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RESEARCH ARTICLE

IMPACT OF HUMAN IMMUNE DEFICIENCY INFECTION ON ORAL MICROFLORA

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ABSTRACT

Aim: To compare levels of *Streptococus mutans* in saliva samples of HIV infected individuals and non-HIV-infected control individuals.
Materials and Methods: Subjects were selected from those attending the Department of microbiology of a tertiary care center
A total of 100 individuals, 50 HIV-seropositive individuals and 50 HIV-seronegative control individuals, men and women, ages 15 yrs and older were selected for the study and divided into 2 groups-Group 1- 50 HIV-seropositive individuals, Group 2- 50 HIV-seronegative control individuals. All the saliva samples were collected using Spitting method. After collection all the saliva samples were cultured using mitis salivarius bacitracin (MSB) agar which is a selective media for the isolation of Streptococcus mutans. Obtained values were analysed using the Mann-Whitney U, Wilcoxon W and Z-tests.
Results: Results showed that *S. mutans* levels were higher in HIV-infected individuals than in the non-HIV-infected control individuals ($p = 0.000$).
Conclusion: It can be suggested that HIV infection accelerates colonization of S. mutans bacteria.

INTRODUCTION

According to various reports, WHO-2010/UNAIDS-2011, an estimated 34 million people worldwide were suffering from AIDS/HIV. Due to improved medical facilities e.g. highly active anti-retroviral therapy (HAART), survival rates of HIV patients have increased drastically, especially during last one decade leading to increased number of people living with HIV virus (Chan *et al*., 2012). It has been demonstrated in various studies that HIV infected individuals showed increased prevalence of dental caries and enlargement and hypofunction of salivary glands, increased incidence of Kaposis sarcoma, non-Hodgkins lymphoma and oral hairy leukoplakia (Mulligan *et al*., 2004; Navazesh *et al*., 2009 and Ramos *et al*., 1999). Investigators have suggested that increased HIV viral loads lead to decreased CD4+ T-lymphocytes, decreased salivary flow, that can lead to increased prevalence of dental caries and other oral diseases (Beena, 2011 and Phelan *et al*., 2004). Out of all pathogenic micro-organisms found in oral micro-flora Streptococcus mutans is considered as the principal pathogen behind dental caries development (De Carvalho Duailibe *et al*., 2007). This study was aimed to compare levels of *S. mutans* in saliva samples of HIV infected individuals and non-HIV-infected control individuals.

MATERIALS AND METHODS

Study subjects were selected from those attending the Department of microbiology of a tertiary care teaching hospital after informed consent in their own language. Permission was obtained from the Medical superintendent to conduct the study. A total of 100 individuals, 50 HIV-seropositive individuals and 50 HIV-seronegative control individuals, men and women, ages 15 yrs and older were selected for the study and divided into 2 groups-

Group 1- 50 HIV-seropositive individuals
Group 2- 50 HIV-seronegative control individuals

Inclusion criteria

- All HIV-infected subjects who were HAART-negative or had been off therapy for at least 6 months and enrolled shortly before the initiation of HAART.

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Exclusion criteria

- Individuals who were pregnant or taking any antimicrobials were excluded from the study.

Data for this study were based on 2 visits, which included two visits within 2 wks for assessment of the variability and reliability of all assays. No changes were made in their oral hygiene and dietary habits.

Standardisation of the saliva collection technique

- Saliva samples were collected from the subjects 1-2 hours after having the breakfast.
- The subject were instructed not to perform any physical exercise prior to collection of saliva (Patil *et al*., 2010).
- After participants chewed a piece of paraffin for 30 sec, whole stimulated saliva samples were collected using Spitting method. After collection all the saliva samples were cultured using mitis salivarius bacitracin (MSB) agar which is a selective media for the isolation of Streptococcus mutans.

Microbiological analysis

A 1-mL quantity of the whole saliva samples were immediately transported in a refrigerated recipient to the laboratory. Saliva samples were vortexed and serially diluted in 10-fold steps in 0.05M phosphate buffer. Aliquots of 100 ul of the appropriate dilutions were cultured into mitis salivarius bacitracin (MSB) agar for the selective isolation and enumeration of S. mutans. The MSB agar contained pancreatic digest of casein, proteose peptone, dextrose, saccharose 20%, dipotassium phosphate, trypan blue, crystal blue, agar, Chapman tellurite, and bacitracin 0.2 U/ml. The MSB agar plates were incubated anaerobically (H2:CO2:N2 10:10:80) for two days at 37^0C. Colony counts with a morphology typical of S. mutans were made on MSB agar. Microbial counts were expressed as colony-forming units (cfu) per ml of unstimulated saliva. Colonies on the MSB agar plates were visualized by Gram's stain and subjected to the specific tests according to the standard guidelines (Gambola *et al*., 2004).

Statistical Analysis

Obtained values were analysed using the Mann-Whitney U, Wilcoxon W and Z-tests. The level of significance was accepted at $P<0.05$.

Hypothesis

Null's hypothesis: No difference in S. mutans concentration in HIV infected individuals and non-HIV-infected control individuals.

Alternate hypothesis: There is difference in S. mutans concentration in HIV infected individuals and non-HIV-infected control individuals.

RESULTS

S. mutans mean levels were higher in the HIV+ participants (68940.00) than in the HIV– participants (29068.00) (Table 1).

