EVALUATION OF ANTIBACTERIAL EFFICACY OF NANOCARE+, A NANOBASED ENDODONTIC IRRIGATION SOLUTION: AN IN-VIVO STUDY

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ABSTRACT

Aim and Objectives: This in-vivo study was conducted to assess the antibacterial efficacy of Nanocare+ as an Endodontic irrigation solution.

Materials and Methods: The study was conducted on sixty permanent Maxillary Central Incisors of patients within age group of 25-35 years. Selected teeth were divided into two groups: Group A: Experimental group (Nanocare+); Group B: control group comprising each of thirty teeth.

The access cavity was prepared and canals were negotiated with path finders. Microbiological sample was collected from the root canal with the help of sterile paper points. Working length was determined following Ingle's method and canals were prepared using Step-Back technique till Master Apical File #60.

After canal preparation, sample was again taken using paper points. Then the teeth in Experimental groups were irrigated with Nanocare+ and with normal saline in the control group. Microbial sample was again taken with paper points both from the Experimental group and Control group. Culture analysis was used to assess the antimicrobial effectiveness of Nanocare+ (Group A) and normal saline (Group B). Microbial count as CFU/ml after access opening, canal preparation and irrigation with final irrigant in each group was determined.

Results: Highly significant absolute and percentage reduction in CFU/ml count was found in the experimental group i.e. Nanocare+.

KEY WORDS

Antibacterial Efficacy, Endodontic Irrigant, Nanocare+

INTRODUCTION

Pulp and periapical diseases occur as a result of bacteria invading into dentin and further into the root canal system of the affected teeth. The main objectives of root canal therapy are removal of infected pulp tissue, eradication of micro-organisms and preventing recontamination of pulp space after treatment. Mechanical preparation alone may not be able to create a sterile space because of complexity of root canal system. To overcome this dilemma, other modalities have been tried in the past, viz. chemical preparation, lasers, irrigants in different forms (single or in combination) etc.

Irrigants play an important role in areas inaccessible to instruments, such as lateral and accessory canals as well as fins and webs throughout the canal. Thus, they are essential for successful debridement of the root canals after mechanical shaping procedures. An ideal root canal irrigant should be biocompatible, non toxic, have broad antimicrobial spectrum, ability to dissolve necrotic pulp tissue and inactivate endotoxin. The routinely used root canal irrigant, Sodium hypochlorite has antimicrobial properties; however, accidental dripping may cause edema, ecchymosis, tissue necrosis and even paraesthesia. Over the years, a great variety of endodontic root canal irrigantshave been introduced having superior antibacterial properties and better biocompatibility.

In the recent past, nanobased materials have shown promising results in different scientific studies. An important group of nanomaterials for biological applications are nanobased antimicrobial agents. The use of nanoparticles in endodontic disinfection, introduced a couple of years back, have shown positive results. Nanosilver or silver nanoparticles consist of nano sized structures that range from 1 to 100nm containing twenty to

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fifteen thousand silver atoms. Due to its strong antibacterial activity, nanosilver coatings are put on certain implants. Furthermore, nanosilver is used for treatment of wounds and burns or as a contraceptive and also marketed as water disinfectant and room spray. At the nanoscale, particles exhibit different physiochemical, biological and optical features. Increased surface area to volume ratio results in the increased reaction between nanoparticles and target molecules in a very short time.(1)

ecently, 'Nanocare+' (Dental nanotechnology, Katowice, Poland) is introduced which is nanosilver and nanogold based root canalirrigant. It is available in liquid form, and is used as final rinse. The manufacturers claim that it leaves a layer of nanoparticles of silver and gold on the canal surfaces, which has a strong bacteriostatic effect and prevents colonization of micro-organisms. Low surface tension of the 'Nanocare+' carrier substance allows nanoparticles to get into the smallest intricacies and even dentinal tubules.

