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Comparative Evaluation of Antibacterial Efficacy and Salivary pH on Consumption of Immunoglobulin Y (IgY) Containing Tablets in Preventing Dental Caries in Children – An in vivo study

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Abstract

Passive Immunization plays an important role in prevention of dental caries. The present in vivo study evaluated the role of oral Immunoglobulin Y (Ig Y) in providing passive immunity, in terms of salivary Streptococcus mutans count and salivary pH. Forty caries-free children, 6-12 year old, were selected and randomly divided into two groups of 20 each. Group A children (experimental group) were instructed to chew Ig Y containing tablets as per recommended dose, along with regular tooth brushing twice daily for a period of 15 days. While Group B children (control group) were advised for regular tooth brushing only twice daily for the same period. Correct tooth brushing technique was demonstrated and the study performed under supervision. Unstipulated saliva samples were collected twice, first at baseline and then at the end of intervention period. Saliva samples from both the Groups A and B, were divided into two subgroups of 10 each, ie, A1, A2 and B1, B2. Samples from A1 and B1 were tested for counting salivary *S. mutans* colonies using a colony counter whereas samples from A2 and B2 were tested for estimating salivary pH using a digital pH meter. Statistical analysis of th

results obtained showed a significant difference among both experimental and control groups when Intergroup and Intergroup comparisons were made. Group A1 and Group B1 showed significant reduction of about 53.07% and 18.69% respectively in S. mutans count while Group A2 and B2 showed an increase of about 9.89% and 1.68% respectively in salivary pH. The present study suggests that Passive immunization with IgY antibodies, when used along with regular tooth brushing, deserves attention in the field of caries prevention in children during the mixed dentition stage and has a much better potential when compared with regular tooth brushing alone. It could also be a promising agent for maintaining oral hygiene in a child who is uncooperative or even in a special child who lacks manual dexterity. Thus IgY containing tablets can be strongly recommended as an adjuvant to regular oral hygiene practice.

Keywords: Dental caries, prevention of dental caries, salivary pH, Streptococcus mutans, passive immunization, oral Immunoglobulin Y (IgY).

Introduction

Millions of children in the world still suffer from dental caries which renders a challenging situation, as once the disease progresses, it is very difficult to revert back to the original healthy state. In this aspect, the age old concept “An ounce of Prevention is worth a pound of cure”, holds good even today, it is thus prudent to address the disease way before its onset. Although there has been a phenomenal decline in prevalence of dental caries with the introduction of various preventive measures, it has not been possible to completely eliminate the disease. Thus the need arises to essentially identify alternative means of preventing the disease altogether, in a way minimizing the future damage it might cause. Here the concept of Passive immunization against dental caries might play an important role which signifies implementation of certain immunological substance designed to produce specific protection against a given disease in humans. [1] Absolute safety should be ensured with respect to immunotherapy but attempts at caries vaccines are still neither successful nor encouraging. [2] Thus in this context Passive immunization might win over active immunization strategies which seem to have more risk over benefits. [3]

Scientific research and technological advances have contributed to a better understanding of the dental disease process and modified dentistry from a purely repairable art to a prevention oriented science. For decades, dental caries have been a multifactorial disease caused by the interplay of four principal factors: host (primarily saliva and teeth), micro flora (cariogenic microorganism), substrate (diet) and time.[3]Thus, if we could intercept even one factor, we will be able to prevent it. Considerable evidence implies that microorganisms play the major role in causing dental caries. Among them, Streptococcus mutans bacteria have been regarded as the key contributor, by virtue of possessing cariogenic determinants such as adhesins, glucosyl transferase enzymes (GTF), mutacin and glucan binding proteins (GBP). [4]Out of these, the cell-associated glucosytransferase enzyme (CA-GTF) of S. mutans plays a vital role in synthesizing water insoluble glucan from fermentable carbohydrates, that traps the acid produced during the process, creating a conducive environment for further growth of the bacteria and subsequent destruction of susceptible dental hard tissues. [4]It also facilitates further accumulation and aggregation of cariogenic bacteria mediated by glucan binding proteins. Furthermore, the metabolic activity of S. mutans can potentiate postprandial pH drop at plaque–tooth interface, eventually leading to dental decay. Hence most of the current treatment regimens are now aimed at either elimination of this bacteria or suppressing their virulence factors. Immunization strategies targeting cariogenic determinants of S. mutans such a

