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Research Article

COMPARISON OF S MUTANS COUNTS AND SIg-A LEVELS IN CESAREAN Vs VAGINALLY DELIVERED CHILDREN AND THEIR ASSOCIATION WITH CARIES INCIDENCE

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ABSTRACT

Background: The extent to which perinatal events influence the acquisition of S mutans and development of immunity is unclear. Mode of delivery may significantly influence the diversity and composition of oral microflora and facilitate the development of caries. Caesarean section, carried out in aseptic conditions accelerate the acquisition of S mutans, while in a normal vaginal delivery, the infant is exposed to variety of bacteria present in mother's Anogenital regions. Post natal development of immunity gets altered by mode of delivery thereby suggesting the need to establish an association between mode of delivery and Caries experience.

Aim & Objectives: To compare the levels of S Mutans and S Ig-A in Cesarean versus vaginally delivered children and incidence of Caries.

Materials & Methods: The prospective experimental study was conducted on 84children aged 6-8 years with Caries born by either cesarean or vaginal delivery. The collected unstimulated whole saliva was subjected for IgA estimation by ELISA and microbial culture on MSKB agar.

Results: The mean S mutans in cesarean and vaginal born children was 238.0±86.67, 113.86±58.85 CFU where as SIgA levels was 72.10±56.91, and 90.52±62.39 µg/ml respectively. In a matched case-control population, children born by cesarean had significantly higher S Mutans count and deft. However, no significant difference between two groups was observed for IgA levels.

Conclusion: A positive association between Cesarean Delivery and increased S mutans and decreased Immunoglobulin's and the converse in vaginally delivered children suggest a link between mode of delivery and occurrence of Caries.

INTRODUCTION

The interaction between the emerging microbial ecology of the oral cavity and the developing immune system serves as backdrop for the relationship between mode of delivery and the emergence of some diseases.[1] Early acquisition of bacteria through vertical transmission in infants may be affected by various neonatal factors. The link between mode of delivery and subsequent childhood pathology is an important one.[2] Caesarean section carried out in aseptic conditions accelerates the acquisition of mutans streptococci, since the infant is less exposed to maternal microbiota; while in a normal vaginal delivery, the infant is exposed to a variety of the bacteria present in the mother's Anogenital regions.[3] The post natal development of immunity gets altered by mode of delivery. If the microflora develops differently depending on the mode of delivery; the postnatal development of the immune system might also be different.[4] Thereby emerges the need to find a link between the mode of delivery and immunity against dental caries. Salivary secretary IgA is the predominant immunoglobulin in whole saliva and is considered to be the main specific defense mechanism of the oral cavity.[5] we found no study that has estimated the levels of SIgA in cesarean and vaginally born children to find a link between dental caries experience and SIgA concentration. Hence the study designed with the following aims and objectives.

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Aims & Objectives: To compare streptococcus mutans counts and SIg-A levels in cesarean versus vaginally delivered children and to find their association with incidence of dental caries.

MATERIALS & METHODS

This experimental study was conducted in the Department of Oral Pathology, after obtaining institutional ethical clearance and written informed consent form parents and children who are participating in the study. The Study sample comprised of 84 children aged 6-8 years with dental caries; born either by Cesearean or normal delivery. Data was collected using a self designed questionnaire with DMFT/deft scores & diet chart. Caries activity was assessed by deft/DMFT index by Gruebbel A.O 1944. Socioeconomic status was assessed by Kuppuswamy socioeconomic status scale 1976. Children with a positive history of fluoride use, those under recent antibiotic coverage, using fixed or removable orthodontic appliances, those with any reported positive medical history and Children with mental/systemic illness were excluded. Unstimulated saliva samples were collected in disposable sterile containers and were immediately transported to the microbiological laboratory for microbial and immunological analyses. 1ml of saliva was transferred from the sterile container by means of a sterile disposable syringe and labeled as Part 2, the left over sample in sterile container was labeled Part I. Part 2- was stored in the ice box and carried immediately for ELISA.

