Relationship between CD4$^+$ count, serum inflammatory cytokines, and oral melanotic hyperpigmentation in newly diagnosed HIV-seropositive patients: a nested case-control study

Elizabeth Oluwatoyin Abe, BDS, MDS, FWACS,[a] Akinyele Olumuyiwa Adisa, BDS, FMCDS,[a] Bukola Folasade Adeyemi, BDS, MSc, FWACS,[a] Olutosin Alaba Awolude, MBBS, MSc, FWACS,[b] and Foluso John Owotade, BChD, FWACS, PhD[c]

Objective. Oral melanotic hyperpigmentation (OMH) in patients with human immunodeficiency virus (HIV) infection has been attributed to the use of antifungal or antiretroviral drugs, as well as HIV-induced cytokine dysregulation. This research aimed to determine the relationship between immunosuppression and cytokine dysregulation in newly diagnosed HIV-seropositive subjects with OMH.

Study Design. The study was conducted among newly diagnosed HIV-seropositive patients at the Infectious Disease Clinic, Ibadan, Nigeria. The cases were patients with OMH matched for age and sex with control subjects without OMH. CD4$^+$ count and cytokine levels (interleukin-6 and tumor necrosis factor-α) were compared between the cases and control subjects. SPSS version 21 software was used for data analysis.

Results. Seventy newly diagnosed HIV-seropositive patients were studied, which comprised of 35 cases and 35 control subjects. The median CD4$^+$ counts for cases and control subjects were 174 cells/mm^3 (interquartile range [IQR], 57-250) and 324 cells/mm^3 (IQR, 107-424), respectively. Severe immunosuppression (CD4$^+$ count, $\leq$200 cells/mm^3) was found in over half of the study participants, being more prevalent among the cases than among the control group (P = .019). Serum cytokine levels did not significantly vary between the cases and control subjects.

Conclusions. There was a significant association between HIV-OMH and severe immunosuppression in the newly diagnosed HIV-seropositive patients. (Oral Surg Oral Med Oral Pathol Oral Radiol 2022;133:182−188)

Oral lesions in human immunodeficiency virus (HIV) infection have been well documented and are of utmost significance in early disease detection as markers of disease progression, disease staging, and determinants for commencement of antiretroviral therapy.[1] Oral health is an important component of overall health status in HIV infection and an essential component of quality of life. Oral lesions are among the early signs of HIV infection, and for individuals with unknown HIV status, they may indicate the need for HIV screening.[2] For patients diagnosed with HIV who are not yet receiving highly active antiretroviral therapy (HAART), the presence of certain oral manifestations may predict progression to acquired immunodeficiency syndrome (AIDS). Furthermore, the presence of certain oral lesions in patients receiving HAART may serve as surrogate markers for the efficacy of antiretroviral therapy. The current classification of the oral manifestations of HIV infection[2,3] groups them into three categories: (1) lesions strongly associated with HIV infection, (2) lesions less commonly associated with HIV infection, and (3) lesions seen in HIV infection. HIV-associated oral melanotic hyperpigmentation (OMH) has been categorized as less commonly associated with HIV infection. It affects any part of the oral mucosa and usually is asymptomatic, single or multiple, well or ill defined, light to dark brown macules of variable sizes and shapes.[4] OMH in patients with HIV has been attributed to the use of antifungal agents or antiretroviral drugs, whereas some have reported HIV-induced cytokine dysregulation causing activation of melanogenesis pathway.[4,5]

HIV infection is characterized by chronic immune activation and is associated with an increase of proinflammatory cytokines.[6] Some authors have documented that patients with HIV infection receiving HAART who

Statement of Clinical Relevance

Oral melanocytic hyperpigmentation has been significantly associated with immune suppression in people newly diagnosed with human immunodeficiency virus (HIV), it could therefore be used in predicting HIV infection among high-risk groups such as commercial sex workers and injection drug users.

