The link between the fibroblast growth factor-23–klotho–vitamin D_3 axis and the renin–angiotensin–aldosterone axis in the development and progression of obesity-related kidney disease

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Received 6 February 2013
Accepted 25 February 2013

Egyptian Journal of Internal Medicine
2013, 25:75–79

Introduction and aim of the work

Obesity is established as an important contributor of increased diabetes mellitus, hypertension and cardiovascular disease, all of which can promote chronic kidney disease (CKD). Recently, there is a growing appreciation that even in the absence of these risks, obesity itself significantly increases CKD and accelerates its progression. The aim of this work is to evaluate the link between Renin-Angiotensin-Aldosterone System (RAAS) and FGF23-Klotho-1,25D3 axis and their impact in obese and non-obese CKD patients.

Patients and methods

In a cross sectional randomized multi centers study, two hundred twenty six CKD patients stage III and IV (eGFR20–60 ml/min/m^2) have enrolled in this study as follows: group I; 87 non diabetic CKD patients aged 20–40 years with body mass index (BMI) between 20–25 kgm/m^2; group II; 130 non diabetic CKD patients aged 20–40 years with (BMI) >30 kgm/m^2 and group III; 89 CKD patients aged >60 years. All patient have been tested for plasma leptinlevels, 1,25-dihydrocholicalciferole (1,25D3), plasmaparathormone (PTH) Serum calcium (Ca), serum phosphorus (PO_4), and plasma FGF-23 , plasma renin activity (PRA), plasma angiotensinogen receptor 1 &2 (AT1 & AT2) and plasma aldosterone (ALD) and pulse wave velocity (PWV).

Results

The eGFR was significantly reduced in the obese group II (eGFR = 37.7 ± 13.6) when compared with eGFR of the lean group I (eGFR = 49.3 ± 7.51) were $P < 0.001$, but not significant when compared with the old age group III (eGFR = 41.0 ± 13.47). The obese group II shows significant increase in the ALD/PRA ratio when compared with the lean group I and old age group III (43.23 ± 14.9) for group II vs 11.29 ± 4.1 for group I, $P < 0.001$ and 24.91 ± 12.1 for group III, $P < 0.05$). Regarding the FGF23-Klotho-vitD3 axis, its components of the obese group II (FGF23 259.55 ± 138.6 Ru/ml; PTH 77.63 ± X32.4 pg/ml; S.PO 4.74 ± 1.61 mg/dl) were significantly elevated when compared to the lean group I (FGF23 132.81 ± 126.1 Ru/ml; PTH 59.18 ± 24.7 pg/ml; S.PO4 3.85 ± 0.92 mg/dl); the P values were <0.001, <0.01 and <0.05 respectively , while when compared with the old age group III (FGF23 179.33 ± 237.4 Ru/ml; PTH 70.94 ± 15.26 pg/ml; S.PO4 4.09 ± 0.42 mg/dl), values were of less significance. Plasma insulin levels were significantly high in the obese group II (insulin = 13.73 ± 2.38fg/l) than the lean group I (insulin = 5.59 ± 2.31 fg/l) and $P < 0.001$ and in group III p. insulin level was 10.7 ± 1.68 ($P < 0.05$).

Conclusion

Obesity per se is an independent risk factor in the development and progression of chronic kidney disease specially in young age patients.

Keywords:
adipose tissue, chronic kidney disease, fibroblast growth factor-23, obesity, renin

Egypt J Intern Med 25:75–79

1110-7782

Introduction

A number of recent clinical and epidemiological studies suggest that obesity itself – independent of its ties to diabetic and hypertensive disease risk – can play an important role in the development of chronic kidney disease (CKD) [1–3]. A multivariate cross-sectional analysis of NHANES data reported a graded association between a higher BMI and reduced kidney function [4].

A number of mechanisms have been proposed as explanations for the obesity–CKD relationship, including chronic inflammation, abnormal vascular remodeling, and renal lipotoxicity [5]. These modes of injury can occur in

the absence of diabetes and hypertension. Perhaps the best-described mechanism of obesity-induced kidney injury involves the adverse effects of increased body mass and subsequent increased glomerular filtration rate (GFR) per intact nephron [6–8].

Another proposed mechanism involves leptin. Leptin is an adipose tissue-derived hormone shown to be associated with several metabolic, inflammatory, and hemostatic factors related to CKD. Recent animal studies have reported that infusion of recombinant leptin into normal rats for 3 weeks fosters the development of glomerulosclerosis [9].

