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Renal tubular function and urinary N-acetyl-β-d-glucosaminidase and kidney injury molecule-1 levels in asthmatic children

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Abstract

Background: Asthma is a chronic inflammatory disorder of the airways which results in chronic hypoxia. Chronic hypoxia and inflammation can affect renal tubular function.

Objectives: The aim of this study was to investigate renal tubular function and early kidney injury molecules such as urinary N-acetyl-betaglucosaminidase (NAG) and kidney injury molecule-1 (KIM-1) excretion in children with asthma.

Methods: Enrolled in the study were 73 children diagnosed with asthma and 65 healthy age- and gender-matched control subjects. Urine pH, sodium, phosphorus, potassium, microalbumin, creatinine, NAG, KIM-1, and serum creatinine, sodium, phosphorus were evaluated. The diagnosis of asthma and classification of mild or moderate were done according to the Global Initiative for Asthma guidelines.

Results: Serum sodium, phosphorus, creatinine, and urinary microalbumin were within normal levels in the both groups. Urinary pH, sodium, potassium, phosphorus, microalbumin, and KIM-1 excretions were similar between the control and study groups. Tubular phosphorus reabsorption was within normal limits in two groups. Urine NAG was elevated in the study group ($P = 0.001$). Urinary KIM-1 and NAG levels were positively correlated ($r = 0.837$; $P = 0.001$). When children with mild and moderate asthma were compared, all of the parameters were similar ($P > 0.05$).

Conclusions: This study showed that chronic asthma can lead to subtle renal impacts. We suggest that in children with asthma, urinary NAG level is a more valuable parameter to show degree of renal tubular injury than markers such as microalbumin and KIM-1. Chronic hypoxy and inflammation probably contributes to these subclinical renal effects.

Keywords

asthma, inflammation, injury, kidney injury molecule-1 (KIM-1), N-acetyl-betaglucosaminidase (NAG), tubulus

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Introduction

Asthma is a chronic disease characterized by airflow obstruction and recurrent episodes of wheeze, cough, and breathlessness. It is a problem worldwide, with an estimated 300 million affected individuals.[1,2]

Novel biomarkers such as urinary N-acetyl-beta-D-glucosaminidase (NAG) and kidney injury molecule-1 (KIM-1) are used to assess acute renal injury. The new biomarkers are used along with

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traditional biomarkers like serum creatinine and urinary microalbumin.[3] The novel renal injury biomarkers show that in many chronic diseases including iron deficiency anemia, obesity, and thalassemia, renal tubular functions are affected without a reduction in glomerular filtration rate.[4–7] To our knowledge, no previous study has investigated the effect of asthma and renal tubular function; however, a retrospective cohort study looking at patients aged over 14 years showed that asthmatic patients have an increased risk of chronic kidney disease independent of risk factors such as obesity and hypertension.[8]

The pathological hallmarks in asthma are airway inflammation and hyper-responsiveness. The inflammatory response results in increased airway mucus secretion, airway wall edema, epithelial damage, and inflammatory cell infiltration.[9] The end result of chronic airway inflammation in asthma is reconstructive lesions which lead to airway remodeling. An imbalance between oxidative-antioxidant systems favoring oxidative injury has been postulated in the pathogenesis of asthmatic children.[10,11] Increased oxidative stress results in exacerbation of inflammation.[12] It is well-known that the renal tubulus is very sensitive and can be affected by acute injury for instance inflammation and oxidative stress.

The aim of the study was to investigate renal tubular function and early kidney injury molecules (urinary NAG and KIM-1) excretion in childhood asthma.

Methods

Study group consisted of 73 children (35 girls, 38 boys) diagnosed with asthma at Bezmialem Vakif University's pediatric allergy outpatient clinic. A second group of 65 healthy children (40 girls, 25 boys) from the pediatrics outpatient clinic of the same hospital was used as a control group.

