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Original Article

Serum and Urinary Levels of Nitric Oxide and Uric Acid in Nigerian Children with Asthma

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Abstract: Background: Asthma is associated with increased production of reactive oxygen and nitrogen species and an alteration in the levels of antioxidants activities in the lung and blood. The increased production of the superoxide anion radicals contributes to airway remodelling and disease severity. Physiologically, the effect of increased free radical generation is eliminated by corresponding activities of a network of antioxidants. Presently, there is the dearth of information on the steady-state concentrations of nitric oxide (NO) and uric acid (UA) in children with asthma. The serum and urinary levels of NO and UA in children with asthma were thus determined in this study. Methodology: Fifty children consisting of 25 children with asthma and 25 age-matched apparently healthy children without asthma were enrolled into this study. Serum and urinary levels of NO and UA were determined using standard methods. Results: Serum levels of NO and UA were significantly higher while the urinary levels of NO and UA were significantly lower in children with asthma compared with the controls. There was no significant correlation between the serum ad urinary levels of NO and UA in children with asthma. Also, gender differences were not observed in the serum and urinary levels of NO and UA in children with asthma. Conclusion: Children with asthma have elevated serum levels of NO and UA accompanied with suboptimal urinary excretion. Therefore, children with asthma might benefit from routine renal function assessment owing to damages that can result from systemic accumulation of UA with concomitant reduction in its urinary excretion.

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INTRODUCTION

Asthma is a heterogenic chronic inflammatory disease involving a variety of gene-environment interactions. Its risk factors, onset, triggers, severity, and response to treatment vary between individuals (Expert Panel Report 3, 2007; Mims, 2015; Schoettler and Strek, 2020). Inflammation of the airways, airflow obstruction and bronchial hyper-responsiveness are the main features of asthma (Prado *et al.*, , 2011; Nanda and Wasan, 2020; Diamant and Vijverberg, 2019).

Asthma is under-diagnosed and under-treated. It creates substantial burden to individuals and families and often restricts individuals' activities for a lifetime (WHO, 2021). Its associated morbidity, mortality and economic costs resulting from productivity loss make it a major public health problem affecting over 300 million people, globally (Masoli *et al.*, , 2004; GBD 2016, 2017; Cloutier *et al.*, , 2020). In Africa, it is an important public health problem which is not attracting the deserved attention. The growing urbanization, population, aging and adoption of western lifestyles are believed to further propel this trend in Africa (Adeloye *et al.*, , 2013).

Childhood asthma is a common chronic disease with profound effects on children's health, their families, and the health care system. The prevalence of asthma in children varies across different countries, but an increased global incidence has been reported (Dharmage et al., , 2019). The lungs and airways of the children are easily inflamed when exposed to triggers and this interferes with play, sports, school and sleep of the children (de Benedictis and Attanasi, 2016; Hoch *et al.*, , 2019). This indicates that many children with asthma especially in Africa, where problems such as overutilization of health services, lack of trained staff and diagnostic apparatus, and non-availability as well as non-affordability of inhaled medications impair early diagnosis and quality management, may fail to achieve their full potential if proper management and control measures are not put in place (Pearce and Strachan, 2011; Uijen *et al.*, , 2008). It is therefore apparent that there is the need for intense research into paediatric asthma with a view to understanding and ameliorating the associated morbidities.

Generally, in asthma, a number of immune cells, infiltrating the lungs, play important roles in the pathogenesis of asthma. These cells include mast cells, eosinophils, neutrophils, macrophages, epithelial cells, and T cells. Activities of these cells result in inflammation which causes persistent occurrences of wheezing, breathlessness, chest tightness, coughing, and an associated increase in the existing bronchial hyper-responsiveness to a variety of stimuli (Saleh *et al.*, , 1998; Mims, 2015).

