Is zinc-α2-glycoprotein a cardiovascular protective factor for patients undergoing hemodialysis?

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ABSTRACT

Background: Zinc-α2-glycoprotein (ZAG) is a lipid mobilizing factor. Its anti-inflammatory action and expression pattern suggest that ZAG could act by protecting against the obesity-associated disorders. In hemodialysis (HD) patients, ZAG levels were described to be elevated but its effects on markers of inflammation and LDL oxidation are still unclear. We investigated the relationship between ZAG and markers of systemic inflammation and LDL atherogenic modification profile in HD patients.

Methods: Forty-three patients regularly on HD were studied and compared to 20 healthy subjects. Plasma ZAG, adiponectin, electronegative LDL [LDL(−)], an atherocleotically negatively charged LDL subfraction, and anti-LDL(−) autoantibodies levels were measured by ELISA. Markers of inflammation and atherogenic cell recruitment (TNF-α, interleukin-6, VCAM-1, ICAM-1, MCP-1 and PAI-1) were also determined.

Results: Inflammatory markers and atherogenic cell recruitment were higher in HD patients when compared to healthy subjects. ZAG levels were also higher in HD patients (151.5±50.1 mg/l vs 54.6±23.0 mg/l; p<0.0001) and its levels were negatively correlated with TNF-α (r=−0.39; p=0.001) and VCAM-1 (r=−0.52; p<0.0001) and, positively correlated with anti-LDL(−) autoantibodies (r=0.38; p=0.016). On multivariate analyses, plasma ZAG levels were independently associated with VCAM-1 (p=0.01).

Conclusion: ZAG is inversely associated with markers of pro-atherogenic factors linked to systemic inflammation and oxidative stress. Thus, this adipokine may constitute a novel marker of a favorable metabolic profile regarding cardiovascular risk factors in HD population.

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1. Introduction

The adipose tissue has been recognized as an active endocrine organ, secreting a large number of bioactive polypeptide known as adipokines [1]. The release of adipokines, as tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6), leads to a chronic subinflammatory state that play a central role in the increased risk of cardiovascular disease associated with obesity [2]. In contrast to other adipokines, adiponectin seems to be related to insulin sensitivity, anti-inflammatory response and vascular-protective effect [3–5]. It has been recently suggested that another adipokine, zinc-α2-glycoprotein (ZAG), may also have a protective role in the prevention of obesity and its associated disorders [6]. This 40-kDa soluble glycoprotein was firstly isolated from human plasma [7], and has been documented to be expressed in adipocytes [8,9]. This adipokine is related to lipid catabolism and adipose tissue atrophy. The biological activity of ZAG is linked to a cyclic AMP-mediated signaling system through interaction with a β3 adrenoreceptor [9,10]. It induces the inhibition of lipogenic enzymes in adipose tissue [11] and activation of uncoupling proteins, increasing thermogenesis and lipid utilization [10,12].

In addition to lipid metabolism, ZAG may be involved in anti-inflammatory actions and the modulation of other adipokines. Studies have described an inhibitory effect of TNF-α on ZAG expression [8,13]. Mracek et al. [13] and Ceperuello-Mallafré et al. [14] showed a positive association between ZAG mRNA and adiponectin mRNA levels in both visceral and subcutaneous fat of human subjects. This parallel expression pattern for the ZAG and adiponectin with comparable positive regulation, suggests that ZAG could act similarly to adiponectin in protecting against obesity and diabetes [6].

Obesity is a well-known independent risk factor for metabolic complications that may lead to cardiovascular disease, as inflammation and dyslipidemia [2]. Obesity, inflammation and dyslipidemia are also

involved on accelerated atherosclerosis and premature death from cardiovascular disease observed in hemodialysis (HD) patients [15–17]. If ZAG is involved in lipid-mobilizing and anti-inflammatory actions, it could be involved in the regulation of some cardiovascular risk factors. In this study, we evaluated ZAG plasma levels and its relationship with a pro-inflammatory and pro-atherogenic profile in HD patients.

2. Material and methods

2.1. Subjects

Forty-three HD patients were studied and compared to 20 healthy subjects. This control group was composed of individuals without any disease and who were not on any medication. The healthy subjects were mainly the staff members of the dialysis unit and presented similar age and body mass index (BMI) when compared to HD patients. Inclusion criteria were age between 18 and 75 y, patients who had been on maintenance HD for at least 6 months and without using lipid-lowering drugs. Patients with cancer, AIDS, inflammatory or liver diseases, and the use of a catheter for dialysis access at the time of blood collection were excluded.

The etiology of chronic kidney disease (CKD) was hypertensive nephrosclerosis (22), diabetic nephropathy (10), chronic glomerulonephritis (4), polycystic kidney disease (3) and other diseases or unknown cause (4). As anti-hypertensive medication, 11 patients (27.9%) were receiving ACE inhibitors and one patient, calcium channel blocker. The average arterial blood pressure at the end of HD session was 122/74 mm Hg. The HD sessions were 3.5–4.5 h three times/week, with a blood flow >300 ml/min, a dialysate flow around 600 ml/min and bicarbonate buffer. The study protocol was approved by the Ethics Committee of the School of Medicine of the Universidade Federal Fluminense. The HD patients and healthy subjects were aware of the study and signed an informed consent after reading such document.

