JURNAL ILMU DAN TEKNOLOGI KESEHATAN
Vol 11, No 2, March 2024
ISSN: 2338-9095 (Print)
ISSN: 2338-9109 (online)

The Decreased of Germs Number Before and After Gargling with Povidone - Iodine

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Article history
Posted, Jan 10th, 2024
Reviewed, Mar 30th, 2024
Received, Apr 7th, 202

ABSTRACT

Povidone-iodine is considered to have the broadest spectrum of antimicrobial action compared with other common antiseptics. Based on the application protocol of commercial Povidone Iodine (PVP-I) mouthwash to support a healthy lifestyle, PVP-I is commonly used in concentrations of 1.0% with a contact time of 30 seconds. The higher the concentration of antiseptic, the smaller the time needed to kill microbes, but the maximum limit of concentration and contact time needs to be considered to minimize the toxicity effects to remain safe, effective, and efficient. This study aims to determine the comparison of germ numbers before and after gargling with povidone-iodine mouthwash based on variations in the concentration of 0.2%, 0.4%, 0.6%, 0.8%, and 1% with variations in contact time of 15 and 30 seconds. The method of this research was an actual experiment with a pre-post-test design. Based on the Non-Parametric Friedman test with a confidence level of 95% (α = 0,05), there is a significant difference between the germs number before and after gargling with PVP-I in every variation of concentration and contact time (p-value = 0.00). This study concludes that PVP-I 0.2% concentration variation with 15 seconds of contact time has been effective in decreasing the number of germs.
Keywords: *Germs Number; Healthy Lifestyle; Mouthwash; Povidone Iodine*

ABSTRAK

Povidone-iodine diakui memiliki spektrum aksi antimikroba terluas dibandingkan dengan antiseptik umum lainnya. Berdasarkan petunjuk penggunaan obat kumur Povidone Iodine (PVP-I) komersial untuk mendukung perilaku hidup sehat, PVP-I umumnya digunakan pada konsentrasi 1,0% dengan waktu kontak 30 detik. Semakin tinggi konsentrasi antiseptik maka semakin kecil waktu yang dibutuhkan untuk membunuh mikroba, namun batas maksimal

DOI: 10.32668/jitek.v11i2.1449

konsentrasi dan waktu kontak perlu diperhatikan untuk meminimalkan efek toksisitas agar tetap aman, efektif, dan efisien. Penelitian ini bertujuan untuk mengetahui perbandingan jumlah kuman sebelum dan sesudah berkumur dengan obat kumur povidone-iodine berdasarkan variasi konsentrasi 0,2%, 0,4%, 0,6%, 0,8%, dan 1% dengan variasi waktu kontak 15 dan 30 detik. Metode penelitian yang digunakan adalah true eksperimen dengan desain pre-post-test. Hasil: Berdasarkan uji Non Parametrik Friedman dengan tingkat kepercayaan 95% (α = 0,05), terdapat perbedaan yang signifikan antara jumlah kuman sebelum dan sesudah berkumur dengan PVP-I pada setiap variasi konsentrasi dan waktu kontak. (nilai p = 0,00). Kesimpulan: Kesimpulan penelitian ini adalah variasi konsentrasi PVP-I 0,2% dengan waktu kontak 15 detik sudah efektif menurunkan jumlah kuman.
Kata Kunci*:* Angka Kuman; Perilaku Hidup Sehat; Obat Kumur; Povidone Iodine

INTRODUCTION

Oral health is an inseparable component of general health. The causes and risks of oral disease often have the same factors as common diseases (Ramadhan, Cholil and Sukmana Indra, 2016). Based on the results of the 2018 Health Research, the prevalence of dental and oral health problems in Indonesia was 57.6%, up from the results of the 2013 report, which was 31.7% (KEMENKES RI, 2018). One of the most common dental and oral health problems is gingivitis. Gingivitis is an inflammatory condition in the gingival tissue, commonly known as the gums (Rathee and Jain, 2022). The prevalence of gingivitis in Indonesia is 96.58% (KEMENKES RI, 2018).

The leading cause of gingivitis is the buildup of microorganisms that form a colony and then form plaque attached to the gingival margin (Diah, Widodorini and Nugraheni, 2018). Gingivitis can also occur as a result of incorrectly cleaning between the teeth, causing an accumulation of bacteria along the gingiva. Types of bacteria in the mouth that can generally cause gingivitis others *Streptococcus sanguis, Streptococcus milleri, Streptococcus mutans, Fusobacterium nucleatum, Actinomyces israelii,* and *Bacteroides intermedius* (Kondo, Wibisono and Ciptaningtyas, 2017).

