

Original Article

Does hepatitis C increase the accumulation of advanced glycation end products in haemodialysis patients?

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Abstract

Background. Hepatitis C may cause increased levels of oxidative stress that contribute to accumulation of advanced glycation end products (AGEs), which increase the risk of cardiovascular disease (CVD). The aim of this study was to determine the influence of hepatitis C on AGE accumulation in haemodialysis patients.

Methods. AGE accumulation was measured by means of skin autofluorescence (AF) in 92 haemodialysis (HD) patients and 93 age-matched healthy controls. In the HD patients, CVD-related biochemical variables were also measured. The HD patients were tested for hepatitis C virus (HCV) antibodies and allocated to a HCV+ or HCV− group.

Results. Skin AF of the healthy subjects was lower than skin AF in the HD patients (3.13 ± 0.95 vs 2.2 ± 0.47 ; $P < 0.001$). We calculated the average increase of skin AF in the healthy subjects to be 0.017 arbitrary units per year, being 14 times lower than in HD patients with CVD only and 20 times lower than in HD patients suffering from combined CVD and diabetes mellitus (DM). Multivariate regression analysis showed that AGE accumulation in HD patients can be described by the independent effects of age, DM, CVD and HD vintage. Although inter-cellular adhesion molecule 1 and liver enzymes were elevated in HCV+ HD patients, levels of oxidative stress markers and skin AF were not significantly different between HCV+ and HCV− HD patients.

Conclusions. AGE accumulation was higher in the HD patients than in the healthy controls. AGE accumulation did not differ in HCV+ and HCV− HD patients. This might be due to the fact that hepatitis C did not cause oxidative stress in our HD population. Independent markers of AGE accumulation were age, HD vintage, DM and CVD, but not hepatitis C.

Keywords: advanced glycation end products; cardiovascular disease; haemodialysis; hepatitis C; skin autofluorescence

Introduction

The close interaction between renal and cardiovascular function makes patients who suffer from chronic renal disease 10–20 times more susceptible for developing cardiovascular disease (CVD) in comparison to a healthy population [1, 2]. Haemodialysis (HD) treatment of end-stage renal disease (ESRD) patients even increases this risk, for example by the HD-related blood–material interactions, haemodynamic instabilities and initiation of anaemia. Because of the high prevalence of CVD in HD patients ($\pm 40\%$), CVD has become the major cause of hospitalization of HD patients, thus reducing the life expectancy of these patients dramatically [3]. High prevalence and high incidence of cardiovascular mortality in HD patients are of multi-factorial origin. Disturbances in the balance between oxidants and antioxidants, the carbohydrate and lipid metabolism, the immuno-inflammatory system and malnutrition are all thought to play a major role in a cascade of organ dysfunction resulting in patient death [4].

Another complicating factor in the HD treatment is the high prevalence of hepatitis C virus (HCV) infection among the HD population. It is estimated that the worldwide prevalence of HCV is around 3% or 200 million people [5], whereas the percentage of HCV+ patients in the HD population is much higher than in the general population and can vary from 5% to 25% in the USA and Western Europe and up to 80% in the Middle East [6]. The association between the prevalence of HCV and overall mortality of HD patients has been proven by others [7–9]. In a meta-analysis study, HD patients with HCV appeared to have an adjusted relative risk of all-cause mortality of 1.34 (1.13–1.59) [10]. Apart from this finding, it should be noted that the relation between CVD and HCV in HD patients is still not fully established, as some researchers had contradictory findings [11–14].

AGEs are formed during glycaemic and oxidative stress, with increased levels in diabetes mellitus (DM), renal failure and chronic inflammatory conditions [15]. AGE accumulation affects the blood vessels and is connected with the development of CVD [16]. Skin autofluorescence (AF) is a non-invasive method to estimate the AGE accumulation in the body [17], which has shown to be a strong non-invasive CVD risk marker in HD patients [18], and has therefore been used in the present study. The aim of this study was to determine the influence of hepatitis C on AGE accumulation and other cardiovascular risk parameters in HD patients. Furthermore, we assessed other factors that possibly influenced AGE accumulation in our patient population.

