Indian J Lepr 2015, 87 : 17-21

http://www.ijl.org.in

Original Article

Saliva as a Diagnostic Tool for Measurement of Total Antioxidant Capacity in Children with Leprosy and Born to Leprosy Parent

V Patni[1], S Baliga[2] and S Sawal[3]

Received : 31.01.2014 Revised : 11.03.2015 Accepted : 12.03.2015

The aim of the study is to assess the role of saliva as a diagnostic tool for measurement of total antioxidant capacity in children with leprosy and children born to leprosy parent. One hundred fifty children in the age group of 4-15 years were split into three equal groups: children with leprosy (CL) and children born to leprotic parents (CLP) and healthy children. Vitamin C level was measured in saliva of children spectrophotometrically at 695nm by Phosphomolybdenum method. Data were determined with student's unpaired t test and one way ANOVA. The result of the study showed that children with leprosy exhibited significantly decreased salivary total antioxidant capacity as compared to healthy controls. Antioxidant Vitamin C was higher in the Paucibacillary leprosy (PB) than those of Multibacillary type (MB) (P<0.001). As age advanced, there was a gradual increase in total antioxidant capacity in both the control and study groups and the results were highly significant statistically. Saliva is an easy medium.

Key words : Leprosy, Total antioxidant capacity, Saliva, Oxidative stress

Introduction

Leprosy, also known as Hansen's disease is caused by *Mycobacterium leprae* and is characterized by damage to the skin, peripheral nerves and the lining of the upper respiratory tract (Mcleod 1984). In 1984, World Health Organization listed India along with other countries such as Bangladesh, Brazil, Nigeria, Indonesia and Myanmar, where the disease was still endemic (Jacobson and Krahenbuhl 1990).

In different forms of leprosy, several substances with recognized antioxidant potential, such as retinol (vitamin A), α-tocopherol (vitamin E), acid ascorbic (vitamin C), zinc, magnesium and selenium have been shown to decrease (Reddy et al 2003). A decrease of antioxidant status can contribute to an increase of oxidative stress and complicate the treatment and the control of these patients (Lima et al 2007) . As a water-soluble antioxidant, vitamin C is in a unique position to

[1] V Patni, MDS Senior Lecturer, Department of Pedodontics & Preventive Dentistry, RR Dental College, Udaipur
[2] S Baliga, MDS, Professor & Head, Department of Pedodontics & Preventive Dentistry, Datta Meghe Institute of Medical Sciences, Sawangi Meghe, Wardha (MS)
[3] S Sawal, MDS, Senior Lecturer, Department of Pedodontics & Preventive Dentistry, Saraswati Dhanwantari Dental College & Hospital, Parbhani
Correspondence to: V Patni **Email:** patni.vaibhav@gmail.com

"scavenge" aqueous peroxyl radicals before these destructive substances have a chance to damage the lipids. It works along with vitamin E, a fat-soluble antioxidant and the enzyme glutathione peroxidase to stop free radical chain reactions. Thus, measurement of Vitamin C levels in association with the determination of the antioxidant status can constitute an important tool in prognosis, treatment, and control of leprosy patients.

Blood serum is used in numerous diagnostic tests, as well as in blood typing and is presently a standard in patients suffering from leprosy. Collection of serum has been an invasive procedure where as saliva, an easily available fluid and by its non-invasive nature of collection has made it a more suitable fluid than blood for diagnostic tests.

Hence, in the present study saliva was analyzed to assess its role as a diagnostic tool for measurement of total antioxidant capacity (TAC) in children with leprosy and child born to leprosy parents.

Materials and Methodology

After approval from the ethical committee [DMIMS (DU)/IEC/2012-13/1055], 150 children between 4 and 15 years of age included in the study were divided into three groups of 50 each. Group I: Children suffering from leprosy. Group II: Child born to leprosy parents and Group III: Healthy children. Children who were reported case of leprosy or were still under treatment and child born to leprosy parent but are healthy were included in the study. Informed written consent was taken from parent/guardian accompanying the child patient. Children with any other systemic diseases were excluded from the study.

Sample Collection

Two milliliter of unstimulated saliva sample was collected by asking the patient to sit in Coachman's position, head titled downwards and saliva was allowed to accumulate in the mouth for 2 minutes. The saliva sample was collected aseptically in sterile receiving vessels and stored in glass vials at a temp of 4 degree Celsius, and analysis was carried out within 3-6 hrs from collection.

