

# Relationships between CD4+ Counts and the Presence of Oral Lesions in Human Immunodeficiency Virus Positive Women in Nigeria

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## Abstract

**Background:** Oral lesions are common findings in human immunodeficiency virus (HIV) infection. The main factor associated with the development of oral lesions is damage to the immune system, specifically loss of CD4+ lymphocytes, which are involved in cell-mediated immunity. **Aim:** This study was aimed to determine the association of oral lesions in HIV/acquired immune deficiency syndrome women patients with the level of immune suppression as measured by the CD4+ counts. **Subjects and Methods:** This was a prospective cross-sectional study with a study population of 191 consecutive female patients seen at the University of Benin Teaching Hospital, Nigeria. Ethical clearance was obtained from the institution of study and informed consent was given by every participant. HIV sero-status was determined for all patients. CD4+ count was analyzed for both the HIV+ and HIV- women with oral lesions. The relationships between oral lesions and CD4+ cell count were investigated. **Result:** About 56.0% (107/191) of the 191 women studied were HIV positive. Age range for the HIV positive women was 18-50 years with a mean age (standard deviation) of 36 (9.2) years. The most common oral lesion observed in the HIV positive women was pseudomembranous candidiasis accounting for 34.6% (37/107). About 68.4% (67/98) of the oral lesions occurred at CD4+ count < 200 cells/ml. Chi-square revealed statistically significant association between the presence of oral lesions and CD4+ count in HIV infected women ( $P = 0.03$ ). **Conclusion:** As the CD4+ count was decreasing the presence of oral lesions was increasing in the study. The presence of pseudomembranous candidiasis was found to be significantly associated with CD4+ count level < 200 cells/ml. This association of oral candidiasis with CD4+ cell counts could be used as additional markers of immunosuppression and progression of HIV infection, particularly in a developing country like Nigeria where CD4+ count cannot be determined routinely.

**Keywords:** CD4+ counts, Human immunodeficiency virus/Acquired immune deficiency syndrome positive women, Oral lesions

## Introduction

The most common oral lesions associated with human immunodeficiency virus/acquired immune deficiency syndrome (HIV/AIDS), according to Greenspan and Greenspan,<sup>[1]</sup> are

candidiasis, hairy leukoplakia, herpetic gingivostomatitis, aphthous ulceration, necrotizing gingivitis, pigmented macules, Kaposi's sarcoma and periodontal diseases. Disease progression is characterized by increased prevalence of oral candidiasis, oral hairy leukoplakia, ulcerative periodontal disease and xerostomia.<sup>[2]</sup>

These oral lesions may cause considerable pain, discomfort, inability to swallow, difficulty in eating and may compromise facial appearance. This may lead to malnourishment, emaciation and stigmatization. Early management of these oral lesions will improve the overall quality of life of the infected individuals. Oral health workers are the most appropriate

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personnel to diagnose and manage the majority of these oral manifestations and are encouraged to do so.<sup>[3,4]</sup> Oral health is an integral component of general health. The inclusion of oral examination is a vital component of an individual's overall health assessment. The oral cavity is among the most biologically dynamic structures of the human body. Any adverse change resulting from immune-suppression predisposes to oral infections.<sup>[5]</sup>

Studies carried out within and outside Nigeria revealed oral candidiasis as the most common oral lesion seen in HIV infected women, with the pseudomembranous variant being the most common.<sup>[6-9]</sup> There are about 40 known oral manifestations of AIDS according to the classification by the European Economic Community.<sup>[10,11]</sup> Apart from their diagnostic importance; oral manifestations may be of prognostic importance for the subsequent development of AIDS. They can also serve as clinical correlates with CD4+ counts.<sup>[12,13]</sup>

CD4+ T-cells are the primary target of HIV; their depletion severely limits the host response capacity.<sup>[14]</sup> The ability of the immune system to mount a specific response against HIV is a key factor in the subsequent disease course.<sup>[15]</sup> Eventually, when a significant number of CD4+ lymphocytes have been destroyed and when production of new CD4+ cells cannot match destruction, then failure of the immune system leads to the appearance of clinical AIDS.<sup>[15]</sup>

The rate of CD4+ lymphocyte destruction correlates with plasma HIV level. A study carried out in our environment revealed that the normal CD4+ count in healthy women ranged between 511 and 920 cells/ml.<sup>[16]</sup> At CD4+ count of 200/ $\mu$ l oral lesions are present.<sup>[17]</sup> Little new information has been presented during the last couple of years of the relationship between oral lesions and CD4+ count in Nigeria.