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glucosyltransferases (GTFs) might effectively interfere with molecular pathogenesis of mutans streptococci, thus resulting in caries prevention. [3&5]

Passive immunization against dental caries in humans involves administration of preformed antibodies generated against specific molecular targets of S. mutans bacteria. The use of chicken egg yolk antibodies, better known as Immunoglobulin Y (IgY), as a novel approach of passive immunotherapy have been discovered in the late 1800s, yet its prophylactic & therapeutic applications have gained attention only in the last two decades. [6]Specific IgY antibodies are obtained by immunizing hen with the antigen of interest. For example, specific anti CA-GTF IgY antibodies are obtained by hyper immunizing hens with the CA-GTF antigen of S.mutans. The IgY antibodies get maternally transferred to the yolk of the eggs laid by these hens, then extracted, purified and given orally to humans. [Figure 1] A small amount of antigen usually elicits enough IgY response in the egg yolk of immunized hens and the IgY-titre remains very high for a certain period of time, over several weeks to few months. [7]Due to convenience of large scale production of chicken eggs, a huge quantity of antibodies could be possibly obtained from hens in an economical way. Because of high yield, sustainable availability and financial viability, IgY could be considered as an ideal alternative to other antibodies. There is apparently no systemic immune response with respect to orally administered antibodies. IgY antibodies are thus highly target specific, easier and cheaper to be produced and shows no cross reactivity with the human complement system. [6]Since poultry eggs are generally considered safe being a normal component of human diet, egg yolk antibodies are considered safe for everyday consumption. [8]IgY is Generally Regarded As Safe (GRAS) by the United States Department of Agriculture (USDA), the Food and Drug Administration (FDA) as well as the Food Safety Standards Authority of India (FSSAI).

Literature supports the role of IgY in preventing a wide variety of infectious diseases in humans including oral diseases like oral candidiasis, gingivitis, periodontitis as well as dental caries. [4,9,10]Various clinical trials revealing encouraging results have given IgY a functional food status and introduced IgY as a novel ingredient in formulations like toothpastes, tablets, lozenges or mouthrinses. Specially the chewable tablet form of oral Ig could provide a painless, hassle free yet effective solution when it comes to treating a child patient. Oral IgY tablets are nowadays extensively used in places abroad like Vietnam, United States of America, Japan as Ovalgen DC, [5] Hakira etc. In India also it is recently available as Nodecay Chewable tablets but so far no data have been available comparing its efficiency with regular tooth brushing alone as a tool for maintaining daily oral hygiene. This has lead to the present study which has been undertaken to evaluate the antibacterial efficacy of orally administered Immunoglobulin Y (IgY) containing chewable tablets for caries prevention in children.

It is already established that the number of mutansstreptococci in saliva is directly linked to its number colonized on dental surfaces. A direct correlation exists between elevated proportions of S.mutans in saliva and a higher frequency of caries activity. [11] Furthermore, salivary pH level also plays a crucial role in establishing a caries conducive acidic environment suitable for further growth of the cariogenic bacteria. Thus in the present study, saliva sample was chosen as a potent medium to check for significant levels of S.mutans as well as pH changes in a given subject. [12]

The present clinical trial was therefore aimed to

1. Evaluate and compare salivary S.mutans count using colony counter device, in children, before and after using oral IgY containing chewable tablets.

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2. Evaluate and compare salivary pH using handheld digital pH meter, in children, before and after using oral IgY containing chewable tablets.