Patients and methods

This was a cross-sectional randomized multicenter study involving 390 stage III and IV CKD patients. Their estimated GFR (eGFR) levels were between 20 and 60 ml/min/m^2. Data were collected from 1 September until the end of October 2012 from outpatient clinics of five hospitals in Egypt. The participants were categorized into three groups: group I included 153 nondiabetic CKD patients aged 20–40 years with a BMI between 20 and 25 kg/m^2; group II included 148 nondiabetic CKD patients aged 20–40 years with a BMI of more than 30 kg/m^2; and group III included 89 CKD patients aged more than 60 years with a BMI of more than 24.

BMI is computed as the weight (kg) divided by the square of the height (m).

The calculated eGFR values were categorized as being less than 60 ml/min/1.73 m^2 on the basis of the Kidney Disease Outcomes Quality Initiative (KDOQI) classification of kidney function. eGFR values less than 60 ml/min/1.73 m^2 were considered abnormal and indicative of moderately reduced kidney function and referred to as prevalent CKD. Estimation of the GFR and the biochemical assessments were performed using an autoanalyzer at a single laboratory [10].

Blood samples were collected in three 10-ml liquid EDTA blood tubes, placed on ice packs, stored in polystyrene foam containers, and returned to the HPFS blood storage facility by means of an overnight courier. More than 95% of the samples arrived within 24 h of collection.

Measurement of plasma leptin levels was carried out by Linco Research Inc. (St. Louis, Missouri, USA). The assay was a radioimmunoassay (RIA) using a polyclonal antibody raised in rabbits against highly purified recombinant human leptin [11,12].

The plasma fibroblast growth factor-23 (FGF23) levels were measured using a second-generation C-terminal human enzyme-linked immunosorbent assay (Immutopics, San Clemente, California, USA). Plasma levels measured twice for each participant were averaged. The coefficient of variation was 9.8%.

The 1,25-dihydrocholicalciferol (1,25-D_3) levels were determined using RIA [Diasorin (Stillwater, Minnesota, USA) and IDS (Tyne and Wear, UK)]. Serum calcium and phosphorus levels were measured using standard methods.

Intact parathormone (PTH) levels were measured using an electrochemiluminescence immunoassay on the 2010 Elecsys Autoanalyzer (Roche Diagnostics, Indianapolis, Indiana, USA).

The plasma renin activity (PRA) and aldosterone (ALD) levels were determined using a RIA. Plasma angiotensin I and angiotensin II levels were measured using a double-antibody RIA; the details of these methods have been described elsewhere [2,3].

The pulse wave velocity (PWV) was measured as a marker of arterial stiffness, a critical determinant of cardiovascular outcomes in CKD. Measurements were obtained for the carotid and femoral arteries. The aortic–femoral PWV was measured for all patients in m/s.

Statistical analysis

The variables were reported as mean and SD if normally distributed or as median and interquartile range if not. The *t*-test was used to compare the groups when the variables were normally distributed and the Mann–Whitney test was used if they were not. The univariable linear regression analysis was used to evaluate the independent associations between the anthropomorphic variables (independent variables). SPSS (version 2010, Microsoft Incorporation, Windows Professional, USA) version 16.0 was used for analysis. A P value less than 0.05 was considered statistically significant.

Results

On comparing the mean age among the groups, there was no significant difference between the mean age of the lean group (group I) (29.7 ± 4.88) and that of the obese group (group II) (31.7 ± 2.41) ($P = 0.362$), whereas the mean age of the old age group (group III) (65.1 ± 4.66) showed a significant difference when compared with the other groups ($P < 0.001$ for both groups). The percentage of hypertensive patients in the lean group (43%) was higher compared with that in the obese group (35%), though the difference was not significant ($P = 267$); however, the percentage in the old age group was highly significantly higher when compared with both the lean group ($P < 0.001$) and the obese group ($P < 0.001$) (Table 1).

The eGFR levels were higher in the lean group (eGFR, 49.3 ± 7.51 ml/min/m^2) compared with those in both the obese group (eGFR, 37.71 ± 13.6 ml/min/m^2) ($P < 0.01$) and the old age group (eGFR, 41 ± 13.47 ml/min/m^2) ($P < 0.05$).