The main factor associated with the development of oral lesions and especially oral candidiasis, is the CD4+ count.<sup>[18]</sup> Laboratory parameters will only partially reflect disease stage and progression. The addition of clinical markers more accurately reflects the overall disease status of the patient.<sup>[19]</sup> Though, the measurement of CD4+ counts requires a blood sample and laboratory analysis, the identification of oral lesions can be made during the course of physical examination. Thus, the aim of this study was to determine the association of oral lesions in HIV/AIDS patients with level of immune suppression as measured by the CD4+ count, in order to use the oral lesions as reference for assessing the CD4+ counts due to the cost implication of running such laboratory test routinely in a developing country like Nigeria.

## Subjects and Methods

This was a prospective cross-sectional study. The study population consisted of 191 women among whom 107 were HIV+ women attending the HIV/AIDS clinic, University of

Benin Teaching Hospital (UBTH). Selection of subjects was based on recently diagnosed HIV positive women attending the HIV/AIDS Clinic (PEPFAR), within the age range of 18-50, who consented to participate in the study and had not commenced antiretroviral therapy. HIV women patients who were not willing to participate in the study and/or had commenced anti-retroviral therapy were excluded from the study. These cases were compared with another cohort of 84 HIV negative women attending Oral diagnosis and Oral medicine clinics, of the same hospital, within the same age group and environment as the HIV patients. The study was carried out between the periods January and March, 2011. The presence of oral lesions and the relationship between oral lesions and CD4+ cell count was investigated. Ethical clearance was obtained from the institution of study and informed consent was given by every participant.

A Questionnaire was used to collect bio data, duration of HIV infection and the type of oral lesions present. The questionnaire was both self-administered and interviewer-administered in English language or via an interpreter of local language of the respondent. A pre-test of the questionnaire was done on HIV infected patients at the HIV/AIDS clinic, UBTH, in order to check for glitches in wording of questions, lack of clarity of instructions and the most efficient way of recording presence or absence of oral lesions in order to achieve the objectives of the study. No patient was on drug therapy for HIV as at the time of study.

A dentist trained in oral medicine with 8 years of experience which prior to conducting the examinations under the study was trained using the diagnostic criteria, systematically examined the patients taking into consideration the site and type of oral lesions present using the criteria established by the European Community Clearing house/World Health Organization, 1993 on oral problems related to HIV. Where multiple lesions were seen (in the same patient) at the time of clinical evaluation, each lesion was considered independently for analysis. The cohort (HIV) women patients were seen at the Oral diagnosis and Oral Medicine clinic, which is a specialist clinic that deals with oral healthcare of medically compromised patients and diagnosis and treatment of oral manifestations of systemic diseases such as diabetes, leukemia, auto-immune diseases and effect of drugs in the oral cavity. These conditions could account for more opportunistic infections in the oral cavity. All the patients seen were screened for HIV using the double enzyme-linked immunosorbent assay method and confirmatory testing was carried out using the Western blot technique for those that were HIV+.

5 ml of blood was drawn from the antecubital vein of both the HIV+ and HIV- women using a sterile 5 ml disposable syringe and needle in a labeled specimen bottle containing the anticoagulant ethylenediaminetetraacetic acid. The samples were analyzed for CD4+ count level by the flow cytometric method at the Federal Government of Nigeria HIV laboratory located in the hospital. CD4+ cell counts  $\geq$  500/ml was classified as "marginally

immunodeficient”, CD4+ cell count of > 200 to < 500/ml as “mildly immunodeficient” and CD4+ cell count of ≤ 200/ml as “severely immunodeficient”<sup>[20]</sup>. Other systemic conditions that could lead to low CD4+ count such as tuberculosis, auto-immune diseases and use of immunosuppressive medications were considered as co-morbidities for CD4+ evaluation.

Data obtained from the study were analyzed using the Statistical Package for Social Sciences (SPSS; version 16.0, Chicago, USA). The statistical variables and data analyzed were the bio data of the recruited population, the types of oral lesions present, the level of CD4+ count and its relationship with oral lesions. Where necessary Chi-square, Student's *t*-test or analysis of variance was performed for the variables, with confidence level set at 95% and *P* < 0.05 considered to be significant.

