on candida albicans biofilms and this study found that the 2.5% NaOCl and 2% CHX endodontic irrigants have antifungal properties. The use of a diode laser in addition to the irrigants can activate the antifungal properties of the 2.5% NaOCl and 2% CHX.

Ahangari Z, Mojtahed Bidabadi M et al (2017)[63] Aimed to compare the antimicrobial efficacy of calcium hydroxide as an intracanal medication and antibacterial photodynamic therapy (aPDT) against Enterococcus faecalis and Candida albicans in teeth with periapical (PA) lesions. And showed that PDT and calcium hydroxide therapy have same antimicrobial efficacy on E. faecalis and C. albicans.

M. Christopher Joel Simon, Pradeep S et al (2018)[2] reviewed that a laser is a device which transforms light of various frequencies into a chromatic radiation in the visible,infrared, and ultraviolet regions with all the waves in phase capable of mobilizing immense heat and power when focused at close range. The purpose of this paper is to summarize laser applications in endodontics, including their use in pulp diagnosis, dentinal hypersensitivity, pulp capping and pulpotomy, sterilization of root canals, root canal shaping and obturation, and apicectomy. The effects of laser on root canal walls and periodontal tissues are also reviewed. This article also discusses whether a laser can provide equal or improved treatment over conventional care.

Sharma N, Kaur J et al (2018)[27] evaluated the efficacy of diode laser in root canal disinfection.In this study found that laser was efficacious in disinfection of the root canal compared to the standard techniques. complete disinfection is mandatory for the success of root canal therapy and laser is an appropriate tool for root canal disinfection.

Njwan F. Shehaba , Nawfal A. Zakaria et al (2018)[23] evaluated the efficiency of diode laser as root canal disinfectant against enterococcus faecalis: an in vitro study and concluded that diode laser 1064 nm has significant antibacterial effect against e. faecalis infected root canals. and bactericidal effect of it depends on the laser parameters and the results vary with different powers. 2. Time of laser irradiation plays a significant role during irradiation with flat fiber optic tip of laser.

Tilakchand M, Singh NN (2018)[4] evaluated the antibacterial efficacy of EZLASE diode LASER on the infected root canal system an in vivo study concluded that combination therapy consisting of irrigation using NaOCl and LASER irradiation, especially at high

output power was an effective treatment option for a reduction in E. faecalis as well as other bacterial flora from the root canal system.

Shaktawat A S ,Verma K G et al (2018)[10] evaluated that antimicrobial efficacy of 980 nm diode laser on Enterococcus feacalis in conjunction with various irrigation regimes in infected root canals: An in vitro study and study concluded that the groups with laser irradiation along with irrigation protocols were more efficient against E. faecalis as comparedwith the groups subjected to irrigation protocol alone .The antimicrobial efficacy of NaOCl+EDTA+Laser was found to be maximum, followed by NaOCl + EDTA, CHX Gluconate + Laser, CHX Gluconate, Neem + Laser, Neem.

Sharma N, Kaur J, et al (2018)[39] evaluated the efficacy of Diode Laser in Root canal disinfection, concluded that laser was efficacious in disinfection of the root canal compared to the standard techniques.

Darmiani S, Salmani F (2019)[8] Compared the antibacterial effect of endo activator and diode laser on root canals infected with entrococcus faecalis an in vitro study and concluded that although 5.25% NaOCl seems to reduce E.faecalis more effectively, EA also reduced the bacterial count. Therefore EA could be considered as a complementary disinfection method in root canal treatment (RCT).

Saleh Z, Mammani I(2019)[19] done comparative evaluation of microbial eradication in root canal by 5.25% sodium hypochlorite and 940 nm diode laser an in vivo study and concluded that both treatments showed a significant difference in eradicating the isolated microorganisms statistically. the 940 nm diode laser was more effective than chemomechanical treatment, as the root canal of more patients were eradicated from root canal bacterial growth. however, combining both treatments will give more effective against the microorganisms present in the root canal.

Walia V,Goswami M et al (2019)[18] done comparative evaluation of the efficacy of chlorhexidine, sodium hypochlorite, the diode laser and saline in reducing the microbial count in primary teeth root canals and based on the results, observations and statistical analysis, the following conclusions can be made 1. chlorhexidine, 1% sodium hypochlorite and laser irradiation succeeded in significantly reducing the root canal microbial count of all the species of bacteria found in the pre-disinfection sample. 2. diode laser irradiation may be used as an alternative to the existing protocols for root canal disinfection in human primary

teeth. however, further clinical trials with the use of the diode laser in primary teeth with a larger sample size are warranted.

Zafar Q A, Malik W J et al (2019)[30] comparison of 980 nm diode laser and q-mix solution alone and in combination on removal of smear layer from root canal surface; a scanning electron microscope study concluded that 980 nm diode laser is an effective tool for irrigant activation and improving the smear layer removal especially from the apical thirds of root canals. this irrigant activation provides better access and penetration into inaccessible areas of root canals. It is recommended that further studies should be conducted to evaluate the interaction with a diode laser with different root canal irrigation solutions.

Hawra Mohammed Al Hamad, Mengari L F et al (2019)[31] studied lasers in endodontics and according to the literature reviewed for this article, when used efficaciously and properly,lasers can be a very useful tool for dentists. With the development of thinner, more flexible and durable laser fibers, laser applications in endodontics will increase. Its better disinfection efficacy, more effective root canal cleaning, reduction of permeability, reduction of micro-leakage, and elimination of the need to use toxic solvents represent the main advantages to dentists, enabling them to provide better treatment for their patients.

Rajakumaran A, Ganesh A (2019)[32] comparative evaluation of depth of penetration of root canal irrigant after using manual, passive ultrasonic, and diode laser–assisted irrigant activation technique within the limitations of the given study, it is concluded that diode laser–assisted irrigant activation technique had better penetration depth in all the three aspects of root dentin.

Abraham S,Vaswani D K et al (2019)[33] done scanning electron microscopic evaluation of smear layer removal at the apical third of root canals using diode laser, endoactivator, and ultrasonics with chitosan an in vitro study .Within the limitations of this study, all tested groups were able to remove the smear layer from prepared root canals to different degrees except the control group. Smear layer was removed more efficiently by the activation of irrigant and with machine-assisted irrigation devices. Diode laser has increased the success rate of endodontic therapy due to its ability to remove smear layer and root canal microbes. This study clearly shows the advantages of laser treatments over currently used conventional irrigation methods and techniques while using 0.2% chitosan as an irrigating solution.

Darmiani S, Salmani F et al (2019)[34] compared antibacterial effect of endo activator and diode laser on root canals infected with entrococcus faecalis an in vitro study and study

showed that 5.25% NaOCl had significantly stronger antibacterial effect compared to a 980-nm diode laser and EA; however, the effectiveness of EA in bacterial reduction was acceptable. EA can be considered as an alternative method for root canal disinfection.

Kihara T, Matsumoto H et al (2019)[35] evaluation of the efficacy of ER:YAG laser–activated irrigation in a simulated accessory canal within the limitations of this in vitro study, we found that LAI possessed higher hydrogel removal capacity from the accessory canals than SI when NaOCl was used as the irrigant. A longer irradiation period influenced cleaning efficacy, but the laser tip position did not.

Mookhtiar H, Hegde V et al (2019)[36] done root canal sterilization using ND: YAG laser and concluded that disinfection of root canal space is of outmost importance for the successof endodontic treatment and prevention of post treatment microbial contamination. Although, completely disinfection of the root canal space is still not possible, the use of lasers has been a boon when it comes to disinfection of root canal space. Further, researchers is still needed when it comes to the use of lasers and its effects in the field of endodontics.

Otaify RR, Roshdy NN (2020)[37] evaluate the efficacy of Sodium hypochlorite activation using two types of laser; Diode laser and Er: YAG laser utilizing PIPS tip as compared to conventional Sodium hypochlorite syringe irrigation on biofilm eradication, smear layer removal and topographic surface changes. Activation of Sodium hypochlorite irrigant using Er: YAG laser utilizing PIPS technique enhanced the biofilm eradication capability and smear layer elimination potentiality. Yet, the dentinal tubules changes remain higher when laser is used.

Dhawan S, Jasuja P et al (2020)[38] done a comparative evaluation of the efficacy of erbium: yttrium–aluminum–garnet and diode lasers in smear layer removal and dentin permeability of root canal after biomechanical preparation – A scanning electron microscopy study Within the limitations of this in-vitro study, it may be concluded that 1. The lowest smear layer scoreand debris score were recorded for Er:YAG laser group when the specimens were prepared with the 17% EDTA followed by the activation with the laser for 30 s 2. Er:YAG laser activation with irrigating solution (17% EDTA) enhances the removal of the smear layer and opening of dentinal tubules even in the apical third area of RCS.

