Arch. Bas. App. Med. 1 (2013) 87 - 91

ARCHIVES OF BASIC & APPLIED MEDICINE

Research Article

Evaluation of Urinary N- acetyl-β-D-glucosaminidase (NAG) and Glutathione-S-transferase (GST) Activities as Early Markers of Occupational Lead Nephropathy in an African Population

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Received: 12 June 2012
Revised version accepted: 31 July 2012

Abstract

Although application of urinary enzymes as markers of diseases in excessive metal exposure is not novel, it is not popular in this part of the world. Urinary NAG and GST activities were evaluated to detect covert kidney damage in Lead (Pb) exposed (LES), chronic renal failure (CRF) and in clinically normal subjects (Control) in a prospective study using standard methods. Concentration of traditional markers of renal function (plasma creatinine, urea and uric acid) for the above group of subjects were found to be grossly normal despite about 18% of the LES showing excessive ingestion of Pb. Mean urinary NAG activities of 10.2±1.7U/l, 12.4±3.8U/l and 14.8±6.3U/l were obtained for the control, LES and CRF subjects respectively. Also, 4.4% of LES and 15.3% of CRF subjects had urinary NAG activity higher than that of the control (17±5.8); the difference was not significant (P≥0.05). Mean urinary GST activity of 39±25.9µg/l, 76±27.7µg/l and 102±54.5µg/l were obtained for the control, LES and CRF subjects respectively. In particular, 91% of LES group and 83% of CRF subjects exhibited higher urinary GST activity relative to the control (39±25.9µg/l); the difference was significant (p≤0.05). These data suggest that Urinary GST has the potential of being a better indicator of early kidney damage in Pb exposed subjects than urinary NAG; its result correlated better with that of blood lead level used as the main marker of occupational Pb exposure in this study.

Keywords: *Urinary enzymes, NAG, GST, Kidney damage, lead*

Introduction

Urinary enzymes (enzymuria) have been widely used in medical diagnoses especially in diseases involving cellular damage, necrosis and increased tubular turnover. Amongst urinary enzymes commonly used in monitoring cellular damage in the kidney especially that due to nephrotoxicity are N- acetyl-β-D-glucosaminidase (NAG) and Glutathione-S-Transferase (GST) (Mutti, 1989). N- acetyl-β-D-glucosaminidase (NAG) is a renal tubular enzyme reported to be produced as an early indicator of renal tubular injury. It is a lysosomal enzyme present in renal tubular cells. Due to its high molecular weight (130000 to 140000), it is not normally filtered at the glomerulus (Meyer *et al*, 1984). Renal proximal tubular cells are rich in N-acetyl-β-D glucosaminidase which is one of lysosomal glucosidases. It has been shown that injury to proximal tubular cells from any toxicological agent causes high urinary excretion of this enzyme (Yuen *et al* (1984); Meyer *et al*, (1984). Demonstration of its activity in the urine has been postulated as an early indicator of renal tubular damage even earlier than most other renal biomarkers especially in cases of toxicological exposure (Kharl-Manesh *et al*, 1994, Bazzi *et al*, 2002). The increased excretion has been postulated to be probably due to renal tubular epithelial cell dysfunction induced by increased protein traffic along the renal tubules especially in renal glomerular dysfunction (Bazzi *et al*, 2002; Vishal *et al* 2006). The problem of developing early markers of renal damage especially in toxicologically induced cases remains a medical challenge even in developed nations. Determination of the activity of this enzyme in the urine of subjects in this study was aimed at establishing the level and extent of secretion of this enzyme in subjects occupationally exposed to lead. Secondly, the estimation was also used to assess its potential as an early sensitive indicator of the presence of tubular or other kidney damage in these subjects compared with other markers of tubular damage in an African population.

On the other hand GST is a dimeric enzyme with many isoforms (Neuefiend *et al* 1997). The Pie isoform of GST has been found to be predominant in human renal tubular cells and play key roles in cellular detoxification. These multifunctional enzymes have been said to be rapidly released in renal tubular cell damage in a number of animal studies and few human experiments (Pranay Kathurai and Paresh Jadav, 2003; Vishal *et al* 2006). Combined estimation of these two urinary enzymes as early indicators of renal tubular damage in subjects occupationally exposed to lead (Pb) was therefore explored in this cohort study with a view to comparing them and evaluate their possible use routinely in the diagnosis of early renal tubular damage due to lead (Pb) poisoning.

