Section A-Research paper
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CORRELATION OF SALIVARY FLOW, SALIVARY pH, BUFFERING CAPACITY OF SALIVA WITH SCARDOVIA WIGGSIAE LEVELS USING REAL TIME POLYMERASE CHAIN REACTION IN CHILDREN WITH SEVERE-EARLY CHILDHOOD CARIES AND WITHOUT EARLY CHILDHOOD CARIES-A MICROBIOLOGICAL STUDY.

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Abstract:

BACKGROUND: Scardovia wiggsiae came into the limelight as potential caries pathogens in 2011, when isolated in microbiota of severe early childhood caries in the absence of S. mutans . Since then, they have been isolated from white spot lesions, deciduous pulps, in patients undergoing fixed orthodontic treatment as well as from cavitated and non cavitated carious lesions in adults . The properties of S. wiggsiae reflect its acidogenic nature.

AIM: Find a correlation between salivary flow , pH, buffering capacity and Scardovia wiggsiae in saliva with children with S-ECC and without ECC.

MATERIALS AND METHODS: Children categorized into 2 group were examined and scored as per WHO criteria (1997) . Saliva samples were collected at least 1 hour after meals . The flow rate (ml/min) was calculated. Immediately after collection, the pH measurement and buffering capacity measurement was done directly, using a digital pH meter. The salivary buffering capacity value was ranked into one of the following three categories: Low buffering capacity (pH < 4.5) , Medium buffering capacity (pH 4.5–5.5),High buffering capacity (pH > 5.5).One ml of unstimulated saliva sample was transferred into microcentrifuge tube containing 1ml of Tris EDTA (T.E) buffer and samples were transported to the Central

Research Laboratory, Maratha Mandal's Nathajirao G. Halgekar Institute of Dental Sciences and Research Centre, Belagavi for quantitative analysis of Scardovia wiggsiae using real time polymerase chain reation. **RESULTS:**The mean values of the un-stimulated salivary flow, pH and salivary buffering capacity in the group 2 were lower than those of the group 1 . However, the amount of Scardovia wiggsiae was higher in the caries patients (p=0.072) when compared to the caries-free population , with p = 0.072 . A statistically significant difference was recorded between the two groups regarding the salivary flow, 0.473 ml/min in group 1 and 0.309 ml/min in group 2 ; p = 0.000**. **CONCLUSION:**Considering the results of this microbiological study it can be assumed that there is a strong correlation of the acidogenic organism Scardovia wiggsiae and salivary pH . Also the S.wiggsiae count was higher in saliva samples of children with S-ECC when compared to children without ECC proving their association with the disease process in a significant manner.

KEYWORDS: Early childhood caries, saliva, Scardovia wiggsiae

1.INTRODUCTION:

A vital and crucial part of good general health is having a healthy mouth. Although enjoying good oral health ensures having more than healthy teeth, many children have inadequate oral and general health because of active and uncontrolled dental caries. Despite the fact that dental caries has been less common over the past few decades, early childhood caries is still one of the most prevalent chronic childhood disorders, particularly in developing countries. [1]

It is widely acknowledged that a natural defence mechanism present in saliva plays an important part in controlling the caries progress. Salivary flow, dilution, pH, buffering, and remineralizing abilities are known to be important variables that influence and, in some cases, regulate the extent to which the caries process advances . If the oral environment is unfavorable, an adequate flow of saliva can help to dilute and buffer the acid challenge, and thus could slow the rate of damage to the tooth or even repair the damage.[2]

Scardovia wiggsiae came into the limelight as potential caries pathogens in 2011, when isolated in microbiota of severe early childhood caries in the absence of S. mutans . Since then, they have been isolated from white spot lesions, deciduous pulps, in patients undergoing fixed orthodontic treatment as well as from cavitated and non cavitated carious lesions in adults . The properties of S. wiggsiae reflect its acidogenic nature. Acid production lowers the intraoral pH causing a microbial dysbiosis towards acidicity. Other properties include an acid producing capacity comparable to or greater than S. mutans. S. wiggsiae are also arginine deaminase negative, thus failing to help produce ammonia to neutralize the lowered intraoral pH .[3] Thus our study aims at finding a correlation between salivary flow , pH, buffering capacity and Scardovia wiggsiae in saliva with children with S-ECC and without ECC.

