Low serum thiol levels predict shorter times-to-death among HIV-infected injecting drug users

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Objectives: To investigate whether serum thiol levels are altered by HIV disease, and whether low serum thiols predict time to death among HIV-infected injecting drug users (IDU).

Design: A cross-sectional study of serum thiol levels among 13 HIV-seronegative IDU, 116 HIV-seropositive IDU, and 17 HIV-seropositive IDU with a history of AIDS, and a cohort study of the 133 HIV-infected IDU who took part in the cross-sectional study.

Methods: Subjects were recruited from a methadone-maintenance treatment program during 1990–1991. Total serum thiols were determined spectrophotometrically at enrolment; low serum thiols were defined as those with an absorbance at 412 nm ≤ 0.46. Deaths through 31 December 1993 were determined from the National Death Index (NDI). Twenty-six HIV-seropositive subjects died during follow up; death certificates, which were obtained for 23 subjects, indicated AIDS or HIV infection for 20. Product-limit estimation was used to calculate survival. Multivariate analyses employed Cox proportional-hazards regression.

Results: Analysis of cross-sectional data showed that serum thiols did not differ significantly among HIV-free subjects, HIV-infected subjects, and HIV-infected subjects with a history of AIDS. Cohort analysis, adjusted for age, revealed that persons with low serum thiols had a significantly increased hazard of death compared with those with high serum thiols (relative hazard = 2.83; 95% confidence interval (CI), 1.15, 6.97); a significant interaction between low serum thiols and a history of AIDS was associated with a relative hazard of 5.65 (95% CI, 1.22, 26.1).

Conclusions: Among HIV-infected persons, low serum thiols, especially in concert with a history of AIDS, predict mortality risk. These findings support the hypothesis that oxidative stress is critical to the pathogenesis of HIV infection.

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Introduction

The hypothesis has been advanced that oxidative stress is critical to the pathogenesis of HIV infection, suggesting that serologic markers of oxidative stress might predict prognosis among HIV-infected individuals [1]. It has been demonstrated previously [2] that reactive oxygen intermediates can induce expression of HIV-1 in the Jurkat human T-cell line. In a short-term study of AIDS patients, total plasma thiol groups increased along with CD4+ counts during the course of antioxidant supplementation therapy [3]. Glutathione and other antioxidants also have been reported to be depleted during HIV infection [4]. An association of HIV infection with reduced levels of acid-soluble thiols (cysteine) has been shown both in HIV-1-infected patients [5] and in macaques infected experimentally with HIV [6].

A significant role for oxidative stress in HIV progression is indicated by the finding that progressively increased plasma concentrations of catalase are found in patients with increasingly more severe disease [7]. Catalase catalyzes decomposition of hydrogen peroxide to oxygen and water. Hydrogen peroxide arises from reactive oxygen intermediates, including superoxide anion and hydroperoxy radical. Neutrophil populations from AIDS patients have elevated numbers of giant cells with high myeloperoxidase levels [8]. Upon activation, neutrophils respond with oxidative burst (a part of the respiratory burst) during which this enzyme is released from neutrophil granules concurrent with generation of superoxide and hydrogen peroxide [9]. Myeloperoxidase catalyzes hydrogen peroxide-mediated oxidation of chloride ions to hypochlorite, a potent oxidizing bactericidal agent. Recently, the interaction of hypochlorite with superoxide was shown to result in production of hydroxyl radicals, the most powerful oxidants [10]. All of these findings point to the potential for a pronounced oxidative stress in HIV disease. In the present study, we have investigated the associations of serum thiols with HIV disease and with time-to-death among HIV-infected injecting drug users (IDU).

Methods

Subjects were 133 HIV-seropositive and 13 HIV-seronegative IDU aged 23 to 62 years (median = 38 years) enlisted from a methadone-maintenance treatment program at a New York City municipal hospital during the period from December 1990 to December 1991. The study population was 22% female and 78% male; it was 24% white, 29% black and 47% Hispanic. Blood was obtained by venipuncture and stored at –70°C.

