

EFFECT OF MOUTHRINSE CONTAINING IMMUNOGLOBULIN-Y ANTI-COMD STREPTOCOCCUS MUTANS + CHITOSAN ON QUANTITY OF SALIVARY STREPTOCOCCUS MUTANS IN CARIES AND CARIES-FREE SUBJECTS

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ABSTRACT

Objective: Immunoglobulin-Y (IgY) anti-ComD *Streptococcus mutans* is expected to be an alternative passive immunization agent against caries. Chitosan which has an antibacterial property is expected to produce a better result. The aim of this study is to evaluate the effect of mouthrinse containing IgY anti-ComD *S. mutans* + chitosan on the quantity of salivary *S. mutans* in caries and caries-free subjects.

Methods: Each subject group was given IgY anti-ComD *S. mutans* mouthrinse and IgY anti-ComD *S. mutans* + chitosan mouthrinse. Mouthrinse was used twice a day for 6 days. Salivary *S. mutans* was cultured in TYS20B agar before and after treatment. The quantity of salivary *S. mutans* colonies was counted manually.

Results: This study showed that mouthrinse containing IgY anti-ComD *S. mutans* + chitosan has the potential to decrease the quantity of salivary *S. mutans* although not significantly.

Keywords: Caries, Chitosan, ComD protein, Immunoglobulin Y, Mouthrinse.

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INTRODUCTION

The most common dental problem in Indonesia is caries, with a 90.05% prevalence according to the Household Health Survey 2004 [1]. This figure is concerning because oral and dental health is very important to support the health of the body.

Microorganisms having a major role in the etiology of caries include *Streptococcus mutans* [2]. *S. mutans* has a mechanism of interspecies (quorum sensing) communication, involving the ComD receptor on the cell surface [3]. Inactivation of the *ComD* gene, which is a gene encoding the ComD receptor that is present on the cell surface, decreases the potential of *S. mutans* to form colonies, and interact with other bacteria [3,4]. *S. mutans* can be found in saliva, and their numbers are related to caries activity in individuals [2].

Research to find alternative methods of caries prevention continues, such as by active and passive immunization. However, because active immunization with the administration of whole *S. mutans* cells would cause side effects of cross-reaction with cardiac muscle tissue [5], researchers are attempting to develop anticaries passive immunization methods [6].

Immunoglobulin-Y (IgY), obtained from chicken eggs, is an inexpensive and easily obtainable source for the production of specific antibodies [7]. IgY does not harm the host, and the presence of IgY in host saliva has no side effects [8].

Many studies identified the effects of IgY on *S. mutans* antigen. Hatta *et al.* developed an IgY anti-*S. mutans* mouthwash and reported decreased numbers of *S. mutans* in saliva [9]. Anggraeni also studied the use of IgY in a gel preparation in Sprague-Dawley rats and reported a significant decrease in *S. mutans* in rat saliva [10]. In addition, Chismirina investigated the effect of IgY as an inhibitor of *S. mutans* adhesion and reported a decrease in *S. mutans* colonization on tooth surfaces [11].

Bachtiar produced the specific IgY ComD *S. mutans* that interfered with the *S. mutans* bacterial communication system. DNA vaccine could be used as an innovative strategy to control the colonization of caries-causing bacteria [12].

The addition of chitosan, which has antibacterial, antifungal, biocompatible, and flexible characteristics, was expected to yield better results than IgY anti-ComD *S. mutans* alone. As noted by Henrietta, IgY anti-cell *S. mutans* and chitosan could suppress the amount of *S. mutans* in biofilms. In addition, chitosan also could act as a preservative [13,14].

Conflicting results could be seen in a study by Febyani, in which autoaggregation of *S. mutans* serotypes c and e actually increased after exposure to IgY anti-*S. mutans* and chitosan [15]. Similar results also were reported by Septryani, who showed that the gel containing the chitosan-nano silver composite with IgY anti-*S. mutans* serotype c actually decreased the effectiveness of IgY anti-*S. mutans* in inhibiting the formation of *S. mutans* biofilm [15]. Therefore, to correct the deficiencies of previous studies, we studied the effect of a mouthrinse containing IgY anti-ComD *S. mutans* + chitosan on the quantity of salivary *S. mutans*.

