

SEM Evaluation of effect of IgY based oral passive immunization against dental caries- An Invitro study

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Citation of this Article: Maj Dr Anubhav Chakrabarty, Lt Col Dr Sonali Sharma, Maj Dr Sourabh Sharma, Maj Sumit Sharma, Maj SGP Sudhir, “SEM Evaluation of effect of IgY based oral passive immunization against dental caries- An Invitro study”, IJDSIR- March - 2021, Vol. – 4, Issue - 2, P. No. 454 – 459.

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Type of Publication: Original Research Article

Conflicts of Interest: Nil

Abstract

Introduction : Dental Caries is an ever growing public health problem. The aim of this study was to evaluate & compare the efficacy of delipidated egg yolk protein tablets in preventing recolonisation of *Streptococcus mutans*.

Materials and methods: 120 freshly extracted molars were collected. Each batch of samples was randomly divided into four groups which are follows- Control, Fluoride and IgY. ATCC stain of *Streptococcus mutans* (25175) were procured and sub-cultured as per

standard guidelines. The organism was then sub-cultured in Brain Heart Infusion broth (BHI) and incubated at 37°C to adjust the suspension to 0.5 Mac Farland standard, which corresponds to 10⁸ colony forming units per milliliter (CFU/mL). The samples were then incubated at 37°C for 48 hours. These plates were incubated at 37°C for 48 hours. Colony counting was done for each group and results were compared with control.

Result: This study has shown the ability of IgY generated against CA-GTF in causing significant reduction in recolonization of bacterial colonies.

Conclusion: The results of the in vitro study reveal that immune IgY has a significant influence in preventing caries development.

Keywords: Dental Caries, Fluorides, IgY

Introduction

Dental Caries is an ever growing public health problem. Along with other treatment modalities, topical fluoride application has been widely practiced to diminish the risk of caries developing.¹ Hence postulation is that fluoride preventive effect is attributed to its propensity to reduce the decalcification of calcified tissues and inhibit growth of bacteria and interfere with its metabolism. Demineralization takes place at a critical pH when the oral habitat is has a low concentration of ions, in comparison to the tooth ionic content.² The enamel crystal or lattice of enamel is constituted primarily of carbonated apatite and in presence of refined carbohydrate, the dissolution of it takes place by the organic acid which are the produce of metabolites of cariogenic biofilm.³ Remineralization is the process by which the weak link i.e carbonated apatite is replaced by acid-resistant crystals of fluoro-apatite by ingress of ions of calcium and phosphate. Fluoride antimicrobial action is targeted towards three main characteristics of oral cariogenic microflora i.e adherence of microorganism to tooth, the acidogenicity and the acidurance or aciduric property. Thus, the game plan would to develop a therapeutic strategy which targets the biofilm, either the integrity of the biofilm or its adherence to the tooth.⁴

Eggs have been deliberated upon as an easily available source for the production of polyclonal lectin which is unique and distinctive to a variety of agglutininogen such as bacteria, hormones, enzymes or virus. Evidence-based studies have shown that yolks from the eggs of *S. mutans* inoculated chickens provide agglutinins which are known as Immunoglobulin Y (IgY). It confers passive immunity

against caries by immobilizing *S. mutans* and disabling *S. mutans* ability to convert refined sugar into acid.⁵ The aim of this study was to evaluate & compare the efficacy of delipidated egg yolk protein tablets in preventing recolonisation of *Streptococcus mutans*.

Materials and methods

The present study was conducted on 120 freshly extracted molars. Each batch of samples was randomly divided into three groups which are follows- Control (Group A), IgY (Group B) and Fluoride (Group C).

ATCC stain of *Streptococcus mutans* (25175) were procured and sub-cultured as per standard guidelines. The organism was then sub-cultured in Brain Heart Infusion broth (BHI) and incubated at 37°C to adjust the suspension to 0.5 Mac Farland standard which corresponds to 10⁸ colony forming units per milliliter (CFU/mL). The teeth samples were immersed in sugary broth in separate sterile containers and agitated at 80 rpm for 90 minutes to promote adhesion of the organism on tooth surface. Teeth samples were washed twice with sterile buffer solution to remove any non-adherent bacteria.

