



Original article

Effects of oral moisturising gel containing egg yolk antibodies against *Candida albicans* in older people

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Effects of oral moisturising gel containing egg yolk antibodies against *Candida albicans* in older people

Objective: The aim of this study was to evaluate the inhibitory effects of oral moisturising gel containing egg yolk antibody against *Candida albicans* (anti-CA IgY) in older people. Therefore, we measured the number of *Candida* CFU present on oral swabs at baseline and after using the gel.

Methods: A randomised, double-blind, placebo-controlled trial was conducted among volunteers living in a nursing home in Japan. The participants were divided into two groups. The group 1 participants received oral care using an experimental oral moisturising gel with anti-CA IgY, and those in group 2 received oral care using a placebo oral moisturising gel without anti-CA IgY. The oral care was performed by care workers three times a day for 4 weeks. The participants' tongues were sampled using a swab method at baseline and after 2 and 4 weeks of using the oral gel, and the number of *C. albicans*, *Candida tropicalis* and *Candida krusei* colonies was counted.

Results: The baseline oral condition of the participants in the two groups did not differ significantly. The experimental gel significantly reduced the number of *C. albicans* colonies from baseline to after 4 weeks of using the oral gel; however, no significant reductions were observed in the number of *C. tropicalis* or *C. krusei* colonies.

Conclusion: The use of oral moisturising gel containing anti-CA IgY for 1 month significantly reduces the number of *C. albicans* CFU present on swabs in older people.

Keywords: *Candida*, candidiasis, egg yolk antibody, oral moisturising gel, randomized controlled trial.

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Introduction

Candida albicans is the species most frequently implicated in oral candidiasis^{1,2}. Affected patients complain of tongue pain, a burning sensation and symptoms of taste disorders; therefore, oral candidiasis has an important effect on the quality of life^{3,4}. The disease is rare in healthy adults and instead tends to develop in older patients with a declining immune function and in patients receiving long-term steroid or antibiotic therapy or those with diabetes or blood diseases^{1,5}. In addition, xerostomia decreases defences due to a decreased level of salivary secretion, thereby allowing for increases in the level of *Candida* colonisation, making it easier to develop tongue atrophy and oral candidiasis^{6,7}.

The application of chicken egg yolk antibodies (IgY) is useful for preventing various diseases due to the simple and low-cost method of manufacturing^{8,9}. In addition, the antibodies are highly safe and do not activate mammalian complement or react with rheumatoid factor^{10,11}. Antibodies against gingipains of *Porphyromonas gingivalis* (anti-GP IgY) and antibodies against *Streptococcus mutans* cell-associated glucosyltransferase (anti-CA-gtf IgY) have been developed to prevent oral diseases, such as periodontal disease and caries^{12,13}. Studies of anti-*C. albicans* egg yolk antibodies (anti-CA IgY) have been performed to evaluate the effects on human oral epithelial cells *in vitro* and in rats¹⁴; however, no research has been conducted to clarify the effects of these antibodies in humans. Therefore, the aim of this

study was to evaluate the effectiveness of using a moisturising gel containing anti-CA IgY in reducing the number of *Candida* CFU present on swabs of the oral cavity.

Methods

Participants

The participants included 30 volunteers living in a special nursing home for the older people in Japan who were not receiving antifungal drug therapy, did not have abnormal oral mucosa and did not have an egg allergy. The participants and their family members received information regarding the study and signed consent forms to participate. A randomised controlled trial was conducted between June and July 2013. The participants were randomly assigned to two groups treated by care workers: group 1, who received oral care using an oral moisturising gel containing anti-CA IgY (experimental group); and group 2, who received care using oral moisturising gel without anti-CA IgY (control group). We applied stratified randomisation according to the oral moisture score. We excluded participants who reported little use of the oral moisturising gel and those with data deficiencies. The final participants included 26 people with a mean age of 89.2 ± 5.4 years (experimental group: two males and 11 females, mean age: 89.2 ± 5.8 ; control group: three males and 10 females, mean age: 89.2 ± 5.3). This study was approved by the Tsurumi University Dental Department Ethics Committee (Approval number 1006).