Materials and Methods

Materials: This was a human experimental research carried out at the University College Hospital, Ibadan, Nigeria after

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obtaining ethical clearance from the Ethical Committee of the hospital. Three categories of subjects were recruited for this study designated as Experimental (LES), Chronic renal failure (CRF) and Control subjects.

The Control: This group consisted of fifty healthy adults (men and women aged between 20 years and 50 years) who were students and civil servants studying and working in the University College Hospital, Ibadan. They had no known previous occupational exposure to lead and had no history or clinical symptoms of renal dysfunction as deducted from the administered questionnaire and had normal renal function biochemical parameters

Experimental Subjects (occupationally exposed (LES) group): One hundred and twenty five subjects aged between 23 years and 47 years consisting of workers in a lead smelting and battery manufacturing plants, automobile-mechanics, battery-repairers, welders, vulcanizers, and vehicle-painters were selected for this group. Averagely, they have worked for periods ranging from 1 to 16yrs as factory workers doing core jobs that exposed them to lead/lead fumes routinely.

Chronic Renal failure (CRF) associated with Pb exposure: Twenty five CRF patients clinically diagnosed at the medical out-patient (MOP) department of the University College Hospital, Ibadan were selected, twenty of these patients were already slated for renal dialysis.

Methods

After the administration of appropriate questionnaire to determine their suitability for the study, venous blood and random urine specimens were collected from subjects for the determination of blood lead (Pb) concentration, urinary microalbumin qualitatively (Mogensen and Christensen (1984) urinary GST and NAG

Blood Lead (Pb) concentration: Blood-lead concentration was determined using Atomic Absorption Spectrophotometry (AAS Model 210VGP manufactured by Buck Scientific, USA) based on the modified method of Hessel (1968).

Urinary microalbumin: Test tablet used in the qualitative determination of urinary microalbumin was based on the method of Mogensen and Christensen (1984). The test material was a preparatory tablet obtained from BAYER Corporation, Diagnostics Division, Elkahart, United States of America.

Urinary N Acetyl-β-D-Glucosaminidase (NAG): N acetyl- β -D-glucosaminidase activity was determined in the urine of the three selected groups using the modified method of Price, (1992)

Urinary glutathione-S-transferase (GST) activity: Urinary Glutamyl-S-Transferase activity was determined using Biotrin GST kit (purchased from Biotrin International Ltd, Dublin, Ireland) based on the principle of enzyme immunoassay (Manning, 1994; Sundberg, 1994).

Statistical analysis: Statistical analysis of the results was done using paired t-test and Pearson's correlation analysis. ANOVA was also used with 0.05 as statistical significance.

Results

Blood Lead Results:

The mean blood lead concentrations obtained were 23.4±19.7µg/100ml, 33.0±20.8 µg/100ml and 90.0±24.0 µg/100ml for the control, the occupationally exposed and the CRF patients groups respectively. On the other hand, the blood lead concentrations ranged between 8.1 µg/100ml and 38.6 µg/100ml in the control, 12.9µg/100ml and 52.0 µg/100ml in the LES and between 64.3 µg/100ml and 114.0 µg/100ml in the CRF groups respectively. From the results, about 18% of the LES showed blood lead concentrations higher than the normal upper limit of 23µg/100ml (Table I).

It was observed that the mean blood lead concentrations obtained for the occupationally exposed and the CRF patients groups were higher than the mean blood lead concentrations obtained for the control group, the difference was statistically significant ($p \leq 0.05$).

Table I:
Mean (±SD) concentrations of Plasma Creatinine, Uric acid, Urea, Blood Lead, Urinary NAG and GST in Control, LES and CRF Subjects.

