A Comparative Evaluation of the Levels of Salivary IgA in HIV Affected Children and the Children of the General Population within the Age Group of 9 – 12 Years – A Cross-Sectional Study

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ABSTRACT

Introduction: The social and health consequences of not breastfeeding and the economic realities associated with expensive diagnostic testing and antiretroviral treatment also play a factor in the present condition. Acquired immunodeficiency syndrome is characterized by profound immune suppression that leads to opportunistic infections, secondary neoplasm and neurologic manifestations. It also leads to a progressive decrease in the number and function of CD4+ T lymphocytes, depressing the immune response.

Objectives: Evaluation of the levels of salivary IgA in HIV affected children and the children of the general population within the age group of 9-12 years.

Methods: 60 children in the age group of 9-12 years were selected for the study, of which 30 children were the HIV affected group (Group A) and 30 were randomly selected children from the general population (Group B). The whole procedure was explained to the child and their parents and written informed consent was obtained from the parents.

Results: 7 showed an increase in the same. Among the 19 children with caries, 7 showed a decrease of SIgA levels and 12 showed an increase of the same. The mean SIgA levels in saliva of HIV affected children was 7.67 mg/dl, which was significantly lower than that of 8.37 mg/dl seen in normal children and a statistical significance of 0.043 was obtained on comparison of the two groups.

Conclusion: SIg A was found to be significantly lower in HIV Affected children, which can increase the risk of caries development. Hence it is essential to know the status of their dental health needs so that it can be addressed promptly. These include the application of preventive methods to control the carious lesions, restoration of existing lesions and implementation of hygiene habits. The adequate management of oral lesions is vital to improving the quality of life of these HIV Affected children.

Key Words: Acquired immunodeficiency syndrome, CD4+ T lymphocytes, Salivary IgA, Spectrophotometer, Turbidometric Immunoassay

INTRODUCTION

In developing countries, HIV infection in children remains a major problem despite the progress that has been made in HIV treatment and prevention during the past two decades. In 2016, India had 80,000 new HIV infections and 62,000 AIDS-related deaths.¹ There were approximately 36.9 million people worldwide living with HIV/AIDS in 2017, of which, 1.8 million were children. Majority of these children live in sub-Saharan Africa and were infected by their HIV-positive mothers at the time of pregnancy, breastfeeding or childbirth. The difficulties in implementation of prenatal HIV screening programs and prophylaxis can be the reason for the higher prevalence of HIV infections in developing countries. The social and health significances, the monetary realism related to costly diagnostic testing and antiretroviral treatment play a role in the present condition.²
Acquired immunodeficiency syndrome is characterized by profound immune suppression that leads to opportunistic infections, secondary neoplasm and neurologic manifestations. It also leads to a progressive decrease in the number and function of CD4+ T lymphocytes, depressing the immune response. According to the widely used definition by UNAIDS, HIV Affected Children are defined as: Children under 18 years of age who are under one of the two categories: children who have lost one/both their parents due to HIV related illness and children living with one/both HIV infected parents. HIV-related stigma is prevalent in many countries and it is a source of constant stress for them and their family members. Stigma experienced by close associates of the infected individuals results in poor mental health outcomes leading to difficulty in psychosocial adjustment and greater delinquent behaviour among the children with HIV-infected parents. Many studies have been conducted on determining the Salivary IgA (SIgA) levels in HIV infected children. But, literature regarding estimation of SIgA levels in HIV affected children is scarce. Hence this study was done to compare the salivary SIgA levels in HIV Affected children with that of normal children.

**MATERIALS AND METHODS**

60 children in the age group of 9-12 years were selected for the study, of which 30 children were the HIV affected group (Group A) from ART centre of Government Hospital in Attur Taluk of Salem district and 30 were randomly selected children from the general population (Group B). The whole procedure was explained to the child and their parents and written informed consent was obtained from the parents. Before the commencement of the study, ethical committee clearance was obtained from the Ethical Committee of the Institution. The Past Medical records were retrieved for every child of the study group to confirm their HIV negativity, CD4 counts along with the history of drug therapy. The personal details such as name, age and sex were noted on a printed pro forma and their DMFT/deft scores were assessed according to the WHO criteria before the start of saliva collection.

