Advanced glycation end-products (AGEs) accumulation in skin: relations with chronic kidney disease-mineral and bone disorder

Acúmulo dos produtos finais da glicação avançada (AGEs) na pele: relações com o distúrbio mineral e ósseo na doença renal crônica

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Abstract

Introduction: Chronic kidney disease (CKD) is associated with high morbidity and mortality rates, main causes related with cardiovascular disease (CVD) and bone mineral disorder (CKD-BMD). Uremic toxins, as advanced glycation end products (AGEs), are non-traditional cardiovascular risk factor and play a role on development of CKD-BMD in CKD. The measurement of skin autofluorescence (sAF) is a noninvasive method to assess the level of AGEs in tissue, validated in CKD patients. **Objective:** The aim of this study is analyze AGEs measured by sAF levels (AGEs-sAF) and its relations with CVD and BMD parameters in HD patients. **Methods:** Twenty prevalent HD patients (HD group) and healthy subjects (Control group, n = 24), performed biochemical tests and measurements of anthropometric parameters and AGEs-sAF. In addition, HD group performed measurement of intact parathormone (iPTH), transthoracic echocardiogram and radiographies of pelvis and hands for vascular calcification score. **Results:** AGEs-sAF levels are elevated both in HD and control subjects ranged according to the age, although higher at HD than control group. Single high-flux HD session does not affect AGEs-sAF levels. AGEs-sAF levels were not related to ventricular mass, interventricular septum or vascular calcification in HD group. AGEs-sAF levels were negatively associated with serum iPTH levels. **Conclusion:** Our study detected a negative correlation of AGEs-sAF with serum iPTH, suggesting a role of AGEs on the pathophysiology of bone disease in HD prevalent patients. The nature of this relation and the clinical application of this non-invasive methodology for evaluation AGEs deposition must be confirmed and clarified in future studies.

Keywords: hemodialysis units, hospital; parathyroid hormone; cardiovascular diseases; bone diseases; glycosylation end products, advanced.

Resumo

Introdução: A doença renal crônica (DRC) apresenta elevadas taxas de morbidade e mortalidade, sendo a doença cardiovascular (DCV) e o distúrbio mineral e ósseo da DRC (DMO-DRC) complicações frequentes. As toxinas urêmicas, dentre elas os produtos finais da glicação avançada (AGEs), são fatores de risco cardiovascular não tradicionais e se encontram envolvidas no desenvolvimento do DMO-DRC na DRC. A medida da autofluorescência da pele (sAF) é método não invasivo para quantificação do acúmulo tecidual de AGEs validado em pacientes portadores de DRC. **Objetivos:** O objetivo deste estudo é avaliar as relações entre os AGEs medidos por sAF (AGEs-AF) e parâmetros de DCV e DMO-DRC em pacientes em hemodiálise (HD). **Métodos:** 20 pacientes em HD (grupo HD) e 24 indivíduos hígidos (grupo controle) foram submetidos à análise bioquímica sérica, medidas antropométricas e de sAF. O grupo HD realizou medida de hormônio intacto da paratireoide (PTHi), ecocardiograma transtorácico e radiografias de pelve e mãos para pesquisa de calcificação vascular. **Resultados:** Os níveis de AGEs-sAF foram elevados para a idade nos grupos HD e controle, porém mais elevados no grupo HD. Sessão única de HD de alto-fluxo não afetou os níveis de AGEs-sAF. Os níveis teciduais de AGEs não se correlacionaram com massa ventricular, espessura de septo interventricular ou calcificação vascular no grupo HD. Os níveis de AGEs-sAF se correlacionaram negativamente com os níveis séricos de PTHi. **Conclusão:** Nosso estudo detectou correlação negativa entre os níveis de AGEs-sAF e os níveis séricos de PTHi, sugerindo que os AGEs estejam envolvidos na fisiopatologia da doença óssea em pacientes em HD. A natureza desta relação e a aplicação clínica deste método não invasivo de avaliação do acúmulo tecidual de AGEs deve ser confirmada e elucidada por estudos futuros.

Palavras-chave: hemodiálise; hormônio intacto da paratireoide; doenças cardiovasculares; doenças ósseas; produtos finais de glicosilação avançada.

