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CARDIOVASCULAR RISK, FERRITIN AND INFLAMMATORY MARKERS IN HIV-SEROPOSITIVE PATIENTS WITH LIPODYSTROPHY

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ABSTRACT - Background & Aims: HIV-seropositive subjects are more susceptible to cardiovascular diseases. Thus, the objective of the present study was to determine the metabolic changes influenced by HIV infection and its treatment and the cardiovascular risk associated with ferritin and inflammatory markers. **Methods:** A cross-sectional study was conducted on 36 adult volunteers of both sexes divided into the following groups: HIV^+ with lipodystrophy (HIV^+LIPO^+), HIV^+ without lipodystrophy (HIV^+LIPO^-) and HIV^- (Control). Anthropometric evaluation was performed and the following analyses were carried out: glycemia, insulin, total cholesterol, LDL-C, HDL-C, triglycerides, iron, ferritin, AST, ALT, alkaline phosphatase, TNF-α and CRP. The Framingham score was calculated and HOMA-IR was determined. **Results:** The HIV^+LIPO^+ group had higher values of HOMA-IR, glycemia, insulin, TNF-α, ferritin and cardiovascular risk and reduced values of HDL when compared to to control group. Cardiovascular risk was positively correlated with ferritin and TNF-α and negative correlated with HDL values.

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Conclusion: Our study is meaningful to provide evidence that HIV asymptomatic patients with lipodystrophy could have higher levels of ferritin and TNF-α and higher risk for cardiovascular diseases.

Keywords: HIV, Lipodystrophy, Ferritin, Inflammation, Cardiovascular.

INTRODUCTION

The natural course of infection with human immunodeficiency virus (HIV) is characterized by intense and continuous viral replication and its consequence is immunodeficiency of the infected individual that causes conditions ranging from an asymptomatic status to the development of serious opportunistic diseases (BARROS et al., 2012). Highly active antiretroviral therapy (HAART) is used promotes significant suppression of viral replication and reduces the occurrence of opportunistic infections (DOURADO et al., 2006; WHO, 2003) Although, HAART is also associated with severe side effects, such as changes in the distribution of body fat and metabolic alterations (dyslipidemias and insulin resistance), defined by lipodystrophy syndrome (BERGERSEN et al., 2006; MARCASON, 2009).

Autopsy studies have demonstrated an association between HIV and coronary artery disease (JOSHI et al., 1987; RERKPATTANAPIPAT et al., 2000). However, with the introduction of HAART and the chronicity of the disease, an increase in the occurrence of cardiovascular involvement has been observed in these patients (HENRY et al., 1998; PATON et al., 1993). The most diverse cardiovascular manifestations have been observed as a consequence of HIV infection itself, of autoimmunity, of the immunological reaction to other viral infections, neoplasias, prolonged immunosuppression, malnutrition and the cardiotoxicity of the medications (ARSHAD et al., 2000; RICKERTS et al., 2000).

At the same time, there is a current discussion about the participation of iron in the inflammatory process. Studies have shown correlations between increased ferritin concentrations and elevated concentrations of inflammatory cytokines, suggesting biological relations between ferritin and the response to inflammatory markers associated with atherosclerosis and its complications (BARBARO, 2002).

Anemia of chronic disease is a frequent hematologic disorder in HIV-infected patients, with its occurrence being related to chronic inflammatory diseases, chronic

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infections or neoplasias, with activation of the immunologic system (SANTOSH et al., 2015).

In contrast to iron deficiency anemia, the clinical manifestations of anemia of chronic disease include reduced serum iron levels despite the presence of adequate reticuloendothelial iron reserves, reduced total iron binding capacity, and increased serum ferritin levels (WEISS AND GOODNOUGH, 2005).

The relation between ferritin and vascular damage was analyzed in a study on patients with hepatic steatosis, in which ferritin was found to be an independent predictor of vascular damage. This result suggests that ferritin may represent a marker of vascular damage (SULLIVAN, 2007).

The objective of the present study was to verify the metabolic changes influenced by HIV infection and its treatment and to determine whether ferritin and the inflammatory markers can also be used as indicators of cardiovascular risk.

MATERIALS AND METHODS

A descriptive, analytical cross-sectional study was conducted on 36 adults of both sexes who were divided into three groups:

- HIV^+LIPO^+ (HIV-seropositive patients taking HAART, with lipodystrophy syndrome)
- HIV^+LIPO^- (HIV-seropositive patients taking HAART, without lipodystrophy syndrome)
- Control (HIV-seronegative subjects).