Children who were mentally compromised or were under any antibiotic treatment, those who had undergone fluoride treatment recently or children who were undergoing orthodontic treatment were excluded from both the study group (Group A) and the control group (Group B).

**Method of Sample collection**

1 ml of unstimulated saliva was collected from each child of both the groups in the morning 1 hour after breakfast. The children were instructed not to indulge in eating or drinking in the interval between breakfast and saliva collection. The children were asked to think about their favourite foods to induce pooling of saliva and then the whole saliva was collected in sterile disposable vials using the drooling method. Saliva samples with blood contamination were discarded. The salivary samples were transferred within 2 hours to a laboratory in chiller boxes. Total SIgA quantification was done by Immunoturbidometry.

**Evaluation of SIgA at the Laboratory**

Salivary IgA was evaluated using Turbidometric Immunoassay. For estimation of salivary IgA, 500 µl of quantic activation buffer was taken in a clean cuvette and 500 µl saliva sample was added to activation buffer. After incubation for 10 min, 50 µl of Antibody (antihuman IgA reagent) was added to sample and reading was recorded using a spectrophotometer at wavelength 340 nm at 37°C.

The Spectrophotometer was connected to a computer so that all the sample readings were displayed in the monitor. The three-time evaluation was done and the mean of the triplicate values was taken as the level of Salivary IgA. The collected data were subjected to statistical analysis using SPSS package version 18.0. Unpaired t-test was used to compare the salivary IgA levels between the two groups and Pearson correlation was used to find any correlation between the Salivary IgA levels and DMFT scores of both the groups. The implication level was set at P<0.05.

**RESULTS**

It can be noted that the IgA levels of Group A showed irregular consistency with their respective DMFT scores. Among the 30 children of Group A, 11 were caries-free among which, 4 children showed a decrease in SIgA levels while 7 showed an increase in the same (Table-1). Among the 19 children with caries, 7 showed a decrease of SIgA levels and 12 showed an increase of the same (Table-2). The mean SIgA levels in saliva of HIV affected children was 7.67 mg/dl, which was significantly lower than that of 8.37 mg/dl seen in normal children and a statistical significance of 0.043 was obtained on comparison of the two groups (Table-3). However, there was no significant difference between the mean DMFT scores of Group A & B, where Group A had a mean DMFT score of 1.87 while Group B had a score of 2.00 and the p-value was found to be 0.775 which was not statistically significant (Table 4). When the correlation of SIgA levels and DMFT scores were assessed in both the groups, a highly negative correlation was found between the SIgA levels and Decayed, Missing, and Filled (DMFT) scores of Group B which indicates that when the salivary IgA levels were low, the DMFT score was found to be high and vice versa. There was no statistically significant difference between the two parameters in Group A (Table 5).
Table 1: Salivary IgA levels and DMFT score

