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Urinary Neutrophil Gelatinase-Associated Lipocalin Levels in Overweight/Obese Adolescents

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ABSTRACT

Background: In recent times, the effect of obesity on the kidneys has become the focus of research, as obesity has been reported to be an independent risk factor for chronic kidney disease. It is well documented that glomerular damage occurs early in the obese; however, it's not certain if tubular damage also occurs early.

Objective: To determine if there is early tubular damage in overweight and obese adolescents using urinary neutrophil gelatinase-associated lipocalin.

Method: Cross sectional cohort study. The subjects were 49 (overweight /obese adolescents) and the controls were 41 normal weight adolescents. Urinary neutrophil gelatinase- associated lipocalin levels were measured using enzyme linked immunosorbent assay.

Results: The median (interquartile range) urinary neutrophil gelatinase- associated lipocalin levels were not elevated in both groups of children, but the overweight /obese adolescents had significantly lower median (interquartile range) levels than the normal weight adolescents 4.3 (2.9 - 6.6) ng/ml vs 7.5 (5.0-8.5) ng/ml (p =0.002).

Conclusion: Early tubular injury as assessed by Urinary neutrophil gelatinase- associated lipocalin is not found in overweight/obese adolescents

Keywords: *Obesity, Overweight, Adolescents, Kidney, Tubular injury, Urinary neutrophil gelatinase- associated lipocalin*

INTRODUCTION

Childhood obesity is on the increase worldwide.[1] In recent times, the effect of obesity on the kidneys has become the focus of research.[2] This is because obesity has been recognized as an independent risk factor for chronic kidney disease (CKD) [2] which is also on the rise globally.[3] The onset of obesity related renal disease is insidious and asymptomatic, thus early markers of renal damage will be useful in its diagnosis and treatment.[4]

Recent studies have demonstrated that renal intracellular lipid accumulation in glomerular endothelial cells, podocytes and proximal tubules cause oxidative stress, endoplasmic reticulum stress, mitochondrial dysfunction and pro inflammatory process that can result in renal damage which will sub sequentially result in Obesity related CKD.[5] Several studies have also shown that glomerular injury occurs in obesity,[6-8] even in the early stage of excessive weight gain.[9, 10] It is however not known how early tubular injury develops in the obesity spectrum.

Urinary biomarkers which are enzymes elaborated as soon as renal tubular damage occurs, [11] have been extensively studied in the assessment

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of kidney injury in various clinical settings.[12, 13] Urinary neutrophil gelatinase-associated lipocalin (uNGAL) is one of the most promising of these urinary biomarkers for the early detection and prediction of renal tubular injury.[14] To determine the presence of early tubular injury in obese state, we evaluated the level of uNGAL in overweight and obese adolescents. In addition, we determined the relationship between uNGAL levels and other markers of glomerular injury - (proteinuria and microalbuminuria).

SUBJECTS AND METHODS

Study setting and subject selection

Forty-nine consecutive subjects were recruited by convenient sampling from a private secondary school in Egor Local Government Area (LGA) of Edo State. The subjects were apparently healthy adolescents aged 10-17 years with body mass index (BMI) e" 85th percentile using the Center for Disease Control (CDC) BMI growth chart. [15] In addition, 49 age and sex matched controls were also recruited. They were apparently healthy adolescents with normal BMI percentile between the 5th - <85th percentile; recruited from the same secondary school as the subjects. Excluded from the study were, adolescents with history of renal disease, endocrine disease, fever and complaints suggestive of symptomatic urinary tract infection (UTI) (such as dysuria, loin pain, suprapubic pain, frequency, urgency) and those on medication that can cause weight gain such as steroid and antipsychotic.

Ethical consideration

Ethical approval was obtained from the University of Benin Ethics Committee. Permission was obtained from the administrative heads of the school and the Ministry of Education. Written informed consent was obtained from the parent(s) of the study participants and verbal assent was given by each of the adolescents recruited.

Data collection and evaluation

Each child's age and gender were noted on a proforma where other information obtained were also recorded. Detailed physical examination of each child was carried out and the following parameters were measured; blood pressure (BP), weight (Wt) and height (Ht).