Methods

Ninety-two patients with ESRD were enrolled in a clinical cross-sectional study on AGE accumulation and CVD risk assessment in HD patients. The study protocol was approved by an ethics committee, and written informed consent was obtained from each patient. The following exclusion criteria were used: <3 months on HD; acute illness or hospitalization 3 weeks prior to the start of the study; neoplasm; kidney transplantation.

The patients were assigned to one of the two groups: HCV+ and HCV-. The determining criterion for the presence of hepatitis C were the anti-hepatitis C virus antibodies measured by an enzyme-linked immunosorbent assay (ELISA) Ortho HCV 3.0 SAvE kit (Ortho Diagnostics, Amersham, UK) as part of the routine blood analysis of the HD patients done on 6-week intervals. Both groups were well matched for age, sex and dialysis vintage.

Ninety-three age-matched healthy control subjects were also enrolled in this study. The controls were selected from a group of people that visited a general practitioners' office in the same area. The inclusion criteria were: control subjects who had an overall American Society of Anesthesiologists [19] physical condition classification score of 1 or 2; and controls should have been registered at that general practitioner for more than a year.

In the healthy controls, age, gender, past and present smoking habits and body mass index (BMI) were recorded, and skin AF was measured.

Dialysis treatment

All patients underwent HD treatment three times per week with a median session duration of 4 h. The duration of the HD session was individually adjusted, according to the Kidney Disease Outcomes Quality Initiative guidelines [20], to maintain an equilibrated Kt/V >1.2. The patients were dialyzed with non-glucose-containing bicarbonate dialysis solutions and low-flux polysulfone (Fresenius, Bad Homburg, Germany) or polyamide (Gambro, Stockholm, Sweden) dialyzer membranes. The device blood flow was 300 ml/min and the dialysate flow was 500 ml/min. Patients received standard medical care as appropriate for HD patients.

Patient characteristics

CVD, hypertension and diabetes were diagnosed by independent specialists, using the following criteria: a patient was considered to have CVD when a history of coronary heart disease, peripheral vascular disease or cerebrovascular disease was present (International Classification of Diseases, Tenth Revision, Clinical Modification codes I20, I21, I63, I70 and I73) [21].

Hypertension was defined as a systolic blood pressure of >140 mmHg or a diastolic pressure of >90 mmHg measured on at least three different occasions [22]. Also, patients under antihypertensive medication were regarded as hypertensive patients. Finally, when nephrosclerosis was present, we also considered hypertension to be the cause of ESRD.

Diabetes was defined by conventional American Diabetes Association criteria [23].

HD vintage was defined as the period between the initiation of long-term HD treatment and the time of the measurement.

Skin autofluorescence

Accumulation of skin AGEs was estimated from skin AF that was measured using the AGE Reader (DiagnOptics Technologies BV, Groningen, The Netherlands) which was manufactured on the basis of the previously described prototype [18]. The AGE Reader has an improved spectrophotometer and a better available UV lamp, which has less excitation <350 nm. Briefly, the AGE Reader illuminates a skin surface of ~4 cm² with excitation light mainly between 350 and 420 nm (peak excitation ~370 nm). The measured skin AF is the average light intensity between 420 and 600 nm, divided by that between 300 and 420 nm, multiplied by 100. Skin AF is measured in arbitrary units (AU). The measurements were performed in triplicate at room temperature in a semi-dark environment.

Skin AF was measured in the HD patients during dialysis. In a group of 24 HD patients, skin AF was measured before and after dialysis. Finally, skin AF was also measured in the control subjects.