3. Compare the mean percentage changes obtained with a control group.

Materials and Method

Forty caries-free healthy institutionalized children in the age group 6-12 years were selected based upon dental examination and relevant case history. Children in this age group have mixed dentition which is a highly dynamic stage as the primary teeth are exfoliating and the permanent teeth are erupting, so this transition period usually have increased susceptibility to dental caries. Consent for participation in the study was obtained. Children having dmft/DMFT scores as 0(zero),according to WHO diagnostic criteria for dental caries, and residing in the institution's hostel thus having similar dietary and oral hygiene habits, were included in the study for proper monitoring and standardization purpose. Unstimulated saliva samples were collected at baseline from the participants before the clinical trial to establish Streptococcus mutans level as well as salivary pH. The children were explained the importance of good oral health and demonstrated with correct method of tooth brushing by a trained professional. All of them were provided with commercially available, conventional and similar tooth pastes and tooth brushes at the beginning of the study. The participants were randomly divided into two groups- Group A and Group B of 20 children each. Group Aor Experimental group children were advised to chew oral Immunoglobulin Y (Ig Y) containing 'No decay' table twice daily, as per recommended dose ie, one 20 mg chewtab (orange in color) in the morning after breakfast and one 40 mg chew tab (white in color) in the night after dinner, for a intervention period of 15 days. As per the manufacturer's guidelines, these children were also advised to continue their regular tooth brushing, twice daily, along with using the tablets, but following correct tooth brushing technique as demonstrated. They were asked not to swallow but chew the tablets, brush their teeth properly before chewing and neither eat nor drink anything for at least half an hour after chewing. Group B or Control group children, were advised to do only regular tooth brushing, twice daily for 15 days, following correct tooth brushing technique. Group B children were not given the Ig Y containing chew tabs. Compliance and effectively of tooth brushing was ensured with the study being performed under supervision. Unstimulated saliva samples were collected again from the participants at the end of intervention period. Saliva samples from both the Groups A and B were again divided into subgroups of 10 each, ie, A1(n=10), A2(n=10) and B1(n=10), B2(n=10). Samples from A1 and B1 were tested for salivary Streptococcus mutans count whereas samples from A2 and B2 were tested for salivary pH changes[Figure 2].The saliva samples were coded during sample collection and microbiological analysis. After being transported in a thermocol ice box to lab for investigation, the saliva samples were immediately subjected for microbiological analysis to assess the *S.mutans* level. The saliva samples were diluted in phosphate buffered saline (pH 7.0) to the serial dilutions of $1/10^3$, agitated for30 seconds on a vortex cyclomixer. One milliliter of saliva sample was inoculated into mites salivarius bacitracin agar(MSB). The agar plates were incubated at 37°C for 48 hours under anaerobic conditions. S.mutans colonies were identified as spherical, raised and dark blue in colour [Figure 3]. The number of S.mutans colonies per milliliter of saliva on each plate was enumerated using the colony counter [Figure 4]. Following this the mean colony forming units (CFU/ml) was determined and semi quantification of the number of colonies was done by multiplying the actual colony count with its dilution factor. Salivary pH was measured with the help of a handheld digital pH meter for both the groups.

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The mean percentage change (between baseline and post intervention) in salivary S.mutans count as well as salivary pH respectively, were evaluated for both the groups and compared with each other using paired and unpaired student 't' test. The data obtained was tabulated and statistically analyzed. Significance level was fixed at $P \leq 0.05$.

Results and Discussion

The mean percentage reduction between baseline and post-intervention showed statistically significant reduction in salivary S.mutans count for both the groups A1 and B1, but it was much higher reduction in Group A1 about 53.07% than in Group B1 about 18.69%. When these percentages were compared with each other, the result was statistically significant (P=0.001). The data has been shown in Table.1 and graphically represented in Graph.1.

The mean percentage reduction between baseline and post-intervention showed statistically significant increase in salivary pH for both the groups A2 and B2, but it was much higher increase in Group A2 about 9.89% than in Group B2 about 1.68%. When these percentages were compared with each other, the result was statistically significant (P=0.001). The data has been shown in Table.2 and graphically represented in Graph.2.