Since the literature is deficient in relation to the antibacterial efficacy of 'Nanocare+' root canal irrigant, the purpose of present study was to evaluate the antibacterial efficacy of this novel root canal irrigant.

MATERIALS AND METHODS

Sixty permanent maxillary central incisors were selected from Department of Conservative Dentistry and Endodontics, Punjab Government Dental College & Hospital, Amritsar to evaluate the antimicrobial efficacy of Nanocare+, an endodontic irrigation solution. Case selection was carried out according to clinical and radiographic evaluation. The whole procedure of root canal preparation and the culture sampling was explained to the patient. After approval from Ethical Committee Board, the written informed consent were obtained from all the individual participants involved in the study. Detailed medical and dental history of the patient was noted to rule out systemic problems, especially the use of antibiotics in the past two months.

INCLUSION CRITERIA

Necrotic teeth confirmed by negative response to pulp vitality test were included. Teeth with periapicalpathosis confirmed by clinical and radiographic evidence were included. Patients within the age group 25-35 year and sex no bars were taken into consideration. Patient who had not consumed antibiotics for two months prior to microbial sampling were selected.

EXCLUSION CRITERIA

Patients with major systemic disorder, teeth with abnormal internal anatomy and teeth with Internal/ external resorption were excluded.

PROCEDURE

To minimize variation in results, patients having both maxillary central incisors with definite periapical radio lucency were selected. Selected teeth were further divided into two groups:

GROUP A (Experimental group)
Thirty teeth were included in this group in which the root canal was irrigated with normal saline as initial irrigant followed by Nanocare+ as final irrigant.

The involved tooth was isolated with rubber dam (Dental Dam Kit, Hygenic, USA). The operative field including the tooth, clamp, and surroundings were cleaned with 30% hydrogen peroxide (J Cheme India) until no further bubbling of the peroxide occurred followed by 2.5% NaOCl for an additional 30 seconds using sterile cotton pellets. Finally, 5% sodium thiosulfate was used to inactivate the disinfecting agent. Access cavity was prepared by using sterile appropriate round (SS-White New Jersey) and en do Z bur (Dentsply International, New York, U.S) following Gross man's principles. The root canals were negotiated with the help of path finders.

After access preparation, the microbiological (pre-irrigation) sample was collected by introducing two sterile paper points (Dentsply Maillefer, Ballaigues, Switzerland) into the root canal. The paper points were kept in the root canal for one minute, and then moved circumferentially along the canal walls as introduced. Then with the help of sterile tweezers, paper points were removed from the canal and immediately placed in air tight test tubes containing thioglycollate broth for anaerobic bacteria and in pep tone water broth for aerobic bacteria.

Finally samples were transferred to the laboratory in respective broth for culturing.

Working length was measured following Ingle's method. The canal preparation was carried out following Step-Back technique by using appropriate Hand .

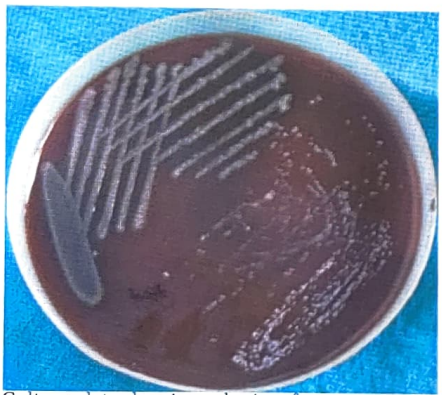
MICROBIOLOGICAL CULTURING PROCEDURE

The microbiological culturing procedure was carried out in the Department of Microbiology, Government Medical College, Amritsar. Each screw capped vial was shaken to disperse the sample content evenly. In the laboratory, a gram stained smear was prepared from each specimen. Samples were directly inoculated with the help of inoculating loop onto Mac Con key agar plates and incubated aerobically at 37o C for 24 hours.