[a]Department of Oral Pathology/Oral Medicine, University College Hospital, Ibadan, Nigeria.
[b]Department of Obstetrics and Gynaecology, University College Hospital, Ibadan, Nigeria.
[c]Department of Oral Pathology/Oral Medicine, Obafemi Awolowo University Teaching Hospital Complex, Ile-Ife, Osun State, Nigeria.
Corresponding author: Elizabeth Oluwatoyin Abe, BDS, MDS, FWACS, Department of Oral Pathology/Oral Medicine, University College Hospital, Queen Elizabeth Road, Agodi-Gate, Ibadan, Oyo 000002, Nigeria E-mail address: eoabe83@yahoo.co.uk
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cease therapy exhibit plasma cytokine and chemokine changes that provide a signature of monocyte/macrophage activation, particularly increased production of interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α).[7] It is possible that HIV-induced upregulation of IL-1, IL-6, and TNF-α promotes the production of α-melanocyte-stimulating hormone (α-MSH) by oral keratinocytes and melanocytes, thus inducing an upregulation of the expression of melanocortin 1 receptor with increased production of melanin, resulting in the development of HIV-associated OMH.[8]

OMH has been suggested as a marker of immune suppression and is associated with low $CD4^+$ T cell count in HIV-seropositive individuals.[9] Also, nail pigmentation and oral pigmentation have both been associated with low $CD4^+$ count.[10,11] The prevalence of HIV-associated OMH varies in different parts of the world and between different ethnic and racial groups. This variation is most probably due to genetic, environmental, biochemical, pathologic, or immunologic factors.[9] A systematic review of 20 Asian studies on oral manifestations of HIV/AIDS reported an overall prevalence of OMH of 22.8%.[12] In West Africa, studies from Nigeria and Ghana reported OMH as the second and third most common oral lesion, respectively, among their newly diagnosed HIV population groups.[13,14] Furthermore, a cross-sectional study done in South West Nigeria revealed the prevalence of HIV-associated OMH to be 14.4% among both HAART-naive and HAART-experienced patients.[15] A cross-sectional study was done among HAART-naive patients and patients receiving HAART in northern Nigeria to assess the relationship between the $CD4^+$ T cell count and the serum levels of IL-6 and IL-10. The authors reported higher serum concentrations of both IL-6 and IL-10 among patients with lower $CD4^+$ T cell counts and those with advanced World Health Organization (WHO) clinical stage of disease, thus conferring higher IL levels in the HAART-naive patients than in HIV-positive participants receiving HAART.[16] Considering the paucity of literature on the pathologic significance of HIV-OMH, we aimed to determine the relationship between immunosuppression ($CD4^+$) and cytokine dysregulation in newly diagnosed HIV-seropositive subjects with OMH.

METHODS

This was a nested case-control study conducted among newly diagnosed HIV-seropositive patients attending the HIV outpatient clinic of the Infectious Diseases Institute of the College of Medicine, University of Ibadan (UI)/University College Hospital, Ibadan, Nigeria. Ethical approval was obtained from UI/ University College Hospital ethics committee (UI/EC/17/0437). The patients were yet to commence HAART and were not receiving any medication implicated in OMH. Sample size was determined using the formula $N = [(1 + r)/r][p \times q(Z\alpha + Z\beta)^2)]/d^2$. Using the prevalence value from a South African study,[4] an approximate of 35 patients per group was calculated. Considering the ratio of cases to control subjects used for the sample size calculation (r = 1:1), 35 patients were enrolled as cases and 35 patients as control subjects. The cases were patients with OMH matched for age and sex with control subjects without OMH. Consecutive consenting patients with OMH among the newly diagnosed HIV-seropositive patients were recruited into the study until the calculated sample size was obtained. The control subjects were newly diagnosed HIV-seropositive patients without OMH who consented to participate in the study. They were age and sex matched with those having HIV-OMH in the ratio of 1:1. The study participants were recruited over a period of 6 months from August 2018 to February 2019. Each study participant was subsequently seen in follow-up for 3 months for the post-HAART initiation assessment. This lasted until May 2019.

