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EFFECT OF IMMERSION TYPE III GYPSUM IN SODIUM HYPOCHLORITE AND POVIDONE-IODINE SOLUTIONS ON THE NUMBER OF CANDIDA ALBICANS COLONIES

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KEYWORDS

candida albicans, disinfectant solution, type III gypsum, sodium hypochlorite, povidone-iodine

ABSTRACT

Introduction: Cross-infection is the transfer of microorganisms that can occur from individual to individual or to object. Candida albicans is one of the infectious pathogens that can cause cross-infection in dentistry. One example is the manufacture of working models of gypsum material, which can cause cross-infection from patients to dentists or technicians, so it is necessary to control infection by means of disinfection of type III gypsum material work models. **Objective:** To determine the effectiveness of soaking type III Gypsum in a 0.5% Sodium hypochlorite and 2% Povidone Iodine solution against the growth of Candida albicans. **Methods:** A laboratory experiment with a total of 21 samples, consisting of 7 samples for each group. The sample is made of a type III gypsum plate measuring 2 x 1 x 0.5 cm. The sample consisted of 3 groups: treatment 1, treatment 2, and treatment 3. The study used the diffusion method. Gypsum plate type III was contaminated with Candida albicans and then immersed in a solution of sterile distilled water (C), 0.5% sodium hypochlorite (P1), and 2% povidone-iodine (P2). The soaking results were planted in SDA media, incubated for 2x24 hours, and then counted using a colony counter. The data were analyzed using Kruskal-Wallis and Mann-Whitney post hoc analyses. **Results:** In the Kruskal-Wallis analysis of significance (p), between each group, there was a significant difference ($p<0.05$). The Mann-Whitney post hoc analysis test showed a correlation between group K and P1 and P2 ($p<0.05$). **Conclusion:** 0.5% Sodium Hypochlorite and 2% Povidone Iodine solutions are effective as disinfectants using the immersion method in Type III Gypsum against the growth of Candida albicans.

INTRODUCTION

According to the WHO, 35 million medical personnel in the world are exposed to cross-infection, 3 million are exposed to blood-borne pathogens, 2 million are infected with *HBV*, 900 thousand are infected with *HCV*, and 170 thousand are infected with *HIV*.[1] There is a study that found 67% of materials sent by dentists to dental laboratories have been infected with pathogenic bacteria and viruses such as *Streptococcus* species, *Staphylococcus* species, *Bacillus* species, *Enterobacter* species, *Hepatitis Virus*, *Herpes simplex Virus*, even the *Human Immunodeficiency Virus (HIV)*.[2] *Candida albicans* is a normal flora in the human oral

cavity that grows commensally and is non-invasive, but in certain circumstances, Candida albicans can become a pathogen and cause infection in humans.[3]

Zilinkas[4] research stated that gypsum is not a suitable medium for multiplying microorganism colonies because the pH of type III gypsum is 6.1, which can cause a decrease in the number of bacteria compared to print results but is still capable of being a suggestion of cross-infection.[5]

Sodium hypochlorite contains hypochlorous acid, which can act as a solvent and release chlorine on contact with organic tissue. Chlorine can combine with amino groups in proteins to produce Chloramide. Chlorine is also a strong oxidant that inhibits bacterial enzymes.[6] Another disinfectant liquid that can be anti-bacterial in the type III gypsum model disinfection process is Povidone iodine. Povidone iodine has a relatively stable concentration of 2%. Povidone iodine is a polymer complex of 1-vinyl-2-pyrrolidinone and iodine, in which iodine has an antibacterial effect.[7]

Based on the above background, the researchers wanted to know the effect of immersing type III gypsum material with 0.5% Sodium hypochlorite solution and 2% Povidone Iodine on the number of Candida albicans colonies.

METHODS

The tools used in this study were test tubes, test tube racks, Petri dishes, 100µm - 1000µm micropipette, incubator, vortex, centrifuge, syringe, autoclave, 0.2µm multipore unit filler, spreader, dropper pipette, printing spoon, bowl, spatula, magnetic stirrer, master model 2 x 1 x 0.5 cm, mask, and gloves. And the materials used are gypsum type III, alginate, Sabouraud Dextrose Agar (SDA) media, phosphate-buffered saline (PBS), 0.5% sodium hypochlorite, 2% povidone-iodine, Candida albicans suspension, and sterile distilled water.