Diagnosis and classification of asthma were determined using the criteria as expressed in the Global Initiative for Asthma guidelines (GINA).[13] Children were defined as asthmatic according to the following criteria: (1) recurrent episodes of at least one symptom of asthma, including cough, wheezing, breathlessness, and chest tightness; (2) an improvement of at least 12% in baseline forced expiratory volume (FEV1) 1 s after bronchodilator use; and (3) a total serum IgE level of over 52I U/mL determined by direct chemiluminescence, plus a positive skin test for at least one allergen. In accordance with the GINA guidelines, the cases were classified as mild or moderate asthma according to daytime symptoms, night-time symptoms, and pulmonary function tests.[13] Members of the control group were selected from those referred to the pediatric outpatient clinic for check-ups (e.g. before swimming in a pool or going through elective surgery like tonsillectomy). Exclusion criteria included: chronic diseases; severe infection; and familial and personal atopy. No children from the control and study groups were taking antibiotics and were free of respiratory tract infections. Prior to providing urine and serum specimens, all subjects had been medication free (athsmatic medication), and symptom- and attack-free for 4 weeks

Renal survey

Mean age was 11.4 ± 2.3 and 10.9 ± 2.6 in the control and study groups, respectively. The renal status was evaluated by urine pH, potassium, microalbumin, creatinine, NAG, KIM-1, and serum creatinine, sodium, phosphorus. Fractional excretion of sodium (FE-NA) and tubulary reabsorption of phosphorus (TRP) were calculated with standard formulas. All tests were measured within 4 h of the sample collection with the exceptions of urinary NAG and KIM-1, which were centrifuged (at 3000 rpm for 10 min) and stored at −80°C until measurement. Glomerular filtration rate was calculated according to the Schwartz formula.[14] Urinary NAG and KIM-1 were measured by the photometric and Elisa methods. Urinary NAG, KIM-1, microalbumin, and sodium excretion were either expressed per gram or by milligrams of urinary creatinine to illiminate the influence of urinary dilution or concentration.

Oral and written informed consent was obtained from all subjects and their parents before the start of the study.

Ethical approval

The study was approved by the Bezmialem Vakif University Ethics Committee.

Statistical analyses

Clinical characteristics are presented as mean ± standard deviation (SD). Statistical analyses were

Table 1. Comparison of demographic, laboratory, kidney injury, and renal tubulary function parameters in asthmatic and control children.

		Asthma (73)	Control (65)	*P*
		Mean ± SD	Mean ± SD	
Age		10.95 ± 2.57	11.40 ± 2.31	0.286*
Gender (female/male)		35/38	40/25	0.110†
Serum creatinine (mg/dl)		0.45 ± 0.10	0.54 ± 0.13	0.001‡
GFR		125.34 ± 23.21	118,84 ± 17,40	0.083*
Urine	Ph	5.61 ± 0.91	5.49 ± 0.76	0.644‡
	Microalbumin (mg/g creatinine)	10.80 ± 17.62	11.22 ± 8.92	0.880*
	KIM-1 (ng/mg creatinine)	0.71 ± 0.79	0.57 ± 0.30	0.373‡
	NAG (U/g creatinine)	91.71 ± 183.23	12.05 ± 6.04	0.001*
Tubulary reabsorption of phosphorus		95.40 ± 2.21	93.80 ± 4.13	0.014*
Fractional excretion of sodium		0.40 ± 0.28	0.51 ± 0.33	0.172‡

KIM1/creatinine and NAG/creatinine were evaluated after logarithmic transformation.
*Student's t-test.
†Pearson Chi-square.
‡Mann–Whitney U test.
GFR, glomerular filtration rate; KIM-1, kidney injury molecule; NAG, N-acetyl-b-D-glucosaminidase.

determined by Student's t-test for parametric analysis and by the Mann–Whitney U test for non-parametric analysis. Categorical data were evaluated using the Chi-square test. For correlation analysis, Pearson correlation analysis and Spearman correlation analysis were used. A P value of <0.05 was regarded as significant. All statistics were performed using the NCSS (Number Cruncher Statistical System) 2007 & PASS (Power Analysis and Sample Size) 2008 Statistical Software (UT, USA).

Results

Serum creatinine levels were all within normal limits in the study and control groups: 0.45 ± 0.10 and 0.54 ± 0.13, respectively. Serum electrolytes were also within normal limits. No differences were found in urine microalbumin/creatinine levels and FE-NA. Although within normal limits in both groups, TRP was higher in the asthma group. The demographic and biochemical data of asthma and control subjects are summarized in Table 1.

Urinary NAG/creatinine was elevated in asthmatic children (Figure 1, P = 0.001). No difference was found in urinary KIM-1/creatinine. Using the Pearson test, urinary NAG/creatinine was well correlated with urinary KIM-1/creatinine (r = 0.837; P = 0.001).

The clinical and biochemical data of mild and moderate asthmatic subjects are summarized in Table 2.