2.MATERIALS AND METHODOLOGY:

The present study is conducted on children coming to the Department of Pediatric & Preventive Dentistry, Bharati Vidyapeeth Dental College & Hospital, Sangli, India. Inclusion criteria of the study was children between 36 months to 71 months of age and children

visiting dentist for the first time. Exclusion criteria was antibiotic usage for at least 2 weeks before sampling , presence of systemic disease and handicapped children.

Minimum sample size calculated by using G. Power software is 21 per group. Therefore, 22 samples per group is suggested that is total 44 samples are selected.

Children categorized into each group were examined and scored as per WHO criteria (1997) .

• Group 1 included 22 children without ECC.

• Group 2 included 22 children with S- ECC.

Saliva samples were collected at least 1 hour after meals . The children were made to sit straight on the chair and allowed to relax for few minutes. The children were advised to spit the saliva into a graduated container continuously, for 5 minutes. [4] The flow rate (ml/min) was calculated. Immediately after collection, the pH measurement was done directly, using a digital pH meter. The electrode was immersed in the sample in a container, the digital reading was allowed to stabilize for few seconds. Final stable reading was taken as the salivary pH value.[5]

After the assessment of salivary pH, 1 ml of the sample was taken in container and 10 µl of 0.1 N hydrochloric acid was added into it using a micropipette. The sample was then gently shaken to homogenously mix the saliva and the HCl. The pH meter was then immersed into the sample and the stable reading was recorded for salivary buffering capacity . [IMAGE 2 ,3]The salivary buffering capacity value was ranked into one of the following three categories: Low buffering capacity (pH < 4.5) , Medium buffering capacity (pH 4.5–5.5),High buffering capacity (pH > 5.5).[5]

One ml of unstimulated saliva sample was transferred into microcentrifuge tube containing 1ml of Tris EDTA (T.E) buffer and samples were transported to the Central Research Laboratory, Maratha Mandal's Nathajirao G. Halgekar Institute of Dental Sciences and Research Centre, Belagavi for quantitative analysis of Scardovia wiggsiae using real time polymerase chain reation.

3.RESULTS :

All the data was entered into a computer by giving coding system, proofed for entry errors . Data obtained was compiled on a MS Office Excel Sheet (v 2019, Microsoft Redmond Campus, Redmond, Washington, United States) . Data was subjected to statistical analysis using Statistical package for social sciences (SPSS v26.0, IBM) . Descriptive statistics like frequencies and percentage for categorical data, Mean ; SD for numerical data has been depicted . Normality of numerical data was checked using Shapiro-Wilk test & was found that the data did not follow a normal curve; hence non-parametric tests have been used for comparisons . Inter group comparison was done using Mann Whitney U test . Bivariate correlation between two numerical variables was checked using Spearman's correlation coefficient . For all the statistical tests, p value less than was considered to be statistically significant, keeping α error at 5% and β error at 20%, thus giving a power to the study as 80%.

The mean values of the un-stimulated salivary flow, pH and salivary buffering capacity in the group 2 were lower than those of the group 1 . However, the amount of Scardovia wiggsiae was higher in the caries patients (p=0.072) when compared to the caries-free population ,

with p = 0.072 . A statistically significant difference was recorded between the two groups regarding the salivary flow, 0.473 ml/min in group 1 and 0.309 ml/min in group 2 ; p = 0.000**. Moreover, a Spearman's correlation coefficient test was performed to calculate the correlations between variables . Both the groups revealed a negative correlation between the total S.wiggsiae count and the salivary pH (Spearman correlation coefficient= -0.885 in group 1 and -0.869 in group 2) . Both the groups revealed a weak negative correlation between the total bacterial count and the salivary buffering capacity. (Spearman correlation coefficient= -0.003 in group 1 and -0.060 in group 2)

4.DISCUSSION:

Early childhood caries (ECC) has been on the rise in numerous countries and has emerged as a serious health concern, particularly in populations that are socially disadvantaged. It has a variety of distinctive clinical features, such as caries that develops rapidly and affects many teeth shortly after they erupt in the oral cavity. These lesions affect tooth surfaces that are less vulnerable to developing caries.[17,18] Although S. mutans is widely acknowledged as the primary cause in severe ECC, the importance of other cariogenic bacteria cannot be disregarded. Our understanding of non-culturable or conditionally restrictive oral bacteria has expanded due to molecular tools and sequencing technologies, and multispecies infection causing dental caries has been recognised . Therefore, bacteria like Lactobacillus, Actinomyces, or Bifidobacterium that do not belong to S. mutans are likely to contribute to the development of caries. Recent research has linked the onset and progression of dental caries, including dental plaque, white spot lesions, and dentinal caries, to Scardovia wiggsiae (S. wiggsiae), "an anaerobic Gram-positive bacillus with acidogenic and aciduric properties in the Bifidobacterium group." S. wiggsiae initially isolated demonstrated acid tolerance. S. wiggsiae strains exhibited comparable growth and acid tolerance to S. mutans when grown on agars at pH 7, pH 5.5, and pH 5. S. wiggsiae was linked to caries regardless of S. mutans was present or not. Acid-production from Scardovia and Scardovia related species indicate that they are strong acid producers, at a similar or greater extent than that of S. mutans. Further S. wiggsiae strains were arginine deaminase negative indicating the inability of this species to raise the pH from ammonia production.[8]

