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Comparative Evaluation of Increase in IgA Levels in Saliva after Corona Vaccination -An In Vivo Study

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Abstract

Introduction: Mucosal immunity against Covid-19 disease obtained through Secretory IgA (sIgA) antibodies, provide a first line of defense. Although Serum IgA is a precursor for sIgA production, the frequency of production could be manifold through vaccination against corona virus. Furthermore, vaccination might indirectly improve oral health by increasing antibacterial activity of saliva and reduction in dental caries. Thus, the present study aimed to evaluate the changes in sIgA levels in saliva of children who received Corona Vaccination with those who did not receive it.

Method: Forty caries free healthy children aged 12–16-year-old with dmft/DMFT=0(zero), were selected and randomly divided into two groups of 20 each. The control group A (n-20) comprised of children who did not receive any vaccination against Corona virus whereas the Experimental group B children (n=20) received 2 doses of Corona vaccination at an interval of 28 days. Unstimulated saliva samples were collected from both groups to evaluate any change in levels of secretory IgA antibodies in saliva, with or without corona vaccination by using ELISA test.

Results: On statistical analysis, it was found that the mean percentage change in the sIgA values between baseline and post-study period for Control group A children (non-vaccinated) showed statistically non-significant increase in salivary IgA antibodies. Whereas, mean percentage change in sIgA levels between pre and post- intervention period for Experimental group B children (vaccinated against Covid-19), showed a statistically significant increase in salivary IgA antibodies. Furthermore, statistically significant results were obtained when intergroup comparisons were made.

Conclusion: The prevention of a contagious infectious disease depends on the local immune response of the population. From the study it can be concluded that, Covid-19 disease can be largely prevented by vaccination of children that imparts improved immunity

primarily by increase in secretory IgA level in saliva. In addition to this, the increased IgA also provides an extended support in controlling dental caries occurrence as well, which is considered a destructive chronic disease worldwide.

Keywords: Children immunity, mucosal immunity, corona vaccination, Covid-19 disease prevention, SARS-CoV-2 virus, salivary antibody or salivary immunoglobulin, Secretory IgA (sIgA)

Introduction

The COVID-19 pandemic has been characterized by rapid global spread and has impacted the life of almost every person on the planet. First reported in the Wuhan province of China in December 2019, the COVID-19 disease reached pandemic status within six months and has spread to nearly every country.[1]

COVID-19 is caused by a novel coronavirus, termed Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) by the WHO in February 2020.[2] Although the exact mechanism behind the increased spread of SARS-CoV-2 remains to be discovered, one hypothesis suggests that the virus primarily spread via respiratory droplets which begin as mucosal secretions in infected individuals, followed by infectivity of asymptomatic or pre-symptomatic carriers. These droplets become aerosolized by coughing, sneezing, or talking and can spread through the air or through contaminating surfaces, making it difficult to contain or prevent the disease transmission.[3]

The COVID-19 disease symptoms may vary widely in severity including sore throat, fever, cough, muscle pain, headache, and a characteristic loss of taste or smell further progressing to Acute Respiratory Distress Syndrome (ARDS).[4] It ranges from asymptomatic to critical disease and, even death in many patients. The rapid spread of the disease worldwide along with the emergence of variants of concern in the subsequent years have thrown a challenge, which has necessitated the discovery of vaccines for providing immunity against the disease spread.