Introduction

Chronic kidney disease (CKD) is associated with high rates of morbidity and mortality. Patients under hemodialysis (HD) treatment have five-fold shorter life expectancy than healthy subjects do at the same age. The main causes of death in patients with end stage kidney disease (ESKD) are associated with cardiovascular disease (CVD) and bone mineral disorder (CKD-MBD).[1,2]

In addition to traditional cardiovascular risk factors, uremic toxins are non-traditional factors associated with mortality in CKD patients. Advanced glycation end-products (AGEs) are uremic toxins which are elevated in CKD because of increased production by oxidative stress, impaired renal excretion and diet consumption.[3] The measurement of skin autofluorescence (sAF) is a noninvasive method used to indirectly evaluate the accumulation of AGEs in this tissue. This methodology was validated in studies, including those with CKD patients, through the comparison between AGEs-sAF values and density of AGEs in skin biopsies processed by immunohistochemistry for AGEs.[3-5]

AGEs accumulation on tissue proteins seems to be a contributing factor in atherosclerosis. Some studies revealed elevated levels of AGEs in patients with coronary artery disease,[6] carotid artery stenosis[7] and peripheral artery disease,[8] irrespective of diabetes *mellitus* (DM) or renal disease.[9] For these reasons AGEs have been described as a predictor of cardiovascular mortality.[3,10]

Another important potential clinical application of AGEs evaluation through sAF is to investigate its relations with CKD-MBD. Of note, AGEs seems to do negative effects on bone quality by mechanisms that are not fully elucidated, being associated with osteoporosis and osteopenia.[11] AGEs can reduce bone formation by interfering with the production of matrix proteins and inducing mesenchymal stem cells apoptosis.[11] Furthermore, AGEs may interfere with the osteoblast differentiation, proliferation and mineralization, actions that were demonstrated in cell culture studies.[11,12]

AGEs seems to be involved in the pathogenesis of adynamic bone disease in CKD patients by inhibiting osteoblastic activity and parathormone secretion.[13] Since AGEs could act on bone turnover by multiple mechanisms, they are probably related to serum intact parathormone (iPTH), the most commonly used biomarker that supports CKD-MBD management,[14] although this issue was not proven yet.

Considering that AGEs can affect both bone tissue and cardiovascular system, this study aim to evaluate the relations between AGEs accumulation in skin measured by sAF (AGEs-sAF) and CKD-MBD parameters through analysis of clinical, biochemical and image studies in a cohort of patients under chronic HD treatment.

Materials and methods

Study design and subjects

This pilot study is an observational, transversal, controlled, single-center study involving CKD patients under HD treatment. Patients included in HD group (N = 20) were recruited from the Nephrology Department's outpatient HD clinic at Hospital das Clínicas, University of Campinas. All patients receiving chronic HD were dialyzed thrice weekly for four hours with high-flux and high-efficiency polysulphone dialyzers. The inclusion criteria for the study are as follows: age > 18 years old; undergoing HD for more than three months. Exclusion criteria were: skin phototypes "V" and "VI" by Fitzpatrick classification[15] (skin colors that not allow the precise measurement of AGE-sAF by AGE-Reader™ following manufacturer's instructions), have clinical instability, cancer or HIV.

In order to compare the measurements of AGEs-sAF values we used a control group with apparently normal healthy subjects (n = 24) that fulfill following criteria: aged between 20 and 70 years; serum creatinine < 1.2 mg/dL; serum albumin > 3.5 mg/dL; do not have the diagnostic of DM, chronic inflammatory disease, not be pregnant or using medicines that exert influences on bone metabolism (i.e., anticonvulsants, bisphosphonates, calcimimetics, calcitonin, corticosteroids, GnRH analogs, vitamin D, dicumarinic, hormone replacement therapy, and thyroid treatments).

Written informed consent was obtained from all subjects, and the ethics committee of the University of Campinas approved the study protocol (CAAE 38406514.6.0000.5404). The study was performed in accordance with the precepts of the Declaration of Helsinki.

Study protocol

All patients were on their HD schedule in the second session of the week in order to perform laboratory blood tests and measurements of anthropometric parameters, blood pressure and AGEs-sAF.