2.2. Anthropometric and biochemical measurements

Anthropometric (weight and stature) measurements were obtained immediately after the HD session by a trained researcher. BMI was calculated as body weight divided by squared stature. Blood samples were obtained from the arterial line, after overnight fasting, immediately before the HD session. The sera were immediately frozen at −80 °C until analyzed. ZAG plasma levels were measured by a commercial enzyme-linked immunosorbent assay (ELISA) (Biovendor, Modrice, Czech Republic) according to the manufacturer’s instructions. The sensitivity of the assay was 0.673 ng/ml and the intra- and interassay CVs were <5 and 6.5%, respectively. Adiponectin levels were also determined by ELISA (RayBiotech, Norcross, GA) and the minimum detectable dose is typically ~25 pg/ml. The intra- and interassay CVs were <10 and 12%, respectively. The concentrations of TNF-α, IL-6, monocyte chemoattractant protein-1 (MCP-1), plasminogen activator inhibitor-1 (PAI-1), vascular cell adhesion molecule 1 (VCAM-1), intercellular adhesion molecule 1 (ICAM) and leptin were measured using routine enzymatic methods. LDL-cholesterol was calculated according to Friedewald’s formula. The electronegative LDL [LDL(−)] and anti-LDL(−) autoantibodies were determined by ELISA as recently described by Lobo et al. [19].

2.3. Statistical analysis

The Kolmogorov–Smirnov normality test was used to characterize the data distribution. Results were expressed as mean ± SD or median (interquartile range), as applicable. Student’s t test was used for the differences between means and the Mann–Whitney test was used for non-parametric data. Pearson or Spearman correlation coefficient was calculated for univariate analyses. Multiple linear regression was performed to determine variables that had independent associations with ZAG (all variables with significant correlations with plasma ZAG levels were included). Statistical significance was accepted as p<0.05.

3. Results

The clinical features and biochemical data of HD patients and healthy subjects are summarized in Tables 1 and 2. The time on dialysis (months) and spKt/V was 66.1±39.9 and 1.39±0.23, respectively. HD patients presented higher levels of LDL(−), VCAM-1, ICAM-1, IL-6, TNF-α and CRP than healthy subjects. MCP-1 and PAI-1 levels were also higher but there was no statistical difference. In contrast, anti-LDL(−) autoantibodies levels were significantly lower. The prevalence of inflammation among HD patients, according to CRP levels (CRP >0.3 mg/dl), was 41.8%.

Eight (18.6%) patients had cholesterol higher than 200 mg/dl and thirty-two (76.7%) triglycerides above 150 mg/dl. Only five (11.6%) patients had LDL-cholesterol higher than 150 mg/dl. The mean of serum albumin was 3.9±0.26 g/l and nine (21%) HD patients had albumin less than 3.8 g/l. Twenty (46.5%) HD patients had BMI between 18.5 and 24.9 kg/m2, 16 (37.5%) were overweight, 5 (11.6%) presented obesity and only two (4.7%) had BMI >18.5 kg/m2.

Circulating ZAG was higher in HD patients as compared to healthy subjects (Table 2). In these patients, serum levels ranged from 55.1 to 258.0 mg/l and there was no difference according to gender (154.9±56.6 mg/l in men and 145.7±37.6 mg/l in women; p=0.56), or the presence of diabetes mellitus (146.4±40.7 mg/l in non-diabetics and 168.2±73.4 mg/l in diabetics; p=0.4).

Plasma ZAG levels were inversely correlated with TNF-α (r=−0.39; p=0.01), but not with IL-6 or CRP. Circulating ZAG was not correlated with lipid profile, adiponectin and LDL(−), but was positively associated with anti-LDL(−) autoantibodies (r=0.38; p=0.01). Among the adhesion molecules, ZAG was inversely correlated with VCAM-1 (r=−0.52; p<0.0001). On multivariate analyses, only VCAM-1 (p=0.01) was independently associated with plasma ZAG levels (Table 3), however, this result for TNF-α was borderline (p=0.05).

4. Discussion

Systemic inflammation and oxidative stress are ubiquitous features of patients with chronic kidney disease. These conditions are associated with progressive cardiovascular disease [16,17]. Oxidative stress seems to be involved in accelerated atherosclerosis and premature death from cardiovascular disease observed in hemodialysis (HD) patients [15–17].

