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OPPORTUNISTIC INFECTIONS WITH ORAL INJURIES IN HIV-INFECTED PATIENTS BEFORE PRESCRIPTION AND AGAINST THE BACKGROUND OF ANTIRETROVIRAL THERAPY

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The study included 181 HIV-infected patients (127 men and 54 women) aged 21 to 55 years (mean 34.6±7.01 years). Follow-up of HIV-infected patients from the time of registration to ART lasted an average of 1.64±0.17 years. The study showed that at the time of ART initiation, the majority of HIV-infected patients – 148 (81.8 %) – were dominated by the 3rd and 4th clinical stages with the registration of opportunistic infections inherent in these stages, respectively. In particular, oropharyngeal candidiasis was observed in half of the examined patients – 76 (42.0 %), and oral hairy leukoplakia in – 49 (27.0 %) patients, and in some of the examined patients, the level of CD4 lymphocytes exceeded 350 cells/μL. Despite antiretroviral therapy's high overall virological and immunological efficacy in 11 (6.0 %) patients, opportunistic infections with oral lesions were recorded at CD4 lymphocyte counts ≥350 cells/μL.

Key words: HIV infection, immunosuppression, oropharyngeal candidiasis, oral hairy leukoplakia, herpes simplex virus.

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ОПОРТУНІСТИЧНІ ІНФЕКЦІЇ З УРАЖЕННЯМ РОТОВОЇ ПОРОЖНИНИ У ВІЛ-ІНФІКОВАНИХ ПАЦІЄНТІВ ДО ПРИЗНАЧЕННЯ ТА НА ФОНІ АНТИРЕТРОВІРУСНОЇ ТЕРАПІЇ

Дослідження включало 181 ВІЛ-інфікованого пацієнта (чоловіків – 127, жінок – 54) віком від 21 до 55 років (середній – 34,6±7,01 роки). Спостереження від моменту встановлення на облік до призначення антиретровірусної терапії, що тривало в середньому 1,64±0,17 років показало, у більшості ВІЛ-інфікованих пацієнтів – 148 (81,8 %) – переважали 3-тя та 4-та клінічні стадії з глибокою імуносупресією та реєстрацією, відповідно, опортуністичних інфекцій з ураженням ротової порожнини, притаманних цим стадіям, зокрема орофарингеального кандидозу у половини обстежених – 76 (42,0 %), та волосистої лейкоплакії язика більше, ніж у чверті – 49 (27,0 %) пацієнтів, причому у частини обстежених – при рівні CD4-лімфоцитів вище 350 кл/мкл. Попри високу загальну вірусологічну та імунологічну ефективність антиретровірусної терапії у 11 (6,0 %) пацієнтів опортуністичні інфекції з ураженням ротової порожнини реєструвались при показниках CD4-лімфоцитів ≥350 кл/мкл.

Ключові слова: ВІЛ-інфекція, імуносупресія, орофарингеальний кандидоз, волосиста лейкоплакія язика, герпесвірусні інфекції.

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Opportunistic infections (OIs) remain the leading cause of treatment and death in HIV patients. Latent OIs are exacerbated against the background of immunodeficiency caused by the human immunodeficiency virus (HIV). Their clinical course becomes life-threatening severity, propensity to disseminate the pathogen with the formation of ectopic foci, low sensitivity to specific therapy with a tendency to multiple relapses, weak specific immune response to the pathogen [14].

One of the main problems with the rapid disease progression and high mortality associated with HIV is late treatment, namely the late presentation of HIV infection [8, 15].

Thus, most HIV patients seek medical care for clinical indications when they are already in the late (3–4) stages of the disease (65.8–80.7 %) and have signs of multiple OIs. Therefore, the timely diagnosis of OIs depends on the success of treatment, duration and quality of life of patients [1].

Oral lesions in HIV infection are among the most common complaints and reasons for seeking medical care in the early stages of HIV-infected patients. Patients with varying degrees of immunosuppression must suffer from various oral cavity viral, bacterial, and fungal lesions. Moreover, one

pathogen may acquire different clinical manifestations in patients with varying degrees of immunosuppression [6].