Height and weight were measured without shoes, and without heavy clothing. Body mass index (BMI) was defined as weight (kg) divided by square of height (m^2). Waist circumference (WC) was measured midway between the last rib and the crest of the ileum and hip circumference (HC) around the pelvis at the point of maximum protrusion of the buttocks, both in a horizontal plane, without compressing the soft tissues. WC and HC were recorded to the nearest cm and waist-to-hip ratio (WHR) was defined as a ratio of WC to HC. Systolic blood pressure was measured on the left arm in control group and on contralateral arm which do not have an arteriovenous fistula in HD patients.

In patients from HD group, transthoracic echocardiogram was performed to analyze left ventricular mass, valve calcification and interventricular septum thickness. Vascular calcification was evaluated in plain radiographic films of pelvis and hands by simple score proposed by Adragão *et al.*[16] ranging from 0 to 8. The pelvis radiographic films were divided into four sections by two imaginary lines: a horizontal line over the upper limit of both femoral heads and a median vertical line over the vertebral column. The films of the hands were divided, for each hand, by a horizontal line over the upper limit of the metacarpal bones. The presence of linear calcifications in each section was counted as "1" and its absence as "0". Vascular calcifications were evaluated only in the follow arteries: iliacs, femorals, radials and digitals by the same observer.

An interview was conducted with all subjects focusing on comorbidities and disease history, both in general and specific previous cardiovascular disease (CVD). History of CVD was considered positive if the following events were present: myocardial infarction, stroke, heart failure, angina *pectoris* or surgical procedures for angina or coronary/peripheral artery disease (including percutaneous-transluminal angioplasty). The patient's medical files were reviewed in order to identify and record any concomitant medications. Patients and healthy subjects were classified according Framingham risk.[17]

Laboratory tests

Blood samples were collected immediately before the second HD session of the week or in a previously scheduled date in control group. Serum creatinine, urea, potassium, hemoglobin, hematocrit, calcium, phosphate, alkaline phosphatase, iron, ferritin, transferrin saturation, fasting glycemia, glycated hemoglobin, total cholesterol, low density lipoprotein cholesterol (LDL), high density lipoprotein cholesterol (HDL), triglycerides, uric acid, C-reactive protein, 25-hydroxyvitamin D (chemiluminescence method), albumin, ß2-microglobulin and bicarbonate levels were assayed in an on-site biochemistry laboratory using standard auto-analyzer techniques (Modular IIPR system, Roche Diagnostics, Basel, Switzerland). Serum intact 1-84-parathormone (iPTH) was determined by a chemiluminometric immunoassay (Liaison N-tact PTH CLIAR, Diasorin, Stillwater).

Measurement of AGEs-sAF

Tissue accumulation of AGEs was indirectly measured by evaluation of sAF with an instrument named AGE-Reader™ according to the supplier's recommendations (DiagOptics BV, Groningen, The Netherlands). This desktop device uses AGEs fluorescent properties to measure sAF, which is calculated as the ratio between the emission light and reflected excitation light, and is expressed in arbitrary units (AU).[4,5] sAF was assessed on the ventral site of the lower arm. Pre- and post-HD evaluations of AGEs-sAF were performed in the second HD sessions of the week, on contralateral arm which do not have an arteriovenous fistula or left arm (control group).

All the measurements were performed in triplicate at room temperature in a room with windows covered by curtains to avoid sun illumination (semi-dark). The mean value of the triple measurement was used for further analysis. To rank AGEs tissue levels according to the age we used the reference values established in a Dutch cohort.[18]

Statistical analysis

Data were expressed as the mean ± SD, median and interquartile range or frequency, as appropriate. For descriptive and analytical purposes, data from patients in HD group were stratified according to the median AGEs-sAF levels (sAF AGEs < 2.7 AU *versus* ≥ 2.7 AU). Intergroup comparisons were performed using a χ^2 test for categorical variables

and Student's t-test or a Mann-Whitney test for continuous variables. Pearson's correlation coefficient or Spearman's rank correlation was used to assess the relationships between AGEs-sAF and selected clinical or biochemical variables. The threshold for statistical significance was set to bicaudal $p < 0.05$. All statistical analyses were performed using SPSS software (version 17.0, SPSS Inc., Chicago).

