ELSEVIER Clinica Chimica Acta 247 (1996) 181–187

Short communication

Increased plasma thiobarbituric acid-reactive substances (TBARS) before opportunistic infection symptoms in HIV infected individuals

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Received 27 July 1995; revision received 21 November 1995; accepted 27 November 1995

Keywords: Thiobarbituric acid-reactive substances; Oxidative stress; Human immunodeficiency virus; Opportunistic infection

1. Introduction

Several groups have provided evidence for oxidative stress during HIV infection, even at the early asymptomatic stages of the disease [1–4]. This condition is characterized by an imbalance between oxidant and anti-oxidant systems, in which oxidative processes are predominant due to immunocompetent and phagocytic cell activation and tumor necrosis factor (TNF) secretion [5]. Intracellular oxidation which induces cell activation and viral replication (including that of HIV) through the activation of a nuclear factor, NFκB, has been described [6]. Among the available markers for oxidative stress detection, we used a thiobarbituric acid-reactive substances (TBARS)

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0009-8981/96/$15.00
SSDI 0009-8981(95)06249-D

assay to evaluate plasma lipid peroxidation [7]. Other oxidative stress markers were also monitored: vitamin E, erythrocyte superoxide dismutase, erythrocyte and plasma glutathione peroxidase and plasma selenium. In previous studies, these markers indicated oxidative stress in HIV-infected individuals compared with healthy seronegative donors [4,8]. We wished to determine whether or not there was a correlation between variations in oxidative stress markers and clinical events such as opportunistic infections.

2. Patients and methods

Sixty-five HIV-seropositive patients (58 male and 7 female), 25–45 years old, were enrolled in this study, which lasted from 1990 to 1993 (Pitié-Salpêtrière General Hospital in Paris). They had clinical and biological status evaluations, with a mean number of five determinations of oxidative stress markers. Patients were divided into two groups (Table 1): (1) 23 HIV-seropositive individuals with no associated infection during the 3-year period of follow-up. These patients were considered as 'asymptomatic' in our study; (2) 42 HIV-seropositive individuals with at least one associated infection during the study. These were considered as 'symptomatic' patients. The different infections that occurred during the study included 23 cases of oral candidiasis, 6 of oral herpes, 4 of genital herpes, 4 of brain toxoplasmosis, 3 of herpes zoster, 2 of tonsillitis, 2 of tuberculosis, 1 of otitis, 1 of pneumocytosis, 3 of bronchitis and 3 of Kaposi Sarcoma. Patients may have had multiple infections during their follow-up.

Treatment was implemented according to disease progression and laboratory results: antiretroviral treatment, opportunistic infection prevention,

Table 1

Clinical CDC stage of the patients at their entrance into the study (note that 29 'symptomatic' patients were in A1, A2 or A3 stage, i.e. without any opportunistic infections at the entrance into the study, but opportunistic infections occurred during the 3-year follow-up of these patients)

CDC	A1	A2	A3	B1	B2	B3	C3
CD4 (/mm^3)	>500	200–499	<200	>500	200–499	<200	<200
No. of 'asymptomatic' patients	7	12	4	0	0	0	0
No. of 'symptomatic' patients	5	14	10	0	8	4	1

vitamin E and selenium which were administered according to recommendations for cancer prevention [9]. Vitamin E and selenium assays were useful to monitor this antioxidant therapy, since these markers were dependent on supplementation. Therefore, their values were not taken into account to evaluate the oxidative stress status and were not reported in the results of this study.