## Results

Of the 191 women studied 56.0% (107/191) were HIV positive while 44.0% (84/191) were HIV negative. The age range for both the HIV+ and HIV- women was 18-50 years with a mean age of 36 (9.2) years for the HIV+ women and 32 (7.9) years for the HIV- women. Table 1 shows *P* values for the relationship between HIV+/HIV- for the various oral conditions studied. The overall prevalence of HIV-related oral lesions in this population was 57.0% (61/107). 25 (23.4%) patients had multiple lesions. The most common oral lesion identified in the HIV+ women was pseudomembranous candidiasis accounting for 34.6% (37/107); this was followed by melanotic pigmentation and xerostomia, each with a prevalence of 10.3% (11/107) respectively. The overall prevalence of oral lesions in the HIV- women patients was 52.4% (44/84). The most common oral lesion identified in the HIV- women was periodontal disease which accounted for 13.1% (11/84). Others were pseudomembranous candidiasis accounting for 11.9% (10/84) and 9.5% (8/84) had recurrent aphthous ulcer [Table 1].

The mean CD4+ count (cells/ml) of HIV+ women was 247.44 (203.2), while the immunologic profile at presentation for the HIV- women showed a mean CD4+ count (cells/ml) of 1020.54 (500.9). The distribution of oral lesions based on the CD4+ count of the HIV infected patients showed that 62.6% (67/98) of the oral lesions occurred at CD4+ count < 200 cells/ml; about 24.5% (24/98) oral lesions were seen at CD4+ count of 200-499 cells/ml, whereas 7.1% (7/98) cases of oral lesions were seen at CD4+ count > 500 cells/ml (*P* = 0.03). The distribution of oral lesions based on CD4+ count shows that 93.2% (41/44) of the oral lesions seen in HIV negative women

occurred at CD4+ cell count of 500 cells/ml and above. There was no statistical significant relationship between the presence of oral lesions and CD4+ count in HIV negative women (*P* = 0.70) [Table 2].

Table 3 shows the prevalence of the specific oral lesions and their relationship with the CD4+ count in HIV positive subjects and the absolute CD4+ counts mean for individual oral manifestations. Among the oral lesions seen in these subjects, the presence of pseudomembranous candidiasis was statistically significant in relation to the level of CD4+ count (*P* = 0.01) with a mean CD4+ count of 156.1 (172.5) cells/ml.

Multivariate logistic regression used to derive predictive odd ratio between the CD4+ count values which had been ranked in the form of categorical variables as shown in Table 4 revealed a significant association between CD4+ count of < 100 cells/ml

**Table 1: Prevalence of oral lesions seen in both HIV positive women and HIV negative women**

| Parameters   | HIV positive women (107)              |                | HIV negative women (84) | P value |
|--------------|---------------------------------------|----------------|-------------------------|---------|
| Oral lesions | Type of oral lesions                  | Prevalence (%) |                         |         |
|              | Patient with at least one oral lesion | 61 (57.0)      | 44 (52.4)               | 0.42    |
|              | Erythematous candidiasis              | 5 (4.7)        | 5 (6.0)                 | 0.69    |
|              | Pseudomembranous candidiasis          | 37 (34.6)      | 10 (11.9)               | 0.01    |
|              | Angular cheilitis                     | 7 (6.5)        | 6 (7.1)                 | 0.87    |
|              | Periodontal diseases                  | 10 (9.3)       | 11 (13.1)               | 0.43    |
|              | Hairy leukoplakia                     | 1 (0.9)        | 0 (0.0)                 | 0.37    |
|              | Kaposi's sarcoma                      | 1 (0.9)        | 0 (0.0)                 | 0.37    |
|              | Cervical lymphadenopathy              | 2 (1.9)        | 0 (0.0)                 | 0.21    |
|              | Melanotic hyperpigmentation           | 11 (10.3)      | 3 (3.6)                 | 0.08    |
|              | Xerostomia                            | 11 (10.3)      | 2 (2.4)                 | 0.03    |
|              | Salivary gland swelling               | 2 (1.9)        | 0 (0.0)                 | 0.21    |
|              | Thrombocytopenic purpura              | 1 (0.9)        | 0 (0.0)                 | 0.37    |
|              | Ulceration NOS                        | 3 (2.8)        | 1 (1.2)                 | 0.44    |
|              | Recurrent aphthous ulcer              | 4 (3.7)        | 8 (9.5)                 | 0.10    |
|              | Facial palsy                          | 1 (0.9)        | 0 (0.0)                 | 0.37    |
|              | Herpes zoster                         | 2 (1.9)        | 0 (0.0)                 | 0.21    |