The samples were then incubated at 37°C for 48 hours, rolled onto sterile blood agar under sterile precautions. These plates were incubated at 37°C for 48 hours. Colony counting was done for each group and results were compared with control (Fig- 1,2,3,4). In case of any contamination, the whole batch was rejected and procedure was repeated. Results were statistically analyzed.

Table 1: Comparison of CFU/ml among patients of all the three study groups

Group	N	Mean	Std. Deviation	Minimum	Maximum	F-value	p-value
Control	40	95060000	264020000	10	1000000000	1.027	0.361
IGY	40	95004000	264040000	100	1000000000		
APF GEL 1.23%	40	30003000	158841000	100	1000000000		
Total	120	73356000	234329000	10	1000000000		

Unit: CFU/ml

Table 1 shows that there were maximum colonies with control however, the difference was non- significant (P> 0.05).

Graph 1: Comparison of CFU/ml among patients of all the three study groups

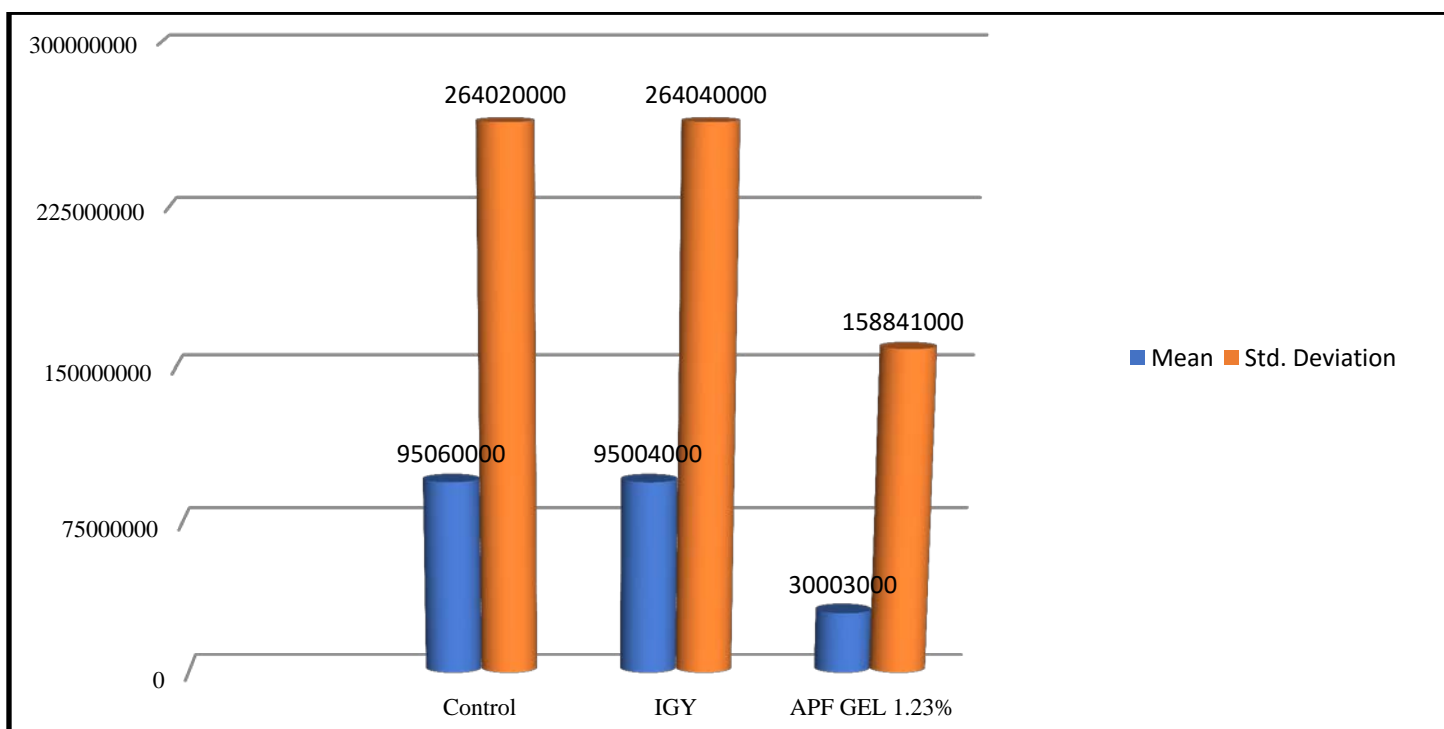


Table 2: Individual group comparison (Post Hoc Tests)

Group	Group	95% CI		p- value
		Lower bound	Upper bound	
Control	IgY	-124300000.0	124410000.0	1.0
	APF gel 1.23%	-59302000.0	189410000.0	0.4
IgY	APF gel 1.23%	-59358000.0	189360000.0	0.4

Table 2 shows individual group comparison by Post Hoc tests, the difference found to be non-significant ($P > 0.05$).

Table 3: Intergroup comparison (Student t test)

Group	N	Mean	SD	t-value	p-value
Control	40	95060000	264020000	0.001	0.999
IgY	40	95004000	264040000		
Control	40	95060000	264020000	1.335	0.186
APF GEL 1.23%	40	30003000	158841000		

Table 3 shows intergroup comparison by Student t test, the difference found to be non-significant ($P > 0.05$).

Discussion

Streptococci Mutans partake in genesis of cariogenic biofilm on ecologically viable niches of the tooth. Cariogenic microbiosis are essentially well-assembled communities of microbes housed in an extracellular gelatinous array. Sucrose is essential for the adhesion and coaggregation of streptococci. Another significant role player are the enzymes known as glucosyltransferases (GTFs), they are intrinsically integrated by strains or serotypes of streptococci. The initial fitment paves way for formation and growth of microbial film.⁶ The aim of this study was to evaluate & compare the efficacy of delipidated egg yolk protein tablets in preventing recolonisation of Streptococcus mutans.