Biochemical blood analysis

The sampling of routine blood analysis was done monthly before and after the HD session. Additional blood samples were taken at the end of the HD session, on the day of the skin AF measurements, for determination of the oxidative stress, inflammation, endothelial activation and organ damage. In order to investigate the effect of the HD treatment on the level of the measured markers, extra blood samples were taken from 24 patients before the same HD session. Albumin levels before and after the HD treatment were measured in order to correct for the possible haemoconcentration after the HD treatment. All blood samples (3 ml) were taken from the vascular access.

The following routine laboratory parameters were used in our study: haemoglobin concentration; platelet count; alkaline phosphates; aspartate aminotransferase (AST); alanine aminotransferase (ALT); gamma glutathione S-transferase (γ -GST); lactate dehydrogenase (LDH); plasma total proteins; plasma albumin; direct and total bilirubin; hepatitis B virus surface antigen (HBsAg) and uric acid.

The concentration of superoxide dismutase (SOD), myeloperoxidase (MPO), inter-cellular adhesion molecule 1 (ICAM-1), C-reactive protein (CRP) and heart-type fatty acid-binding protein (H-FABP) in blood plasma was measured, using the following methods:

- SOD, which catalyzes the dismutation of the superoxide anion (O₂⁻) into hydrogen peroxide and molecular oxygen, is one of the most important antioxidative enzymes. In order to determine the SOD activity in plasma, the water-soluble tetrazolium 1 microtiter plate method [24] was used.
- MPO is a leukocyte-derived enzyme that catalyzes the formation of a reactive oxidant species like hypochlorous acid (HOCl) and tyrosyl. Increased level of MPO is a sign of increased oxidative stress. MPO was measured by ELISA (Hytest, Turku, Finland).
- CRP is an acute phase plasma protein produced by the liver and adipocytes. CRP is mainly used as a marker of inflammation. We used a high sensitivity CRP ELISA (Dakopatts, Glostrup, Denmark).
- ICAM-1 is a cell adhesion molecule. ICAM-1 is a ligand for the lymphocyte function-associated antigen-1 (LFA-1), a part of the leukocyte integrin family of molecules. When leukocytes are activated, they bind increasingly to endothelial cells via ICAM-1/LFA-1 and then transigrate into the tissue. Plasma concentrations of ICAM-1 are indicative of endothelial activation. ICAM was measured by ELISA (R&D Systems, Minneapolis, USA).
- H-FABP is a protein with a size of 15 kDa. H-FABP is abundantly expressed in cardiomyocytes as well as in distal tubular cells of the kidney and skeletal muscle. H-FABP is used as a marker of kidney and heart injury. H-FABP was measured by ELISA (Hytest).

Statistical analyses

Comparisons between the four groups were performed with Student's *t*-test. The skew-distributed variables were log transformed. The correlations were analyzed with the Spearman rank method. Multivariate regression

analyses were performed for determination of independent relationships between the risk markers and the presence of CVD, AGE accumulation and its potential causes. SPSS statistical software (version 14.0, SPSS Inc., Chicago, IL) was used for the analysis; two-tailed $P < 0.05$ was considered significant. Data are shown as mean \pm standard deviation.

Results

The 92 patients treated by maintenance haemodialysis were divided into two groups according to their anti-HCV antibody status: an HCV+ group of 48 patients and an HCV- group consisting of 44 patients. The age of all studied patients ranged from 21 to 86, with a mean of 58. The mean HD vintage was 4.28 years (range 0.25–8.92). The weekly average session of all patients was 12.2 h. Twelve (13%) were active smokers. The patient characteristics are listed in Table 1.

The 93 age-matched control subjects had an average age of 54 ± 17 years (vs 56 ± 13 years for the HD patients; $P = 0.83$); 31 (33%) were smokers and 62 were non-smokers from which 20 were ex-smokers. The average smoking vintage of combined current and ex-smokers was 20 ± 12 years. The controls' characteristics are listed in Table 2.