Table 1

| General characteristics and lipid profile of hemodialysis patients and healthy subjects. |
|---------------------------------|---------------------------------|------------------|
| **Age (y)**                     | **HD patients**                 | **Healthy subjects** |
| (N=43)                          | (N=20)                          | p value          |
| 54.1±12.6                       | 49.5±15.2                       | NS               |
| Gender (male/female)            |                                 |                  |
| 27/16                           | 11/9                            |                  |
| BMI (kg/m²)                     |                                 |                  |
| 24.4±4.2                        | 25.6±4.1                        |                  |
| Triglycerides (mg/dl)           |                                 |                  |
| 192.7±77.9                      | 137.0±32.2                      | 0.001            |
| Total cholesterol (mg/dl)       |                                 |                  |
| 159.4±34.2                      | 190.0±38.3                      | 0.006            |
| LDL-cholesterol (mg/dl)         |                                 |                  |
| 104.4±28.4                      | 121.5±24.2                      |                  |
| HDL-cholesterol (mg/dl)         |                                 |                  |
| 30.0±8.2                        | 33.0±8.5                        |                  |
| LDL(−) (U/l)                    |                                 |                  |
| 0.17±0.12                       | 0.10±0.08                       | 0.020            |
| Anti-LDL(−) autoantibodies (mg/l)| 0.01 (0.02)                     | 0.06 (0.08)      | 0.002 |

BMI, body mass index; LDL, low density lipoprotein; HDL, high density lipoprotein; LDL(−), electronegative low density lipoprotein.
to be the link between inflammation and cardiovascular risk in HD patients [20]. In fact, despite normal LDL levels [20], the presence of increased plasma concentrations of modified LDL (LDL-\(fl\)–), an important factor in the initiation and progression of atherosclerotic plaques [22], was described in HD patients [23]. In addition, the release of adipokines from adipose tissue leads to a chronic inflammatory state that could play a role in the increased risk of cardiovascular disease in patients with high body fat.

Zinc-\(\alpha\)-2-glycoprotein is an adipokine with anti-inflammatory properties [6,13]. In HD patients, only the study by Phillip et al. [24] evaluated the relationship among ZAG, inflammation and lipid profile. These authors reported higher circulating ZAG values but no association between ZAG and these parameters. In the present study, we observed high ZAG levels in HD patients, also not related with LDL, HDL, TG or cholesterol levels. In these patients, dyslipidemia has special features being characterized by hypertriglyceridemia, low plasma HDL-cholesterol concentration, and total and LDL-cholesterol values frequently within or below the normal limits [21]. This profile was also observed with the patients in our study. However, a possible role of increased plasma ZAG levels on this characteristic lipid pattern cannot be explained at this moment.

Atherosclerosis is associated with high concentrations of LDL(−) due to its ability to modulate the expression of molecules involved in inflammation, apoptosis, and angiogenesis [23,25–29]. High plasma levels of LDL(−) were positively correlated with IL-6 and ICAM-1, whereas levels of anti-LDL(−) antibodies were negatively correlated with TNF-\(\alpha\), ICAM-1 and VCAM-1 [19]. The present study showed that anti-LDL(−) autoantibodies were positively associated with ZAG, and several studies have demonstrated that anti-LDL(−) autoantibodies display a protective antiatherogenic role [19,23,30–34]. Taken together, it can be postulated that both proteins are associated with a better outcome in renal patients. A possible role of ZAG on anti-LDL(−) autoantibodies modulation warrants further studies.

Although the factors that downregulate ZAG are not well understood, obesity-related inflammation could be involved in the ZAG regulation. Gao et al. [35] showed that chronic treatment with TNF-\(\alpha\) led to a significant decrease in ZAG expression and secretion in adipocytes. Bao et al. [8] and Mracek et al. [13] also found a chronic and dose-dependent decrease in ZAG mRNA levels after treatment with TNF-\(\alpha\). Inhibition of ZAG expression and release by TNF-\(\alpha\) suggests that reduced ZAG production may increase the susceptibility to lipid accumulation in adipose tissue and liver in obesity [13], and that these two mediators of cachexia have different effects on lipid metabolism within adipose tissue. Moreover, the parallel expression pattern of ZAG and adiponectin, similarly regulated by rosiglitazone and TNF-\(\alpha\) [8,36,37], suggest that ZAG could act like adiponectin in protecting against the obese state [6].

In fact, the multivariate analysis performed in the present study showed that ZAG was independently negatively associated with VCAM-1, a potential marker of atherosclerosis in HD patients. Adiponectin has also been reported to have direct antiatherogenic effects, as inhibition of the expression of adhesion molecules, including ICAM-1, VCAM-1 and selectin [3]. Although adiponectin could be the link between ZAG and markers of a pro-atherogenic state, in our study circulating ZAG was not correlated with adiponectin, in agreement with findings from healthy individuals [38,39] and also in HD patients [24].

5. Conclusions

In summary, this study evaluated plasma ZAG levels and its correlation with pro-atherogenic factors closely linked to inflammation and oxidative stress in renal patients on regular HD. Our results showed that ZAG was not correlated with lipid profile and adiponectin but, we observed an inverse correlation between ZAG and inflammatory proatherogenic markers as TNF-\(\alpha\), VCAM-1 and anti-LDL(−) antibodies in HD patients. These findings introduce zinc-\(\alpha\)-2-glycoprotein as a marker of prognostic value in the evaluation of the pro-atherogenic status on renal patients undergoing chronic hemodialysis. The mechanistic interactions between ZAG secretion by adipocytes and its regulation by pro-inflammatory cytokines need to be deeply examined.

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