METHODS

After obtaining ethical clearance, 24 FKG UI students with and without caries provided signed informed consents to participate. Subjects were divided into two groups given mouthwash containing IgY anti-ComD *S. mutans* (Group 1) and IgY anti-ComD *S. mutans* + chitosan (Group 2).

The mouthwash was made by adding IgY anti-ComD *S. mutans* to total care non-alcohol mouthwash to a concentration of 0.01% and chitosan to a concentration of 2% in a 120 mL mouthwash bottle. Before treatment, 1 mL of the subjects' saliva was collected. Subjects then were asked to rinse 2 times a day in the morning and evening for 30 s using 10 mL of mouthwash for 6 days. After the treatment period, 1 mL samples of saliva were collected again.

Each saliva isolate then was centrifuged for 2 min at 13,000 g pressure. Then, the salivary supernatant was removed, and the remaining pellet was added to 1 mL phosphate-buffered saline and pipetted. A total of 15 μ L were cultured from each sample in duplicate on medium agar TYS20B and incubated at 37°C for 3×24 h. Bacterial colonies of *S. mutans* were calculated manually.

The data then were analyzed statistically. Data in group 1 had a normal distribution and were tested with the paired t-test. Data in Group 2 had an abnormal distribution and were tested with the Wilcoxon signed-rank test. The numbers of bacterial colonies in both groups were compared and tested by an independent t-test. The statistical significance level was set at 0.05 ($p=0.05$).

RESULTS

Mean values decreased in both groups after treatment (Figs. 1 and 2). The paired t-test and Wilcoxon signed-rank test in Groups 1 and 2, respectively, revealed no significant difference in mean numbers of *S. mutans* colonies between before and after treatment ($p=0.218$ and $p=0.583$, respectively). The decrease in *S. mutans* colonies was greater in Group 2.

DISCUSSION

After the discovery of the association of *S. mutans* with dental caries, various studies have been conducted to determine alternative caries prevention methods using antibacterial agents. We used IgY anti-ComD *S. mutans* as antibodies and chitosan, which had antimicrobial effects.

IgY inhibits caries development by inhibiting *S. mutans* colonization. IgY works by binding to *S. mutans* antigens and neutralizing the

biological activity of the antigen. Specific IgY antigen I/II given locally may bind *S. mutans* because the I/II antigen resides on the cell wall [16]. This causes the *S. mutans* bacteria to fail to proliferate. In addition to its antigens, virulence of *S. mutans* bacteria also involves intercultural and interspecies communication mechanisms (quorum sensing). Communication of *S. mutans* involves the role of the autoinducer molecule and its receptor (ComD) on the cell surface [4]. IgY anti-ComD *S. mutans* interferes with this communication system to control the colonization of the bacteria that cause caries. On the basis of the inactivation ComD study of Loo *et al.*, the gene encoding ComD decreases the ability of *S. mutans* to colonize and interact with other bacteria [4]. The IgY anti-ComD *S. mutans* used in this study were produced by Bachtiar [12]. We used chitosan because, in addition, its antibacterial properties, chitosan also can bind well to proteins, in this case, IgY anti-ComD *S. mutans* [17]. In addition, Costa *et al.* showed that chitosan is capable of interfering with *S. mutans* adhesion and biofilm formation and capable of decreasing mature biofilm defenses by as much as 94% [18]. Other chitosan benefits are that it is nontoxic, biodegradable, and biocompatible [19].

Samples were taken from the saliva of the subjects because the number of *S. mutans* bacteria in saliva described the number of bacteria in the biofilm and was related to caries levels in adults [20]. In this study, a mouth rinse was chosen because it was used commonly in caries prevention. We wished to determine the effect of combined IgY anti-ComD *S. mutans* + chitosan on the amount of *S. mutans* saliva in subjects with and without caries. After treatment, the number of *S. mutans* in the saliva decreased in both test groups. A larger decrease occurred in Group 2 compared with Group 1.

