Chairside Sterilization Of Guttapercha Cones With Sodium Hypochlorite(2.5%) Glutaraldehyde(2%) And Ethyl Alcohol(70%)-An Invitro Comparative Study.

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Abstract

Objective – 1) The study aimed to - Compare the effectiveness of chemical agents - Sodium hypochlorite (2.5%), Gluteraldehyde (2%), Ethyl alcohol (70%) for chair side sterilization of gutta-percha cones – An *In Vitro* study. 2) To find out which disinfectant solution requires less time for effective sterilization of gutta-percha points.(4,9)

Methodology – The test solutions used were devided into 3 groups - (2.5%) Sodium hypochlorite, (2%) Glutaraldehyde, (70%) Ethylalcohol. Exposure times were - 1, 2, 5, 10, 15 min for each group. A total of 93 G.P cones were used in each group. Of these 31 cones used per group, 25 cones were then immersed in a petridish containing 20 ml of the chemical agent (5 cones for each time period) being tested and incubated at 37°C for 24 hours. After 24, 48, 72 hours and plated on 5 % sheep blood - BHI agar plates to support growth of aerobic bacteria. All blood agar plates and test tubes were incubated at 37°C for 24, 48, 72 hours to investigate any possible microbial growth. Microbial growth were analyzed after 24, 48, 72 hours by visualization of turbidity of the culture medium bacterial colonies in blood agar plates were the signs of bacterial growth, was tested in three parallel studies.(1,6,8,11)

Results - There was a statistically decrease in bacterial count in Sodium hypochlorite 2.5% effective in chair side disinfect of the G.P cones at 1min, 2% Glutaraldehyde taken 10min for disinfect and Ethyl alcohol 70% unable to disinfect G.P cones after 15min time interval.(1,5,11)

Conclusion – Within the limitations of the study, it can be concluded that -

1. Sodium hypochlorite 2.5% (Group -1) was effective in chair side disinfection of the gutta-percha cones and killing *Bacillus. Subtillis* microorganism in 1minute, where as 2% Glutaraldehyde (Group -2) was taken 10 minutes for disinfection of the gutta-percha cones and Ethyl alcohol 70% (Group -3) was unable to disinfect gutta-percha cones even after 15minutes time interval.

2. Ethyl alcohol was ineffective for chair side disinfection of gutta-percha cones in the present study.(1,5,7,11)

Keywords - Sodium hypochlorite, Glutaraldehyde, Ethyl alcohol, *B. Subtilis*, G.P cones.

Introduction –

According to pyramid of endodontic treatment plan three dimensional obturation of root canals is one of the most important step for success. Obturation is frequently performed with the use of G.P cones removed directly from factory sealed containers, from which they are purchased, with no concern for their sterility. G.P cones contaminated by handling, aerosols and physical sources during the storage process.
Hence the objective of this present study was to assess the effectiveness of the chemical agents such as Sodium hypochlorite (2.5%), Glutaraldehyde (2%) and Ethyl alcohol (70%) for decontaminating gutta-percha cones, which are frequently used in dental practice for chair side sterilization. In addition, time periods required for decontamination of gutta-percha cones in the clinical environment were checked and assessed in the study.(4,6,9,11)

Aim and objectives -

The aim of this present *In Vitro* study was to assess the effectiveness of -
1. Sodium hypochlorite (2.5%), Glutaraldehyde (2%) and Ethyl alcohol (70%) chemical agents for decontaminating gutta-percha cones, frequently used in dental practice for chair side sterilization.
2. In addition time periods required for decontamination of gutta-percha cones in the clinical environment was checked and assessed in the study.(1,7,11)

Materials -

Source of data -
Pre-sterilized 93 gutta-percha cones of ISO size No-40 were previously sterilized by ethylene oxide gas selected from the Department of Conservative dentistry and Endodontics of P.M.N.M Dental College and hospital, Bagalkot.

Criteria for selection of data -
Presterilized 93 gutta-percha cones of size No-40 will be selected.

Methodology –

Pre-sterilized total 93 gutta-percha cones of size No-40 were divided into 3 groups based on the agents used for decontaminating (31 gutta-percha cones in each group).

Test microorganism and growth conditions –
Certain species of Bacillus and Clostridium can form ‘‘cells of repose’’ called as spores (endospores). *Bacillus. subtilis* spores were selected for the study, because they are more resistant to physical and chemical agents than vegetative forms and represent a form of survival and not a form for bacterial reproduction. A variable spore suspension of *B. subtilis* was obtained commercially. A strain of *B. subtilis* (ATCC 6633) was grown to exponential phase in nutrient broth medium (NBM) at 37°C for 24 hours. After 24 hours test tube was showed turbidity as an indicator of bacterial growth.