The blood pressure was determined using mercury sphygmomanometer. The adolescent sat restfully for 3-5mins; thereafter an appropriate cuff (covering 2/3 of their right upper arm) was applied. Blood pressure measurements were according to the recommendation of the National Blood Pressure Education Programme [16] Three blood pressure readings were measured and the average determined. The weight of each adolescent was measured using a Seca® weighing scale (Seca gmbh & co, Germany) with a sensitivity of 0.1kg. The weights were measured after heavy clothing and accessories such as school cardigans or blazers, belt, wrist watches and shoes had been removed and the pockets of their school uniform emptied. A stadiometer was used to measure the height of each adolescent. Each child stood erect on the stadiometer looking straight ahead with hands on the sides, feet (without shoes) placed together and his/her occiput and buttock resting on the stadiometer.

The lever was then lowered to the vortex and the height read off the calibrated scale. All measurements were in centimeters (cm) to the nearest 0.1cm.

Body mass index was determined using the formula weight (Kg)/Height^2 (m^2). [15] Overweight was defined as a BMI e" 85th but d" 95th percentile on the CDC BMI chart while obesity was defined as a BMI e" 95th percentile.[15]

Laboratory procedures

Ten milliliters (mls) of clean catch mid-stream sample of urine was obtained from each enrolled child. Five milliliters of the obtained urine sample was used for microalbuminuria determination using Micral test strips. Presence of >20mg/L was considered as microalbuminuria. The same urine sample was used in urinalysis, using the combi 9 multitest strips to determine presence of protein in urine. The other half of the urine sample was used for uNGAL determination.

The urine for uNGAL determination was preserved at -70ºC till the day of analysis; on that day, the urine specimens were allowed to thaw to room temperature. Thereafter, they were centrifuged at 2500rpm for 10 minutes and the clear supernatant was used in the analysis. Urinary NGAL was measured by the enzyme-linked immunosorbent assay (ELISA) method (BioPorto Diagnostics kit 036RUO) according to the manufacturer's instructions. The intra and inter assay coefficient of variations was

4% and 5% respectively. Urinary NGAL levels were expressed as ng/ml.

In addition, 5mls of blood was obtained from each child by aseptic technique. One milliliter was kept in sample bottles containing fluoride oxalate anticoagulant and was used for determination of random blood sugar. The remaining blood sample obtained from each child was kept in a in a sterile bottle containing no anticoagulant and were subsequently centrifuged. The obtained sera samples were used for creatinine levels determination. The serum creatinine obtained was used in the calculation of estimated glomerular filtration rate (eGFR) by the Schwartz formula.[17]

Statistical analysis

The data was analysed using IBM SPSS version 20 (SPSS for Window Inc; Chicago, LL, USA) Statistical software. Continuous data such as child's age and all measurements obtained were summarized as mean (±SD) except uNGAL levels which was summarized in median (interquartile range). Categorical data such as age group and gender were represented as proportions. Fisher's Exact test was used to test strength of association between categorical data. Strength of association between continuous data expressed in mean ± standard deviation (SD) were analysed with Student t test while those expressed in median and interquartile range were analysed by the Mann Whitney U test and One-Way Analysis of Variance (ANOVA) for comparing multiple medians. The level of significance of each test was set at $P < 0.05$.

RESULTS

Forty nine subjects were recruited for this study. Of these 28(57.1%) were overweight while the obese adolescents were 21(42.9%). In addition, 49 age and sex matched normal weight adolescents were recruited as controls. However, laboratory data were incomplete for 8 controls and such individuals were excluded from the study.

Clinical and biochemical characteristics of the study population

The subjects and controls were well matched for age and gender

(p =0.745 and p = 0.663 respectively). Majority of the overweight/obese children were from the early adolescent age group 44(89.8%). The mean BMI (25.0 ± 3.4) and mean systolic blood pressure (110.1 ± 12.5) were significantly higher in the overweight/obese adolescents $p =< 0.0001$ and $p =$ 0.0003 respectively.

