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Original article

IL-6, D-DIMER AND HIGH-SENSITIVITY C-REACTIVE PROTEIN IN HIV INFECTION – PRELIMINARY STUDY

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ABSTRACT:

Combined antiretroviral therapy (cART) provides HIV-infected people life expectancy comparable with HIV-uninfected people and turns the disease into a manageable chronic condition necessitating the need for innovative inflammatory markers.

Our **purpose** was to determine the correlation between IL-6, D-dimer and high-sensitivity C-reactive protein (hsCRP) levels among HIV-infected and the presence of chronic inflammation during general and immunological aging and drug exposure.

Material and methods: Comparative prospective study was conducted at 37 HIV-positive persons from the Center for Monitoring and Treatment of HIV-positive Patients at the Clinic for Infectious Diseases, UMBAL "Dr. G. Stranski" – Pleven (target group) and 18 HIV-negative individuals from outpatient practice (control group), aged e"18 years.

Results: The median age of seropositive persons was 40 years (24÷70 years), of the control group – 51 years (29÷72 years); 78% of the target group and 61% of the controls are men. The average duration of ART is 4 years (1÷9 years). The study of specified biomarkers in the target group found increased IL-6 in 8.11% of patients (mean 3.67±1.86 pg/mL; range 1.5÷8.62; 95% CI 3.11-5.02), increased D-dimer in 8.11% (mean 0.37±0.28 μg/mL; 0.21÷1.96; 95% CI 0.3691-0.37459) and increased hsCRP in 10.81% (mean 2.10±1.99 μg/mL; 0.19÷7.0; 95% CI 1.89-2.31). In the control group IL-6 was not increased (mean 2.75±1.67 pg/mL; 1.5÷6.91), D-dimer was increased in 16.67% (mean 0.37±0.17 μg/mL; 0.09÷0.8) and increased hsCRP – in 5.56% (mean 1.76±1.75 μg/mL; 0.19÷5.66). Il-6 was significantly higher in the target group.

Conclusion: The implementation of sensitive biomarkers is crucial in the general diagnostic-therapeutic approach in aging with HIV.

Keywords: IL-6, D-dimer, hsCRP, chronic inflammation, HIV,

INTRODUCTION

HIV is a major global public health issue, with 36.3 million deaths so far [1]. The increased access to effective prevention, diagnosis and treatment transformed HIV infection into a manageable chronic health condition. Combined antiretroviral therapy (cART) provides HIV-infected people life expectancy comparable with HIV-uninfected people [2, 3]. But in conditions of aging and immunological dysfunction, hypercoagulation and persistent chronic inflammation associated with HIV, the risk of developing non-AIDS conditions (cardiovascular, liver, kidney complications; tumors) increases. This necessitates the need to supplement the diagnostic panel with innovative markers of inflammation [3]. According to the Strategies for Management of Antiretroviral Therapy (SMART) trial, elevated levels of three inflammatory markers were independently and strongly associated with a subsequent risk of death during follow-up: interleukin-6 (IL-6) – pro-inflammatory cytokine that is a proximal mediator or upstream inflammatory marker; high-sensitivity C-reactive protein (hsCRP) – downstream, acute-phase reactant whose hepatic production is stimulated by IL-6; and D-dimer – degradation product of intravascular fibrin that is a marker of hypercoagulation [4].

Our **purpose** was to determine the correlation between IL-6, D-dimer and hsCRP levels among HIV-infected and the presence of chronic inflammation during general and immunological aging and drug exposure.

MATERIAL AND METHODS

A comparative prospective study was conducted at 37 HIV-positive persons from the Center for Monitoring and Treatment of HIV-positive Patients at the Clinic for Infectious Diseases, UMBAL "Dr G. Stranski" – Pleven (target group) and 18 HIV-negative individuals from outpatient

practice (control group), aged ≥18 years.