The human oral cavity provides nutrition and facilitates growth of microorganisms. Daily tooth brushing, although considered the basic and most extensively used oral hygiene method worldwide still provide inadequate result sometimes in terms of controlling dental caries in a child patient because of various factors like incorrect tooth brushing technique, frequency, duration etc. Thus supplementary or adjunctive measures might be essential for an added effect in sufficiently decreasing the cariogenic bacterial load from the oral cavity. Now, if these adjuvants are also able to provide immunity against potentially cariogenic species of mutans streptococci which is the chief caries causing agent, and significantly inhibit the cariogenic process thus preventing the disease occurrence, it is invariably a boon to the overall caries preventive protocol. Hence Immunotherapy against dental caries has been considered as one of the appropriate approaches to combat the dental disease and the need for a safe and effective method of caries prevention led to the development of passive immunization with chicken egg yolk antibodies (IgY), which seems to be a good choice. [1,7,13-16]But there is a relative dearth of studies regarding the fact that how much extra benefit this oral IgY could provide over regular tooth brushing alone. Keeping this in mind, the present in vivo study has been undertaken to evaluate the efficacy and efficiency of oral Immunoglobulin Y (IgY) containing chewable tablets generated against CA-GTF antigen of S.mutans bacteria.

The prophylactic effects of a chewing agent may be due to mechanical cleaning as well as potential release of any biologically active ingredient if used. In the present study chewable tablet form has been chosen since oral Immunoglobulin Y antibody if delivered in slowly melting tablet form, facilitates a more thorough contact with the oral components like teeth surfaces, fissures and grooves, saliva etc where the cariogenic bacteria resides. In a study, it was found that children who used chewing agent for oral hygiene had fewer caries lesions than children who brushed their teeth with a conventional toothbrush and paste. [17]Moreover flavored chewtab can be easily administered in pediatric patients just like their favorite toffee. Since children are usually unable to hold liquids in their mouth, flavored lozenge or chewtab are a preferable option. [3]

In the present study, the clinical significance of the decreased levels of salivary mutans streptococci shown by Group A1 (oral IgY chewtab with regular tooth brushing) might be due to the following reasons. It is known that Immunoglobulin Y confers passive immunity against dental caries[5] by immobilizing *S.mutans* and disabling its ability t

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convert sugar into acid. The adherence of *S.mutans* bacteria on the dental surface required for caries development is mediated by the enzymatic action of microbial glucosytransferase (GTFs). [3]CA-GTF has catalytic and glucan binding domains and antibody to these domains can block the synthetic ability as well as other aspects of protein glucan interactions. Past research have shown that antibody against CA-GTF is effective in preventing adherence of S.mutans to tooth surfaces. A short term study showed decrease in the ratio of the percentage of *S.mutans* per total streptococci in saliva in a 4 hour test using a mouth rinse containing 10% sucrose and immune IgY. [4] An effective local protection against plaque formation related to dental caries was achieved with anti-S.mutans IgY. [18] Tooth paste incorporated with anti S. mutans IgY was found to be effective in reducing caries in deciduous teeth in human volunteers. [6] Anti S. mutans IgY spray in adult volunteers produced significant decrease in S. mutans colonies in the test group after three weeks of IgY application, which correlates well with the present study. [10]A five-day trial in young adults have also demonstrated significant decrease in salivary mutans streptococci scores in participants treated with lozenges containing anti-CA-GTF IgY. [3]Similarly in the present study, chewtab containing specific IgY antibodies derived against CA-GTF enzyme of S.mutans have been able to markedly reduce the S.mutans count in saliva. The reason could be formation of antigen- antibody complexes that caused bacterial agglutination and hence reduction in their viable count. Oral IgY shows antimicrobial effect by prevention of bacterial adherence, agglutination, opsonization followed by phagocytosis, neutralization of toxins and inactivation of microbial enzymes. The various mechanism of action could be the following: First, the antigen binding domain of IgY antibody might have compelled the target bacterial cells to aggregate into large clusters, making them susceptible to displacement by salivary movement and rendering them unable to anchor to and colonize tooth surface. Second, the anti-CA-GTF IgY is seen to effectively abolish the GTF catalytic site of the bacteria, thereby inhibiting the ability of CA-GTF enzyme to synthesize water insoluble glucan that promotes *S.mutans* adherence to teeth enamel. Third, the anti CA-GTF IgY molecule could have blocked the glucan binding site of the target bacteria, resulting in failure of *S.mutans* cells to re-colonize existing dental plaque thereby limiting the total number of S.mutans in the oral cavity. Thus tablets containing target specific oral IgY, if administered daily for a particular time period, impedes the survival of *S.mutans* bacteria in the oral environment, reduce their overall count and render them virtually incapable of causing dental caries. Hence this study suggests that tooth brushing itself is a good oral hygiene measure but when aided with oral IgY therapy, provides a huge beneficial effect.

