

Correlation of Oral Manifestations with Circulating CD4+ T Lymphocytes in Patients with HIV/AIDS in Indian Subpopulation

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ABSTRACT

Background and objectives: Oral mucosal lesions have been described in HIV infected individuals since the beginning of the epidemic. Occurrence of these oral manifestations has been associated with lower CD4+ cell counts. The present study was undertaken to assess the relevance of using oral manifestations as markers of immune deterioration by correlating the oral manifestations to the levels of circulating CD4+ T lymphocyte counts in HIV/AIDS patients.

Study design: A complete oral examination was carried out for 96 HIV-infected individuals whose CD4+ counts were available within 1 month of oral examination. The CD4+ counts were estimated using a FACSCount machine. The data collected was subjected for statistical analysis using student t-test and Chi-square test.

Results: The prevalence of oral manifestations was 57.3%, i.e. amongst the 96 patients 55 had oral manifestations. The mean CD4+ count when any oral manifestation present was 158 ± 187 . The mean CD4+ count of patients in whom there were no oral manifestations was 397 ± 256 .

Conclusion: Oral manifestations of HIV are associated with lower CD4+ count and can be used as clinical markers of immune deterioration.

Keywords: Oral manifestations of HIV, CD4+ count.

INTRODUCTION

The human immunodeficiency virus (HIV) infection and acquired immunodeficiency syndrome (AIDS) represent an unprecedented epidemic form of immunodeficiency involving defects of the T lymphocyte arm of the immune system.¹

The rate of progression of HIV disease that varies substantially among different patients is indicated by certain clinical and laboratory markers. CD4+ count has long been used by AIDS clinicians to track HIV disease progression and is still the best surrogate marker for immune function. Several research groups have noted that when the CD4+ count begins to diminish below 400 cells/mm³, oral lesions develop in the compromised host and when the CD4+ count goes below 200 cells/mm³, frequently concurrent, multiple oral lesions develop that suggest progressive immune deterioration.²⁻⁸ As the development of these oral lesions has been found to be highly correlated with immunosuppression, the oral lesions could also be used as markers of immunosuppression.

MATERIALS AND METHODS

The study was conducted in the Department of Oral Medicine and Radiology, Government Dental College, Bengaluru and

Seva Clinic, run by Samraksha, a nongovernmental organization at Shivaji Nagar, Bengaluru. The study group was composed of 96 HIV-positive men and women in the age group of 18 to 70 years, whose CD4+ counts were available within 1 month of oral examination. Of the 96 patients, 85 patients had not received antiretroviral therapy (ART) and 11 of them had received ART for not more than 1 month. All the patients signed a written informed consent form and consent from the treating physician was also obtained. The protocol received approval from the Institutional Ethical Committee, Samraksha, Bengaluru and Ethical Committee of Government Dental College, Bengaluru.

Information regarding demographic features, high-risk behaviors and the history of contracting HIV infection and duration was obtained from the patients. The medical records were reviewed for the information about current and previous HIV-associated opportunistic infections or malignancies, laboratory data inclusive of CD4+ count and treatment received for opportunistic infections.

CD4+ T LYMPHOCYTE COUNTS

The CD4 counts within 1 month of oral examination were recorded from the patient's medical records. The CD4 counts

were done at NIMHANS (National Institute of Mental Health and Neurosciences), Bengaluru at the Department of Neurovirology, using a nonlysis method on a fluorescent activated cell sort count (FACSCount) flow cytometer (Becton Dickinson, USA). This machine uses single platform and a dual fluorescent dye-based flow cytometric analysis to enumerate CD4 cells. Before every run of the assay the FACSCount machine was calibrated using high, medium, low and zero control beads provided by the manufacturer in order to ensure linearity and accuracy in the readings. In addition, a blood sample obtained from a healthy volunteer was also tested every day as quality control measure to establish the proper working of the FACSCount.

CLINICAL ORAL EXAMINATION

Oral examination was carried out by one calibrated examiner in a conventional dental chair with artificial illumination using a sterile mouth mirror and probe. Oral lesions were diagnosed clinically, according to the presumptive diagnostic criteria established by the European Community Clearinghouse on oral problems related to HIV infection in August 1990 and September 1992.⁹

Statistical Analysis

The collected data was analyzed by the student t-test and Chi-square test using SPSS software (Version 10.1) package. p-value less than 0.05 was taken to be statistically significant.