One of the most common OIs is oropharyngeal candidiasis (OPC). It is caused primarily by Candida albicans, a ubiquitous polymorphic fungus that is part of the normal microbiota of the gastrointestinal tract and acquires pathogenic properties when the local defences of the host are weakened. This allows the fungus to penetrate and damage the epithelial cells of the oral cavity [12].

It was found that in the late stages of HIV infection, the colonisation of yeast fungi in the oral cavity of patients reaches 81.1 %. In contrast, symptomatic disease occurs in a third and is characterised by a recurrent course [13].

With the progression of immunodeficiency, cases of clinical manifestation of the Herpes simplex virus (HSV) with lesions of the skin and mucous membranes, particularly herpes labialis, are becoming more frequent. It is established that in the stage of acquired immunodeficiency syndrome (AIDS) herpesvirus lesions acquire a continuous recurrent course, atypical localization with the possibility of dissemination and generalization, and lead to a deterioration in the quality of life of patients. Epstein-Barr virus oral hairy leukoplakia (OHL) occupies an essential place among herpesvirus lesions of the oral cavity. Data on the prevalence and incidence of OHL in HIV-infected people vary significantly from 20 % in patients with asymptomatic HIV to 36 % in patients with AIDS [7, 9].

Lesions of the oral mucosa may be the earliest and first available signs of HIV infection, particularly during a dental examination.

Despite the proven direct link between the level of immunosuppression and the development of opportunistic infections [10], the data on the preconditions for their occurrence is pretty contradictory. The development of OPC and OHL in patients without diagnosed immunodeficiency and HIV infection indicates that they are not always a pathognomonic sign of profound immunosuppression [11].

Diagnosis of HIV infection in the early stages is an important task. It will allow an HIV-infected patient to enter the cascade of medical services promptly.

The purpose of the study was to provide an assessment of opportunistic infections with oral injuries in HIV-infected patients before prescription and against the background of antiretroviral therapy.

Materials and methods. A retrospective and prospective cohort study was performed to assess the manifestations and determine the clinical and immunological characteristics of the course of HIV infection on the background of antiretroviral therapy (ART). We studied 181 HIV-infected patients (men – 127, women – 54) aged 21 to 55 years (median – 34.6±7.01 years), who were registered at the dispensary at the Regional Center for HIV/AIDS Prevention and Control of the Poltava Regional Council (RC HIV/AIDS PC) for the period from 2013 to 2017. Patients were observed in dynamics from 2 to 14 years. The mean follow-up duration was 4.3±3.4 years. A retrospective cohort study was performed from the time of registration to the ART prescription and lasted an average of 1.6 years. After ART was prescribed, the dynamics of HIV infection and the occurrence of OIs were assessed in a prospective cohort study that lasted an average of 3.4 years.

All medical and diagnostic procedures were carried out with the informed consent of patients.

The diagnosis of HIV infection was established according to the International Classification of Diseases 10 revisions and confirmed by detecting specific serological and molecular biological markers. Antibodies to HIV were detected in the blood by ELISA (test system "Labsistem", the Netherlands), viral load (VL) of HIV-1 –in a polymerase chain reaction (PCR) (Real-Time HIV-1 "About", USA). The state of cellular immunity was assessed by the total number of white blood cells, lymphocytes and their subpopulations (absolute and relative content) by immunophenotyping with monoclonal antibodies, followed by an analysis of test samples on a current cytofluorimeter FACSCailbur (Becton Dickinson, USA).

All patients underwent a general clinical examination and analysed case history data. The analysis of the obtained clinical data, the establishment of the clinical stages of HIV infection, the prescription and evaluation of the effectiveness of ART were performed according to standard protocols using the classifications given in ICD-10 (WHO, 2006). Patients started ART depending on the level of CD4 cells (before the introduction of new WHO recommendations in 2015). ART was performed using first-line regimens – 2 nucleoside and 1 non-nucleoside reverse transcriptase inhibitors: Zidovudine/Lamivudine + Efavirenz or Tenofovir/Emtricitabine + Efavirenz.

Analysis of the obtained clinical data and diagnosis of opportunistic infections were carried out in accordance with the standard protocols in force at the time of the study (Order of the Ministry of Health of Ukraine No. 182 of 13.04.2007) using the classifications given in ICD-10 (WHO, 2006) with amendments and additions.

