INFLAMMATION BIOMARKERS IN HIV SEROPosITIVE PATIenTS UNDERGOING ANTiRETROvIRA l THERAPY

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Abstract. Background. Under combined antiretroviral therapy, HIV infection has become a chronic condition, still accompanied by a proinflammatory status with a consequent impact on multiple organs. We evaluated the levels of inflammatory biomarkers in HIV-infected patients undergoing specific therapy and their association with the presence of metabolic syndrome. Methods. A non-interventional study was conducted on HIV infected patients undergoing antiretroviral therapy in a tertiary care hospital in 2008. Besides routine laboratory assays, TNF alpha, IL 6 and MCP 1 were tested, by means of BioSource EASIA (Enzyme Amplified Sensitivity Immunoassay). Metabolic syndrome was defined according to International Diabetes Federation. Results. A total of 106 patients were included, with a M:F ratio =1.4; median age of 31 years, mode age of 20 years; mean CD4 cell count of 530/mmc; undetectable HIV viremia found in 69% of the subjects. In detectable versus undetectable HIV viremia samples, a pathological level of biomarkers was found in the following proportion: 53.1 % versus 39.1% for TNF alpha, 16.1% versus 13.2% for IL 6, and 9.7% versus 4.3% for MCP 1 respectively. Metabolic syndrome was identified in 10% of the cases (15.2% versus 8.2% in detectable versus undetectable HIV viremia samples). In multivariate analysis, patients with pathological values of MCP 1 are 11.7 times more likely to develop metabolic syndrome than those with normal values (CI 95% 2-71, p=0.008). Conclusions. In our young study population, MCP 1 is significantly associated with the presence of metabolic syndrome. Identifying key subclinical changes would help to anticipate the developing of metabolic syndrome in HIV infected patients. Once the practical utility of these biomarkers is well understood, it might also call for adequate therapy in order to reduce the incidence of polypathology associated with HIV infection. Keywords: HIV, MCP 1, TNF alpha, IL 6

Introduction

HIV infection has become a chronic condition due to the advent of combined antiretroviral therapy (cART) in 1996 [1]. Even now when HIV is supressed, HIV presence, yet below the detectability threshold of conventational laboratory assays, seems to lead to chronic inflammation. Both HIV itself and cART increases the risk of cardiovascular disease, metabolic disturbances, kidney impairement, osteoporosis and premature ageing [2-6]. In these circumstances, nowadays, these mechanisms are under intense investigation in order to decrease the non-AIDS morbidity and mortality in HIV seropositives.

The markers of inflammation which have recently been taken into discussion are: tumor necrosis factor alpha (TNF alpha), interleukin 6 (IL 6), monocyte chemoattractant protein 1 (MCP 1). As they are secreted by adipocytes among other cells, they can influence the lipidic and glucidic metabolism.

We evaluated the levels of inflammatory biomarkers in HIV-infected patients undergoing combined antiretroviral therapy and their association with the presence of metabolic syndrome.

Materials and methods

A non-interventional study on HIV seropositive patients undergoing cART was conducted in National Institute of Infectious Diseases „Prof. Dr. Matei Bals”, Bucharest, Romania, as part of research
grant PNCDI no.62077/ 2008 and later of doctoral project FEST POSDRU /89/1.5/S/64331. The current article presents partial results, based on data registered at enrollment only. Patients signed an informed consent and filled in a questionnaire on demographics, anthropological data and medical history. They underwent a clinical examination and routine laboratory assays, including CD4 cell count, HIV viremia, fasting blood glucose and lipogram. Also specific markers were tested by means of BioSource EASIA (Enzyme Amplified Sensitivity Immunoassay): tumor necrosis factor-alpha, interleukin-6, monocyte chemotactic protein 1. Normal values, expressed as pg/ml, are within the following range for each parameter: 4.6 – 12.4, 0-50 and 74-760 for TNF alpha, IL 6 and MCP 1, respectively.

The presence of metabolic syndrome was defined according to the International Diabetes Federation [7], as:
- central obesity (abdominal circumference ≥ 94 cm in men and ≥ 80cm in women) or Body Mass Index > 30
- plus two of the following criteria:
  - triglycerides ≥ 150 mg/dL or specific treatment for this lipid abnormality;
  - HDL cholesterol < 40 mg/dL in males and < 50 mg/dL in females or current hypolipemiant therapy;
  - blood glucose a jeune ≥ 100 mg/dL or Diabetes Mellitus II already diagnosed;
  - systolic blood pressure ≥ 130 mm Hg or diastolic blood pressure ≥ 85 mm Hg or therapy of previously diagnosed hypertension.

The design of the study was approved by the Local Ethical Committee. All data were kept confidential.

Statistics

We processed the study data in SPSS software (Statistical Package for the Social Sciences version 17, USA).

First Mann-Whitney tests were used to compare age, CD4 cell count, levels of inflammatory markers in people having detectable versus undetectable HIV viremia.

Then we performed chi-square tests to investigate any association between the metabolic syndrome on one side and the inflammation markers, age and sex on the other side. In this section, we used the inflammatory markers as categorical variables, marked by the threshold between normal and pathological values. Those variables which proved significantly linked to the metabolic profile of the patients were introduced in the multivariate regression, together with age to identify the independent factors.

Statistical significance was defined as two-tailed p<0.05.

Results

There were 106 subjects, with: M:F ratio of 1.4; median age of 31 years and mode age of 20 years; mean CD4 cell count of 530/mmc; undetectable HIV viremia in 69% of the cases. TNF alpha was elevated in 43.6% of the cases, while IL 6 in 14% – and MCP 1 in 6% only.