In this study, we adhered to ethical guidelines and obtained ethical approval from the Ethical Commission of Medical University - Pleven (Protocol No.72/2023). Prior to participation, written informed consent was obtained from all participants after providing them with comprehensive information about the research objectives, procedures, and potential risks or benefits involved. We ensured that participants had a clear understanding of their rights and responsibilities as research participants. In order to be involved in the study, the individuals from the control group were screened for HIV with a rapid qualitative antibody test after signing a consent form. To maintain confidentiality, the identities of participants were treated with utmost confidentiality and were kept anonymous. Data were entered into an electronic database (Microsoft Excel v. 2010) and analyzed using statistical software (IÂÌ SPSS Statistics 19.0). The variables were analyzed by t-test and χ^2 test (for parametric and non-parametric distributions, respectively; $p<0.05$ was considered as significant). The Odds Ratio (OR) was calculated.

RESULTS

The age distribution revealed that the mean age of the subjects in the target group was 42±11.5 years (24-70 years; median age was 40 years), with prevalent ages 31-40 and 41-50 years (43.24% and 29.73%, respectively) (Figure 1). Individuals in the control group were significantly older than the target group; the mean age of the subjects was 51.5±13.2 years (29-72 years; median age was 51.5 years) ($p<0.025$) with prevalent age >60 years (33.33%) (Figure 2).

Fig. 1. Age distribution in the target group (number and percentage of the subjects)

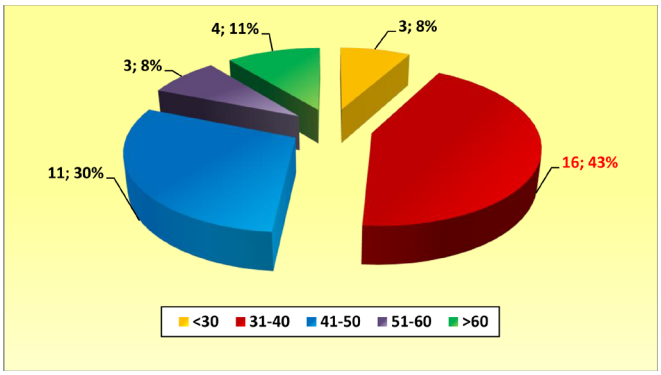
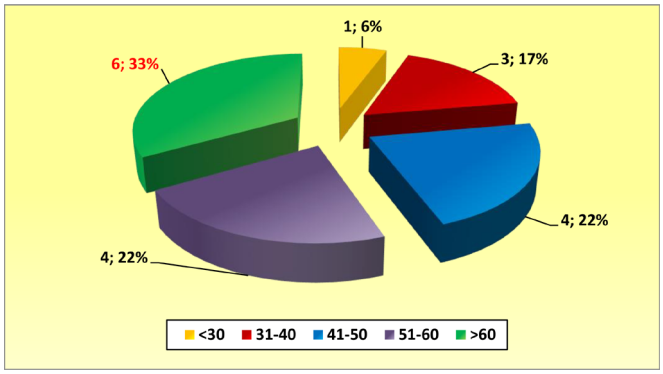


Fig. 2. Age distribution in the control group (number and percentage of the subjects)



Males were 78% of the target and 61% of the control group ($p<0.05$). Comorbidity with different chronic diseases was registered in 20 cases in the target group (54.05%) and in 8 (44.44%) in the control group ($p>0.05$).

Thirty-six HIV-positive subjects (97.30%) receive ART. The average duration of ART is 4 years (1÷9 years). There are three regimens of ART – 1) integrase inhibitor based ART regimen (INI + 2NRTI), 2) boosted protease inhibitor based ART regimen (PI + 2NRTI) and 3) non-nucleoside reverse transcriptase inhibitor based ART regimen (NNRTI). The distribution of HIV-positive people on these regimens is 21 (58.33%), 9 (25.00%) and 6 (16.67%), respectively ($p<0.05$).

The study of specified biomarkers in the target group found increased IL-6 in 8.11% of patients (mean 3.67 ± 1.86 pg/mL; range 1.5÷8.62; 95% CI 3.101-5.022), increased D-dimer in 8.11% (mean 0.37 ± 0.28 µg/mL; 0.21÷1.96; 95% CI 0.3691-0.375) and increased hsCRP in 10.81% (mean 2.10 ± 1.99 µg/mL; 0.19÷7.0; 95% CI 1.89-2.313). In the control group IL-6 was not increased (mean 2.75 ± 1.67 pg/mL; 1.5÷6.91; 95% CI 2.018-3.472), D-dimer was increased in 16.67% (mean 0.37 ± 0.17 µg/mL; 0.09÷0.8; 95% CI 0.366-0.371) and increased hsCRP – in 5.56% (mean 1.76 ± 1.75 µg/mL; 0.19÷5.66; 95% CI 1.496-2.019). Il-6 is significantly higher in the target group ($p<0.05$). The comparison of investigated biomarkers in target and control groups has been shown in Table 1.