The first line of defense against a viral respiratory infection is the mucosal immune system in the respiratory tract, which plays critical roles in both innate and adaptive immunity. Upon detection of a pathogen, the immune cells trigger innate responses, which include the generation of reactive oxygen species and anti-microbial peptides targeted against the pathogen. This protects the respiratory tract and facilitates muco-ciliary pathogen clearance. When the viral inoculum is large enough, it can trigger participation by the mucosal adaptive immune system, through generation of antibodies or Immunoglobulins against the pathogen, such as IgA, specifically Secretory IgA, that plays a crucial role in immune defense of mucosal surfaces, being the first point of entry of SARS-CoV-2.[5]

There are different types of Immunoglobulins (IgA, IgG, and IgM) among which IgA, is the antibody class produced in largest quantities by the human body. Many researchers have already proven that IgA antibodies play an important role in immune response against the SARS-CoV-2 virus. Serum IgA has been detected in COVID-19 patients and appears to be detectable earlier than IgM or IgG antibodies, possibly as early as two days after onset of symptoms (versus five days for either IgM or IgG). [6] Secretory IgA (sIgA), which is a dimeric structure, is predominantly found in mucosal secretions in comparison to its predecessor molecule Serum IgA, which is a less abundant monomeric form primarily found in serum. The dimeric structure of sIgA consists of two IgA molecules covalently linked along the Fc region via J-chain, and this covalent linkage confers protease resistance to the IgA molecule. Many mucosal

pathogens secrete a group of proteolytic exoenzyme known as IgA protease that have the potency to protect the bacteria or virus from host immune responses by cleaving the hinge region of IgA, which is the primary secreted antibody active at mucosal surfaces. Viral proteases are therefore essential for replication, which makes them ideal therapeutic targets. Medications that inhibit the cleavage of polyprotein into functional proteins are called protease inhibitors. A coronavirus protease inhibitor helps to fight Covid-19 virus by blocking or preventing the functions of viral proteases, that have an important role in the SARS-CoV-2 life cycle.[7]

The Secretory IgA (sIgA) molecule has a number of essential functions with regard to mucosal immunity. A major function is to prevent the cognate pathogens from infecting host cells via immune exclusion, by competing for the host-cell ligands that trigger viral entry. The sIgA further contributes to viral clearance via agglutination and shielding of microbial adhesins for later clearance via ciliary activity. In the case of SARS-CoV-2, sIgA antibodies may prevent adhesion to target epithelial cells via neutralization of the coronavirus spike protein and thus inhibiting its interaction with the host ACE-2 receptor or binding to the SARS-CoV-2 nucleocapsid protein. Beyond this role, sIgA can initiate and regulate the process of myeloid immune responses through the FCAR receptor to the IgA Fc region that is found on multiple immune and epithelial cells, resulting in a broad range of effector functions involving both humoral and cellular responses. Thus, IgA is considered the first line of defense against pathogenic viruses and bacteria, hence suggesting that IgA may be the first antibody to appear in response to SARS-CoV-2 infection. But research into the role of IgA, especially mucosal IgA in COVID-19 has lagged.[8]

Beyond its important role in mucosal immunity, IgA is also valued for another reason: its relationship with saliva and dental caries. Salivary IgA contributes 60% of the total immunoglobulin count in saliva and also helps in prevention of dental caries by its antibacterial effect. Dental caries being considered the most common chronic childhood disease in children, affecting about 60-90% of school-aged children worldwide. Among multiple factors, Salivary components play a major role in the prevention, initiation, and progression of the disease. Salivary IgA contributes to the antibacterial action of the saliva by neutralizing the bacterial toxins and enzymes, preventing the adherence of the bacteria to the tooth surface by blocking of bacterial adhesins, and agglutination of the bacteria, thus providing oral immunity.[9]

As sIgA gets localized in the mucosa, being a secreted antibody, it is easily accessible in saliva, hence salivary testing could be considered an ideal method due to its ease of collection. So here, IgA test was performed using the ELISA testing method, which is validated for both qualitative and quantitative detection of any changes in salivary IgA levels in accordance with FDA guidelines.[10]

Understanding the protective capacity and the duration of humoral immunity during SARS-CoV-2 infection or after vaccination is critical for managing the pandemic and would also provide more evidence about the efficacy of SARS-CoV-2 vaccines. This research would help to analyze whether vaccination improves salivary IgA production, that would impart immunity against Covid-19, as well as help in prevention of dental caries. Such knowledge should optimize vaccination strategies and public health decisions.[11]