The mean random blood sugar and mean serum creatinine of the overweight/obese adolescents were significantly lower than those of the normal weight adolescents 91.5± 10.5 v 98.0±8.1 $p =< 0.002$ and 0.69 ± 0.18 v 0.9 ± 0.16 $p =< 0.0001$ respectively. The mean estimated glomerular filtration rate was significantly higher in the overweight/obese adolescents compared with the normal weight adolescents. Table 1 shows the clinical and biochemical characteristic of the overweight/obese adolescents and the normal weight adolescents.

Table 1: Clinical and biochemical characteristic of the overweight/obese children and normal weight adolescents

Characteristics	Overweight/Obese Adolescents n = 49	Normal Weight Adolescents n = 41	P-Value
Mean age	12.3 ± 1.4	12.4 ± 1.5	0.745
Age group			
10-14	44(89.8)	36(87.8)	
15-17	5.0(10.2)	5.0(12.2)	1.0000
Gender			
Female/Male	29/20(59.2/40.8)	27/14(65.9/34.1)	0.663
Mean body mass index	25.0 ± 3.4	17.2 ± 1.9	**<0.0001**
Mean systolic blood pressure	110.1 ± 12.5	101.1 ± 9.3	**0.0003**
Mean diastolic blood pressure	64.7 ± 13.1	60.5 ± 11.2	0.110
Proteinuria			
Present	2(4.0)	2(4.9)	
Absent	47(96.0)	39(95.1)	1.0000
Microalbuminuria			
Present	11(22.4)	10(24.4)	
Absent	38(77.6)	34(75.6)	1.0000
Mean random blood sugar	91.5 ± 10.5	98.0 ± 8.1	**0.002**
Mean serum creatinine	0.69 ± 0.18	0.9 ± 0.16	**<0.0001**
Mean eGFR	141.4 ± 46.2	100.2 ± 17.7	**<0.0001**

eGFR= Estimated glomerular filtration rates

Urinary Neutrophil gelatinase- associated lipocalin levels between overweight/obese adolescents and the normal weight adolescents.

The urinary levels of neutrophil gelatinase-associated lipocalin were within normal range in both groups of children. However, the median uNGAL level was significantly lower in the overweight/obese adolescents than the normal weight adolescent.

4.3 (2.9 - 6.6) ng/ml vs 7.5 (5.0-8.5) ng/ml $p =< 0.002$).

