ORIGINAL RESEARCH

Efficacy of Immunoglobulin Y Chewable Tablets on *Streptococcus mutans* Count in Patients Undergoing Orthodontic Treatment

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Abstract

Aim: The aim of the study was to determine the efficacy of commercially available immunoglobulin Y (IgY) chewable tablets on *Streptococcus mutans* (*S. mutans*) count in patients undergoing orthodontic treatment.

Materials and methods: Participants aged between 12 and 19 years who had fixed, nonextraction orthodontic treatment with no carious lesion were included in the study and advised to take IgY chewable tablet for 15 days (one course) and saliva samples were collected from the patients on day 61, 91, and 121 days from the placement of fixed appliance, to assess its ability in reduction of *S. mutans* count. The collected samples were processed, and *S. mutans* levels were estimated. The results were tabulated and subjected to statistical analysis using Statistical Package for the Social Sciences (SPSS) version 20, and the repeated measures test was used to compare different groups.

Results: Immunoglobulin Y (IgY) chewable tablet was found to be significantly effective in decreasing *S. mutans* counts in patients undergoing orthodontic treatment during the study period.

Conclusion: Oral passive immunotherapy *via* egg yolk antibody IgY effectively decreased the *S. mutans* level, which was found to increase during orthodontic fixed appliance treatment.

Clinical significance: The IgY chewable tablets can be used as an adjuvant to reduce *S. mutans* counts and provide basic oral hygiene measures.

Keywords: Caries prevention, Children, Dental caries, Immunoglobulin Y, Immunization, Orthodontic treatment, *Streptococcus mutans*.

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Introduction

Dental caries is a common and widespread disease in adults and children.[1] Dental caries is a multifactorial, irreversible disease of the calcified tissues of teeth that demineralize the inorganic portion and destroy the tooth's organic substance, which may or may not lead to cavitation.[2] Caries are caused by the interplay between the host factors such as saliva and teeth, the oral microflora, the diet, and the time.[1] It is prevalent throughout the world, affecting people of all age-groups.[3] In India, the National Oral Health Survey reported a 58% prevalence of dental caries among schoolchildren.[2] Hence, with such a prevalence rate, this disease has gained the interest of the scientific community in the prevention of the disease. It is unlikely that all the preventive measures would be sufficient to eradicate the multiple factors responsible for causing the disease.[4]

Streptococcus mutans (*S. mutans*) are the primary microbial agents that induce transmissible dental caries. The primary colonization of *S. mutans* is by vertical transmission and secondarily due to an imbalance in the oral environment that would lead to the early colonization of *S. mutans*.[5] *S. mutans* does not colonize the oral cavity until the emergence of teeth as it requires a nonshedding surface, to sustain itself.[6] *S. mutans* require a virgin habitat to colonize easily without competing with the already established bacteria.[6–8]

Acquisition of *S. mutans* in the oral cavity is influenced by various host factors, such as hereditary, altered mucosal surfaces, reduced quantity and quality of saliva, reduced oral immunity from congenital to acquired conditions, frequent ingestion of sweets, snacks and sugary drinks, and poor oral hygiene.[9] Fixed appliances used during orthodontic therapy make maintenance of oral hygiene difficult, as the complex design of orthodontic bands and brackets tends to accumulate plaque, facilitating the growth of bacteria.[10] According to a study by Lundström and Krasse in 1987, patients undergoing orthodontic appliance therapy have changes in oral microflora, of which *S. mutans* count in saliva and plaque were found to have increased.[11] Common methods employed for maintaining oral hygiene in patients undergoing fixed orthodontic treatment include dietary advice, proper brushing techniques, the use of mouthwashes and fluoride application which were aimed at eliminating or suppressing the caries-causing bacteria and thereby preventing dental caries. Recently, antibodies against the bacteria's

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virulent factors *via* immunotherapy (vaccination/immunization) have gained interest. Various caries vaccine techniques are available to induce a response against the caries-causing bacteria.[12]