The PRA was significantly lower in the obese group II (2.62 ± 1.45 ng/ml/h) compared with both the lean group (3.39 ± 2.07 ng/ml/h) ($P < 0.05$) and the old age group (3.46 ± 2.82 ng/ml/h) ($P < 0.05$); however, the difference between the activity in the lean and the old age groups was not significant ($P = 346$). The ALD levels showed a

Table 1 Patient clinical data

	Group I Lean (20–40 years)	Group II Obese (20–40 years)	Group III Old (>60 years)
Number	87	130	89
Sex male (%)	47.70	46.60	42.70
Age (years)	29.7 ± 4.9	31.7 ± 4.21	65.1 ± 4.66
BMI (kg/m^2)	22.73 ± 3.2	37.3 ± 8.2	29.2 ± 6.4
SBP (mmHg)	133 ± 25.8	138 ± 36.8	146 ± 41.5
DBP (mmHg)	80 ± 16.9	82 ± 24.7	81 ± 11.4
Hypertension (%)	23	35	72

DBP, diastolic blood pressure; SBP, systolic blood pressure.

significant elevation in the obese group (89.91 ± 22.6 pg/ml) when compared with the lean group (47.55 ± 21.8 pg/ml) ($P<0.001$), whereas there was no significant difference on comparison with the old age group (86.18 ± 16.8 pg/ml) ($P=0.34$).

In contrast, on evaluating the renin–angiotensin–ALD system (RAAS) activity using the ALD/PRA ratio, it was observed to be markedly elevated in the obese group (ALD/PRA, 37.8 ± 14.9) compared with both the lean group (ALD/PRA, 14.0 ± 4.1) ($P<0.001$) and the old age group (ALD/PRA, 24.91 ± 12.1) ($P<0.01$).

The level of angiotensin receptors (AT1 and AT2) were significantly higher in the obese group (AT1, 653.28 ± 352.6 pg/ml; AT2, 48.1 ± 9.8 pg/ml) compared with the lean group (AT1, 290.29 ± 210.6 pg/ml; AT2, 25.46 ± 11.4 pg/ml) ($P<0.001$ and $P<0.001$, respectively); however, when the levels in the obese were compared with those in the old age group (AT1, 407.91 ± 370.4 pg/ml; AT2, 33.68 ± 7.63 pg/ml), the significance values were less ($P<0.01$ and $P<0.01$, respectively). The power of significance between the lean and the old age groups were $P<0.05$ and $P<0.05$, respectively.

Significant elevation was observed in the aortic–femoral PWV in the old age group (PWV, 10.21 ± 0.68 m/s) when compared with the obese group (PWV, 9.31 ± 0.9 m/s) ($P<0.5$) and the lean group (PWV, 9.48 ± 0.8 m/s) ($P<0.5$); however, no significant difference was observed on comparing the aortic–femoral PWV between the obese and lean groups ($P=0.334$).

Hyperphosphatemia occurred significantly in the obese group [serum phosphate ($S.PO_4$), 4.74 ± 1.61 mg/dl] when compared with the lean group ($S.PO_4$, 3.85 ± 0.92 mg/dl) ($P<0.05$). However, in the old age group, the occurrence of hyperphosphatemia ($S.PO_4$, 4.09 ± 0.42 mg/dl) was not significantly higher when compared with the other groups. The serum calcium levels were almost within the normal range for all the groups. The PTH levels were elevated in both the obese group (PTH, 77.63 ± 32.4 pg/ml) and the old age group (PTH, 70.94 ± 15.26 pg/ml) when compared with the lean group (PTH, 59.18 ± 24.7 pg/ml) ($P<0.1$ for the obese group and $P<0.05$ for the old age group); however, the levels in the obese group were significantly higher when compared with the old age group ($P=0.336$). The levels of 1,25-D_3 were significantly lower in the obese group (1,25-D_3, 19.85 ± 3.6 ng/ml) compared with the lean group (1,25-D_3, 24.42 ± 5.41 ng/ml) and the old age group (1,25-D_3, 25.0 ± 5.81 ng/ml) ($P<0.05$ for both groups).

The leptin levels were markedly and significantly elevated in the obese group (leptin, 24.13 ± 7.81 fg/l) compared with the lean group (leptin, 1.92 ± 1.61 fg/l) ($P<0.001$) and the old age group (leptin, 5.51 ± 3.21Fg/l) ($P<0.01$). The plasma insulin levels were also significantly elevated in the obese group (insulin, 13.73 ± 2.38 μU/ml) compared with the lean group (insulin, 5.59 ± 2.31 μU/ml) ($P<0.01$) and the old age group (insulin, 10.7 ± 1.68 μU/ml) ($P<0.05$).