The clinical details (HAART regimen, WHO staging, and $CD4^+$ count) of the study participants were accessed from the database of the clinic. Data collection was done using an interviewer-administered questionnaire. Information obtained were sociodemographic characteristics, social habits (alcohol intake and smoking), and other comorbidities. Oral examination was carried out by an oral medicine specialist using normal clinical examination procedures. Clinical diagnosis of HIV-related oral lesions was made according to the criteria proposed by the European Community Clearinghouse (Table I). The participants who had symptomatic oral lesions were referred to the tertiary dental clinic, which is in collaboration with the HIV clinic, for appropriate management. Clinical oral photographs were acquired with a digital camera to demonstrate OMH lesions at presentation (i.e., before commencement of antiretroviral therapy) (Figure 1). Serum levels of IL-6 and TNF-α were determined by using enzyme-linked immunosorbent assay kits (Qiagen, Valencia, CA). Clinical features, $CD4^+$ count, and cytokine levels (IL-6 and TNF-α) were compared between the cases and control groups.

SPSS version 21 was used for data analysis. Quantitative variables were summarized using the median, mean, and standard deviation, whereas qualitative variables were expressed as frequency and percent. $CD4^+$ counts were categorized according to WHO immunologic classification of HIV. The categories include none/not significant, mild, advanced, and severe with their respective $CD4^+$ counts of $\geq$500, 350-499, 200-349, and <200 cells/mm^3. Conditional logistic regression analysis was used to further analyze the relationship between matched cases and control groups using

Table I. Classification of the oral manifestations of HIV disease in adults by European Community Clearinghouse criteria

Group 1: Lesions strongly associated with HIV infection
- Oral candidiasis: erythematous, pseudomembranous
- Oral hairy leukoplakia
- Periodontal disease: linear gingival erythema, necrotizing ulcerative gingivitis, necrotizing ulcerative periodontitis
- Non-Hodgkin's lymphoma
- Kaposi sarcoma

Group 2: Lesions less commonly associated with HIV infection
- Melanotic hyperpigmentation
- Bacterial infections: *Mycobacterium tuberculosis, Mycobacterium avium- intracellulare*
- Necrotizing ulcerative stomatitis
- Salivary gland diseases
- Viral infections: herpes simplex virus, human papillomavirus lesions (*Condyloma acuminatum*, focal epithelial hyperplasia, *Verruca vulgaris*), varicella zoster virus
- Ulceration (not otherwise specified)
- Thrombocytopenic purpura

Group 3: Lesions seen in HIV infection
- Bacterial infections: *Actinomyces israelii, Escherichia coli, Klebsiella pneumoniae*
- Cat scratch disease
- Neurological disturbances: facial nerve palsy, trigeminal neuralgia
- Fungal infections other than *Candida: Aspergillus flavus, Histoplasma capsulatum, Cryptococcus neoformans*
- Viral infections: cytomegalovirus, molluscum contagiosum
- Drug reactions: erythema multiforme, toxic epidermolysis, lichenoid reaction, ulcerative
- Epithelioid (bacillary) angiomatosis

their CD4 count and serum cytokine levels. The level of significance at 5% was used.

RESULTS

The ages of the study participants ranged from 22 to 68 years with a mean of 42.4 ± 10.7 years. The majority of the study participants were in the fourth and fifth decades of life (Table II).

The tongue (75.5%) was the most common site affected, whereas multiple sites of involvement were seen in 9 (25.7%) cases. Eighteen (51.4%) had HIV-OMH as localized pigmented macules, and 22 (62.9%) of the pigmentation cases were categorized as being light in intensity (Table III). About half of the study participants were categorized as WHO stage 3, with a ratio of 2:1 between cases and control subjects ($P = .009$) (Figure 2).

