End Stage Renal Disease Associated with Metabolic Syndrome: A study of Biomarkers Utilizing Biochip Array Profiling Methods

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Abstract

Introduction: Metabolic syndrome (MS) represents a cluster of cardiovascular risk factors which contribute to myocardial infarction and stroke in end stage renal disease (ESRD) patients. Several metabolic biomarkers have been identified and their circulating levels provide a better understanding of the pathogenesis of MS/ESRD. The purpose of this investigation is to profile biomarkers of MS in ESRD patients.
Materials and Methods: Plasma samples from 87 patients with ESRD undergoing maintenance hemodialysis and 50 normals were collected prior to a routine session. These samples were profiled for metabolic biomarkers using multiple protein chip bioarray technology. The protein chip array was comprised of several biomarkers of MS. All results were compiled in terms of group means +/- 1 SD (SEM) and statistically analyzed.
Results: In comparison to the normal samples, the ESRD group showed marked increase in circulating levels of all biomarkers. TNFa (47.4 +/- 18.3 vs 4.1 +/- 1.1 ng/mL) and IL-6 (7.8 +/- 9.1 vs 0.8 +/- 0.4 ng/mL) showed the most pronounced increase. C-peptide (14.5 +/- 4.1 vs 2.8 +/- 1.6 ng/mL), leptin (29.7 +/- 29.2 vs 5.2 +/- 7.9 ng/mL), resistan (16.1 +/- 6.0 vs 2.3 +/- 0.7 ng/mL) and ferritin (274 +/- 57 vs 57 +/- 63 ng/mL) showed a 5-fold increase in the ESRD group compared to normal. PAI-1 (5.4 +/- 5.2 vs 2.9 +/- 2.5 ng/mL), IL-1a (1.3 +/- 1.7 vs 0.35 +/- 0.07 ng/mL) and insulin (32.1 +/- 17.3 vs 17.1 +/- 1.7 ng/mL) showed modest increase in the ESRD patients. The increase in all of the MS biomarkers in the ESRD patients were highly significantly elevated, $p<0.05$. *Discussion:* The specific biochip array for MS allows the selective determination of various biomarkers associated with this syndrome in the ESRD patients and normals. Parallel increase in resistan, insulin, C-peptide, and leptin points to the derangement of glucose metabolism in these patients. Increase IL-1a, TNFa, and ferritin suggests the upregulation of an inflammatory process. PAI-1 increase suggests a fibrinolytic deficit. All of these biomarkers stimulate multiple pathways through signal transduction processes.

Introduction

CKD not only puts patients at risk for renal failure, but also for co-morbidities such as cardiovascular disease (CVD) and stroke, and increases all-cause mortality. As MetS and CKD share many of the same risk factors and similar inflammatory pathogenesis, multiple studies have suggested a correlation between CKD and MetS. The purpose of this study is to investigate the prevalence of MetS in ESRD patients. Furthermore, through increasing our understanding of the pathogenesis, disease state, and biomarker profiles of patients with ESRD, we may be able to predict the development of comorbidities, treat aggressively, and offer patients with ESRD a better chance of survival.