In the current study, the Scardovia wiggsiae count was quantitatively analysed using real time PCR technique. The results showed that there was a significant difference in the bacterial count in the saliva samples of children with S-ECC and children without ECC. Both the groups showed the presence of Scardovia wiggsiae. The bacterial count was much higher in children with S-ECC (Mean value -1.47E8) and was much lower in caries free children. (Mean value -67683341.32). S. wiggsiae were isolated from all samples of children, both caries free and those suffering from S-ECC. A study done by Matondkar et al (2019) quantified S. wiggsiae from dental plaque samples of children suffering from severe early childhood caries and children who were caries free by employing a real time DNA polymerase chain reaction method. Both the groups showed the presence of the organism S. wiggsiae, however there was a significant difference in its quantification between groups, with the median number being 1.49 X 108 per ml in caries free samples compared to 1.40 X 109 per ml in S-ECC samples.[3]

Chandna et al (2018)10 selected 45 children aged 71 months. Unstimulated saliva was taken from the participants and analysed for microbes using RT-PCR. The mean relative 16s rRNA expression of S. wiggsiae was found to be considerably greater in the SECC group (n = 15) than in the ECC group (n = 15) and controls group (n = 15). (1.69 and 0.85, respectively). In both ECC and SECC patients, the relationship between decayed, missing, or filled surface levels and 16S rRNA levels was significantly positive. Children's ECC was substantially correlated with S. wiggsiae salivary levels.[9]

Vacharaksa et al (2015) evaluated the incidence and quantity of S. wiggsiae and S. mutans using quantitative PCR. Dental plaque, as well as infected dentine was obtained from children of 2-6 years of age who were caries-free (n = 30) or detected with early childhood caries (n = 30). The prevalence of S. wiggsiae and S. Mutans levels were higher in diseased dentine than in young children's dental plaque.[10]

Dental caries are believed to have a complex multifactorial aetiology and pathophysiology. Saliva is given a significant role, with its rate and composition playing a key part in the initiation and development of the cariogenic process. Being the bodily fluid that constantly comes into contact with the teeth and soft oral tissues, it is held accountable for maintaining both their structural integrity and the ongoing remineralization of the dental structures. A healthy balance of the oral environment depends on the volume of salivary flow, the chemical composition of saliva, and its ability as a buffer. Any changes to these traits have the potential to affect the demineralization process, which in turn causes caries . The salivary components may have a substantial implication in the reduction of the risk factors involved in dental caries incidence .[11]

In the present study , correlation of buffering capacity of saliva with Scardovia wiggsiae count was examined . According to our knowledge ,this is the first ever study to check the correlation of buffering capacity of saliva with Scardovia wiggsiae count .Our study concluded that in children with S-ECC Scardovia wiggsiae count was higher where the buffering capacity was lesser and in children with no caries showed lesser quantity of Scardovia wiggsiae and the buffering capacity was more but was not statistically significant .There was a weak negative correlation seen between Scardovia wiggsiae and buffering capacity in both the groups.

(correlation coefficient = -0.003 in group 1 and -0.060 group 2).

In our study, there was a highly statistical difference in the salivary pH values of children with S-ECC and children with no caries with p value of 0.000**. Salivary pH in children with S-ECC was much less (Mean value-6.609) than those with no caries (Mean value -7.041).There was a statistically significant negative correlation between the salivary pH and the Scardovia wiggsiae count in both the groups . In children with no caries the pH value was higher . A higher salivary flow and an increased salivary pH seem to represent protective factors against caries in children, while high levels of Scardovia wiggsiae are correlated with caries active lesions. This data demonstrates their strong inverse correlation with the disease process.