Several studies have shown that allergen-induced inflammatory responses in asthma is associated with increased production of reactive oxygen and nitrogen species and an alteration in the levels of antioxidants activities in the lung and blood of asthmatics (Bowler, 2004; Caramori and Papi, 2004; Sackesen *et al.*, , 2008). Increased production of the free radicals contributes to airway remodelling and disease severity (Godard *et al.*, , 1987; Dut *et al.*, , 2008). The report of Cluzel *et al.* (1987) showed that inflammatory cells from the peripheral blood and broncho alveolar lavage (BAL) fluid of asthmatics generate more superoxide anion radicals than those from non-asthmatics. Also, Baraldi *et al.* (2003) and Corradi *et al*. (2003) reported that exhaled air of patients with asthma contains elevated levels of various markers of oxidative stress. In addition, an association between airway obstruction/severity of asthma and systemic oxidative stress has been reported (van Aalderen, 2012).

Nitric oxide (NO) is a free radical released by immune, epithelial and endothelial cells upon enzymatic breakdown of L-arginine to NO and L-citrulline (Moncada and Higgs, 1993; Prado *et al.*, , 2011). Its various roles in the pathophysiology of asthma have been reported (Ricciardolo *et al.*, , 2004; Morris *et*

al., , 2004; Prado *et al.,* , 2011). The report of Batra *et al*. (2007) showed that elevated NO levels is associated with risk genotypes and could be responsible for the pro-inflammatory role of NO in asthma patients. Physiologically, the effect of increased free radical generation is eliminated by corresponding activities of a network of antioxidants including the uric acid.

Uric acid (UA) is the most predominant antioxidant molecule in human plasma (Lima *et al.,* , 2015; Wang *et al.,* , 2020). It is synthesized in the liver, intestines, muscles, kidneys and vascular endothelium as an exogenous waste product of nucleotide metabolism in food and as an endogenous product of damaged, dying and dead cells whose nucleic acids are degraded into uric acids (El Ridi and Tallima, 2017; Shojaei-Far *et al.,* , 2017). Measurement of UA levels is usually used as a diagnostic tool in screening for purine metabolism abnormalities (Simoni *et al.,* , 2007). UA has been reported to be an inflammatory mediator of allergic asthma and a trigger for acute neutrophilic inflammation (Martinon *et al.,* , 2006; Kool *et al.,* , 2011). Li *et al*. (2014) and Abdulnaby *et al*. (2016) also showed that there is an increase in serum level of UA at the onset of asthma exacerbation and that the increase had a negative impact on spirometric pulmonary functions. Recently, Wang *et al*. (2020) showed that serum UA level is an independent risk factor for incident asthma.

Although reports on the serum levels of NO and UA in patients with asthma are abound, there is little information on their urinary excretion and hence, their steady-state concentrations. This study was thus designed to determine the serum and urinary levels of nitric oxide and uric acid as well as possible gender differences in their levels in children with asthma.

MATERIALS AND METHODS

Study participants

A total of 50 children between the ages of 6 and 14 years were enrolled into this study. They consisted of 25 (11 males, 14 females) children with asthma and 25 (11 males, 14 females) age-matched apparently healthy children without asthma who served as controls. All the children with asthma were enrolled from the Paediatric Clinic, University College Hospital, Ibadan. Asthmatics with any other underlying disease conditions were excluded from the study.

Ethical Consideration

Ethical approval was obtained from the University of Ibadan/University College Hospital Joint Research Ethics Committee before the commencement of the study. Also, informed assent was obtained from parents/guardians before the children were enrolled into the study.

Blood sample collection and laboratory analyses

Five millilitres (5 ml) of venous blood was obtained from each study participant and dispensed into plain sample bottles. The samples were allowed to retract, centrifuged and serum obtained as appropriate. The serum samples were stored at -20^{0}C until analysed. Similarly, 5 ml of random urine was obtained from each participant into a clean universal bottle and refrigerated until analysed within 48 hours.

Laboratory analyses

Serum and urinary levels of nitric oxide (NO) were estimated using the Griess Reagent which is based on the conversion of nitrite to a purple-coloured azo-dye that is assayed spectrophotometrically (Green *et al.,* , 1982; Csonka *et al.,* , 2015). The analysis was carried out as previously described by Arinola (2020). Similarly, the serum and urinary levels of uric acid were estimated using the enzymatic colorimetric method.