MATERIAL AND METHOD

Place of the study :

This in vivo study was undertaken in Babu Banarasi Das College of Dental Sciences, Department of Conservative Dentistry and Endodontics, Lucknow U.P. in collaboration with Ram Manohar Lohia Institute of Medical Sciences, Department of Microbiology, Lucknow U.P.

The Institutional Ethical Committee Review Board of Babu Banarasi Das College of Dental Sciences, Lucknow U.P. gave the ethical clearance for study.

ARMAMENTARIUM-

1. For screening-

1. Mouth mirror (API,India)
2. Explorer (API, India)
3. Tweezer(API, India)
4. Gloves (Hand pro , India)
5. Mouth mask (Kashi surgicals, India)
6. Electric pulp tester (Parkell pulptester gentle plus , USA)
7. Cold test (Coltene, Switzerland)

FIG.1 COTTON ROLLS, ELECTRIC PULP TESTER, COLD TEST (ENDO -FROST)

2. Pre-operative radiography-

1. RVG (Unicorn, India)

3. **Intra-operative-**

Root canal procedure-

1. Rubber dam (API ,India)
2. Cotton and gauge pieces
3. Air rotor handpiece (NSK, Japan)
4. Endo access bur (Mani, Japan)
5. Endodontic explorer DG-16 (API, India)
6. Endoblock (API ,New Delhi, India)
7. Endomotor (X-Smart, Dentsply, U.S)
8. Stainless steel k files (Mani, Japan)
9. Hyflex EDM (Coltene, Switzerland)
10. Electronic apex locator (Morita Root ZX mini Apex locator,USA)
11. Irrigation needle (Indo-dent , India)
12. Diode laser (Dentsply Sirona , North carolina)

Fig.2 RUBBER DAM KIT

13. 5.25% sodium hypochlorite (Davis chemicals private, New Delhi)
14. Normal Saline (0.9%w/v NaCl) (Baxter ,India)
15. Absorbent paper point (Diadent, South Korea)
16. Cavit G (3M™ ESPE Minnesota U.S.)

FIG. 3 SALINE AND SODIUM HYPOCHLORITE

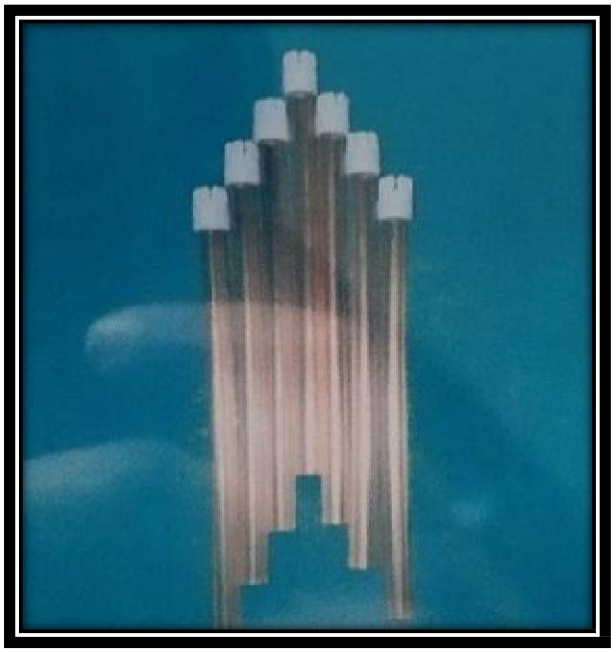


FIG. 4 SUCTION TIPS

Microbiology Armamentarium-

1. Disposable petri dishes- 90mm in size (Borosil, India)
2. Glass test tubes (Borosil, India)
3. Test tube stand (Laboratory supplies and company ,India)
4. Micropipette and pipette tips (Borosil, india)
5. Streak wire loop (Laboratory supplies and company, India)
6. Spirit lamp (COMET, India)
7. Incubator(Swastik , India)
8. Autoclave (Confident, India)
9. Laminar air flow (Hasthas scientific Instruments, India)

FIG.5 INCUBATOR

Fig.6 REFRIGERATOR

FDDFG

Fig.7 AUTOCLAVE

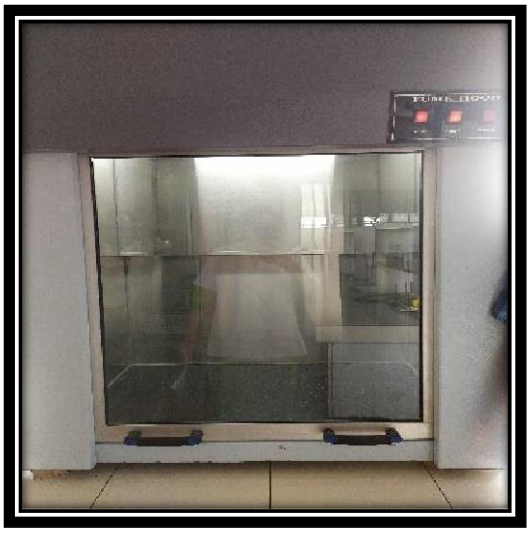


FIG.8 LAMINAR AIR FLOW

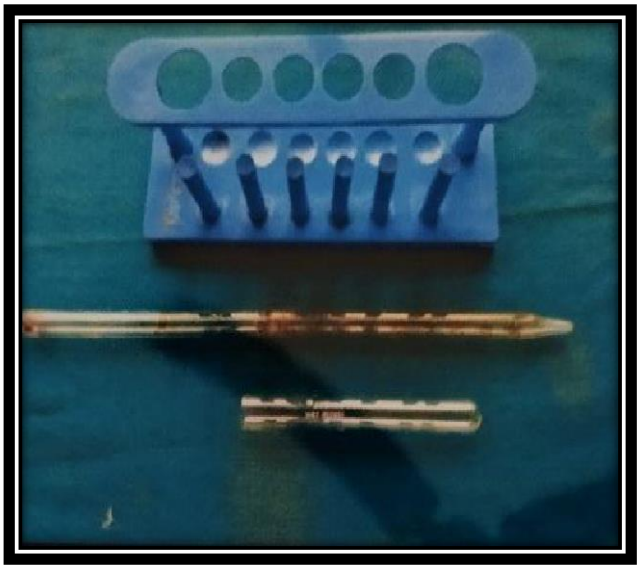


Fig. 9 TEST TUBE STAND AND PIPETTE, TEST TUBE

FIG. 10 STREAK WIRE LOOP

FIG.11 TRANSPORT MEDIA

FIG.12 PETRI DISH

SAMPLE-

This present study included patients of age 20-50 years who visited for root canal treatment at the hospital. Total 60 patients after radiographic examination were selected. The pulp vitality was performed using either electric pulp test or cold test.

Patients were first explained about the procedure and after their approval written consent was taken before treatment. A detailed medical and dental history was taken.

SELECTION OF CASES-

Clinically intraoral examination was done and radiograph was taken to follow the inclusion and exclusion criteria.

Inclusion criteria

- Patients who are willing for the treatment and giving written informed consent.
- Permanent anterior maxillary teeth with acute irreversible pulpitis.
- Teeth with Vertucci's type I root canal configuration.
- Completely developed single root with closed apex.

Exclusion criteria

- Patients who are not compliant with the terms of the study.
- Pregnant women and lactating mothers.
- Patients with systemic conditions.
- Patients allergic to any components of materials being used in study.
- Patients who are on antibiotic treatment.
- Teeth with root resorption and calcified canals.
- Teeth having dental anomalies.
- Non vital teeth.
- Patients who are having any sort of periodontal disease with clinically and radiographic changes suggestive of periodontal/combined lesion.
- Teeth which have been previously endodontically treated.

METHODOLOGY-

Preparation for access cavity-

Povidine iodine solution 5% was used for disinfecting tooth and surrounding area. Local anaesthesia was administered, and isolation was done with rubber dam. A high-speed handpiece and sterilized round bur was used for access opening. Debridement was done using saline. working length was determined radiographically and confirmed by apex locator which was kept 0.5mm short of the apex .The root canal patency was checked by size 10K-file.

Collection of the pre-treatment samples-

The presterilized paper points were used for collection of initial pre-treatment root canal samples.

One paper point was placed in the canal for 60 seconds and then transferred into presterilized tube. This was designated as the first sample (Sample 1).

All the samples were transferred directly in thioglycolate broth and cultured in blood agar and were kept in an incubator at 37°C for 24 hours. Colonies were counted with colony counter manual method.

Chemicomechanical preparation-

Hyflex EDM rotary file system at 450 rounds per minute at a torque of up to 2.5 Ncm was used for cleaning and shaping except the glidepath files which was used at 300 rpm and at a torque of up to 1.8 Ncm and then patients were randomly divided into three groups –

Group A- saline along with diode laser combination was used on 20 patients

Group B- 5.25% NaOCl was used alone on 20 patients.

Group C - 5.25%% NaOCl and diode laser on 20 patients.