The study conducted by Anita et al (2022) on a population consisting of 400 school going children in the age group of 6 to 12 years. Testing of resting saliva was done for evaluation of

visual inspection of the level of hydration, saliva consistency, pH measurement, saliva quantity, and buffering capacity . The mean buffering capacity of stimulated saliva was found to be significantly more among children with DMFT scores less than 5 than children with DMFT scores of 5 or more.[12] A similar study was conducted by G.M.Abbate et al (2014) wherein they evaluated the correlations between un-stimulated salivary flow, pH and level of S. mutans, and analysed through real time PCR, in caries-free and caries-active children. Results showed that an inverse correlation was present between pH and S. mutans (p = 0.088). [4] In the present study, we found a negative correlation between the salivary pH and the Scardovia wiggsiae count which indicates that in children with S-ECC, the salivary pH was decreased significantly and it also inversely correlated with the Scardovia wiggsiae count. Ericson and Makinen (1986) have substantiated an inverse relationship between salivary buffering capacity and caries activity .[13] Gopinath and Arzreanne (2006) found that salivary flow rate, viscosity, pH and buffering capacity were lower in subjects with high dental caries .[14] Lamberts et al (1983) studied the salivary pH rise activities in caries free and caries active naval recruits, and found no significant relationship between salivary pH rise activity and caries experience; but, there existeda significant positive correlation between the minimum pH values and bicarbonate content of the samples.[15] The present study also shows a weak negative correlation between caries activity and salivary buffering capacity .

An insufficient salivary flow rate causes an inadequate salivary buffering action, which results in decreased oral pH. Consequently, the inability of the host to counter balance the acidic environment creates an ideal condition for cariogenic bacteria.[16] In the current study ,the salivary flow of the caries free group was higher than those affected by S-ECC .There was a statistically significant increase in salivary flow in caries free children with p value of 0.004. This indicates that caries activity has a great influence on the salivary flow of an individual . A higher salivary flow seem to represent protective factors against caries in children but there was no correlation found between the salivary flow and Scardovia wiggsiae count .

Hence, the current study reveals a strong association of Scardovia wiggsiae with S-ECC. Scardovia wiggsiae count and salivary pH show a statistically significant negative correlation in children with S-ECC and children without ECC . No correlation was found in the salivary flow and Scardovia wiggsiae count in both the groups . We found a weak negative correlation between the salivary buffering capacity and the Scardovia wiggsiae count .

The present intervention was not conducted as a long term longitudinal analysis. A cross-sectional study design was followed. However, this type of a study design does not necessarily reflect the complete oral environment accurately and comprehensively. It probably indicates only the microbial count at a certain point of time giving us a snapshot view after which the bacterial counts could perhaps change in response to the changing oral environment. Therefore, to capture the complete caries development cycle, more longitudinal study designs should be used, where the microbial samples are taken at regular intervals. Also, the data collected is representative of children of Indian ethnicity only. However , further studies are required to clarify the correlation of Scardovia wiggsiae counts and the salivary parameters in children with ECC and without ECC .

5.CONCLUSION:

Recently discovered Gram-positive cariogenic pathogen Scardovia wiggsiae is closely linked to both early and advanced carious lesions. As a result, Scardovia wiggsiae has been classified as an anaerobic bacillus. According to genetic testing, this organism is saccharolytic and has the potential to ferment lactic and acetic acids. These potential traits showed that this organism may be a component of the oral biofilm causing the onset or progression of dental caries. In the present study, a real-time polymerase chain reaction was used to examine the relationship between unstimulated salivary flow, pH, and buffering capacity and Scardovia wiggsiae levels in children with and without S-ECC. Considering the results of this microbiological study it can be assumed that there is a strong correlation of the acidogenic organism Scardovia wiggsiae and salivary pH . Also the S.wiggsiae count was higher in saliva samples of children with S-ECC when compared to children without ECC proving their association with the disease process in a significant manner. However, the caries microbiome is diverse and complex which needs to be explored and the association with S. wiggsiae with the salivary parameters other than salivary flow , pH and buffering capacity in children needs to be investigated further.