	Control	LES	CRF
Creatinine mg/100ml	0.8±0.4	0.4±0.2	8.8±5.2
Uric acid mg/100ml	3.1±1.1	3.7±1.8	8.1±3.8
GSTµg/l	39.5±25.9	75.9±27.7	102.2±54.5
NAG U/L	10.3±1.8	12.4±3.8	14.8±6.3
Urea mg/100ml	21.9±1.1	13.0±8.0	94.9±43.9
Lead µg/100ml	23.4±15.2	33.0±20.8	89.9±23.9

Key to the Tables:
Creat= creatinine, GST= Glutathione-S-transferase, NAG= N-acetyl-β-D-glucosaminidase, Pb= Lead, U/Acid= uric acid, LES= Lead Exposed subject, CRF= chronic Renal failure patients

Although about 18% of the occupationally exposed subjects had excessive blood lead level, none of the traditional biochemical indicators of renal function investigated showed evidence of kidney dysfunction. However, low haematocrit and reduced haemoglobin levels were observed in about 40% of the subjects indicating anaemia.

Urinary microalbumin Investigation: To further confirm the presence of glomerular dysfunction in the subjects selected for this study, presence of urinary microalbumin was investigated. 60% of the CRF patients group had microalbuminuria while no microalbuminuria was observed in the urine samples from both the control and occupationally exposed subjects. This further confirmed absence of any evidence of kidney glomerular damage based on traditional renal function markers.

Urinary N Acetyl-β-D-Glucosaminidase (NAG)

The results obtained showed mean NAG values of 10.3±1.8U/l, 12.4±3.8U/l and 14.8±6.3U/l for the control, the occupationally exposed and the CRF patients groups respectively. The urinary NAG values obtained ranged from 8.6 U/l to 15.1U/l in the control, from 8.6 U/l to 23.6U/l in the LES and 9.0 U/l to 32.6U/l in the CRF

groups respectively. On further analysis, 4.4% of the LES and 15.3% of the CRF patients groups had urinary NAG activity higher than the expected normal range of 17±5.8U/l (Meyer *et al* 1984). However, the difference in the urinary NAG values of the occupationally exposed and CRF patients groups was not statistically significant (P>0.05) (Table 2).

Table 2:
Descriptive Statistics and indices of plasma Creatinine, Uric acid, Urea, Blood Lead and Urinary NAG and GST in Control, LES and CRF subjects

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
CREAT	Between Groups	768.567	2	384.284	289.201	.000
	Within Groups	239.180	180	1.329		
	Total	1007.747	182			
Uric Acid	Between Groups	222.265	2	111.133	8.300	.000
	Within Groups	2369.827	177	13.389		
	Total	2592.092	179			
Pb.	Between Groups	2515.015	1	2515.015	12.540	.001
	Within Groups	30486.024	152	200.566		
	Total	33001.039	153			
GST	Between Groups	28123.288	2	14061.644	17.409	.000
	Within Groups	55734.324	69	807.744		
	Total	83857.611	71			
NAG	Between Groups	121.864	2	60.932	1.858	.164
	Within Groups	2262.254	69	32.786		
	Total	2384.118	71			
UREA	Between Groups	57231.752	2	28615.876	70.247	.000
	Within Groups	15072.248	37	407.358		
	Total	72304.000	39			

Urinary glutathione-S-transferase (GST) activity
The results of GST activity obtained showed mean urinary GST activity of 39.5±25.9µg/l, 75.9±27.7µg/l and 102.2±54.5µg/l for the control, the LES and the CRF patients groups respectively. The urinary GST values ranged between 7.5µg/l and 90µg/l for the control, between 14µg/l and 193µg/l in the LES and 42µg/l and 170µg/l in the CRF groups respectively. (Table I). In particular, 91% of the LES and 83% of the CRF patients group exhibited higher urinary GST activity relative to the control. There was also a statistically significant difference between the mean urinary GST values obtained for the LES and the CRF patients groups ($p \leq 0.05$) (Table II). The result of urinary GST activity showed that 91% of the occupationally exposed had overt kidney dysfunction as shown by increased secretion of this enzyme into the urine. Expectedly, about 83% of the CRF subjects also showed increased secretion of this enzyme into the urine.
Correlation analysis (Table 3) shows a positive correlation between lead and urinary GST secretion.