Analytical Procedure

The antioxidant activity of the saliva was evaluated by the phosphomolybdenum method (Prieto et al 1999). The assay was based on the reduction of molybdenum (Mo VI) - (Mo V) by the reducing agents like antioxidants and subsequent formation of green phosphate complex at acidic pH. A 100µL of clear supernatant solution of the sample (saliva) was combined with 1ml of reagent (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tube was capped and incubated in water bath at 95 ºC for 90 minutes. Following this, the absorbance of the solution was measured at 695 nm using a spectrophotometer (SHIMADZU UV-VIS) against blank after cooling to room temperature. 100µl of distilled water was used as the blank. The antioxidant activity is expressed as the number of gram equivalents of ascorbic acid. All the data obtained were subjected to statistical evaluation using student's unpaired *t* test and one way ANOVA.

Results

The children of the control group and the study groups were compared for the total antioxidant capacity of saliva. It was found that the antioxidant capacity of saliva was lower in the study groups when compared with the control group. The antioxidant capacity of saliva was lower in children with leprosy when compared with child born to leprotic parents. The mean Total antioxidant capacity of saliva was compared and the results were statistically very highly

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

Group	N	Mean	Standard Deviation	p-value
Children with Leprosy	50	4.71	2.50	0.000 (vhs)
Child born to leprotic parents	50	12.28	2.56	
Control Group	50	21.65	8.116	

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 : Comparison of mean antioxidant capacity in Paucibacillary and Multibacillary leprosy

Group	N	Mean	Standard Deviation	p-value
PB	30	13.29	2.29	0.021 (vhs)
MB	20	11.61	2.54	

Table 3 : Comparison of mean antioxidant between different age groups

Age Group	N	Mean	Standard Deviation	F-value	p-value
Upto 8 yrs	55	10.07	7.06	4.96	0.008 (vhs)
8 to 10 yrs	42	14.00	6.70		
>10 yrs	53	14.90	10.61		

significant (Table 1). A closer look at the two disease type revealed that the antioxidant Vitamin C was higher in the Paucibacillary leprosy (PB) than those of Multibacillary type (MB) (P<0.001) (Table 2). The total antioxidant capacity of saliva when observed in relation to age showed a gradual increase in both the control and study groups and the results were statistically very highly significant (Table 3).

Discussion

Leprosy is a debilitating disease and is considered important mainly because of its potential to cause permanent and progressive physical deformities with serious social and economic implications. Of the various mechanisms that influence the pathogenesis of leprosy, oxidative stress caused by reactive oxygen species (ROS) plays an important role (Vijayaraghavan et al 2005). Oxidative stress, an expression used to describe various deleterious processes resulting from an imbalance between free radical generating and scavenging systems can lead to metabolic impairment and cell death (Jyothi et al 2008).

In the present study, primarily unstimulated saliva was preferred over stimulated saliva, as it is claimed that total antioxidant capacity is higher in unstimulated saliva (Pereslegina 1989). Secondly, Free radical/Reactive oxygen species and antioxidant system appear to act in concert rather than alone. Hence, the total antioxidant capacity of saliva was evaluated as investigating individual antioxidants is expensive, difficult, misleading and may be of less representative of the whole antioxidant status. The result of this study is in

accordance with the studies which have used serum as medium of evaluating level of antioxidants in patients with leprosy (Lima et al 2007). In the present study, TAC was also found to be low in children suffering from leprosy as compared to child born to leprosy parents. The primary reasons for the lower antioxidant capacity might be because of inter-relationship between poor nutritional supply, compromised immunity and low economic status.

Adequate nutritional supply can result in elevated antioxidant levels which in turn can enable the individual to combat infections. Dietary supplements like micronutrients (vitamins and minerals) which form an important group of dietary antioxidants can help elate the TAC of saliva. Children belonging to our study group were institutionalized, belonged to low socio-economic status and hence the importance of diet not being considered by them could be one of the reasons for the low antioxidant level. 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. This might be because of the difference in the level of nutritional supply at different ages. The volume of food consumed by children of younger age group is lesser than older children. Hence, we can assume that these younger children could be consuming lower level of micronutrients like Vitamin A, C &E which constitute a good volume of dietary antioxidants and therefore accounting for lower level of antioxidants in this group. Another reason for the increase levels of antioxidants with age may be attributed to the fact that as the age advances the immune status of the child improves (Padmanabhan et al 2010).

The children included in this study were on multiple drug therapy (MDT) and there is an overwhelming evidence to prove that dapsone, one of the MDT drugs for leprosy is strongly oxidative and induces hemolysis. This increases free radicals and subsequently reduces the oxidants (Kelly et al 1984, Lardo et al 1997).