Results

Twenty-four healthy subjects (control group) and 20 patients (HD group) had completed the study. Clinical and demographic characteristics, as well biochemistry results are shown on Tables 1 and 2.

Table 1 Clinical and demographic characteristics according to control and hemodialysis groups

Parameters	Control (n = 24)	HD (n = 20)	*p*
Age (years)	38 ± 9	38 ± 19	0.85
Female gender, n (%)	14 (58)	9 (43)	0.3
BMI (Kg/m²)	25 ± 4	23.5 ± 6	0.3
Waist circunference (cm)	83.5 ± 14	87 ± 17	0.47
Waist-to-hip ratio	0.84 ± 0.1	0.92 ± 0.1[a]	0.009
SBP (mmHg)	110 ± 9	132 ± 18[a]	0.001

HD: Hemodialysis; BMI: Body mass index; SBP: Systolic blood pressure. [a] $p < 0.05$.

In control group, AGEs-sAF according to their age was higher in 22 (91%) subjects (2.3 ± 0.4 AU). AGEs-sAF were positively correlated with age (R = 0.77; $p = 0.001$), as well the serum C-reactive protein (R = 0.48; $p = 0.03$), triglycerides (R = 0.43; $p = 0.03$), total cholesterol (R = 0.54; $p = 0.006$) and glycated hemoglobin levels (R = 0.46; $p = 0.02$). Systolic blood pressure (R = 0.46; $p = 0.02$) and BMI (R = 0.45; $p = 0.02$) were clinical parameters positively associated with the parameter "age". The Framingham cardiovascular (CV) risk score were intermediate in 2 (8%) and low in 18 (91%) subjects.

In HD group, AGEs-sAF according to their age was high in all HD patients (2.6 ± 0.4 AU). Pre- and post-HD session AGEs-sAF measurements did not differ between them (2.6 ± 0.4 *vs.* 2.6 ± 0.4; $p = 0.18$), and were positively correlated (R = 0.862; $p = 0.0001$). Serum levels of iPTH were 397 ± 386 pg/mL, 25-hydroxyvitamin D 36.6 ± 11 ng/mL, bicarbonate 19 ± 3 mmol/L, alkaline phosphatase 94 ± 64 IU/mL and ß2-microglobulin 4.6 ± 0.9 mg/mL. The Framingham cardiovascular (CV) risk score were high in 1 (5%), intermediate in 2 (10%) and low in 17 (85%) patients.

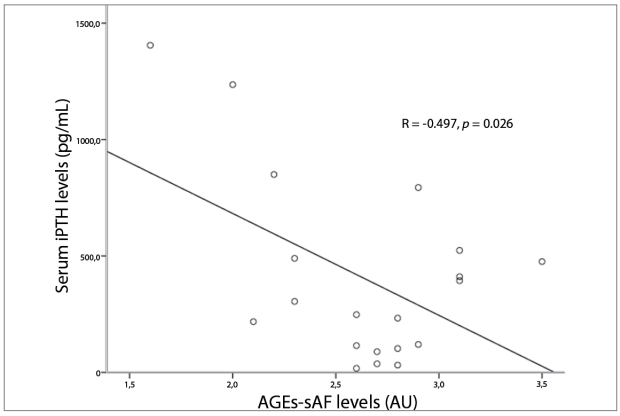
We found a negative correlation between serum iPTH levels and AGEs-sAF levels in patients in HD group (R = -0.497; $p = 0.026$) (Figure 1). Parameters

Table 2 Biochemical and laboratory parameters according to control and hemodialysis groups

