Total Antioxidant Capacity of Saliva in Children with HIV

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Several recent reports have indicated high levels of reactive oxygen species, causing oxidative stress, in the pathogenesis of HIV infection. Oxidative stress may lead to enhanced HIV replication in infected cells and may also aggravate the immunodeficiency by reduction of cellular immunity and possibly by increased programmed cell death of lymphocytes. Saliva can constitute a first line of defense against free radical mediated oxidative stress. The use of saliva as a diagnostic fluid has become somewhat of a translational research success story. Technologies are now available enabling saliva to be used to diagnose disease and predict disease progression.

Purpose:*The antioxidant capacity of saliva was investigated in 68 children who were divided into two groups. 34 children who were investigated were diagnosed as having HIV infection and the other group consisted of children who reported to the department and served as healthy controls. Total antioxidant capacity of saliva was evaluated by spectrophotometric assay.*

Conclusions:The results indicated that the total antioxidant capacity (TAC) of saliva decreased in children with HIV infection. TAC was seen to increase with the age of the children.

Keywords: *HIV; Oxidative stress; total antioxidant capacity (TAC).*

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INTRODUCTION

Oxygen is required for all mammalian energy needs. Oxygen is used to oxidize molecules rich in carbon and hydrogen (i.e., nutrients) to produce the different forms of energy needed for life. The reduction of molecular oxygen to water is accompanied by a large free energy release that can give rise to Free Radicals (FR) and/or Reactive Oxygen Species (ROS). The most important FR in biological systems are radical derivatives of oxygen. Other highly reactive compounds are known as ROS. ROS include not only oxygen FR but also non-radical oxygen derivatives involved in oxygen radical production. The reactivity and associated toxicity of these may be major contributors to the pathogenesis of several chronic degenerative diseases.[1, 2]

Nature has endowed us with protective antioxidant mechanisms against these FR and/or ROS. These antioxidants include superoxide dismutase (SOD), catalase, glutathione, glutathione peroxidases and reductase, vitamin E (tocopherols and tocotrienols), vitamin C etc., apart from many dietary components. There are epidemiological evidences correlating higher intake of components or foods with antioxidant abilities to lower incidence of various human morbidities or mortalities. Current research reveals the different potential applications of antioxidant/free radical manipulations in prevention or control of disease.[3]

Several recent reports have indicated high levels of reactive oxygen species, causing oxidative stress, in the pathogenesis of HIV infection. Oxidative stress may lead to enhanced HIV replication in infected cells and may also aggravate the immunodeficiency by reduction of cellular immunity and possibly by increased programmed cell death of lymphocytes. Moreover, reduced levels of antioxidants in the serum have been found in patients with HIV infection.[4]

In a previous study of the same authors, we had described the relationship between TAC of saliva and ECC and Rampant caries.[5] This study was done to evaluate the relationship between TAC of saliva and HIV infection.

MATERIALS AND METHOD

Sixty eight children between 4 and 14 years of age were included in the study. The study group containing thirty four children belonged to an HIV foundation. These children

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were diagnosed as having HIV infection and were on antiretroviral therapy (ART), the other thirty four were the children who reported to our department and served as healthy controls. The study group had twenty two females and twelve males and the control group had twenty females and fourteen males. In the study group informed consents were obtained from the children and also from the organization and in case of the control group the informed consents were obtained from the children and the parents. The study was also approved by the ethical committee of our institution.

A regularized diet was given to these children 24hrs before the saliva collection for investigation so as to rule out any bias in the results. Children were brought in for saliva collection 2hrs after the morning breakfast.

Unstimulated saliva was collected using the method given by Sreebny LM *et al* [6] The saliva was collected into a precalibrated collecting vessel. Immediately after the collection of saliva it was maintained at a temperature of 4ºC until it was transported to the laboratory and there it was maintained at a temperature of -80ºC until analyzed. The total antioxidant capacity of saliva was evaluated using the spectrophotometric assay.[7]

RESULTS

The children of the study and control groups were compared for the total antioxidant capacity of saliva. It was found that the antioxidant capacity of saliva was lower in the study group when compared with the controls. The mean TAC of saliva was compared and the results were statistically significant. (Table 1)

Table 1. Comparison of mean TAC of saliva levels between study and control groups

Group	N	Mean TAC (mol/l)	Std. Deviation	Sig. P Value
Study	34	29.32	16.13	0.0005 **(vhs)**
Control	34	51.55	19.45	

P> 0.05 Not Significant (ns)
P<0.05 Significant (s)
P<0.01 Highly Significant (hs)
P<0.001 Very Highly significant (vhs)

Table 2. Table for correlation
Comparison of Mean Age between Study and Control Group and Its Correlation with TAC of Saliva.

Group	N	Mean TAC(µmol/l)	Mean age (yrs)	Correlation between TAC and Age
Control	34	51.55	8.94 SD ± 3.25	
Study	34	29.32	7.11 SD ± 2.64	
Total	68	40.44	8.02 SD± 3.08	r = .773, p =0.01 (significant)

The total antioxidant capacity of saliva when observed in relation to age, it was observed that the antioxidant levels gradually increased with age both in the study and control groups and the results were statistically significant (Tables 2 and 3).

However, when the antioxidant capacity of saliva was compared between the sexes of the population included in the study, the results were not found to be statistically significant.