A comparison between the people with detectable as opposed to undetectable HIV viremia indicated a significant difference in terms of CD4 cell count, as Table 1 shows. In detectable versus undetectable HIV viremia samples, a pathological level of biomarkers was found in the following proportion of the cases: 53.1% versus 39.1% for TNF alpha, 16.1% versus 13.2% for IL 6, and 9.7% versus 4.3% for MCP 1 respectively. Only TNF alpha was slightly higher in detectable HIV viremia samples than in the undetectable ones with a border-line statistical significance.

Metabolic syndrome was found in 10% of the study population, varying between individuals with detectable HIV plasma load (15.2%) and those with undetectable viremia (8.2%). It was also found more frequently in men (14.5%) than in women (4.5%).

Univariate analysis showed an important association between the presence of metabolic syndrome and MCP 1 (p=0.01). In the multivariate model, this
link remained significant statistically, as described in Table 2. Patients with pathological values of MCP 1 are 11.7 times more likely to develop metabolic syndrome than those with normal MCP 1.

**Discussions**

This study revealed a significant association between monocyte chemoattractant protein 1 and the presence of metabolic syndrome among HIV-infected patients undergoing antiretroviral therapy. Nowadays, data on inflammatory markers and their clinical relevance in HIV pathology are still controversial; these laboratory assays are not yet introduced in routine practice as they need further investigation. To our knowledge, this current project is the first Romanian one conducted on specific biomarkers, as TNF alpha, IL 6 and MCP 1, in HIV seropositive people.

International reports indicate that HIV infection results in activation of inflammatory pathways that may impact multiple organs [2]. On the other hand, cART extended considerably the lifespan of seropositives, but brought some adverse effects patients should fight with on long term. Regarding the topic of the present article, TNF alpha and IL 6 are associated to changes in glucidic metabolism especially in HIV-infected people with lipodistrophic syndrome. It is rather cART which perturbates the endocrine activity of adipose tissue and consequently the secretion of inflammatory adipocytokines (TNF-alfa, IL6) [8-10]. Samaras et al. [11] found that subjects with treated HIV infection had an inflammatory profile (TNF alpha, IL-6, hsCRP) equivalent to that found in insulin-resistant obesity, despite lower body fat. There are cited proportional correlations between proinflammatory molecules and lipid profile as well [12]. IL 6 is also strongly associated to non-AIDS morbidity and mortality [13]. In our study, IL 6 was within normal range in most of the patients undergoing cART, but it should be monitored in dynamics. Levels of TNF alpha was elevated in almost half of the population, but without an obvious clinical impact on metabolism. Its levels were slightly lower in patients with undetectable viremia under cART. HIV-related inflammation was thus corrected by cART to some extent.

MCP 1 is a chemokine secreted by different cells, as macrophages, monocytes, fibroblasts, endothelial and epithelial cells, adipocytes, smooth muscle, mesangial, astrocytic and microglial cells [14]. It is subsequently involved in inflammation, atherosclerosis, metabolic disturbances, including insulin-resistance [10, 15]. Macrophages exposed to viral envelope glycoprotein 120 belonging to HIV stimulates the production of MCP 1 [16]. But as mentioned above, cART could influence the synthesis of MCP 1 in particular tissues. In our study, all patients were administered cART. There were no significant differences in terms of MCP 1 between the 2 groups of subjects. However, the number of patients with pathological levels was double in people with detectable compared with undetectable HIV plasma load.

Moreover, metabolic syndrome was 11.7-fold more frequently found in individuals with abnormal MCP 1. According to the International Diabetes Federation, people diagnosed with metabolic syndrome are 5 times more likely to develop diabetes mellitus, 3 times more likely to have acute coronary syndrome and 2 times more likely to die from a disease related to metabolic syndrome than general population [7, 17]. In addition to the above-mentioned risks, our patients bear the burden of the HIV infection also. It is known that both HIV itself and certain cART are independent risk factors for metabolic changes, atherosclerosis and cardiovascular disease [3-5].

Age itself did not influence the presence of the 3 criteria which defined the metabolic syndrome. But in our population age was relatively condensed in the 3rd and 4th decade of life.

The percentage of people diagnosed with metabolic syndrome in our study is not negligible, taken into consideration their young age and their long-term perspective. This study group mirrors the characteristics of Romanian HIV seropositives: young patients with a mode age of 20 years, balanced sex distribution and multi-experience to antiretroviral therapy [18]. In other countries, metabolic syndrome among people living with HIV was superior than ours, fluctuating from 10-15% in France [19] and Spain [19-20] to 20-25% in Italy [19] and USA [21]. The age of studied populations - higher than in Romanian cohort - could offer an explanation.

There are also some limitations to consider: this current sub-study was cross-sectional; the number of subjects was modest, but the price of the investigational biomarkers was restrictive; we lacked

<table>
<thead>
<tr>
<th>Variable</th>
<th>Presence of metabolic syndrome</th>
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<tbody>
<tr>
<td></td>
<td>OR</td>
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<tr>
<td>Age</td>
<td>1</td>
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<tr>
<td>MCP 1 pathological versus normal values</td>
<td>11.7</td>
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Table 1. Multivariate analysis on risk factors for metabolic syndrome
HIV-negative and naïve HIV-positive comparators; therapy regimens were heterogeneous in terms of duration and combinations. Nevertheless, the main novelty is that this project covered an area incompletely explored in international research.

Conclusions

Under antiretroviral therapy, there is still a residual HIV replication below the level of detection of standard plasma assays and subsequently, a proinflammation status. MCP 1 is significantly associated with the presence of metabolic syndrome in our young study population.

Identifying key subclinical changes would help to anticipate the developing of metabolic syndrome in HIV infected patients. Once the practical utility of these biomarkers is well understood, it might also call for adequate therapy in order to reduce the incidence of polypathology associated with HIV infection.

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References


