Levels of Salivary Immunoglobulin A (SIgA) in HIV Infected Children

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Secretory IgA is the main type of immunoglobulin in saliva and is considered to be the main secretion factor of adaptive immunity in the mouth. ***Objective:*** *To assess the effect of Anti Retroviral Therapy on SIgA levels in saliva of HIV infected children.* ***Study Design:*** *A cross-sectional sample of 50 HIV infected children aged 6-8 years were divided into 2 groups ; Group 1: children prior to onset of anti-retroviral therapy and Group 2: children undergoing anti-retroviral therapy. Stimulated whole saliva samples were collected from each child following I hour of breakfast. The samples were placed on ice packs and immediately transferred to a laboratory, processed and total SIgA quantification was estimated using ELISA. Data obtained was statistically analyzed.* ***Results:*** *Among HIV infected children, significantly low SIgA levels of 6.2 mg/dl was seen in children prior to ART.* ***Conclusion:*** *Salivary IgA levels were significantly low in HIV infected children, particularly in children prior to ART.*

Key words: *ART, salivary IgA, dental caries, HIV*

INTRODUCTION

Acquired immunodeficiency syndrome (AIDS) was first recognized in 1981 and the virus responsible for this syndrome, Human Immunodeficiency Virus (HIV), was first isolated in 1983.[1] HIV and AIDS have made a huge global impact permeating the social, cultural and economic fabric of almost all nations. It affects residents of all countries of the world, but the vast majority of affected individuals live in the developing world. The HIV epidemic has been so dramatic and devastating that it has been described as the "epidemic of our century".[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. [3]

Pediatric HIV is a major world health problem, which is progressing at an alarming rate. In most cases it is due to transmission from HIV seropositive mothers during pregnancy, delivery or breast feeding. The global estimate of children living with HIV infection in 2007 was 2.7 million.[4] AIDS has become one of the leading causes of death among children worldwide. The impact of AIDS on children is not limited to their increasing rate of infection, but also to the fact that in a decade's time, over 40 million children are expected to be orphaned as a result of AIDS.[5]

HIV infection in children presents different features from those observed in adults, mainly due to earlier acquisition of the virus, immature immune system and other body structures.[3,6,7] Most children infected by HIV manifest the initial symptoms of AIDS before their first birthday, and oral manifestations are the first signs of the disease for nearly half of them.[8,9] The oral cavity is particularly susceptible to infection since it harbors numerous micro-organisms that thrive in conditions of immunosuppression and cause characteristic fungal, viral, bacterial and neoplastic lesions. The early recognition of oral signs associated with AIDS may facilitate therapeutic interventions aimed at reducing the clinical impact of the disease and can predict progression of HIV disease to AIDS.[10]

In addition to the overall improvement of survival and quality of life, the introduction of new antiretroviral drugs reduces the frequency of some oral manifestations in HIV-infected adult patients.[11] Certain studies that examined the presence of oral lesions in HIV- infected children have suggested a decrease in their prevalence following introduction of anti-retroviral treatment.[5,12] Studies on oral conditions in HIV infected individuals have primarily focused on their immune status.[9-11] However, information is lacking on the correlation between the use of antiretroviral therapy and salivary IgA in HIV infected children. Hence, the objective of this study was to assess influence of Anti Retroviral Therapy (ART) on Salivary Immunoglobulin A (SIgA) levels in HIV infected children, and to compare it with that of normal healthy children.

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MATERIALS AND METHOD

The study protocol was approved by the Ethical Committee of the Institutional Review Board. Information was obtained regarding the number of HIV infected children from ACCEPT Society and Desire Society, Bangalore, India. Prior to conducting the study, nature of the study was explained and written permission was obtained from authorities of these HIV centers. An undertaking was given to these authorities stating that the identity of children will not be disclosed.

The study group consisted of a cross-sectional sample of 50 HIV infected children aged 6-8 years. Their CD4 cell count on the day of saliva collection ranged from 200 to 500 cells. They were divided into two groups: Group 1 consisting of children prior to onset of anti-retroviral therapy and Group 2 consisting of children undergoing anti-retroviral therapy for more than 3 years.