The FGF23 levels were significantly elevated in the obese group (FGF23, 259.55 ± 138.6 U/ml) when compared with the lean group (FGF23, 132.81 ± 126.1 U/ml) ($P<0.001$) and the old age group (FGF23, 179.33 ± 237.4 U/ml) ($P<0.01$) (Table 2).

Discussion

The aim of this study was to determine whether obesity *per se* – independent of its ties to hypertension and/or diabetes – has a positive relationship with the parameters and markers of renal and vascular injury.

We selected an obese group (group II) of patients with CKD (BMI > 30 kg/m^2; eGFR, 20–60 ml/min/1.73 m^2); none of them were diabetic to exclude diabetes as a possible cause. We compared the laboratory findings of the obese group with those of another group (group I) of patients whose demographic features were more or less similar to those of the obese group except that they were lean (BMI 20–24.9 kg/m^2), hence, any significantly different values can be attributed merely to obesity. In addition, we also compared the results of the obese group with those of another old age CKD-matched group of patients (age > 60 years), in whom cardiovascular insults were more marked and more prominent, to statistically exclude any age-related cardiovascular disease impacts on the kidneys.

Although the obese patients showed low plasma renin levels, low PRA, and elevated plasma ALD levels compared with the lean patients because of the negative feedback loop interaction between them, the level of each at any given time is not an accurate marker of the activity of the RAAS axis; however, the ALD/PRA ratio might be a more reliable marker for this activity [13]. The significant elevation in the ALD/PRA ratio in obese patients compared with lean and old patients is explained, at least in part, by the increased adipose tissue. The old age group showed a slight elevation in their ALD/PRA ratios because the individuals in this group were slightly overweight (BMI < 29.9 kg/m^2); yet, this elevation was far less than that observed in the obese group, confirming this proposal. There are nonepithelial injurious effects of ALD that occur in the presence of normal to high levels of the sodium cofactor, defined as a high sodium intake with an increased extracellular volume [14]. As our CKD patients usually had a positive sodium balance and extracellular volume expansion, the harmful interaction between ALD and the expanded

Table 2 Statistical analysis of all parameters among the different groups of the study

	Group I Lean (20–40 years) *N*=87	Group II Obese (20–40 years) *N*=130	Group III Old age (>60 years) *N*=89	*P* value (group I vs. group II)	*P* value (group II vs. group III)	*P* value (group I vs. group III)
eGFR (ml/min/m^2)	49.3±7.51	37.71±13.6	41.0±13.47	<0.01	<0.05	<0.05
Diabetes (%)	Nil	Nil	36%	–	–	–
PRA (ng/ml/h)	4.21±2.07	2.08±1.45	3.46±1.36	<0.05	<0.05	346
ALD (pg/ml)	47.55±21.8	89.91±22.6	86.18±16.8	<0.001	0.34	0.05
ALD/PRA ratio	11.29±4.1	43.23±14.9	24.91±12.1	<0.001	<0.01	<0.01
AT1 (pg/ml)	290.3±210.6	653.3±352.6	407.1±370.4	<0.001	<0.01	<0.05
AT2 (pg/ml)	25.46±11.4	48.1±4.8	33.68±7.63	<0.001	<0.01	<0.05
PWV (m/s)	9.48±0.8	9.31±0.9	10.21±0.68	0.334	<0.05	<0.05
SCa (mg/dl)	9.19±0.64	9.27±0.88	9.24±0.49	NS	NS	NS
S.PO_4 (mg/dl)	3.85±0.92	4.74±1.61	4.09±0.42	<0.05	NS	NS
PTH (pg/ml)	59.18±24.7	77.63±32.4	70.9±15.3	<0.01	336	<0.05
1,25-D_3 (ng/ml)	24.4±4.51	19.85±3.6	25.0±5.81	<0.05	<0.05	NS
S leptin (fg/l)	1.92±1.61	24.13±7.81	5.51±3.21	<0.001	NS	<0.01
FGF23 (RU/ml)	132.8±126.1	259.6±138.6	179.3±237.4	<0.001	<0.01	<0.05
P insulin (μU/ml)	5.59±2.31	13.73±2.38	10.7±1.68	<0.01	<0.05	<0.01
S albumin (gm/l)	37.53±4.5	39.1±3.81	37.48±4.2	NS	NS	NS

ALD, aldosterone; AT1 and AT2, angiotensin receptors 1 and 2; 1,25-D_3, 1,25-dihydrocholicalciferol; eGFR, estimated glomerular filtration rate; FGF23, fibroblast growth factor 23; PRA, plasma renin activity; PTH, parathormone; PWV, pulse wave velocity; P insulin, plasma insulin; S albumin, serum albumin; SCa, serum calcium; S leptin, serum leptin; S.PO_4, serum phosphate.