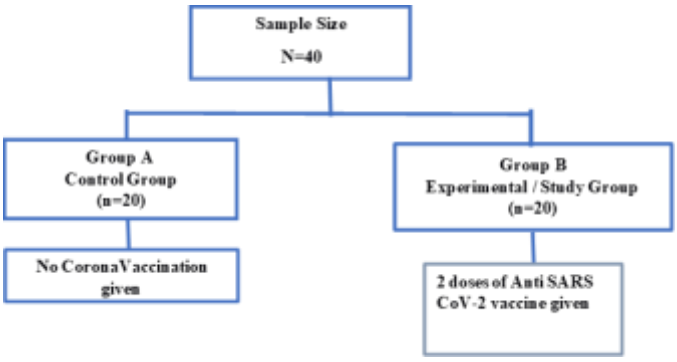
Keeping this in mind, this study, is therefore aimed to evaluate and compare the changes in level of in secretory

IgA antibodies in saliva of individuals with or without corona vaccination, using ELISA test. This is Experimental clinical research based in vivo study.

Materials and Methods

Forty caries-free healthy institutionalized children in the age group 6-12 years were selected based upon dental examination and relevant case history.[12] 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 has increased susceptibility to dental caries. Informed consent for participation in the study was obtained from their parents. 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.[13] Children who were found to be affected by upper respiratory tract infection, during the last week prior to data collection, or if they were suffering from any medical illness that could affect saliva flow rate or saliva composition were excluded from joining the study.[14]

The samples were further divided into two groups of 20 members each where Group A (Control group, n=20) comprised of children who were not vaccinated against SARS-Cov-2 virus whereas Group B (Experimental group, n=20) consisted of children who were vaccinated against SARS-Cov-2 virus. Group B children were given two doses of Anti SARS CoV-2 vaccination. 1st dose was given after the beginning of the study and 2nd dose was given after 28 days of 1st dose. For Group B children saliva samples were collected at the beginning prior to 1st vaccine dose and again after 21 days of 2nd vaccine dose. For Group A children there was no vaccination at any point during the study period and saliva samples were collected at beginning and end of study period (Flowchart. 1).



Flowchart 1: Division of samples

Salivary samples were selected as the media for carrying out this in vivo study. Saliva collection is a simple and well-established non-invasive method to measure the quantity of sIgA produced in the oral cavity after vaccination. Since saliva is present at the portal of entry of the corona virus, ie, oral cavity and nasopharynx, saliva containing sIgA could be considered an effective medium to control or prevent corona viral disease. [15]

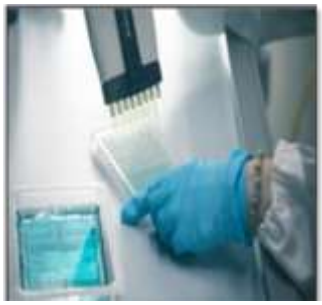
Unstimulated saliva samples were collected for the entire study group at the beginning and end of the study period under standard temperature and humidity conditions. The children were told not to ingest anything by mouth for 2 hours before saliva collection and not to use mouthwash during the study period. Just before saliva collection the children were directed to gargle with sterilized water. [16] The saliva collection was done by Spitting method during morning hours. Each subject was instructed to spit into sterile container to collect about 5 ml volume of saliva over 1-10 minutes. The saliva was collected directly into sterilized tubes, appropriately sealed and labeled and transported to clinical laboratory for evaluation of anti-Covid- 19 specific salivary IgA antibody. The saliva samples were surrounded by ice packs while transportation, that helped to protect salivary proteins from bacterial degradation until further processing. [17]

In the microbiological lab, the salivary samples were evaluated for changes in quantity of sIgA antibodies.