A study was conducted by Kruger et al⁷ to ascertain the virtue of chicken egg yolk lectins against GTF's dispensed as a drinking infusion experimented in a desalivated animal archetypal model. Evaluating the inhibition of assays, it was observed that the chicken anti-GTF antibodies diminished the activity of both GtfC and GtfB factions in mixture. IgY antibodies against Cell associated Glucosyl transferase (CA-GTF) of S mutans are obtained by immunizing hens with CA-GTF antigen, causing them to produce antibodies as part of their normal immune response. The antibodies thus produced are transferred to the egg yolk through maternal transfer. These IgY antibodies which are proteins are then

extricated from the yolk of eggs laid by the hens, extracted and purified by the classic process of protein purification. Once formulated it is given orally to humans, where they are reported to diminish the quantity of habitant S mutans colonies by forming antigen-antibody complexes of mature dental plaque. Several mechanisms have been postulated for the anticaries mechanism of IgY. The most accepted is the prevention of bacterial adherence, agglutination, opsonization followed by phagocytosis, neutralization of toxins and inactivation of enzymes.

We observed that there is ability of IgY generated against CA-GTF in causing significant reduction in recolonization of bacterial colonies. IgY has been observed to aggregate S mutan cellules into extensive assemblages, making them vulnerable to deracination by salivary clearance and tendering them incapable of anchoring on and eventually to colonize on enamel surface.⁸ It has also been noted that anti-CA-GTF moiety of IgY efficaciously abolishes the GTF catalytic locus, thereby inhibiting the ability of CA-GTF enzyme to produce water-insoluble glucan as well as impede various facets of protein glucan synergy. This results in ineptitude of streptococci mutans to affix to teeth enamel with support of glucan reliant cohesion and disrupts early stage of biofilm formation.

