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Effect of Streptococcal IgY on Quantity of *Streptococcus mutans* in High Caries Risk Children

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Authors' contributions

This work was carried out in collaboration between both authors. Author DJM designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author MGM managed the analyses of the study. Author DJM managed the literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

Early childhood caries (ECC) is defined as a condition of one or more of decayed, missing, and filling in teeth (dmft) of children aging less than 71 months or even younger. Prevalence and severity of ECC is still high in some countries. Aim of this study to evaluate the effect of streptococcal IgY on the quantity of *Streptococcus mutans* in high caries risk children. 20 children aged 3-5 years were selected from the Department of Pedodontics of which 10 children were with high caries experience and 10 were controls who were caries free. Saliva swabs were taken from the occlusal surface of primary second molars and microbial count was counted using mitis salivarius bactaracinagar. High risk caries were given one No decay™ tablet morning and evening for 15 days. Swabs were taken before intervention and than 1 month, 2 months and 3 months post intervention. *Streptococcus mutans* count was significantly reduced in patients who have taken no decay tablets. Within the limits of the study, we found that streptococcal IgY was able to reduced *streptococcus mutans* count after 2 months. There was no statistically significant difference in *Streptococcus mutans* count between high caries risk children and caries free children after 3 months.

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1. INTRODUCTION

Early childhood caries (ECC) is defined as a condition of one or more decayed, missing, and filling in teeth (dmft) of children aging less than 71 months or even younger [1]. Prevalence and severity of ECC is still high in some countries. The definition of ECC is the presence of one or more decayed (non-cavitated or cavitated lesions), missing (due to caries), or filled tooth surfaces in any primary tooth in a child under the age of 6 years. This condition will lead to a decrease in children's health quality, where ECC can decrease the chewing ability of the children, so that it can interfere in growth and development of children [2,3].

ECC is a multifactorial disease that occurs due to host, environment, and microorganism as the etiology factors [4], Mutans Streptococci (MS) is the main microorganism that causes ECC. In the past decade, several researches conducted in the relationship between the occurrence of caries and the presence of salivary MS [5]. Saliva may transmit the bacteria and plays a role as a reservoir for the colonization of the bacteria. If the cariogenic bacteria predominate in saliva and plaque, it will increase the acids, which are produced by them through the fermentation process of carbohydrate [6]. This will also increase the colony of the bacteria and start creating virulence biofilm on the tooth surface by quorum-sensing mechanism [7] One of the main factors of MS virulence is the ability to produce glucan synthesised by glumly transferase, which mediated microorganism attachment to the tooth surface along with other protein, such as I/II antigen 185, PAc 190, GbpA 74, GbpB 41,3 kDa, etc. The proteins affect biofilm formation and increase caries activity in children (ECC) [8].

Hence, we are interested to observe the difference of MS amount and protein profile isolated from saliva in ECC subjects compared to caries-free children. Passive immunity is the transfer of active humoral immunity in the form of ready-made antibodies from one individual to another. As such, passive immunotherapy by antigen-specific IgY acquires a special value as a tool for infection control and immunologic research with global commercial application as raw material for nutraceutical and pharmaceutical products and for applications in numerous medical and research fields since the 1980s.

IgY immunotherapy has several attractive features including: lack of reactivity with the human complement system and human Fc-receptors thereby preventing non-specific inflammation [9] excludes the use of toxic compounds or additives for their preparation from egg yolks egg cholesterol and triglycerides can be controlled to infinitesimally low levels and IgY exerts beneficial antimicrobial and immuno-stimulatory effects in conjunction with other egg proteins [10]. Egg allergies usually involve egg albumin components which may explain why no reactivity issues have been encountered in consumer use of several products now in the market containing purified IgY. Compared with vaccination, passive immunotherapy using IgY has distinct advantages such as: rapid and local onset of action, highly specific activity, applicability to a broader age range of patients from infants to adults including immunodeficient patients and it is nontoxic being a normal part of the human diet. While immunity derived from passive immunization lasts for only a short period of time coterminous with the presence of antibodies in the recipient, it nonetheless provides immediate and efficient host protection when given in proper concentration onto the target organ [11,12,13]. In this study we have evaluated the effect of streptococcal IgY on the quantity of *streptococcus mutans* in high caries risk children.

2. MATERIALS AND METHODS

20 patients, aged 3-5 years who visited the Department of Pedodontics and Preventive Dentistry were included in the study in which 10 were patients who had high caries experience and 10 were controls who were caries free. Patients with systemic diseases or those on any long term medications. Both test (Children with caries) and controls (children without caries) were age and gender matched. Salivary swabs were taken from the occlusal surface of primary second molars. Sterile vials containing 1 ml thioglycollate broth as transport media were used for sample collection and transportation. The sample was transported to the laboratory immediately after collection at 37°C and processed on the same day. The sample was vortexed (15 seconds) and serial dilution up to 10^{-3} was done. Using micropipette, 0.1 ml (100 µl) of this diluted sample was inoculated on Mitis Salivarius Bactracin Agar plates. The sample was uniformly spread on the agar plates using a

sterile swab. The petri plates were incubated at 37°C in a 5-10% CO_2 (candle) jar for 48 hours. After 48 hours colony characteristics were studied and the number of colony forming units (CFU/ml) *S. mutans* in the samples were counted using a digital colony counter. One No Decay Tablet™ (Inzpera Healthsciences Ltd) each was given to children with high caries risk in the morning and evening respectively after a meal. Swabs were taken before treatment and then 1 month, 2 months and 3 months post treatment. Data was statistically analysed using Student T test with SPSS version 23.