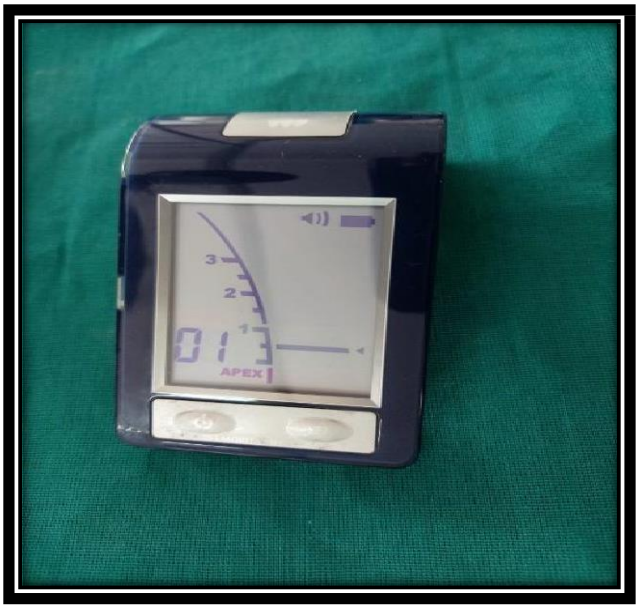


FIG.13 ELECTRONIC APEX LOCATOR

(Morita Root ZX mini Apex locator,USA)

FIG. 14 PAPER POINT

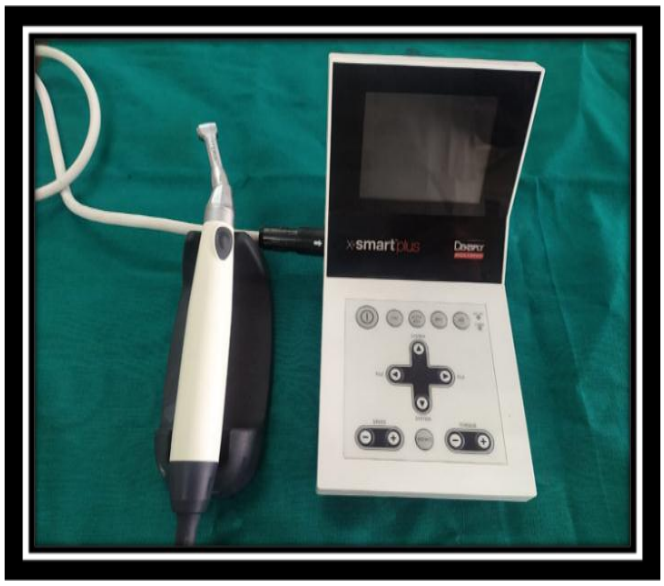


FIG. 15 ENDOMOTOR (X SMAT PLUS)

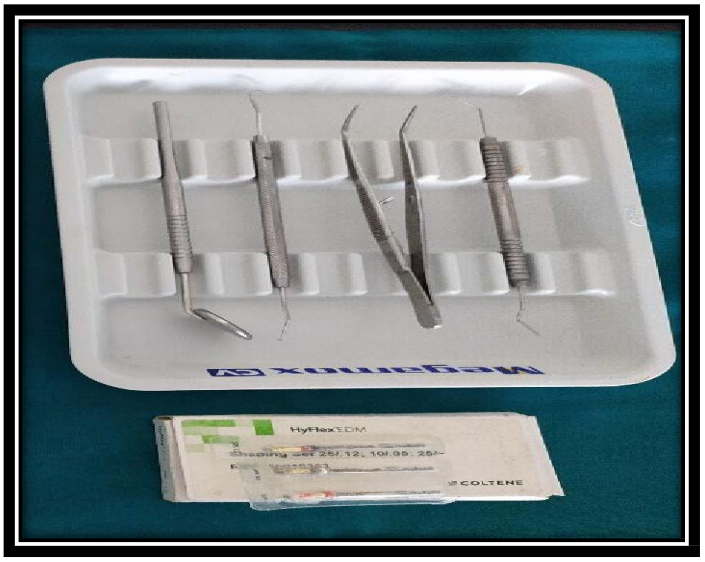


FIG. 16 HYFLEX EDM FILE SYSTEM AND DIAGNOSTIC INSTRUMENTS

FIG.17 ISOLATION WITH RUBBER DAM

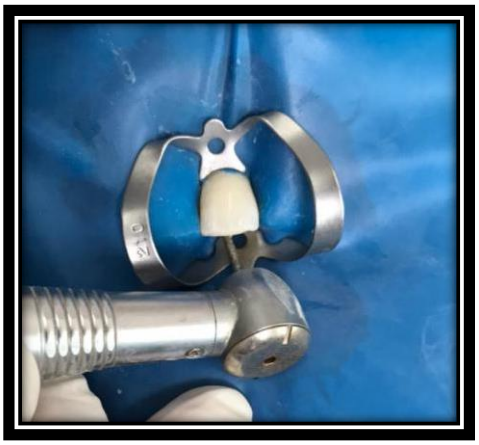


FIG. 18 ACCESS PREPARATION WITH ROUND BUR

Post treatment sample collection-

Disinfection was done according to selected protocol and the canals were treated according to the groups given below.

Group A-

Irrigation was done with 5ml of saline and then canal irradiated with Diode laser wavelength of 980nm was used at 1.5 watt power, frequencies 15 Hz, energy 21.2 j , average power-0.7watt, then, the fiber optic tip 200 micrometer was placed 1 mm short of the working lengh and reccess in circular movement with speed of 1 mm/sec and repeated six times at intervals of 10 seconds between each one. After completion of irradiation samples was taken with sterile paper point and designated as (Sample A2) .

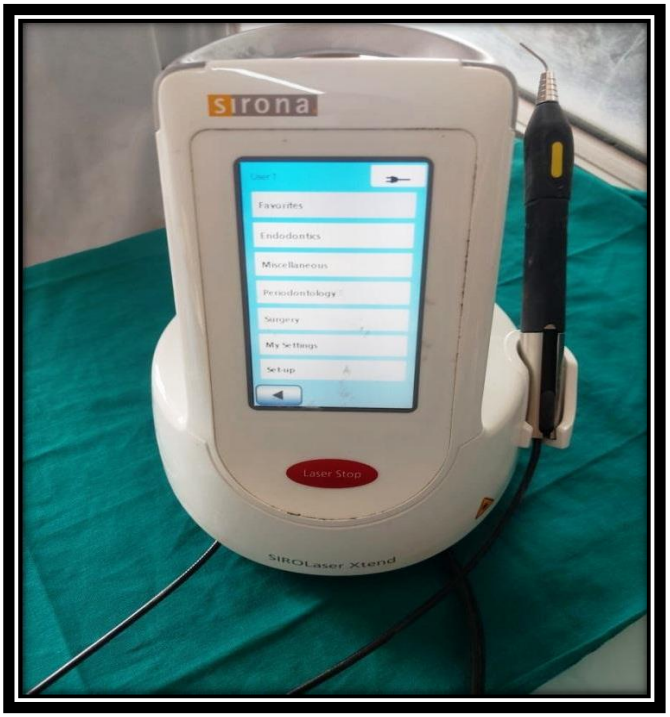


FIG. 20 Diode Laser (Densply, Sirona)

Group B-

The Canal was irrigated using 5ml of 5.25% NAOCL irrigating solution with 30 gauge side vented needle which was kept 1mm short of the apex. Then canal was flushed with saline and sample was taken with sterile paper point and designated as (Sample B2).

Group C-

First the canal was irrigated with 5.25% sodium hypochlorite and then irradiated with diode laser wavelength of 980nm was used with 1.5 watt power, frequencies 15 Hz, energy 21.2 j , average power- 0.7watt, then the fiber optic tip 200 micrometer was placed 1 mm short of the working lengh to recesse in circular movements with speed 1 mm/sec, and repeated six times at intervals of 10 seconds. And then sample collection was done with sterile paper point and designated as (Sample C2)

All the samples were transferred directly in thioglycolate broth and cultured in blood agar and were kept in an incubator at 37°C for 24 hours.

