Lipid Oxidation Kinetics in Hemodialysis Patients With and Without History of Myocardial Infarction

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Abstract

Background: In lipid oxidation kinetics studies, prevalent cardiovascular disease has been associated with shortened lag phase, the length of time preceding the onset of oxidation.

Objectives: To examine, *in vitro*, copper-induced lipid oxidation kinetics in unfractionated serum from hemodialysis patients and to determine differences in kinetic parameters between patients with and without a history of CVD.

Methods: Of the 76 patients enrolled in a study of oxidative stress in hemodialysis (44/76 with prevalent CVD, 53/76 males), 9 males with a history of myocardial infarction were selected and matched for age, diabetes and smoking status with 9 males from the non-CVD group. The kinetics of lipid oxidation was studied. Blood chemistry determinations including serum lipids, lipoproteins, hemostatic factors and serum malondialdehyde were obtained. Variables were compared using the *t*-test for independent samples with history of MI entered as the categorical variable.

Results: Tmax, the oxidation kinetic parameter defined as the time at which the rate of absorbing product accumulation was maximal, was significantly shorter in dialysis patients with a history of MI than in those without (115.2 $\pm$ 38.5 vs. 162.7 $\pm$ 48.9 minutes, $P = 0.04$). Further, Tmax and MDA were negatively correlated to one another ($r = -0.47$, $P = 0.04$). Odds ratios indicate that each 1 minute increase in Tmax was associated with a 3% decrease in odds that a subject had a history of MI.

Conclusions: These findings indicate the presence of increased oxidative stress in hemodialysis patients with a history of MI.

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Patients on chronic hemodialysis treatment have an age-adjusted mortality rate 3.5–4 times that of the general population [1]. This excess mortality is largely accounted for by increased cardiac mortality, which is estimated to be 5–20 times that in the general population [2].

In the general population, oxidized low density lipoprotein has been implicated in the pathogenesis of cardiovascular disease. Oxidized LDL has been demonstrated in atherosclerotic plaques in both humans and animals [3]. The titer of autoantibodies to an epitope of oxidized LDL (malondialdehyde) has been shown to be an independent risk factor for carotid atherosclerosis, an association as strong as that of smoking and serum LDL concentrations [4]. Furthermore, angiographically demonstrated atherosclerosis was shown to be inversely correlated to oxidation lag phase (a marker of LDL susceptibility to oxidation) in humans [5].

LDL oxidation kinetics studies in hemodialysis patients have yielded conflicting results. Panzetta et al. [6], and independently, Maggi et al. [7], reported enhanced LDL susceptibility to peroxidation in hemodialysis patients compared to controls, as measured by decreased *in vitro* lag time. Conversely, Sutherland et al. [8] found that the maximum rate and extent of LDL oxidation were significantly lower in patients with renal disease compared to healthy controls, and proposed increased oleic acid and decreased linoleic acid LDL content as a mechanism to explain this observation. Schulz and colleagues [9] found that LDL oxidation lag time was not shortened in comparison to healthy controls despite leukocyte activation; moreover, lag time was actually prolonged at the end of cellulose acetate and cuprophane dialysis.

LDL oxidation kinetics parameters have been compared between healthy persons and dialysis patients to make inferences about CVD pathogenesis in the hemodialysis group [7]. Loughrey et al. [10], however, observed abnormal LDL composition in hemodialysis patients, and proposed that oxidation kinetics studies in this group may be highly dependent on the method used to induce oxidation. If so, comparisons of LDL oxidation kinetics parameters between hemodialysis patients and healthy controls for the purposes of hypothesis testing in the area of CVD pathogenesis may be of

CVD = cardiovascular disease
MI = myocardial infarction
MDA = malondialdehyde

LDL = low density lipoprotein

limited usefulness. Comparing hemodialysis patients with and without prevalent CVD is another approach to explain excess cardiac morbidity and mortality in this patient group, because risk factor identification requires demonstration of its ability to discriminate for prevalent CVD within this population. The present study examined lipid oxidation kinetics parameters in unfractionated serum in a group of hemodialysis patients with a documented history of myocardial infarction compared to hemodialysis patients without MI history, matched for age, gender, and smoking status.