Part-1 used for Microbiological evaluation

Mitis salivarius Kanamycin Bacitracin agar was prepared and poured in 10 Petri dishes of size 19mm/15mm and allowed to set. Part 1 of the sample was inoculated on the culture media with 1% potassium tellurite. The petridish containing the media and inoculated saliva samples were placed in an atmosphere of carbon dioxide in a candle jar**,** then left to incubate for 48 hours at 37 degree centigrade. Dark blue, round, elevated colonies of Streptococcus mutans were formed which were counted using a magnifying glass. Pure strain of S mutans (ATCC 25175) was used as a reference.

Figure1 Armamentarium for Immunological Assay **1a.** ELISA Kit DRG Diagnostics Germany, **1b**.Reagents added in Micro wells using Micropipettes, **1c.** Micro wells with added Sulphuric acid to stop the TMB reaction, **1d**. Micro plate Reader

Sample from the established colonies were smeared on the slide, air dried and fixed shortly over the flame. Gentian violet was allowed to act for one minute over the prepared smear. Stain was poured off and the remaining stain was washed away by Gram's iodine. Smear was covered with gram's iodine, which was allowed to act for 2 -3 minutes. Iodine was washed off with alcohol by adding it drop by drop till no more violet color comes off the smear. Smear was washed with tap water and counter stained with saffranin for one minute. Smear was again washed with tap water, and observed under the microscope. (Figure-2a, b, c, and d)

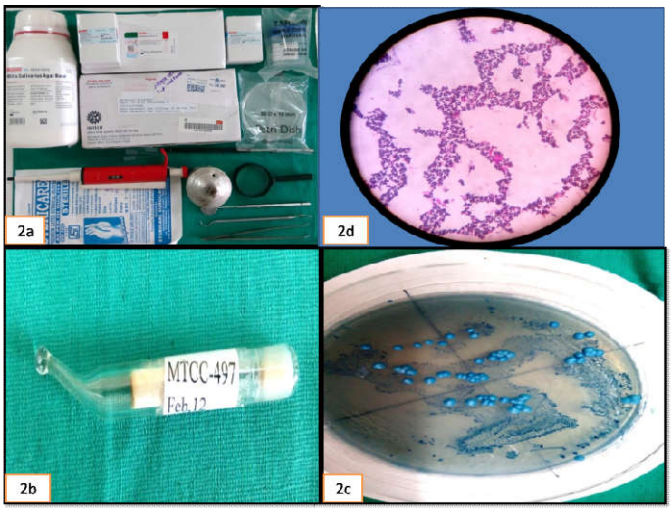


Figure 2 Microbiological Isolation, **2a.**Materials used for Microbiological assay, **2b**.Freeze dried pure form of Streptococcus Mutans **MTCC 497**, **2c**.Colony Growth on MSKB agar Media, **2d**. Cultured S.Mutans Confirmed by Positive Grams Staining.

Part 2 of the sample was subjected to the immunological assay for the estimation of salivary immunoglobulin A by ELISA. The h-IgA saliva ELISA TEST is based on simultaneous binding of human IgA to two antibodies, one monoclonal immobilized on micro well plates, the other polyclonal conjugates with horseradish peroxidase (HRP). After incubation the bound/free separation is performed by a simple solid-phase washing. The enzyme in the bound-fraction reacts with the Substrate (H2O2) and the TMB Substrate and develops a blue color that changes into yellow when the Stop Solution (H2SO4) is added. The color intensity is proportional to the IgA concentration in the sample. The IgA concentration in the sample is calculated based on a standard curve.

Preparation of the Standards (S0 - S4), IgA Assay Buffer, Diluted Conjugate and Wash solution were first carried out and the assay was done on the same day of sample collection. A standard curve was plotted on a graph and the IgA levels were noted down. (Figure-1a, b, c, and d)

Statistical Analysis

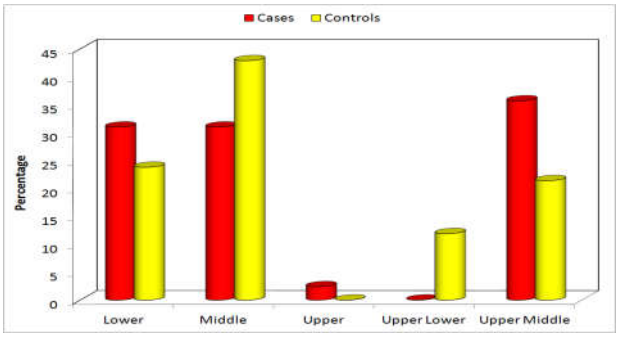
SPSS (Statistical Package for Social Sciences) Version 15.0 statistical Analysis Software was used; the values were represented in Number (%) and Mean±SD. The Statistical formulas used were Mean, Standard Deviation, Chi square test, Student 't' test, Analysis of Variance (ANOVA), Bivariate correlation and Level of significance.