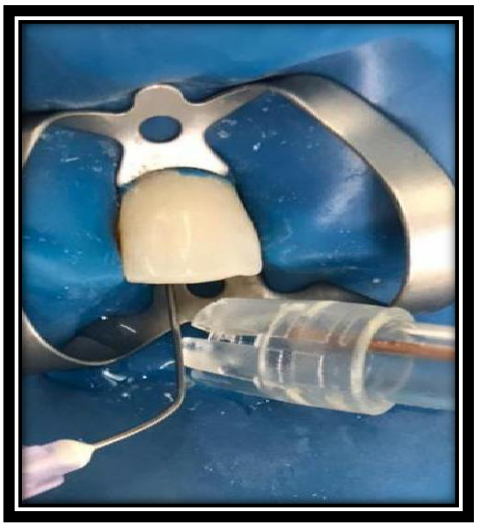


FIG.21 IRRIGATION

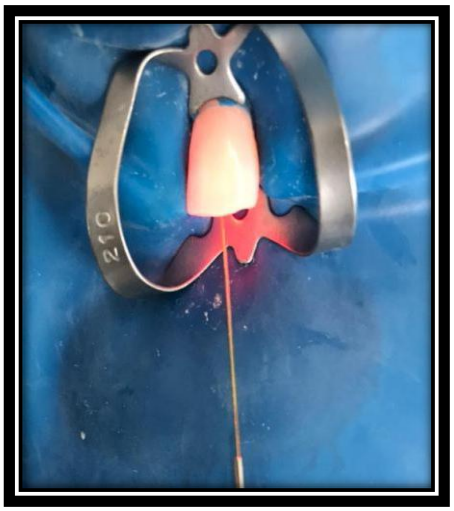


FIG. 22 DISINFECTION OF ROOT CANAL WITH DIODE LASER

FIG.23 COLLECTION OF SAMPLE WITH PAPERPOINT

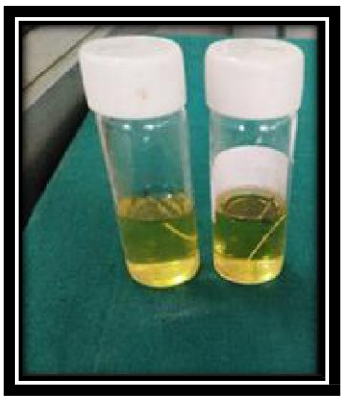


FIG.24 COLLECTED SAMPLES

FIG. 25 CULTURE PLATES

Lab procedure-

Department of Microbiology, Dr. Ram Manohar Lohia Institute of Medical Sciences ,Lucknow carried out microbiological culturing of the collected samples (paper point), inoculated in nutrient broth and Thioglycolate broth were vortexed and then incubated at 37degree Celsius for 24 hours to 48 hours to observe the turbidity.Calculation was done for counts per ml of diluted broth with multiplication by dilution factor.

Sample was plated on MacConkey agar and blood agar plates (HiMedia Lab Pvt. Ltd. India) with a calibrated loop . Incubation of agar plate was done for 24-48 hours at 37 degree celsius . and counted for the growth of bacteria and followed with manual colony counting for observing the efficacy of A, B, and C solutions. The similar procedure was performed in both the groups pretreatment and post treatment samples in order to figure out the effectiveness of the solution against the bacteria.

Gram staining and biochemical tests were used for identification of the bacteria

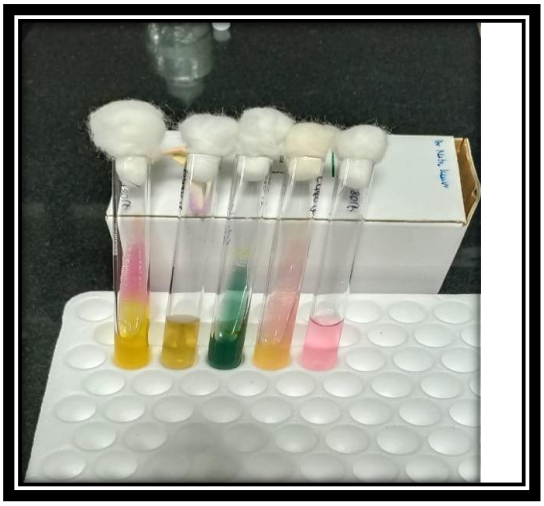


FIG. 26 ISOLATION OF BACTERIA **FIG.27 BIOCHEMICAL TESTS**

OBSERVATION AND RESULTS

Table 1: Comparison of colony count manually in three groups

Descriptive Statistics

GROUP	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
A. (Saline along with diode laser)	20	710890	752689	168306	358620.53	1063200	48200.00	3250000
B. (5.25% NaOCl alone)	20	478755	424803	94988.83	279941.07	677568.92	32400.00	1209600
C. (5.25% NaOCl and diode laser)	20	81766.75	87856.27	19645.26	40648.74	122884.75	3240.00	330800

- Krushkal Wallis Chisquare value = 8.04 ,p-value=0.001, Significant

Mean colony count manually in Saline along with diode laser group was 710890±752689, in 5.25% NaoOCL alone group it was 478755±424803 and in 5.25% NaOCL laser group it was 81766.75±87856.27. By using Krushkal Wallis Chisquare test statistically significant variation was found in mean colony count manually in three groups ofpatients(χ2-value=8.04,p-value=0.001).

Mann Whitney U Test

Group		Mean Difference (I-J)	Std. Error	p-value	95% Confidence Interval	
					Lower Bound	Upper Bound
Saline along with diode laser	5.25% NaOCl alone	232135	158610	0.316,NS	-149547.90	613817.90
	5.25% NaOCl and diode laser	629123	158610	0.001,S	247440.34	1.0108E6
5.25% NaOCl alone	5.25% NaOCl and diode laser	396988	158610	0.040,S	15305.34	778671.15

On comparing mean colony count manually count in three groups by using Mann Whitney U Test statistically significant difference was found between saline along with diode laser group and 5.25% NaOCL and diode laser group(p=0.001) and between 5.25% NaOCL alone and 5.25% NaOCL and diode laser group(p=0.040) and no significant difference was found between saline along with diode laser and 5.25% NaOCL along group (p=0.316).

Graph 1: Comparison of colony count manually in three groups

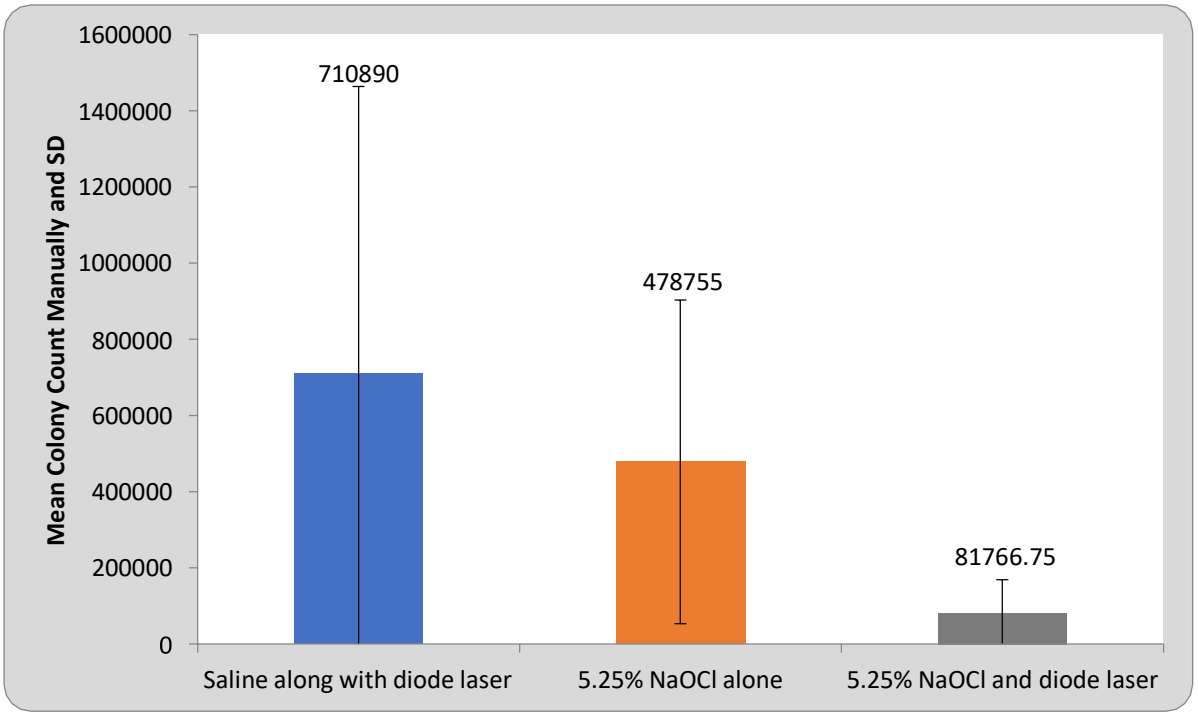


Table 2: Comparison of Reduction in Bacterial Colonies in three groups

Descriptive Statistics

Group	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Saline along with diode laser	20	320449.50	270677	60525.14	193768.91	447130.08	20610	826800
5.25% NaOCl alone	20	239735.00	263889	59007.30	116231.28	363238.71	9800	865700
5.25% NaOCl and diode laser	20	9984.00	15823.56	3538.25	2578.34	17389.65	0.00	54310