Methods

Study population

The population for this clinic-based cross-sectional study represents a subgroup (n=18) of 76 adult maintenance hemodialysis patients reported on earlier [11]. In the original population, all adult maintenance dialysis patients with a documented medical history of CVD (n=44) were identified from the entire hemodialysis population treated in the Department of Nephrology at Wolfson Medical Center, Holon, Israel. CVD was defined as documentation in the medical record of a history of one or more of the following: MI, ischemic stroke, angina pectoris, transient cerebral ischemia, or peripheral vascular disease (not including the arteriovenous fistula). The presence in the patient medical record of hospitalization and/or treatment for one or more of the aforementioned conditions was followed by the location of supporting diagnostic criteria including electrocardiographic, angiographic, biochemical (enzyme alterations) and surgery dates. These patients were matched for gender, age (in 5 year categories) and smoking status (present smoking, yes/no) with hemodialysis patients from the same center with no evidence of CVD in the medical record (n=32).

From this population, nine male patients with a history of MI only were identified for the lipid oxidation kinetics study. MI was defined as documentation in the medical record of at least two of the following: pain of typical intensity and duration, enzyme elevation (at least twice the upper normal laboratory value), or diagnostic electrocardiographic changes. Nine males from the non-CVD group matched for age and smoking status were selected as controls.

All the included hemodialysis patients had received a minimum of 12 hours per week of maintenance dialysis therapy (three treatments of 4 hours each) for a minimum of 3 months prior to the study. Dialysis was performed in all patients on AK100 dialyzers by Gambro (Lund, Sweden) using Nipro Sureflux (Osaka, Japan) cellulose triacetate hollow-fiber dialyzer filters sized 1.1 or 1.5 m^2. Participants had no known history of malignancy, were not insulin-dependent, had no active liver disease, and were not seropositive for hepatitis B virus, hepatitis C virus or human immunodeficiency virus. All participants signed a statement of informed consent and the study was approved by the Helsinki Committees at Wolfson Medical Center, Tel Aviv University, and the Israel Ministry of Health.

Blood chemistry

All plasma and serum determinations were carried out on samples taken immediately prior to the midweek dialysis session. Fasting (14 hour) blood was drawn through antecubital venipuncture into a syringe. The tourniquet was released immediately after blood began to enter the syringe, thus avoiding venous stasis. Approximately 14 ml of blood were removed from the syringe to separation gel-containing test tubes with no additive for chemistry and lipoprotein analysis. Serum was separated by centrifugation within 2 hours of being drawn and was stored at 4°C for not more than 2 days before analysis. The following determinations were made on samples according to standard protocol at the Biochemistry Laboratory of the Wolfson Medical Center: glucose, phosphorus, creatinine, urea, albumin, potassium, calcium, uric acid, total cholesterol, high density lipoprotein cholesterol, and triglycerides. All analyses were carried out using products by Boehringer-Mannheim, GmbH (Mannheim, Germany), and analyzed on a Hitachi 704 autoanalyzer. Serum intact parathyroid hormone level was determined in the hospital's Endocrinology Laboratory using the Nichol method. LDL cholesterol levels were calculated using the Friedewald equation. The following determinations were made in the laboratory of the hospital's Institute of Physiologic Hygiene: apolipoprotein A-1, apolipoprotein B and fibrinogen were measured using kits by Boehringer-Mannheim; tissue plasminogen activator, plasminogen activator inhibitor, and factor VII were measured using the Asserachrom kits from Diagnostica Stago (Asnieres Sur-Seine, France).

Serum malondialdehyde

To determine serum MDA levels, approximately 7 ml of blood were removed from the syringe to a foil-wrapped test tube with no additive. Blood was immediately taken to the Biochemistry Laboratory where centrifugation at 1,500 g for 10 minutes at 4°C was carried out, after which serum was removed for MDA determination. This was performed spectophotometrically with 2-thiobarbituric acid solution. After boiling in a water bath and cooling, the absorbance was measured at 532 nm. The concentration of lipid peroxides in plasma was expressed in terms of MDA, with 1,1,2,2,-tetramethoxypropane as standard (nanomolar).