HCV status and routine biochemical parameters

The prevalence of CVD ($P = 0.01$) and diabetes ($P < 0.001$) reduced with the increase of the HD vintage, whereas the HCV prevalence increased ($P < 0.001$). Table 3 shows the results for the patients of both groups. The mean blood pressure was lower (114 ± 33 vs 133 ± 35 mmHg, $P = 0.02$) in the HCV+ HD group. Although the routine biochemical parameters haemoglobin, alkaline phosphatase, ALT and AST, direct bilirubin, LDH, γ -GST, plasma globulins and uric acid were increased in the HCV+ HD patients, all measured values remained within their reference ranges.

Multiple regression analysis showed that HCV+ had a direct effect on the following diagnostic parameters (given with their odds ratios and confidence intervals in brackets): blood pressure 0.973 (0.951–0.995, $P = 0.016$), bilirubin direct 3.062 (1.03–9.13, $P = 0.045$), LDH 1.02 (1.01–1.03, $P = 0.001$), log(γ -GST) 12.84 (1.53–107.44, $P = 0.019$) and globinaemia 1.23 (1.03–1.46, $P = 0.023$).

Table 1. Demographic and clinical parameters of the haemodialysis patients enrolled in the study

Variable	N = 93
Gender (male)	58 (61%)
Age (years)	58 ± 13
Current smokers	12 (13%)
Diabetic	32 (35%)
CVD	23 (25%)
Average weekly duration of HD session (h)	12.2 ± 0.62
Hemodialysis vintage (years)	4.28 ± 2.49
Hypertension as a cause of ESRD	26 (28%)
Anti-HCV (+)	48 (52%)
HbSAg (+)	13 (14%)

ESRD = end-stage renal disease; HBsAg = hepatitis B surface antigen; HCV = hepatitis C virus.

Table 2. Demographic parameters and skin AF results of the healthy subjects

Variable	N = 93
Gender (male)	48 (52%)
Age (years)	54 ± 17
Smokers (current/ex/never)	31/20/42 (33/21/45%)
Average smoking vintage–current and ex (years)	20 ± 12
Amount of cigarettes (per day)	21 ± 9
BMI (kg/m^2)	27.8 ± 18
Skin AF (AU)	2.2 ± 0.47

AF=autofluorescence; BMI=body mass index.

Skin autofluorescence

Skin AF in the HD patients was higher than in age-matched controls (3.13 ± 0.95 vs 2.20 ± 0.47 AU; $P < 0.001$). There was no significant difference between skin AF values of HCV+ and HCV- HD patients (3.20 ± 0.96 vs 3.03 ± 0.96 AU; $P = 0.39$) as shown in Figure 1. In the control group, skin AF correlated strongly with age ($R = 0.55$; $P < 0.001$) and smoking vintage combined for current and ex-smokers ($R = 0.50$; $P < 0.001$). In the HD patients, skin AF correlated with age ($R = 0.24$; $P < 0.05$) and with HD vintage ($R = 0.22$; $P < 0.05$). Diabetic HD patients had increased skin AF compared to non-diabetics (3.48 ± 0.95 vs 2.93 ± 0.90 AU; $P = 0.01$), and patients with hypertension as a cause of ESRD had higher skin AF than HD patients with other cause of ESRD (3.28 ± 0.92 vs 2.81 ± 0.99 AU; $P < 0.02$).

Multivariate regression showed that AGE accumulation in HD patients could be described by the independent effects of age ($P < 0.05$), diabetes ($P < 0.01$) and HD vintage ($P = 0.03$; $R^2 = 0.23$), whereas in the healthy subjects, skin AF could be described by age ($P < 0.001$) and smoking vintage ($P < 0.001$; $R^2 = 0.61$).