RESULTS

The demographic data of our study population revealed that maximum number of patients belonged to the age group of 30 to 39 years, the mean age being 32.53 ± 6.62 years. Maximum numbers of the patients were males (59/96, 61.5%) as compared to females (37/96, 38.5%) (Table 1). It was observed that the mean age for male patients was higher 34.17 ± 6.43 than for females 29.92 ± 6.13 .

Table 1: Age and gender distribution of the study population

Age group (in years)	Male (%)	Female (%)	Total
20-29	13 (22.0)	20 (54.1)	33 (34.4)
30-39	37 (62.7)	14 (37.8)	51 (53.1)
40-49	9 (15.3)	3 (8.1)	12 (12.5)
Total	59 (100.0)	37 (100.0)	96 (100.0)

With regard to the immune status 49% of the patients had CD4+ count of <200, 34.4% had CD4+ between 200 to 500 and 16.6% had >500 CD4+ counts (Table 2).

The prevalence of oral manifestations was 57.3%, i.e. among the 96 patients 55 had oral manifestations. The total number of oral manifestations observed in this study was 73. 43/55, 78.2% of patients had single oral manifestation, 12/55, 21.8% of patients had more than one oral manifestation.

Table 2: Distribution of the population among CD4+ count groups

CD4+ count	No. of patients (%)
<200	47 (49.0)
200-500	33 (34.4)
>500	16 (16.6)

Table 3: Mean CD4+ counts for presence/absence of oral manifestations

Oral manifestation	No. of patients	Mean CD4+ count \pm SD	p-value	Inference
Present	73	158 \pm 187	0.001	Significant
Absent	41	397 \pm 256		

The mean CD4+ count when any oral manifestation present was 158 ± 187 . The mean CD4+ count of patients in whom there were no oral manifestations was 397 ± 256 which was significantly higher ($p < 0.05$) than the mean CD4+ count of patients with oral manifestations (Table 3).

The frequency with which particular oral manifestations were noted in the study population and mean CD4+ counts of particular oral manifestations are given in Table 4. Forty-two patients had candidiasis with mean CD4+ count of 139 ± 274 . Eight patients had periodontal involvement with CD4+ count of 96 ± 56 .

Among the 73 oral manifestations 55 belonged to the strongly associated category of 1993 European Community Clearinghouse on oral problems related to HIV (75.3%), 15 belonged to the less commonly associated category (20.6%), three belonged to the lesion seen category (4.1%) (Table 5). Among the 55 patients having strongly associated lesion 36, 65.5% had CD4+ count of less than 200 cells/mm³, 15, 27.3% had CD4+ count of 200 to 500 cells/mm³ and 4, 7.3% had CD4+ count of more than 500 cells/mm³. Of 15 patients having less commonly associated lesion, 12, i.e. 80% belong to the category of less than 200 CD4+ count with only three, i.e. 20% belonging to the category of 200 to 500 CD4+ counts. All the three patients having oral manifestations belonging to the category of lesions seen had a CD4+ count of less than 200 cells/mm³. It can also be observed that more number of oral manifestations had a CD4+ count of less than 200 cells/mm³.

DISCUSSION

Since the first reports of HIV infection in 1981, the importance and frequency of the associated oral manifestations have been recognized.¹⁰⁻¹² It is generally agreed that the recognition of some oral manifestations of HIV disease is of great significance because they may represent the first sign of the disease and they have been shown to be highly predictive markers of severe immune suppression and disease progression.^{4,5} But most of the studies in this regard have been from the developed countries. Very few reports are from the Indian subcontinent. Hence this study was undertaken to correlate the oral manifestations of HIV/AIDS to the levels of circulating CD4+ T lymphocytes, so that the oral manifestations can be used as markers of immune suppression, where resources are limited

Table 4: Frequency and mean CD4+ count of particular oral manifestations

Oral manifestations	Frequency	%	Mean CD4+ count	SD
Pseudomembranous candidiasis	26	35.6	120	120
Erythematous candidiasis	10	13.7	213	137
Angular cheilitis	6	8.2	98	114
Linear gingival erythema	2	2.7	100	2
Necrotizing ulcerative periodontitis	6	8.2	95	67
Herpes zoster	4	5.5	787	151
Herpes simplex	1	1.4	241	
Ulceration (Nos)	9	12.3	103	120
Aphthous (Minor)	2	2.7	125	10
Hyperpigmentation	6	8.2	58	28
Depigmentation	1	1.4	15	