Lipid oxidation kinetics

For each participant, 1 ml of serum obtained for MDA analysis was frozen at -70°C until analysis. The kinetics of lipid oxidation was studied spectrophotometrically on thawed samples, as described by Schnitzer et al. [12]. Briefly, 30 µl serum was added to 1.5 ml of sodium phosphate-buffered solution containing 720 µM sodium citrate, in 1 cm quart cuvettes. Oxidation was then monitored at 37°C using a Kontron double beam spectrophotometer (Uvikon 933) equipped with a 12 position automatic sample changer. At time zero, freshly prepared $CuCl_2$ solution was added to a final concentration of 100 M and the formation of reaction products was monitored continuously from the ultraviolet absorption at 245 nm. The kinetics of oxidation was characterized by three parameters: a) ODmax, defined as the maximal accumulation of oxidation products (measured in OD units); b) Vmax, defined as the maximal rate of accumulation of absorbing products (expressed in OD units per minute); and c) Tmax, defined as the time at which the rate was maximal. Odmax was obtained directly from the OD

measured 5 hours after $CuCl_2$, whereas the other two factors were determined from the first derivative of the OD vs. time (i.e., from the time dependence of the rate). The "lag" preceding lipid oxidation, as derived from the intercept of the tangent at the point of maximal accumulation with the time axis, has been reported to strongly correlate with Tmax [13]. Oxidation kinetic parameters in hemodialysis patients were also compared to those of a laboratory reference population of age-matched males 6–12 months post-recovery from MI.

Measurement of hemodialysis adequacy

The delivered dose of dialysis was described as the fractional clearance of urea as a function of its distribution volume (Kt/V) and was determined using the Kt/V natural logarithm formula. The slow-flow sampling technique was used, in which the blood pump was slowed to 50 ml/min and, after 50 seconds had elapsed, blood was drawn from the arterial sampling port closest to the patient.

Statistical analysis

Data were stored on spreadsheet using Microsoft Excel 97 Hebrew Edition software (Microsoft Corporation, Seattle, WA, 1985-97). Data analysis was carried out on SPSS for Windows, Version 9.0 (SPSS Inc., Chicago, IL, 1999). Descriptive statistics are reported as mean ± standard deviation for biochemical data, years of dialysis treatment, age, and lipid oxidation kinetics parameters. Frequency counts and cross-tabs by MI history were obtained for dialyzer filter size, smoking status and diabetes. The *t*-test for independent samples was used to detect differences in biochemical and lipid oxidation kinetics parameters by MI history. One-way analysis of variance (ANOVA) with Bonferroni's comparison of means was used to simultaneously compare means of Tmax in hemodialysis patients with and without history of MI to laboratory reference values. The Fisher exact test was used to detect differences in smoking, filter size and diabetes by MI history. Pearson's correlation coefficients were calculated to describe associations between lipid oxidation kinetics parameters, biochemical variables, age, and years of hemodialysis treatment. The Spearman rank test was used to detect correlations between parameters of lipid oxidation kinetics and MI history, diabetes, and filter size. Odds ratios with 95% confidence intervals for history of MI were calculated.

Table 1. Characteristics of study population by history of myocardial infarction

	No history of MI (n=9)	Previous MI (n=9)
Variable		Mean
Age (yrs)	64.9 ± 6/5	62.7 ± 13.1
Kt/V	1.3 ± 1.3	1.6 ± 0.4
Years of dialysis treatment	4.6 ± 5.8	3.4 ± 2.5
Selected blood chemistry		
Albumin (mg/dl)	4.1 ± 0.34	4.1 ± 0.4
Calcium (mg/dl)	9.6 ± 0.4	8.3 ± 2.9
Creatinine (mg/dl)	8.7 ± 1.8	9.3 ± 1.8
Glucose (mg/dl)	112.3 ± 32.7	107.1 ± 29.3
Potassium (mg/dl)	5.2 ± 0.7	4.97 ± 0.6
Phosphorus (mg/dl)	6.6 ± 1.6	6.5 ± 2.1
Parathyroid hormone (pg/ml)	107.7 ± 89.2	171.6 ± 137.6
Urea (mg/dl)	156 ± 28.9	149.4 ± 41.7
Uric acid (mg/dl)	5.9 ± 1.8	5.7 ± 1.3
Lipids, lipoproteins, hemostatic factors		
Total cholesterol (mg/dl)	158.1 ± 29.7	169.5 ± 33.8
HDL cholesterol (mg/dl)	28.1 ± 5.6	33.7 ± 4.5
LDL cholesterol (mg/dl)	98.5 ± 19.3	106.04 ± 25.4
Triglycerides (mg/dl)	157.9 ± 95.8	148.9 ± 75.9
Apolipoprotein A (mg/dl)	89.9 ± 8.6	96.7 ± 9.5
Apolipoprotein B (mg/dl)	74.1 ± 12.9	77.5 ± 13.3
Hemostatic factors		
Factor VII (%)	88.5 ± 22.2	92.8 ± 30.8
Fibrinogen (mg/dl)	343.8 ± 45.2	327.4 ± 62.8
Plasminogen activator inhibitor 1 (g/dl)	30.9 ± 19.6	27.8 ± 2.9
Tissue plasminogen activator (g/ml)	5.7 ± 4.8	5.5 ± 4.1
Blood pressure		
Diastolic (mmHg)*	77.5 ± 5.9	77.3 ± 8.1
Systolic (mmHg)*	122.5 ± 15.6	138.2 ± 25.1
Oxidative stress		
Malondialdehyde (nmol/ml)	2.5 ± 0.2	2.8 ± 0.8
Maximum optical density (ODmax)	0.47 ± 0.2	0.4 ± 0.1
Maximum time (Tmax)**	162.7 ± 48.9	115.2 ± 38.5
Maximum velocity (Vmax)	0.0041 ± 1.50E–03	0.0044 ± 1.10E–03