A 20 µl portion of this culture was then inoculated on 5 % sheep blood - Brain Heart Infusion (BHI) agar plates (soybean-casein digest agar base) and incubated at 37°C for 24 hours to investigate any possible bacterial growth.(4,6,9,11)

Evaluation of prepackaged gutta-percha cones –
A total of these 31 cones used per group, 3 cones were inserted into the nutrient medium and incubated at 37°C for 24 hours to confirm its initial sterility

(**Negative control**). Then with 10µl solution from test tube not showing turbidity with 10 fold dilution technique was plated on 5 % sheep blood - Brain Heart Infusion (BHI) agar plate to support the growth of aerobic bacteria. Then blood agar plate was incubated at 37oc for 24 hours to investigate any possible bacterial growth. After 24 hours there was an absence of bacterial growth, indicating initial sterility of gutta-percha cones (**Negative control**). (4,6,9,11)

Evaluation of artificial contamination of gutta-percha cones –
The remaining 28 cones were contaminated by the bacteria *Bacillus. Subtilis*; of these 3 cones were inserted into a test tube containing nutrient broth and incubated at 37°C to confirm its contamination (**Positive control**). Then with 10µl solution from test tube showing turbidity with 10 fold dilution technique was plated on 5 % sheep blood - Brain Heart Infusion (BHI) agar plate to support growth of aerobic bacteria. Then blood agar plate was incubated at 37oc for 24 hours to investigate any possible microbial growth. After 24 hours there was presence of bacterial growth indicating contamination of gutta-percha cones (**Positive control**). (4,6,9,11)

Evaluation of test solutions -
The test solutions used for sterilization were –
Group 1 – Sodium hypochlorite (2.5%)
Group 2 – Glutaraldehyde (2%)
Group 3 – Ethyl alcohol (70%).

Exposure times periods were - 1, 2, 5, 10 and 15 minutes for each group.
The remaining 25 cones were then immersed in a petridish containing 20 ml of the chemical agent (5 cones for each time period) being tested and incubated at 37°C for 24 hours. To eliminate any residual effects of the tested agent, then cones were immersed for 4 minutes in 10 ml of sterile distilled water and will be transferred to test tubes containing 10 ml of nutrient medium.

Test tubes were vortexed, incubated at 37°C for 24, 48 and 72 hours. Test tubes were checked daily for turbidity as an indicator of microbial growth. After 24, 48 and 72 hours test tubes showing turbidity were inoculated with 10µl solution was diluted with 10 fold dilution technique from each test tube and plated on 5 % sheep blood - Brain Heart Infusion (BHI) agar plates (soybean-casein digest agar base) to support growth of aerobic bacteria. All blood agar plates and test tubes were incubated at 37°C for 24, 48 and 72 hours to investigate any possible microbial growth.
Microbial growth were analyzed after 24, 48 and 72 hours by visualization of turbidity of the culture medium the bacterial colonies in blood agar plates were the signs of bacterial growth, is tested in three parallel studies. The time spent for each test solution to produce total microbial inhibition growth was recorded.(1,6,8,11)

Results –

The bacterial quantitative readings are shown in Table – 1. The values are mentioned in terms of percentage of growth of microorganism for their respective groups and time intervals as per Table. Statistics were considered significant at p value ≤ 0.05. The inter group variation in bacterial growth

were compared by using **Kruskal Wallis ANOVA** post hoc procedure Table and these are Graphically represented in Graph-1. Three groups were compared using Newman- Keuls post hoc procedure with respect to their change in bacterial growth Table - 1. There was a significant difference ($p \leq 0.05$) in bacterial growth between different groups on pair wise comparison except for sodium hypochlorite group.