Stimulated saliva (1 ml) was collected from each child in the morning at least 1 hour after breakfast. Children were instructed not to indulge in eating or drinking in the interval between breakfast and saliva collection. The initial saliva produced after 1 minute of paraffin chewing was discarded. The child then chewed for an additional 5 minutes, during which whole saliva was collected using sterile disposable vials. Saliva samples clearly contaminated with blood were discarded. The samples were transferred within 2 hours to a laboratory in ice packs at -15°C. The samples were kept at -85°C in order to prevent degradation of protein that might affect the estimation of IgA levels in the samples. Total SIgA quantification by ELISA was carried out according to Castro *et al* .[13]

In order to establish values in normal children of similar age and geographic surrounding, SIgA levels in saliva of 50 normal children, aged 6-8 years was assessed using the same methodology as described above. Both caries free children and those having dental caries with deft ≥ 4 and DMFT≥ 2 were selected. Children with long standing medical history and chronic illness were not included. Children with a drug history of prolonged intake of antibiotics or on any medications in recent two weeks were excluded. Data obtained was tabulated and subjected to statistical analysis. Student t test (two tailed, independent) was used to find significance of study parameters on a continuous scale between two groups. ANOVA was used to find the significance of study parameters on a categorical scale between three groups. Level of significance was considered at $p < 0.001$ is highly significant. Data was analyzed using Statistical Package of Social Sciences (SPSS) 18.0 for windows.

RESULTS

There was a significant difference in SIgA levels between children who were on antiretroviral drugs when compared to those who were not on ART. (Table 1) Mean SIgA levels in saliva of caries free children was 14.7 ±1.56 mg/dl, which was significantly lower than that of 17.6 ±1.52 mg/dl seen in children with dental caries. Saliva of HIV infected children had significantly lower mean SIgA levels when compared to that of normal children with dental caries. (Table 2)

Table 1: Comparison of SIgA levels in HIV infected children prior to and on ART

Groups	N	Mean ± SD(mg/dl)	p value
Group 1	25	6.2 ±1.7	0.000**
Group 2	25	8.6 ±2.9	

p<0.001 is highly significant

Table 2: Comparison of SIgA levels between HIV infected children and children with dental caries

Groups	N	Mean ± SD(mg/dl)	p-value
Group 1	25	6.2 ±1.7	0.000**
Group 2	25	8.6 ±2.9	
Children with dental caries	25	17.6 ±1.52	

p<0.001 is highly significant

DISCUSSION

Human immunodeficiency virus is a retrovirus, which on entry into the host's body, attacks and disturbs the delicate balance, thereby rendering the host susceptible to a lot of life-threatening opportunistic infections, neurological disorders, unusual malignancies and oral lesions. Major systemic findings in pediatric HIV infection include: chronic pneumonitis, recurrent bacterial infections including otitis media, persistent oral candidiasis, chronic diarrhoea, lymphadenopathy, hepatosplenomegaly and failure to thrive. Certain clinical findings are typical in the pediatric age group, such as salivary gland enlargement, pyogenic bacterial infections, developmental delay and dysmorphic craniofacial features.[14]

Most children with perinatally acquired HIV infection are treated early with Highly Active Anti Retroviral Therapy (HAART). Such therapy, consisting of a combination of 3 or more potent anti retroviral drugs, has been shown to dramatically modify the course of HIV infection in children, reducing mortality by fivefold or more and resulting in high survival rates (>90%) into adulthood. [15] HIV infection appears to have both direct and indirect effects on oral mucosal immunity, affecting both cellular and humoral immunity as well as both specific and innate immunity. [16]

The impact of HIV infection on salivary IgA levels is unclear, with reports of both decreased and increased titers.[17-21] From a humoral perpective, IgA2 subclasses are reduced in HIV infection in saliva, and total secretory IgA levels are reduced later in the progress of disease. There is now convincing evidence that salivary IgA can be neutralizing to HIV 1 and HIV 2, as well as block epithelial transmigration.[16] HIV infection results in a progressive decrease in the levels of CD4+ T lymphocytes. Since this cell has a pivotal role in the maturation of the secretory immune system, it is expected that this system would be altered in these patients.

Opportunistic infections and other related infections are uncommon in children in the HAART era compared to pre-HAART era.[22] Two mechanisms account for this finding: the immune reconstitution induced by HAART and an antifungal effect. [23] HAART results in an increase in the levels of CD4+ T lymphocytes. Protease inhibitors present in HAART the drug cocktail could interfere with secreted aspartic proteinases, hampering its proliferation and pathogenicity.[24]

It has been reported that the salivary secretory immune system maintain its responsiveness despite HIV infection.[25] Although several studies [22,26-29] have examined the presence of oral candidiasis in HIV infected children and suggested a decrease in the prevalence of these lesions after the introduction of HAART, there are only few studies in the literature examining effect of anti retroviral therapy on salivary levels of SIgA. The present study assessed the impact of anti retroviral therapy on the salivary levels of SIgA.