- Krushkal Wallis Chisquare value = 10.87 ,p-value=0.0001,Significant

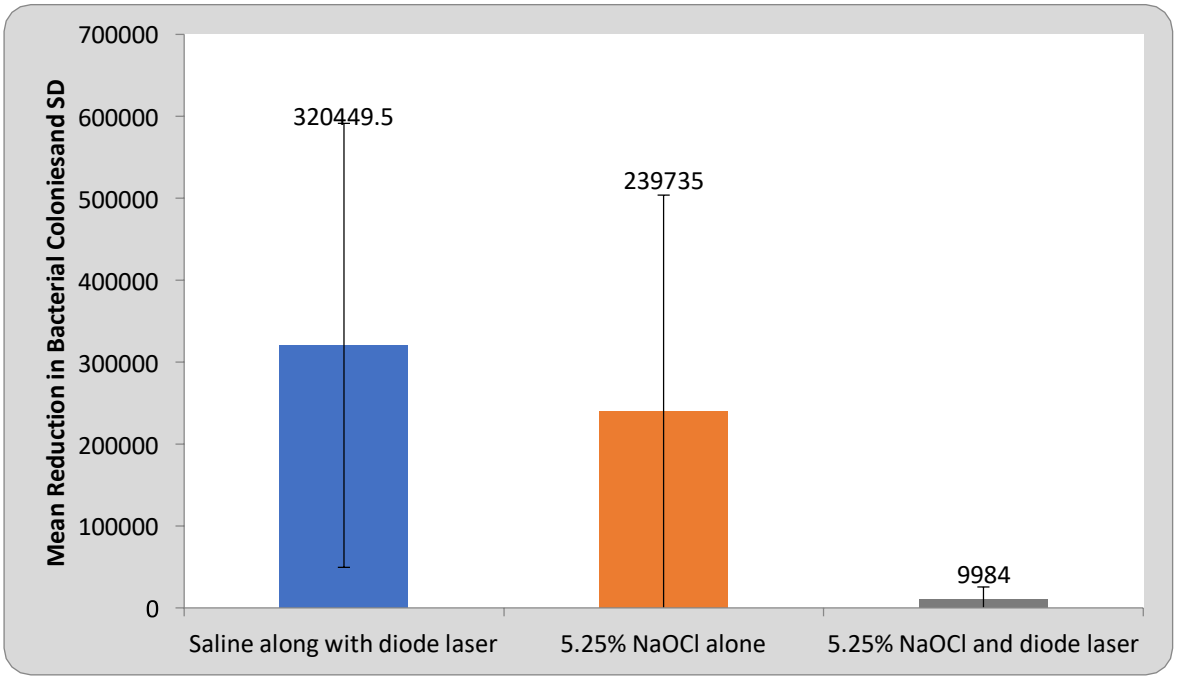
Mean reduction in bacterial colonies in Saline along with diode laser group was 320449.50±270677, in 5.25% NaoOCL alone group it was 239735±263889 and in 5.25% NaOCL laser group it was 9984±15823.56. By using Krushkal Wallis Chisquare test statistically significant variation was found in mean reduction in bacterial colonies in three groups of patients (χ2-value =10.87,p-value=0.0001).

Mann Whitney U Test

Group		Mean Difference (I-J)	Std. Error	p-value	95% Confidence Interval	
					Lower Bound	Upper Bound
Saline along with diode laser	5.25% NaOCl alone	80714.50	69078.09	0.477,NS	-85516.28	246945.28
	5.25% NaOCl and diode laser	3466	69078.09	0.0001,S	144234.71	476696.28
5.25% NaOCl alone	5.25% NaOCl and diode laser	2751	69078.09	0.004,S	63520.217	395981.78

On comparing mean reduction in bacterial colonies in three groups by using Mann Whitney U Test statistically significant difference was found between saline along with diode laser group and 5.25% NaOCL and diode laser group(p=0.0001) and between 5.25% NaOCL alone and 5.25% NaOCL and diode laser group(p=0.004) and no significant difference was found between saline along with diode laser and 5.25% NaOCL along group (p=0.477).

Graph 2: Comparison of Reduction in Bacterial Colonies in three groups



DISCUSSION

In Endodontic treatment for successful root canal therapy disinfection of the root canal system is an essential step.[4] Successful root canal treatment is a result of a combination of mechanical preparation by instrumentation and the use of disinfecting irrigating solutions. Therefore, root canal irrigation is essential, during and after instrumentation, to facilitate the proper removal of microorganisms, residual tissue, and debris from the root canal.[59]

For successful debridement and disinfection of the root canal system irrigation is an essential step.[30] The most frequently used irrigants are sodium hypochlorite, chlorhexidine, citric acid ethylenediaminetetraacetic acid, tetraclean and MTAD [mixture of doxycycline, citric acid, and a detergent] etc. [3]

Ideal property of an irrigant is it should be bactericidal, germicidal, and fungicidal, ability to serve as a lubricant during instrumentation, also dissolve organic and inorganic dentinal tissues (pulp tissue, collagen, and biofilm)

Non irritating to periapical tissues, Prolonged and sustainable antibacterial activity after use, activity in an environment in which blood, serum, and tissue protein products are present. And it should remove the smear layer completely.[60]

sodium hypochlorite and hydrogen peroxide, or the combined use of both are the most frequently used irrigation solutions (**Grossman 1981**). Their benefits, good tissue dissolving and disinfecting capability, NaOCl reacts with organic tissue, resulting in saponification, amino acid neutralization, and chloramine reactions. Owing to its solvent effect on necrotic tissues. **(Senia et al .1975, Zielke et al. 1976)**.NaOCl has become the most widely used irrigation solution in endodontics have been demonstrated in several investigations.

The concentration of the irrigants is still a matter of debate and remains controversial; many authors recommend a 5.25% concentration of sodium hypochlorite **(Harrison 1984**), others prefer a lower concentration of 3% or even 0.5% **(Baumgartner & Cuenin 1992 et al**).Sodium hypochlorite is highly effective antimicrobial agent, but it has some drawbacks also.[33]

Pashley et al (1985) concluded that it has many disadvantages such as unpleasant flavor, cytotoxicity, and potential for irritation in periapical tissues, especially at high concentrations.. However, it has been shown that NAOCL has toxic effects on vital organs and tissues, which can lead to haemolysis of blood, ulceration and necrosis of skin and mucosa.[30]

Because of the undesirable effects of this agent, the need of alternating irrigating agents has increased in recent years.

As adjunct to currently used disinfection methods in root canal disinfection various LASER systems have been examined to improve the efficacy of dynamic irrigation techniques.

The most recent development in endodontic treatment is the use of lasers. Since the development of the ruby laser by Maiman in 1960 and the application of the laser for endodontics by Weichman in 1971, a variety of papers on potential applications for lasers in endodontics have been published.[42]

The first laser use in endodontics was reported by Weichman& Johnson. The impact of the laser light depends on the interaction of the light quanta and the molecules and the molecular formations in the target material.[43]

Working of the laser and its effect on biological tissue is determined by interaction of laser radiation parameters, such as: wavelength, physical characteristics of the illuminated tissue, energy radiation, continuous or pulsed mode, diameter of the laser beam, and the exposure time.

As absorption of the LASER beam by both hydroxyapatite and water is less, the penetration of diode LASER energy into the dentinal tubules is better than erbium, chromium: yttrium-scandium-gallium-garnet laser. Diode laser works efficiently at low power with less heat production with power output from 0.5 Watt to 7 Watt.[50]

The diode LASER causes a thermal and photodisruptive action which leads to superior bactericidal effect and greater depth of penetration of laser beam (more than 1000 micro meter into dentinal tubules). In addition to the diode laser leads to enhanced bactericidal effect in unreachable parts of dentin [51]

Further studies done by **Tilakchand, et al, Sonarkar SS et al , Mehta et al.** have revealed that LASER is more efficient than commonly used irrigants, such as 5.25% sodium hypochlorite , 10% citric acid and 17% Ethylenediaminetetraacetic acid).[22] The use of diode LASER in endodontic treatment has been increasing to enhance the efficiency of these irrigating solutions.

To evaluate the antimicrobial efficacy of diode laser and conventional chemomechanical treatment a number of in-vitro studies are available but there are limited studies about their efficacy in vivo.

So the present study was designed to comparatively evaluate the effectiveness of 980nm diode laser, 5.25% sodium hypochlorite separately and in combination.

The present study included 60 patients as they showed statistical significance and reliable results. After selecting the patients ,they were randomly divided into three groups, then access opening was done with endo access bur (Mani, Japan) and biomechanical preparation performed using Hyflex EDM file system.

Hyflex EDM (COLTENE) rotary is a one file system, which has controlled memory, greater flexibility and extreme fracture resistance ,retain their shape in curved canals and do not possess the „Spring back action, thereby avoiding any perforation. The conventional NiTifiles have another drawback of not giving a „warning sign" before breakage (unlike stainless steel files, which show such signs in the form of unwinding of flutes, or presence of a shiny spot on the file, indicating that the file should be discarded).[62]

After biomechanical preparation disinfection was done according to the protocol. In this present study 30 gauge needle was used for irrigation, researchers have found that thechemical agents have a limited effect distal to the tip of the needle as the air in the apex prevents the solution to reach the apical tip. conventional syringe irrigation transmitted solutions go no more than 0-1.1 mm beyond the needle tip.[6] hence preventing effective cleansing. However, smaller needles allow the solution to enter the apex of root canal but with safety concerns and facilitate effectiveness and minimize safety risks.[61]

Sodium hypochlorite was used as the irrigant of choice was NAOCL because of its wide use in endodontics, it has broad spectrum antimicrobial action, and function to dissolve and hydrolyse organic tissue in the root canal.