Parameters	Control (n = 24)	HD (n = 20)	*p*
AGEs-sAF (AU)	2.3 ± 0.4	2.6 ± 0.4	0.031
Glycemia (mg/dL)	86 ± 9	77 ± 23	0.12
Glycated hemoglobin (%)	5.1 ± 0.2	5.1 ± 0.7	0.54
Ferritin (ng/mL)	129 (103-171)	519 (414-750)	0.001
C-reactive protein (mg/dL)	0.2 ± 0.2	1.7 ± 3	0.03
Albumin (g/dL)	4.4 ± 0.2	3.8 ± 0.5	0.001
Total cholesterol (mg/dL)	185 ± 37	145 ± 33	0.001
LDL-cholesterol (mg/dL)	106 ± 29	77 ± 23	0.01
HDL-cholesterol (mg/dL)	54 ± 12	39 ± 13	0.001
Triglycerides (mg/dL)	133 ± 102	143 ± 61	0.68
Uric acid (mg/dL)	4.9 ± 1.2	6.7 ± 1.1	0.001
Urea (mg/dL)	29 ± 7	136 ± 38	0.001
Creatinine (mg/dL)	0.79 ± 0.14	11 ± 2.8	0.001
Hemoglobin (g/dL)	14 ± 1	10.3 ± 1.7	0.001
Hematocrit (%)	43 ± 3	32.2 ± 5	0.001

HD: Hemodialysis; AGEs-sAF: Advanced glycation end products; sAF: Skin autofluorescence; AU: Arbitrary unit; LDL: Low density lipoprotein; HDL: High density lipoprotein.

Figure 1. Relations between advanced glycation end products measured by skin autofluorescence (AGEs-sAF) and serum parathormone levels. AU, arbitrary units.



of CVD and bone and mineral metabolism were also analyzed in HD group as a function of the median of AGEs-sAF levels (i.e., < 2.7 and ≥ 2.7 AU) (Table 3). In this analysis serum iPTH levels in patients with AGEs-sAF < 2.7 AU were almost twofold higher from those with AGEs-sAF ≥ 2.7 AU, although this difference do not reach statiscal significance (543 ± 503 *vs*. 292 ± 246 pg/mL, p = 0.16). All others parameters related with bone metabolism, like serum alkaline phosphatase (96 ± 88 *vs*. 97 ± 43, p = 0.98), total calcium (9 ± 1.2 *vs*. 9.2 ± 0.5, p = 0.65) and phosphate levels (4.3 ± 1.2 *vs*. 4.9 ± 2, p = 0.46) were similar. In regard of CVD parameters, interventricular septum (9.7 ± 2.7 *vs*. 8.2 ± 1 mm, p = 0.11), left ventricular mass (193 ± 87 *vs*. 135 ± 42 g, p = 0.08) and vascular calcification score (2 ± 2.5 *vs*. 1.7 ± 1.9, p = 0.76) did not differ according to median AGEs-sAF levels.

The comparisons between groups revealed that patients in HD group had significantly higher mean AGEs-sAF levels than did control group (2.6 *vs*. 2.3; p = 0,031). As expected, most part of traditional cardiovascular risk factors was noted in HD group. For example, they had higher WHR (0.92 ± 0.1 *vs*. 0.84 ± 0.1; p = 0.009), systolic blood pressure (132 ± 18 *vs*. 110 ± 9; p = 0.001), higher levels of inflammatory markers like serum ferritin [519 (414-750) *vs*. 129 (103–171); p = 0.001] and C-reactive protein (1.7 ± 3 *vs*. 0.2 ± 0.2; p = 0.03), anemia (10.3 ± 1.7 *vs*. 14 ± 1; p = 0.001), as well creatinine and urea levels (p = 0.001). Despite the presence of three (15%) patients with DM in HD group, no differences was noted on serum glycemia (86 *vs*. 77; p = 0.12) and glycated hemoglobin levels (5.1 *vs*. 5.1; p = 0.54) between the groups, which could be explained by low prevalence of DM.

Discussion

Our study demonstrates three main findings. First, AGEs-sAF levels ranged according age are elevated both in HD and control groups, although it was significantly higher in HD group. Second, high-flux HD single session did not affect AGEs-sAF levels. Third, AGEs-sAF was negatively associated with serum iPTH levels, the main bone turnover marker used in CKD-MBD management.