Data presented as mean ± SD

* Measured at the end of the hemodialysis treatment; all other variables were measured prior to the midweek treatment

** $P = 0.038$. Hypothesis testing was carried out using the Mann-Whitney U; all tests are significant at $P < 0.05$.

Results

Of the 18 subjects in this study, only 1 was diabetic (non-insulin dependent), and this person was in the group with no MI history. Five patients smoked (present smoking, yes/no): two with no MI history and three with MI history. Seven patients in each group were dialyzed using the 1.1 m^2 filters and two in each group used the 1.5 m^2 filters. Fisher's exact test indicated that patients did not differ by MI history on prevalent diabetes, smoking status or filter size.

The characteristics of the 18 male study participants by MI history are described in Table 1. As can be seen, participants with and without a history of MI differed significantly only by Tmax. Table 2 compares lipid oxidation kinetics parameters in dialysis patients with and without MI history to those of a laboratory reference population. Tmax is elongated in dialysed patients without MI history compared to the reference values, while Tmax in dialysed patients with MI history is similar to laboratory reference levels. Figure 1 shows the lipid oxidation kinetics for one dialysed patient with and one without MI history. At time zero, $CuCl_2$ solution was added to unfractionated serum and absorbance was monitored at 245 nm as a function of time. Marked are Tmax, Vmax and ODmax.

Tmax was associated with MI history (r = -0.5, P = 0.03). Additionally, a strong, inverse association between Tmax and MDA

Table 2. Means of measured kinetic parameters by MI history status and compared to laboratory reference population

Kinetic parameter	MI history Mean ± SD	No MI history Mean ± SD	Laboratory reference population Mean ± SD
Tmax (min)	115 ± 39*	163 ± 49*	110 ± 10
Vmax (OD/min)	0.0036 ± 0.001	0.0041 ± 0.002	0.0045 ± 0.002
Odmax (OD units)	0.43 ± 0.11	0.469 ± 0.15	0.5 ± 0.1

* $P = 0.03$. No other means were significantly different from one another

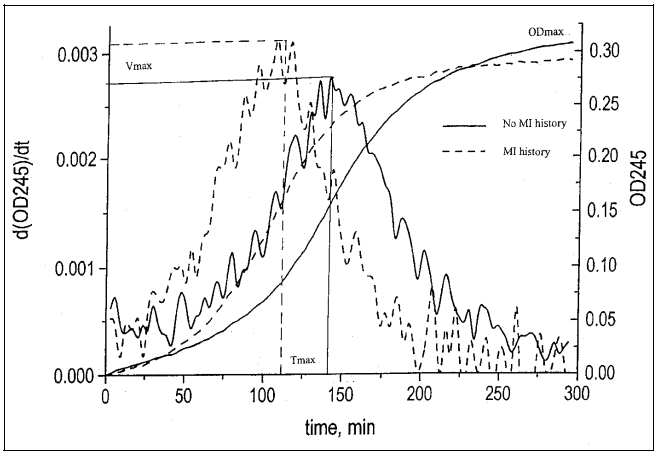

Figure 1. Lipid oxidation kinetics for one patient with and one without MI history

was observed ($r = -0.5$, $P = 0.03$). In this population, MDA was not significantly associated with MI history ($r = 0.24$, $P = 0.3$).

