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Hyperhomocysteinemia in human immunodeficiency virus-infected patients.

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Abstract

Objective: Evaluation of Hyperhomocysteinemia (HHCY) in HIV-infected patients.
Methods: Retrospective cohort study of risk factors for HHCY (>14 mmol/L) in patients infected with HIV for more than three years and treated, or not, with different combinations of HAART. Eighty cases were selected for this study.
Results: 46.3% (n=37) of HIV infected have HHCY. Hepatitis B or C co-infection and lower levels of Total Antioxidative System (TAS) are the most important factors associated with HHCY (OR=3.2, p=0.021 and OR=63.9, p=0.027, respectively). Although not statistically significant, the HHCY individuals have more cases of overweight and obesity, IP HAART scheme, lipodystrophy, and higher levels of Homa index. The sample with 2 criteria for Metabolic Syndrome (MS) and with MS, has significantly higher mean values of HCY compared to those without MS group.
Conclusions: The presence of hepatitis B or C co-infection and lower levels of TAS are the most important factors associated with HHCY in a group of patients infected with HIV, with or without HAART. Within this group, those with 2 criteria for SM or with SM have higher values of HCY.

Keywords: Risk factors, Hyperhomocysteinemia, HIV-infected, HAART.

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Introduction

Homocysteine (HCY) is a sulfur amino acid produced during conversion of methionine to cysteine. Once generated, HCY is either remethylated to methionine via remethylation pathway (by homocysteine methyltransferase or methionine synthase) or metabolized to cysteine via the transsulfuration pathway, controlling this way the blood concentration [1]. Hyperhomocysteinaemia (HHCY) has been recognised as an independent risk factor for cardiovascular diseases, stroke, hypertension and insulin resistance [2-6] and consequently to the Metabolic Syndrome (MS). HHCY can result from genetic mutations, nutritional deficiencies (vitamins B6, B12 and folate), hypothyroidism, some carcinoma, and the presence of some drugs which inhibit key enzymes in its metabolism (e.g. methotrexate, a potent inhibitor of folate reductase) [1,7,8].

Several mechanisms have been proposed to explain the association between HHCY and increased risk of cardiovascular disease, such as:

- Increased oxidative stress due to the production of Reactive Oxygen Species (ROS) resulting from HCY autoxidation [9] and interaction of ROS with vascular nitric oxide, blocking its vasoactive effects [6].
- Oxidation of LDLc [10], an important condition for the genesis of atheromatous plaques, stimulation of cell proliferation of vascular smooth muscle and other pathophysiological mechanisms of inflammatory nature [11].
- The increased propensity for thrombus formation resulting from endothelial damage, reduction of anticoagulant factors and increased aggregating platelet factors [12].

HIV infection also results in an increased risk of cardiovascular disease due to metabolic changes, especially in lipids (with an increase in triglycerides and LDLc), associated with HAART therapy, particularly in combinations involving protease inhibitors (PIs) [7,13]. The HIV infection can also change the non-traditional risk factors (or emerging) for coronary artery disease, including the aforementioned

inflammatory markers, coagulation factors, as well as apolipoproteins, lipoprotein-a (Lp (a)), the esterified fatty acids, and HCY [14,15]. At present, it seems undeniable that HIV infection increases oxidative stress in the host and this is an independent risk factor for cardiovascular disease [14]. Moreover, ROS derived from HCY and the infection itself, activate nuclear transcription factor NF-kB, contributing to power the replicative cycle of the virus [16].

Despite the conflicting results [5,17] some evidence suggests that there is a prevalence of HHCY, mild to moderate, in individuals infected with VIH [15]. Different pathophysiological processes may be behind this HHCY, including the effects of HIV infection and its replication, the antiretroviral agents and/or other medications administered, as well as decreased folate levels and/or B group vitamins [1]. The aim of this work is to identify the risk factors for HHCY in a population (general and separated according to the presence or not of metabolic syndrome) of patients infected for more than three years with HIV and treated, or not, with different combinations of HAART medications at the Hospital Joaquim Urbano (HJU), Oporto, Portugal.