Peripheral blood was collected in lithium heparin containing tubes (Vacutainer®, Becton-Dickinson) and centrifuged at 4°C within 30 min of sampling. Erythrocytes were washed three times with 0.15 mol/l NaCl, then plasma and washed erythrocytes were frozen at −80°C until measurement. Plasma TBARS were determined by the Yagi spectrofluorometric method with a spectrofluorometric measurement (λexc.: 515 nm, λem.: 548 nm) [7]. We calculated minimal and maximal mean values for plasma TBARS by taking into account, for each patient, the lowest and highest values obtained during follow-up. Erythrocyte superoxide dismutase (SOD) activity was measured using the colorimetric method of Hyland from a 1:10 dilution of hemolysates [10]. Erythrocyte and plasma glutathione peroxidase (GPx) were evaluated by spectrophotometry at 340 nm according to the Beutler method with *t*-butyl-hydroperoxide as substrate [11]. For each of these markers, we noted the values corresponding to the samples which had the minimal and maximal values of TBARS, as defined above. Steady-state normal values were initially determined in 30 healthy individuals (15 males, 15 females) with ages ranging from 20 to 40 years. Statistical analysis of results was performed with the Student *t*-test.

3. Results and discussion

As an example, Fig. 1 represents TBARS values obtained for one asymptomatic and one symptomatic patient during follow-up. It should be noted that in the latter patient, high TBARS values coincide with the onset of an opportunistic infection. Fig. 2 shows the plasma TBARS concentration for all patients during longitudinal follow-up over a 3-year period. In 30 seronegative donors, plasma TBARS were levelling at a mean value of 0.90 ± 0.30 μmol/l (mean ± S.D.) [4]. The mean maximal value for TBARS in symptomatic patients was significantly higher than in asymptomatic patients: respectively, 2.73 ± 0.94 μmol/l versus 1.64 ± 0.35 μmol/l ($P < 0.001$). Conversely, the mean minimal TBARS value in symptomatic patients (1.47 ± 0.34 μmol/l) was not significantly different from that of asymptomatic patients (1.48 ± 0.36 μmol/l). Nevertheless, these minimal values were significantly higher than the mean value found in HIV-seronegative donors ($P < 0.001$). Moreover, the variation in the range of TBARS concentrations was far more important in symptomatic than in asymptomatic pa-