The oral cavity is the part of the body that contains the most microorganisms; more than 500 microorganisms have been isolated from the mouth (Widani and Nasution, 2015). Andreas (2019) revealed that microbes live in cavities mouth at first, as many as 20 billion, but the microbes continue to multiply five times within 24 hours (Zilan, 2020). The number of germs in normal saliva is 108 to 109 CFU/ml (Vesna, 2018).

Dental and oral hygiene is very important to maintain and prevent oral cavity

DOI: 10.32668/jitek.v11i2.1449

diseases. The mouth is not always in a clean condition, plaque sometimes appears on the teeth which is the result of the colonization and growth of various microorganisms. Nearly 70-80% of dental plaque consists of bacteria, and 1 mm^3 contains more than 10^8 bacteria. Maintaining oral hygiene can be done in various ways, namely by brushing teeth, gargling with antiseptic fluids, and cleaning teeth with dental floss (Mariyam and Alfiyanti, 2016). Gargling with an antiseptic can eliminate the bacteria in interdental areas that a toothbrush cannot reach. As for the mechanism, mouthwash works to help clean the oral cavity thoroughly through mechanical and chemical action (Talumewo, 2015).

Gargling with an antiseptic can eliminate the bacteria in interdental areas that a toothbrush cannot reach. As for the mechanism, mouthwash works to help clean the oral cavity thoroughly mechanical and chemical action. Antiseptics are chemical compounds that can kill, destroy and inhibit the growth of microorganisms in creature life. Antiseptic mouthwash commonly uses active ingredients such as *Povidone Iodine, Chlorhexidine, Cetylpyridinium Chloride,* etc., as a material to kill germs (Zilan, 2020).

Povidone Iodine (PVP-I) is an antiseptic with the broadest antimicrobial spectrum compared to general antiseptics. It is said to be effective in killing gram-positive and gram-negative bacteria, bacterial spores, fungi, protozoa, and viruses. Based on the commercial Povidone Iodine mouthwash protocol, PVP-I is usually used at a concentration of 1.0% with a contact time of 30 seconds (Kanagalingam *et al.*, 2015).

WHO recommendations for the maximum use of Iodine for adults in one day is 0.15 mg. 10% PVP-I contains the equivalent of 11 mg/mL Iodine, so 1% PVP-I contains 1.1 mg/mL Iodine (Kirk-Bayley, Sunkaraneni and Challacombe, 2020). Even while it is below the maximum daily use restriction, this statistic can be deemed fairly high. The concentration of PVP-I that is typically available on the market is 1.0%, but using it for an extended period of time increases the danger of toxic effects because most protocols prescribe a concentration of 0.2% to 0.5% to limit the toxic effects of PVP-I in repeated usage (Frank *et al.*, 2020).

This research was conducted by varying the concentration of PVP-I and the contact time of gargling, which effectively reduced the number of germs. The Centers for Disease Control and Prevention states that the higher the concentration, the less time it

DOI: 10.32668/jitek.v11i2.1449

takes to kill microbes (CDC, 2016). The maximum limit of PVP-I concentration and contact time must be considered to reduce toxic effects, so the choice of concentration and contact time for mouthwash must be adjusted to remain safe, effective and efficient.

MATERIALS AND METHODS

This research was conducted in an actual experiment using a pre-post-test design to see a comparison of the number of germs before and after gargling using Povidone Iodine with varying concentrations of 0.2%, 0.4%, 0.6%, 0.8% and 1.0% with contact times of 15 and 30 seconds. The subjects in this study were 12 experiment samples who met the inclusion and exclusion criteria. Retrieval of research data using a questionnaire and examination of the number of germs in the laboratory. This research was conducted based on ethical clearance from the Ethics and Research Committee of the Budhi Asih Regional General Hospital No: 152/KEP-ETIK/V/2023.

All research subjects will rinse their mouths using 15 mL of sterile aquadest as a sample before rinsing their mouths with PVP-I. Furthermore, the subject will gargle once daily using one type of PVP-I concentration with a contact time of 15 and 30 seconds. Various concentrations of gargling were used, namely, 0.2%, 0.4%, 0.6%, 0.8% and 1.0%, so that the subject would rinse his mouth for five days, used as a sample after gargling with PVP-I-Based on variations in concentration and contact time. All samples obtained were then incubated in PCA media for 24 hours at 37°C. Data analysis was performed using Friedman's non-parametric test because the data distribution was abnormal.