Material and Methods

Sample selection

Data collection for this study took place between 2010 and 2012, after having been approved by the Ethical Committee of the Hospital, and corresponds to 80 Caucasian patients (total sample studied) diagnosed with HIV randomly selected from 173 patients followed at the HJU, to whom the homocysteine level was quantified, taking account the following inclusion criteria: a) HIV-positive, and b) the existence of data analysis results after at least 3 years of infection, with or without ARV treatment, for glucose, insulin, Total Cholesterol (TC), Triglyceride (TG), Viral Load (VL) and $CD4^+$ T-lymphocyte ($CD4^+$), Interleukin-6 (IL-6), high-sensitivity CRP (hsCRP), the Total Antioxidant Status (TAS), Manganese (Mn), and folic acid. Using the ATPIII guidelines [18], the selected sample was subdivided in three groups: one without Metabolic Syndrome (MS), another with 2 criteria for MS, and another one with MS.

Laboratory methods

Most of the blood tests were performed with an automated analyzer (Abbott®, ARQUITECH ci 8200). Measurements of glucose and lipid parameters (TC and TG) were made in serum by an enzymatic colorimetric method; insulin by a chemiluminescence microparticle immunoassay; hsCRP by an immunoturbidimetric method; and folates by chemiluminescence. HCY has been determined by fluorescence polarization immunoassay using AXSYM analyser and HCY <14 µmol/L were considered as reference normal values [19]. The final HOMA Index (HI) has been calculated through the equation: [(µU/mL of insulin × mg/dL of glucose)/405] [20]. The TAS has been determined in plasma with the commercial kit TAS (Randox Lab. Ltd UK) according to Miller et al. [21] adapted to automated analyzer Architect® ci8200, Abbott. The determination of Interleukin-6 (IL-6) has been made by electrochemiluminescence Roche Diagnostic device in the Elecsys 2010 and Mn has been determined in whole blood by plasma-mass spectrometry.

The quantification of $CD4^+$ T-cells has been performed in venous blood collected in EDTA, by flow cytometry (Coulter®, EPICS® XL-MCL Flow cytometry System) with final determination by fluorimetry. The method of quantification of VL in plasma has been VERSANT® HIV-1 3.0 (bDNA) test, through the amplification of the signal emitted by the nucleic acid, using the System 340 bDNA analyzer (Bayer®). The remaining data (sex, age, BMI, AIDS phase, lipodystrophy, HCV/HBV co-infection and therapeutic scheme) has been obtained from patients clinical processes. The BMI has been evaluated in routine hospital consultations during the last year of the follow-up. Lipodystrophy (including facial and limb lipoatrophy and/or abdominal and cervical lipo-accumulation) has been assessed through clinical observation. Lipodystrophy and the viral co-infections (HBV and HCV) were assumed to be present if diagnosed during the follow-up period. B group vitamins were not measured because the blood samples had to be prepared soon after harvesting or having a special conservation, which was not possible.

Statistical methods

Categorical variables were expressed as counts and percentages and continuous variables as mean (± standard deviation) or median and interquartile range, unless otherwise specified. Continuous variables have been compared between groups using t-test (for normally distributed variables) or the Mann-Whitney U-test (for non-normally distributed variables). The difference or association between categorical variables has been assessed by the Chi-square test or the Fisher's exact test (when criteria for using the Chi-square test were not found).

After univariate analysis, the stepwise binary logistic multivariate regression model (Wald backward stepwise method, p=0.05 for covariate inclusion and 0.2 for exclusion) has been performed to identify independent factors associated with the HCY >14 µmol/L outcome. The models validity has been evaluated through the determination coefficients of Cox and Snell, NagelKerke, and the Hosmer and Lemeshow test. Furthermore, it has been assessed using the area under the Receiver Operating Characteristic (ROC) curve (AUC).

All reported probability values are two-tailed, and $P < 0.05$ have been considered statistically significant. Analyses were performed with the IBM®SPSS® Statistics software package version 21.0 (IBM Corporation, USA).