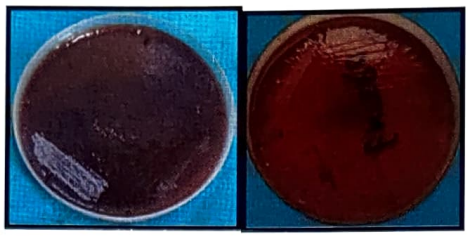
For anaerobes, specimens were directly inoculated on blood agar and incubated in anaerobic jar at 37o C for 48 hours. This was used as a selective medium for obligate anaerobes.

After incubation period, the plates were examined and the colonies were counted with digital colony counter (Khera Company, India). The collected data was subjected to statistical analysis.

RESULTS



Culture plate showing colonies after access cavity preparation



The mean absolute reduction in CFU/ml count from canal preparation (P2) to final irrigation (P3) for experimental group was 43.02 and for control group was 1.13. The mean percentage reduction in CFU/ml count from canal preparation (P2) to final irrigation (P3) for experimental group was 94.08 and for control group was 2.55.

The mean absolute reduction in CFU/ml count from access opening (P1) to final irrigation (P3) for experimental group was 90.62 and for control group was 49.53. The mean percentage reduction in CFU/ml count from access opening (P1) to final irrigation (P3) for experimental group was 97.25 and for control group was 49.85.

Mann-Whitney U test was used to compare the mean absolute reduction and mean percentage reduction in CFU/ml count at different follow-up points. Inter-group comparison of absolute reduction at different follow-up points revealed that both P2-P3 and P1-P3 was found to be significantly higher in experimental group as compared to that in control group ($p<0.001$).

Inter-group comparison of percentage reduction at different follow-up points revealed that both P2-P3 and P1-P3 was found to be significantly higher in experimental group as compared to that in control group ($p<0.001$).

Inter-group comparison of absolute reductions at different follow up points revealed that (P1-P2) was higher in control group than experimental group but the difference was not found to be statistically significant. Both (P2-P3) and (P1-P3) was found to be significantly higher in experimental group as compared to that in control group.

Inter-group comparison of percentage reductions at different follow up points revealed that (P2-P3) and (P1-P3) was found to be significantly higher in

TABLE 1: Inter-group Comparison of absolute reductions in CFU/ml count among Experimental group and control group

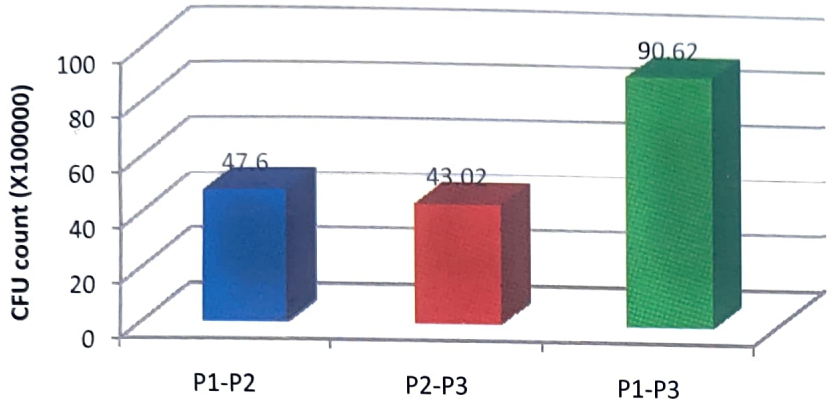
		Absolute Reduction in CFU count from access opening to canal preparation P1 – P2	Absolute Reduction in CFU count from canal preparation to final irrigation P2 – P3	Absolute Reduction in CFU count from access opening to final irrigation P1 – P3
Experimental Group	Mean	47.60	43.02	90.62
	SD	18.27	14.12	24.43
Control Group	Mean	48.20	1.13	49.53
	SD	15.20	0.57	15.36
Pc value, Significance		0.451, NS	<0.001, S	<0.001, S

experimental group as compared to that in control group.