Discussion

The objective of this study was to detect early kidney damage, which often-times traditional renal function markers fail to detect until the chronic stage. The study was also meant to investigate which portion of the nephron was more prone to toxicity of the trace metal (Pb) under reference. However, it was evident from the results that about 18% of the LES subjects exhibited high blood lead (Pb) level as a consequence of exposure in their occupation. However, none was shown to have any glomerullar dysfunction as suggested by the grossly normal traditional kidney function markers -plasma creatinine, urea and uric acid concentrations- and urinary microalbumin level. The insensitivity of these traditional kidney function markers to indicate the presence of renal dysfunction early enough necessitated the search for more sensitive markers in form of urinary enzymes- GST and NAG.

Table 3: Correlation analysis table

		Pb	PCV	Hb	GST	Urea	Creat 1
Pb	Pearson Correlation	1.000	-.194*	-.104	-.471*	-.071	.256**
	Sig. (2 - tailed)	.	.039	.271	.036	.766	.008
	N	123	114	114	20	20	108
Pcv	Pearson Correlation	-.194*	1.000	.903**	.091	.075	-.246*
	Sig. (2 - tailed)	.039	.	.000	.694	.747	.013
	N	114	116	116	21	21	101
Hb	Pearson Correlation	-.104	.903**	1.000	.000	.083	-.222*
	Sig. (2 - tailed)	.271	.000	.	.999	.721	.026
	N	114	116	116	21	21	101
GST	Pearson Correlation	-.471	.091	.000	1.000	.719**	.036
	Sig. (2 - tailed)	.036	.694	.999	.	.000	.900
	N	20	21	21	21	20	15
Urea	Pearson Correlation	-.071	.075	.083	.719**	1.000	.102
	Sig. (2 - tailed)	.766	.747	.721	.000	.	.707
	N	20	21	21	20	21	16
Creat 1	Pearson Correlation	.256**	-.246*	-.222*	.036	.102	1.000
	Sig. (2 - tailed)	.008	.013	.026	.900	.707	.
	N	108	101	101	15	16	109
Uric	Pearson Correlation	.190	.122	.182	.300	.329	-.067
	Sig. (2 - tailed)	.060	.244	.081	.319	.251	.509
	N	99	93	93	13	14	100
NAG	Pearson Correlation	-0.17	.160	-.094	.088	-.050	-.155
	Sig. (2 - tailed)	.942	.476	.679	.704	.831	.567
	N	21	22	22	21	21	16

Most experimental work on enzymuria in the diagnosis of covert renal disease have been on investigating levels of NAG in the urine especially in humans (Wellwood *et al*,1975; Meyer *et al*, 1984; Yuen *et al*., 1984; Khalil-Manesh *et al* 1993a and b).The few reports on GST have been on experimental animals (Hirsch, 1973; Feinfeld *et al*, 1977).

In this study, pie GST secretion reported to be localized to the S_3 portion i.e. the distal end of the proximal tubule was investigated in both the LES and CRF patients groups along with those of the control. Using a mean of 38.17±6.11 obtained for the control group, ligandin (GST) activity could be seen to be high in both the LES group (76%) and the CRF groups (80%) (relative to the control). There was also a positive correlation between the levels of urinary GST and blood lead in the LES and CRF patients groups (Table III).

As stated earlier, the role of GST is in cellular detoxification leading to the formation of glutathione and subsequently mercapturic acid before being excreted. Thus, the observed increased urinary GST activity observed in LES subjects in this study was likely to be induced by the presence of lead along the renal tubules of these subjects. That clinically diagnosed CRF patients included in the study also exhibited similar results clearly supports this. It is established that the S_3 portion of the renal distal tubule is associated with the reabsorbtion and concentration of small molecular substances hitherto filtered by the glomerulus (Khalil-Manesh Farhad I *et al* 1993). Therefore, the consistent and rapid release of this enzyme from its intracellular space into the extracellular area and ultimately into the urine is likely a consequence of the toxic action of the Pb on the enzyme that energizes this pump (Na^+/K^+ATPase). Derangement of this function could likely be a result of the breakdown of the usual membrane transport barrier which is maintained by the various membrane pumps one of which is the Na^+/K^+ pump (Omotosho and Olorunsogo, 2012; Fernando Magro *et al* 2004;). Alternatively, the toxic action of the metal could have indirectly induced oxidative stress through a disruption in ATP production in the mitochondria with consequent adverse effect on the pumping action of the enzyme (Omotosho and Olorunsogo, 2012). The possibility of this as the molecular basis of the tubular damage in lead and other metal toxicities is imminent since most heavy metals accumulate in segments of the proximal nephron where transport or binding sites such as the sulphydryl (SH) groups are abundant. The theory on oxidative stress being a possible basis of the observed renal tubular dysfunction has also been proposed, (Olorunsogo *et al*; 1991). It can thus be inferred that the disturbance in intracellular Ca^{2+} (and other cations) homeostasis and subsequent disruption in cytosolic Ca^{2+} (and other cations) concentration could have led to the disruption in the normal plasma membrane barrier and the attendant increased release of GST into the extracellular space. It is thus likely that if the disturbance and its attendant disruption remain unabated, a gradual death of the renal tubular cells leading to end stage renal disease (ESRD) will ensue.