Our results are supported by those of several studies. Studies on the effects of IgY in mouthwash conducted by Hatta *et al.* demonstrated a decrease in the ratio of *S. mutans* percentage per total streptococci to saliva after subjects used a mouth rinse containing IgY and 10% sucrose twice in 4 h [9]. In the same study, subjects who used a nonsucrose IgY mouth rinse for 6 days experienced a decrease in *S. mutans* salivary levels that were not statistically significant. Nguyen *et al.* also reported a significant decrease in the number of *S. mutans* in the saliva of subjects given lozenges containing Gtf-specific IgY [21]. Smith *et al.* reported that specific IgB GbpB may provide protection from the mutagenicity of *S. mutans* and prevent caries [22]. Their research showed that molar caries levels in mice given GbpB-specific IgY decreased significantly within 78 days, and this decrease was related to the amount of IgY administration. In addition, the effect of IgY on the amount of *S. mutans* in saliva also was investigated by Yonezu *et al.*, indicating that topical application of IgY significantly decreased the amount of *S. mutans* in saliva [23].

Our study provided an overview of the effect of a mouthwash containing a combination of IgY anti-ComD *S. mutans* + chitosan on the number of *S. mutans* saliva in subjects with and without caries. Our results showed no significant difference in the decreased numbers of *S. mutans* in subjects who used a mouth rinse with and without chitosan. Further research must be done to determine the effectiveness of chitosan as an anti-ComD IgY binding agent in the form of mouthwash and to determine the concentration of IgY anti-ComD *S. mutans* and chitosan effective for use as an alternative to caries prevention.

CONCLUSIONS

Mouthwash containing a combination of IgY anti-ComD *S. mutans* + chitosan could decrease the number of *S. mutans* in saliva, but not significantly. More research is needed on the concentration of IgY anti-ComD *S. mutans* and chitosan effective to decrease the amount of *S. mutans* in saliva significantly.

CONFLICTS OF INTEREST

Declared none.

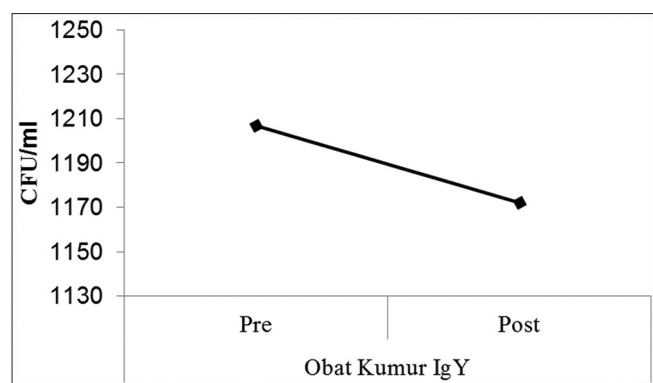


Fig. 1: Mean number of *Streptococcus mutans* colonies group in Group 1 before and after treatment

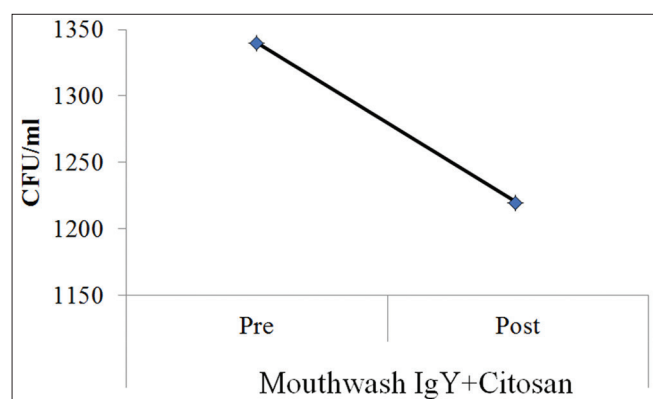


Fig. 2: Mean number of *Streptococcus mutans* colonies in Group 2 before and after treatment

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