Among all the study participants, the $CD4^+$ count ranged between 5 and 1,612 cells/mm^3 with a mean value of 247.73 ± 252.91cells/mm^3, whereas the median value was 192 cells/mm^3 (interquartile range

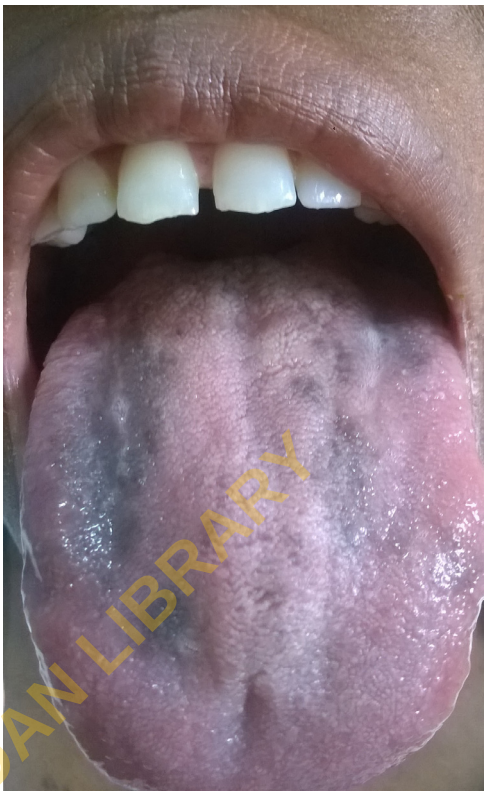

Fig. 1. A 44-year-old newly diagnosed female HIV-seropositive patient with multiple maculopapular darkly pigmented lesions and whitish coating on the dorsum of the tongue.

Table II. Sociodemographic profile of the study participants

Category	*Group*		*Total N (%)*
	Cases, n (%)	*Controls, n (%)*	
Sex			
Male	5 (14.3)	5 (14.3)	10 (14.3)
Female	30 (85.7)	30 (85.7)	60 (85.7)
Age group, y			
21-30	5 (14.3)	6 (17.1)	11 (15.7)
31-40	11 (31.4)	10 (28.6)	21 (30.0)
41-50	11 (31.4)	13 (37.1)	24 (34.3)
51-70	8 (22.9)	6 (17.1)	14 (20.0)
Marital status			
Single	4 (11.8)	3 (8.6)	7 (10.1)
Married	20 (58.8)	18 (51.4)	38 (55.1)
Divorced/separated	3 (8.8)	9 (25.7)	12 (17.4)
Widow	7 (20.6)	5 (14.3)	12 (17.4)
Education			
Primary	8 (29.6)	6 (17.1)	14 (22.6)
Secondary	14 (51.9)	19 (54.3)	33 (53.2)
Tertiary	4 (14.8)	7 (20.0)	11 (17.7)
Nil	1 (3.7)	3 (8.6)	4 (6.5)
Occupation			
Trader	17 (48.6)	19 (54.3)	36 (51.4)
Civil servant	6 (17.1)	3 (8.6)	9 (12.9)
Artisan	10 (28.6)	11 (31.4)	21 (30.0)
Clergy/student	2 (5.7)	2 (5.7)	4 (5.7)

Table III. Distribution, extent, and intensity of HIV-OMH among the cases

Category	*n (%)*
OMH site	
Palate	9 (20)
Tongue	34 (75.5)
Buccal mucosa	2 (4.5)
Extent	
Localized	18 (51.4)
Generalized	17 (48.6)
Intensity	
Light	22 (62.9)
Dark	13 (37.1)
Number of anatomical sites	
Single	26 (74.3)
Multiple	9 (25.7)
Margin of pigmented lesion	
Discrete	8 (22.9)
Diffuse	27 (77.1)