In this study, volunteers in the control Group B1 (regular tooth brushing only), also showed a significant reduction in salivary mutans streptococci count. The resultant decline could be attributed to the fact that all children were staying in an institution, so their dietary habits and oral hygiene maintenance could be monitored. Moreover mechanical tooth cleaning on a routine basis twice daily, maintaining proper technique, might have disrupted dental plaque formation as well as reduced the number of total *S.mutans* colonies. However, the reduction in *S.mutans* count was much higher in Group A1 when compared to Group B1. This could be possibly because for Group A1 children, once the bacteria has been displaced by physical tooth cleaning, application of IgY antibodies locally, prevented their immediate re-colonization and suppressed their re-emergence for a comparatively longer period than the antibody actually persisted in the mouth. Thus correct tooth brushing technique added with IgY antibody activity might have complimentary effect with each other.

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The findings of this clinical study revealed a statistically significant increase in salivary pH for Group A2(oral IgY chewtab with regular tooth brushing). This could be probably because, the daily consumption of IgY containing tablets might have hampered the cariogenic potential of S. mutans and also influenced the amount of acid released by the microorganisms on fermentation of sugars. This modified the oral environment to less acidic and made it less favourable for growth of the bacteria. This might have helped to increase the salivary pH level which resulted in reduced demineralization of teeth and subsequent prevention of dental caries. The results were quite similar to another study where reduced *S.mutans* count was related to increased salivary pH, but in contrast to another study, where no significant correlation has been shown. [19&20]

In this study, children in the control group B2 (regular tooth brushing only), also showed a significant increase in salivary pH, after 15 days intervention period. This could be because all the participants were tooth brushing properly under supervision. So there might be disruption in dental plaque formation and reduced activity of cariogenic bacteria leading to inadequate acid production. All these sequence of events led to increase in salivary pH that is non-conducive for further growth of bacteria necessary for causing dental decay. However, this increment in salivary pH was much higher in Group A2 when compared to Group B2. This was because IgY tablets were chewed on a daily basis along with performing routine tooth brushing twice daily. Here both IgY and good oral hygiene maintenance together have played their role.

Thus daily consumption of IgY containing tablets for about two weeks was proved to be effective enough to prevent dental caries in 6-12 year old children. In a nutshell, regular tooth brushing when performed in combination with oral IgY therapy helps in achieving a much better outcome in terms of preventing dental caries. But IgY should be used in synergism with daily tooth brushing and not as a substitute for it.

In spite of this, it is worth mentionable that passive administration of preformed antibodies does not induce any immunological memory and can persist in the mouth for a short period of time, approximately 90 days, after which the antibody titer starts to decline gradually. Among few studies that have been reported, one short term topical treatment with the antibody resulted in an apparent long term interference with S.mutans decolonization, [14]and another one demonstrated about 4 to 6 months protective effect of anti CA-GTF IgY against dental caries, [1] still the need to provide a continuous source of antibodies at certain intervals, in order to maintain protection over a prolonged time period remains a major challenge. Hence further clinical studies are recommended using different strengths of immune IgY, involving different age groups across the population, with larger sample size and longer follow ups, for successful application of chicken egg yolk derived antibodies and determining its long term efficacy and substantivity in providing immunity against dental caries.