AGEs-sAF levels ranged according to age were higher in HD group than control group. This finding was supported by some studies which have demonstrated that AGEs accumulation occurs in uremic patients, independently of the presence of DM or their serum glucose levels.[3,10,19] Possible mechanisms of this uremic toxin accumulation in ESKD are the reduced metabolic clearance and the increased oxidative stress, in addition to higher rate of reactive carbonyl compounds formation.[3,20] AGEs tissue accumulation can also explain, at least partially, the aging phenotype of HD patients, since is known that AGEs cause widespread damage to tissues through upregulation of inflammation and cross-linking of collagen and other proteins.[21]

In our study we observed high values of AGEs-sAF, both in healthy and HD subjects, using as reference values obtained from a Dutch study in caucasian population.[18] We cannot exclude that different skin types and eating habits in our healthy subjects (control group) can at least partially, explain these results, since we know that diet-derived AGEs is an important source of this toxins[22] and that skin pigmentation can influence AGEs-sAF lecture.[5] Mook-Kanamori *et al.*[23] demonstrate that the ethnicity exerts influence on AGEs-sAF, with higher levels in Arabs and Filipinos. Limited data exists on sAF in non-caucasian population and in the best of our knowledge, no prior data exist in South Americans, as well in Brazilians sub-groups. More studies including Brazilians are needed to determine if the actual AGEs-sAF values of reference range values are applicable in our population.

In our study, AGEs-sAF pre- and post-HD high flux single session were similar. The use of different HD techniques to counteract AGEs accumulation in HD patients has been investigated, with evidences of free plasma AGEs and AGEs peptides removal by low or high flux HD during a single session. High flux and more frequent HD regime, as daily or home

Table 3 Clinical, demographic, biochemical and imaging characteristics of hemodialysis patients according to median AGEs-SAF levels

		AGEs-sAF (AU)		
		< 2.7	≥ 2.7	*p*
	(n = 20)	(n = 10)	(n = 10)	
Age (years)	38 ± 19	40 ± 24	36 ± 26	0.67
BMI (Kg/m^2)	23.5 ± 6.3	21.3 ± 5.2	25.5 ± 7	0.15
WC (cm)	87.3 ± 17.3	82.1 ± 16.4	91 ± 19	0.30
HD vintage (months)	46 ± 21	42 ± 47	52 ± 53	0.66
Kt/V	1.5 ± 0.61	3 ± 0.5	1.6 ± 0.6	0.3
UF (L)	2.7 ± 0.7	2.6 ± 0.9	2.8 ± 0.8	0.57
Creatinine (mg/dL)	11 ± 2.8	12.4 ± 2.5	10 ± 2.7	0.06
Urea (mg/dL)	136 ± 38	138 ± 54	135 ± 24	0.84
Potassium (mEq/L)	5 ± 0.7	5.3 ± 0.5	4.9 ± 0.7	0.27
Bicarbonate (mmol/L)	18.7 ± 2.7	19.2 ± 3.5	18.3 ± 2.2	0.45
Hematocrit (%)	32.2 ± 5	31.2 ± 6.5	33 ± 3.9	0.50
Calcium (mg/dL)	9.2 ± 0.8	9 ± 1.2	9.2 ± 0.5	0.65
Phosphate (mg/dL)	4.6 ± 1.6	4.3 ± 1.2	4.9 ± 2	0.46
iPTH (pg/mL)	397 ± 385.7	543 ± 503	292 ± 246	0.16
25-OH-D (ng/mL)	36.6 ± 10.9	34.3 ± 11.6	38 ± 11	0.53
ALP (UI/L)	95 ± 64	96 ± 88	97 ± 43	0.98
Ferritin (ng/dL)	575 ± 257	620 ± 226	545 ± 296	0.53
CRP (mg/dL)	1.73 ± 3.1	2.9 ± 4.4	0.9 ± 1.1	0.18
Albumin (g/dL)	3.8 ± 0.5	3.7 ± 0.6	3.8 ± 0.3	0.67
Glycose (mg/dL)	77.4 ± 24.9	89 ± 27	68 ± 21	0.07
HbA1c (%)	5.1 ± 0.7	4.9 ± 0.5	5.2 ± 0.8	0.30
IVS (mm)	8.9 ± 2	9.7 ± 2.7	8.2 ± 1	0.11
LV mass (g)	161.4 ± 68	193 ± 87	135 ± 42	0.08
Total cholesterol (mg/dL)	145 ± 33.5	133 ± 22	155 ± 40	0.16
LDL-cholesterol (mg/dL)	77.1 ± 23.5	67 ± 18	84 ± 26	0.12
HDL-cholesterol (mg/dL)	39 ± 13.4	37 ± 15	41 ± 13	0.54
Triglycerides (mg/dl)	143.3 ± 61.7	141 ± 59	148 ± 68	0.82
VC score	1.8 ± 2.1	2 ± 2.5	1.7 ± 1.9	0.76
VC score ≥ 3 (N)	6	3	3	0.62