HIV: Human immunodeficiency virus, NOS: Not otherwise specific

**Table 2: Distribution of oral lesions based on CD4+ count in the study population**

| HIV sero-status | Number of oral lesions based on CD4+ count/ml |      |         |      | Total number of lesions | $\chi^2$ | P value |
|-----------------|---|------|---------|------|-------------------------|----------|---------|
|                 | Mean CD4 (SD)                                 | <200 | 200-499 | >500 |                         |          |         |
| Positive        | 247.44 (202.2)                                | 67   | 24      | 7    | 98                      | 10.295   | 0.03    |
| Negative        | 1020.54 (500.9)                               | 0    | 3       | 41   | 44                      | 0.714    | 0.70    |

SD: Standard deviation, HIV: Human immunodeficiency virus

**Table 3: Prevalence of specific oral lesions and their relationship with CD4+ count in HIV positive subjects**

| Oral lesions                         | Number of oral lesions based on CD4+ count/ml (%) n (%) |           |          | Mean CD4+ count (SD) | P value |
|--------------------------------------|---|-----------|----------|----------------------|---------|
|                                      | <200  | 200-499   | >500     |                      |         |
| Erythematous candidiasis             | 3 (60.0)  | 1 (20.0)  | 1 (20.0) | 200.0 (194.8)        | 0.29    |
| Pseudomembranous candidiasis         | 27 (73.0)   | 8 (21.6)  | 2 (5.4)  | 156.1 (172.5)        | 0.01    |
| Angular cheilitis                    | 5 (71.4)  | 2 (28.6)  | 0 (0.0)  | 137.9 (152.6)        | 0.48    |
| Necrotizing ulcerative gingivitis    | 1 (100.0)   | 0 (0.0)   | 0 (0.0)  | 179.0 (0.0)          | 0.64    |
| Necrotizing ulcerative periodontitis | 1 (50.0)  | 1 (50.0)  | 0 (0.0)  | 169.0 (0.0)          | 0.16    |
| Linear gingival erythema             | 5 (62.5)  | 2 (25.0)  | 1 (12.5) | 182.1 (154.8)        | 0.83    |
| Hairy leukoplakia                    | 0 (0.0)   | 1 (100.0) | 0 (0.0)  | 258.0 (0.0)          | 0.38    |
| Kaposi's sarcoma                     | 1 (100.0)   | 0 (0.0)   | 0 (0.0)  | 148.0 (0.0)          | 0.64    |
| Cervical lymphadenopathy             | 2 (100.0)   | 0 (0.0)   | 0 (0.0)  | 360 (199.4)          | 0.43    |
| Melanotic hyperpigmentation          | 5 (45.5)  | 5 (45.5)  | 1 (9.1)  | 228.1 (145.5)        | 0.72    |
| Xerostomia                           | 7 (63.6)  | 3 (27.3)  | 1 (9.1)  | 201.0 (172.9)        | 0.77    |
| Salivary gland swelling              | 2 (100.0)   | 0 (0.0)   | 0 (0.0)  | 173.0 (0.0)          | 0.43    |
| Thrombocytopenic purpura             | 1 (100.0)   | 0 (0.0)   | 0 (0.0)  | 164.0 (0.0)          | 0.64    |
| Ulceration NOS                       | 3 (100.0)   | 0 (0.0)   | 0 (0.0)  | 36.0 (28.6)          | 0.26    |
| Herpes zoster                        | 1 (50.0)  | 0 (0.0)   | 1 (50.0) | 345.0 (220.6)        | 0.21    |
| Facial palsy                         | 1 (100.0)   | 0 (0.0)   | 0 (0.0)  | 28.0 (0.0)           | 0.64    |
| Recurrent aphthous ulcer             | 3 (75.0)  | 1 (25.0)  | 0 (0.0)  | 135.8 (94.4)         | 0.61    |

SD: Standard deviation, HIV: Human immunodeficiency virus, NOS: Not otherwise specific

**Table 4: Logistic regression table showing predictive odds of CD4 count values for oral candidiasis**

|                | B      | SE    | P value | OR   |
|----------------|--------|-------|---------|------|
| CD4 count <100 | -1.222 | 0.559 | 0.03    | 3.39 |
| CD4 count <200 | -0.012 | 0.696 | 0.99    | 2.36 |
| CD4 count <300 | -0.260 | 0.887 | 0.77    | 1.30 |
| CD4 count <400 | -0.857 | 1.033 | 0.41    | 1.01 |
| CD4 >400       | 0.000  | 1.087 | 1.000   | 1.00 |

SE: Standard error, OR: Odds ratio

and oral candidiasis ( $P = 0.03$ ). Adjusted OR for developing oral candidiasis at < 100 cells/ml was found to be 3.39. Adjusted OR for patients with CD4+ count > 400 cells/ml was found to be 1.00 revealing no added risk.