Statistical Analysis

Statistical analyses were carried out using the Statistical Package for Social Sciences (SPSS) version 20. Variables were tested for normalcy using the Smirnov Kolmogrov test of normality. Results are presented as median (interquartile range) and mean ± standard deviation. Independent Student's t-test was used to compare group means for parametric variables while Mann Whitney *U* test was used for non-parametric group comparison. Correlation between the variables was performed using the Spearman rank correlation coefficient test and $P<0.05$ was considered as statistically significant.

RESULTS

The age, serum and urinary levels of NO and UA are shown in Table 1. The mean ages of the children with asthma and the controls were not significantly different. The serum levels of NO and UA were significantly higher in children with asthma compared with the controls. In contrast, the urinary levels of NO and UA were significantly lower in children with asthma compared with the controls.

Table 1: Age and levels of nitric oxide and uric acid in the serum and urine of children with asthma and controls

Variables	Asthma (n = 25)	Controls (n = 25)	*P*-value
Age (years)	10.32 ± 0.44	10.16 ± 0.57	0.825
Serum			
NO (µmol/L)	21.81 ± 2.72	9.24 ± 0.87	0.000*
UA (mg/dL)	5.30 ± 0.33	4.19 ± 0.16	0.023*
Urine			
NO (µmol/L)	1.37 (0.48 - 5.23)	2.31 (1.79 - 3.25)	0.029*
UA (mg/dL)	7.58 (4.61 - 9.21)	37.38 (30.34 - 48.48)	0.000*

*Significant at $P<0.05$, NO = Nitric oxide, UA = Uric acid

No significant correlation was observed between the serum levels of NO and UA and the urinary levels of NO and UA in children with asthma (Table 2).

Table 2: Correlation between serum and urinary levels of nitric oxide and uric acid in children with asthma

Correlating pair		Cases r-value	*P*-value	Controls r-value	*P*-value
Serum NO	Urine NO	-0.104	0.622	-0.234	0.260
	Serum UA	0.345	0.091	0.036	0.864
	Urine UA	0.210	0.313	-0.126	0.547
Serum UA	Urine UA	-0.016	0.940	-0.010	0.963

NO = Nitric oxide, UA = Uric acid

In order to identify possible gender differences in the serum and urinary levels of NO and UA in the children with asthma, the children were categorized into two groups based on gender. The serum and urinary levels of NO and UA were similar in male and female children with asthma (Table 3).

Table 3: Serum and urinary levels of nitric oxide and uric acid in male and female children with asthma

Variables	Male Asthmatics (n = 11)	Female Asthmatics (n = 14)	*P*-values
Serum			
NO (µmol/L)	22.67 ± 3.38	21.13 ± 4.18	0.352
UA (mg/dL)	5.00 ± 0.46	5.53 ± 0.47	0.443
Urine			
NO (µmol/L)	1.37 (0.48 - 5.23)	1.20 (0.44 - 6.09)	0.956
UA (mg/dL)	7.58 (4.61 - 9.21)	7.59 (4.88 - 9.21)	0.784

NO = Nitric oxide, UA = Uric acid

In Table 4, differences in serum and urinary levels of NO and UA in male children with asthma and male controls are shown. The mean serum level of NO was significantly higher in male children with asthma compared with the male controls. In contrast, the median level of UA was significantly lower in male children with asthma compared with the male controls. Similar observations were observed when female children with asthma were compared with the female controls (Table 5). In addition, the mean level of serum UA was significantly higher in female children with asthma compared with the female controls.