Materials & Methods

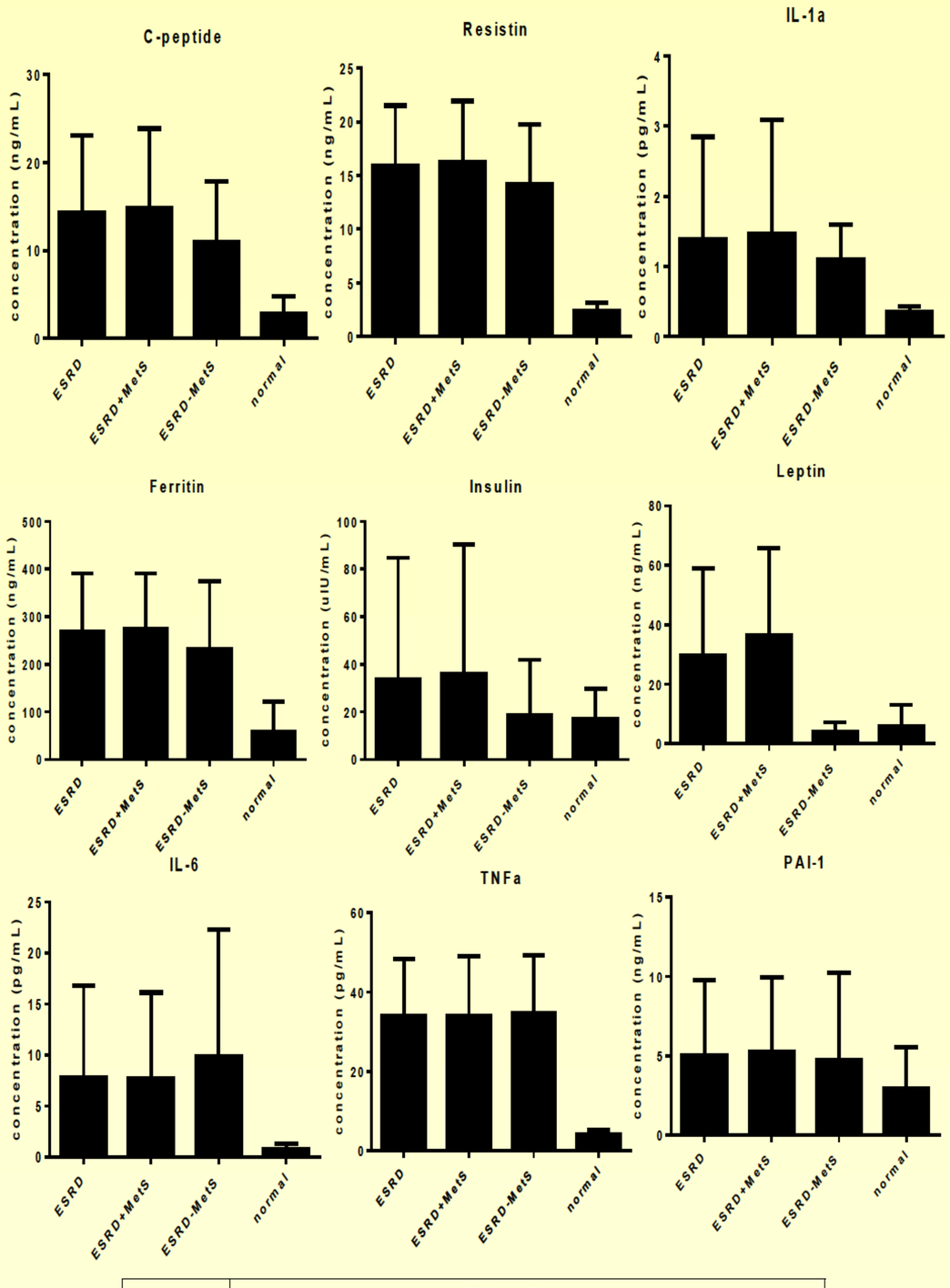
Biomarker evaluation
Under IRB approval, plasma samples collected from 89 patients with ESRD prior to hemodialysis on November 1st and 2nd, 2013. Normal human plasma samples (female & male, 18-35 years old) were purchased from George King Biomedical Inc. (Overland Park, KS). Samples were stored at -80° C. Metabolic biochips were purchased from RANDOX (Co. Antrim, Northern Ireland) to test include C peptide, ferritin, IL-6, resistin, insulin, TNFα, IL-1a, leptin, PAI-1. These biomarkers were tested on 82 ESRD and 17 normal samples.