Comparison between the two disease types revealed that oxidative stress was found to be increased in MB types, which is in accordance with the study by Jyothi et al (2008), who reported a similar finding in serum of the leprosy patient. Hooper et al 2000 had shown that PB leprosy patients have higher immunity when compared with the MB group. This could be as a result of the low bacterial load of the causative organism M.*leprae*. Also, PB patients are endowed with the ability to eliminate bacteria through cell mediated immunity, next to resistant normal controls (Kelly et al 1984, Hooper at al 2000).

An important factor which needs to be studied is the inter-relationship of diet and leprosy. As leprosy is known to occur in group of individuals with low socio-economic status, shortage of balanced diet might play an important role in worsening the immune response of these patients. Henceforth when ever implementing a program to study leprosy patients, diet should be considered as an important parameter.

Due to the smaller sample size, a definite opinion regarding oxidative stress in children suffering from leprosy and child born to leprosy parents could not be formed. Detailed studies with more cases are required to evaluate if saliva could be used as a diagnostic tool for the assessment of oxidative stress.

Conclusion

From the study we derived the following:

1. Saliva can serve as a diagnostic tool to estimate antioxidant capacity.
2. TAC is decreased in children suffering from leprosy.
3. TAC of saliva has a linear relation with age.

Recommendation

The introduction of diet rich in antioxidant by various vitamins and mineral supplements could be beneficial attenuating the toxic actions caused by ROS and propitiating an adjusted treatment and recovery improving the quality of life of these patients.

Acknowledgement

The authors are grateful to the Department of Biochemistry at Institute of Pharmaceutical Education and Research Centre, Borgaon Meghe, Wardha, Civil hospital - Wardha and Anandwan Ashram - Warora for the assistance given in all phases of this research.

References

1. Hooper DC, Scott GS, Zborek A et al (2000). Uric Acid, a peroxynitrite scavenger inhibits CNS inflammation, blood - CNS barrier permeability changes and tissue damage in a mouse model of multiple sclerosis. *FASEB J*. **14**: 691-698.
2. Jacobson RR and Krahenbuhl JL (1990). Leprosy. *Lancet*. **20**: 655-660.
3. Jyothi P, Riyaz N, Nandakumar G et al (2008). A study of oxidative stress in paucibacillary and multibacillary leprosy. *Indian J Dermatol Venereol Leprol*. **74**: 80.
4. Kelly JW, Scott J, Sawland M et al (1984). Vitamin E and Dapson Induced Hamolysis. *Arch. Dermatol.* **120**: 1582-1584.
5. Lardo MM, Diaz NB, Artaza JR et al (1997). Vitamin E as a Protective Agent against Hamolysis in Leprosy Patients under Dapsone Treatment. *J Medicina (B Aires)*. **57**: 150-154.
6. Lima ES, Roland IA, Maroja MF et al (2007). Vitamin A and lipid peroxidation in patients with different forms of leprosy. *Rev Inst Med Trop S Paulo*. **49**: 211-214.
7. Macleod J (1984). Leprosy In: Davidson's Principle and Practice of Medicine. Longman Group LTD, pp758-762.
8. Padmanabhan V, Rai K, Hegde AM et al (2010). Total Antioxidant Capacity of Saliva in Children with HIV. *J Clin Pedia Dentistry*. **34**: 347-350.
9. Pereslegina IA (1989). The activity of antioxidant enzymes in the saliva of normal children. *Lab Delo*. **11**: 20-23.
10. Prieto P, Pineda M, Aguilar M (1999). Spectrophotometric quantitation of antioxidant capacity through the formation of a Phosphomolybdenum Complex: Specific application to the determination of vitamin E. *Ana Biochem.* **269**: 337-341.
11. Reddy YN, Murthy SV, Krishna DR et al (2003). Oxidative stress and anti-oxidant status in leprosy patients. *Indian J Lepr*. **75**: 307-316.
12. Vijayaraghavan R, Suribabu CS, Sekar B et al. (2005). Protective role of vitamin E on the oxidative stress in Hansen's disease (Leprosy) patients. *Eur J Clin Nutr*. **59**: 1121-1128.

How to cite this article : Patni V, Baliga S and Sawal S (2015). Saliva as a diagnostic tool for measurement of total antioxidant capacity in children with leprosy and born to leprosy parent. *Indian J Lepr.* **87** : 17-21.