| Samples | Triplicate Values | Mean ± S.D | DMFT | Samples | Triplicate Values | Mean ± S.D | DMFT |
|---------|-------------------|------------|------|---------|-------------------|------------|------|
| A01     | 7.49              | 7.46       | 7.53 | 7.49± 0.03 | 4 | B01              | 11.24       | 11.2 | 11.21 | 11.22± 0.02 | 0 |
| A02     | 6.2               | 6.21       | 6.27 | 6.23± 0.03 | 2 | B02              | 8.13        | 8.32 | 8.34  | 8.26± 0.09  | 2 |
| A03     | 10.11             | 10.12      | 10.1 | 10.12± 0.01 | 0 | B03              | 7.83        | 7.3  | 7.31  | 7.48± 0.25  | 2 |
| A04     | 5.14              | 5.48       | 5.42 | 5.35± 0.15  | 5 | B04              | 6.94        | 6.92 | 6.95  | 6.94± 0.01  | 4 |
| A05     | 8.08              | 8.09       | 8.11 | 8.09± 0.01  | 3 | B05              | 10.29       | 10.21| 10.24 | 10.25± 0.03 | 0 |
| A06     | 7.42              | 7.49       | 7.46 | 7.46± 0.03  | 2 | B06              | 6.24        | 6.25 | 6.26  | 6.25± 0.01  | 2 |
| A07     | 8.13              | 8.26       | 8.27 | 8.22± 0.06  | 1 | B07              | 11.24       | 11.2 | 11.21 | 11.22± 0.02 | 0 |
| A08     | 6.25              | 6.27       | 6.29 | 6.27± 0.02  | 1 | B08              | 8.13        | 8.32 | 8.34  | 8.26± 0.09  | 1 |
| A09     | 9.24              | 9.38       | 9.37 | 9.33± 0.06  | 0 | B09              | 7.83        | 7.3  | 7.31  | 7.48± 0.25  | 1 |
| A10     | 6.2               | 6.27       | 6.29 | 6.25± 0.04  | 2 | B10              | 6.94        | 6.92 | 6.95  | 6.94± 0.01  | 3 |
| A11     | 8.95              | 8.96       | 8.94 | 8.95± 0.01  | 8 | B11              | 10.29       | 10.21| 10.24 | 10.25± 0.03 | 0 |
| A12     | 10.08             | 10.84      | 10.85| 10.59± 0.36 | 0 | B12              | 6.24        | 6.25 | 6.26  | 6.25± 0.01  | 5 |
| A13     | 6.58              | 6.95       | 6.98 | 6.84± 0.18  | 0 | B13              | 6.24        | 6.25 | 6.26  | 6.25± 0.01  | 4 |
| A14     | 7.48              | 7.43       | 7.03 | 7.31± 0.20  | 0 | B14              | 11.24       | 11.2 | 11.21 | 11.22± 0.02 | 0 |
| A15     | 9.24              | 9.2        | 9.21 | 9.22± 0.02  | 0 | B15              | 8.13        | 8.32 | 8.34  | 8.26± 0.09  | 1 |
| A16     | 8.28              | 8.15       | 8.19 | 8.21± 0.05  | 3 | B16              | 7.83        | 7.3  | 7.31  | 7.48± 0.25  | 1 |
| A17     | 8.27              | 8.25       | 8.21 | 8.24± 0.02  | 2 | B17              | 6.94        | 6.92 | 6.95  | 6.94± 0.01  | 3 |
| A18     | 8.08              | 8.09       | 8.11 | 8.09± 0.01  | 3 | B18              | 10.29       | 10.21| 10.24 | 10.25± 0.03 | 0 |
| A19     | 7.42              | 7.49       | 7.46 | 7.46± 0.03  | 2 | B19              | 6.24        | 6.25 | 6.26  | 6.25± 0.01  | 2 |
| A20     | 8.13              | 8.26       | 8.27 | 8.22± 0.06  | 1 | B20              | 7.42        | 7.45 | 7.46  | 7.44± 0.02  | 3 |
| A21     | 6.25              | 6.27       | 6.29 | 6.27± 0.02  | 1 | B21              | 8.23        | 8.26 | 8.25  | 8.24± 0.01  | 3 |
| A22     | 9.24              | 9.38       | 9.37 | 9.33± 0.06  | 0 | B22              | 7.56        | 7.53 | 7.65  | 7.58± 0.05  | 2 |
| A23     | 6.2               | 6.27       | 6.29 | 6.25± 0.04  | 2 | B23              | 6.85        | 6.55 | 6.35  | 6.58± 0.25  | 3 |
| A24     | 8.95              | 8.96       | 8.94 | 8.95± 0.01  | 8 | B24              | 10.9        | 10.58| 10.8  | 10.76± 0.14 | 0 |
| A25     | 10.08             | 10.84      | 10.85| 10.59± 0.36 | 0 | B25              | 8.98        | 8.3  | 8.55  | 8.61± 0.37  | 1 |
| A26     | 6.58              | 6.95       | 6.98 | 6.84± 0.18  | 0 | B26              | 7.55        | 7.42 | 7.49  | 7.48± 0.07  | 4 |
| A27     | 7.48              | 7.43       | 7.03 | 7.31± 0.20  | 0 | B27              | 9.1         | 9.25 | 8.95  | 9.1± 0.15   | 0 |
| A28     | 9.24              | 9.2        | 9.21 | 9.22± 0.02  | 0 | B28              | 6.25        | 6.33 | 6.35  | 6.31± 0.05  | 3 |
| A29     | 8.28              | 8.15       | 8.21 | 8.21± 0.05  | 3 | B29              | 8.95        | 8.91 | 8.86  | 8.91± 0.05  | 3 |
| A30     | 8.27              | 8.25       | 8.21 | 8.24± 0.02  | 2 | B30              | 6.74        | 6.88 | 6.63  | 6.75± 0.13  | 2 |