Saliva is an alternative biological fluid to serum and can be analyzed for diagnostic purposes. Whole saliva contains locally produced as well as serum-derived markers that have been found to be useful in the diagnosis of a variety of systemic disorders.[30] As compared with serum, the sensitivity and specificity of antibody to HIV in saliva for detection of infection are between 95% and 100%.[31] Salivary collection is a simple, non-invasive method that is not associated with risk of infection to subjects under study. SIgA synthesis rate is high and its half life is short32 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, decreasing from the highest levels in the morning to the lowest in the evening[32] In our study, total salivary IgA was quantified in whole saliva, collected in the morning after stimulation with paraffin chewing. Saliva collected in this manner would not have included contribution of non salivary IgA, arising from the circulation through gingival crevicular fluid and lesions present in the oral cavity. This is particularly relevant, since oral mucosal lesions are highly prevalent in HIV-infected children. [33,34] Sweet *et al* have reported that despite raised titers of serum IgA, HIV infected patients presented with reduced salivary IgA concentrations.[19] In contrast, HIV seropositive children presented a significantly higher mean concentration of total salivary IgA [13, 21,25] which suggested that increased salivary IgA production is most probably a response to the elevated stimulus provided by the opportunistic infections that accompany the progression of the HIV infection. In addition, it is possible that compensatory mechanisms for IgA production may be in place in these patients, such as polyclonal B-cell activation. However in our study, HIV infected children presented with low salivary IgA levels.

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 [35-40] regarding SIgA levels and dental caries have given contradictory results with reports of both an increase and decrease in caries activity associated with SIgA levels in saliva. Lower mean antibody levels have been observed in AIDS patients compared with HIV+ subjects. [13,41] In an earlier study on 150 children aged 6 to 18 years, SIgA levels were found to be low and showed a significant inverse relation oral mucosal lesions. 42

Potent antiretroviral therapy has become the standard of care for people with HIV infection. Although incomplete, considerable immune recovery occurs, sufficient, in most cases, to provide adequate protection. Suppression of viral replication after administration of potent antiretroviral therapy that includes inhibitors of the HIV-1 protease is associated with quantitative and qualitative changes in the immune system. In patients with relatively advanced disease, there is a first-phase rise (during the initial 3 months) in both naive and memory CD4+ and CD8+ T lymphocytes and B lymphocytes. This is followed by a slower second-phase increase (after 3 months) in cells primarily of the naive CD4+ and CD8+ phenotypes. These quantitative changes are associated with qualitative improvements in host immune responses, best characterized by dramatically reduced risk of opportunistic infection. Restoration of the immune system during the first year of potent antiretroviral therapy is partial at best. [43]

This was similar to our observations, where saliva of HIV-infected children who were on ART, had significantly higher SIgA levels. There is a beneficial impact of ART initiated during acute stages of HIV infection on the $CD4^+$ T cell population. Recent studies [44,45] suggest that ART may have similar positive effects if initiated during the first 90 days post-infection . With respect to duration of therapy, even a short course of ART treatment initiated during the acute stages of infection and continued for up to 24 weeks post-infection could exert beneficial effects on the longevity and breadth of $CD8^+$ and $CD4^+$ T cell responses. [46] The prevalence of orofacial lesions has been found to be lower in children on highly active anti retroviral therapy.[26,29,47]

The use of antiretroviral therapy in HIV infected children was associated with immune reconstitution, decreases in the prevalence of oral candidiasis, and a lower Candida species carriage. The decrease in exposure to Candida species was accompanied by a decrease in levels of total SIgA and Candida-specific SIgA.[48]

Salivary analysis may be used for assessing patients' condition before initiation of ART, during active treatment and thereafter. Salivary IgA levels to HIV decline as infected patients become symptomatic. It was suggested that detection of IgA antibody to HIV in saliva may, therefore, be a prognostic indicator for the progression of HIV infection.[49] It has been demonstrated that the diagnosis of HIV infection based on specific antibody in saliva is equivalent to serum in accuracy, and is therefore applicable for both clinical use and epidemiological surveillance.[50]

It is important for pediatric dentists to incorporate such simple, non-invasive diagnostic methods in their care for HIV-infected children. Implementing oral health risk assessment complemented by periodic follow-up visits based on individual risk determination can prevent opportunistic infection in these children, thus improving their quality of life.[51] It is essential to be familiar with the early oral findings and their etiological factors in order to understand the patient's dental health needs.[52,53]

The long term impact of anti-retro viral therapy on oral microbiota and consequently oral health and immune status of HIV positive children needs further investigation.

CONCLUSION

Salivary SIgA levels were significantly low in HIV infected children. Anti Retroviral Treatment (ART) appears to have a significant beneficial effect on levels of SIgA in saliva of HIV-infected children.

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