Table 5: ECC categories of oral manifestations and CD4+ counts

Oral manifestation	N (%)	Mean CD4+ count ± SD	CD4+ count			Total
			<200	200-500	>500	
Strongly associated	55 (75.3)	182 ± 205	36 (65.5)	15 (27.3)	4 (7.3)	55 (100.0)
Less commonly associated	15 (20.6)	79 ± 97	12 (80.0)	3 (20.0)	–	15 (100.0)
Lesion seen	3 (4.1)	118 ± 14	3 (100.0)	–	–	3 (100.0)
Total	73 (100)	158 ± 187	51(69.9)	18 (24.7)	4 (5.4)	73 (100.0)

and the costs of tests like CD4+ counts are not afforded by majority of patient population.

Most of the patients in our study group belonged to the age group of 30 to 39 years. The male to female ratio was 1.6: 1. The mean age of our study population was 32.5 years. Similar age and gender incidence was observed by K Ranganathan, M Umadevi et al¹³ in their study of 100 HIV-positive patients in South India. The male to female ratio was 3.1:1 and mean age of patients found in their study was 34.5 years.

Fifty-five of the 96 patients in our study population had oral manifestations, i.e. the prevalence rate was observed to be 57.3%. Several investigators have reported the prevalence of oral lesions to range from 40 to 70%. Roberto Barone MD et al¹⁴ observed a prevalence rate of 41% among 217 patients. Ramirez-Amador V et al¹⁵ reported a higher prevalence of 75% whereas Aguirre-Urizar JM¹⁶ observed oral lesions in all but two patients (99.5%) in a Spanish population.

These substantial differences in the prevalence of HIV-related oral lesions may be explained by many factors.

1. As reported by Ira B Lamster et al¹⁷ lifestyle, access to healthcare and the condition of the oral cavity before infection influence the development of oral lesions in persons with HIV infection.
2. Recruitment of an appropriate study population is a crucial point in any study design. Different populations, clinical settings used for evaluation of clinical stage, distribution of risk groups, race and socioeconomic status are the major confounding factors.
3. Other factors that may have an influence are the examiner’s clinical experience and use of different diagnostic criteria.

Because the entry criteria in our study population did not select or exclude patients on the basis of either lesion status or CD4+ T cell count, we believe that estimates presented herein

are largely unbiased. However, the conditions of the oral cavity and habits like smoking and tobacco chewing, socioeconomic status have not been considered in our study.

Oral manifestations have been reported to appear more frequently below a CD4+ count of 200 cells/mm³. This result is expected because the association between progression of HIV disease and CD4+ depletion is well-established. Accordingly, in our study also the mean CD4+ count was less, i.e. 158 when any oral lesion was present and compared to the mean CD4+ count of 397 when no oral lesions were reported. Similar observations were reported in a study population of 606 patients by Lauren L Patton and Chapel Hill of North Carolina.⁸ The mean CD4 count for any lesion present was 243 as compared to 416 when lesion absent in homosexual men and 332 as compared to 411 among intravenous drug users.¹⁷

In our study 69.9% of the oral manifestations occurred in patients with a CD4+ count of <200 cells/mm³. Among the 24.7% of oral manifestations occurred with patients having a CD4+ count of 200 to 500 cells/mm³. Only 5.4% of oral manifestations were seen in patients having a CD4+ count of >500 cells/mm³. Anwar R Tappuni et al¹⁸ showed that subjects with a CD4+ count of <200 cells/mm³ and viral load >3000 copies/ml, whether the patients were on ART or not were significantly more likely to experience oral manifestations of HIV than the other study subjects with CD4+ count of >200 cells/mm³. In a study conducted by G Sharma et al¹⁹ four or more concurrent oral lesions were statistically significant with low CD4+ counts <200 cells/mm³.

The oral manifestations found in our study were categorized according to 1992 EC Clearinghouse—WHO classification system.⁹ Strongly associated lesions (SA): Oral candidiasis, HIV-associated periodontal conditions. Less commonly associated lesions (LCA): Herpes simplex, herpes zoster and ulceration NOS. Lesion seen (LS): Aphthous ulcers and hyperpigmentation.

The cases of oral hairy leukoplakia, Kaposi's sarcoma and Non-Hodgkin's lymphoma, HIV-associated salivary gland diseases were not observed in our study population. This could be attributed to smaller sample size of our study and may be related to the higher heterosexual transmission category of HIV infection as compared to the homosexual transmission categories prevalent in other study groups.