RESULTS AND DISCUSSION

The study's findings can be seen in Table 1:

Table 1. Oral Hygiene Behavior and Mouth Conditions of Respondents

No	Behavior & Oral Condition	Observations	N (%)	
1	Daily tooth brushing	< 3 times/day	2	(16.7%)
		Three times per day	9	(75.0%)
		> 3 times/day	1	(08.3%)
2	Routine Using Mouthwash	Yes	1	(08.3%)
		No	11	(91.7%)
3	History of PVP-I allergy	Yes	0	(0.00%)
		No	12	(100%)

DOI: 10.32668/jitek.v11i2.1449

4	Teeth Holes	Yes	0	(0.00%)
		No	12	(100%)
5	Wounds in the Oral Cavity	Yes	0	(0.00%)
		No	12	(100%)

In Table 1, the results obtained for routine tooth brushing are the most (75%), namely three times a day. The majority of respondents, as many as 91.7%, did not use mouthwash in their oral hygiene routine. All respondents (100%) did not have a history of allergies to the use of PVP-I, did not have cavities, and did not have mouth sores.

The results of observations in Table 1 regarding oral hygiene behaviour by brushing their teeth show that the majority of respondents, namely 75%, have a habit of brushing their teeth three times a day. This aligns with WHO recommendations to brush your teeth at least twice daily (World Health Organization, 2023). Ngatemi, Erna Sariana, and Silwa, in 2020 in their research stated that the frequency of brushing your teeth should be done three times a day, namely after eating and before going to bed.

Based on this, the oral hygiene behaviour of TLM semester six students in brushing their teeth was appropriate, primarily based on recommendations. In Tince Arniati Jovina's research on the influence of tooth brushing habits, it was found that people tend to brush their teeth more frequently when bathing in the morning and evening and before going to bed.

The observation results also stated that most respondents (91.7%) did not routinely use mouthwash in their daily oral hygiene behaviour. This can be influenced by several aspects, including economics (Lestari *et al.*, 2022). Non-working people, such as students, tend not to use mouthwash due to the price factor of the mouthwash itself. Maintaining oral hygiene by brushing alone may be sufficient, but in some cases, brushing alone is less effective at reducing plaque accumulation that causes problems with teeth and gums.

None of the respondents had a history of allergies to PVP-I. Bigliardi et al., 2017 stated that PVP-I is an antiseptic that rarely causes allergic effects. In addition, PVP-I also has a minimal possibility of causing an irritating impact compared to other antiseptics (Lachapelle, 2014). Observations regarding dental and oral health, such as holes in the teeth and sores in the oral cavity, showed that all respondents had healthy teeth and mouth conditions. well indicated by the absence of cavities and sores in the oral cavity, this could be related to the respondent's habit of

DOI: 10.32668/jitek.v11i2.1449

brushing his teeth three times a day, so that the respondent's oral hygiene was quite good and had no problems.

Table 2 shows the results of calculating the number of germs before and after rinsing with PVP-I concentration variations of 0.2%, 0.4%, 0.6%, 0.8%, and 1.0% and contact times of 15 and 30 seconds.