AGEs: Advanced glycation end products measured by skin autofluorescence; AU: Arbitrary unit; BMI: Body mass index; WC: Waist circumference; HD: Hemodialysis; UF: Ultrafiltration; iPTH: Intact parathormone; 25-OH-D = 25-hydroxyvitamin D; ALP: Alkaline phosphatase; CRP: C-reactive protein; HbA1c = glycated hemoglobin; NR: Not rated; IVS: Interventricular septal thickness; LV: Left ventricular; LDL: low density lipoprotein; HDL: High density lipoprotein; VC: Vascular calcification.

dialysis, has been suggested to be modalities capable of reducing serum AGEs levels in the long-term.[24,25] However, these previous studies had demonstrated HD influence on plasma AGEs levels, but not on skin accumulation, a tissue with low turnover. Probably, if there is, it is necessary months on HD treatment to note changes in its levels on skin.

It is suggested that AGEs-sAF is an independent predictor of overall and CVD mortality in HD patients.[4,26] AGEs and their receptor (RAGE) play an important role in the pathogenesis of vascular damage and cardiovascular disorders, especially in patients with diabetes and CKD. Recently, an *in vivo* study had demonstrated the association of AGEs accumulation in vessel tissues and medial arterial calcification severity in patients with renal failure.[27]

Despite these evidences in the literature, our subjects in HD group did not shown significantly differences in CV parameters according AGEs-sAF levels, namely, interventricular septum thickness,

left ventricular mass, and vascular or valve calcification. Since AGEs seems to be associated with atherosclerosis, further studies including carotid ultrasound in HD population can improve findings of vascular calcification. Furthermore, the present study did not associated AGEs-sAF to metabolic stress markers, glycated hemoglobin, C-reactive protein and hyperlipidemia, in opposition to previous studies, probably due our study sample size.[5,28]

AGEs negative effects on bone tissue have been described since is known your association with osteoporosis.[29] Many studies using cell culture experiments have been done to clarify AGEs effects on bone tissue, demonstrating multiple possible mechanisms as following: stimulating osteoblasts apoptosis,[30] impairing growth factors or osteogenic cells adhesion to bone matrix,[11] inhibiting osteoblastic differentiation,[31] stimulating osteoblasts to secrete bone-resorbing cytokines,[32] and enhancing osteoclast bone resorption.[33]

Recently, is speculate AGEs participation on CKD-MBD pathophysiology, mainly in adynamic bone disease.[34] Yamamoto *et al.*[13] had described that AGEs can inhibit osteoblastic activity and parathyroid hormone secretion in response to hypocalcemia. In our study we detected a negative correlation of AGEs -sAF with serum iPTH, suggesting a role of AGEs on the pathophysiology of bone disease in HD prevalent patients. These findings must be confirmed in future studies with bone biopsy and serum dosage of AGEs to clarify AGEs effect on CKD-MBD.

This pilot study has several limitations. Potential influences of ethnicity and diet limit definitive conclusions. In addition, we did not have performed AGEs analysis on serum or bone histomorphometry studies. However, to the best of our knowledge, this is the first Brazilian study performed in HD patients using sAF to analyze AGEs tissue accumulation and their relations with CVD parameters and markers of bone metabolism.

In conclusion, AGEs-sAF levels were higher in HD group than control. Surprisingly, some apparently healthy subjects had high AGEs-sAF levels expected according to their age. Single high-flux HD session seems not affect AGEs-sAF levels. A negative relation between AGEs-sAF levels and serum iPTH levels was observed suggesting a role of AGEs on the pathophysiology of bone disease in prevalent HD patients. The nature of this relation and the clinical application of this non-invasive methodology for evaluation AGEs deposition must be confirmed and clarified in future studies.

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