Conclusion and Summary

The results of the present study has clearly shown that passive immunization with IgY generated against CA-GTF of S.mutans has the ability to inhibit decolonization of Streptococcus mutants bacteria on teeth surfaces, reduce their count in saliva as well as maintain a pH that is unfavorable for causing dental decay, thereby interferes with caries development much effectively. Hence, there is a need to emphasize the importance of incorporating oral IgY therapy in addition to regular tooth brushing as a oral hygiene measure in daily oral care. Although passive immunization delivers a comparatively short-term immunity, however it has rapid and local onset of action and also provides immediate and efficient host protection when given in proper concentration onto the target site. Therefore

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it can be concluded that, Passive immunotherapy with chicken egg yolk derived specific oral IgY tablets is a desirable option for prevention of dental caries in children and could be considered as a promising alternative strategy in future caries control. More appropriately, Oral IgY is an efficient, easily administered and cost-effective therapy in prevention of dental caries. Hence can be strongly recommended as an adjuvant to regular oral hygiene practice. Furthermore oral IgY chewtab could also be used in case of uncooperative children as well as a special child, for whom their parents have a tough time in properly maintaining their daily oral hygiene.

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List of Figures, Tables and Graphs

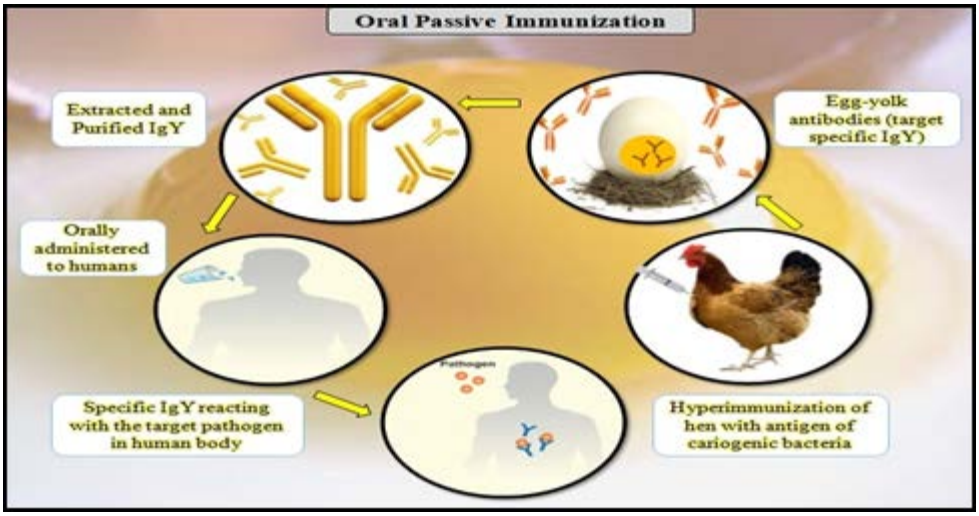

Figure 1: Oral Passive immunization using target specific Immunoglobulin Y (IgY) antibodies

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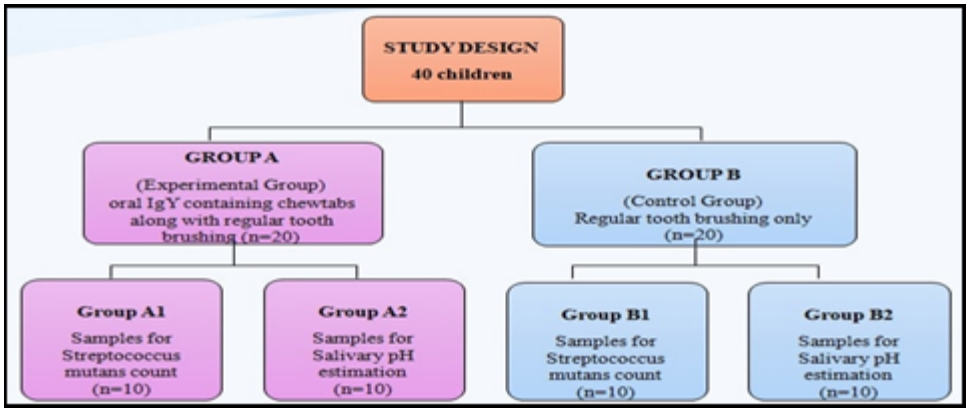