From the results of the control group, we fitted skin AF from healthy subjects as a function of age ($R = 0.55$; $P = 0.01$). To calculate the additional skin AF value of the HD subjects, we subtracted the values obtained from the fit of the controls as a function of age from the real measured value of a HD subject. The additional skin AF increase correlated with HD vintage only in CVD patients with diabetes and showed to be 0.34 AU per year for the diabetes patients ($R = 0.70$; $P < 0.01$) as seen in Figure 2. A significant trend of 0.021 AU per year for CVD patients without DM ($R = 0.54$; $P = 0.05$) was calculated. For the non-CVD patients, no significant trend line was obtained. Furthermore, we calculated the average increase of skin AF in healthy subjects to be 0.017 AU per year, which was 14 times lower than in HD patients with CVD only and 20 times lower than in HD patients with CVD and DM.

Additional biochemical markers

The measured values of SOD, MPO, ICAM-1, CRP and H-FABP were higher than the normal reference values listed in Table 3. Log SOD was significantly lower in the HCV+ HD patients compared with HCV- (2.59 ± 0.22 vs 2.68 ± 0.25 ng/ml; $P = 0.067$), whereas ICAM-1

Table 3. Clinical, demographic and biochemical parameters of HCV+ and HCV– haemodialysis patients

Variable	Reference values	HCV+ 48 (52%)	HCV– 44 (48%)	<i>P</i> value
<i>Demographic and clinical parameters</i>				
Age (years)		56 ± 13	59 ± 13	0.255
Sex–males		28 (58%)	28 (64%)	0.672
Average weekly duration of the haemodialysis treatment (h)		12.3 ± 0.8	12.1 ± 0.3	0.092
Haemodialysis vintage (years)		4.6 ± 2.4	4.0 ± 2.5	0.260
Hypertension as a cause of ESRD		13 (27%)	13 (30%)	0.820
Mean blood pressure (mmHg)	75–135	114 ± 33	133 ± 35	0.020
Diabetes mellitus		19 (40%)	13 (30%)	0.383
CVD		11 (23%)	12 (27%)	0.810
<i>Biochemical parameters</i>				
Routine blood analysis				
HBsAg (+)		7 (15%)	6 (14%)	0.999
Haemoglobin (g/l)	120–180	116 ± 13	109 ± 12	0.028
Ferritin (mg/l)	0–300	436 ± 261	496 ± 263	0.450
Platelet count (10 ⁹ /l)	140–340	201 ± 84	208 ± 76	0.701
Log alkaline phosphatase (U/l)	1.57–2.10	2.10 ± 0.27	1.85 ± 0.2	0.001
Log AST (U/l)	1–1.53	1.36 ± 0.25	1.17 ± 0.16	0.001
Log ALT (U/l)	1–1.66	1.44 ± 0.31	1.24 ± 0.21	0.001
Bilirubin total (mmol/l)	6.8–20.5	7.25 ± 1.68	7.0 ± 2.4	0.588
Bilirubin direct (mmol/l)	1.5–6.8	3.41 ± 0.75	3.05 ± 0.69	0.037
LDH (U/l)	213–423	281 ± 82	198 ± 71	0.001
Log γ-GST (U/l)	0.96–1.81	1.62 ± 0.37	1.20 ± 0.38	0.001
Plasma proteins (g/l)	63–83	71.5 ± 8.7	68 ± 5.2	0.039
Plasma albumin (g/l)	35–50	36.5 ± 5.0	36.3 ± 5.1	0.905
Plasma globulins (g/l)	27–35	35.7 ± 7.2	31.6 ± 5	0.006
Uric acid (mmol/l)	143–446	434 ± 76	385 ± 82	0.013
<i>Additional blood analysis</i>				
Skin AF (AU)	2.10 ± 0.45	3.20 ± 0.96	3.03 ± 0.96	0.391
Log SOD (ng/ml)	1.68 ± 0.21 (unlogged 48 ± 2) [34]	2.59 ± 0.22	2.68 ± 0.25	0.067
MPO (ng/ml)	43.80 ± 23.30 [35]	674 ± 311	598 ± 248	0.213
Log CRP (μg/ml)	<0.69 (unlogged <5)	0.55 ± 0.98	0.74 ± 0.97	0.262
ICAM-1 (ng/ml)	0.111 ± 0.017 [36]	2.20 ± 0.81	1.84 ± 0.62	0.022
H-FABP (ng/ml)	4.4 ± 3.9 [37]	36.7 ± 21.8	36.5 ± 28.7	0.975