Table 2. Calculation of Germ Numbers Before and After Gargling with PVP-I

No	Before	PVP-I 0.2%		PVP-I 0.4%		PVP-I 0.6%		PVP-I 0.8%		PVP-I 1.0%	
		15s	30s	15s	30s	15s	30s	15s	30s	15s	30s
1	$2{,}9 \times 10^2$	1,3 x 10	0,9 x 10	0,3 x 10	0,1 x 10	0,1 x 10	0 x 10	0,1 x 10	0 x 10	0 x 10	0 x 10
2	$2{,}6 \times 10^2$	1,1 x 10	0,6 x 10	0,4 x 10	0,1 x 10	0 x 10	0 x 10	0 x 10	0 x 10	0 x 10	0 x 10
3	$3{,}4 \times 10^2$	1,9 x 10	0,8 x 10	0,2 x 10	0,1 x 10	0 x 10	0 x 10	0 x 10	0 x 10	0 x 10	0 x 10
4	$2{,}3 \times 10^2$	1,4 x 10	0,8 x 10	0,2 x 10	0,1 x 10	0,1 x 10	0 x 10	0 x 10	0 x 10	0,1 x 10	0,1 x 10
5	$3{,}0 \times 10^2$	1,4 x 10	0,7 x 10	0,3 x 10	0,2 x 10	0,1 x 10	0 x 10	0 x 10	0 x 10	0 x 10	0 x 10
6	$3{,}0 \times 10^2$	1,0 x 10	0,6 x 10	0,5 x 10	0,2 x 10	0 x 10	0 x 10	0 x 10	0 x 10	0 x 10	0 x 10
7	$2{,}3 \times 10^2$	1,2 x 10	0,7 x 10	0,2 x 10	0,1 x 10	0 x 10	0 x 10	0 x 10	0 x 10	0 x 10	0 x 10
8	$2{,}9 \times 10^2$	1,4 x 10	0,9 x 10	0,4 x 10	0,3 x 10	0,1 x 10	0 x 10	0,1 x 10	0 x 10	0 x 10	0 x 10
9	$2{,}5 \times 10^2$	1,1 x 10	0,8 x 10	0,4 x 10	0,2 x 10	0,1 x 10	0 x 10	0 x 10	0 x 10	0 x 10	0 x 10
10	$3{,}2 \times 10^2$	1,9 x 10	0,6 x 10	0,2 x 10	0,1 x 10	0,1 x 10	0 x 10	0 x 10	0,1 x 10	0 x 10	0 x 10
11	$2{,}9 \times 10^2$	1,7 x 10	0,8 x 10	0,2 x 10	0,1 x 10	0,1 x 10	0 x 10	0 x 10	0 x 10	0 x 10	0 x 10
12	$2{,}9 \times 10^2$	1,9 x 10	0,7 x 10	0,2 x 10	0,1 x 10	0,1 x 10	0,1 x 10	0 x 10	0 x 10	0 x 10	0 x 10

In Table 2, most of the germ numbers (75%) obtained results of more than 250 CFU/mL before intervention. The highest germ rate was 3.4 x 102 CFU/mL, and the lowest was 2.3 x 102 CFU/mL. After rinsing, the whole number of germs obtained results of less than 30 CFU/mL starting from the lowest concentration of 0.2% and the growth of the number of germs was not found at a concentration of 0.6%.

From the results of the calculation of the germ count in Table 2, it was found that the germ count before rinsing was 230-300 colonies, while the germ count after rinsing was very low, with the highest number of colonies, namely 19 colonies. A very significant decrease was found in the sample group rinsing with PVP-I with the lowest variation in concentration and contact time, namely a concentration of 0.2% for 15 seconds, where the number of germs before rinsing was hundreds, while the number of germs after rinsing with this treatment only numbered dozens. The unit number of germs was found in the gargling

DOI: 10.32668/jitek.v11i2.1449

treatment with PVP-I concentration variations of 0.2% with a contact time of 30 seconds, while the treatment group with a concentration of 0.6% with a contact time of 15 seconds to the treatment group with a concentration of 1.0% with a contact time of 30 seconds, the number of germ colonies that grew even only 0-1 colonies. In theory, PVP-I is an antiseptic with a broad spectrum; PVP-I effectively kills gram-positive, gram-negative, and even spore bacteria (Eggers *et al.*, 2018). This is in line with research conducted by Betadion Rizki in 2014, which stated that PVP-I is effective in killing mixed bacteria (more than one species) so that all types of bacteria in the mouth can be killed by PVP-I mouthwash. Besides killing many species of bacteria, PVP-I mouthwash can also reduce bacteria in saliva (Fauzia, Hartman and Jeffrey, 2021).

The Friedman test was then used for multivariate analysis. The result is Table 3 below.

Table 3. Friedman Test Analysis

Non-Parametric *Friedman*	
n	12
p-value	0.00

After the Friedman test was carried out with a confidence level of 95% ($\alpha = 0.05$, a p-value of 0.000 was obtained (p-value $\leq$ 0.05), there was a difference in the number of germs before and after rinsing with PVP-I in all variations of concentration and contact time. The significant decrease in germ numbers before and after rinsing using PVP-I at all variations in concentration and contact time can be seen statistically in Table 3. The reduction of germ numbers was due to PVP-I having anti-bacterial properties. The antiseptic activity of Povidone Iodine is based on the ability to penetrate free Iodine, which will oxidize amino acids, nucleotides and unsaturated fatty acids in microorganism cells. This causes Povidone Iodine to damage protein and microbial DNA, so the microbial cells will die (National Center for Biotechnology Information, 2022).