Figure 1

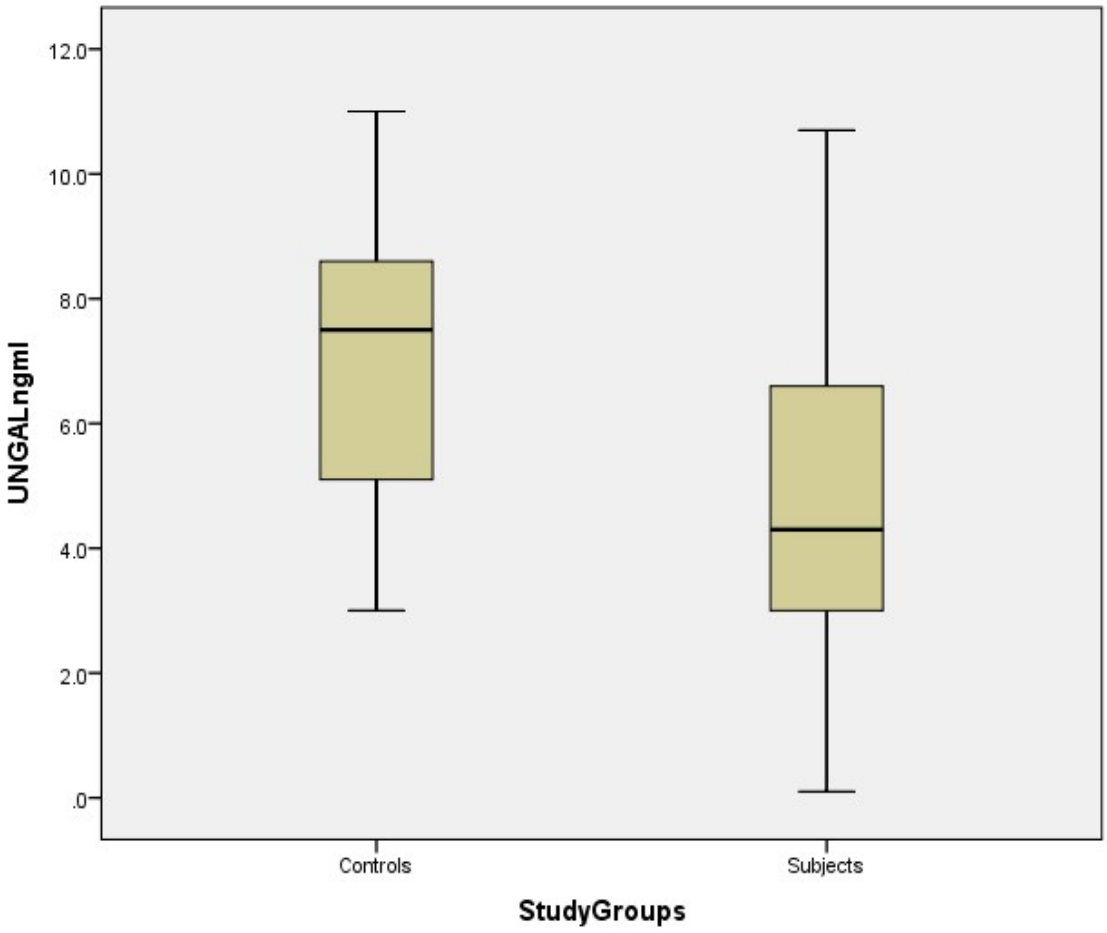

Fig. 1: *Comparison of Urinary neutrophil gelatinase-associated lipocalin levels between subjects and controls*

Relationship between Urinary neutrophil gelatinase- associated lipocalin levels and other glomerular markers in overweight/obese adolescents.

The median uNGAL level of the overweight/ obese adolescents with proteinuria was higher than that in those with microalbuminuria and those with normoalbuminuria. However, the differences were not statistically significant. 6.5(6.3-6.5) ng/ml vs 4.0(2.9-5.2) ng/ml vs 4.3(2.9-7.0) ng/ml respectively ($p = < 0.333$).

DISCUSSION

In this study we measured uNGAL, a marker of proximal tubular damage to determine the presence of early tubular injury in overweight/obese adolescents and we found that uNGAL levels in the overweight obese adolescents were not elevated but were rather reduced compared to the normal weight adolescents. This finding is similar to the report by Goknar *et al* [18] who studied a panel of urinary renal injury markers in obese children which included NGAL, *N* - acetyl-beta-D-glucosaminidase (NAG) and Kidney injury molecule- 1 (KIM-1).

The observation that uNGAL levels were not elevated in the overweight/ obese adolescents from this study may imply that, lipotoxicity that occur due

to renal intercellular lipid accumulation is not an early event in the pathogenesis of renal tubular injury in obesity. It may also mean that excessive weight gain, alone, may not be responsible for development renal tubular injury. It is possible therefore, that factors such as diabetes mellitus which propagates progressive excessive weight gain is required for the development of obesity induced renal tubular injury. Support for the above statement is that, from several studies [19, 20] on diabetic individual, uNGAL amongst other urinary biomarkers of tubular injury were observed to be significantly elevated even before the markers of glomerular injury such as microalbuminuria and proteinuria were observed. Thus, the absence of diabetes in the adolescents we studied which was also the case in the study by Gokner *et al* [18] is another reason why uNGAL levels were not elevated in this study.

In this study, although uNGAL levels were not elevated, we observed that the levels were higher in the overweight/ obese adolescents with proteinuria than those with microalbumiuria and normoalbuumiuria. The small number of adolescents in this subset makes inference difficult. However, this finding may support the notion that when obesity is associated with proteinuria tubular injury could also ensue as it has been reported in persons with diabetics.[19] To substantiate this notion large population studies would be required.

In conclusion this study revealed that uNGAL levels are not elevated in overweight/obese adolescents; however, the levels appear to be higher in overweight/obese adolescents who have proteinuria. Thus, uNGAL may be a useful marker for monitoring progression of established obesity associated renal disease.

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