AF=autofluorescence; ALT=alanine aminotransferase; AST=aspartate aminotransferase; CRP=C-reactive protein; CVD=cardiovascular disease; ESRD=end-stage renal disease; GST=glutathione *S*-transferase; HBsAg=hepatitis B surface antigen; H-FABP=heart-type fatty acid-binding protein; ICAM-1=inter-cellular adhesion molecule-1; LDH=lactate dehydrogenase; MPO=myeloperoxidase; SOD=superoxide dismutase.

was higher (2.20 ± 0.81 vs 1.84 ± 0.62 ng/ml; $P = 0.022$). SOD, MPO and ICAM-1 were also included in the multiple regression analysis, but did not appear to be independent markers of the presence of the HCV infection. Also, no significant differences were found between the HCV+ and HCV– patients with regard to the CVD markers.

We questioned whether the plasma markers used were substantially affected by the proceeding dialysis procedure itself. Therefore, SOD, ICAM-1, CRP and H-FABP were measured before and after a single dialysis session. The MPO molecule is too large (140kDa) to pass through the dialysis membrane. No significant changes in SOD, CRP, ICAM-1 and H-FABP were measured in these samples (see Table 4).

Discussion

Our results indicate that hepatitis C prevalence increased with HD vintage, whereas diabetes and CVD prevalence dropped at the same time. Most likely, the inverse associ-

ation between HCV+ and the prevalence of CVD and diabetes was due to the high early mortality of CVD and diabetes HD patients. This higher mortality was also illustrated by the AGE accumulation per year in HD CVD patients, which was 14–20-fold higher than in the healthy subjects.

AGE accumulation was higher in the HD patients than in the healthy controls. The level of AGE accumulation in HD patients was mostly due to their age, HD vintage and diabetes, whereas in the healthy subjects it was due to their age and smoking vintage. Although the level of enzymatic activity and ICAM were higher in HCV+ than in HCV– patients, AGE accumulation did not differ between the HCV+ and HCV– patients.

Skin AF, a marker of tissue AGE accumulation, was much higher in HD patients compared to the age-matched controls. Skin AF and AGE accumulation are markers of glycaemic and oxidative stress or of reduced clearance and have been found to be independent, strong predictors of CV mortality in diabetes and renal failure. A comparison of skin AF in healthy subjects and HD patients was for the first time done by Meerwaldt *et al.* [18] who found a 2.5-