Caries vaccine was first introduced by William Bowen in 1969. Whole-cell *S. mutans* were injected in *Macaca fasicularis* monkeys to induce an immune reaction which showed successful results against caries.[7] As of late, various cariogenic components, such as adhesins, glucosyltransferase (GTF) enzymes, dextranases, and glucan-binding proteins (GBPs) of *S. mutans* are employed in the production of antibodies.[13] Vaccines target the initial stages of host colonization by inhibiting the adhesion of bacteria to the tooth surface, inactivation of enzymes and blockage of surface receptors.[14] Prevention of dental caries by vaccination includes active and passive immunotherapy. The focus has been shifted to passive immunotherapy, which has more merits than demerits in its usage. Passive immunization against *S. mutans* includes bovine milk antibodies, monoclonal antibodies, plant antibodies, and a newer approach from egg yolk, which has been of great interest in recent years.[15] Immunoglobulin Y (IgY) (IgY, egg yolk antibodies) was discovered as early as the 1800s, but the recent alternative method of IgY production has gained attention due to its many benefits.[16] Various studies have shown the effectiveness of IgY used in various forms in caries prevention, both in animals and in humans. Hatta et al., in 1996, reported the antibacterial efficacy of mouth rinse containing IgY against *S. mutans* in humans.[17–19] Bachtiar et al., in 2016, showed the effect of topical IgY anti-*S. mutans* gel reduces *S. mutans* count on the rat's tooth surface and the persistence of IgY in saliva.[18]

The possible effect of these immunotherapies on maintaining oral hygiene and maintaining balance in an oral environment in patients undergoing orthodontic treatment has been less reported. In India, IgY is made available commercially in the form of flavored chewable tablets to reach out to children. Hence, the proposed study is to find the efficacy of the commercially available IgY chewable tablets on *S. mutans* counts in children/adolescents undergoing orthodontic treatment.

Materials and Methods

The present follow-up study was planned and carried out in the Department of Pedodontics and Preventive Dentistry along with the Department of Orthodontics and Microbiology at Sree Balaji Dental College and Hospital, Chennai, Tamil Nadu, India, following approval from the Institutional Ethics Committee (Ref No: SBDCH/IEC/10/2018/12).

Subjects aged 12–19, reporting to the Department of Orthodontics, Sree Balaji Dental College and Hospital, Chennai, Tamil Nadu, India, who had planned to undertake fixed orthodontic treatment, were recruited for the study. For this study, 58 subjects were screened and examined clinically by the chief investigator and the proforma containing the following details were recorded, *viz*, the demographic information, such as name, age, sex, contact number and address; medical history of any recent illness and history of antibiotic therapy in past 3 months, long-term medications; dental history regarding restorations, extractions, oral prophylaxis, topical fluoride application; personal history regarding diet (vegetarian or nonvegetarian), hypersensitivity to egg, and brushing habit. Intraoral assessment of caries was done using the International Caries Detection and Assessment System II (ICDAS II) scoring system. The presence of interproximal caries/secondary caries was investigated radiographically with an orthopantomogram taken for diagnostic purposes for orthodontic treatment. Parents/guardians were explained about the study in detail. A total of 21 subjects (1–21) who fulfilled the following selection criteria were included in the study following written informed consent from the parents/guardians. Subjects in the age-group of 12–19 years, subjects who planned for fixed orthodontic appliance treatment without extraction, subjects with no active carious lesion (examined clinically with ICDAS II score = 0 and radiographically with orthopantomogram), parents/guardians who were ready to participate in the study were included. Subjects who planned for removable orthodontic appliance treatment, subjects with carious dentition, subjects planned for orthodontic extraction, subjects who underwent oral prophylaxis or topical fluoride application within 3 months, subjects under antibiotic therapy in past 3 months, subjects with systemic disease, long-term medication, physically and mentally challenged subjects with a history of hypersensitivity to egg were excluded.