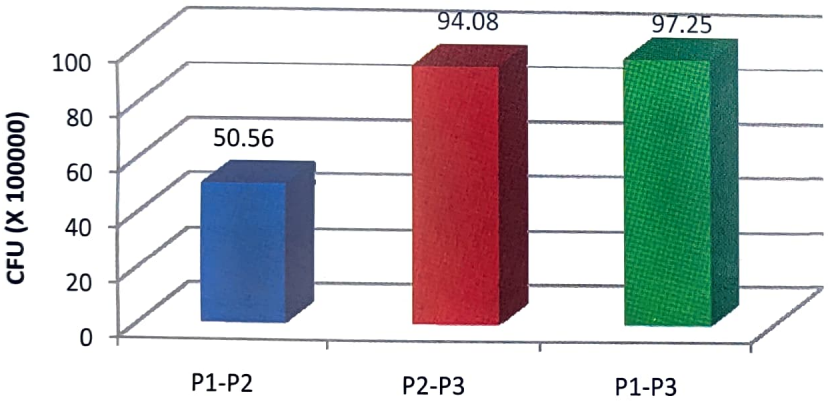
DISCUSSION

One of the greatest challenges of the root canal treatment is the effective chemo-mechanical preparation of the root canal in order to eliminate pulp remnants, bacteria, smear layer, and other organic material; facilitating three-dimensional obturation. In infected root canal system, bacteria grow mostly in sessile biofilms, aggregates, and congregates in which they are embedded in an extra cellular matrix. All forms of bacterial aggregates need to be removed. Irrigation is an important procedure, which help eliminating both forms of microorganisms (planktonic & biofilms) from the root canal system. Ideal root canal irrigant should prevent formation of smear layer during instrumentation or dissolve the latter once it has formed, have a broad antimicrobial spectrum, allow penetration of antimicrobial agents present in solution into the dentinal tubules, non toxic to surrounding tissue and have minimal effect on physical properties of the tooth in addition to dissolving necrotic pulp tissue remnants, disinfecting root canal system and dentinal tubules.

Absolute reductions at different points of times in experimental group



Percent reductions at different points of times in experimental group



Over the years, various root canal irrigants have been used, viz. normal saline, sodium hypo chlorite, EDTA, hydrogen peroxide, chlorhexidine, MT AD, urea, etc; however, none of these irrigants meet all requirements of an ideal endodontic irrigant. The newer irrigants, Ozone, Nanocare+ and Plasma etc. have shown some promising results in the recent past; however, limited studies had been documented so far.

The purpose of present study was to assess the antimicrobial efficacy of Nanocare+, a novel root canal irrigant.

Nanocare+ is nanosilver and nanogold based root canal irrigant. It is available in liquid form, and is used as final rinse. The manufacturers claim that it leaves a layer of nanoparticles of silver and gold on the canal surfaces, which has a strong bacteriostatic effect and prevents colonization of microorganisms. Low surface tension of the Nanocare+ carrier substance allows nanoparticles to get into the smallest intricacies and even dentinal tubules.

Upon reaching nanoscale, like other nanomaterials, silver particles exhibit remarkably unusual physicochemical properties such as high catalytic capabilities and ability to generate reactive oxygen species (ROS) and biological activities.(2) Silver ions interact with three main components of the bacterial cell

to produce the bactericidal effect: the peptidoglycan cell wall and the plasma membrane, cytoplasmic DNA and bacterial proteins.(3) It also interacts with the ribosome and inhibits the expression of the enzymes and proteins essential to ATP production.(4) Also, application of silver nano-coating directly on dentin can successfully prevent the biofilm formation on dentin surfaces as well as inhibit bacterial growth in the surrounding media5.Bednarski M et al (2013)6 observed promising antimicrobial properties when Nanocare+ was used as an intracanal medicament.