Serum creatinine and electrolytes levels were all within normal limits in the study and control groups. No differences were found in urine microalbumin levels, TRP, FE-NA, urinary NAG/creatinine, and urinary KIM-1/creatinine between asthmatic subgroup subjects (P >0.05).

Discussion

In this study, we investigated renal tubular injury molecules and renal tubular functions of children with mild or moderate asthma. We discovered that children with asthma had elevated urinary NAG levels (an early kidney injury marker) while renal tubular function had not deteriorated in the study group. In addition, serum electrolyte and creatinine levels remained within normal limits in both the control and study groups No differences were found in other kidney injury molecules such as urine KIM-1 and microalbumin levels. Likewise, no differences were observed between mild and moderate asthmatic children.

Bronchial asthma has become an important public health problem, especially in industrialized countries. The prevalence of asthma has increased significantly, particularly among children, and is now the most frequent chronic medical condition in the pediatric age group. At present, the two primary reasons for kidney damage in patients with asthma are chronic hypoxia and increased oxidative stress as a result of chronic inflammation. It is

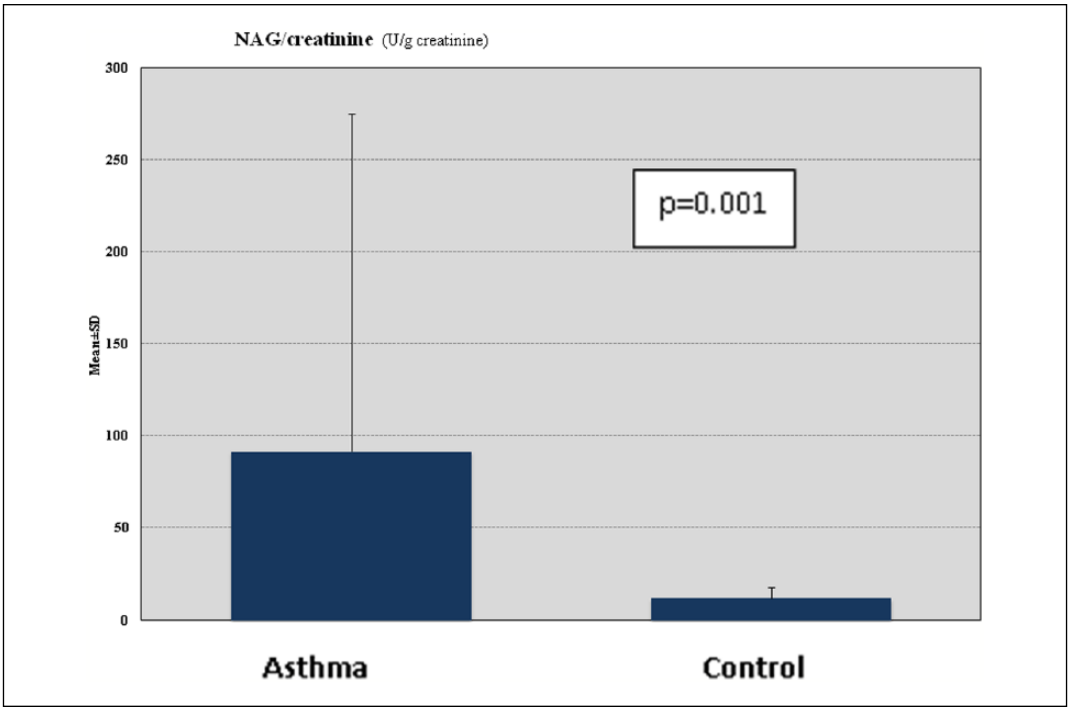

Figure 1. Urinary NAG levels in asthmatic children and control groups.

Table 2. Laboratory data of mild and moderate asthma.

		Asthma (mild) (n = 38)	Asthma (moderate) (n = 35)	*P*
		Mean ± SD	Mean ± SD	
Urine	Microalbumin (mg/g creatinine)	13.54 ± 19.95	7.83 ± 14.30	0.194*
	KIM-1 (ng/mg creatinine)	0.66 ± 0.91	0.75 ± 0.67	0.373*
	NAG (U/g creatinine)	116.61 ± 256.32	67.31 ± 83.45	0.305*
Tubuler reabsorption of phosphorus		95.42 ± 2.17	95.35 ± 2.31	0.903*
Fractional excretion of sodium		**0.45 ± 0.31**	0.49 ± 0.37	0.905†