All HIV-positive patients had an undetectable viral load, i.e., less than 50 copies/ml and a $CD4^+$ cell count of more than 200 cells/mm^3, had been taking HAART for at least 6 months and had no signs or symptoms of opportunistic infections.

To be included in the HIV^+LIPO^+ group, an individual had to report loss and/or accumulation of fat in specific regions of the body and these changes were confirmed by clinical examination performed by the senior author (SUTINEN AND YKI-JÄRVINEN, 2007). Thus, it was an essential requirement to detect lipoatrophy, i.e., loss of visible fat in peripheral regions (arms or legs) or in the face accompanied or not by lipohypertrophy, i.e. accumulation of fat in an abdominal region, dorsal gibbosity or gynecomasty (SUTINEN AND YKI-JÄRVINEN, 2007). Exclusion criteria were the presence of severe renal, cardiac and hepatic changes and the use of ferrous sulfate supplementation.

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HIV-seropositive volunteers were recruited at the Special Unit for the Treatment of Infectious Diseases of the University Hospital, Faculty of Medicine of Ribeirão Preto, University of São Paulo (HCFMRP-USP), while control subjects were selected from individuals of the different specialties of HCFMRP-USP with HIV-negative serology in an exam performed for a different reason. The data of the participants and a blood sample were collected in the Unit of Clinical Research of HCFMRP-USP. The study was approved by the Research Ethics Committee of HCFMRP-USP (protocol nº 3900/2011) and all subjects gave written informed consent to participate.

Anthropometric evaluation

Weight and height were measured and body mass index (BMI) was calculated as weight (kg) divided by height (m) squared and classified according to the World Health Organization (WHO, 2000).

Biochemical analyses

Blood samples were collected in the morning into 5 mL SST® II Advance® BD vacutainer® tubes after a 12 hour fast. The samples were then centrifuged in a Universal 320R Hettich® centrifuge for 10 minutes at 23ºC and at 3500 rpm. The sera obtained were stored at -20ºC until the time for analysis. The biochemical analyses were performed in the Multiuser Laboratory of Nutrition and Metabolism of HCFMRP-USP.

Serum iron and ferritin were determined for the evaluation of iron metabolism. Serum iron was determined by colorimetry using a Labtest® kit (Labtest Diagnóstico S. S., Lagoa Santa, Minas Gerais, Brazil) and the Spectro Max M5 apparatus of Molecular Devices®. Ferritin was determined by a solid phase chemiluminescent immunometric assay (IMMULITE®, DPC, Los Angeles, CA, USA).

The hepatic profile of the patients was evaluated based on the serum determination of the enzymes aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase by colorimetry using Labtest® kits and the Spectro Max M5 apparatus.

Insulin resistance was analyzed using the homeostasis model assessment–insulin resistance (HOMA-IR) index, calculated by the formula: fasting glycemia (mmol/l = mg/dl ÷ 18) x fasting insulinemia (µU/ml)/22.5 (STERN et al., 2005).

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Fasting glycemia was determined by colorimetry using Labtest® kits (Labtest Diagnóstico S. S.) and the Spectro Max M5 instrument of Molecular Devices®, and fasting insulinemia was determined by solid phase chemiluminescent immunometric assay using a comercial kit (IMMULITE®, DPC).

In order to determine the lipid profile, total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C) and triglycerides (TG) were measured by colorimetry using Labtest® kits and the Spectro Max M5 instrument of Molecular Devices®. Low-density lipoprotein cholesterol (LDL-C) was obtained from the combined TC, HDL-C and TG results using the Friedewald equation (CORDOVA et al., 2004; FEI et al., 2000).

Serum concentrations of tumor necrosis factor alpha (TNF-α) and C-reactive protein (CRP) were determined by solid phase chemiluminescent immunometric assay using a commercial kit (IMMULITE®, DPC).

Cardiovascular risk

The Framingham score was calculated for all patients in order to determine the probability of absolute risk of infarction and death within 10 years (XAVIER et al., 2013).

Statistical analyses

Statistical analyses were obtained in Statistical Package for the Social Sciences (SPSS), version 15. An exploratory analysis of the data was first carried out. The basic objective of this methodology is to synthetize a series of values of the same nature in order to obtain a global view of the variation of these values by organizing and describing the data with tables and graphs.