The frequency of SA lesions was 75.3% with a mean CD4+ count of 182 cells/mm³ that of LCA and LS were 20.5%, 4% and 79 and 118 cells/mm³ respectively. Margiotta et al⁷ in a cohort of 104 Italian subjects infected with HIV found that having one or more lesions strongly associated with HIV infection was just at the level of significance for having a CD4+ count of <200 cells/mm³. In our study the mean CD4+ counts of LCA and LS category of oral manifestations are lower when compared to the mean CD4+ count of SA lesions whereas in their study the mean CD4+ counts of SA lesions was lowest. But frequency of occurrence of SA, LCA and LS oral manifestations was similar.

In our study, the most frequent lesion was oral candidiasis with a frequency of 57% with pseudomembranous subtype being the most frequent. This finding is most consistent with other clinical studies where pseudomembranous and erythematous candidiasis were predominantly present.^{2,14,20} The mean CD4 count of 139 in patients with oral candidiasis of our study is similar to the mean CD4 count of 149 cells/mm³ as reported by Michel Glick et al.³ The mean CD4+ count of patients with pseudomembranous candidiasis and erythematous candidiasis was 120 and 213 respectively. As reported by Schiodt and Pindborg² from Copenhagen, the mean CD4+ count of patients with pseudomembranous and erythematous candidiasis were 220 and 320 respectively. Angular cheilitis was present in 8.2% of our study population and the mean CD4+ count of patients with angular cheilitis was 98. The frequency observed is similar to that seen in other series, Laskaris et al²¹ (11%) and Moniaci et al²² (13.8%). A higher prevalence (25.25%) was noted by Aguirre-Urizar in a Spanish population.¹⁶

The prevalence of HIV-associated periodontal conditions in our study population was 10.9%. The prevalence of periodontitis in HIV-infected patients as reported in the literature varies between 5 and 17%. We observed two cases of linear gingival erythema and all the necrotizing ulcerative lesions had involved the bone. Necrosis confined to gingiva was not found in our study. The prevalence rate of necrotizing ulcerative periodontitis (NUP) was 8.2% and the mean CD4+ count of patients with NUP was 95. Michel Glick and Brian C Muzyka²³ have investigated the association between NUP diagnosis and CD4 count <200 cells/mm³. The prevalence of NUP as reported by them was 6.3% and the mean CD4 count was 51 cells/mm³. It was suggested by them that NUP should be considered to be included as a clinical marker for staging of HIV disease and AIDS.

Atypical presentation of ulcers was noted in nine patients (12.3%). On biopsy the histological features were those of

nonspecific ulcers and cultures failed to identify specific etiologic agents. The mean CD4+ count in these patients was 103 cells/mm³. The reported prevalence of these ulcers in the literature ranges from 1.1 to 12%.²⁴

A prevalence of 5.1% and CD4+ cell count of <100/mm³ for herpes simplex virus has been reported by Michel Glick et al³ however, only one patient had herpes simplex with a CD4+ count of 241 cells/mm³.

A frequency of occurrence of 5.5% was noted with herpes zoster in our study. Multidermatomal involvement and a higher rate of complications have been reported in the literature. Of four patients one had extensive osteonecrosis of the maxilla with a CD4+ count of 926 cells/mm³.

Altered pigmentation of the skin, nails and mucous membranes has been observed in HIV-infected patients. In our study populations 6 (8.2%) patients had hyperpigmentation and depigmentation was noted in a single patient. A prevalence of 38% for hyperpigmentation has been reported by Bravo IM et al²⁵ in Venezuelan population. Drugs like clofazamine, ketoconazole, pyrimethamine and zidovudine, adrenocortical destruction, inflammation caused by immunologic mediators or infectious agents have been considered as possible etiologic agents.

CONCLUSION

Our study serves once more to show that most oral lesions associated with HIV infection increase in frequency as the CD4+ counts decrease. The prevalence becomes particularly important when the count falls below 200 cells/mm³. Though CD4+ count is the principal laboratory marker, comparisons with other laboratory parameters like viral load, p24 antigen, β 2 microglobulin, etc. are recommended in future.

In conclusion, oral manifestations are highly predictive markers of severe immune deterioration. Oral examinations are an essential component for early recognition of disease progression and comprehensive evaluation of HIV-infected patients.

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