The level of anti-covid-19 salivary IgA antibodies were measured using Elabscience Human sIgA ELISA kit and Tecan ELISA Reade (Fig. 1, Fig. 2). The selected ELISA kit could be used for in vivo quantitative determination of sIgA concentrations in Human saliva (Fig. 3, Fig. 4). ELISA detects and measures antibodies in human samples produced against specific antigens by enzymatic reaction. ELISA has been considered as a reliable, highly- sensitive, confirmatory and cost-effective technique for quantification of both serum and salivary constituents, hence more frequently used. [18]

Figure 1: ELISA Kit

Figure 2: ELISA Reader



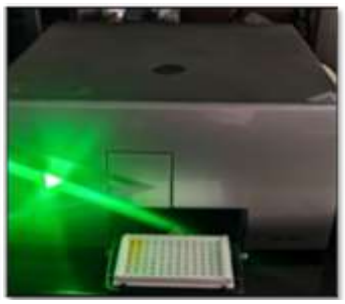


Figure 3, Figure 4: Evaluation of sIgA in microbiology lab using ELISA Kit & ELISA Reader

Test principle

This ELISA kit uses the Sandwich-ELISA principle. The micro-ELISA plate provided in this kit is pre-coated with an antibody specific to Human sIgA. Saliva samples were serial diluted till $1/10^5$ in Phosphate buffered saline (pH 7.0) and added to the micro-ELISA plate wells and combined with the specific antibody, covered with a sealer and then incubated for 90 mins at 37^0C. Then a biotinylated detection antibody specific for Human sIgA and Avidin-Horseradish Peroxidase (HRP) conjugate were added successively to each micro plate well and incubated for 60 mins and 30 mins respectively. Finally, substrate reagent was added to each well and incubated for 25 mins. Only those wells that contain Human sIgA, biotinylated detection antibody and Avidin-HRP conjugate appeared blue in color. The enzyme-substrate reaction was terminated by the addition of stop solution and the color turned yellow. The optical density (OD) was determined for each well spectrophotometrically with a micro plate reader set at a wavelength of 450 ± 2 nm. The OD value was proportional to the concentration of Human sIgA. The concentration of Human sIgA in the samples was calculated by comparing the OD of the samples to the standard curve using Beer's Law. [19]

Calculation of results

Beer's Law is used for calculation of antibody levels or concentration. The law states that the concentration of a

chemical solution is directly proportional to its absorption of light. The various absorbance corresponding to different concentrations were plotted against a graph, where the X-axis is Concentration of the sample and Y-axis is the Absorbance or Optical density (OD) of the sample. The actual concentration is the calculated concentration multiplied by the dilution factor. The dilution factor is 5. As the OD values of the standard curve may vary according to the conditions of the actual assay performance, a standard curve is established for each test (Fig. 5).

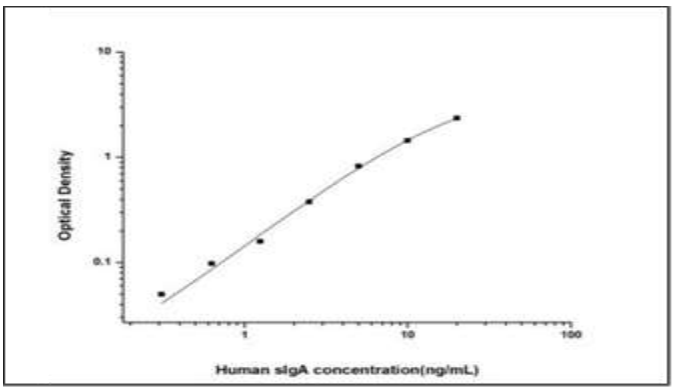


Figure 5: Standard curve

Statistical analysis

The data for the present study was entered into Microsoft Excel 2007 and analyzed using the SPSS statistical software 23.0 Version. The level of the significance was fixed at $P < 0.05$. Intergroup and Intergroup comparisons were made using the independent t tests and the Paired t test respectively. The Shapiro–Wilk test was used to investigate the distribution of the data and Levene's test to explore the homogeneity of the variables.