Guzman M et al (2012) 7 demonstrated the antibacterial activity of silver nanoparticles against gram-positive and gram-negative bacteria.

Kangarlou A et al (2013)8compared the antimicrobial activity of new irrigation solution containing nanosilver particles with that of sodium hypochlorite and chlorhexidine. The nanosilver containing irrigation solution at different concentrations upto 100ppm did not show a significant difference with sodium hypochlorite against various bacterial species.

Makkar S et al (2014) 9 found that the number of colony forming units dropped to zero after 3 minutes contact time with silver nanoparticle based irrigant and showed large inhibition zones as compared to 3% sodium hypochlorite group.

Gomes-Filho JE et al (2010)10 assessed the tissue reaction to silver nanoparticles dispersion as an alternative irrigating solution and found it to be biocompatible especially in lower concentrations. Chan ELK et al (2015)11 demonstrated the non-cytotoxic effects of novel nanosilver particle endodontic irrigant to both mouse fibroblast and primary human periodontal ligament stem cell.

In the present study, culture analysis was used to assess the antimicrobial effectiveness of Nanocare+ (Group A) and Normal saline (Group B). Culture analysis of endodontic infections have always provided substantial information about the ecology of apical periodontitis, composition of micro biota in different conditions, effects of treatment procedure in microbial elimination and also susceptibility of endodontic microorganisms to antibiotics. Culture analysis, like any other method, has its own advantages and limitations. It is considered as one of the most reliable methods to detect viable bacteria; and also it provides the identification of broad range of unexpected microbial species. The limitation of the method refers to the fact that about one half of the endodontic bacteria have not been cultivated by standard culture techniques. Also, because of the method's low sensitivity, some bacteria may pass unnoticed in the event that the number of bacterial cells sampled is below the detection limits of the culture. However, various studies using culture-dependent techniques have shown a correlation between negative cultures and optimal treatment outcome (Siqueria JF et al, 2007)12. A negative culture is likely to mean that the bacterial reduction in the canal has reached levels below the detection limits of culture procedures, and these levels are compatible with periradicular tissue healing.

In our study, the number of microorganisms was counted using digital colony counter (Khera Company, India). The count per ml of diluted broth was calculated and multiplied by dilution factor. The microbial count as CFU/ml after access opening, canal preparation and irrigation with final irrigant in each group was determined and tabulated. The mean of absolute and percentage reduction in CFU/ml count at different follow-up points of all groups was computed.

The results of present study demonstrated that the mean absolute reduction in CFU/ml count from canal preparation (P2) to final irrigation (P3) for experimental group was 43.02 and for control group was 1.13. The mean percentage reduction in CFU/ml count from canal preparation (P2) to final irrigation (P3) for experimental group was 94.08 and for control group was 2.55. The mean absolute reduction in CFU/ml count from access opening (P1) to final irrigation (P3) for experimental group was 90.62 and for control group was 49.53. The mean percentage reduction in CFU/ml count from access opening (P1) to final irrigation (P3) for experimental group was 97.25 and for control group was 49.85.

Mann-Whitney U test was used

to compare the mean absolute reduction and mean percentage reduction in CFU/ml count at different follow-up points. Inter-group comparison of absolute reduction at different follow-up points revealed that both P2-P3 and P1-P3 was found to be significantly higher in experimental group as compared to that in control group ($p<0.001$).

Inter-group comparison of percentage reduction at different follow-up points revealed that both P2-P3 and P1-P3 was found to be significantly higher in experimental group as compared to that in control group ($p<0.001$).

CONCLUSION

The results of the present study imply that highly significant absolute and percentage reduction in CFU/ml count was found in the experimental group i.e. Nanocare+. Therefore, within the limitations of present in-vivo study, Nanocare+ can be recommended as an effective root canal irrigant.

However, before any definite conclusion can be drawn, larger number of samples coupled with longer duration of clinical evaluation should be carried out to evaluate the efficacy of this novel root canal irrigant.

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