IMAGES:

IMAGE 1.Salivary pH Estimation

IMAGE 2. Measurement of salivary buffering capacity

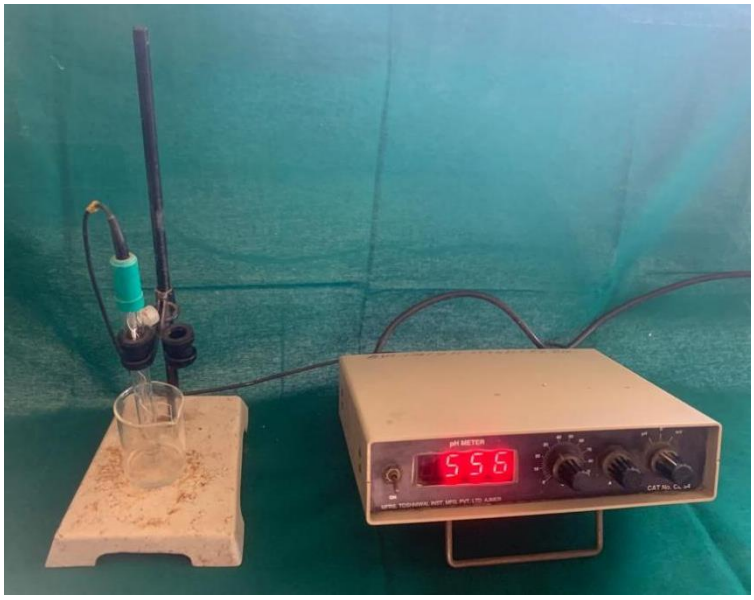


IMAGE 3- Measurement of salivary buffering capacity

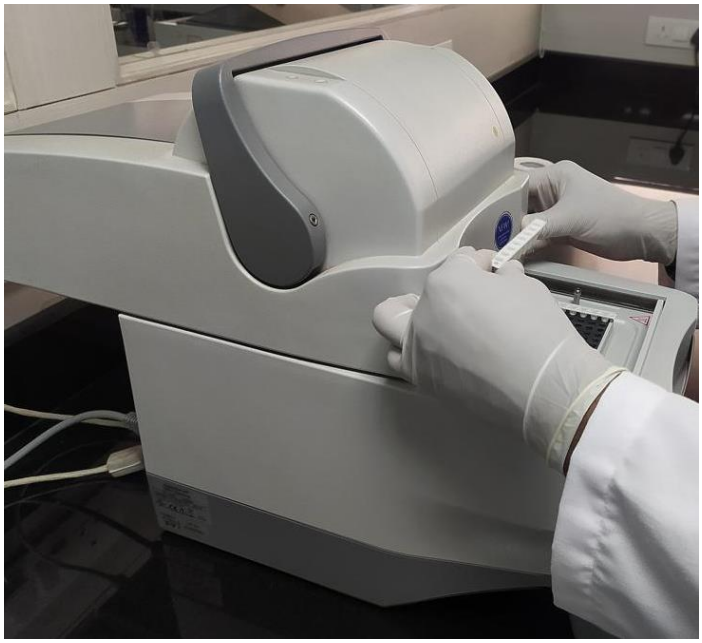
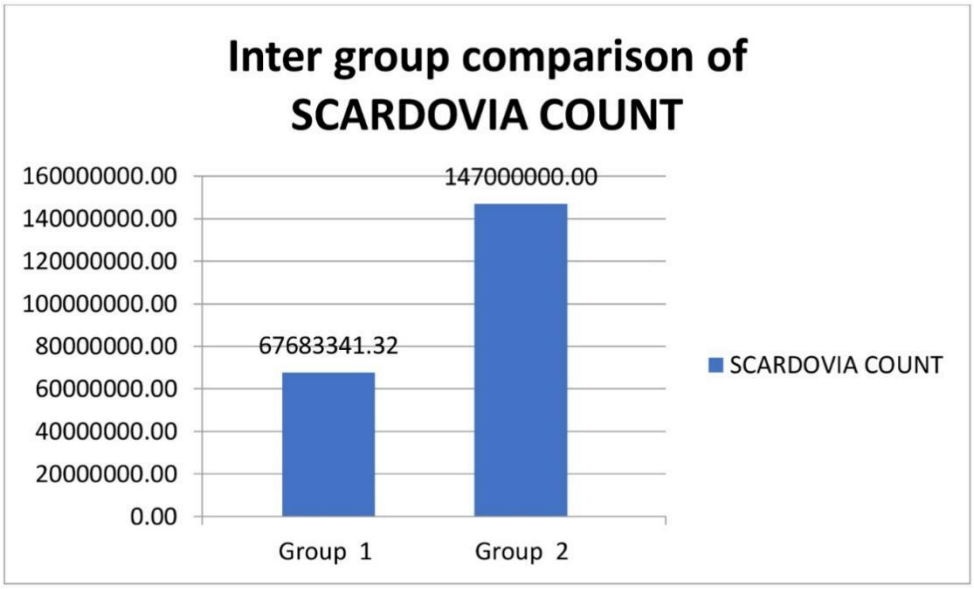
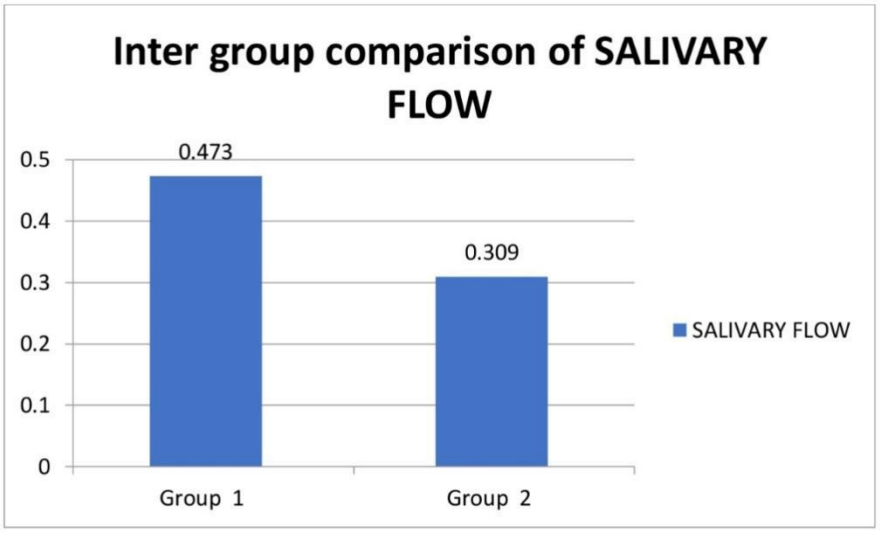