Hatta et al⁹ it was observed that Ig Y passive immunization was as effective as laser caries inhibition method in reducing adherence of streptococci mutans.

Further, both laser group and IgY group alone or in combination fared better than fluoride application group. Systemic inoculation of cows with a vaccine from whole-cell of *Streptococci mutans* cells generated IgA lectins in serum as well as the milk which is incorporated in daily diet. In a animal study on rats, the use of immunized whey foot printed in undiminished *S. mutans*, minuscule biofilm formation and truncated caries activity. Another development in field of passive inoculation documented was the trial of transgenic plants to yield the indispensable agglutinin. Transgenic plants proposes countless merits as compared to immunogens procured from zoological sources, the construction of multimeric forms of immunoglobulins and indeed, a secretory IgA has in recent times been fabricated from tobacco plants by cross-hybridization of 4 chains of plants.¹⁰ This immunoglobulin exhibits anti-*S. mutans* properties and prevents the agglutination of the cell.

Conclusion

The results of the in vitro study reveal that immune IgY has a significant influence in preventing caries development.

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Legend Figure

Fig. 1: Color Plates & Sem Results



Fig 2



Fig. 3

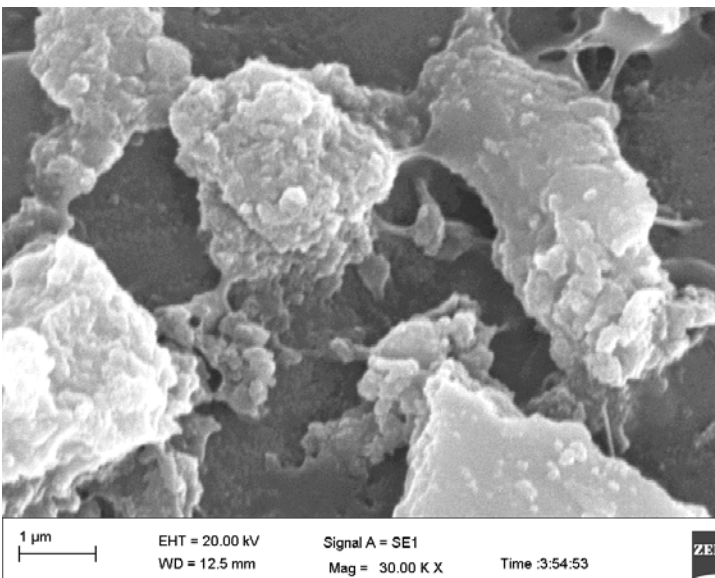


Fig. 4

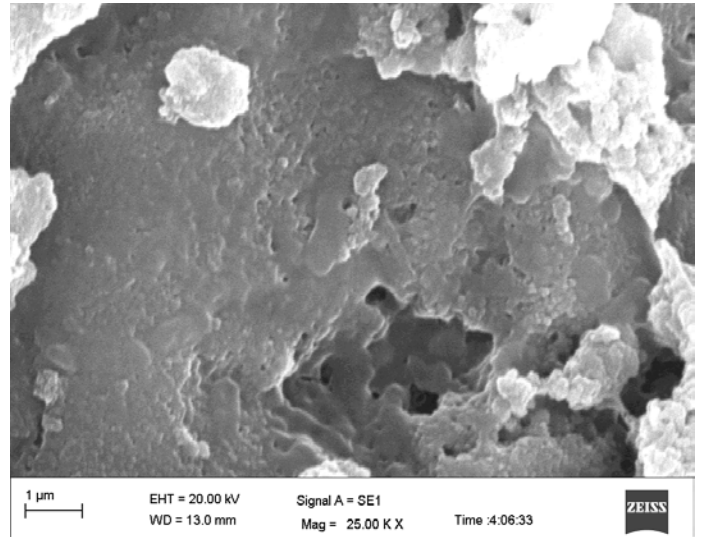


Fig.5