RESULTS & OBSERVATIONS

Out of 84 children enrolled in the study, 50% were children who were delivered through cesarean section whereas remaining 50% were delivered through normal vaginal route.

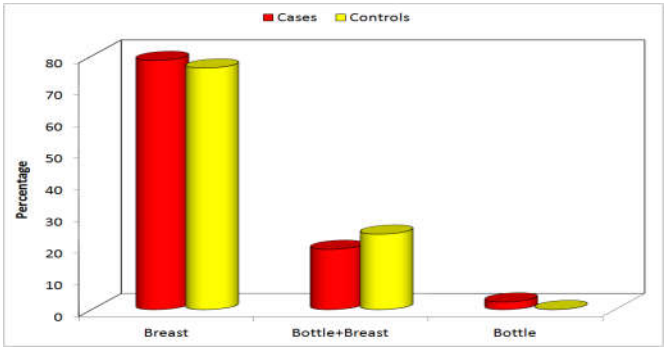
Among cesarean born children, a total of 28.6% children each were aged 6 and 8 years whereas remaining 42.9% were aged 7 years whereas in children born through vaginal route, a total of 31% were aged 6 years, 45.2% were aged 7 years and remaining 23.8% were aged 8 years. Statistically, the two groups did not show a significant difference for age (p=0.883). Majority of cesarean born children were males (54.8%) whereas majority of children born through vaginal route were females (57.1%). Overall, majority was female (51.2%). Male to female ratio of study subjects was 0.95:1. Statistically, the difference between two groups was not significant (p=0.275).

Socioeconomic status assessed by Kuppuswamy scale showed (36.9%) Children from middle class, upper middle class (28.6%), lower class (27.4%), upper lower class (6%) and upper class (1.2%). Among cesarean born children, maximum were from upper middle class (35.7%) followed by those in lower and middle class (31% each) and minimum were from upper class (2.4%). Children born through vaginal route were from middle class (42.9%), lower class (23.8%), upper middle (21.4%) and upper lower (11.9%) respectively. Although, there were proportional differences between two groups yet this difference was not significant statistically (p=0.069). GRAPH-1



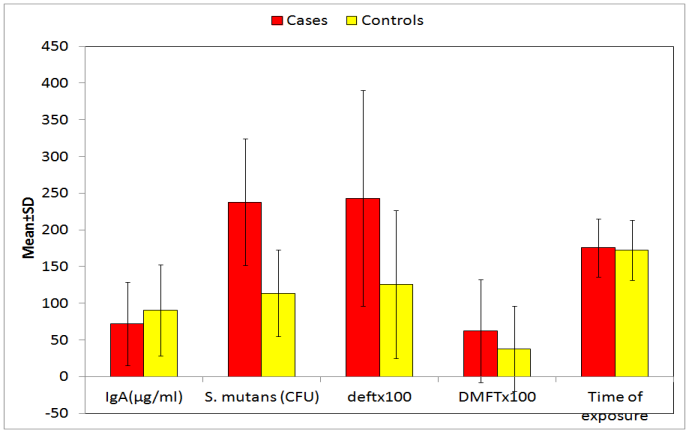
Graph 1 showing comparison of subjects in two groups according to Socioeconomic Status

Majority of subjects in both the groups had a history of breast feeding. 19.0% cesarean and 23.8% vaginally born children were fed in combination, whereas only 2.4% had a history of exclusive bottle feeding. Statistically, there was no significant difference between two groups with respect to feeding history (p=0.539). GRAPH-2