[IQR], 77.5-299.25). The mean values were 174.57 ± 131.36 cells/mm^3 and 324.66 ± 316.44 cells/mm^3 for cases and control subjects, respectively. The median CD4$^+$ counts for cases and control subjects were 174 cells/mm^3 (IQR, 57-250) and 324 cells/mm^3 (IQR, 107-424), respectively. The CD4$^+$ count mean rank value was lower for the cases than for the control subjects, and the difference was statistically significant (P = .03). Severe immunosuppression (CD4$^+$ count, ≤200 cells/mm^3) was found in over half (52.9%) of the study participants, being greater among the cases than among the control group (P = .019). Thirty-three (94.3%) of the participants with HIV-OMH and 23 (65.7%) of those without OMH had a CD4$^+$ count ≤350 cells/mm^3 (P = .003) (Table IV).

The median values of serum IL-6 for cases and control subjects were 4.07 pg/mL (IQR, 3.48-5.31) and 4.23 pg/mL (IQR, 3.64-4.66), respectively. The median values of serum TNF-α for cases and control subjects were 3.47 pg/mL (IQR, 2.43-5.43) and 4.10 pg/mL (IQR, 2.43-4.16), respectively (Table V). Conditional logistic regression was performed to assess the relationship of OMH, cytokine levels, and CD4$^+$ count among the study participants. The analysis revealed the odds of having OMH in cases using control subjects as a reference and