Results

The characterization of our samples (total sample studied and sample groups according to HCY cut-off) is depicted in table 1 and corresponds to results obtained after at least 3 years of HIV infection, with or without HAART. All patients are

Caucasian and have been diagnosed with HIV between 1988 and 2008.

Table1. *Clinical and biological characterization of the sample and with or without homocysteine >14 µmol/L.*

Variable	Total study population (n=80)	Population according to homocysteine cutoff		p*
		HCY<14 µmol/L (n=43)	HCY>14 µmol/L (n=37)	
Gender, Male, n (%)	61 (76.3)	35 (81.4)	26 (70.3)	0.244
Age (at the end of follow-up), med (IQR)	44.0 (38.8-50.3)	44.0 (37.5-55.0)	44.0 (39.0-52.6)	0.737§
Follow-up time (years), mean ± SD	9.5 ± 4.6	9.7 ± 4.4	9.4 ± 4.8	0.824®
Body mass index (Kg/m^2), n (%)				
Underweight (<18.5)	1 (1.3)	1 (2.4)	0 (0)	0.598
Normal weight (18.5-24.9)	39 (52.0)	22 (53.7)	17 (50.0)	
Overweight (25.0-29.9)	27 (36.0)	16 (39.0)	11 (32.4)	
Obesity (>30.0)	8 (10.7)	2 (4.9)	6 (17.6)	
HAART scheme:				
NRTI+NNRTI, n (%)	35 (43.8)	23 (53.5)	12 (32.4)	0.167
NRTI+PI, n (%)	36 (45.0)	16 (37.2)	20 (54.1)	
HAART-naïve group, n (%)	9 (11.3)	4 (9.3)	5 (13.5)	
Lipodystrophy, n (%)				
Yes	6 (42.9)	2 (28.6)	4 (57.1)	0.592ε
No	8 (57.1)	5 (71.4)	3 (42.9)	
AIDS, n (%)				
Yes	22 (78.6)	11 (73.3)	11 (84.6)	0.655ε
No	6 (42.9)	4 (57.1)	2 (28.6)	
Hepatitis C or B co-infection#, n (%)	30 (37.5)	11 (25.6)	19 (51.4)	0.018
Viral load (103 RNA /mL), med (IQR)	0.015 (0.015-0.08)	0.015 (0.015-0.11)	0.015 (0.015-0.035)	0.915§
CD4$^+$ count (cell/mL), mean ± SD	543.1 ± 289.7	553.7 ± 288.7	530.3 ± 294.5	0.723®
Folate (ng/mL), med (IQR)	8.2 (7.4-11.0)	8.4 (6.8-9.9)	8.0 (7.9-14.6)	0.227§
Total cholesterol (mg/dL), mean ± SD	186.6 ± 44.5	190.4 ± 47.6	182.2 ± 40.9	0.416®
Triglycerides (mg/dL), mean ± SD	143.4 ± 93.3	147.7 ± 85.9	138.4 ± 102.3	0.660®
Glucose (mg/dL), mean ± SD	89.8 ± 21.6	86.8 ± 17.4	93.2 ± 25.5	0.193®
Homa, mean ± SD	3.5 ± 5	3.0 ± 4.4	4.1 ± 5.6	0.340®
PCRus (mg/dL), med (IQR)	2.1 (0.9-3.7)	1.8 (0.7-6.5)	2.1 (1.0-2.9)	0.533§
IL-6 (pg/mL), med (IQR)	2.9 (1.8-4.3)	3.4 (1.8-5.1)	2.7 (1.7-3.6)	0.144§
TAS (mmol/L), med (IQR)	1.95 (1.87-2.01)	1.96 (1.91-2.02)	1.91 (1.78-1.98)	0.017§
Mn (µg/L), mean ± SD	8.4 ± 3.0	8.5 ± 2.3	8.3 ± 2.4	0.565®

n (%): number (percentage), med (IQR): median (Inter-Quartile Range), SD: Standard Deviation #Includes those patients who have at least one of the hepatitis. HAART: Highly Activity Anti-Retroviral Therapy, NRTI: Nucleotide Reverse Transcriptase Inhibitors, NNRTI: Non Nucleotide Reverse Transcriptase Inhibitors, PI: Proteases inhibitors 1: Chi-square test-in the case of BMI, with two BMI cathegories: Underweight or Normal weight (<25) and Overweight or Obesity (>25) §: Mann-Whitney Test, ε: Fisher test, ®: Student t-test, *Comparation of HCY<14 and HCY>14 populations