Oral hairy leukoplakia was verified based on objective examination and biopsy of the tongue mucosa (if necessary).

Oropharyngeal candidiasis was verified using microscopy of the obtained material and subsequent culture examination to identify characteristic fungi of the genus *Candida*.

Laboratory tests for the diagnosis of opportunistic infections were performed based on the RC HIV/AIDS PC laboratory.

Statistical processing was performed using the SPSS statistical program, version 17. Baseline values at the time of the prescription and on the background of ART were presented as the mean (and standard deviation of the mean) frequency (%).

The frequencies of binary signs in the group of patients in the dynamics before the prescription and on the background of ART were performed using McNemar's test. Differences were considered plausible with a generally accepted probability of error $p < 0.05$. At p in the range from 0.05 to ≤0.1 there was a tendency to probability.

Results of the study and their discussion. Follow-up of HIV-infected patients from the time of registration to ART lasted an average of 1.64±0.17 years. Patients were included in the study in 2013–2015 when the criteria for ART were determined on the basis of Order No. 551 in the presence of a history of any AIDS-indicating disease and/or a decrease in CD4 lymphocytes less than 350 cells/μl. To exclude immune reconstitution syndrome, the emergence or recurrence of OIs was assessed no earlier than six months after the onset of ART.

The study showed that at the time of ART initiation, the majority of HIV-infected patients – 148 (81.8 %) – were dominated by the 3rd and 4th clinical stages with the registration of opportunistic infections inherent in these stages, respectively. The data obtained were confirmed by the analysis of the distribution of stages of the disease at the time of initial treatment. Thus, at the time of registration, the 3rd and 4th clinical stages were diagnosed in 129 (71.3 %) patients, which corresponds to global trends. Thus, according to preliminary estimates, half of the patients diagnosed with HIV seek help late, with a CD4 cell level <350 cells/mm^3 or in the presence of AIDS-indicating OIs within 6 months after diagnosis of HIV. This is evidence of the late presentation of the disease [5] and has a colossal clinical and epidemiological impact in terms of contribution to the growth of the HIV epidemic [15].

In the study of HIV viral load and CD4 lymphocyte levels at the beginning of ART, viral load of HIV above 1.0×10^5 copies/ml was registered in 59 (32.5 %) people, and the average CD4 lymphocyte count was 152.0 (60.0–283.5) cells/μL. Distribution by CD4-lymphocyte levels showed that the proportion of patients with CD4-lymphocyte levels <200 cells/μL was 110 (61.3 %), respectively, with CD4-cell levels >500 cells/μL – 6 (3.3 %), 200–349 cells/μL – 45 (24.3 %) and 350–499 cells/μL – 20 (11.0 %) individuals. Thus, individuals with high VL and deep immunosuppression predominated among the patients.

Thus, oropharyngeal candidiasis at the time of ART was diagnosed in half of the subjects– 76 (42.0 %), and oral hairy leukoplakia was detected in more than a quarter – 49 (27.0 %) patients. Signs of herpetic lesions of the oral mucosa were registered less often, in 26 (14.3 %) patients.

Given the high registration of OIs with oral mucosa lesions, it was advisable to analyze their registration depending on the level of immunosuppression. The study found that at the time of ART initiation in the majority (n=78, 43.0 %) of patients, OIs were registered at the level of CD4 lymphocytes below 200 cells/μL.