Evaluating prevalence of MetS in patients with ESRD
National Cholesterol Education Program (NCEP/ATP III) guidelines were used to evaluate which patients met MetS criteria. Patients who meet three or more of the following criteria meet the requirements for metabolic syndrome:

-- Abdominal obesity, defined as a waist circumference in men ≥102 cm (40 in) and in women ≥88 cm (35 in)
-- Serum triglycerides ≥150 mg/dL (1.7 mmol/L) or drug treatment for elevated triglycerides
-- Serum HDL cholesterol <40 mg/dL (1 mmol/L) in men and <50 mg/dL (1.3 mmol/L) in women or drug treatment for low HDL-C
-- Blood pressure ≥130/85 mmHg or drug treatment for elevated blood pressure
-- Fasting plasma glucose (FPG) ≥100 mg/dL (5.6 mmol/L) or drug treatment for elevated blood glucose

Statistical Analysis
All data will be analyzed using GraphPad Prism Software (San Diego, CA). Unpaired, non-parametric t-tests were conducted to determine the significance of patterns among variables. Paired, non-parametric correlations were also computed between select variables.

Results



	P-values			
	ESRD vs. normal	ESRD+MetS vs. ESRD-MetS	ESRD+MetS vs. normal	ESRD-MetS vs. normal
C-peptide	<0.0001	0.2	< 0.0001	0.002
Resistin	<0.0001	0.2	< 0.0001	< 0.0001
IL-1a	<0.0001	1	< 0.0001	< 0.0001
Ferritin	<0.0001	0.4	< 0.0001	0.005
Insulin	0.4	0.09	0.2	0.6
Leptin	0.01	0.0002	0.0003	0.6
IL-6	<0.0001	0.9	< 0.0001	< 0.0001
TNFα	<0.0001	1	< 0.0001	< 0.0001
PAI-1	0.05	0.4	0.04	0.5

	% difference from normal		
	ESRD	ESRD +MetS	ESRD-MetS
C-peptide	120	122	99
Resistin	140	143	131
IL-1a	92	92	90
Ferritin	133	123	168
Insulin	79	72	104
Leptin	130	139	90
IL-6	129	141	85
TNFα	158	148	181
PAI-1	69	70	74

Summary

1. **All biomarkers, except insulin, were significantly elevated in patients with ESRD compared to normal.**
2. **Patients with ESRD+MetS, as compared to ESRD-MetS, had significantly elevated leptin. Furthermore, ESRD+MetS vs. normal was significant for leptin, but ESRD-MetS vs. normal was not.**
3. **ESRD+MetS and ESRD-MetS populations were not statistically different for all other biomarkers.**
4. **83.5% of the patients with ESRD meet the criteria for metabolic syndrome.**

Conclusion

Biomarker levels in ESRD vs. normal
Elevated levels of C-peptide, resistin, IL-1a, IL-6, TNFα, ferritin, leptin, and PAI-1, are consistent with literature. Elevated biomarkers suggest an ongoing inflammatory processes in patients with ESRD. The lack of significant insulin increase in patients with ESRD may be attributed to the high circulating levels of leptin, which has been shown to disrupt insulin secretion.

Prevalence of Metabolic S yndrom in patients with ESRD
Based on NCEP/ATP III guidelines, 83.5% of the patients with ESRD meet the criteria for metabolic syndrome, which is greatly increased from the 42% in a representative population over 70 years old. When broken down by criteria, hypertension and elevated glucose were the two criteria most prevalent in the ESRD population.

Biomarker levels in ESRD+MetS vs. ESRD-MetS
Lack of a significant difference between ESRD-MetS patients and normal Leptin levels suggests that the significantly elevated Leptin levels in the ESRD population as a whole, may be attributed to MetS, which is highly prevalent in the ESRD population. ESRD+MetS and ESRD-MetS populations are not statistically different for all other biomarkers, suggesting biomarker elevation is due to ESRD pathogenesis, rather than due to MetS as a comorbidity.

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