Figure 2: Division of samples

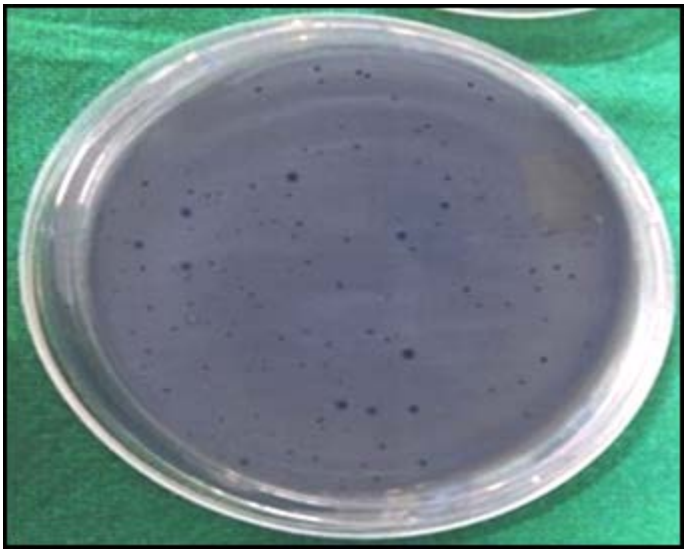

Figure 3: Streptococcus mutants colonies as seen with naked eye post incubation

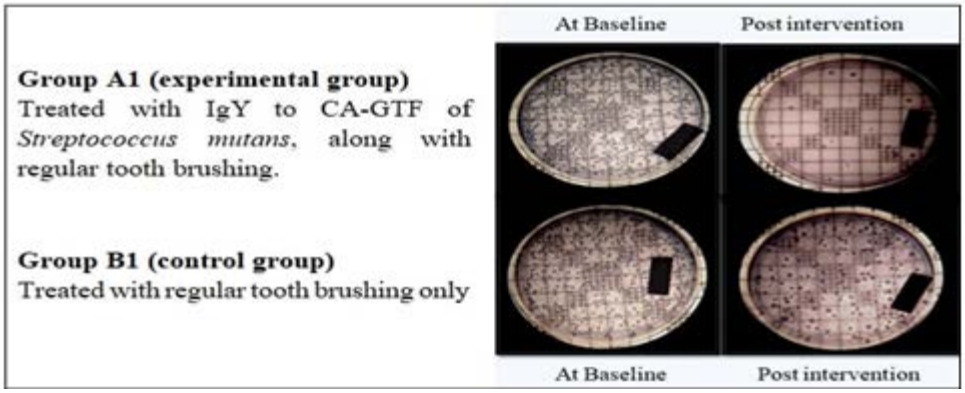

Figure 4: Streptococcus mutans colonies as seen under colony counter showing reduction incount between baseline and post-intervention