Salivary Sample Collection

Six salivary samples (A–F) were collected from each subject during the course of this study. On the day of saliva collection, subjects were asked to refrain from eating, tooth brushing, and mouth rinsing for at least 2 hours. The subject was made to sit upright in the dental chair with the head bent down and mouth open to allow the saliva to drip passively from the lower lip into the sterile plastic container (M B Laboratory, Chennai, Tamil Nadu, India) until 2 mL of unstimulated saliva was collected (A). The collected sample was stored in an icebox and transferred immediately to the microbiology laboratory for processing. On the same day, the patient underwent ultrasonic scaling, after which the second saliva sample (B) was collected and transferred according to the above procedure. On the scheduled appointment, the fixed orthodontic appliance was placed. The subject was instructed to follow oral hygiene instructions as advised and was asked to report back on the 15th day from the placement of the orthodontic appliance. On the 15th day, a third saliva sample (C) was collected according to the procedure stated above. From the 16th day, the subject was instructed to take one course (15 days: day 16–30) of IgY chewable tablets according to the instructions stated below—to take a dose of 40 mg after breakfast and 80 mg after dinner; to chew the tablet before swallowing; not to eat or drink for one hour after intake. The subject was asked to report back after 1 month (61st day), 2 months (91st day), and 3 months (121st day) for the collection of fourth (D), fifth (E) and sixth saliva sample (F), respectively, according to the procedure stated above. Salivary samples (A–F) from all the participants (1–18) were collected as stated above. Three study participants withdrew from the study after the collection of the second sample.

Processing of Saliva Sample and Enumeration of *S. mutans* Count

The saliva samples were processed immediately in the microbiology laboratory. Around 10 µL of each salivary sample was inoculated onto a sterile Mutans-Sanguis Agar plate (HiMedia). The inoculum was uniformly seeded on the agar surface using the spread plate method. The plate was incubated at 37°C in 5% carbon dioxide for 24 hours. *S. mutans* appear as 3–5 mm, round, convex, glossy, gummy white colonies on Mutans-Sanguis Agar. The number of colony-forming units (CFU) of *S. mutans* was counted using a digital colony counter (deep vision).

Statistical Analysis

The number of *S. mutans* colonies was recorded, and the results were statistically analyzed using Statistical Package for the Social Sciences (SPSS) version 20. The repeated measures test was used to compare different groups. A *p*-value of ≤0.05 was considered statistically significant.

Results

The results obtained are presented in Figure 1, which shows the mean *S. mutans* count of six salivary samples (A–F). There was a significant reduction in the mean *S. mutans* count from 98222.22 CFU/mL (sample C), which was taken before the course of the tablet, to 55622.222 CFU/mL (sample D), 46366.667 CFU/mL (sample E), and 303000 CFU/mL (sample F) in the subsequent samples taken after one course of IgY chewable tablet on day 61, 91, and 121 with *p*-value of 0.029, 0.014, and 0.003, respectively (Table 1). The result implies the effectiveness of IgY chewable tablets in decreasing *S. mutans* counts in patients undergoing orthodontic treatment. The baseline *S. mutans* counts before scaling (sample A), after scaling (sample B) and after initiation of orthodontic treatment (sample C) had a mean count of 274022.222 CFU/mL, 88022.222 CFU/mL, and 98222.22 CFU/mL, respectively.

Discussion

Dental caries occur due to an imbalance created among the host (saliva and teeth) and environmental factors (oral microflora, substrate). *S. mutans* is considered the chief etiological agent responsible for the initiation of dental caries, which is referred to as incipient caries/white spot lesions.[20,21] A major side effect of orthodontic treatment with fixed appliances is the demineralization of enamel, leading to the formation of white spot lesions on the labial surfaces of the teeth. A study done by Boersma et al. in 2005 showed that up to 97% of the patients who underwent fixed orthodontic appliance therapy had white spot lesions.[22] Peros et al. in 2011 studied the levels of *S. mutans* and *Lactobacillus spp* in children who were undergoing fixed orthodontic appliance therapy and found an increase in salivary *S. mutans* count.[23]