## Discussion

Oral lesions are common findings in HIV infection. The main factor associated with the development of oral lesions is damage to the immune system, specifically loss of CD4+ lymphocytes, which are involved in cell-mediated immunity.<sup>[18]</sup> Earlier studies have reported that HIV patients with a CD4+ count of < 200 cells/ml had more oral lesions.<sup>[12,18,21]</sup> A similar finding was recorded in this study in which 68.4% of the total HIV-related oral lesions were in the group of patients with CD4+ count of < 200 cells/ml.

The prevalence of 57.0% of HIV related-oral lesion as shown in this study, is in keeping with Campisi *et al.*,<sup>[7]</sup> which reported a prevalence of 56.5% in HIV+ Italian women, but higher than the 43.9% reported among HIV+ Nigerian women by an earlier study.<sup>[9]</sup> These discrepancies might be as a result of

other demographic and/or clinical factors. The prevalence of 52.4% oral lesions in HIV negative women reported in this study compared with earlier report of 57.7%.<sup>[22]</sup>

The inverse relationship between the CD4+ counts and the prevalence of oral mucosal lesions in HIV infected patients has been previously reported.<sup>[1,21,23,24]</sup> Similarly, this study observed a significant inverse relationship between CD4+ counts and the prevalence of oral lesions in HIV infected patients. These findings indicate that the occurrence of oral lesions in HIV patients could be a useful guide in determining a reduction in the immunological status of HIV patients. This agrees with reports that CD4+ depletion was strongly associated with a high level of viral load.<sup>[18]</sup> Therefore, clinicians and researchers are advocating oral lesions as a useful tool for the diagnosis and detection of the progression of HIV infection.<sup>[18,24]</sup>

The main factor associated with the development of oral opportunistic lesions is the CD4+ count.<sup>[18]</sup> The onset of oral candidiasis and oral hairy leukoplakia is heralded by a sustained reduction in the CD4+ blood cell count associated with a sharp increase in viral load.<sup>[25]</sup> An earlier study<sup>[23]</sup> observed that oral lesions found among a cohort of 737 persons in Italy infected with HIV were significantly associated with CD4+ count of < 300 cells/ml. In a study done on a population of 43 subjects in Greece,<sup>[26]</sup> oral hairy leukoplakia was found to be associated with CD4+ counts < 200 cells/ml. Analysis of oral lesions in 81 HIV-positive subjects and 31 HIV-negative subjects and their CD4+ counts in a study done at Oyo, Nigeria has shown that CD4+ counts < 500 cells/ml were significantly associated



with having pseudomembranous candidiasis and angular cheilitis.<sup>[27]</sup> Another study,<sup>[21]</sup> reported oral candidiasis and melanotic hyperpigmentation could be used as markers of immunosuppression depicted by CD4+ counts < 200 cells/ml while oral hairy leukoplakia could indicate HIV-ribonucleic acid  $\geq 20,000$  copies/ml in an adult Nigerian population. Similarly, this study revealed that the presence of oral candidiasis and hairy leukoplakia were associated with CD4+ counts < 200 cells/ml. Both oral candidiasis and oral hairy leukoplakia have been accepted to be of value in staging and classification schemes for HIV disease.<sup>[2,12,18]</sup> In addition, they are also used as clinical correlates of CD4+ count.<sup>[2]</sup> However, a study<sup>[28]</sup> reported that HIV-related oral lesions may not be diagnostic of immunodeficiency, as some patients may have these lesions at high CD4+ counts while others do not have them despite low CD4+ counts. Nevertheless, a study conducted on sero-positive Hemophiliacs has shown that advanced stage of immune suppression and presence of oral lesions were significantly associated ( $P = 0.04$ ).<sup>[29]</sup>

Thus far, CD4+ cell count is recognized and widely used as a marker for HIV-related disease progression. Accordingly, the Centers for Disease Control and Prevention has proposed a revised classification system and AIDS case surveillance definition that incorporates both clinical signs and symptoms as well as one laboratory marker, CD4+ cell count.<sup>[30]</sup>

## Conclusion

As the CD4+ count was decreasing the presence of oral lesions was increasing in the study.

The presence of pseudomembranous candidiasis was found to be significantly associated with CD4+ count level < 200 cells/ml in this study. This association of oral candidiasis with CD4+ cell counts could be used as additional markers of immunosuppression and progression of HIV infection, particularly in a developing country like Nigeria where CD4+ count cannot be determined routinely.

However, the study limitation was the unavailability of detailed bio data and past medical history of patients.

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