Cross-sectional analysis compared serum thiol levels among the 13 HIV-seronegative IDU and the 133 HIV-seropositive IDU, including 116 HIV-seropositive without a history of AIDS and 17 HIV-seropositive with a history of AIDS. A cohort analysis of time-to-death focused on the 133 HIV-seropositive subjects who were followed through 31 December 1993. Mortality was determined from a search of the National Death Index (NDI). Matches were found for 26 subjects (19.5%). For 23 (19 males, four females) of the 133 HIV-infected subjects, death certificates were obtained from the state where death was recorded; for the present analysis the other three presumed decedents were censored (i.e., withdrawn alive) on the date of

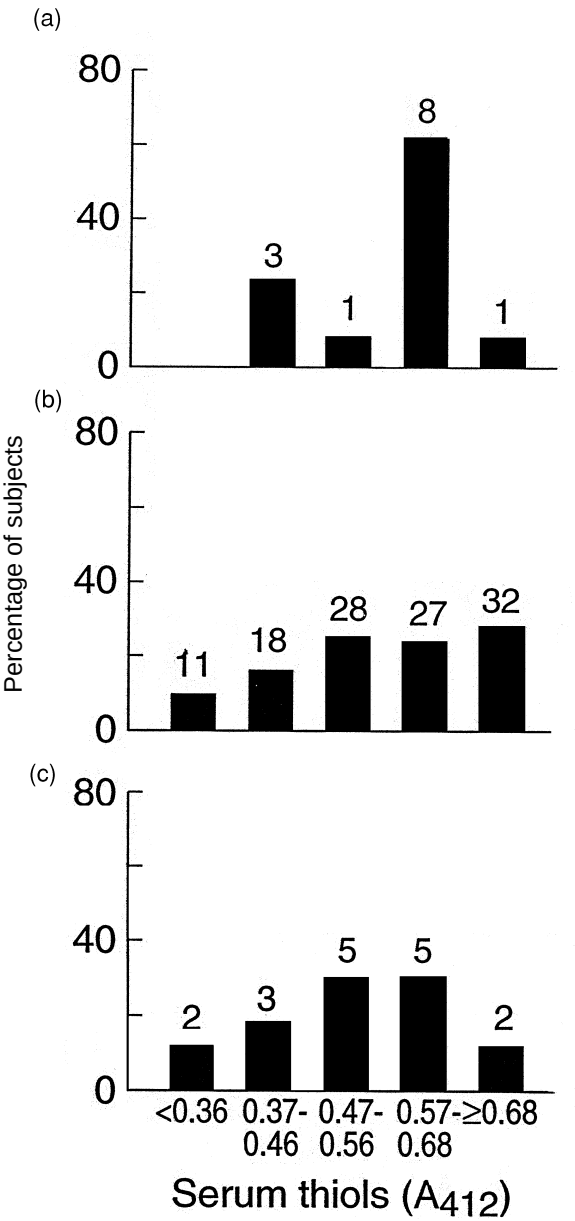

Fig. 1. Distributions of serum thiols measure (absorbance at 412 nm) among HIV-seronegative subjects (a), HIV-seropositive subjects (b) and HIV-seropositive subjects with a history of AIDS (c).

death as indicated in the NDI search. None of the 23 certified deaths was from traumatic injury; 20 of the 23 death certificates listed HIV infection and/or AIDS as a factor contributing to the causes of death.

Total serum thiols were assayed in a 1 : 4 dilution of serum in water (1 ml total) to which 30 μl of 5,5′-dithio-bis-2-nitrobenzoic acid (DTNB, Ellman's Reagent: 9.5 mg DTNB solution dissolved in 1 ml of 0.1M K_2HPO_4, 17.5 mM EDTA, pH 7.5) was added. The reaction mixtures were incubated at room temperature for 1 hour, at which time the absorbance at 412 nm (A_{412}) was measured. Total serum thiols were calculated by subtraction of reagent blank values from the values of total DTNB-reactive thiols.

Product-limit estimation was used to calculate survival [11]. The Mantel–Haenszel log-rank test was used to assess the evidence for differences in time-to-death. Multivariate analyses employed Cox proportional-hazards regression. The proportional-hazards model provides estimates of the relative mortality hazard conferred by a given set of covariates adjusted for all other covariates in the regression equation. The relative hazard can be interpreted as an adjusted relative mortality risk, taking account of differences in follow-up time.