Results

The study aimed to evaluate the effects of COVID-19 vaccination on salivary IgA levels in children, revealing significant insights into mucosal immunity enhancement through vaccination. In the control group (Group A), which consisted of children who did not receive the COVID-19 vaccination, there was no significant change ($P<0.001$) in mean salivary IgA levels from baseline (A1) to the end of the study period (A2). This suggests that in the absence of vaccination, the natural secretion of IgA remains relatively constant over the period observed. The data is shown in Table. 1 and graphically represented in Graph. 1.

In contrast, the experimental group (Group B), which included children who received the COVID-19 vaccination, showed a statistically significant increase ($P=0.001$) in mean salivary IgA levels between pre-intervention (B1) and post-intervention (B2) stages. The increase was particularly pronounced after the second dose, indicating a boosted immune response due to the vaccination. The data is shown in Table. 2 and graphically represented in Graph. 2

When comparing the control and experimental groups, at pre-intervention level, there was a statistically non-significant difference between the sIgA values. While when intergroup comparison was made at non-intervention level for control group and post-intervention level for test group, a statistically significant difference was found, with vaccinated children having notably higher levels of salivary IgA compared to their unvaccinated counterparts. The data is shown in Table. 3 & Table. 4 and graphically represented in Graph. 3 & Graph. 4.

Table 1: Mean IgA Antibody Concentration in the Control Group

Control Group (Group A)	Mean sIgA concentration	Std Dev	Std Error	P value	Significance
Pre-Intervention	16.612	1.523	0.123	0.194	Non-Sig
Post Intervention	17.771	2.253	0.134		

Graph 1: Mean IgA Antibody Concentration in the Control Group

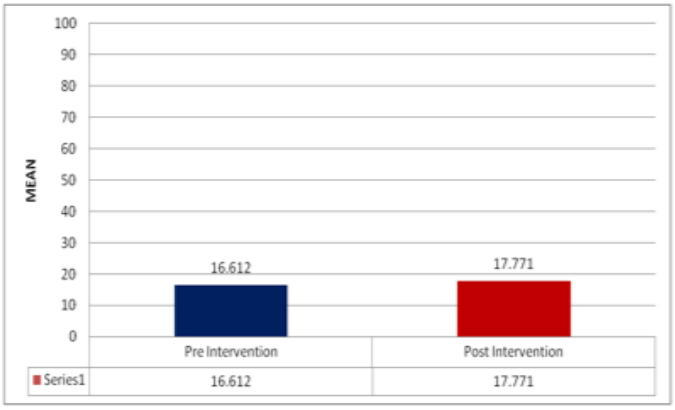


Table 2: Mean IgA Antibody Concentration in the Test Group

Test Group (Group B)	Mean sIgA concentration	Std Dev	Std Error	P value	Significance
Pre-Intervention	17.830	2.601	0.121	0.001	Sig
Post intervention	77.26	10.14	2.16		

Graph 2: Mean IgA Antibody Concentration in the Test Group

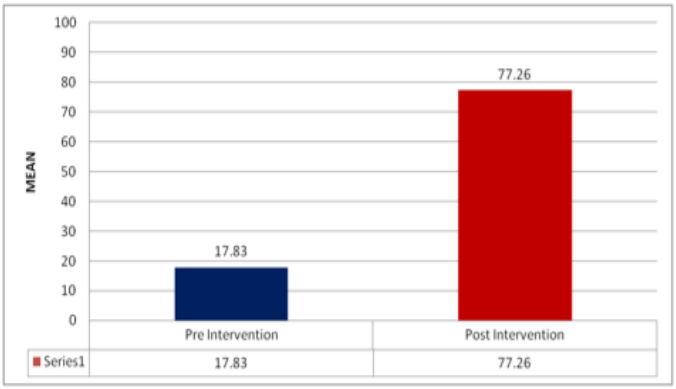


Table 3: Intergroup Comparison Between Control Group and Test Group at Pre-Intervention Levels