Effectiveness of NAOCL is dependent on various factors such as concentration, contact time ,increasing temperature ,volume, and surface tension of solution **(Nio, 2017)** [19]

5.25% NaOCl is significantly more efficient in eliminating microorganisms and in particular the resistant Enterococcus faecalis as compared to the antimicrobial efficacy of 1.3% NaOCl (Kho and Baumgartner, 2006).[30]

The highest number of isolates with no microbial growth (CFU = 0) was observed with NAOCL in a study done by **Ashofteh et al. and Sohrabi** et al. and study done by Baumgartner et al also agrees with this.[28]

Similar study done by **Waltimo, Kudva et al., and Marcia et al (2014) also** considered NAOCL as most potent irrigant for its capacity to kill microorganism when NAOCL was used for sufficient time (Gołąbek et al., 2019; Battista and Pantera, 2019; Gazzaneo et al., 2019; Mohmmed, 2017).

Raphael, et al. use of 5.25% NAOCL on S. faecalis, S. aureus, and Pseudomonas aeruginosa at 21 degree celsius and 37 degree celsius and observed that increase in temperature has no difference on bactericidal effect of irrigation solution and may even decrease it.

Buttler and Crawford, using E. coli and S.typhi , studied 0.58%, 2.7%, and 5.20% NAOCL for its ability to nutrilize endotoxin. Effectiveness of all 3 concentrations were equal, although large amounts of E. coli endotoxin could not be neutrilize by 0.58% or 2.7% NAOCL.

Many studies have showen that the infected pulp tissue, and layers of root canal dentin can only be removed to a certain extent by conventional root canal treatment. While lateral and accssesory canal of the root canal system are limited to the extent of mechanical preparation. Chemical irrigation solutions are only effective in dentin layers adjacent to the canal walls.

Parhar (2012) found that the infection (bacteria) may occur around 1000 μm but depth of penetration of chemical disinfectants is limited to a range about 130 μm.[36]

According to **Gunwal et al.** to 5.25% NaOCl reduces microbial count more significantly as compared to 810nm diode laser. This result is similar to other studies whether ex-vivo or in-vivo, which were done using culture dependent or independent methods, stating that chemomechanical treatment is very effective in reducing microbial growth in most of the cases (Gazzaneo et al., 2019). However, in spite of the instrument used, sodium hypochlorite concentration, exposure time, and volume it is not possible to render the root canal bacteria free **(Gazzaneo et al., 2019).**[64]

So to overcome these drawbacks Lasers have become recent choice to disinfect root canals especially lateral dentinal tubuli. This eradication of microorganism has been achieved by a fiber delivery system. [2]

So in the present study saline along with diode laser was used in group A. saline was used as an irrigating solution. Saline will not synergistically help in antimicrobial action and will not act as a lubricant but it will help in flushing the debris out of the canal during chemomechanical preparation.

Study done by Moritz et al . showed that treatment of root canals with 2W diode laser (810nm), when the irradiation was repeated 5 times at each laser treatment, each time for a period of 5 sec with short breaks in-between, a maximum of two irradiations resulted in nearly complete elimination of E.faecalis.**Gutknecht et al.** also demonstrated that diode lasercan eradicate microbes that have migrated deep into the dentine and more specifically Enterococcus faecalis (Gutknecht et al.,2004).[52]

An early study by **Gutknecht et al**. (**1996)** reported that 83% of infected cases were treated successfully, after been unsuccessfully treated by conventional chemomechanical method.[55]

Olivi, 2013 who concluded that Diode laser at 810 nm , achieved a microbial reduction of 74%, whereas, 980 nm achieved a maximum microbial reduction**.**[64]

Also Njwan F. Sehaba et al done a study and showed that 10 sec (6 cycles) exposure time to laser, was more effective than 5 sec (6 cycles) at each output power, and no significant difference between 2W and 2.5W at 5 sec (6 cycles). These results indicated that time plays an important role in root canal disinfection when using laser. So in this present study we have used 1.5 WATT at 10 seconds (6cycles).[12]

The study done by Lee and his colleagues (2006) found that continuous movement of fiber tip reduced the thermal effect and simultaneously reached high bactericidal efficiency . **Radaelli et al**. study showed that maximum temperature changes of 7.45°C (ΔT) following the application of 830 nm diode lasers (3 and 2.5 W) (CW) in the safe area. **Gutknecht et al.** During the procedure, the fiber optic tip was directed out of the canal with a helicoidal motion in the speed of 2 mm/s. Moreover, the tissues underwent a relaxation time to modulate temperature changes.[41]

So in our study the fiber optic of laser tip was constantly kept with continuous movements inside the root canal to reduce the thermal effect of laser.

Therefore, it can be used as an adjuvant to root canal disinfection treatment. Considering all available results only **Jha et al.** study was inconsistent with our results on the use of the

diode lasers. Jha et al noted the inability of laser and rotary instrumentation in disinfecting the root canal.

Mashalkar et al. and Agrawal AA et al. also concluded from their in-vivo comparative study that conventional method of root canal disinfection using sodium hypochlorite and hydrogen peroxide as irrigating solutions were highly effective, however lasers when used can also reduce the bacterial load of the infected root canal.[7]

In present study in **group C** we have used 5.25% sodium hypochlorite along with diode laser. Laser irradiation is done at the conclusion of endodontic shaping just prior to obturation as a final means of decontamination. Clinically a fine 200µm diameter fiber optic tip is positioned 1mm short of the apical terminus and using a helical movement the tip is retracted coronally at 10 seconds(6 cycles). The lasing procedure is done in canal filled with an irrigant such as NaOCl to avoid undesirable thermal effects and to enhance the antibacterial efficacy .[49]

The result of the present study are accordance with the studies done by

Kreisler et al., (2003) investigated the bactericidal effect of a semiconductor laser used in combination with NaOCl/ hydrogen peroxide (H2 O2) irrigation, or saline alone, and found that the former resulted in significant bactericidal reduction compared to the use of laser alone.[57]

Tilakchand et al done "Evaluation of the antibacterial efficacy of EZLASE diode LASER on the infected root canal system:" An in vivo study and concluded that Combination therapy consisting of irrigation using NaOCl and LASER irradiation is an effective treatment option for reduction in E. faecalis as well as other bacterial flora from the root canal system.[4]

According to Thomas et al., Mithra et al., Castelo-Baz et al., and Krishna Shetty et al. which concluded that the diode used in conjunction with conventional chemomechanical techniques demonstrated potential antimicrobial activity against E. faecalis in root canals.[14]

Collection of sample was done with presterilized paper point , because Paper point cultures of the root canal detected bacteria more frequently than dentine filling cultures on the reamers ,and hence it was the preferred mode of sample collection throughout the present study.

All the samples were transferred in thioglycolate broth and cultured at 37 degree celsius for 24 hours.

Another Limitation in laser application is rise in temperature which eventually damage to the periapical area, especially when the roots are close to proximity of mental foramen, canal of the inferior alveolar nerve or maxillary sinus. Passage of laser beam through the apex of the roots can damage this anatomic region.[24]

On the basis of this study it can be concluded that Diode laser with Sodium hypocloride is more effective in disinfection of the root canal., Further studies are required to investigate theclinical effectiveness of this approach due to limitations and complications during treatment procedures in a clinical situation.

CONCLUSION

The chemomechanical preparation forms an integral part of root canal treatment. The eradication of persisting microorganisms in distant areas of the tubular system is a major challenge in the present-day treatment regimen.

Sodium hypochlorite has been demonstrated to be most effective irrigant against broad spectrum bacteria.

Findings of this study also indicate that sodium hypochlorite and diode laser had antimicrobial effect. However, combined effect of diode laser with NaOCl found to be more significant in bacterial colony count reduction.

Due to limitations and complications during treatment procedures in a clinical situation, further studies are required to investigate the clinical effectiveness of this approach.

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ANNEXURES

ANNEXURES-1

BABU BANARASI DAS COLLEGE OF DENTAL SCIENCES (FACULTY OF BBD UNIVERSITY), LUCKNOW

INSTITUTIONAL RESEARCH COMMITTEE APPROVAL

(Revised)

The project titled **"Comparative evaluation of root canal disinfection with different techniques" An *In-Vivo* Study."** submitted by **Dr Ruchi Gupta** Post graduate student from the **Department of Conservative Dentistry and Endodontics** as part of MDS Curriculum for the academic year 2018-2021 with the accompanying proforma was reviewed by the Institutional Research Committee present on **28th January, 2021** at BBDCODS.