Results

The distributions of serum thiol levels among HIV-seronegative subjects, HIV-seropositive AIDS-free subjects and HIV-infected subjects with AIDS are shown in Fig. 1. Mean serum thiol levels, as indicated by A_{412}, were similar in the three groups (HIV-seronegative patients' mean = 0.58; HIV-seropositive/AIDS-free patients' mean = 0.57, and HIV-infected/AIDS patients' mean = 0.49. These differences were not statistically significant [analysis of variance (ANOVA), F = 1.44, $P = 0.24$]. The histograms shown in Fig. 1, however, suggest a shifting of the distribution downward from HIV-seropositive/AIDS-free patients to HIV-seropositive patients with a history of AIDS.

Additional analyses considered only HIV-infected subjects with or without histories of AIDS. Among these subjects, all of whom had histories of injecting drug use, there were no significant differences in serum thiol levels associated with gender or frequency of drug injection (Table 1). There was a striking and highly significant inverse association between serum thiol levels and age, however. After correction for age, there were no differences in serum thiol levels according to race.

Mortality rates among HIV-seropositive subjects increased as A_{412} decreased. Annualized death rates/100 persons were 4.59 among those with $A_{412} > 0.46$; 14.3 among those with $0.36 < A_{412} \leq 0.46$; and 13.0 among those with $A_{412} \leq 0.36$. When A_{412} was dichotomized at 0.46, the relative mortality risk associated with $A_{412} \leq 0.46$, unadjusted for confounders, was 2.97 [95% confidence interval (CI), 1.36, 6.47] compared to $A_{412} > 0.46$. Survival at 2 years was 93.9% among subjects with $A_{412} > 0.46$ compared with 76.5% among those with $A_{412} < 0.46$ (Fig. 2, logrank $P = 0.007$).

Table 2 shows estimated relative hazards from two multivariate regression models, intended to adjust for potential confounders of the relation of serum thiols to mortality risk. Model A is the best-fitting parsimonius main-effects-only model. This model shows that the age-adjusted relative mortality risk conferred by low serum thiols was 3.8 for $A_{412} \leq 0.46$. The model deviance (2 × the model log-likelihood) was 207.5, giving a likelihood-ratio statistic for the model of 10.7 (3 degrees of freedom, $P = 0.014$).

Model B includes terms for effect modification by prior AIDS diagnosis. Low serum thiols increased mortality

Table 1. Serum thiol levels in HIV-infected injecting drug users.

	n	Mean	95% CI	Range	*P*-value
Gender					
Males	104	0.55	0.52–0.59	0–0.93	0.24*
Females	29	0.59	0.53–0.66	0–0.92	
Injection frequency					
≥ 1 x /day	11	0.55	0.44–0.66	0.12–0.86	0.88*
< 1 x /day	78	0.56	0.52–0.60	0–0.93	
Race (age corrected)					
White	31	0.52	0.46–0.57		0.22 (F = 1.52†)
Black	40	0.52	0.46–0.59		
Hispanic	62	0.60	0.56–0.65		
Age (years)					
< 30	14	0.71	0.64–0.79§		0.0001 (F = 7.49‡)
30–39	70	0.57	0.53–0.61‖		
40–49	42	0.52	0.47–0.57		
≥ 50	7	0.37	0.25–0.50		

*Student t test. †Analysis of covariance. ‡Analysis of variance. §Significantly different from other groups at $P < 0.05$. ‖Significantly different from ≥ 50 years group at $P < 0.05$.

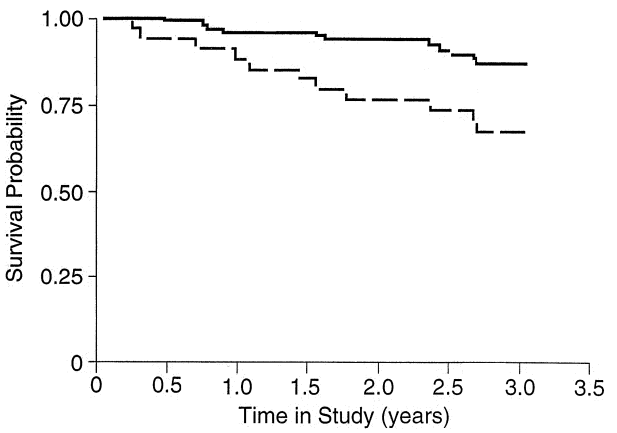

Fig. 2. Survival, estimated by product-limit method, among 133 persons with total serum thiols measure (absorbance at 412 nm; A_{412}) dichotomized at $A_{412} \leq 0.46$ (---) versus $A_{412} > 0.46$ (—).