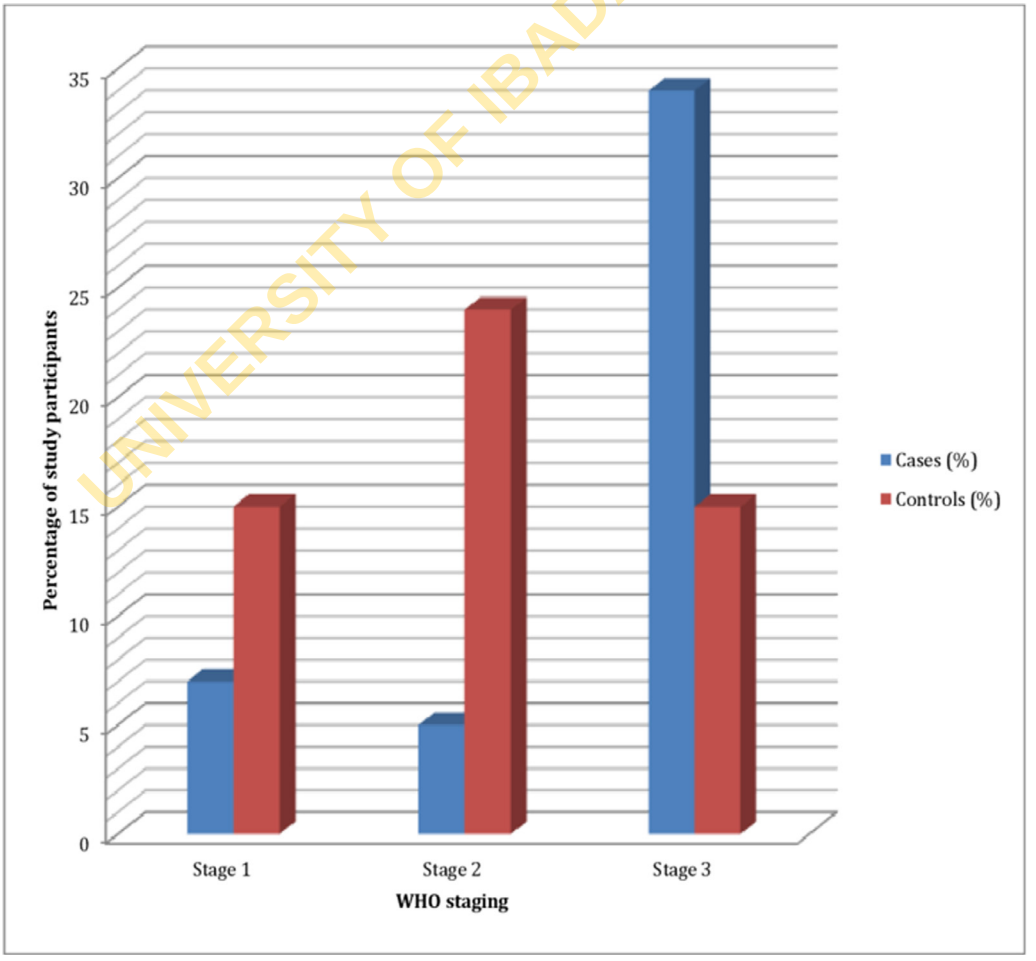

Fig. 2. Staging of the study participants according to World Health Organization criteria.

Table IV. Comparison of CD4+ mean, median and categories between the cases and control groups

Groups	*Mean (SD) CD4⁺ count (cells/mm³)*	*Median (IQR) CD4⁺ count (cells/mm³*	*Mean rank*	P *value*
Cases	174.57 ± 131.36	174 (57-250)	30.26	
Control subjects	324.66 ± 316.44	251 (107-424)	40.74	.03*
Category	Cases N (%)	Control subjects N (%)	Total N (%)	*P* value
$CD4^+$ count				
<200 cells/mm³	20 (57.1)	17 (48.6)	37 (52.9)	.019*
200-349 cells/mm³	13 (37.1)	6 (17.1)	19 (27.1)	
350-499 cells/mm³	1 (2.9)	6 (17.1)	7 (10.0)	
≥500 cells/mm³	1 (2.9)	6 (17.1)	7 (10.0)	
$CD4^+$ count				
≤350 cells/mm³	33 (94.3)	23 (65.7)	56 (80.0)	.003*
>350 cells/mm³	2 (5.7)	12 (34.3)	14 (20.0)	

*Statistically significant value.

Table V. Comparison of serum cytokine levels between cases and control subjects

Cytokine levels (pg/mL)	*Cases*	*Control subjects*
Mean (SD) IL-6	6.32 ± 7.38	4.66 ± 2.58
Median (IQR) IL-6	4.07 (3.48-5.31)	4.23 (3.64-4.66)
Mean (SD) TNF-α	8.52 ± 16.70	4.16 ± 2.43
Median (IQR) TNF-α	3.47 (2.43-5.43)	4.10 (2.43-4.16)

SD, standard deviation.

adjusting for $CD4^+$, IL-6, and TNF-α. Odds ratios of 0.997, 0.811, and 1.193 for $CD4^+$ count, IL-6, and TNF-α, respectively, were obtained (Table VI).

DISCUSSION

Over the past few decades, several local and international studies have shown candidiasis as the most prominent oral lesion associated with HIV infection as well as being a marker of immunosuppression.[2,17-19] The prevalence of candidiasis among other oral lesions associated with HIV infection is notably high, regardless of race, age group, or sex; however, OMH has also been reported more commonly among the black population and adults than in other groups.[19] More than half of the participants in this study who were severely immunosuppressed were significantly more represented among the cases than among the control group. This finding was consistent with studies by Taiwo and Hassan,[13] Namakoola et al.,[10] and Pillai et al.[20] among newly diagnosed HIV-seropositive participants. The majority of the participants in this study had stage III disease with a ratio of 2:1 between those having HIV-OMH and those without. Tamí-Maury et al.[21] and Eweka et al.[22] similarly reported a late presentation among their study participants, with most categorized as WHO stage III. Late presentation at HIV diagnosis has been defined as persons presenting for care with a $CD4^+$ count <350 cells/mm³ or presenting with an AIDS-defining event, regardless of the $CD4^+$ cell count.[23]

Table VI. Estimates of $CD4^+$ count and cytokines by logistic regression between cases and control groups