Preparation and schedule of application of the oral moisturising gel

The oral moisturising gel was composed of sorbitol, glycerine, carboxymethylcellulose, xylitol, sodium citrate, hydrolysed collagen, monolaurin acid polyglyceryl, citric acid, dipotassium glycyrrhizinate and cetylpyridinium chloride. The experimental group received an oral moisturising gel containing 1% anti-CA IgY which is Ovalgen[®] CA (Ew Nutrition Japan, Gifu, Japan); control group received the gel without anti-CA IgY. The participants received the oral moisturising gel three times a day (after each meal) for 4 weeks. After meals, the participants received teeth cleaning using tooth brushes, with the exception of those who were edentulous. Denture wearers also received denture cleaning after removing the dentures. The care workers were instructed to place

the gel on a sponge brush measuring length of the sponge 1 cm and apply it in a thin layer while rubbing softly across the entire oral mucosa, palate, buccal mucosa and tongue. After application, the denture wearers again wore their dentures.

Oral examinations

Three oral examinations were conducted to evaluate changes in the oral health status and the level of *Candida* colonisation. The first oral examination was performed before using the gel at baseline, the second oral examination was performed 2 weeks after baseline, and the third oral examination was performed 4 weeks after baseline. The oral examinations included assessments of the number of teeth present, presence or absence of dentures, presence of a tongue coating, oral moisture status and the level of *Candida* colonisation. The presence of tongue coating was evaluated according to a modification of the criteria proposed by Oho et al¹⁵. The tongue was divided into six regions, and the presence of a tongue coating was recorded in each region using a score of 0–2, as follows: 0 = no coating; 1 = slight coating; and 2 = significant coating (total score: 0–12). The oral moisture status was evaluated using a moisture meter mucus[®] (Life Co., Ltd., Saitama, Japan) device¹⁶. The left buccal mucosa was measured five times. We calculated the average value after excluding the maximum and minimum values. The presence of *Candida* was sampled using the swab method. The surface of the dorsum of the tongue was swabbed 10 times with a cotton swab (Nippon Becton Dickinson Co., Ltd., Tokyo, Japan), which was then directly inoculated onto CHROMagar™ *Candida* medium (Nippon Becton Dickinson Co., Ltd., Tokyo, Japan). The number of *Candida* colonies on the agar was counted following incubation at 37° for 48 h¹⁷. The *Candida* species were identified based on the colour of each colony according to the manufacturer's chart.

Statistical analysis

The mean and standard deviation of each index were calculated. The Wilcoxon rank-sum test was used to compare the oral health status of the experimental and control groups at baseline (5% significance level). The chi-squared test was used to compare the presence or absence of dentures in the experimental and control groups (5% significance level). The differences in the longitudinal data for the oral examination scores obtained at baseline and after using the gel at each

Table 1 Characteristics and oral health status of participants at the baseline.

| Parameter | EXP | CTL | p-Value |
|--|------------|------------|---------|
| Teeth present | 5.2 ± 8.3 | 3.8 ± 4.9 | 0.960 |
| Oral moisture status | 27.7 ± 3.5 | 27.3 ± 1.6 | 0.762 |
| Tongue coating | 5.4 ± 3.3 | 5.0 ± 2.9 | 0.960 |
| <i>Candida albicans</i> (Log ₁₀ CFU/swab) | 1.3 ± 1.1 | 0.7 ± 0.8 | 0.223 |
| <i>Candida tropicalis</i> (Log ₁₀ CFU/swab) | 0.1 ± 0.5 | 0.1 ± 0.2 | 1.000 |
| <i>Candida krusei</i> (Log ₁₀ CFU/swab) | 0.6 ± 0.9 | 0.7 ± 1.0 | 0.650 |
| Total <i>Candida</i> (Log ₁₀ CFU/swab) | 1.5 ± 1.2 | 1.0 ± 1.0 | 0.336 |

EXP, experimental group; CTL, control group.

examination point were analysed using the Friedman test (5% significance level) and post hoc Wilcoxon signed-rank test with Bonferroni adjustment (1.67% significance level) in each groups. Due to the non-normal distribution of the number of *Candida* colonies, logarithmic transformation was applied to the variables. The statistical analysis was performed using the SPSS (Tokyo, Japan) 19.0 software package.