Graph 2 showing comparison of subjects in two groups according to Feeding type

On Comparing subjects in both groups for IgA levels, *S. mutans* count, deft, DMFT and time of exposure values, we found Mean IgA levels were 72.10±56.91 µg/ml in cesarean born children as compared to 90.52±62.39 µg/ml in vaginally born children, thus showing statistically no significant difference between two groups. Mean *S. mutans* colony count was 238.0±86.67 CFU in cesarean born children as compared to 113.86±58.85 CFG in vaginally born children, thus showing that the mean count in cesarean born children was significantly higher as compared to that of vaginally born ($p<0.001$). Mean deft was 2.43±1.47 in cesarean born children as compared to 1.26±1.01 in vaginally born, thus showing that the mean value in cesarean born children was significantly higher as compared to that of vaginally born children ($p<0.001$). For DMFT too, mean value in cesarean born children was higher (0.62±0.70) as compared to that in vaginally born (0.38±0.58), however, this difference was not significant statistically (p=0.093). The time of exposure to sugar was 175.71±39.64 min in cesarean born children as compared to 172.38±40.71 min in vaginally born, thus showing no statistically significant difference between two groups (p=0.705). GRAPH-3



Graph 3 showing comparison of subjects in two groups according to IgA levels, S. mutans count, deft, DMFT and time of exposure values

IgA levels were minimum at age 6 years and maximum at age 7 years. Statistically there was no significant difference in mean IgA levels among different age groups when evaluated independently for both groups.

All the Bivariate correlations were random, weak and mostly non-significant. A weak positive and statistically significant correlation was observed between deft and S. mutans count for overall assessment only (r=0.217; p=0.048). TABLE-1

Table 1 Bivariate correlation of different variables studied

Correlation	Cases (n=42)		Control (n=42)		Total (n=84)	
	"r"	"p"	"r"	"p"	"r"	"p"
deft with *S. mutans*	-0.147	0.352	0.053	0.739	0.217	0.048
DMFT with *S. mutan.*	0.006	0.972	-0.027	0.866	0.115	0.299
deft with S. IgA	0.164	0.299	0.032	0.842	0.028	0.801
DMFT with S. IgA	-0.040	0.803	-0.071	0.653	-0.081	0.463
S. mutans with S. IgA	0.025	0.877	0.175	0.266	-0.035	0.755

DISCUSSION

Caries is a dynamic process with Multifactorial etiology results from the complex interactions among the microbial species adhering to the tooth surface, with dietary, salivary, and genetic influences. The infectious nature of caries supports the hypothesis that some form of host immunity can regulate its

activity. If so SIgA, a predominant immunoglobulin in the saliva can give a clear correlation. It has been suggested that salivary SIgA generated by the mucosal immune system play an important role in the immune response against Caries.[6]

In the present study the overall deft score increased with age in both groups suggesting that caries experience increases with age. The findings are in accordance with the observations of Kumar A , Dutta S, Namdev R, Mehta R, Hooda A, Goel M (2014)[7]

Females had greater caries experience in both groups of the present study and were statistically insignificant. Our findings are in accordance with Kumar A ,Dutta S, Namdev R, Mehta R, Hooda A, Goel M[7] and in contrast to Moses J, Rangeeth BN, Guruinathan D(2011)[8]; Arora SA, Setia S, Ahuja P, Singh D, Chandna A(2012)[9]

According to Li Y, Caufield PW(1995)[10] a high degree of fidelity was observed between strains of Streptococcus mutans in mothers and their female infants (88%), in contrast to those isolated from male infants (55%), indicating that the conservation of Streptococcus mutans within mother-infant pairs is gender specific. They observed that the infants whose teeth emerged early were significantly more likely to acquire MS from their mothers than infants whose teeth emerged later.

Caries experience increased progressively in upper, upper middle, middle, and lower class in Group-II, whereas in Group-I upper lower class, lower, middle and upper middle class respectively. Our observations are in accordance with Popoola BO, Denloye OO, Iyun OI [11], and Reisine ST, Psoter W [12]

Caries experience was less in breast fed when compared to breast &/ bottle fed children in both the groups. According to Michalek SM and Childers NK [13] milk antibodies interfere with binding of microorganisms with mucosa/tooth and prevents exposure to food proteins in the suckling infant, thereby preventing microbial invasion. Whereas Smith DJ, Taubman MA[14] stated that maternal milk and colostrum provide the infant with multitude of innate and specific immune components. Colostrum is the initial mammary secretion after birth, which contains IgA in high concentrations (10 to 20 mg/ml).