Intergroup comparison (Pre-intervention level)	Mean sIgA concentration	Std Dev	Std Error	P value	Significance
Control Group	16.612	1.523	0.123	0.173	Non-Sig
Test Group	17.830	2.601	0.121		

Graph 3: Intergroup Comparison Between Control Group and Test Group at Pre-Intervention Levels

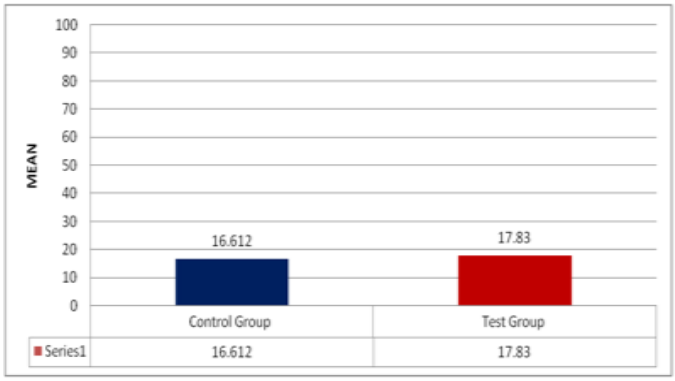
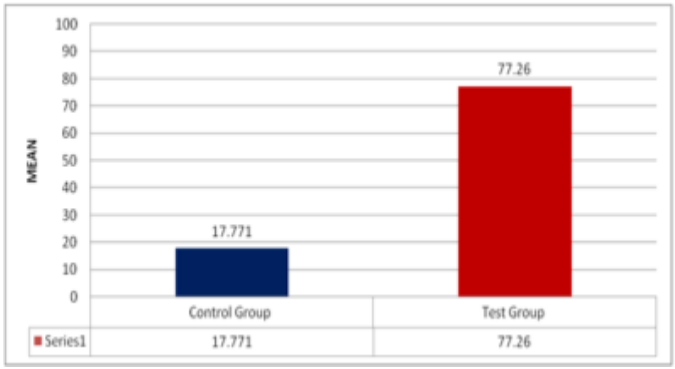


Table 4: Intergroup Comparison Between Control Group and Test Group at Post Intervention Levels

Intergroup comparison (Post-intervention level)	Mean sIgA concentration	Std Dev	Std Error	P value	Significance
Control Group	17.771	2.253	0.134	0.001	Sig
Test Group	77.26	10.14	2.16		

Graph 4: Intergroup Comparison Between Control Group and Test Group at Post Intervention Levels



Discussion

The difference in significance level underlines the impact of vaccination in enhancing mucosal immunity, specifically through the elevation of salivary IgA levels. The significant increase in salivary IgA levels in vaccinated children aligns with the primary goal of vaccination: to enhance the body's immune defense against SARS-CoV-2. [20] Secretory IgA (sIgA) plays a critical role in mucosal immunity, particularly in the oral and nasopharyngeal regions, which are primary entry

points for respiratory pathogens like SARS-CoV-2. The rise in IgA levels following vaccination suggests a stronger first line of defense, potentially reducing viral load and preventing the establishment of infection. [21]

These findings underscore the importance of mucosal immunity in preventing respiratory infections. By elevating sIgA levels, the COVID-19 vaccine may provide not only systemic immunity but also enhanced localized mucosal protection. This dual-layer protection could be crucial in preventing both symptomatic and asymptomatic spread of the virus among children, who are often vectors of transmission within communities. [22] Moreover, the increase in salivary IgA has implications beyond COVID-19 prevention. Given IgA's antibacterial properties, the enhanced salivary IgA levels could help reduce the incidence of dental caries, a common chronic disease in children. Thus, vaccination might indirectly contribute to better oral health outcomes by increasing the antibacterial activity of saliva and reducing the burden of caries in this population. [23]