Table 1. IL-6, D-dimer and hsC-reactive protein in HIV-infected and control subjects

Subjects / Markers (reference)	Target group			Control Group			OR	P
	n %	mean ± sd	95% CI	n %	mean ± sd	95% CI		
IL-6 **(0.00-7.00** pg/mL)	3 8.11	3.67±1.86	3.101-5.022	0	2.75±1.67	2.018-3.472	1.41176	**<0.05**
D-dimer **(< 5.00** µg/mL)	3 8.11	0.37±0.28	0.369-0.375	3 16.67	0.37±0.17	0.366-0.371	0.47059	>0.05

hsCRP (**0.00-5.00** μg/mL)	4 10.81	2.10±1.99	1.890-2.313	1 5.56	1.76±1.75	1.496-2.019	1.93939	>0.05

n – number of cases with increased levels

DISCUSSION

Chronic inflammation is one of the major characteristics of aging in people with HIV (PWH). Inflammatory markers such as IL-6, D-dimer and hsCRP are elevated in some PWH. This elevation continues even after the of virologic suppression [5]. In some studies, the levels of these markers differ among different ART regimens. Many factors may influence the level of systemic inflammation associated with different ART regimens. In the first place, a low level of HIV transcription in any tissue reservoirs could trigger immune activation despite suppression of viral RNA levels in peripheral blood [6]. The extent of this process could be affected by the pharmacokinetics of ART regimens [5, 7]. Except for this, the toxicity of certain ART regimens could increase the level of systemic inflammation to a greater extent than others, independent of the number of antiviral drugs. Also, suboptimal adherence may lead to more prolonged periods of low-level viremia in plasma, and this may vary according to the pharmacokinetic characteristics of ART regimens. Suboptimal adherence has been associated with increased inflammation, even among individuals with stable virologic suppression [8]. Over the long term, high levels of inflammation have been associated with adverse clinical outcomes among PWH. This motivated the study of Serrano-Villar S. et al. for modeling potential clinical differences in suppressive regimens and based on observed differences in inflammatory markers [5]. Higher levels of IL-6 and/or D-dimer have been reported to confer an increased risk of serious non-AIDS events (SNAEs) and death [9]. To the present time, our study confirms the significant elevation of IL6 levels in the group of HIV-infected subjects compared with the control group ($p<0.05$), but there is a need for further investigations to assess the impact of different ART regimens on these inflammatory biomarkers.

IL-6 and other inflammatory markers are not routinely assessed in PWH. The impact of inflammation on clinical outcomes is not clearly understood. The international guidelines emphasize the importance of inflammation in PWH but do not define specific recommendations for the measurement, prevention, or treatment of inflammation [5]. This is because there is not enough evidence to show that interventions intended to change heightened inflammation levels affect clinical outcomes. This also is due to the relatively small number of studies that investigate eventual differences in inflammatory markers with different ART regimens. Our study is focused on the initial investigation of the levels of high-sensitive inflammatory markers by comparative analysis of PWH and HIV-negative people. At the same time, the principal aim of this preliminary study was to open a pathway for primary orientation about the predictive value of three non-routinely used inflammatory biomarkers. Of course, there is a need for further investigations into the use of long-term results from large, parallel-group studies conducted in PWH. Another challenge for explanation is the assumption of no changes in D-dimer levels in our study. It is in contrast with the differences in D-dimer levels observed in other studies that consider elevations of this inflammatory biomarker as a result of longer-term consequences of changes in inflammation [10, 11].

In conclusion, the implementation of sensitive biomarkers is crucial in the general diagnostic-therapeutic approach in aging with HIV. Future clinical studies covering a larger cohort of PWH and additional analysis would clarify the prognostic significance of inflammatory biomarkers.

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