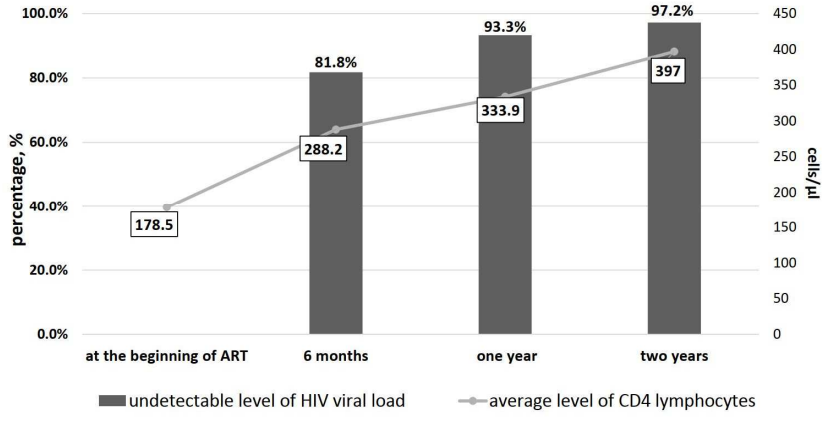

Fig. 1. Virological and immunological efficacy of ART in dynamics

However, in some patients, opportunistic lesions were also observed at CD4 lymphocyte levels above 350 cells/μL. Thus, OHL was diagnosed in 10 (5.5 %), OPC – in 9 (4.9 %), herpesvirus lesions of the oral cavity – in 4 (2.2 %) patients with relatively sufficient levels of CD4 cells.

Since ART is currently the only effective tool, it is advisable to evaluate the clinical effectiveness of ART in this cohort of patients in the fight against immunosuppression and prevent the development/reactivation of OIs in the future [3, 5].

Observation of this group of patients during the period against the background of ART revealed high virological and immunological effects of treatment. Thus, the average level of CD4 lymphocytes during 6 months of ART increased from 178.5±10.8 cells/μL at the time of ART initiation to 288.2±13.3 cells/μL, after 1-year ART – up to 333.9±13.9 cells/μL, and after 2 years – up to 397±16.7 cells/μL. Overall, an adequate immunological response during the first year of ART was achieved in 147 (81.2 %) HIV-infected people. The level of HIV VL was indeterminate after 6 months from the

beginning of ART in 148 (81.8 %) patients, 1 year later in 169 (93.3 %), and 2 years later in 176 (97.2 %) individuals (fig. 1).

In addition, the frequency of registration of opportunistic infections of the oral cavity on the background of treatment decreased significantly, which confirmed the analysis of their prevalence. Thus, OPC on the background of ART was registered by 3.8 (p=0.000), OHL – 5.4 (p=0.000), and HSV – 2.8 (p=0.0056) times less often than before the therapy prescription (Table 1). However, in 24 (13.2 %) patients from the general ART study group, OIs with oral damage still developed/reactivated. During the follow-up period with ART, OPC was diagnosed in 16 (8.8 %) patients, and OHL and HSV – in 9 (4.9 %) patients.

Table 1

Analysis of the structure of opportunistic infections with oral lesions in HIV-infected patients at the time of prescription and on the background of ART

Opportunistic infections	At the beginning of ART (n=181)		Against the background of ART (n=181)		p
	Abs. number	%	Abs. number	%	
Oropharyngeal candidiasis	62	42.0	16	8.8	0.000
Oral hairy leukoplakia	49	27.0	9	4.9	0.000
Herpesvirus lesions of the skin and mucous membranes of the oral cavity	26	14.3	9	4.9	0.005

Notes: p is a significant difference taking into account the McNemar's test

Analysis of the level of CD4 lymphocytes revealed that in 11 (6.0 %) patients, OIs on the background of ART was registered with CD4 lymphocytes ≥350 cells/μl: OHL was diagnosed in 3 (1.6 %), OPC and HSV – in 4 (2.2 %) patients (fig. 2).

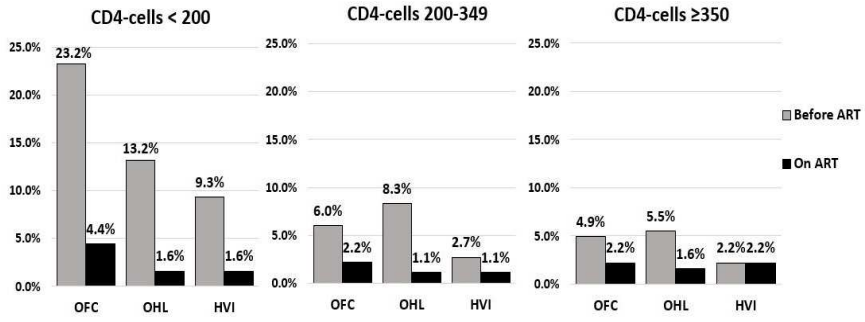

Fig. 2. Analysis of the structure of opportunistic infections with oral lesions in HIV-infected patients before prescription and on the background of ART depending on the level of CD4 lymphocytes