The Committee has granted approval on the scientific content of the project. The proposal may now be reviewed by the Institutional Ethics Committee for granting ethical approval.

Prof. Vandana A Pant
Co-Chairperson

Prof. B. Rajkumar
Chairperson

ANNEXURES-2

Babu Banarasi Das University
Babu Banarasi Das College of Dental Sciences,
BBD City, Faizabad Road, Lucknow – 226028 (INDIA)

Dr. Lakshmi Bala
Professor and Head Biochemistry and
Member-Secretary, Institutional Ethics Committee

Communication of the Decision of the VIIth Institutional Ethics Sub-Committee

IEC Code: 25 (Revised) **BBDCODS/02/2021**

Title of the Project: "Comparative evaluation of root canal Disinfection with Different techniques"

Principal Investigator: Dr. Ruchi Gupta **Department:** Conservative Dentistry and Endodontics

Name and Address of the Institution: BBD College of Dental Sciences Lucknow.

Type of Submission: Revised, MDS Project Protocol

Dear Dr. Ruchi Gupta,

The Institutional Ethics Sub-Committee meeting comprising following four members was held on 2nd February, 2021.

1.	Dr. Lakshmi Bala Member Secretary	Prof. and Head, Department of Biochemistry, BBDCODS, Lucknow
2.	Dr. Amrit Tandan Member	Prof. & Head, Department of Prosthodontics and Crown & Bridge, BBDCODS, Lucknow
3.	Dr. Rana Pratap Maurya Member	Reader, Department of Orthodontics & Dentofacial Orthopedics, BBDCODS, Lucknow
4.	Dr. Akanksha Bhatt Member	Reader, Department of Conservative Dentistry & Endodontics, BBDCODS, Lucknow

The committee reviewed and discussed your submitted documents of the current MDS Project Protocol in the meeting.
The comments were communicated to PI thereafter it was revised.

Decisions: The committee approved the above protocol from ethics point of view.

Forwarded by:

Lakshmi Bala 02/02/21

(Dr. Lakshmi Bala)
Member-Secretary
IEC

Member-Secretary
Institutional Ethics Committee
BBD College of Dental Sciences
BBD University
Faizabad Road, Lucknow-226028

(Dr. B. Rajkumar)
Principal
BBDCODS

ANNEXURES-3

CONSENT FORM

Title of the study…….

Study Number…….

Patient Full Name……

Date of Birth/Age…….

Address …….

Phone No. and email address……...

Occupation: Student/Self employed/Service/Housewife/Other

1. I confirm that I have read and understood the Participant Information Document dated ……. for the above study and have had the opportunity to ask questions

OR

I have been explained the nature of the study by the investigator and had the opportunity to ask questions.

2. I understand that my participation in the study is voluntary and given with the free will without any duress and that I am free to withdraw at any time, without given any reason and without my medical care or legal rights being affected.

3. I understand that the sponser of the project, others working on the sponsor's behalf, the Ethics Committee and the regulatory authorities will not need my permission to look at my health records both in respect of the current study and any further research that may be conducted in relation to it, even if I withdraw from the trail. However, I understand that my identity will not be revealed in any information released to third parties or published.

4. I agree not to restrict the use any data or results that arise from this study provided such a use is only for scientific purpose(s).

5. I agree to participate in the above study for the future research

Yes [ ] No [ ] Not Applicable [ ]

6. I have been explained about the study, and have fully understood them. I have also read and understand the participant/volunteer's information document given to me.

Signature/Thumb impression of the subject/Legally acceptable

Representative…….

Signatory's Name……..Date…….....

Signature of Investigator's Name……

Study Investigator's Name…….Date……..

Signature of the witness……

Name of witness……..Date……..

Received a signed copy of the duly filled consent form

Signature/Thump Impression of the subject/Legally acceptable

representative…….Date……

ANNEXURES-4

सहमति पत्र

अध्ययन का शीर्षक .....।
अध्ययन संख्या .....।
विषय का पूरा नाम .....
जन्म तिथि / आयु .....।
विषय का पता .....।
फ़ोन नंबर और ईमेल पता ....।
योग्यता .....
व्यवसाय: छात्र / स्वयं कार्यरत / सेवा / गृहिणी / अन्य
1. मैं पुष्टि करता हूं कि मैंने प्रतिभागी सूचना दस्तावेज को पढ़ा और समझा है .....। उपरोक्त अध्ययन के लिए और प्रश्न पूछने का अवसर मिला है

या

मुझे अन्वेषक द्वारा अध्ययन की प्रकृति के बारे में समझाया गया है और मुझे प्रश्न पूछने का अवसर मिला है।
2. मैं समझता हूं कि अध्ययन में मेरी भागीदारी स्वैच्छिक है और बिना किसी ड्यूरेस् के स्वतंत्र इच्छा के साथ दी गई है और मैं बिना किसी कारण के बिना किसी भी समय वापस लेने के लिए स्वतंत्र हूं, और मेरी चिकित्सा देखभाल या कानूनी अधिकारों को प्रभावित किए बिना।
3. मैं समझता हूं कि परियोजना के प्रायोजक, प्रायोजक की ओर से काम करने वाले अन्य लोग, आचार समिति और नियामक अधिकारियों को वर्तमान अध्ययन के संबंध में मेरे स्वास्थ्य रिकॉर्ड को देखने के लिए मेरी अनुमति की आवश्यकता नहीं होगी और आगे कोई शोध हो सकता है इसके संबंध में आयोजित किया जाता है, भले ही मैं निशान से वापस लेता हूं। हालाँकि, मैं समझता हूँ कि मेरी पहचान तीसरे पक्ष को जारी किसी भी सूचना में या प्रकाशित होने पर प्रकट नहीं होगी
4. मैं इस अध्ययन से उत्पन्न किसी भी डेटा या परिणामों के उपयोग को प्रतिबंधित नहीं करने के लिए सहमत हूं, बशर्ते ऐसा उपयोग केवल वैज्ञानिक उद्देश्य के लिए हो।
5. मैं भविष्य के अनुसंधान के लिए उपरोक्त अध्ययन में भाग लेने के लिए सहमत हूं
हाँ [] नहीं [] लागू नहीं []
6. मुझे अध्ययन के बारे में समझाया गया है, और उन्हें पूरी तरह से समझा है। मैंने प्रतिभागी / स्वयंसेवक के सूचना दस्तावेज को भी पढ़ और समझ लिया है।

विषय के हस्ताक्षर / अंगूठे का निशान / कानूनी रूप से स्वीकार्य
प्रतिनिधि .....।
हस्ताक्षरकर्ता का नाम .....
अन्वेषक के नाम पर हस्ताक्षर .....
अन्वेषक का नाम ..... .डेट ....
साक्षी का हस्ताक्षर .....
साक्षी का नाम ....
विधिवत भरे हुए सहमति फॉर्म की एक हस्ताक्षरित प्रति प्राप्त की
विषय / कानूनी रूप से स्वीकार्य प्रतिनिधि के हस्ताक्षर / गांठ छाप ..... .Date .....

To
The HOD,
Department of Microbiology
Ram Manohar Lohia Institute of Medical Sciences,
Lucknow.

Subject- Regarding Culture of root canal samples

Respected Madam,

I Dr. Tanushri Chatterji, Department of Microbiology, **Babu Banarasi Das College of Dental Sciences Lucknow,** would like to collaborate with your department to conduct culture of root canal samples for the thesis work of one of my MDS student. The thesis work entitled ***"Comparison of disinfecting potential of chemical method and combination of laser with chemical method AN IN VIVO study"***. I would like to carry out the work under the guidance of Dr. Manodeep Sen. Kindly grant permission for the same. I am enclosing the work plan for your reference.

Thanking you.

Dr. B.Rajkumar
Principal
BBD College of Dental Sciences,
Lucknow

Dr. Tanushri Chatterji
Reader
Department of Microbiology
BBD College of Dental Sciences
Babu Banarasi Das University , Lucknow

Enclosure: work plan

Forwarded

Prof. JYOTSNA AGARWAL
Professor and Head
Department of Microbiology
Dr. R.M.L.I.M.S., Lucknow

ANNEXURES-5

Babu Banarasi Das University

Babu Banarasi Das College of Dental Sciences

DEPARTMENT OF CONSERVATIVE DENTISTRY & ENDODONTICS
CASE HISTORY PROFORMA AND PATIENT INFORMATION

NAME :
AGE / SEX :
MARITAL STATUS :
ADDRESS :
PHONE NO :
OCCUPATION :

Date
O.P.D. No.