Various preventive measures against cariogenic *S. mutans* are reported, of which vaccinations *via* active or passive immunization have gained interest.[5] Passive antibody administration has been examined for effects on indigenous mutants streptococci. Hatta et al. in 1997 studied the effect of hen egg yolk IgY antibody to *S. mutans* cells which led to modest decreases in the numbers of indigenous mutans streptococci in saliva or dental plaque.[17–19]

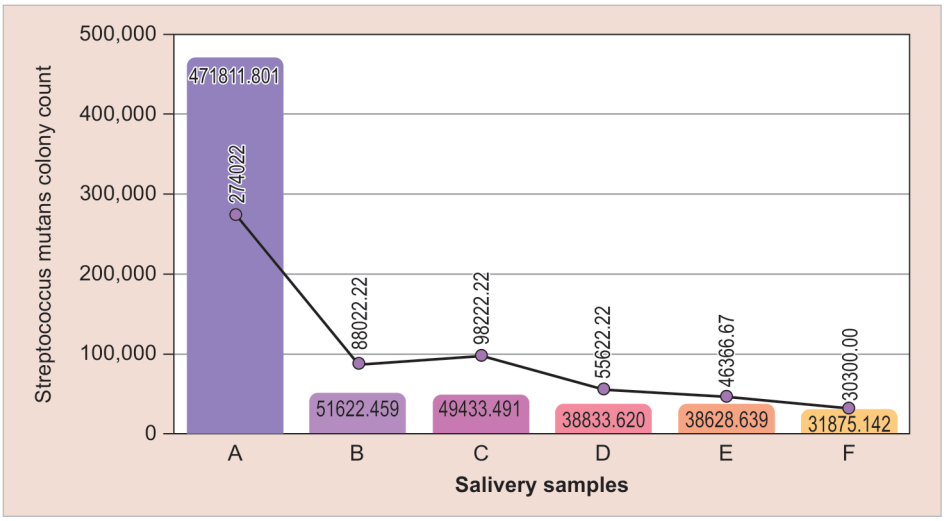

Fig. 1: The standard deviation and the mean of *S. mutans* count of six salivary samples (A–F) taken during the study period

Table 1: Intercomparison of samples collected during the course of the study, which showed a variable mean difference ranging from a minimum of 9255.556 CFU/mL to a maximum of 67922.222 CFU/mL. Intercomparison of samples C vs D, C vs E, and C vs F showed a mean difference of 42,600.000 CFU/mL, 51855.556 CFU/mL, and 67922.222 CFU/mL, which were statistically significant with a *p*-value of 0.029, 0.014, and 0.003, respectively

				95% confidence interval for difference	
Inter sample comparison	*Mean difference CFU/mL*	*Standard error*	*p-value*[+]	*Lower bound*	*Upper bound*
B vs C	−10200.000	14211.058	1	−58662.620	38262.620
C vs D	42600.000	11610.174	**0.029***	3006.928	82193.072
C vs E	51855.556	12925.256	**0.014***	7777.786	95933.325
C vs F	67922.222	14202.855	**0.003***	19487.578	116356.87
D vs E	9255.556	9108.106	1	−21804.952	40316.063
D vs F	25322.222	11329.791	0.587	−13314.684	63959.129
E vs F	16066.667	6332.399	0.319	−5528.1 12	37661.445

[+], Repeated measures; *, Bold values show statistically significant *p* values ($p \leq 0.05$) significance

Hence, in this study, the effectiveness of chewable tablets containing IgY against *S. mutans* was analyzed in patients who were planned for fixed orthodontic treatment.

Subjects who had undergone oral prophylaxis, fluoride therapy or antibiotic therapy in the past 3 months were excluded from this study as it could have an effect on salivary *S. mutans* count. Subjects with active carious lesions were excluded from the study because of the direct correlation that exists between a higher frequency of active caries and elevated proportions of *S. mutans* count, as reported by Ali et al. in 1998. Subjects who were hypersensitive to eggs were excluded from the present study.[24] Hoffman in 1983, stated that almost all patients with food allergies to eggs were reactive to white protein.[25] IgY though derived from egg yolk, for the safety of the participants and to avoid any possible side effects during the course of the study, this criterion was taken into consideration. In this study, dietary instructions were given before saliva collection to avoid the influence of food consumption. Unstimulated saliva collected by the draining method reflects the basal salivary flow, and it's preferred to use overstimulated saliva since the latter contains only a diluted concentration.[26] In this study, saliva was collected as the patient sat upright with the head slightly tilted forward, as stated ideal by Shannon and Chauncey in 1967.[27,28]