Thus, the registration in patients of oral opportunistic infections for late clinical stages of HIV infection is evidence of late presentation of the disease. Thus, the diagnosis of OPC at the time of ART was established in half, and OHL was detected in more than a quarter of respondents. At the same time, the relatively low prevalence of herpes lesions of the oral mucosa in patients compared to other lesions – 26 (14.3 %) can be explained by the fact that patients sought medical help mainly in recurrent and common forms of herpes simplex in the form of gingivostomatitis.

Damage to the immune system in HIV infection is systemic in nature, manifested by an imbalance and deep suppression of the T-and B - links of cellular immunity. This manifests itself in the form of changes in hypersensitivity reactions of immediate and delayed type, humoral immunity, factors of nonspecific protection, functional activity of lymphocytes. It has been established that HIV replication takes place mainly in CD4 lymphocytes, on which the virus has a cytopathic effect with cell lysis or fusion into syncytium. Therefore, the initial link of disorders of the interaction of the immune response in HIV infection is a decrease in the level of this population of lymphocytes. In the future, it seemed appropriate to analyze the registration of OIs depending on the level of CD4 cells. The analysis showed that at the time of ART initiation, the vast majority of OIs was recorded at low CD4 cell counts. This is natural, as the clinical manifestations of OIs are inversely correlated with changes in immune status. 200 cells/μL is the generally accepted limit of CD4-lymphocyte levels, below which most opportunists are activated [2]. However, it is known that even with a sufficient level of CD4 cells, activation of such OIs as tuberculosis, HSV, some forms of candidiasis, etc. is possible. Thus, in the group of patients we examined, opportunistic lesions were also observed with relatively sufficient CD4 lymphocyte counts above 350 cells/μL. This may be due to the fact that dependence on the level of CD4 lymphocytes is inherent in those opportunists, protection from which is determined by cellular adaptive immunity. In cases where the primary protective role belongs to phagocytosis, the activity of the complement system, the barrier function of the skin and mucous membranes, OIs may manifest with sufficient values of CD4 cells [4]. In addition, CD4 lymphocyte levels may vary from person to person depending on age, gender, and HIV viral load.

Follow-up of this cohort of patients from the moment of ART initiation in order to assess the effectiveness of treatment revealed not only a high virological and immunological response in patients, but also a decrease in the incidence of opportunistic oral infections during treatment, which emphasizes the need for early initiation of ART for HIV-infected patients.

However, the development/reactivation of OIs with oral damage in 24 (13.2 %) patients from the general group of the ART study, and in half of them (N=11 (6.0 %)) with CD4 lymphocyte counts ≥350 cells/μL, indicates partial clinical efficacy of ART.

The appearance of opportunists characteristic of late clinical stages of HIV infection with CD4 lymphocyte counts ≥350 cells/μL both before prescription and against the background of ART indicates that such OIs with oral damage, such as OPC and OHL, can not always be considered as a pathognomonic sign of profound immunosuppression. The results obtained dictate the need to search for risk factors for clinical progression of HIV infection in this group of patients against the background of ART: biological (age, gender, body mass index), behavioral (route of transmission, smoking, alcohol abuse, adherence to treatment) and genetic characteristics.

The results obtained correspond to the literature on the prevalence of these OIs in oral lesions [7, 9, 13].

These results underscore the need for timely diagnosis of OIs with oral lesions in HIV-infected patients with sufficient levels of CD4 lymphocytes to allocate a priority group of patients to increase the frequency of dispensary supervision in the particular dental examination.

Conclusion

Patients with late clinical stages of HIV infection predominated among patients both at registration and at the time of onset of ART, indicating late treatment for clinical signs of the disease. Half of the patients in the study group were diagnosed with OPC at the time of ART initiation, more than a quarter with OHL, and 26 (14.3 %) people with HSV. In some patients, OIs with oral lesions was recorded at CD4 lymphocyte levels ≥350 cells/μl both before ART prescription and during the treatment, despite its overall immunological and virological efficacy.

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