Table 2: Comparison of S IgA levels in HIV affected children and children of general population

| Group | N   | Salivary levels of IgA | t  | p   |
|-------|-----|------------------------|----|-----|
|       |     | Mean       | SD | SE  |    |    |
| Group A | 30  | 7.67        | 1.04 | 0.19 | 2.07 | 0.043 |
| Group B | 30  | 8.37        | 1.54 | 0.28 |    |    |

Table 3: Comparison of DMFT scores in HIV affected children and children of general population

| Group | N   | DMFT | t  | p   |
|-------|-----|------|----|-----|
|       |     | Mean | SD | SE  |    |    |
| Group A | 30  | 1.87 | 2.15 | 0.39 | 0.287 | 0.775 |
| Group B | 30  | 2.00 | 1.36 | 0.25 |    |    |
Saliva is an alternative biological fluid to the serum which can be evaluated for the diagnostic reason for the variety of systemic disorders. As compared with serum, the sensitivity and specificity of the antibody to HIV in saliva for detecting infection are between 95% and 100%. The salivary collection is a simple, non-invasive method that does not cause any risk of infection. SIgA synthesis rate is high and its half-life is short. Therefore, any small variation in SIgA concentration can be observed immediately and related to the causative factor. Salivary SIgA concentration is also a good index of mucosal immune function. SIgA synthesis is T cell-dependent and any change in its synthesis can be related to T or B cell activation. SIgA exhibits a diurnal rhythm, having the highest levels in the morning and the lowest in the evening. In our study, total salivary IgA was quantified in the whole saliva, collected in the morning using the drooling method. Saliva collected in this manner would have the highest levels in the morning and the lowest in the evening. Track and Haffajee, 1981 observed higher levels of SIgA concentration in non-caries subjects than in subjects with caries. Salivary SIgA levels are measured in mg/dL which lies within this range but were on the relatively low end of the normal range of SIgA when compared to the results obtained by Jyoti Chawda et al (2011) where the values obtained ranged from 11 – 32mg/dL. When a comparison was made for the salivary SIgA levels between the two groups, the results obtained showed that the SIgA levels ranged from 5.14 – 10.85 for Group A and 6.24 – 11.24 for Group B. The difference in SIgA levels of Group A and B were not statistically significant.

Irregular SIgA levels were noted among the children of Group A irrelevant to their caries status. This can be postulated to the fact that the levels of IgA can be altered by the psychological state of the individual since the HIV affected children might be under stress due to the unfavourable living conditions arising from the stigma experienced due to their parents’ HIV positivity when compared to their normal counterparts. In contrast to our study, De Farias et al. and Thaweboon et al. found that the presence of dental caries was associated with an increase of total salivary SIgA in normal children. However, Koga, et al. (2004) and Shifa, et al. found no correlation between dental caries and SIgA levels.