Prepare a master model of stainless steel in the shape of a box with dimensions of 2 x 1 x 0.5 cm (p x w x h). The master model was printed with alginate in a ratio of powder to distilled water, namely, 10 gr/10 cc, and then the main model was removed from the mold. The alginate impression was filled with type III gypsum dough with a ratio of powder to distilled water, namely 100 gr to 30 cc, and then waited for 60 minutes until it reached the setting time. Remove the type III Gypsum model from the mold and then rinse it with 10 ml of sterile distilled water using a syringe.[8]

Candida albicans were obtained from preparations in the microbiology laboratory of the Faculty of Dentistry, Hang Tuah University, and then incubated for 24 hours at 37°C. Candida albicans was taken as much as 1 ose of (1), put in a solution of $BaCL_2$ 0.048 M 0.5 ml and H_2SO_4 0.18 M 9.95 ml (1x 106 CFU/ml), which is equivalent to McFarland 0.5, and incubated for 24 hours at 37°C, adjusted for gypsum plate contamination.

Furthermore, the gypsum plate was contaminated with Candida albicans suspension and then incubated at 37 °C for 24 hours. Gypsum plates are divided into three groups. The first group (C) was soaked in sterile distilled water for 10 minutes. The second group (T1) was soaked in 0.5% sodium hypochlorite for 10 minutes. The third group (T2) was soaked in 2% povidone-iodine for 10 minutes. After immersion, the gypsum plate was rinsed with PBS twice, and then the gypsum plate was put into 5 ml of Sabouraud Dextrose Agar Plate media and vibrated using a vortex for 30 seconds with the aim that Candida albicans attached to the gypsum plate could be released. Candida albicans seeding was carried out by spreading 0.1 ml of Candida albicans suspension on Sabouraud agar dextrose, then incubating for 2x24 hours at 37 °C. After 2x24 hours, Candida albicans colonies were counted in colony-forming units per milliliter (CFU/ml) using a colony counter.

RESULT

The data obtained were analyzed using descriptive statistics and then statistical data were analyzed with SPSS 20. Then a hypothesis was tested using Kruskal-Wallis and a posthoc analysis was performed with the Mann-Whitney test.

Table 1. The mean value and standard deviation

Group	N	Mean±Std.Deviation
Aquades	7	137,86 ± 48,66
Sodium hypochlorite	7	1,43 ± 1,13
Povidone iodine	7	3,14 ± 3,34

Table 2. Normality test with Shapiro-Wilk test results

	Shapiro-Wilk
Aquades	0.2*
Sodium hypochlorite	0.000004*
Povidone iodine	0.01*

Note: * p value <0.05 (not significant)

Based on Table 2 of the results of the normality test with Shapiro-Wilk, it can be seen that each treatment group has a significance value of p<0.05. This shows that the data is not normally distributed, so the Kruskal Wallis test was carried out to test the hypothesis of this study.

Table 3. Kruskal Wallis test results

	p
Kruskal-Wallis	0.00*

Note: * p value <0.05 (significant)

Based on Table 3, the results of the Kruskal-Wallis test showed that the significance value was 0.000, less than 0.05 (P <0.005), so it could be concluded that there was a significant difference in the anti-*Candida albicans* power between the treatment group and the control group. To see the significant differences in anti-*Candida albican*'s power between groups, a post hoc analysis was performed using the Mann-Whitney test.

Table 4. Mann-Whitney test results

Grup	C	T1	T2
C		0,001*	0,001*
T1			0,383
T2			

Note: * p value <0.05 (significant)

Based on Table 4, the results of the Mann-Whitney test show that there is a significant difference in the number of *Candida albicans* colonies in the negative control group and all treatment groups. This is evidenced by the significance value of each group, namely P <0.05. However, there was no significant difference between treatment group 1 and treatment 2 with a significant value of 0.383 (P>0.05).