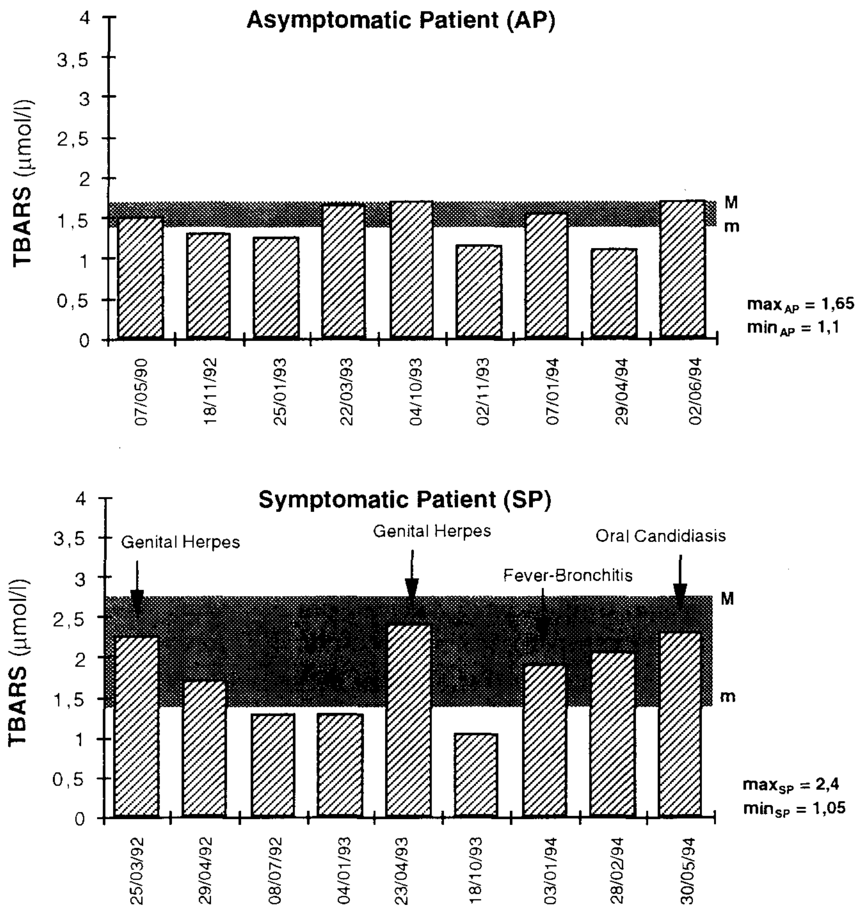

Fig. 1. Example of longitudinal follow-ups of an asymptomatic patient and a symptomatic patient: the shaded area behind the bars represents the mean values (m for minimal and M for maximal) for the entire cohorts of asymptomatic and symptomatic patients.

tients. In fact, the difference between TBARS mean maximal concentration and TBARS mean minimal concentration was 1.26 ± 0.93 μmol/l in symptomatic patients and 0.18 ± 0.14 μmol/l in asymptomatic patients ($P < 0.001$). These results indicate that plasma TBARS concentrations did not vary extensively in asymptomatic individuals but increased significantly in patients with opportunistic infections. This increase of TBARS preceded or was concomitant with the onset of opportunistic infection. The more important enhancements in plasma TBARS were observed in the four cases of

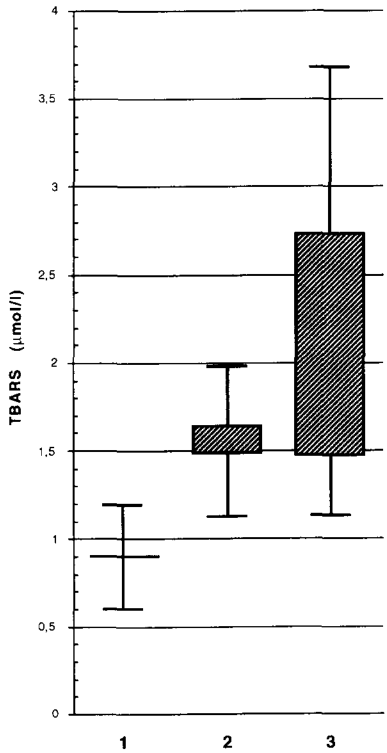

Fig. 2. Longitudinal follow-up of 65 seropositive patients over a 3-year period (plasma TBARS values in comparison with those from seronegative healthy donors). 1: plasma TBARS mean value in healthy seronegative donors; 2: minimal and maximal mean values with corresponding standard deviation for 23 HIV-seropositive patients without any associated infection symptom during the 3-year period; 3: minimal and maximal mean values with corresponding standard deviation for 42 HIV-seropositive patients with at least one associated infection during the 3-year period.

cerebral toxoplasmosis (mean maximal value: 4. 18 ± 2.75 μmol/l) and occurred 1–3 months before the clinical symptoms. TBARS decreased after antitoxoplasmosis treatment. As regards SOD and GPx (reported in Table 2) from samples corresponding to maximal and minimal TBARS concentrations (mean ± S.D.), no significant differences were observed between patients with or without associated infection. Moreover, no statistically significant correlation was observed in the 65 patients, within the symptomatic or asymptomatic patient subpopulations, between TBARS values and other oxidative stress parameters. On the other hand, no correlation between TBARS (or the other oxidative stress markers) and CD4 count was observed.

In seropositive asymptomatic patients, an increase in TBARS has already

Table 2
Superoxide dismutase (SOD) and glutathione peroxidase (GPx) activities (mean ± S.D.) for samples corresponding to minimal (m index) and maximal (M index) TBARS concentrations, in 65 HIV-seropositive patients over a 3-year period (NS, non-statistically significant difference between symptomatic and asymptomatic patients)