Results

Characteristics and oral health status of the participants at baseline

The average number of teeth present was 5.2 ± 8.3 in the experimental group and 3.8 ± 4.9 in

the control group. The average oral moisture score was 27.7 ± 3.5 in the experimental group and 27.3 ± 1.6 in the control group. The average tongue coating score was 5.4 ± 3.3 in the experimental group and 5.0 ± 2.9 in the control group. The percentage of participants using dentures was 46.2% ($n = 6$) in the experimental group and 38.5% ($n = 5$) in the control group. There were no statistically significant differences in the oral health status or percentage of participants with dentures between the two groups at baseline.

The average (mean ± SD) number of *C. albicans* colonies (Log₁₀ CFU/swab) was 1.3 ± 1.1 in the experimental group and 0.7 ± 0.8 in the control group. The average number of *Candida tropicalis* colonies was 0.1 ± 0.5 in the experimental group and 0.1 ± 0.2 in the control group. The average number of *Candida krusei* colonies was 0.6 ± 0.9 in the experimental group and 0.7 ± 1.0 in the control group. The average number of all *Candida* colonies was 1.5 ± 1.2 in the experimental group and 1.0 ± 1.0 in the control group. There were no statistically significant differences in the number of *Candida* colonies between the two groups at baseline (Table 1).

Changes after using the oral moisturising gel

Changes in the oral moisture status and presence of a tongue coating. The changes in the oral moisture status and presence of tongue coating are shown in Table 2. At baseline and after 2 and 4 weeks, the oral moisture scores were 27.7 ± 3.5, 26.7 ± 3.6 and 27.9 ± 3.3 in the experimental group, and 27.3 ± 1.6, 25.8 ± 3.3 and 27.3 ± 2.5 in the control group, respectively. There were no significant

Table 2 Changes in the oral moisture and tongue coating score.

| Parameter | Baseline (0 week) | After 2 weeks (2 weeks) | After 4 weeks (4 weeks) | Friedman test p-Value |
|--|-----------------------|-------------------------|-------------------------|-----------------------|
| Oral moisture status | | | | |
| EXP | 27.7 ± 3.5 | 26.7 ± 3.6 | 27.9 ± 3.3 | 0.794 |
| CTL | 27.3 ± 1.6 | 25.8 ± 3.3 | 27.3 ± 2.5 | 0.291 |
| Tongue coating | | | | |
| EXP | 5.4 ± 3.3 | 3.1 ± 2.0 | 2.0 ± 1.4 | < 0.001 |
| CTL | 5.0 ± 2.9 | 3.2 ± 2.4 | 2.2 ± 2.0 | 0.004 |
| Wilcoxon signed-rank test ^a | | | | |
| | 0 vs. 2 weeks p-Value | 0 vs. 4 weeks p-Value | 2 vs. 4 weeks p-Value | |
| Tongue coating | | | | |
| EXP | 0.007 | 0.003 | 0.046 | |
| CTL | 0.039 | 0.009 | 0.027 | |

^a1.67% significance level, due to Bonferroni adjustment.

improvements in each group from baseline to after using the gel according to the Friedman test.

At baseline and after 2 and 4 weeks, the tongue coating scores were 5.4 ± 3.3 , 3.1 ± 2.0 and 2.0 ± 1.4 in the experimental group, and 5.0 ± 2.9 , 3.2 ± 2.4 and 2.2 ± 2.0 in the control group, respectively. There were significant improvements from baseline to after 2 and 4 weeks in the experimental group and from baseline to after 4 weeks in the control group according to the Friedman test and post hoc Wilcoxon signed-rank test with Bonferroni adjustment (Table 2).

Changes in the number of Candida colonies. The changes in the number of *Candida* colonies (Log_{10} CFU/swab) and participants who are yeast positive are shown in Table 3. At baseline and after 2 and 4 weeks, the number of *C. albicans* colonies was 1.3 ± 1.1 , 1.2 ± 1.1 and 0.6 ± 0.9 in the experimental group. There was a significant reduction of the number of *C. albicans* colonies from baseline to after 4 weeks in the experimental group according to the Friedman test and post hoc Wilcoxon signed-rank test with Bonferroni adjustment. However, there were no significant