The HCY overall level was 15.0 ± 4.8 µmol/L, ranging from 7.28 to 32.17 µmol/L. From the total sample studied, 46.3% (n=37) had HCY >14 mmol/L (HHCY) (Table 1). In univariate analyses, we have noticed that the group of patients with

HHCY has significantly more cases of hepatitis B or C co-infection and lower values of TAS compared to the group of patients with HCY <14 mmol/L (19 vs 11 cases, p=0.018 and 1.91 vs 1.96 mmol/L, p = 0.017, respectively) (Table 1). Although not statistically significant, the HHCY sample has also a greater number of individuals with overweight and obesity (50.0% vs 43.9), IP HAART scheme (54.1 vs 37.2%), lipodystrophy (57.1 vs 28.6%), and higher levels of Homa index (4.1 vs 3.0) (Table 1).

In multivariate binary logistic regression analysis, gender, HAART scheme, coinfection, folato, glucose, IL-6, and TAS entered the model in the first step, but only hepatitis C or B co-infection and TAS were significant independent risk factors associated with HCY >14 µmol/L (OR=3.2 and p=0.021; and OR=63.9 and p=0.027, respectively) (Table 2). Hepatitis B or C co-infection, BMI and TAS (although the latter not significant) are the most important risk factors for HCY > 14 mmol/L in the sample without MS (Table 2). In the sample with 2 criteria for MS and MS the unique risk factor (although not significant) is hepatitis B or C co-infection. Although not significant (p=0.195), the sample with MS and with 2 criteria for MS have higher mean values of HCY comparing to the sample without MS (Table 3).

Table 2. *Multivariate bynary logistic regression for independent factors associated with homocysteine (HCY) >14 µmol/L for total study population, population without metabolic syndrome, and population with metabolic syndrome and with 2 criteria for metabolic syndrome.*

Sample	Independent factors associated with HCY >14 µmol/L	p	OR	95%CI
Total (n=80)*	Hepatitis C or B co-infection (yes)#	0.021	3.2	1.2-8.6
	TAS (1 unit decrease)	0.027	63.9	1.6-2523.4
Without MS (n=54)**	Hepatitis C or B co-infection (yes)#	0.017	5.7	1.4-23.5
	BMI (1 unit increase)	0.018	1.3	1.1-1.6
	TAS (1 unit decrease)	0.069	115.3	0.7-19232.4
With MS and with 2 criteria for MS (n=26)***	Hepatitis C or B co-infection (yes)#	0.135	3.7	0.7-20.2

MS: Metabolic Syndrome, **TAS:** Total Antioxidant Status #includes those patients who have at least one of the hepatitis *AUC (area under curve)=0.746 (95%CI: 0.637-0.855) **AUC (area under curve)=0.776 (95%CI: 0.652-0.901) ***AUC (area under curve)=0.657 (95%CI: 0.432-0.882)

Table 3. *Homocysteine concentrations according to metabolic syndrome classification.*

Sample	n	HCY (µmol/L) mean ± SD	p*
Without MS	54	14.5 ± 4.8	0.195
With 2 criteria for MS	11	17.4 ± 5.1	
With MS	15	15.0 ± 4.7	