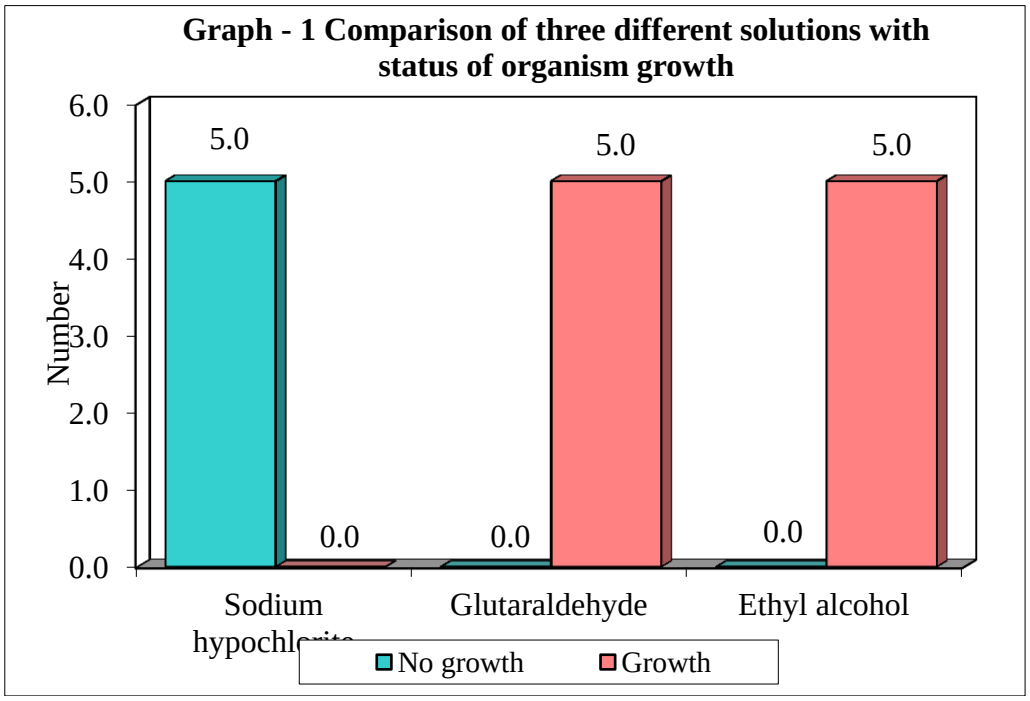
In the present study, based on the recorded data and statistical analysis Graph and Table summarizes Sodium hypochlorite (**Group -1**) is effective in chair side disinfection of the gutta-percha cones and killing *Bacillus. Subtillis* microorganism at 1minute which was statistically significant ($p \leq 0.05$), where as Glutaraldehyde (**Group -2**) was taken 10 minutes for disinfection of the gutta-percha cones was statistically significant than Ethyl alcohol.

Ethyl alcohol (**Group -3**) was unable to disinfect gutta-percha cones even after 15minutes time interval which was statistically not significant. All positive controls showed positive results during the first 24 hours. Negative controls were followed up for 48 and 72 hours and no microbial growth was observed in any of the groups tested, demonstrating the efficacy of previous sterilization.(1,5,7,11)

Table - 1 Comparison of three different solutions with status of organism growth by Kruskal Wallis ANOVA -

Solutions	No growth	%	Growth	%
Sodium hypochlorite	5	100.00	0	0.00
Glutaraldehyde	0	0.00	5	100.00
Ethyl alcohol	0	0.00	5	100.00
H-value	14.0000			
P-value	0.0009*			
Pair wise comparison by Mann-Whitney U test				
Sodium hypochlorite vs Glutaraldehyde	P=0.0090*			
Sodium hypochlorite vs Ethyl alcohol	P=0.0090*			
Glutaraldehyde vs Ethyl alcohol	P=1.0000			

*p<0.05



According to study is justified sodium hypochlorite has best effectiveness of disinfection.

Discussion –

Sterilization of endodontic instruments and materials is an important step during the endodontic treatment. These commercially available gutta-percha cones have several advantages: they do not stain the tooth structure; they are biocompatible, radio opaque, dimensionally stable, easily removed from root canal and are also antibacterial 1. Even though gutta-percha cones are produced under aseptic conditions and possess potential antimicrobial properties, especially due to their zinc-oxide component, they can still be contaminated by handling, aerosols and physical sources during the storage process. Previous studies found that 8% of the commercially available cones when tested showed bacterial growth.3 Because it is difficult to determine the number of accessory cones to be used during lateral condensation before initiating the procedure, an effective, quick-acting chemical agent is needed to prevent any possible surface contamination by microorganisms.(1,8,11). Several studies have examined cold sterilizing agents for gutta-percha. According to previous studies, gutta-percha cones sterilization can be done by means of chemical agents such as - glutaraldehyde, ethyl alcohol, povidine-iodine, sodium hypochlorite, chlorhexidine are utilized in clinical practice.(3,7,11) The appropriate disinfectant should be the one that can be used routinely in dental clinics, providing a fast disinfection without modifying the structure of the gutta-percha cone. But only few chemical agents sterilize effectively, inexpensively and rapidly. (3,5, 6,)