CHIEF COMPLAINT :

HISTORY OF PRESENT ILLNESS :

Pain :

Symptoms	Location	Quality	Frequency	Affected by	Prior Rx
	Localised	Sharp Intensity	Constant	Hot / Palpation / Cold	Restoration
	Diffuse	Dull +++ ++++	Momentary	Manipulation - Head position - Any activity	Emergency
	Referred	Pulsating Spontaneous	Intermittent	Biting	R.C.T.
	Radiating	Steady Provoked Occasional	Lingering	Chewing Percussion	

[1]

MEDICAL HISTORY :

Cardiac disorder -
Hypertension -
Diabetes Mellitus -
Renal Disorder -
Bleeding Disorder -
Drug Allergy -
Any Other -

PAST DENTAL HISTORY -

PERSONAL HISTORY -

Habits -
Brushing Schedule -

Clinical Examination :

Extraoral Examination :

Lips -
Lymph Node -
T.M.J. -
Swelling -
Fistula / Sinus -
Any Other -

[2]

Intraoral Examination -

Soft Tissue -

Labial Mucosa -

Buccal Mucosa -

Tongue -

Palate -

Floor of the mouth -

Gingiva -

Hard Tissue Examination (DMFT INDEX)-

18	17	16	15	14	13	12	11	21	22	23	24	25	26	27	28
48	47	46	45	44	43	42	41	31	32	33	34	35	36	37	38

NUMBER OF DECAYED TEETH

NUMBER OF MISSING TEETH

NUMBER OF FILLED TEETH (TYPE OF RESTORATION I.E. SILVER AMALGAM/GIC/COMPOSITE OR PROVISIONAL RESTORATION / CAST RESTORATION AND CROWN)
ANY OTHER FINDING (**supernumerary teeth, deciduous teeth**)

Tooth / Teeth : Discolouration Caries Pulp exposure

Attrition / Abrasion Fracture

Missing Supernumerary

Restoration Amalgam Composite Glass Ionomer

Inlay / Onlay Temporary Crown Faulty

[3]

Periodontal Status: Mobility - Nil / Grade

Vitality Test
- Thermal
  - Hot Test
  - Cold Test
- Electric Pulp Test
- Test cavity

Provisional Diagnosis -

[4]

Radiograph -

Findings -

Normal -
Widened Periodontal Space -
Apical Rarefaction -
Faulty R.C.T. -
Root / Crown Fracture -
Curved Root -
Internal Resorption -
Pulp Calcification -
Incomplete Root Formation -
Periapical Pathology -

Final Diagnosis :

Treatment Plan :

Prognosis : Favourable Questionable Poor Hopeless

Case Alloted to : :

- 3rd Year students for class I/ Class V
- 4th Year Students for Class II / Class III / Class IV
- Interns for Anterior RCT / Composite
- Post Graduate for all cases including emergency cases

SIGNATURE -

[5]

ANNEXURE-6

Formula used for the analysis

Arithmetic Mean

The most widely used measure of central tendency is arithmetic mean, usually referred to simply as the mean, calculated as

$$X = \frac{\sum_{i=1}^{n} X_i}{n}$$

Standard deviation and standard error

The standard deviation (SD) is the positive square root of the variance, and calculated as

$$SD = \sqrt{\frac{\sum X_i^2 - \frac{(\sum X_i)^2}{n}}{n-1}}$$

and SE (standard error of the mean) is calculated as

$$SE = \frac{SD}{\sqrt{n}}$$

where, n= no. of observations

Minimum and Maximum

Minimum and maximum are the minimum and maximum values respectively in the measure data and range may be dented as

Range = Min to Max or Min-Max

and also evaluated by subtracting minimum value from maximum value as

Range = Maximum value-Minimum value

Mann Whitney U Test

The modules on hypothesis testing presented techniques for testing the equality of means in two independent samples. An underlying assumption for appropriate use of the tests described was that the continuous outcome was approximately normally distributed or that the samples were sufficiently large (usually $n_1 \geq 30$ and $n_2 \geq 30$) to justify their use based on the Central Limit Theorem. When comparing two independent samples when the outcome is not normally distributed and the samples are small, a nonparametric test is appropriate.

A popular nonparametric test to compare outcomes between two independent groups is the Mann Whitney U test. The Mann Whitney U test, sometimes called the Mann Whitney Wilcoxon Test or the Wilcoxon Rank Sum Test, is used to test whether two samples are likely to derive from the same population (i.e., that the two populations have the same shape).Some investigators interpret this test as comparing the medians between the two populations. Recall that the parametric test compares the means (H_0: $\mu_1=\mu_2$) between independent groups.

In contrast, the null and two-sided research hypotheses for the *nonparametric test* are stated as follows:

H_0: The two populations are equal versus

H_1: The two populations are not equal.

This test is often performed as a two-sided test and, thus, the research hypothesis indicates that the populations are not equal as opposed to specifying directionality. A one

sided research hypothesis is used if interest lies in detecting a positive or negative shift in one population as compared to the other. The procedure for the test involves pooling the observations from the two samples into one combined sample, keeping track of which sample each observation comes from, and then ranking lowest to highest from 1 to n_1+n_2, respectively.

Test Statistic for the Mann Whitney U Test

The test statistic for the Mann Whitney U Test is denoted **U** and is the ***smaller*** of U_1 and U_2, defined below.

$$U_1 = n_1 n_2 + \frac{n_1(n_1+1)}{2} - R_1$$

$$U_2 = n_1 n_2 + \frac{n_2(n_2+1)}{2} - R_2$$

where R_1 = sum of the ranks for group 1 and R_2 = sum of the ranks for group 2.

Kruskal–Wallis one-way analysis of variance

The **Kruskal–Wallis test** by ranks, **Kruskal–Wallis *H* test** (named after William Kruskal and W. Allen Wallis), or **one-way ANOVA on ranks** is a non-parametric method for testing whether samples originate from the same distribution. It is used for comparing two or more independent samples of equal or different sample sizes. It extends the Mann–Whitney *U* test, which is used for comparing only two groups. The parametric equivalent of the Kruskal–Wallis test is the one-way analysis of variance (ANOVA).

A significant Kruskal–Wallis test indicates that at least one sample stochastically dominates one other sample. The test does not identify where this stochastic dominance occurs or for how many pairs of groups stochastic dominance obtains. For analyzing the specific sample pairs for stochastic dominance, Dunn's test, pairwise Mann–Whitney tests with Bonferroni correction, or the more powerful but less well known Conover–Iman test are sometimes used.

Since it is a nonparametric method, the Kruskal–Wallis test does not assume a normal distribution of the residuals, unlike the analogous one-way analysis of variance. If the researcher can make the assumptions of an identically shaped and scaled distribution for all groups, except for any difference in medians, then the null hypothesis is that the medians of all groups are equal, and the alternative hypothesis is that at least one population median of one group is different from the population median of at least one other group.

ANNEXURES-7

Table 1: Reduction of number of bacterial colonies in Group A (Saline along with diode laser)

patients no.	Tooth No.	Colony count Manually	Reduction in bacterial colonies
1	21	336000	224000
2	22	378000	328800
3	21	182600	164200
4	11	48200	30200
5	21	818400	425200
6	23	202800	83000
7	21	1260000	826800
8	11	1411200	655200
9	12	1008000	604000
10	21	503200	236200
11	21	182600	94200
12	22	1411200	755400
13	11	173600	93100
14	23	504000	239100
15	21	672000	236200
16	11	162400	75280
17	22	1209600	826800
18	11	50400	20610
19	21	453600	362100
20	22	3250000	128600

Table 2: Reduction of number of bacterial colonies in Group B (5.25%NaOCl alone)

Patient no.	Tooth No.	Colony count Manually	Reduction in bacterial colonies
1	21	273600	98000
2	22	173600	52400
3	21	162400	78400
4	11	32400	9800
5	11	604800	326900
6	22	173800	81600
7	23	1158100	765700
8	11	1209600	513000
9	13	957600	246000
10	21	460200	210800
11	11	174800	60400
12	23	1209600	715000
13	11	86600	41500
14	12	403200	210800
15	11	554400	157500
16	12	134400	76300
17	23	1159200	865700
18	22	41300	21700
19	12	431900	201700
20	22	173600	61500

Table 3: Reduction of number of bacterial colonies in Group C (5.25%NaOCl along with diode laser)

Patient No.	Tooth No.	Colony count Manually	Reduction in bacterial colonies
1	21	173600	0
2	22	7360	0
3	22	16245	2150
4	11	3240	1200
5	21	70490	41250
6	12	73800	15000
7	22	158100	26200
8	23	219600	0
9	12	5760	0
10	23	46020	0
11	11	17480	0
12	21	120960	54310
13	11	8660	0
14	12	40320	10150
15	22	54400	31500
16	12	24400	2520
17	11	169200	5700
18	23	21300	0
19	12	330800	5600
20	23	73600	4100

URKUND

Urkund Analysis Result

Analysed Document:	plagrism report.docx (D110227249)
Submitted:	7/7/2021 9:30:00 AM
Submitted By:	1180322004@bbdu.ac.in
Significance:	9 %

Sources included in the report:

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Instances where selected sources appear:

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ANNEXURES

ANNEXURES