3. RESULTS AND DISCUSSION

The study included 20 children of which 10 were tests and 10 were controls. There was no statistical difference between the groups in age or gender. There was a statistically significant difference in dmft between both groups ($p<0.001$) (Table 1).

There was a very statistically significant difference before treatment in which the test group had a colony count of 44.87±2.87 compared to the control group 25.73±1.49 ($p <0.001$). 1 month after treatment there was a decrease in the microbial count of the test group but still a statistically significant difference existed between the groups ($p <0.05$). At 2 months (p: 0.981) and 3 months (p value: 978) there was no statistical significant difference between controls and tests.

A large number of studies previously have established a positive correlation between MS and ECC [14]. The results of this study also confirmed that MS was associated with ECC as indicated by the number of colonies (ECC children had more MS colonies) [15]. A positive correlation was also found between severity of ECC as measured by the dmft index and high levels of MS counts, indicating that as the number of colonies increased, the number of teeth and surfaces affected by caries also increased [16].

It has been proven by numerous experts in cariology that saliva is an agent for caries prevention [17] According to Edgar ,saliva contains, among other things, various immunoglobulin and non-globulin anti-bacterial agents such as amylase, lysosomes, lactoperoxidase, lactoferrin, apolactoferrin, and histatins [18]. Saliva prevents the formation of biofilms that can cause caries. IgY gel could inhibit the growth of *S. mutans* [19].

Generally the action of orally administered IgY is intended to be achieved within a specific localized site along the alimentary tract, is highly target specific and relies on the largely predictable and usually efficient antigen-antibody interaction [20]. Several mechanisms of action is proposed in host protection inhibition of microbial adhesion to cell surfaces, suppression of viral colonisation by preventing cell-to-cell spread, bacterial agglutination with resulting microbial immobilisation and death or ease of being

Table 1. Demographic data

Parameter	Study group	Control group	P value
Age(Years)	4.2±0.9	4.1±0.7	0.712
Gender	Male - 6 Female - 4	Male - 5 Female - 5	0.795
Dmft	9(5-17)	0	<0.001

Table 2. *S. mutans* levels in test and control

Colonies	Mean		P value
Before treatment	Test	44.87±2.87	<0.001
	Control	25.73±1.49	
1 month	Test	35.87±2.59	<0.05
	Control	26.00±2.03	
2 months	Test	25.47±3.02	0.981
	Control	25.93±1.67	
3months	Test	25.21±2.33	0.978
	Control	25.33±1.45	

flushed down the gut, inhibition of enzyme activity and neutralisation of toxin activity [21]. The cumulated literature on IgY covers in vitro and animal model studies which comprised the foundation upon which current mucosal disease protection among domestic animals were based [22]. The initial success of IgY in animal subjects has provided impetus toward human clinical trials and immunotherapeutic applications [23].

There are several properties that make IgY attractive for oral immunotherapy. While the mouth is the portal of entry for many infectious agents, it is therefore logical to use this as the route for IgY to target specific infectious entities within the alimentary tract. IgY does not pass as intact molecules from the intestines to the blood circulation thus precluding any systemic effect [24]. IgY use is associated with a much lower risk of inducing specific resistance among pathogenic microorganisms since it is directed to multiple antigenic targets that require multiple genes for their synthesis. Being an ingredient in our regular diet, poultry eggs are considered generally safe [24] Ma and Allergic reactions may occur upon ingestion of egg-derived components particularly those that contain appreciable amounts of egg white. However, the water-soluble IgY materials purified from egg yolk (devoid of lipids) are not usually associated with allergic reactions based on our own experience, which conforms to the general perception that egg white materials are the ones responsible for the common egg allergy [25]. The risk of allergy is lower when administering the antibodies orally than by other routes [25,24,26] Moreover, oral administration of egg protein (mainly ovalbumin) has been shown to induce systemic tolerance [27].

4. CONCLUSION

Within the limits of our study we found that Streptococcal IgY reduced the microbial count in high caries risk patients. There was no significant difference in microbial colonies of the test and control groups even after 3 months of intervention showing that these tablets can help to reduce the count of S. mutans and thus reduce the microbial risk of caries. However, long term studies with larger sample size will allow us to understand the long term efficacy of Streptococcal IgY.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT AND ETHICAL APPROVAL

The study was initiated after ethical approval was obtained from the institutional ethics committee. Consent was obtained from the parents of participating children.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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