Sodium hypochlorite has powerful antibacterial and sporicidal activities, antibacterial activity of sodium hypochlorite is mainly due to hypochlorous acid (HClO) in solution which has oxidative action on sulphydryl groups of bacterial enzymes. Its concentration ranges from 0.5% (Dakin solution) to 5.25%. One of the most widely used endodontic solutions, either as an irrigant or for intermediate level disinfection of rubber dam and gutta-percha cone decontamination. Besides no literature reference was found regarding the effectiveness of 2.5% sodium hypochlorite for rapid chair side decontamination of gutta-percha cones, the reason that led us to undertake the present investigation. So in this present study, the use of 2.5% sodium hypochlorite and not 5.25%, as recommended by several authors, was based on the fact that the weaker concentration is used by many endodontists during chemo-mechanical preparation of the root canal system, use sodium hypochlorite in lower concentrations as it is biocompatible. In the present study, its efficacy as a chair side sterilizing solution have been demonstrated at the concentration of 2.5% (Group – 3) within the first 1 minute of contact with contaminated gutta-percha cones (Colour plate – 7). Our results confirmed the efficiency of sodium hypochlorite (2.5%) solution for chair side sterilization of gutta-percha cones.(3,7,11)

The council on Dental therapeutics recognized chlorine compounds as intermediate level disinfectants which are suitable for the use on environmental surfaces and instruments that cannot be sterilized by heat. The council has recognized only liquid preparations of formaldehyde and glutaraldehyde as high-level disinfectants or sterilizing agents. Glutaraldehyde is an important dialdehyde that has found usage as a disinfectant and sterilant, in particular for low-temperature. Glutaraldehyde has a broad spectrum of activity against bacterial spores, fungi and viruses. Low concentrations of the glutaraldehyde (0.1%) inhibit germination, whereas much higher concentrations (2%) are sporicidal. This is the reason which led us to undertake this investigation. So in the present study, 2% gluteraldehyde was used. (1,511)

Gutta-percha cones decontamination method with glutaraldehyde described as comprises of immersion of the gutta-percha cones in a 2% glutaraldehyde solution for 5 minutes. In a quantitative study these authors compared the bactericidal and sporicidal action of two chemical solutions of 2% glutaraldehyde, who detected no bacterial growth after 10 minutes of contact with gutta-percha

cones contaminated with *Bacillus subtilis*. In the present study, its efficacy as a chair side sterilizing solution was demonstrated at the concentration of 2% (Group - 2) for 10 minutes contact with contaminated gutta-percha cones (Colour plate -8). Our results confirmed the efficiency of glutaraldehyde (2%) solution for chair side sterilization of gutta-percha cones.(1,5,11)

Alcohols are lipid solvent and exert their effects on bacteria by disorganizing the lipid structure of membranes. They cause membrane damage, denaturation of cellular proteins, with subsequent interferences with metabolism and cell lysis. They are however, known to inhibit sporulation and spore germination. This activity is greater in the presence of water. Alcohols exhibit rapid broad-spectrum antimicrobial activity against vegetative bacteria, viruses and fungi. Ethanol in a concentration of 70% by volume is widely used in dentistry for disinfection of instruments and surgical equipments. It has been claimed that it may be a reasonable choice for intermediate-level disinfection provided the items can be submerged for an adequate contact time. This is reason led us to undertake this investigation. So in the present study 70% ethyl alcohol was used. (1,6,8)

In the present study ethyl alcohol 70% (Group -3) was not able disinfection of gutta-percha cones even after 15minutes time interval (Colour plate – 9). Our results confirmed the ethyl alcohol (70%) solution was not effective for chair side disinfection of gutta-percha cones. (1, 6, 8)
In the present study, it is assessed that the effectiveness of chemical agents such as Sodium hypochlorite (2.5%), Glutaraldehyde (2%) and Ethyl alcohol (70%) for decontaminating gutta-percha cones, which are frequently used in dental practice for chair side sterilization. In addition, time periods required for decontamination of gutta-percha cones in the clinical environment has also been checked and assessed in the study. The selection of chemical agents for de-contamination of gutta-percha cones in the present study was based on literature data.(1,6,8,11)

Conclusion –

Even though gutta-percha cones are usually sterile during storage, they can be easily contaminated if incorrectly manipulated. This study was undertaken in an attempt to develop a clinically effective and rapid chair side procedure for the sterilization of this material. Based on the recorded data and statistical analysis, the following conclusions can be drawn. Within the limitations of the study, it can be concluded that -

1. Sodium hypochlorite 2.5% (Group -1) was effective in chair side disinfection of the gutta-percha cones and killing *Bacillus. Subtillis* microorganism in 1minute, where as 2% Glutaraldehyde (Group -2) was taken 10 minutes for disinfection of the gutta-percha cones and Ethyl alcohol 70% (Group -3) was unable to disinfect gutta-percha cones even after 15minutes time interval.
2. Ethyl alcohol was ineffective for chair side disinfection of gutta-percha cones in the present study. (1,5,7,11)

INTEREST OF CONFLICT-NIL

SOURCE OF FUNDING-NIL

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