Lipid and oxidative profile in hemodialysis patients: Clinical follow-up for three years.

Profil lipidique et oxydatif chez les patients hémodialysés: suivi clinique pendant trois ans

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RÉSUMÉ

Objectif: La principale cause de mortalité chez les patients hémodialysés (HD) est la morbidité cardiovasculaire ; qui est en effet, étroitement liée au stress oxydatif (OS). Le but de cette étude était d'une part, d'évaluer les biomarqueurs de la peroxydation lipidique chez ces patients en mesurant les taux de malondialdéhyde (MDA) et des diènes conjugués (DC) et ensuite, de suivre ces paramètres après trois ans d'hémodialyse.
Méthodes: Une centaine de patients atteints d'insuffisance rénale terminale recevant une hémodialyse régulière et 100 volontaires sains ont été inclus dans cette étude. Les données chimiques de routine, le profil lipidique et les taux de MDA et de CD ont été mesurés.
Résultats : Les taux de MDA plasmatique et érythrocytaire étaient significativement plus élevés chez les patients HD que chez les sujets sains (p <0,001). Cependant, seulement une augmentation du taux des DC érythrocytaire a été observée entre les deux groupes d'étude (p <0,001). Après 3 ans, une augmentation significative des biomarqueurs de la peroxydation lipidique a été observée.
Conclusion: La perturbation de l'état de peroxydation lipidique chez les patients HD a été observée. Après trois ans de suivi, le stress oxydatif est plus prononcé avec une augmentation significative des taux des MDA et DC.

Mots-clés
Stress oxydant; peroxydation lipidique; malondialdéhyde; diènes conjugués; hémodialysés

SUMMARY

Background: The major cause of death for hemodialysis (HD) patients was cardiovascular morbidity; it was closely related to oxidative stress (OS).
Aim: Firstly, to evaluate lipid peroxidation biomarkers on HD patients through measuring malondialdehyde (MDA) and conjugated dienes (CD), and secondly, to follow these parameters after three years undergoing HD.
Methods: One hundred patients with end stage renal diseases receiving regular hemodialysis and 100 healthy volunteers were included in this study. Routine chemical data, lipid profile and levels of MDA and CD were measured.
Results: The plasmatic and erythrocyte MDA levels were significantly increased in the HD patients compared to healthy subjects (p <0.001). However, an increased level on erythrocyte CD was only observed between the two study groups (p<0.001). After 3 years, a significant increased level of lipid peroxidation biomarkers was observed.
Conclusion: The disturbance in lipid peroxidation state in HD patients was observed. At three years follow-up, oxidative stress is more pronounced with a significant increase in MDA and CD.

Key-words
Oxidative stress; lipid peroxidation; malondialdehyde; conjugated dienes; hemodialysis patients

INTRODUCTION

End Stage Renal Diseases (ESRD) is an irreversible and terminal state that cause serious problems and consequences for the whole organism, related to uremic intoxication and the failure of renal endocrine functions (1). Hemodialysis (HD) is the most therapy on renal replacement can used for removing metabolites and excess fluid (2). The dialysis through artificial membranes contributes to activation of the immune system caused by overproduction of reactive oxygen species and induce the oxidative stress (OS) (3,4). The excess radicals were formed when the available antioxidant defenses was less than the free radical production. These radicals react with many classes of biological molecules such as lipids, causing lipid peroxidation (5,6).

The most popular methods for evaluation of the lipid peroxidation is determination of products of the oxidation of polyunsaturated fatty acid: the malondialdehyde (MDA) and the conjugated Dienes (CD) levels (2,4,7). Increased OS contribute to atherosclerosis and lead to higher incidence of cardiovascular morbidity (1,8), which are known to be the major cause of death for HD patients (9,10). For this reason, evaluation of oxidative stress is crucial and especially to follow this evolution during a few years. The aim of our study is to evaluate the oxidative profile through measuring plasma malondialdehyde levels and erythrocytes of conjugated dienes levels in hemodialysis patients follow three years.

METHODS

Study population

The study was carried out in hemodialysis department in the Taher Sfar hospital in Mahdia (Tunisia). This study was involved a total of 200 participants; 100 with end stage renal diseases receiving regular hemodialysis and 100 healthy volunteers appeared in age and gender. All participants had given their written consent before the enrolment. We excluded patients under than 18 years old, less than 6 months in HD, provisory hemodialysis having malignancy, acute or chronic hepatic disease, unconscious and patients from others dialysis center. Hemodialysis treatment was performed three times per week for 4 hours until a low-flux dialyser (Fresenius Medical Care, Germany) using a polysulfone membrane (surface area of 1.4 m2, Fresenius Medical Care) through an arteriovenous fistula. The dialysate flow rate was 500 mL/min and the blood flow rate was 250-300 mL/min. For anticoagulation, heparin was used.