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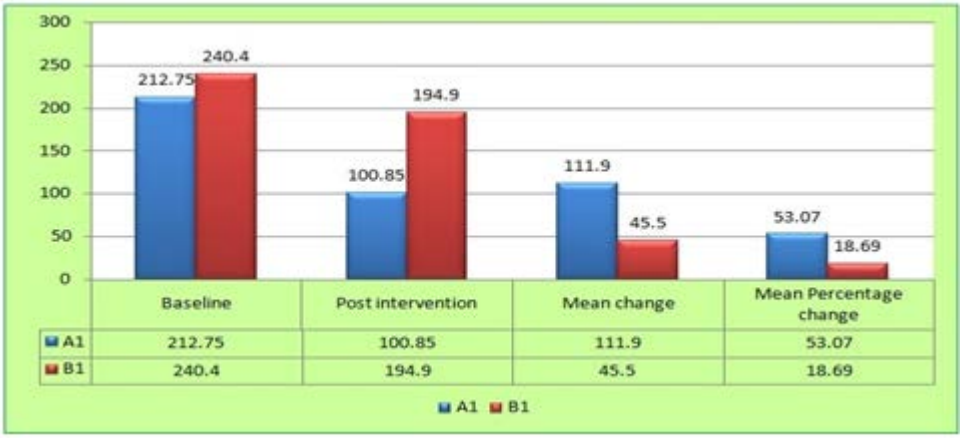
	GROUP	Baseline	Post Intervention	Mean Change	Mean Percentage Change	P value	Significance
Streptococcus mutans count	Group A1	212.75±38.31	100.85±30.24	111.90±20.47	53.07±7.57	0.001	Statistically significant
	Group B1	240.40±56.57	194.90±43.76	45.50±14.54	18.69±2.97	0.001	

Table 1: Mean percentage change in salivary Streptococcus mutans count between baseline and post- intervention for Group A1 (Experimental) & Group B1 (control)



Graph 1: Graphical representation of the Mean percentage reduction in salivary Streptococcus mutans count between baseline and post- intervention for Group A1 (Experimental) & Group B1 (control)

	GROUP	Baseline	Post Intervention	Mean Change	Mean Percentage Change	P value	Significance
Salivary pH	Group A2	5.99±0.23	6.58±0.22	0.59±0.14	9.89±2.56	0.001	Statistically significant
	Group B2	5.97±0.15	6.07±0.15	0.10±0.07	1.68±1.22	0.001	

Table 2: Mean percentage change in salivary pH between baseline and post- intervention for Group A2 (Experimental) & Group B2 (control)

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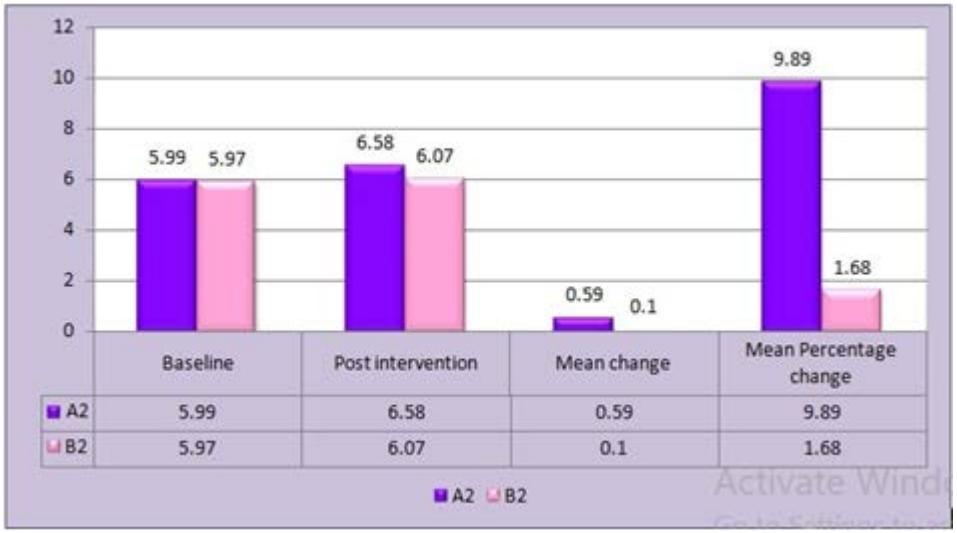

Graph 2: Graphical representation of the increase in salivary pH between baseline and post- intervention for Group A2 (Experimental) and Group B2 (control)

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