IMAGE 4 – Real time PCR

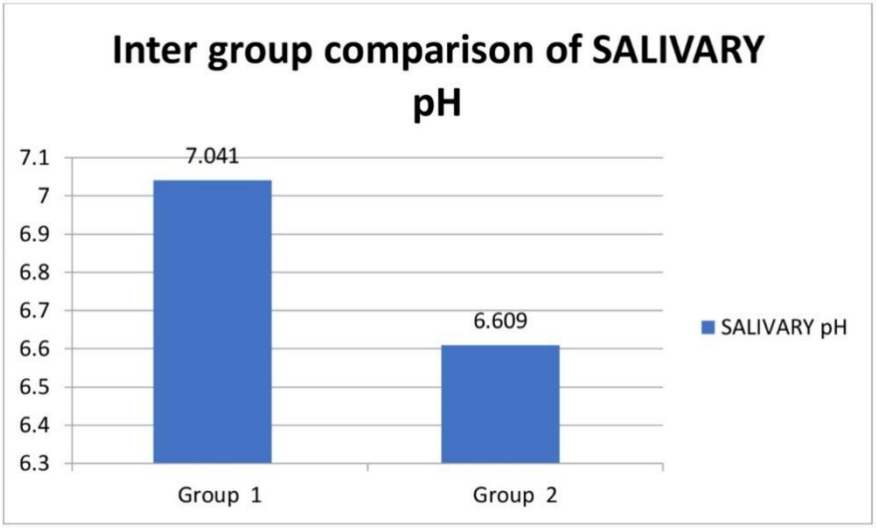
4.GRAPHS:



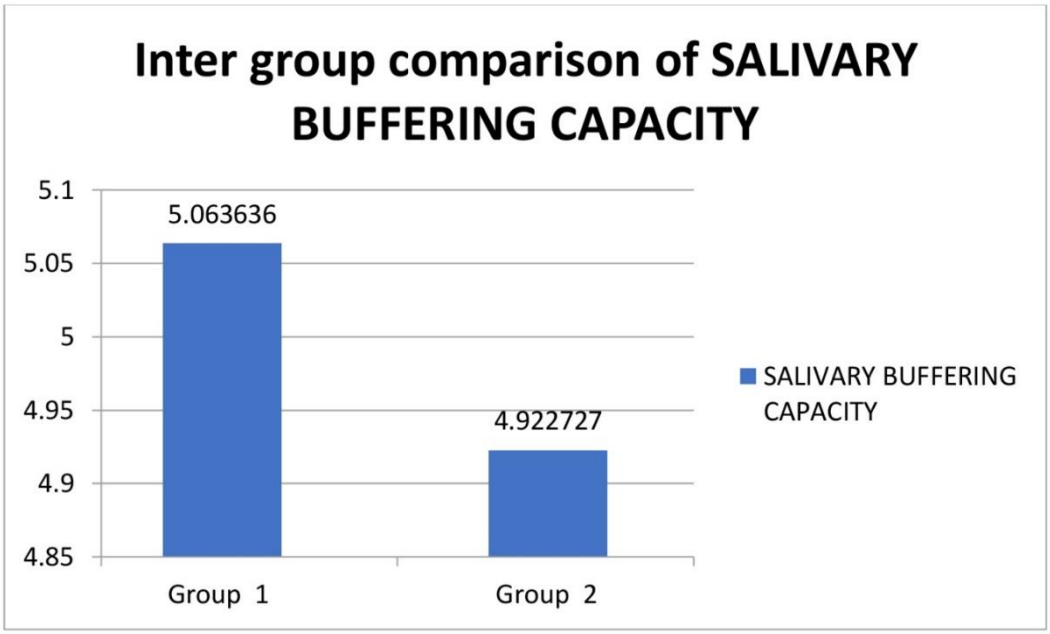
GRAPH 1:Inter group comparison between Scardovia wiggsiae count



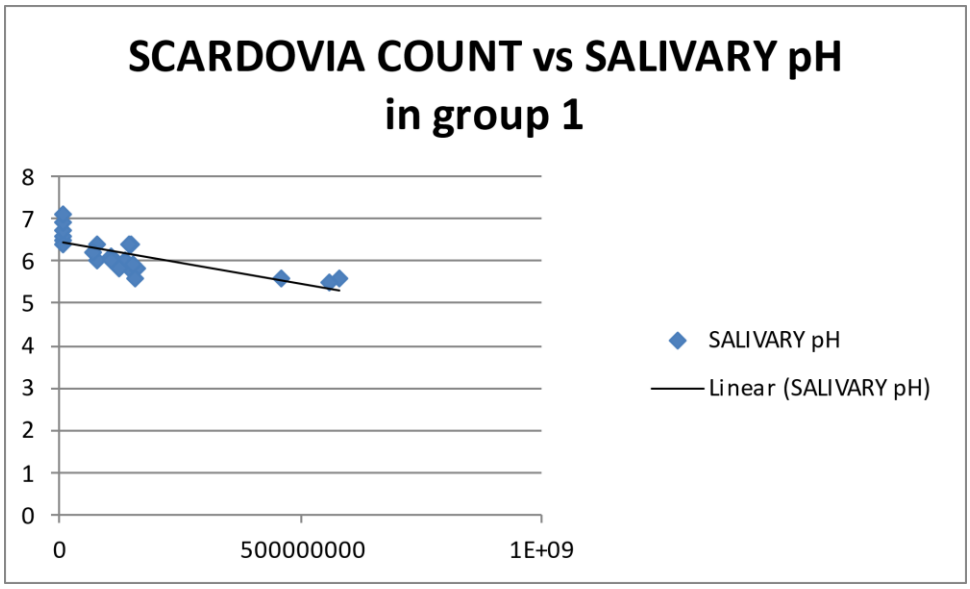
Graph 2 Inter group comparison between salivary flow



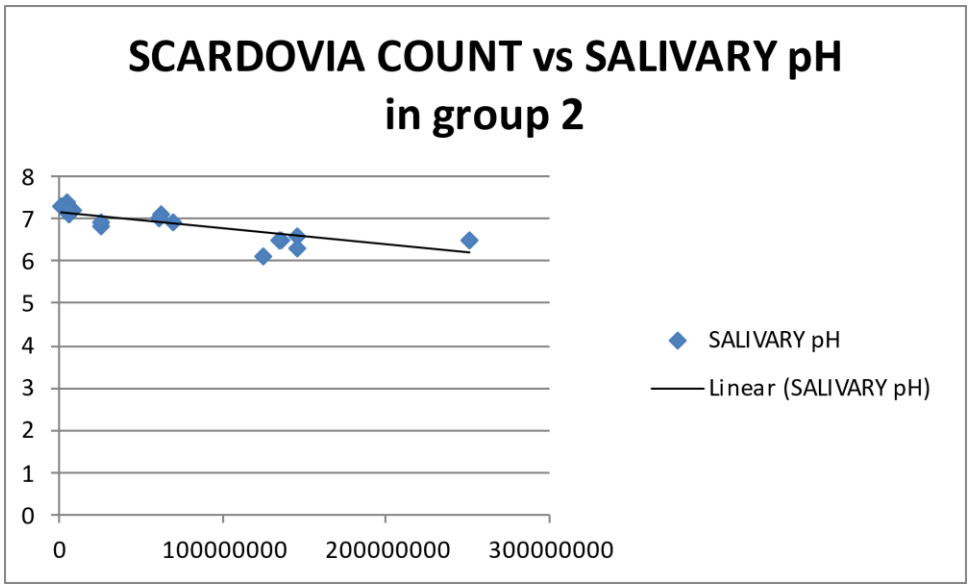
Graph 3 Inter group comparison between salivary Ph