Biochemical investigations

Fasting venous blood samples were collected for healthy control and in pre-hemodialysis period for HD patients. Plasma and serum collected after centrifugation at 3000 rpm for 10min at 4°C were stored at -80°C until analysis. Hemoglobin A1c (HbA1c) was measured using G7 HPLC Analyser (Tososh Europe N.V., Belgium). Enzymatic methods (DX600 Auto-chemical analysis instrument;Beckman coulter, USA) were used for measuring glucose, serum creatinine, uric acid, and lipid levels [cholesterol (TC), triglyceride (TG), high-density lipoprotein (HDL) and low-density lipoprotein (LDL)]. The atherogenic index was obtained through those ratios TC/HDL and TG/HDL. Lipid peroxidation was estimated by the measurement of the malondialdehyde levels according to the method of Yoshioka and al. (11). The precipitation of protein was carried out by the addition of thiobarbituric acid to plasma or erythrocyte. Reading absorbance was done in the wavelengths of 532 nm and concentration of MDA was expressed in mmol/L. The method elaborated by El-Saadani and al. (1989) (12) was used for estimation of Conjugated Dienes levels according to the reading absorbance at 234 nm. Concentration of CD was expressed in µmol/mL.

Statistical analysis

Statistical Package for the Social Sciences (SPSS Software, version 21) for windows was used for all the data analysis. All values are expressed as mean ± standard deviation (SD). The independent sample Student's t-test was used for analyzing differences between the two groups. A p value less than 0.05 was considered as statistically significant. Correlations between levels of lipid peroxidation and different variables of HD patients were determined by a Spearman test. The variables were estimated as well as the correlation coefficient r, with significance accepted at $p < 0.05$.

RESULTS

Clinical characteristics of the studied participants

Two hundred subjects were enrolled and agreed to participate in the study. Among these, one hundred were healthy volunteers and one hundred patients undergoing HD for at least six months with an average age of 52.87 ± 9.25 years and 54.17 ± 17.62 years respectively. There were no statistically significant differences between the

HD patients and healthy control groups regarding age, gender and BMI. More demographics and characteristics of the study participants were summarized in Table1.

Table 1. Demographic and clinical characteristics of study population**.**

	HD patients (n = 100)	Control subjects (n = 100)
Age (years)	54.17 ± 17.62	52.87 ± 9.25
Female (%)	62	71
Diabetes (%)	18	-
Hypertension (%)	73	-
Smoking (%)	41	-
Hyperuricemia (%)	11	-
BMI (kg/m²)	24.5 ± 4. 8	23.82 ± 2.7
Underlying renal disease (%)		-
Hypertensive Nephrosclerosis	9	
Diabetic nephropathy	25	
Interstitial Nephropathy	11	
Chronic Glomerulonephritis	9	
Polycystic kidney disease	7	
unknown cause	39	
Duration of hemodialysis (months)		-
< 12	18	
12 - 47	52	
48 - 83	12	
84 - 119	7	
120+	11	

Data represent means ± SD or percentages.
BMI= body mass index

Table 2. Lipid profile and lipid peroxidation biomarkers of study population.

Parameters	HD patients (n = 100)	Healthy subjects (n = 100)	p
Triglycerides (mmol/L)	1.84 ± 0.93	1.08 ± 0.51	< 0.001
Total cholesterol (mmol/L)	3.94 ± 1.23	4.55 ± 0.73	< 0.001
HDL cholesterol (mmol/L)	1 ± 0.33	1.31 ± 0.33	< 0.001
LDL cholesterol (mmol/L)	2.11 ± 1.1	2.8 ± 0.72	< 0.001
TC/HDL	4.33 ± 2.19	3.68 ± 1.1	0.012
TG/HDL	2.11 ± 1.52	0.91 ± 0.68	< 0.001
MDA-p (mmol/L)	0.95 ± 0.49	0.52 ± 0.16	< 0.001
MDA-e (mmol/L)	28.19 ± 13.37	14.59± 2.49	< 0.001
DC-p (µmol/mL)	137.27 ± 35.8	125.3 ± 32.6	NS
DC-e (µmol/mL)	170.35 ± 50	131.73 ± 21.6	< 0.001

The values are expressed as means ± standard error.
NS: not significant.
TG: Triglycerides; TC: Total cholesterol; HDL-cholesterol: high density lipoprotein cholesterol; LDL-cholesterol: low density lipoprotein cholesterol; MDA: Malondialdehyde; CD: Conjugated dienes. p: plasmatic; e: erythrocyte.

Lipid profile and oxidative parameters in studied population

Table 2 showed results of lipid profiles, lipid peroxidation biomarkers and heterogenic index in patients and control subjects. According to our results, we found statistically significant difference between levels of TG, TC, HDL and heterogenic index between the two groups. The plasmatic and erythrocyte MDA levels were significantly increased in the HD patients compared to healthy subjects (p <0.001). However, an increased levels on erythrocyte CD was only observed between the two study groups (p <0.001).