| Parameter | Baseline | After 2 weeks | After 4 weeks | Friedman test |
|---|---|--|--|---------------|
| | Mean \pm SD Min–Max Yeast positive ^a | Mean \pm SD Min–Max Yeast positive | Mean \pm SD Min–Max Yeast positive | p-Value |
| <i>Candida albicans</i> (Log_{10} CFU/swab) | | | | |
| EXP | 1.3 ± 1.1 0–1.9 10/13 | 1.2 ± 1.1 0–2.9 8/13 | 0.6 ± 0.9 0–2.2 6/13 | 0.028 |
| CTL | 0.7 ± 0.8 0–2.8 9/13 | 0.5 ± 0.9 0–2.9 8/13 | 0.7 ± 0.7 0–2.2 9/13 | 0.168 |
| <i>Candida tropicalis</i> (Log_{10} CFU/swab) | | | | |
| EXP | 0.1 ± 0.5 0–1.9 1/13 | 0.1 ± 0.2 0–0.5 2/13 | 0.1 ± 0.5 0–1.8 1/13 | 0.867 |
| CTL | 0.1 ± 0.2 0–0.7 2/13 | 0 0–0 0/13 | 0.0 ± 0.2 0–0.6 1/13 | 0.368 |
| <i>Candida krusei</i> (Log_{10} CFU/swab) | | | | |
| EXP | 0.6 ± 0.9 0–2.8 5/13 | 0.6 ± 0.9 0–2.4 6/13 | 0.4 ± 0.7 0–2.4 6/13 | 0.846 |
| CTL | 0.7 ± 1.0 0–2.7 6/13 | 0.6 ± 0.8 0–2.1 6/13 | 0.7 ± 1.0 0–2.8 7/13 | 0.607 |
| Total <i>Candida</i> (Log_{10} CFU/swab) | | | | |
| EXP | 1.5 ± 1.2 0–2.9 10/13 | 1.4 ± 1.1 0–2.9 10/13 | 0.8 ± 1.0 0–2.6 9/13 | 0.132 |
| CTL | 1.0 ± 1.0 0–3.1 11/13 | 0.9 ± 0.9 0–2.6 9/13 | 1.0 ± 0.9 0–2.8 10/13 | 0.273 |
| <i>Wilcoxon signed-rank test^b</i> | | | | |
| | 0 vs. 2 weeks p-Value | 0 vs. 4 weeks p-Value | 2 vs. 4 weeks p-Value | |
| <i>Candida albicans</i> (Log_{10} CFU/swab) | | | | |
| EXP | 0.484 | 0.011 | 0.069 | |

Table 3. Changes in the number of *Candida* colonies.

^aThe number of participants who were yeast positive.

^b1.67% significance level, due to Bonferroni adjustment.

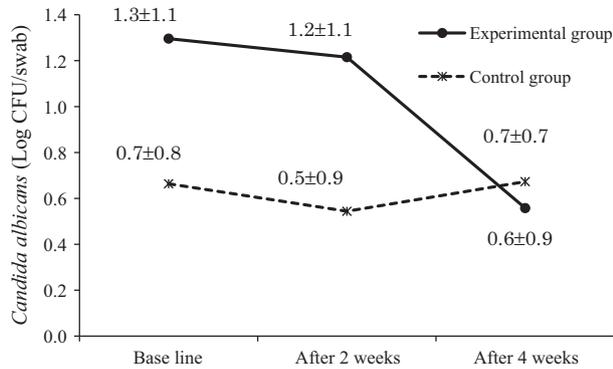


Figure 1 Comparison of changes in the number of *Candida albicans* colonies. The results are presented as the mean \pm SD of the number of *C. albicans* colonies (Log_{10} CFU/swab) in each group.

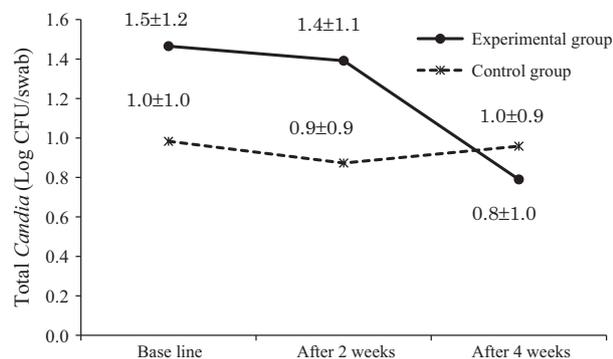


Figure 2 Comparison of changes in the total number of *Candida* colonies. The results are presented as the mean \pm SD of the total number of *Candida* colonies (Log_{10} CFU/swab) in each group.

improvements from baseline to after using the gel in the control group (Table 3, Figs 1 and 2).