Christoper et al., in 2020 in their research explained that the release of the active ingredient Iodine from the PVP-I complex and optimal bactericidal activity occurs in the concentration range of 0.1% to 1.0%. Maren Eggers of 2018 said that PVP-I with a concentration variation of 0.23% with a contact time of 15 seconds was proven to

DOI: 10.32668/jitek.v11i2.1449

be quite effective in reducing the number of germs. In Table 4, it can be seen that the PVP-I concentration of 0.2% has been effective in reducing the number of germs, but statistically, it has a significant difference when compared to the reduction in the number of germs in PVP-I concentrations of 0.4%, 0.6%, 0.8% and 1.0% in the entire contact time was either 15 or 30 seconds. Research conducted by Kanagalingam in 2015 shows that PVP-I is faster in killing germs. PVP-I can kill all tested strains of germs within 30 seconds of contact time, while other mouthwashes, such as Cetylpyridinium Chloride (CPC), are only effective within 60 seconds. This can happen due to the bactericidal effect of each mouthwash.

Analysis in the follow-up test (Post Hoc Test) using the Bonferroni test was also carried out to determine variations in the difference in germ numbers between treatment groups, the results of the analysis can be seen in Table 4 below.

Table 4. Bonferroni Test Analysis

	0%	0.2% 15s	0.2% 30s	0.4% 15s	0.4% 30s	0.6% 15s	0.6% 30s	0.8% 15s	0.8% 30s	1.0% 15s	1.0% 30s
0%	-	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.2% 15s	0.00	-	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.2% 30s	0.00	0.00	-	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.4% 15s	0.00	0.00	0.00	-	0.01	0.00	0.00	0.00	0.00	0.00	0.00
0.4% 30s	0.00	0.00	0.00	0.00	-	0.29	0.00	0.00	0.00	0.00	0.00
0.6% 15s	0.00	0.00	0.00	0.00	0.29	-	0.37	1.00	0.37	1.00	1.00
0.6% 30s	0.00	0.00	0.00	0.00	0.00	0.37	-	1.00	1.00	1.00	1.00
0.8% 15s	0.00	0.00	0.00	0.00	0.00	1.00	1.00	-	1.00	1.00	1.00
0.8% 30s	0.00	0.00	0.00	0.00	0.0	0.37	1.00	1.00	-	1.00	1.00
1.0% 15s	0.00	0.00	0.00	0.00	0.00	1.00	1.00	1.00	1.00	-	1.00
1.0% 30s	0.00	0.00	0.00	0.00	0.00	1.00	1.00	1.00	1.00	1.00	-

Based on the results of the follow-up test (Post Hoc Test) using the Bonferroni test with a confidence level of 95% ($\alpha = 0.05$), the results in Table 4 show that not all germ numbers in each treatment group have a significant difference. An insignificant difference in germ numbers began to be seen in the treatment group gargling with a PVP-I concentration variation of 0.6%

DOI: 10.32668/jitek.v11i2.1449

contact time of 15 seconds compared to the treatment group gargling with a PVP-I concentration variation of 1.0% contact time of 30 seconds.

In Table 4, it can also be seen that the use of PVP-I based on variations in contact time did not show many different results, but the results of significant differences occurred in variations in concentration 0.2% contact time 15 seconds and concentration 0.2% contact time 30 seconds with a p-value < 0.05. Pattana-Arun and Wolff, 2008 said that the bactericidal effect caused by free iodine can work within 15 seconds. These results align with Daniela P's research in 2017, namely that 15 seconds was not inferior in reducing the number of germs, but with a contact time of 30 seconds, it reduced the number of germs. This is also in accordance with the statement from the CDC, 2016 that the higher the concentration, the faster the contact time required, conversely if the concentration is lower, the required contact time will be longer.

In the use of antiseptics, concentration and contact time need to be considered. Selection of concentration and contact time can affect the toxic effects that arise. The harmful effects of using antiseptics can increase with increasing concentration and contact time (Zhang *et al.*, 2023). PVP-I is known to have the lowest toxic effect when compared to other antiseptics such as Octenidine, Chlorhexidine and Hydrogen Peroxide (Bigliardi *et al.*, 2017), but the toxicity of PVP-I as an antiseptic can occur when it is used excessively and in large amounts/concentrations. Excessive use of Povidone Iodine can cause a corrosive effect caused by the Iodine content (National Capital Poison Center, 2021).

CONCLUSION

This study concludes that there is a significant difference between the number of germs before and after rinsing using PVP-I in all variations of concentration and contact time with a p-value of 0.00 <95%. Variation of concentration and lowest contact time of 0.2% for 15 seconds is effective enough in reducing the number of germs in the mouth so that the toxic effects caused by using PVP-I can be minimized.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

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DOI: 10.32668/jitek.v11i2.1449

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DOI: 10.32668/jitek.v11i2.1449