The nonparametric Kruskal-Wallis test, which does not require assumption about data distribution, was applied to compare the 3 groups (HIV+LIPO+, HIV+LIPO- and control). Partial Spearman correlations and its p value were used to summarize the association between cardiovascular risk and others variables when adjusting for age.

RESULTS

The 36 subjects studied, 12 women and 24 males, were divided into three groups of 12 subjects each. The sex distribution of the groups is presented in Table 1. Evaluation

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of nutritional status based on the BMI revealed that all groups had mean BMI values below 24.9 kg/m^2, i.e., within healthy limits (Table 1).

Among the three groups, age was significant different. The HIV+LIPO+ group had longer time of HIV infection and of HAART use (Table 1). Data regarding the biochemical profile of the groups are reported as mean ± standard deviation (SD) for each group (Table 2). Insulin, Glycemia HOMA-IR and TNFα values were higher for the HIV$^+$LIPO$^+$ group compared to Control group. Both HIV+LIPO+ and HIV+LIPO- had lower levels of HDL in contrast to the control group (Table 2).

The mean levels of the hepatic enzymes AST and ALT were inside reference values (YOUNG, 1987). There was difference between the HIV$^+$LIPO$^-$ and control groups for ALT. Mean alkaline phosphatase concentration was higher in the HIV$^+$ groups compared to control (Table 2).

Considering the biochemical iron markers, HIV-seropositive individuals of both groups had higher mean ferritin levels compared to control, with emphasis on mean ferritin concentration in the HIV$^+$LIPO$^+$ group, which was higher than the reference value (YOUNG, 1987). Mean serum iron, although lower in the HIV-seropositive groups compared to control, did not differ significantly between groups ($p>0.05$) (Table 2).

The absolute risk of infarction and death within 10 years was significant higher in the groups of HIV-seropositive individuals comparing to control group (Figure 1). The absolute risk of infarction and death within 10 years was positively correlated with ferritin and TNF-α and negatively correlated with HDL (Table 3).

DISCUSSION

The present study investigated the possible changes in glucide, lipid, hepatic and iron metabolism influenced by HIV infection and time of HAART use. The presence of inflammatory markers, cardiovascular risk and iron metabolism variables were also investigated in HIV-infected individuals with and without lipodystrophy.

Considering metabolic variables, in the present study, HOMA-IR, glycemia and insulin were higher in the HIV$^+$LIPO$^+$ group when compared to control group. This was expected like fact previous studies, insulin resistance is part of metabolic alterations present in lipodystrophy syndrome (MONTESSORI et al., 2004; NOOR et al., 2006; VASSIMON et al., 2011).

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Regarding the lipid metabolism, alterations were observed only in HDL concentrations. Studies emphasizes that the dyslipidemia associated with HIV infection with the use of HAART is characterized meanly by low serum HDL-C levels and increased TG levels, representing a lipid profile known to be atherogenic (MONTESSORI et al., 2004; PODZAMCZER, 2013).

Studies have shown that disturbances in iron homeostasis and anemia are associated with advanced HIV infection, in patients with aids disease (DRAKESMITH AND PRENTICE, 2008; WISAKSANA et al., 2013). To our knowedge, there have been no studies on the relationship between serum ferritin levels and HIV lipodystrophy. These results provide novel evidence of ferritin as predictive factor to cardiovascular risk in this group. The hiperferritinemia found in HIV+LIPO+ was correlated with higher Framingham score. Also TNFα, an inflammatory cytokine, was higher in this group. The presence of inflammatory cytokines and the activation of macrophages in an inflammatory process act directly on iron metabolism, with a consequent inhibition of erythropoiesis and a reduced bioavailability of iron reserves (WEISS AND GOODNOUGH, 2005). Patients with chronic infections, inflammations or neoplasias with persistent activation of the immunologic system, on a long-term basis develop anemia of chronic disease, which is characterized by reduced serum iron levels despite adequate reticuloendothelial iron reserves, with a reduction of total iron binding capacity and an increase in serum ferritin (WEISS AND GOODNOUGH, 2005). Important to highlight that higher levels of ferritin, TNF and cardiovascular risk was observed in assintomatic HIV individuals and not patients with aids. Since a while HIV infection have been considered as a chronic disease, frequently with no detectable viral load and higher levels of CD4 count, although metabolic alterations have been raised with alterations of glicemic and lipid metabolism. Some authors defined the HIV lipodystrophy as Metabolic Syndome of HIV (BERGERSEN et al., 2006; MARCASON, 2009). This could be associated with inflammation state of the individuals from the present study. Elevated serum ferritin concentrations have been implicated in the pathogenesis of many chronic inflammatory diseases including the metabolic syndrome (CHANG et al., 2013).