	Erythrocyte SOD (μg/g Hb)		Erythrocyte GPx (units/g Hb)		Plasma GPx (units/l)		Plasma TBARS (μmol/l)	
Steady state values (controls[a])	816 ± 258		26 ± 4		334 ± 41		0.90 ± 0.30	
TBARS	m	M	m	M	m	M	m	M
Asymptomatic patients ($n = 23$)	725 ± 280	709 ± 191	25.1 ± 9.2	24.7 ± 6.7	281 ± 39	281 ± 50	1.48 ± 0.36	1 ± 0.30
Symptomatic patients ($n = 42$)	753 ± 209	710 ± 182	27.2 ± 8.0	25.5 ± 7.3	269 ± 42	267 ± 45	1.47 ± 0.34	2.73 ± 0.94
Significance	NS	NS	NS	NS	NS	NS	NS	$P < 0.001$

[a]Seronegative donors, $n = 30$.

been described [4,12]. The reason for the TBARS increase, which usually correlates with lipid peroxidation, remains to be determined. In brain toxoplasmosis, the necrosis of nervous tissues could partially account for this increase, since nervous tissues are particularly rich in lipids. Several research groups have underlined a correlation between oxidative stress and HIV replication [6,13]. The follow-up of plasma TBARS, even if they are considered as non-specific markers of oxidative stress, could allow for early detection and treatment of opportunistic infections in HIV-infected individuals. In addition, the determination of oxidative stress as an indicator of potential cell activation and viral replication could help in the definition of antioxidant molecule combinations in the treatment of HIV infection.

References

[1] Halliwell R, Cross CE. Reactive oxygen species, antioxidants and acquired immunodeficiency syndrome: sense or speculation? Arch Intern Med 1991;151:29–31.

[2] Revillard JP, El Habib R, Dupuy JM. Oxidative stress and HIV infection. In: Girard M, Valette L, eds. Retroviruses and related diseases. Proceedings of the Cinquième Colloque des Cent Gardes, 29–31 October 1990. Fondation Mérieux, Lyon, France, 1990;5:299–305.

[3] Staal JT, Ela WS, Roederer M, Anderson MT, Herzenberg LA. Glutathione deficiency and human immunodeficiency virus infection. Lancet 1992;339:909–912.

[4] Coutellier A, Bonnefont-Rousselot D, Delattre J et al. Stress oxydatif chez 29 sujets séopositifs. Resultats à deux ans d'une étude en double insu diéthyldithiocarbamate versus placebo. Presse Méd 1992;21:1809–1812.

[5] Jarstrand C, Akerlund B. Production of oxygen radicals by PMN of HIV infected patients. Winter Research Conferences of CERLIB, Les Deux Alpes, 12–23 January 1993 CHW, Grenoble, France.

[6] Schreck R, Rieber P, Rauerle PA. Reactive oxygen intermediates as apparently widely used messengers in the activation of NFκ3 transcription factor and HIV 1. EMBO J 1991;10:2247–2258.

[7] Yagi K. A simple fluorometric assay for lipoperoxide in blood plasma. Biochem Med 1976;15:212–216.

[8] Malvy DJM, Richard M-J, Arnaud J, Favier A, Amédée Manesme O. Relationship of plasma malondialdehyde and antioxidant micronutrients to HIV-1 seropositivity communication. Clin Chim Acta 1994;224:89–94.

[9] Watson RR, Leonard TK. Selenium and vitamins AEC nutrients with cancer preventious properties. J Am Diet Assoc 1986;86:505–510.

[10] Hyland K, Voisin E, Banoun H, Auclair C. Superoxide dismutase assay using alkaline dimethylsulfoxide as superoxide anion-generating system. Anal Biochem 1983;135: 280–287.

[11] Beutler E. Glutathione peroxidase. In: Beutler E, ed. Red cell metabolism. Grüne and Stratton, New York, 1975; 71–73.

[12] Sonnerborg A, Carlin U, Akerlund B, Jarstrand C. Increased production of malondialdehyde in patients with HIV infection. Scand J Infect Dis 1988;20:287–290.

[13] Staal FJT, Roederer M, Herzenberu LA, Herzenberg LA. Intracellular thiols regulate activation of nuclear factor kB and transcription of human immunodeficiency virus. Proc Natl Acad Sci USA 1990;87:9943–9947.