Difference in S. mutans levels among HIV infected individuals and non-HIV-infected control individuals was found to be statistically significant (p= 0.000) (Table 2).

Table 1. Comparison of *S. mutans* Colonization

		N	Mean	Std. Deviation
CFU*	HIV_Positive	50	68940.00	16150.959
	Control	50	29068.00	19744.329
	Total	100	49004.00	26898.297
Log_CFU	HIV_Positive	50	4.825563	.1100964
	Control	50	4.355691	.3265228
	Total	100	4.590627	.3384106

*CFU- Colony forming units

Table 2. Showing "*P*" value

	CFU	Log_CFU
Mann-Whitney U	181.000	181.000
Wilcoxon W	1456.000	1456.000
Z	-7.370	-7.370
P value	.000	.000

DISCUSSION

According to some studies, it is proposed that decrease in CD4+ T-lymphocytes count and subsequent immunosuppression could lead to compromised immunity against oral micro-organisms, leading to increased concentration of cariogenic bacteriae like S. mutans, resulting in increased prevalence of dental caries and other HIV associated pathological conditions (Aas *et al*., 2007 and Saxena *et al*., 2012). Another study co-related decreased immune response in HIV patients to decreased CD8+ counts (Gulzar and Copeland, 2004). Present study also revealed findings in accordance to the above mentioned studies. Our study revealed that mean S. mutans concentration was more in HIV infected individuals. Also, the difference between S. mutans concentration was found to be statistically significant in HIV infected and non-HIV-infected control individuals (p = 0.000). We did not consider dental caries score of the study subjects, gender and age based differentiation of study subjects was not performed, these are the potential limitations of the present study.

Conclusion

Our study showed increased concentration of S. mutans among HIV infected individuals which proves null's hypothesis wrong. Within the limitations of our study we suggest that-

- HIV infection accelerates colonization of S. mutans bacteria.
- Increased concentration of S. mutans could be contributed to immunodeficiency caused by HIV infection.

Additional studies are required to understand the correlation between the colonization of cariogenic microbes, including *S. mutans,* and the status of immunosuppression at the advanced stages of HIV infection.

REFERENCES

Aas, J.A., Barbuto, S.M., Alpagot, T., Olsen, I., Dewhirst, F.E. and Paster, B.J. Subgingival plaque microbiota in HIV positive patients. *J. Clin. Periodontol.,* 2007. 34:189-195.

Beena, J.P. Prevalence of dental caries and its correlation with the immunologic profile in HIV-infected children on antiretroviral therapy. *Eur. J. Paediatr Dent.,* 2011. 12:87-90.

Chan, S., Sidibé, S. and Lake, A. GLOBAL HIV/AIDS RESPONSE - Epidemic update and health sector progress towards Universal Access - Progress Report 2011. [Internet] Malta: WHO, UNAIDS, UNICEF; [updated 2011 November 30; cited 2012 Mars 18]. Available from: http:// www.who.int/hiv/pub/progress_report2011/en/index.html

De Carvalho Duailibe, S. A., Gonçalves, A. G. and Ahid, F. J. M. Effect of a propolis extract on *streptococcus Mutans* counts *in vivo*. *J. Appl. Oral Sci*., 2007. 15(5):420-3.

Gambola, F., Estupinan, M. and Galindo, A. Presence of streptococcus mutans in saliva and its relationship with dental caries: Antimicrobial susceptibility of the isolates. Universitas Scientiarum 2004. 9: 23-27.

Gulzar, N. and Copeland, K.F. CD8+ T-cells: function and response to HIV infection. *Curr. HIV Res.,* 2004. 2:23-37. http://whqlibdo*C*.who.int/publications/2011/9789241502986_eng.pdf

Mulligan, R., Phelan, J.A., Brunelle, J., Redford. M., Pogoda, J.M. and Nelson, E. *et al*. Baseline characteristics of participants in the oral health component of the Women's Interagency HIV Study. *Community Dent Oral Epidemiol,* 2004. 32:86-98.

Navazesh, M., Mulligan, R., Karim, R., Mack, W.J., Ram, S. and Seirawan, H. *et al*. Effect of HAART on salivary gland function in the Women's Interagency HIV Study (WIHS). *Oral Dis.,* 2009. 15:52-60.

Patil, S., Venkataraghavan, K., Anantharaj, A. and Patil, S. Comparison of two commercially available toothpastes on the salivary streptococcus mutans count in urban preschool children- an in vivo study. *International dentistry SA,* 2010; 12(4): 72-82.

Phelan, J.A., Mulligan, R., Nelson, E., Brunelle, J., Alves, M.E. and Navazesh, M. *et al*. Dental caries in HIV-seropositive women. *J Dent Res., 2004.* 83:869-873.

Ramos-Gomez, F.J., Flaitz, C., Catapano, P., Murray, P., Milnes, A.R. and Dorenbaum, A., Collarobative Workgroup on Oral Manifestations of Pediatric HIV infection, Oral AIDS Center, University of California. Classification, diagnostic criteria, and treatment recommendations for orofacial manifestations in HIV-infected pediatric patients. *J Clin Padiatr Dent*., 1999. 23(2):85-96.

Saxena, D., Li, Y., Yang, L., Pei, Z., Poles, M. and Abrams, W.R. *et al*. Human microbiome and HIV/AIDS. *Curr HIV/AIDS Rep.,* 2012. 9:44-51.

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