Variables	*Odds ratio*	*95% Confidence interval*		P *value*
		Lower	*Upper*	
$CD4^+$ count	0.997	0.994	1.000	.05
Interleukine-6	0.811	0.481	1.368	.43
TNF-α	1.193	0.870	1.635	.27

More commonly, oral lesions associated with HIV infection have been identified as markers of immunosuppression and thus are a reliable predictor of the $CD4^+$ count.[9,19] OMH, one of the HIV-associated oral lesions, has been postulated as a marker of immune suppression, being associated with a low $CD4^+$ T cell count in HIV-seropositive individuals.[10,11] In this research, there was a statistically significant difference in the mean $CD4^+$ count and $CD4^+$ mean rank values of participants with and without HIV-associated OMH, being lower in those with OMH. This research finding of low $CD4^+$ count and melanotic pigmentation is in tandem with those of Sud et al.[11] and Namakoola et al.[10] from India and Uganda, respectively.

Also, the mean $CD4^+$ count among participants in this study was lower than counts reported by Taiwo and Hassan[13] and Hegde et al.[24] but higher than the mean values reported by Nanteza et al.[18] and Frimpong et al.,[14] all being HAART-naive subjects. These variations could be due to the complex relationship between genetic composition and immunologic status, which is uniquely different in each individual.[25] In addition, most of the study participants in this research were at WHO stage III on presentation, which is indicative of the low $CD4^+$ level found.

A postulate that HIV-induced upregulation of IL-6 and TNF-α promotes the production of α-MSH by oral keratinocytes and melanocytes has been well documented. This induces upregulation of the expression of melanocortin 1 receptor of melanocytes with increased

production of melanin, resulting in the development of HIV-associated OMH.[26,27] The mean values of IL-6 and TNF-α in this study were similar to those reported by Nyambura Mugwe et al.[28] and Akase et al.[29] among HAART-naive subjects, who also had higher values of these cytokines than those receiving HAART. Also, Akase et al.[16] found a significant association between high levels of IL-6 and WHO stages III and IV, which was in tandem with our study finding. In this study, the cytokine levels were further categorized into low or high categories using the values for healthy Nigerian individuals as obtained in a study by Osuji et al.[30] A high level of IL-6 and a low level of TNF-α were noted among the study participants, regardless of their status as cases or control subjects. Likewise, the majority of the study participants with a $CD4^+$ count $\leq$350 cells/mm^3 had high levels of IL-6 and low levels of TNF-α. This finding of high IL-6 is in agreement with that of French et al.,[7] who noted this cytokine as the most informative biomarker associated with increased risk of mortality in HIV-1 infection. Therefore, differences in the cytokine levels may be due to the severity of immunosuppression and corresponding cytokine dysregulation, thus producing clinical manifestations of HIV/AIDS, including OMH.

Most of the participants in this study were within the fourth and fifth decades of life, similar to the findings from South Africa and Ghana by Moodley and Wood[31] and Frimpong et al.,[14] but a decade lower than that from Ethiopia and Morocco reported by Diro et al.[32] and Admou et al.,[33] respectively.

With respect to the outcome from this study, we strongly recommend a review of the classification of HIV-related oral lesions according to European Community Clearinghouse classification. The oral lesions strongly associated with HIV or commonly seen in HIV may vary slightly on the basis of geographic location as well as genetic factors. Thus, we recommend that more studies be done to verify lesions to be classified into each of the 3 groups in Black individuals and not just assumed to be same across the globe.

CONCLUSIONS

There was a significant association between HIV-OMH and severe immunosuppression in the newly diagnosed HIV-seropositive patients. Thus, OMH could serve as a clinical marker of immunosuppression in HIV infection. Serum cytokine levels did not significantly vary between the cases and control subjects.

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PRESENTATION

A modified version of this research was presented at the virtual conference of the International Association of Oral Maxillofacial Pathologists/Medicine (IAOP) in June 2021.

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