Table 4: Serum and urinary levels of nitric oxide and uric acid in male children with asthma and male controls

Variables	Male Cases (n = 11)	Male Controls (n = 11)	*P*-value
Serum			
NO (µmol/L)	22.67 ± 3.38	10.04 ± 1.53	0.002*
UA (mg/dL)	5.00 ± 0.46	4.26 ± 0.23	0.3000
Urine			
NO (µmol/L)	1.37 (0.69 - 6.82)	2.36 (1.80 - 3.60)	0.123
UA (mg/dL)	7.58 (3.79 - 17.88)	36.50 (33.86 - 46.58)	0.008*

*Significant at $P<0.05$, NO = Nitric oxide, UA = Uric acid

Table 5: Serum and urinary levels of nitric oxide and uric acid in female children with asthma and female controls

Variables	Female Cases (n = 14)	Female Controls	*P*-value
Serum			
NO (µmol/L)	21.13 ± 4.18	8.62 ± 1.00	0.000*
UA (mg/dL)	5.53 ± 0.47	4.14 ± 0.21	0.041*
Urine			
NO (µmol/L)	1.20 (0.44 - 6.09)	2.18 (1.76 - 3.00)	0.154
UA (mg/dL)	7.23 ± 0.91	40.68 ± 3.40	0.000*

*Significant at $P<0.05$, NO = Nitric oxide, UA = Uric acid

DISCUSSION

A number of mediators including leukotrienes, prostaglandins and reactive oxygen and nitrogen species have been implicated in the pathophysiology of asthma (Martinez, 2006). The involvement of inflammatory cells and epithelial cells in the pathogenesis of asthma indicates that the airways of asthmatic patients are subject to increased levels of free radicals due to increased production of reactive oxygen and nitrogen species by the cells (Busse and Lemanske, 2001).

NO plays active roles in host defence system as it is involved in phagocytosis and intracellular killing (Webb *et al.*, , 2001; Tümer *et al.*, , 2007). It reacts with superoxide to form peroxynitrite anion which has potent antimicrobial activity mediated by its ability to react with microbial proteins and lipid molecules (Beckman and Siedow, 1985; Zhu *et al.*, , 1992; Ischiropoulos *et al.*, , 1992; Radi *et al.*, , 1991). The observed elevated serum level of NO in children with asthma corroborates the report of Kong *et al*. (2002). Similarly, Morris *et al*. (2004) reported elevated level of NO, albeit insignificant, in patients with asthma. The observed elevation in serum NO levels in this study might be of beneficial and adverse effects as NO plays dual roles in asthma pathology. NO produced by the constitutive isoforms of nitric oxide synthase (cNOS) has been shown to relax the smooth muscle of airways and vessels via cyclic guanosine 3′,5′-monophosphate (cGMP) regulation which induces bronchodilation and vasodilation. In contrast, NO produced by the inducible isoforms of NOS (iNOS), when in high concentrations, induces chemotaxis of inflammatory cells and reacts with superoxide anions to form peroxynitrites and other nitrogen intermediates thereby increasing the oxidative stress with consequent airway hyper-responsiveness and induction of cell necrosis or apoptosis resulting in cellular injury (Brüne *et al.*, , 1998; Kröncke *et al.*, , 1997).

Furthermore, our observed elevation in serum NO concentration in children with asthma could be an indication of arginase inhibition since L-arginine is a common substrate for NOS and arginase. It is established that arginase regulates NO production and in turn, NO regulates arginase activities via substrate competition (Prado *et al.*, , 2011; Fujihara *et al.*, , 1995; Sanders, 1999). This interrelationship between NOS and arginase is shifting research focus towards the use of arginase inhibitors in the treatment of asthma (Maarsingh *et al.*, , 2009). Report has shown that arginases are involved in airway remodelling and that arginase inhibition attenuated antigen induced chronic inflammation culminating in reduced smooth muscle mass growth, airway bronchial reactivity, and hydroxyproline content involved in lung fibrosis (Maarsingh *et al.*, , 2009).