The mean S. mutans in GROUP II with deft (2.43±1.47) and DMFT (0.62±0.70) was 238.0±86.67 CFU; whereas in GROUP I it was 113.86±58.85 CFU with deft 1.26±1.06 and DMFT 0.38±0.58. The S.mutans and deft in Group-II was significantly higher as compared to that of Group-I ($p<0.001$). The difference was statistically insignificant for DMFT ($p=0.093$) between the two groups. The findings of our study support the view that S. mutans is the primary causative agent of caries in human beings and are in agreement with Barfod MN, Christensen LB,Twetman S, Lexner MO [15]; though in contrast to Pattanaporn K *et al* [16] who found increased caries prevalence in vaginally born children.

A weak positive and statistically significant correlation was observed between deft and S. mutans. Studies by Guglielmo C, Aurea L and Lai SB [17], Hegde PP, Ashok Kumar BR and Ankola VA [18], Loyola *et al* [19] have indicated a highly significant statistical relation between Streptococcus mutans and dental caries experience.

Variations in S. mutans colony counts can be attributed to several complex interrelated phenomena such as sucrose content of the diet, eating habits, brushing habits, fluoride content of the tooth and plaque, possible immune mechanisms in the saliva, genetic factors, and inherent characteristics of S. mutans.

The mean SIgA levels in Group II with deft 2.43±1.47 and DMFT 0.62±0.70 was 72.10±56.91 µg/ml, whereas in Group I SIgA levels were 90.52±62.39 µg/ml with deft 1.26±1.06 and DMFT 0.38±0.58, showing statistically insignificant difference. Though the levels are lower in cesarean born children when compared to vaginally born children suggesting its protective role, but a weak correlation was observed between IgA levels and caries score. ($r=0.028$,$p=0.801$)

According to Thaweboon S *et al* [5] the levels of SIgA in Korean children ranged between 0.025-0.186mg/ml, whereas the findings of Rashkova M *et al* [20], the average values of S-IgA in the saliva of the healthy children was 121.3 ±15.0 µg /ml by ELISA.

Koga Ito *et al* (2004) [21] and Dofoide D and Damle SG (2011) [22] demonstrated a lower incidence of caries as a result of high salivary IgA concentration; whereas Bagherian A *et al* (2008)[23], Thaweboon S *et al*(2008)[5] and Randheer E *et al* (2011)[24] demonstrated salivary IgA levels to be significantly higher in caries active mouths. Our findings are in accordance with Shifa S *et al* (2008) [25] who reported statistically insignificant correlation between dental caries and salivary IgA levels.

A weak correlation ($r<0.30$) between SIgA and S.mutans was observed in the present study. Many factors can influence the concentration of SIgA; the most important factor being the salivary flow rate which was not assessed in our study. Studies which do not assess the rate of salivary flow, fail to correlate caries incidence to variations in SIgA or flow rate. It should also be kept in mind that all antimicrobial factors in the oral cavity interact with each other. [11]

Not all data are supportive of the role of secretions in prevention of dental caries. Geetha Priya PR, Asokan S, Karthick K, Reddy NV, Rao VA (2013)[26] stated that mere quantification of SIgA might have no reflection on the functional antibodies involved in caries process, and successful dental treatment alone does not alter the SIgA levels, suggesting a multifaceted approach to combat the cariogenic challenge.

In the present study, mean SIgA levels were found to be significantly higher among those aged 7 years supporting the view of Thaweboon. S. et. al. (2008)[5], who concluded that SIgA has a parabolic relationship with age. At birth, SIgA is undetectable, but there is a consistent increase in their levels with age. By the age of 7 the levels of SIgA reach their peak and remain consistently high during mid-life only to decline during old age.

Thus, in a matched case-control population, children born by cesarean section had significantly higher S. mutans count and deft. However, no significant difference between two groups was observed for IgA levels or any other parameter studied; indicating that difference in deft and S. mutans count can be just because of difference in mode of delivery.

In a developing country like India, where dental caries is the most prevalent oral disease, sincere efforts have to be put to improve preventive and curative dental services among children so that they have a healthier dentition. Questions on role of immunity in dental caries are yet to be explored and answered. The results of the present study can be used as a powerful aid for assessing caries risk factors. The mode of delivery may be an appropriate question to be included in past medical history for further determination of why some children are at greater risk for caries than others.

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