Discussion

This study sought to evaluate the inhibitory effects of oral moisturising gel containing anti-CA IgY in older people. Therefore, we measured the number of *Candida* CFU present on swabs at baseline and after using the oral gel. Our study findings demonstrated that the number of *C. albicans* colonies present on the swabs was significantly reduced by using the oral moisturising gel containing anti-CA IgY in the older participants after 1 month. Previous studies of anti-CA IgY have been performed to evaluate the effects on human oral epithelial cells *in vitro* as well as in rats; however, no research has been conducted with respect to the effects of these antibodies in humans. Our findings suggest the possibility of lowering the risk of oral candidiasis,

although further studies are needed to evaluate these effects on a long-term basis and in oral candidiasis patients.

Anti-CA IgY is specific to *C. albicans*. In the present study, there were no statistically significant reductions in the number of *C. tropicalis* or *C. krusei* colonies. However, in a previous study, Fujibayashi *et al.*¹⁸ reported that anti-CA IgY cross-reacts with various *Candida* spp. and the anti-CA IgY reduces the level of adherence to human oral epithelial cells *in vitro*. One cause of these differences in results may be differences in the preparation of the anti-CA IgY; however, further research is needed to address these issues.

Age is one of the risk factors for oral candidiasis, especially in the older person in need of nursing care. In the present study, oral care was provided three times a day both in the experimental and in the control groups by caregivers. By the virtue of oral care, the tongue coating significantly reduced even in the control group. It seems that the reduction in tongue coating is due to tongue rubbing with the sponge brush rather than due to the oral gel. The formation of a tongue coating occurs during the solid interactive phase in the oral cavity primarily involving large salivary proteins and large amounts of desquamated epithelial cells and bacterial and blood components¹⁹. Approximately one-third of the bacteria in the oral cavity are found on the surface of the tongue²⁰. Providing oral care is important for preventing oral malodor and pneumonia in the older people by reducing the number of bacteria in the oral cavity^{21–23}. In the present study, the amount of tongue coating significantly decreased in both the experimental and control groups after using the gel. The tongue coating scores in both groups decreased by 50% or more after 4 weeks, and we confirmed that oral care using the oral gel contributed to reducing the incidence of a tongue coating. However, one previous study reported that the oral care with oral moisturising gel alone does not reduce the number of *Candida* colonies²⁴. Therefore, the use of oral care with an oral moisturising gel containing anti-CA IgY may act to lower the risk of oral candidiasis. In addition, the cost of oral moisturising gel containing anti-CA IgY is not particularly high compared with other types of oral moisturising gel.

Dry mouth is another risk factor for oral candidiasis. Although moistening mouth gel has known to be effective to alleviate dry mouth²⁵, there were no significant changes on the oral moisture score in the present study. One of the possible reasons might be that the participants enrolled in

the present study did not include severe dry mouth patients. The baseline moisture score was more than 27 in most of the cases; the cut-off point ≥ 26 was set on the dry mouth according to the manufacturer's data sheet.

Denture wearers also possess the risk of oral candidiasis. Denture care is important for the prevention of oral candidiasis. When treating patients who have candidiasis, removed denture should be treated as well. Many studies investigated the prevention of candidiasis; these are adhesion of *C. albicans* to the denture materials and effective cleaning of dentures^{26–29}. Anti-CA IgY-containing oral moisturising gel must be useful tool to prevent the adhesion of *Candida* to the denture, and/or removal of biofilms comprised of fungi from the denture surface. The products containing anti-CA IgY can be applied not only in the oral cavity, but also on the denture surface.

The present study investigated the effects of the oral gel for an observation period of only 1 month.

In addition, we simply measured the number of *Candida* CFU present on the swabs. It is not known whether the same results would have been obtained in a long-term study which investigated oral candidiasis patients. Further research is therefore needed to examine the long-term effects of using the oral gel, as well as the efficacy of this method in oral candidiasis patients.

Conclusion

The present study demonstrated that the use of oral moisturising gel containing anti-CA IgY for 1 month significantly reduces the number of *C. albicans* CFU present on swabs in older people. The findings of this study suggest the possibility of lowering the risk of oral candidiasis, although further studies are needed to evaluate the long-term effects of using oral gel in oral candidiasis patients.

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