extracellular volume may be a key component to the pathogenesis of obesity-induced kidney injury [15]. AT1 and AT2 were highly significantly increased in obese patients compared with lean patients. It is possible that the protective function of the AT2 receptor is induced when there is a renal injury, and this effect would not be necessary in the absence of renal pathologies. Angiotensinogen is highly expressed in adipose tissues and is constitutively secreted by mature adipocytes in animal models and humans [16]; this is consistent with the concept that the adipose tissue is an endocrine organ and that adipose angiotensinogen has a paracrine role. Adipose angiotensinogen may also have autocrine effects [17].

ALD blockade reduces renal injury [18]. These benefits are independent of the antihypertensive effects and instead may relate to the blocking effects of ALD on the plasminogen activator inhibitor 1 and transforming growth factor-β [19,20]. Elevated ALD levels, which prevail during obesity, may be injurious to the glomeruli through indirect effects increasing the GFR and through direct effects on the podocytes [21].

The levels of 1,25-D_3 were significantly reduced in obese compared with lean patients. The plasma PTH and S.PO_4 levels were significantly elevated in the obese group, which is a classic pattern usually met with in CKD. The first clinical studies suggesting an inverse relationship between calcitriol and renin levels were published two decades ago [17,22] and were recently confirmed in a large cohort study [23]. Deficiency of vitamin D, defined as having calcidiol levels below 15 ng/ml, associates with a reduced renal plasma flow that responds to infused angiotensin II, suggesting endogenous intrarenal RAAS activation in vitamin D-deficient patients [16].

The FGF23 levels were significantly elevated in the obese group compared with the other two groups; this dramatic elevation should not be explained only on the basis of the degree of CKD as both the other two groups did not show elevated levels. Whether the elevation in the levels of FGF23 is a cause or a result of a decreased GFR and decline in calcitriol levels has not been elucidated. There is increasing evidence that the interactions between vitamin D, FGF23, and klotho form an endocrine axis for calcium and phosphate metabolism, and derangement of this axis contributes to the progression of renal disease [24]. Several recent studies also demonstrate negative regulation of the renin gene by vitamin D. In CKD, low levels of calcitriol, due to the loss of 1-α-hydroxylase, increase renal renin production and activation of the RAAS, in turn, reducing the renal expression of klotho, a crucial factor for proper FGF23 signaling. The resulting high FGF23 levels suppress the activity of 1-α-hydroxylase, further lowering the calcitriol levels. This feedback loop results in a vitamin D deficiency, RAAS activation, high FGF23 levels, and renal klotho deficiency, all of which associate with progression of renal damage [25].

Leptin has also been shown to serve as a cofactor for transforming growth factor-β activation, promotes renal endothelial cell proliferation, and potentially may play a role in renal glomerulosclerosis [9,26,27]. The plasma levels of leptin were significantly elevated in obese compared with both lean and old patients; this elevation in the levels of leptin was associated with elevated plasma insulin levels, both of which are byproducts of adipose tissue remodeling.

Okpechi *et al.* [28] reported that plasma leptin levels were inversely related to the levels of eGFR. In another study, common polymorphisms in the *LEP* gene were found to be associated positively with serum creatinine levels and inversely with eGFR levels [29].

Despite the statistically significant different GFR values between the lean and obese groups, it is still most likely that obesity *per se* is the main risk factor for the differences in the levels of biomarkers responsible for renal and vascular injury. In our study, we found that the results of the obese group were similar to those of the old age group, with the

exception of PWV results. The more obese we become, the shorter our life. This leads to the question 'How many days of our life does one kilogram of overweight cost?'

Conclusion

Obesity *per se* is an independent risk factor for the possible development and progression of CKD, especially in young age patients. The mechanisms by which obesity induces these effects are through the activation of the RAAS and FGF23–klotho–1,25-D_3 axes by overexpression of adipocytokines and insulin resistance, both of which start early in the course of obesity. Preventing and managing obesity should start early enough to halt, if not prevent, the development and progression of CKD and cardiovascular disease. It would be attractive to hypothesize that targeting optimization of these axes could enhance the therapeutic efficacy, either by titrating the dose–effect of presently available drugs or introducing novel agents.

Acknowledgements

Conflicts of interest

There are no conflicts of interest.

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