Graph 4 Inter group comparison between salivary buffering capacity



Correlation between Scardovia wiggsiae and salivary pH in Group I



Correlation between Scardovia wiggsiae and salivary pH in Group II

REFERENCES-

1. Seow WK. Biological mechanisms of early childhood caries. Community Dent Oral Epidemiology. 1998;26:8–27

2.McDonald RE, Avery DR, Stookey GK. Dentistry for the child and adolescent. 8th ed. St. Louis: Mosby Co; 2005. Dental caries in the child and adolescent, 203–235.

3.Sucheta Prabhu Matondkar, Chandrashekar Yavagal, Manohar Kugaji, Kishore G. Bhat. Quantitative assessment of Scardovia wiggsiae from dental plaque samples of children suffering from severe early childhood caries and caries free children. J Indian Soc Pedod Prev Dent.,66, 123: 312–318.

4.G.M. Abbate, D. Borghi, A. Passi, L. Levrini, Correlation between un-stimulated salivary flow, pH and streptococcus mutans, analysed with real time PCR, in cariesfree and caries-active children. European Journal of Paediatric Dentistry Vol. 15/1- 2014.

5.Jayaraj D, Ganesan S. Salivary pH and Buffering Capacity as Risk Markers for Early Childhood Caries: A Clinical Study. Int J Clin Pediatr Dent 2015;8(3):167-171.
6. De Grauwe A, Aps JK, Martens LC. Early childhood caries (ECC): what's in a name? Eur J Paediatr Dent (2004) 5(2):62–70.
7. Feldens CA, Giugliani ER, Duncan BB, Drachler Mde L, Vitolo MR. Long-term effectiveness of a nutritional program in reducing early childhood caries: a randomized trial. Community Dent Oral Epidemiol (2010) 38(4):324–32.
8.Tanner AC, Mathney JM, Kent RL, Chalmers NI, Hughes CV, Loo CY, Pradhan N, Kanasi E, Hwang J, Dahlan MA, Papadopolou E, Dewhirst FE. Cultivable anaerobic microbiota of severe early childhood caries. J Clin Microbiol. 2011; 49:1464–74.
9.Chandna P, Srivastava N, Sharma A, Sharma V, Gupta N, Adlakha VK. Isolation of Scardovia wiggsiae using real-time polymerase chain reaction from the saliva of children with early childhood caries. J Indian Soc Pedod Prev Dent 2018;36:290-5.
10.Vacharaksa A, Suvansopee P, Opaswanich N, Sukarawan W. PCR detection of Scardovia wiggsiae in combination with Streptococcus mutans for early childhood caries-risk prediction. Eur J Oral Sci 2015; 123: 312–318.
11.Finlayson TL, Siefert K, Ismail AI, Sohn W. Psychosocial factors and early childhood caries among low-income African-American children in Detroit. Community Dent Oral Epidemiol (2007) 35(6):439–48.
12.Choudhary A, Bhat M, Choudhary H, et al. (August 08, 2022) Prevalence of Dental Caries With Salivary Assessment in Six to Twelve Years Old School-Going Children in Shahpura Tehsil, Jaipur. Cureus 14(8): e27802.
13.Ericson T, Makinen KK. Textbook of clinical cariology. Copenhagen: Munksgard; 1986 Saliva formation, composition and possible role. pp. 28–45.
14.Gopinath VK, Arzreanne AR. Saliva as a diagnostic tool for assessment of dental caries. Arch Orofac Sci. 2006;1(1):57–59.
15.Lamberts BL, Pederson ED, Shklair IL. Salivary pH-rise activities in caries-free and caries-active naval recruits. Arch Oral Biol. 1983;28(7):605–608.
16.Marsh PD. Microbiology of dental plaque biofilms and their role in oral health and caries. Dent Clin North Am. 2010; 54:441–54.