MS: metabolic syndrome, n: number, mean ± SD: mean ± Standard Deviation,*ANOVA

Discussion

The main purpose of our study was that the presence of hepatitis B or C co-infection and lower levels of TAS are the most important factors associated with HHCY in patients infected with HIV, with or without HAART (Tables 1 and 2). Roca et al. [22] and Raiszdeh et al. [23] also found a positive relationship between co-infection with HCV and increased levels of HCY. This may be explained by the fact that hepatic transsulfuration pathway get even more constrained due to liver damage caused by this co-infection [1]. Roca et al. [22] found that in addition to co-infection, a significant relationship with a family history of cardiovascular disease and hypertension, suggesting a genetic component in the changes of HCY metabolism and BMI in addition to the known relationship with low values of folic acid and vitamin B12 [23]. In our study, the group with HHCY also seems to shows more cases of overweight and obesity (50.0% vs 43.9%) (Table 1) although this risk factor for HHCY may only be significantly relevant in the group without MS (Table 2). This may be related to the small size of the sample with MS.

Such as in our study, in which the reduction of TAS is associated with HHCY, several authors also associate HIV infection and HHCY to increased production of ROS, thus contributing to an oxidative stress environment [9,14,24]. The decrease in TAS will be mainly the consequence of chronic inflammation caused by HIV infection and the autoxidation of higher levels of HCY [1]. Although not completely conclusive, some studies have associated increased HCY values with HAART [15,25]. Bongiovanni et al. [26] also found higher values of HCY in the treatment group compared to the untreated one, but concluded that only age and low folate concentrations were significantly associated with HHCY. In our study, we have found a relationship, although not significant, between HHCY and PIs treatment.

Raiszadeh et al. [23] studied a female population of 249 HIV infected and 127 healthy women and concluded that 16.9% and 13.4% had HHCY, respectively. In HIV infected group, there was a significant association between HHCY and age, serum creatinine, low values of $CD4^+$, and vitamin B12. In our study, the group of HHCY showed also lower CD4 values, though not significant. In our sample the presence of HHCY was 46.3%, higher than that found in this study, which is probably related to the small size of our sample and because it was mainly

based in men and aged people, factors that are, in general, associated with higher values of HCY.

Guaraldi et al. [27] studied the prevalence of MS in a group of HIV patients on therapy, combining traditional risk factors (age, BMI, diabetes, hypertension), with insulin resistance (through the HOMA index) and plasma HCY, which makes it consistent with our study. They have concluded that HCY levels were higher in individuals who had MS. In our study higher HCY levels were present in individuals with high risk of MS (with 2 criteria for MS) (Table 3). These higher values were mainly found in individuals showing high blood pressure, high HOMA index and TG, obesity and large waist circumference, finding no direct association of HCY with HAART [27].

Unfortunately our study suffered of almost no access to clinical data of cardiovascular disease. However our results as well as in other studies show that HIV-infected patients have an increased risk of cardiovascular disease (inflammation, HHCY, coinfection, and BMI) [22,23,27]. The known two-way relationship with mutual influence between markers of inflammation and markers directly associated with heart disease, allow us to conclude/assume that these patients, add the particularities and synergism of infection factor, co-infection, therapy, and HHCY (independent risk factor for cardiovascular disease), an increased risk of cardiac pathologies compared to the uninfected ones.

Another limitation was the size of our sample. The fact that we needed to have HCY values having only 80 available tests, has significantly reduced the number of cases studied. The fact that we have not detected other risk factors for HHCY (besides those two which have been reported) may be a result of insufficient data. These reported conclusions should be considered as provisional and more work is needed to determine if these estimates may be widely, consistently, and usefully assumed.

Conclusion

We have found that after having followed up along 3 years a group of patients infected with HIV-with or without HAART-the presence of hepatitis B or C co-infection and lower levels of TAS may be considered the most important factors associated with HHCY. Within this group, those with 2 criteria for SM or with SM have higher values of HCY, so there is an association between oxidative stress and HHCY along with other changes in metabolic pathways that directly influence MS (dyslipidemia and insulin resistance) and consequently, cardiovascular disease. So it is important to intervene counteracting the inflammatory mechanism base by treating co-infections, increasing $CD4^+$ values, lowering the VL and having a diet (or supplement) rich in trace elements, for instance, in oligoelements, which are important cofactors of antioxidant enzymes.

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