A study has confirmed that chronic diseases are accompanied by inflammatory processes and that the presence of inflammation can precede the future development of other diseases such as atherosclerosis. The inflammation reaction and the associated immunological response are the main events leading to the process of atherogenesis in conjunction with metabolic syndrome (YOUSUF et al., 2013). In the present study there was a clearly higher risk of infarction and death within 10 years inboth HIV-infected

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individuals. This result agrees with data from other studies which reported an increase of coronary artery disease in these group (HENRY et al., 1998; RICKERTS et al., 2000).

In HIV-infected patients taking HAART, dyslipidemia levels associated with increased risk of cardiovascular diseases occur in approximately 70% of cases (MONTESSORI et al., 2004). However, a study has shown that elevated CRP levels are associated with an increased risk of cardiovascular diseases even in the absence of hyperlipidemia (YOUSUF et al., 2013). The present study is limited by the small sample size.

In conclusion, our study shows that ferritin can be considered as inflammatory marker and to predict a higher cardiovascular risk. Therefore, our study is meaningful to provide evidence that HIV asymptomatic patients with lipodystrophy had anemia of chronic disease, with higher levels of ferritin and TNF-α and higher risk for cardiovascular diseases.

REFERENCES

ARSHAD, A., BANSAL, A., & PATEL, R. C. (2000). Cardiac complications of human immunodeficiency virus**.** Diagnostic and therapeutic considerations. **Heart Disease**, 2(2), 133-145.

BARBARO, G. (2002). Cardiovascular manifestations of HIV infection. **Circulation**, 106(11), 1420-1425.

BARRO, N. B., GUIMARÃES, C. M., & BORGES, O. S. (2012). Políticas de Saúde e Prevenção Ao HIV/AIDS No Brasil 1982-2012. **Estudos**, 39(4), 537-546.

BERGERSEN, B. M., SCHUMACHER, A., SANDVIK, L., BRUUN, J. N., & BIRKELAND, K. (2006). Important differences in components of the metabolic syndrome between HIV-patients with and without highly active antiretroviral therapy and healthy controls. **Scandinavian journal of Infectious Diseases**, 38(8), 682-689.

CHANG, J- S., LIN, S-M., HUANG, T-C., CHAO, J. C-J., CHEN, Y-C., PAN, W-H., et al. (2013). Serum ferritin and risk of the metabolic syndrome: a population-based study. **Asia Pacific Journal of Clinical Nutrition**, 22(3), 400-407.

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CORDOVA, C. M. M., SCHNEIDER, C. R., JUTTEL, I. D., & CORDOVA, M. M. (2004). Avaliação da dosagem direta do cholesterol-LDL em amostras de sangue de 10.664 pacientes em comparação com o uso da fórmula de Friedewald. **Arquivos Brasileiros de Cardiologia**, 83(6), 476-481.

DOURADO, I., VERAS, M. A., BARREIRA, D., BRITO A. M. (2006). Aids epidemic trends after the introduction of antiretroviral therapy in Brazil. **Revista de Saúde Pública**, 40: 9-17.

DRAKESMITH, H., & PRENTICE A. (2008). Viral infection and iron metabolism. **Nature Reviews. Microbiology**, 6(7), 541-552.

FEI, H., MAEDA, S., KIRII, H., FUJIGAKI, S., MAEKAWA, N., FUJII, H., et al. (2000). Evaluation of two different homoge-neous assays for LDL-cholesterol in lipoprotein-X-positive serum. **Clinical Chemistry**, 46(9), 1351-1356.

HENRY, K., MELROE, H., HUEBSCH, J., HERMUNDSON, J., LEVINE, C., & SWENSEN, L. (1998). **Severe premature coronary artery disease with protease inhibitors. Lancet**, 351(9112), 1328.

JOSHI, V. V., PAWEL, B., CONNORM E., SHARER, L., OLESKE, J. M., MORRISON, S., ET AL. (1987). Arteriopathy in children with acquired immune deficiency syndrome. **Pediatric Pathology**, 7(3), 261-275.

MARCASON, W. (2009). What does the term “HIV-associated lipodystrophy” mean? **Journal of the American Dietetic Association**, 109(2), 364.