DISCUSSION

Cross-infection is a high risk that can endanger medical personnel. Cross-infection can occur in almost every process of patient management, one of which is of concern to WHO is cross-infection that occurs from the post-impression process of the patient's oral cavity.[1] One effort to reduce the high risk of cross-infection of medical personnel is by carrying out a disinfection process impressions of the patient's oral cavity. There are several types of disinfection techniques that can be used, such as immersion techniques, spraying techniques, and disinfection techniques using microwaves. Disinfection steps can be carried out using a disinfectant liquid, for example, 0.5% sodium hypochlorite and 2% povidone iodine.[4]

In this study, the disinfection stage of the immersion technique was carried out to compare the effectiveness of 0.5% sodium hypochlorite and 2% povidone iodine on the growth of the number of *Candida albicans* colonies in the Gypsum III study model. This effectiveness test used the agar diffusion method and the results of research on the effectiveness of 0.5% sodium hypochlorite and 2% povidone iodine were seen from the number of colonies that formed after the soaking process with a disinfecting agent.

In the control group, a comparison was made with group P1 which was given a sodium hypochlorite solution. It was found that a significant value comparison indicated that a 0.5% sodium hypochlorite solution inhibited the growth of the *Candida albicans* fungus. This is in accordance with the research by Syaula[9] which showed that 0.5% sodium hypochlorite was more effective in inhibiting *Candida albicans* than distilled water. Research by El Sayed[10] showed that sodium hypochlorite was effective in inhibiting *Candida albicans* compared to chlorhexidine, calcium hydroxide, 2% chlorhexidine gel, antibiotic-corticosteroid mixture, calcium hydroxide-iodoform mixture, and sodium hypochlorite gel.

In comparison with the control group P2 which was given a 2% povidone iodine solution, there was a significant difference. This indicates that the povidone iodine solution inhibits the growth of the *Candida albicans* fungus. Research according to Putri[11] showed 2% povidone iodine was effective in inhibiting *Candida albicans* compared to aquades. Povidone iodine with a minimum concentration of 0.45% was able to inhibit and kill fungal cells for 180 seconds.[12]

The results of the comparison between the P1 and P2 groups in this study found no significant difference. Therefore, it can be concluded that sodium hypochlorite and povidone iodine are both effective in inhibiting the growth of the *Candida albicans* fungus. Research according to Rondhianto[13] which compared the antibacterial effects of sodium hypochlorite and povidone iodine on *Staphylococcus aureus* bacteria showed that povidone-iodine was better. Bacteria and candida have different structures that affect different results.

0.5% sodium hypochlorite solution is an effective and safe concentration for use as a disinfectant. When sodium hypochlorite is added to water it forms hypochlorous acid which can act as a solvent and release chlorine. This chlorine will join the amino group of proteins to form Chloramide. Chlorine is also a strong oxidant inhibiting fungal enzymes.[13]

Povidone iodine has anti-bacterial properties with the mechanism of povidone carrying free iodine compounds to penetrate cell membranes and the cytotoxic properties of povidone-iodine which is capable of killing bacterial cells.[14] Povidone iodine is complex iodine that is capable of killing microorganisms such as bacteria, fungi, viruses, protozoa, and bacterial spores. The iodine content combined with polyvinyl pyrrolidone produces an iodophor complex.[13] Povidone-iodine has a mechanism of action by inhibiting the synthesis of glucosyltransferase (GTF) and fucosyltransferase (FTF) by *S. mutans* bacteria. GTF and FTF play a role in the process of attachment of the *Candida albicans*.[14]

When mixing the gypsum material with water there is a risk of trapping air bubbles which can cause porosity on the surface of the gypsum model. This porosity can become an attachment site for pathogenic bacteria and microorganisms.[15] The nature of the porosity makes it susceptible to attachment of pathogenic bacteria from saliva and other fluids which can cause cross-infection because they contain microorganisms that can move from the mold and attach to the gypsum model.[16] Presence of *Candida albicans* in the oral cavity occurs through several stages, namely the acquisition of *Candida albicans* from the environment, growth stability, attachment and penetration of *Candida albicans* in the oral tissue. *Candida albicans* virulence tools include the ability to transform yeast into pseudohyphae or hyphae, biofilm formation, and hydrolytic enzymes such as aspartyl proteinases and phospholyphases.[17]

CONCLUSION

In this study, it can be concluded that immersing the type III Gypsum model in a disinfection solution of 0.5% Sodium hypochlorite and 2% Povidone-iodine has the effect of reducing the number of Candida albicans fungal colonies.

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