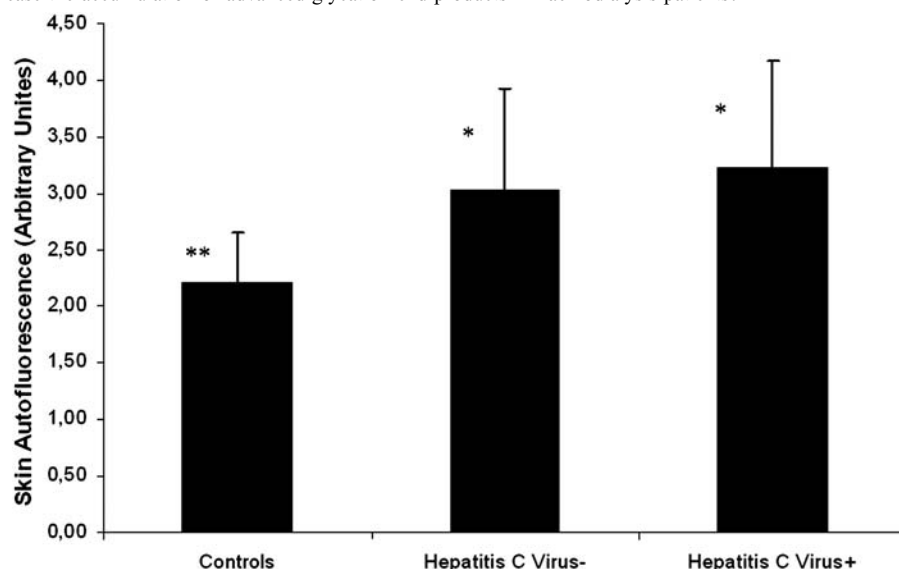


Fig. 1. The skin AF in age-matched controls and hepatitis C+ and hepatitis C- haemodialysis patients. The skin autofluorescence is significantly lower in the controls compared with the haemodialysis patients in both groups. * $P < 0.001$.

fold higher skin AF in HD patients, whereas Hartog *et al.* [25] and Matsumoto *et al.* [26] found 1.5 times higher values of skin AF, which is similar to our study. In all of these studies [18, 25, 26], skin AF in HD patients was strongly correlated with age and HD vintage as in our study. We confirmed the observation of Meerwaldt *et al.* [18] that skin AF correlates with the presence of diabetes. Moreover, we found that the AGE accumulation per year in HD patients was 14–20 times higher than in healthy subjects depending on the presence of CVD and diabetes.

HCV+ HD patients presented higher enzymatic activity, but all elevated parameters were within reference value range except for ICAM-1. This is in line with the findings

of others on the progress of HCV infection in HD patients. Okuda *et al.* [27] compared the progress of hepatitis C in HD patients with non-uraemic controls over a period between 4 and 23 years. During the first 4 years, 25% of the controls developed cirrhosis whereas the HD patients did not. Also, it is interesting to note that Okuda *et al.* [27] found that all of the patients that were followed for >15 years had asymptomatic HCV infection. These results were confirmed by our study as all the HCV+ patients that were >9 years on HD were having asymptomatic HCV infections. This means that HCV infection is not a strong factor for the development of liver dysfunction in patients that were on HD for a longer time.