The *S. mutans* count of sample B taken after scaling in all subjects showed a decrease in count. This is similar to the study done by Devishree in 2015, which reported that ultrasonic vibration from the scalers definitely reduced the bacterial load.[29] *S. mutans* count of sample C taken after initiation of orthodontic treatment on the 15th day, 10 subjects showed an increase in *S. mutans* count, which correlates with the study done by Ahn et al. in 2005, had reported that the complex features of orthodontic bands and brackets may create an ecological imbalance that would pave the way for the establishment and growth of *S. mutans*.[20] Also it can be attributed to subject's inability to maintain proper oral hygiene. Few subjects had shown a decrease in *S. mutans* count after the initiation of fixed orthodontic treatment, which can be related to the result in the study done by Papaioannou et al. in 2007 had reported that the presence of a salivary pellicle on the brackets and other bacterial species seem to have a significant effect on the adhesion of *S. mutans* which has low binding affinity, thus reducing their number.[30] The subjects were asked to take one course of IgY chewable tablets as per the manufacturer's instruction for 15 days from the 16th to the 30th day.

When comparing sample C with samples D, E, and F, 15 subjects in the 1st month 16 subjects in the second and 3rd months had shown a decrease in *S. mutans* count. When comparing sample D with samples E and F, 16 subjects in the 2nd month and 14 subjects in the 3rd month had shown a decrease in *S. mutans* count. On comparing sample E with sample F, 10 subjects in the 3rd month had shown a decrease. Reduction in *S. mutans* count in our study could be attributed to the ability of IgY to intervene in the binding process by either blocking the adhesins, which are the receptors necessary for colonization or *via* glucan-binding domains of GBPs and GTF that aid in the accumulation of bacterias within the dental biofilm. Kruger et al. 2004 showed the effect of IgY to GTF and the reduction in caries induction in rat models.[31] Jain et al. 2022, in a study, reported that IgY chewable tablet had the efficacy to decrease *S. mutans* count and has also mentioned that IgY confers extended immunity by preventing recolonization of *S. mutans* onto the tooth surface by the antibodies persisting in saliva.[32] The results of the present study are well in line with the Nguyen et al. study reported in 2011, a 5-day randomized, double-blind, placebo-controlled trial used lozenges containing IgY, which has significantly ($p < 0.001$), suppressed oral colonization of *S. mutans*.[16]

The significant decrease in the last three samples (D, E, and F) reveals the efficacy of IgY chewable tablets in reducing the *S. mutans* count at the end of 3 months following one course of the tablet. The finding of this study was similar to a long-term study done by Gandhimathi et al. in 2015, which tested the efficacy of mouth rinse with IgY antibody, used two times a day for 15 days, wherein there was a decrease in *S. mutans* count until 7 months.[4] Hence, from this study, it is evident that the egg yolk antibodies (IgY), taken as chewable tablets for 15 days, significantly reduced *S. mutans* count for 3 months in patients undergoing fixed appliance therapy. Future studies with larger sample sizes, wider age-group ranges, and long-term studies would probably strengthen the research on the efficacy of IgY chewable tablets in reducing *S. mutans* count.

Conclusion

The IgY chewable tablets were effective in decreasing *S. mutans* counts in patients undergoing orthodontic treatment. Hence, IgY chewable tablets can be used as an adjuvant to reduce *S. mutans* counts along with basic oral hygiene measures in subjects undergoing orthodontic treatment, thus reducing the risk of developing dental caries.

Manufacturer Name

Mutans-Sanguis Agar (HiMedia Lab Pvt Ltd, Thane, Maharashtra, India).

IgY Chewable Tablets (Nodecay™ Inzpera Healthsciences Ltd, India).

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