DISCUSSION

In the last 10 years, the use of saliva as a diagnostic fluid has become somewhat of a translational research success story. Technologies are now available enabling saliva to be used to diagnose disease and predict disease progression.[8]

Saliva has being researched as a diagnostic tool for identifying diseases such as HIV and hepatitis. Scientists have discovered a strong correlation between virus-specific antibodies for HIV present in saliva and other oral fluids. Saliva testing also reduces the risk of contact with blood.[9]

Unstimulated saliva samples was used in this study as it is preferred in determination of antioxidant defense parameters to stimulated saliva and moreover it is claimed that Total Antioxidant Capacity is higher in unstimulated saliva.[10] The total antioxidant capacity of saliva was evaluated as it is suggested that FR/ROS and antioxidant system appear to act in concert rather than alone. Investigations of individual antioxidant activity may be misleading, and the measurement of any individual antioxidant may be less representative of the whole antioxidant status. Moreover, the number of different antioxidants makes it difficult, and also expensive, to measure each of them separately.[11, 5]

The results of this study are in accordance with the studies which have used serum as their mode of evaluating levels of antioxidants in patients with HIV infection.[3] Our study showed that in the group of HIV-positive subjects, oxidative stress was significantly higher (i.e., TAC of saliva was lower in children with HIV infection) than in seronegative control subjects as determined from the TAC of saliva.

The serum antioxidant levels observed in many former studies of HIV-infected populations is low and this is hypothesized largely due to an increase in oxidative stress. Oxidative stress is defined as a disturbance in the equilib-

Table 3. Comparison of mean TAC of saliva between different age groups

Age groups (yrs)	Mean TAC Study (µmol/l)	Mean TAC Control (µmol/l)	Std. Deviation Control	Std. Deviation Study	Sig p value
4-6	21.74	31.22	6.79	6.07	Study Group .032 **(s)**
6-8	33.35	41.83	2.78	18.82	Control group 0.0005 **(vhs)**
8-10	27.08	46.50	7.03	8.27	
10-12	39.10	62.67	8.43	1.55	
12-14	47.65	80.87	6.91	30.34	
Total	29.32	51.59	19.45	16.13	

rium status of pro-oxidant/antioxidant systems of intact cells.[12] In HIV infection, oxidative stress may be caused by both overproduction of reactive oxygen intermediates (ROIs) and a simultaneous deficiency of antioxidant defenses.[13] This antioxidant deficiency in HIV population is probably due to increased utilization of antioxidant micronutrients because of increased oxidative stress rather than due to inadequate dietary intake or malabsorption.[14,15]

The immune status of the children with HIV could have been compromised sufficiently enough which already could have consumed the important antioxidants which would fight this lowered immunity. As a result of an overwhelming depression of the immunity and the inability of the antioxidants available to strike a balance with the oxidative stress produced, could result in a lowered TAC of saliva as seen in the study group.

The children included in this study were on antiretroviral therapy (ART).It has been suggested that the use of ART has significantly improved the prognosis of HIV infected patients but is associated with significant side effects such as diabetes, atherosclerosis, and cardiovascular complications. Studies have though cautiously suggested that the effects of ART drug combinations increase the oxidative stress seen in these individuals.[16] Though this needs to be proved further but could also result in lowered TAC of saliva.

Nutrition is very important and can result in elevated antioxidant levels which in turn can enable the individual to combat infections. Micronutrients like vitamins and minerals (which form an important group of dietary antioxidants), which usually are given as dietary supplements can help elate the TAC of saliva. As these children belonging to our study group were institutionalized and the importance of diet not being considered, it could be one of the reasons for a low antioxidant level. Even the quantity of micronutrients consumed which could have a linear correlation with the levels of antioxidants could be a reason for altered antioxidant levels.

The overwhelming infection itself which has compromised upon the immune status of these children compounded by the fact that even the nutrition of these children is inadequate could well explain the lowered TAC of saliva.

Though the aforementioned could be the true causes of decreased TAC of saliva, what needs to be explained or rather answered is, if antioxidant supplements can virtually increase the TAC of these individuals thereby giving their immune system a boost to fight this dreaded infection with some respect. Although no clinical trials have yet been reported on this, it is necessary that such studies be implemented.

It was also observed in the results that the TAC of saliva increased with the age respectively regardless of the presence or absence of the infection and this was similar to the findings in a previous study by the same authors.[5]

The increase in levels of TAC could be due to the levels of nutrition that can vary between the ages. Children of the younger age group could be having adequate nutrition for their age but the volume of food consumed is lesser than older children. Hence, we can assume that these younger children could be consuming lower levels of micronutrients like Vitamin A, C and E which constitute a good volume of dietary antioxidants and therefore accounting for the lower levels of antioxidants in these younger age group children.

Another plausible reason for the increasing levels of antioxidants with age may be attributed to the fact that the immune status of a child improves with age and therefore children who are older can have higher antioxidant levels when compared with the children of the younger age group. This reason may not hold very good in the study group of our population as these children already have a compromised immune status on account of the HIV infection. But this reason holds well for the control population of this study who were normal healthy children.

When the TAC of saliva was compared with the gender of the children included in this study, no statistically significant results could be seen.

Studies need to be done to evaluate if the oxidative stress of these HIV infected individuals can be improved upon by the administration of antioxidant supplements and also that if the same thing can be evaluated in saliva samples.

CONCLUSIONS

Total antioxidant capacity of saliva is reduced in children with HIV infection, and has a linear relationship with age. No significant relationship with sex was seen in this study.

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