risk in two ways. As before, the main effect was seen at $A_{412} \leq 0.46$. Additionally, there was an interaction of low serum thiol levels with prior AIDS diagnosis. The relative hazard of death, adjusted for age, was 2.83 (95% CI, 1.15, 6.97) among persons still free of AIDS with $A_{412} \leq 0.46$ compared with those free of AIDS with $A_{412} > 0.46$. For individuals already diagnosed with AIDS, the age-adjusted risk of death was 5.65 times higher in those with $A_{412} \leq 0.46$ compared with those with $A_{412} > 0.46$ (95% CI, 1.22, 26.1). This means that an individual with $A_{412} \leq 0.46$ who had been diagnosed with AIDS had about 16 times the risk of death ($2.83 \times 5.65 = 15.99$) of an HIV-seropositive person of the same age with $A_{412} > 0.46$ and no history of AIDS.

Discussion

These results show that low serum thiols are associated with increased mortality risk among IDU infected with HIV. Since serum thiol levels correlate inversely with oxidative stress, these data support the hypothesis linking oxidative stress with progression of HIV disease [1]. Importantly, in the preliminary analysis here, the effect of low serum thiols on mortality risk was stronger in persons already diagnosed with AIDS than in HIV-infected individuals without AIDS. Our analysis did not adjust for potential confounding by the degree of immune dysregulation because of the unavailability of appropriate markers of infection maturity (e.g., viral load or CD4+ cell counts). Further analysis should take account of these markers and consider potential effect modification of the serum thiols–mortality link by infection maturity.

Table 2. Multivariate proportional-hazards regression models of the relationship between low serum thiols measure ($A_{412} \leq 0.46$) and mortality risk in HIV-infected injecting drug users.

Covariate	Relative hazard*	95% CI
Model A		
History of AIDS	2.59	0.92, 7.32
Low serum thiols ($A_{412} \leq 0.46$)	3.76	1.59, 8.91
–2 log (likelihood) = 207.5		
Model B		
Low serum thiols ($A_{412} \leq 0.46$)	2.83	1.15, 6.97
History of AIDS x low serum thiols	5.65	1.22, 26.06
–2 log (likelihood) = 206.1		

*Adjusted for age. CI, Confidence interval.

In addition, our results reveal an unexpected age-related alteration in serum thiol levels. There was a statistically significant decrease in serum thiol levels with age among HIV-infected IDU (Table 1). We do not know whether this response is unique to subjects in this study or is characteristic of all HIV-infected IDU. It is likely to be not simply a characteristic of aging populations, *per se*, however. Analysis of serum thiol levels among a cohort of 63 non-HIV infected, non-IDU (36 male, ages 31–69 years; 27 female, ages 25–88 years) showed no age-related decrease in serum thiol levels (data not presented).

A recent report has provided further evidence to support our findings [12]. In this study, the acid-soluble thiols glutathione and cysteine were reduced significantly in the plasma of HIV/AIDS patients compared with seronegative controls. This reduction was attributed to increased oxidative stress.

Several caveats should be recognized. First, the data reported here were from a relatively small number of IDU in a methadone-maintenance program in New York City. Thus, inferences should not be generalized to IDU subject to different mortality risks (e.g., in jails or hospitals), or to those not in treatment or those in non-urban environments. Second, serum thiol levels were measured at only one time point. Therefore, the potential effect of intra-individual variability on serum thiol values cannot be stated. For the same reason, we could not assess the prognostic utility of serum thiol levels when considered as a time-varying covariate. Third, follow-up times were relatively short (~2.6 years of observation/person) so long-term effects of covariates could not be studied.

These data suggest that low serum thiol levels affect HIV progression both before and after AIDS diagnosis, and that this effect is magnified after AIDS onset. In the studies reported here, data on AIDS diagnosis were by self-report only. Further investigations should be carried out on individuals whose viral load levels or serial CD4+ counts are known independently and for whom validated information on disease status is available. Finally, the simplicity of the serum thiol assay

combined with its minimal invasiveness suggest that it would be worthwhile to determine whether levels of serum thiols correlate well with therapy and whether alterations in serum thiol patterns are reversible.

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