Table 4: Correlation of SIgA levels and DMFT scores of Group A & B

| Salivary levels of IgA | Pearson Correlation | P     | N   |
|------------------------|---------------------|-------|-----|
|                        |                     |       | 30  |

DISCUSSION

The impact of HIV infection on salivary IgA levels is unclear, with reports of both decreased and increased titers. IgA2 subclasses are reduced in HIV infection in saliva, and total secretory IgA levels are reduced later in the progress of the disease. Salivary IgA is reported to be able to neutralize HIV 1 and HIV 2, as well as block epithelial transmigration. HIV infection results in a progressive decrease in the levels of CD4+ T lymphocytes. Since the epithelial cell has a pivotal role in the maturation of the secretory immune system, its is expected that this system would be altered in these patients. The infectious nature of dental caries accepts the supposition that some form of host immunity can regulate caries activity. SIgA might give a strong association if immunity can control caries activity. It has been recommended that salivary SIgA antibodies produced by the mucosal immune system play a significant role in the immune reaction against dental caries. Bagherian, et al observed greater levels of SIgA in the saliva and lower DMFT scores in children who were colonized for less than 6 months with S. mutants compared to those who had S. mutans for a more than 24 months.

A high antigenic load can result in depressed SIgA, even in healthy, asymptomatic individuals. In our study, normal and healthy children with dental caries showed significantly higher levels of SIgA than caries-free children. Earlier studies showed that there was a decrease in SIgA levels in children with dental caries, while a contradictory result has noted another study done by Gregory et al which showed that SIgA levels in saliva are not associated with a decrease in caries activity.

The levels of IgA can be affected by stress and this is an environmental and psychological stimulus that may create a mental or physiological response and that leads to disease in individuals. Valdimarsdottir et al. in their study, showed that acute stress can reduce the performance of the immune system and suppress the production of immunoglobulin. Recently, the measurement of salivary immunoglobulin A (slgA) is an additional possible non-invasive method for the evaluation of stress. The IgA is the quite common class of antibodies in the mucous membrane, which is a very significant factor in the safety against allergy, infectious agents, and external proteins and has a concentration that can be affected by stress. It is reported that salivary IgA level changes in response to psychological factors, such as needed or disagreeable daily events, daily hassles, positive and negative mood, short-term stressful cognitive tasks and presentation. The levels of SIgA in subjects without any systemic or immunological diseases range from 4-30 mg/dL. Systemic conditions like protein-energy malnutrition, obesity, infections, psychological stress, cigarette smoking can affect the SIgA levels. The levels of SIgA detected in our study was 5 – 11 mg/dL which lies within this range but were on the relatively low end of the normal range of SIgA when compared to the results obtained by Jyoti Chawda et al (2011) where the values obtained ranged from 11 – 32mg/dL. When a comparison was made for the salivary SIgA levels between the two groups, the results obtained showed that the SIgA levels ranged from 5.14 – 10.85 for Group A and 6.24 – 11.24 for Group B. The difference in SIgA levels of Group A and B were not statistically significant.

Irregular SIgA levels were noted among the children of Group A irrelevant to their caries status. This can be postulated to the fact that the levels of IgA can be altered by the psychological state of the individual since the HIV affected children might be under stress due to the unfavourable living conditions arising from the stigma experienced due to their parents’ HIV positivity when compared to their normal counterparts. In contrast to our study, De Farias et al. and Thaweboon et al. found that the presence of dental caries was associated with an increase of total salivary SIgA in normal children. However, Koga, et al. (2004) and Shifa, et al. found no correlation between dental caries and SIgA levels.
Variation in our result compared to other studies could be due to different criteria for patient selection, different sampling methods, and different laboratory tests used.

**CONCLUSION**

Based on the findings of the present study, SLgA was found to be significantly lower in HIV Affected children, which can increase the risk of caries development. Hence it is essential to know the status of their dental health needs so that it can be addressed promptly. These include the application of preventive methods to control the carious lesions, restoration of existing lesions and implementation of hygiene habits. The adequate management of oral lesions is vital to improving the quality of life of these HIV Affected children.

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**Authors contribution**

1. PDG: Investigation
2. SKV: Data Collection
3. VD: Analysis
4. RK: Manuscript Writing
5. JBJ: Editing

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