KIM1/creatinine and NAG/creatinine were evaluated after logarithmic transformation.
*Student-t Test.
†Mann Whitney U Test.
KIM-1, Kidney injury molecule; NAG, N-acetyl-b-D-glucosaminidase.

well-known that hypoxia plays a crucial role in the pathogenesis of kidney injury. Güneş et al. demonstrated that urinary kidney injury molecules were increased in children with iron deficiency anemia. They suggested a possible subclinical injury due to hypoxia.[15] In asthmatic patients, increased production of reactive oxygen species results in the imbalance of oxidative status and an increase of in oxidant status.[10] Oxidative damage is important in the pathogenesis of asthma. Increased levels of PON1 and TOS levels were found in asthmatic children with poor control of the disease.[11]

Known kidney biomarkers are serum creatinine, blood urea nitrogen, urinary microalbumin, and total volume of urinary excretions. In our study, renal tubular functions and GFR were not affected because neither creatinine nor BUN can change quickly enough during injury due to the fact that individuals with normal renal function have a functional reserve which compensates for nephron injury.[16] In numerous studies evaluating renal hypoxic injury, these values were also within normal limits.[4–7] The currrent opinion is that serum creatinine is considered to be a poor indicator of acute renal damage or injury. Novel biomarkers of

tubular injury (the most metabolically active segment of the nephron) were investigated because of their unique properties of susceptibility to ischemic and nephrotoxic insults.[3] In other studies regarding renal injury secondary to systemic problems (drugs, anemia, hypoxia, etc.), new biomarkers were used.[4–7] This study utilizes urinary NAG, KIM-1, and microalbuminuria to detect possible early renal effects of asthma.

Having a biomarker which directly measures injury and can also be easily detected from body fluids like blood or urine can help to monitor injury. This marker could provide detailed data before the occurrence of late consequences of injury like decrease in GFR.[16] Urinary NAG is a lysosomal enzyme that plays a role in the breakdown of glycoproteins in proximal renal tubular cells. It is a high molecular-weight lysosomal enzyme found in many tissues in the body. It cannot pass into glomerular ultrafiltrate due to its high molecular weight. Increased concentrations of urinary NAG indicate a loss of lysosomal integrity of the tubular epithelial cells. In nephrotoxic drugs exposure, delayed allograft nephropathy, diabetic nephropathy, and acute kidney injury, urinary NAG levels were found to be elevated. Also in upper urinary tract infections, nephrolithiasis and reflux nephropathy, etc., urinary NAG was found elevated. High levels of urinary NAG in our study confirmed the presence of proximal renal tubular damage.[17–19] In the present study, we demonstrated elevated levels of urinary NAG in asthmatic children. On the other hand, no differences were found between mild and moderate cases, perhaps because no patient suffered from acute exacerbation at the time of study: all were under control with appropriate medication according to the GINA guidelines.

Microalbuminuria, an early marker of chronic kidney disease, is also considered to be an early marker of renal injury in nondiabetic patients.[20] In our study however, urinary microalbumin levels were similar between the control and study cases. Microalbuminuria is not effective for measuring renal impact of asthma in mild and moderate cases. This study did not include severe cases, which may give different results.

The kidney injury molecule (KIM-1) is a type-1 transmembrane glycoprotein which is not detectable in normal kidneys. The function of KIM-1 is to make epithelial cells recognize and phagocyte dead cells due to acute kidney injury. The soluble form of KIM-1 can be easily detected in patients' urine.[21] In this study, urinary KIM-1 levels of the control subjects were not significantly different from those of the asthmatic children, possibly because KIM-1 is directly affected by acute injury, whereas our patients were not in acute exacerbation phases of the disease.

The limitations of this study are that we did not analyze the effect of asthma medication on renal tubular function and injury molecules. In addition, no patient had severe asthma. Further analyses with greater patient numbers and different stages of asthma could give more precise data about the effects of asthma on renal tubular function.

In conclusion, this study showed that minor abnormalities on kidneys and subclinical renal effects may occur even in mild to moderate asthma. We also conclude that increased urinary NAG levels in children with asthma may help to detect subtle and early alterations in renal integrity. Urinary measurement of NAG is a safe and simple method for detection of renal tubular injury secondary to hypoxy and chronic inflammation. The cause of this dysfunction is not known, but chronic hypoxia and inflammation may be key factors. Further studies are required to verify the presence of renal effects of chronic hypoxy in children who have chronic asthma, particularly in those children that have persistent asthma.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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