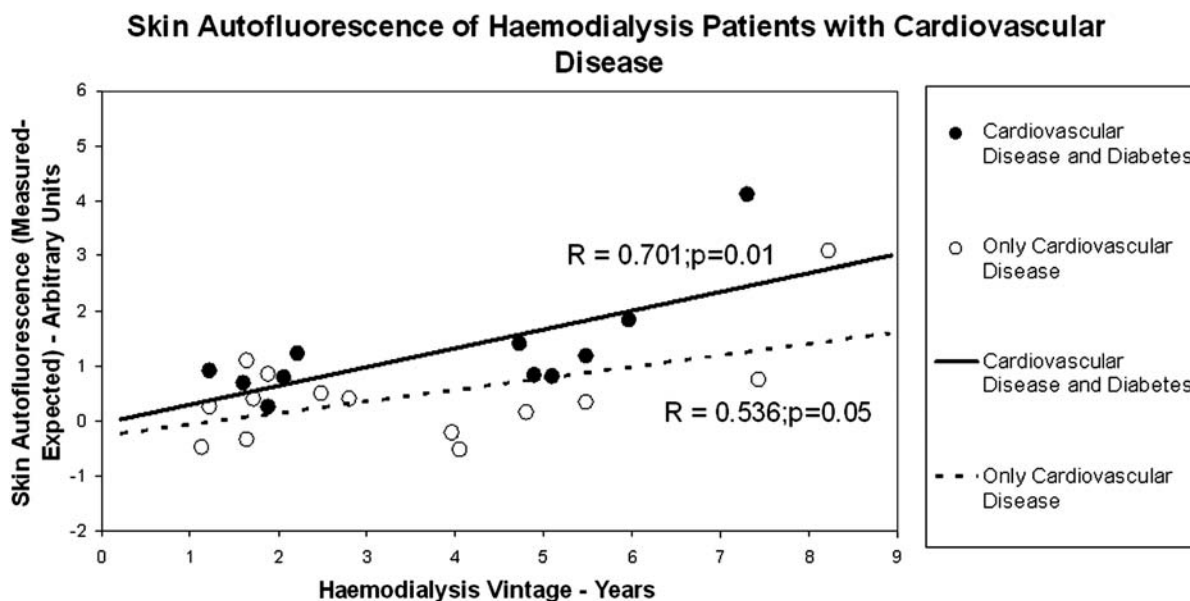


Fig. 2. The skin AF difference between healthy controls and haemodialysis patients with cardiovascular disease of the same age, given according to the duration of HD.

Table 4. Validation study of the biochemical markers in 24 patients

Variable	Before	After	P value
Skin AF (AU)	3.01 ± 0.89	2.94 ± 0.90	0.89
Log SOD (ng/ml)	2.67 ± 0.28	2.66 ± 0.28	0.95
Log CRP (µg/ml)	0.73 ± 1.12	0.77 ± 1.11	0.67
ICAM-1 (ng/ml)	2.2 ± 0.89	2.25 ± 0.94	0.31
H-FABP (ng/ml)	38.1 ± 3	40.5 ± 2	0.68

The markers were measured before and after a single hemodialysis session. AF=autofluorescence; SOD=superoxide dismutase; CRP=C-reactive protein; ICAM-1=inter-cellular adhesion molecule-1; H-FABP=heart-type fatty acid-binding protein.

The most important factor for AGE accumulation is the level of the oxidative stress for which we used the SOD and MPO as major markers. Akiyama *et al.* concluded that SOD is up-regulated and can thus be used as a marker of increased oxidative stress, especially when leucocytes are activated, like during HD treatment by the membrane [28]. The findings of other studies on the oxidative stress in HCV+ HD subject are controversial. There are studies that found that HCV infection in HD patients is responsible for increased [29, 30] oxidative stress, whereas others suggest that HCV infection can be even protective [31]. However, those studies were either underpowered or did not measure oxidative stress markers directly. We found nearly significant reduction in the level of SOD and no changes of MPO in the HCV+ HD patients, which indicated the low influence of HCV on oxidative stress. The AGE accumulation in our study reflected the oxidative stress over a long period of time and agreed with those results, as it did not show differences between HCV+ and HCV− HD patients either. To our knowledge, we were the first to investigate the influence of hepatitis C on AGE accumulation in HD patients. The studies that investigated the influence of HCV on circulatory AGEs in non-HD patients did not show differences between healthy subjects and HCV+ patients [32, 33] as well. Regarding HD patients, Nascimento *et al.* [30] found higher levels of plasma pentosidine in HCV+ compared to HCV− HD patients. In contrast, we did not find higher AGE accumulation in HCV+ compared to HCV− HD patients.

The limitations of our study are that we cannot exclude the influence of uraemic toxins or skin fluorophores other than AGEs on skin AF measurements. Furthermore, it should be noted that most AGEs present in the human body are non-fluorescent. However, previous results showed that skin AF may function as a marker of the AGE pool, based on the strong correlations with both fluorescent and non-fluorescent skin AGE levels measured by conventional biochemical means in skin biopsies of HD and diabetes patients [17, 18].

Our result of SOD, MPO and skin AF measurement clearly showed that the oxidative stress was not higher in the HCV+ HD patients. Additional research on the influence of HCV on oxidative stress in HD patients is needed due to the conflicting results between various studies.

In conclusion, AGE accumulation is higher in HD patients than in healthy subjects, but does not differ between HCV+ and HCV− hemodialysis patients. This is probably

a result of the similar level of oxidative stress between the HCV+ and HCV− patients. The major predictors of the AGE accumulation are the HD treatment itself, diabetes, CVD and age of the patient. HCV does not influence AGE accumulation in HD patients.

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