The rate of formation and the rate of decomposition of NO determine its steady-state concentration (Kelm, 1999). NO and nitrite are converted to nitrate which is the final metabolite of NO in human urine following glomerular filtration and reabsorption in the renal tubules (Green *et al.*, , 1982; Wennmalm *et al.*, , 1993; Kelm, 1999). In this study, the urinary level of NO was significantly lower in children with asthma compared with the controls. Our observation presents a biological paradox in that urinary level of NO was lower despite the observed higher serum levels in children with asthma compared with the controls. This observation might indicate that there is impaired urinary excretion of NO in children with asthma leading to increased systemic retention which could partly explain our observed serum NO elevation in children with asthma. The reduction in urinary excretion could be a physiologic mechanism aimed at maintaining optimal plasma NO level with a view to relaxing the smooth muscle of airways and vessels which will induce bronchodilation and vasodilation. In contrast, the retention might indicate impairment in urinary excretion. Trachtman (2004) reported that elevation in NO level can cause glomerular injury. This report and ours thus suggest that there could be the need for routine renal function assessment in children with asthma.

Uric acid is an antioxidant with strong scavenging activities for reactive oxygen species (ROS) and peroxynitrites (Ames *et al.*, , 1981; Sautin and Johnson, 2008; Glantzounis *et al.*, , 2005). However, alteration in the production or renal excretion of UA has been associated with a number of pathological conditions including asthma (El Ridi and Tallima, 2017; Shojaei-Far *et al.*, , 2017; Wang *et al.*, , 2020). The observed elevated serum level of UA in children with asthma contradicts the reports of Anetor *et al*. (2003) and Al-Abdulla *et al*. (2010) in asthmatic adults and children with acute asthma respectively. Our observation could indicate a physiologic response to increased inflammation and oxidative stress linked to tissue hypoxia in children with asthma (Holgate and Polosa, 2006). It has been shown that inflammation associated with cell apoptosis and necrosis can cause increased metabolism of purine nucleotides and enhance the activity of xanthine oxidase (XO) with a resultant increase in serum uric acid concentration (Wu and Wu, 2007).

Increase in serum UA production, reduced urinary excretion of UA or a combination of both result in hyperuricaemia (Fahlen MT, 2019; Bobulescu and Moe, 2012; Maiuolo *et al.*, , 2016). In this study, reduced urinary excretion was observed in children with asthma. Taken this observation together with the concomitant elevated UA level in the children, it is apparent that children with asthma suffer from oxidative stress and are at increased risk of developing kidney injury. Avalanche of studies have shown that hyperuricemia increases the risk of acute kidney injury and impairs the contractile activity of the intraglomerular mesangial cells thereby inducing mesangial and proximal tubules epithelial cells damage (Convento *et al.*, , 2011; Xiao, Fu, *et al.*, , 2015; Xiao, Zhang, *et al.*, , 2015; Xu *et al.*, , 2017). Children with asthma could also be at increased risk of developing kidney stones as uric acid could accumulate in the kidney causing formation and deposition of stones in the kidney. Additionally, uric acid may accumulate in the kidney, leading to formation and deposition of stones (El Ridi and Tallima, 2017). This observed alteration in the steady-state concentration of uric acid in children with asthma further substantiates our earlier suggestion that children with asthma might benefit from routine renal function assessment.

Reports have shown that there is gender disparity in serum UA levels (Casiglia *et al.*, , 2020). Fujikawa *et al*. (2020) reported that pathological decline of lung function in females was protected against by elevated blood UA; this effect was however not observed in the males. Similarly, Wang *et al*. (2020) showed that serum UA cut-off level that could predict incident asthma is predictable in men but not in women. In this study, serum and urinary levels of UA and NO were similar in male and female children with asthma. However, gender-based differences were observed when male and female children with asthma were compared with male and female controls. The observed reduced urinary excretion of UA with concomitant elevation is serum UA and NO in female children with asthma compared with female controls suggests increased oxidative stress requiring counterbalance by serum UA. The reduced urinary level could be a physiological mechanism necessitated by the need to maintain commensurate level of antioxidant against the oxidative stress. This probably explains the similar observation in reduction in urinary UA excretion accompanied by elevated level of serum NO in male children with asthma compared with the male controls.

It could be concluded from this study that children with asthma have elevated serum levels of NO and UA accompanied with suboptimal urinary excretion. Therefore, children with asthma might benefit from routine renal function assessment owing to

damages that can result from systemic accumulation of UA with concomitant reduction in its urinary excretion.

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