MONTESSORI, V., PRESS, N., HARRIS, M., AKAGI, L., & MONTANER, J. S. G. (2004). Adverse effect of antiretroviral therapy for HIV infection. **Canadian Medical Association Journal**, 170(2), 229-238.

NOOR, M. A., FLINT, O. P., MAA, J. F., & PARKER, R. A. (2006). Effects of atazanavir/ritonavir and lopinavir/ritonavir on glucose uptake and insulin sensitivity: demonstrable differences in vitro and clinically. **AIDS**, 20(14),1813-1821.

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Volume 17, Número 4
ISSN 2447-2131
João Pessoa, 2017

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PATON, P., TABIB, A., LOIRE, R., & TETE, R. (1993). Coronary artery lesions and human immunodeficiency virus infection. **Research in Virology**, 144(3), 225-231.

PODZAMCZER, D. (2013). Lipid metabolism and cardiovascular risk in HIV infection: new perspectives and the role of nevirapine. **AIDS Reviews**, 15(4), 195-203.

RERKPATTANAPIPAT, P., WONGPRAPARUT, N., JACOBS, L. E., & KOTLER, M. N. (2000). Cardiac manifestations of acquired immunodeficiency syndrome. **Archives of Internal Medicine**, 160(5), 602-608.

RICKERTS, V., BRODT, H., STASZEWSKI, S., & STILLE, W. (2000). Incidence of myocardial infarctions in HIV-infected patients between 1983 and 1998: the Frankfurt HIV-cohort study. **European Journal of Medical Research**, 5(8), 329-333.

SANTOSH, H. N., NAGARAJ, T., & SASIDARAN, A. (2015). Anemia of chronic disease: A comprehensive review. **Journal of Medicine, Radiology, Pathology & Surgery**, 1, 13-16.

STERN, S. E., WILIAMS, K., FERRANNINI, E., DEFRONZO, R. A., BOGARDUS, C., & STERN, M. P. (2005). Identification of individuals with insulin resistence using routine clinical measurements. **Diabetes**, 54(2), 333-339.

SULLIVAN, J. L. (2007). Macrophage iron, hepcidin, and atherosclerotic plaque stability. **Experimental Biology and Medicine**, 232(8):1014-1020.

SUTINEN, J., & YKI-JÄRVINEN, H. (2007). Increased resting energy expenditure, fat oxidation, and food intake in patients with highly active antiretroviral therapy-associated lipodystrophy. American journal of physiology. **American Journal of Physiology. Endocrinology and Metabolism**, 292(3), 687-692.

VASSIMON, H. S., JORDÃO, A. A., ALBUQUERQUE DE PAULA, F. J., MACHADO, A. A., & MONTEIRO, J. P. (2011). Comparison of bioelectrical impedance with skinfold thickness and x-ray absorptiometry to measure body composition in HIV-infected with lipodistrophy. **Nutricion Hospitalaria**, 26(3), 458-464.

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ISSN 2447-2131
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Artigo

WEISS, G. AND GOODNOUGH, L. T. (2005). Anemia of Chronic Disease. **N Engl J Med**, 352(10), 1011-1023.

WHO (World Health Organization). (2000). **Obesity: Preventing and managing the global epidemic. Report of a WHO consultation on obesity**. Geneve - Suice.

WHO, World Health Organization. (2003). **Nutrient requirements for people living with HIV/AIDS Report of a technical consultation**. Geneve - Suice.

WISAKSANA, R., DE MAST, Q., ALISJAHBANA, B., JUSUF, H., SUDJANA, P., INDRATI, A. R., et al. (2013). Inverse relationship of serum hepcidin levels with CD4 cell counts in HIV-infected patients selected from an Indonesian prospective cohort study. **PLoS One**, 8(11), e79904.

XAVIER H. T., IZAR M. C., FARIA NETO J. R., ASSAD M. H., ROCHA V. Z., SPOSITO A. C. et al. (2013). V Diretriz Brasileira de Dislipidemias e Prevenção da Aterosclerose. **Arquivos Brasileiros de Cardiologia**, 101(4 Suppl 1), 1-20.

YOUNG, D. S. (1987). Implementation of SI Units for Clinical Laboratory Data. **Annais of Internal Medicine**, 106(1), 114-129.

YOUSUF, O., MOHANTY, B. D., MARTIN, S. S., JOSHI, P. H., BLAHA, M. G., NASIR, K., et al. (2013). High-sensitivity C-reactive protein and cardiovascular disease: a resolute belief or an elusive link? **Journal of the American College of Cardiology**. 62(5), 397-408.