Among different types of microbes, the viruses target the immune system of an individual that plays a vital role in fighting back and control of a disease severity and spread. Most viral diseases tend to have both systemic and oral manifestations, thus it is important to monitor the changes happening in the oral cavity once the pathogen enters the body, because oral cavity represents preliminary signs of the disease and suggests how the disease could be controlled in a better way. [24]

The Covid-19 disease was rarely diagnosed in children at the beginning; hence it was first thought that pediatric population is less susceptible to it. However, in the later phase, it came into picture that children could also be seriously affected by SARS-CoV-2, but interestingly, a major proportion of them might be asymptomatic while others can be symptomatic, leading to a delayed diagnosis and questionable cure of the disease. [25] As per data obtained by WHO, children under the age of 5 years accounted for 1.8% cases, between the age of 5 to 14 years accounted for 6.3% cases, and from age 15 to 24 years represented 14.5% of the total reported cases of COVID-19 worldwide. [1] Since it is always better to prevent a disease than to cure it, considering the serious life- threatening results of the Covid-19 pandemic, vaccination is the gold standard for disease prevention and an optimal solution for effectively controlling and eliminating the disease by increasing the herd immunity of the total population. Hence immunization protocol involving only adults will not suffice rather it demands increase in coverage which includes protecting the children as well. As recommended by CDC, children older than six months should get a COVID-19 immunization, including those who have been infected with COVID-19 in the past. [26]

In addition, multiple cross-sectional studies have revealed that salivary IgA antibodies also prevent dental caries, a chronic infectious microbial disease with high prevalence in children worldwide. Reported data accounts for caries prevalence rate of 84% among children 5-12 years and 72% among children 12–15 years. Children in this highly dynamic age are more susceptible to dental caries due to frequent intake of refined sugars, soft and sticky foods. [14] Hence it is important to control the disease, and salivary factors could play an important role in this regard by interfering microbial adherence on tooth surface, through neutralization of enzymes, toxins and viruses. This prevents demineralization of tooth enamel and hence helps to control the initiation of caries process. Some studies have also shown a lower incidence of caries due to a high salivary IgA concentration. In addition, low levels of salivary IgA have been considered as a risk

factor for upper respiratory infection and associated with an increased risk for caries and periodontal disease. [10]

Childers et al have reported that the levels of serum IgA increased with age in children (aged 6-12 years) and adults (aged 22-51 years) after recent exposure to the virus or post- vaccination. [27] This was probably because vaccination with a particular viral antigen induces immune response inside human body, both in serum as well as mucosa, through production of antibodies or immunoglobulins specific to the target antigen for example viral spike protein, nucleocapsid protein etc. Among these, IgA production is maximum noted in saliva of any individual. [28]

Overall, Covid-19 disease can be largely prevented by restricting the entry of the pathogen, through vaccination of the children that imparts herd immunity in the entire population. Also, the presence of sIgA in saliva plays a crucial role and contributes to the maintenance of oral health by preventing the colonization and growth of cariogenic bacteria. Thus, corona vaccination indirectly helped in reducing the incidence a dental caries in children as well.

Conclusion

In conclusion, the study demonstrates that COVID-19 vaccination significantly enhances mucosal immunity in children, as evidenced by increased salivary IgA levels. This enhancement provides a primary defense against SARS-CoV-2 and contributes to improved oral health. The results support the inclusion of children in vaccination programs as a strategy to curb the spread of COVID-19 and improve overall health as well as oral health outcomes. Further investigations into the long-term effects of vaccination on mucosal immunity and cross-protection against other pathogens are warranted.

However, the study has some limitations. It was conducted on a small, homogeneous population of caries-free children, limiting the generalizability of the findings. Future research should include diverse populations with varying dental health statuses and different geographic or socio-economic backgrounds. Additionally, this study only measured short-term changes in salivary IgA levels. Long-term studies are needed to evaluate the persistence of elevated IgA levels and their correlation with ongoing protection against SARS-CoV-2 and other respiratory pathogens.

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