Each 1 minute increase in Tmax was associated with a non-significant 3% decrease in the odds of an individual having a history of MI (OR 0.97, 95%CI 0.94–1.0, $P = 0.07$). MDA did not significantly predict a history of MI in this sample (OR 2.2, 95%CI 0.5–10.5, $P = 0.3$).

Discussion

In the present study, Tmax was found to be prolonged in hemodialysis patients compared to the laboratory standard population. This is consistent with findings by Schulz and co-workers [9] who observed that LDL oxidation lag time was not shortened or was even prolonged in dialysis patients compared to controls. In a study of females only, Westhuyzen et al. [14] detected no significant differences between dialysed patients and controls with regard to lag time, propagation phase or maximal rate of oxidation. Similarly, the present study, carried out in males only, did not detect significant differences between dialysed patients and controls with regard to ODmax or Vmax. These observations appear to suggest that hemodialysis patients have decreased susceptibility to lipid peroxidation compared to controls. Hemodialysis patients have been reported to have higher LDL densitometric analysis scores, implying smaller LDL particle size, than controls, and triglyceride enrichment of very low density lipoprotein and LDL has been demonstrated in dialysis patients [15]. These alterations in LDL size and composition are associated with increased susceptibility to oxidation; however, they have been shown to confer no change in oxidation kinetics parameters in other patient groups characterized by increased oxidative stress, such as diabetics [16]. Elevated LDL oleic acid content has been observed in dialysis patients compared to controls [8], and LDL oleic acid content has been shown to be negatively correlated to the rate of *in vitro* LDL oxidation [17]. Another possible explanation for these observations is that serum nitrite/nitrate concentrations, which have been shown to be elevated in dialysed patients compared to controls [18], may have inhibited *in vitro* oxidation. This finding has been reported in other patient populations with elevated nitric oxide levels, including Bartter's and Gittleman's syndromes [19]. Finally, in a study of copper-induced plasma oxidation kinetics, Spranger and associates [20] noted that plasma levels of sulfhydryl groups correlated negatively with oxidizability. This finding is meaningful in the context of uremia and its concomitant sulfuric acid accumulation characteristic of hemodialysis patients [21], especially since samples were drawn prior to the midweek dialysis session.

It cannot be concluded from these findings that dialysis patients are in less oxidative stress than controls. First, other markers of oxidative stress – including serum MDA, advanced glycation end-products, advanced oxidation protein products, and antioxidant enzyme systems – have all been shown to be altered in dialysis patients [22–25]. Second, *in vitro* oxidation kinetics parameters in dialysed patients have been shown to be sensitive to the method of induction used [10].

To make inferences about potential risk factors for CVD in dialysis patients, however, the more meaningful comparison is between dialysis patients with and without a history of CVD. In the present study, only Tmax discriminated for MI history in adult hemodialysis patients. The strong, inverse association between Tmax and MDA further implies the presence of enhanced oxidative stress in hemodialysis patients with a history of MI. This study lacked the power to detect differences in other variables by MI history. However, in a previous study using the full study population (n=76) a power study was performed, the results of which suggested that "traditional" risk factors simply did not discriminate for prevalent CVD in this population [11].

The odds ratio for Tmax suggested that increasing Tmax reduced the likelihood that a study participant was in the MI history group. Small sample size contributed to the wide confidence intervals in this case, but the trend observed is consistent with findings in non-dialysis populations; specifically, that lag phase is shortened in patients with a history of cardiovascular disease [5].

The present study indicates that markers of oxidative stress, such as shortened Tmax, are more pronounced in hemodialysis patients with a history of MI than in those without such a medical history. These findings are consistent with but do not prove the hypothesis that excess CVD in dialysed patients is due to oxidative stress. Like any cross-sectional study, the present study cannot detect time sequence and thus cannot determine whether oxidative stress preceded or resulted from MI. Inferences about causality cannot be made on the basis of the observations reported here.

OR = odds ratio
CI = confidence interval

Nevertheless, these finding indicate an association between history of MI and oxidative stress in hemodialysis patients that merits exploration in a prospective design.

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