Lipid profile and oxidative parameters in HD patients after 3 years

Table 3 showed results of lipid profile, lipid peroxidation biomarkers and heterogenic index in HD patients before and after 3 years. Before 3 years HD patients were 100 and after 3 years 27 HD patients were death and the lipid profile, lipid peroxidation and heterogenic index were only analyzed on 43 HD patients. A significant increased level of these parameters was observed after 3 years.

Table 3. Lipid profile and lipid peroxidation biomarkers in hemodialysis patients before and after 3 years.

Parameters	HD patients Before 3 years	HD patients After 3 years	p
Triglycerides (mmol/L)	1.84 ± 0.93	2.15 ± 0.83	< 0.05
Total cholesterol (mmol/L)	3.94 ± 1.23	4.42 ± 1.20	< 0.05
HDL cholesterol (mmol/L)	1 ± 0.33	0.88 ± 0.24	< 0.05
LDL cholesterol (mmol/L)	2.11 ± 1.1	1.38 ± 0.97	< 0.001
TC/HDL	4.33 ± 2.19	5.47 ± 2.5	< 0.01
TG/HDL	2.11 ± 1.52	2.83 ± 2.02	< 0.05
MDA-p (mmol/L)	0.95 ± 0.49	1.10 ± 0.37	NS
MDA-e (mmol/L)	28.19 ± 13.37	36.85 ± 10.50	< 0.001
CD-p (µmol/mL)	137.27 ± 35.8	149.82 ± 24.79	< 0.05
CD-e (µmol/mL)	170.35 ± 50	193.63 ± 29.88	< 0.05

The values are expressed as means ± SD.
NS: not significant.
TG: Triglycerides; TC: Total cholesterol; HDL-cholesterol: high density lipoprotein cholesterol; LDL-cholesterol: low density lipoprotein cholesterol; MDA: Malondialdehyde; CD: Conjugated dienes. p: plasmatic; e: erythrocyte.

DISCUSSION

Currently, nephrology research has focused on causes of cardiovascular diseases in the end stage renal disease receiving hemodialysis, because its becomes the extensive epidemiology and the crucial cause of morbidity and mortality in those patients (9,10). To develop new strategies for treatment and prevention of cardiovascular diseases, researchers was no longer interested in traditional risks, such as hypertension, diabetes, physic inactivity and hyperlipidemia, but new research focused on oxidative stress (6,13,14). In fact, many investigations have shown evidence that the oxidative stress is highly prevalent in HD patients (15–17). OS occurs when the production of oxidants exceeds the capacity of local antioxidants and it results the oxidation of various macromolecules such as lipids (2,13,18).

Lipid peroxidation generates primary products as peroxides and they are broken down in secondary products with a shorter hydrocarbon chains (4,12). The most important biomarkers used for the quantification of those products were, respectively, conjugated dienes (CD) and malondialdehyde (MDA). In the present study we examined, firstly, lipid profile plasma and erythrocyte levels of MDA and CD and atherogenic index in HD patients. We showed an increased level of lipid parameters, MDA and CD, and atherogenic index in HD patients. Many studies confirmed that, the lipid profile on hemodialysis patients was classically characterized by a high prevalence of dyslipidemia ranged from 40% to 80% (19) with predominance of hypertriglyceridemia, hypo-HDL cholesterol and a high atherogenic index (2,18,20). This has been confirmed in our study.

Secondly, we are interested to follow-up the level of MDA, CD and atherogenic index in HD patients after 3 years. We showed a significant increased of these parameters in HD patients. Unfortunately, these results were observed in 43 HD patients only survived after 3 years. Increased oxidative stress, lipid peroxydation and atherognic index may increase the risk of vascular complication in HD patients. Free radical induced damages are thought to be involved in chronic kidney disease, especially in patients who are one hemodialysis for prolonged periods. A loss or inactivation of antioxidant factors, coupled with increased lipid peroxydation were observed in patients with HD (21). In addition, to vascular complications, Kim et al. showed that the prevalence of pulmonary hypertension was higher in HD patients and was associated with higher rates of major cardiovascular events (22).

In conclusion; our findings confirm the disturbance observed in lipid peroxidation state in HD patients. Three years follow-up, oxidative stress is more pronounced with a significant increase in MDA and CD. In order to establish prevention strategies for these patients, it is necessary to monitor and treat lipid abnormalities.

Competing interest: No authors declared any potential conflicts of interest.
Funding: This work was supported by funds from the Research Laboratory LR12ES05 Lab-NAFS 'Nutrition - Functional Food & Vascular Health', Faculty of Medicine, University of Monastir.
Guarantor: Pr Mohamed Hammami

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