Aside from the aforementioned, associating the observed increased urinary GST activity in the occupationally exposed subjects with a disturbance in renal distal proximal tubular dysfunction is further supported by the results of both the urinary protein and plasma uric acid determination on the subjects. It is established that proteinuria, when observed, is most probably an indication of glomerular dysfunction and usually not tubular dysfunction except in cases like Fanconi syndrome where generalized aminoaciduria is a complication of chronic renal disease. Bazzi (2002) lent credence to this assertion by his observation that such renal disease exhibiting tubulotoxicity of proteinuria is usually due to glomerular damage. The observation that all the occupationally exposed subjects showed absence of microalbuminuria in contrast to 60% of the CRF patients showing microalbuminuria in this study may be an indication of little or no glomerular dysfunction in the occupationally exposed subjects. However, that the results of both plasma creatinine and urea concentrations in the occupationally exposed subjects which are known glomerular function indicators were grossly normal in this study (Table I) affirms the functionality of the glomerulli .

Additionaly, hyperuricaemia which was observed in about 13% of the occupationally exposed subjects was very significant ($F=8.3$, $P<0.05$). Unlike the absence of proteinuria, hyperuricaemia is a far more prominent feature of both acute and chronic lead toxicity. The mechanism of hyperuricaemia in lead nephropathy include glomerular filteration, reabsorption and tubular secretion and post secretory reabsorption (Hirsch, 1973). Hence, normal glomerular function as demonstrated from the results of plasma creatinine, uirea and also urinary microalbumin clearly confirmed that the derangement was most likely localized to the renal tubular region. As a correlate to this, it has been postulated that hyperuricaemia is possibly the link between lead intoxication and its eventual progression to renal insufficiency and subsequently hypertension in lead toxicity (Sanches Fructuoso *et al*, 1996; Mountokalakis, 2001; Vupputuri *et al* 2003). The persistent hyperuricaemia observed in the occupationally exposed subjects is most likely due to loss of resorptive and secretory functions at the proximal end of the nephron.

It can therefore be concluded that the increased GST secretion and its consequent excretion into the urine was essentially an indication of the loss or deranged function of the distal portion of the proximal tubule.

On the other hand, the results of urinary NAG activity in both the LES and CRF subjects showed that about 6.7% and 7.7% respectively exhibited increased urinary excretion of this enzyme relative to the control group. This was markedly different from the 60% and 77% ratio obtained for the CRF and LES subjects respectively using GST as a marker of toxicity. Essentially, the molecular weight of NAG is large enough to preclude its passage through the normal glomerular membrane. It is therefore expected that NAG should not be filtered through an intact glomerulus. Results of both the urinary protein and traditional glomerular function markers (plasma creatinine and urea) have shown that the glomeruli were normal in both the control and LES subjects, an increased excretion of this enzyme would be a reflection of active tubular damage. The enzyme level was therefore prognostically expected to be high in the group of subjects studied if there was any overproduction due to kidney damage occasioned by lead poisoning. However, majority of the subjects exhibited normal urinary NAG activity relative to the control group. Apart from the non-statistically significant relationship observed between the level of urinary NAG and the marker

of toxicity (lead), there was equally no dose-response relationship as depicted by the almost similar mean values obtained for the control, the occupationally exposed and even the CRF patients studied (10.3 Vs 12.4 Vs 14.8u/100ml; P>0.05 respectively). Additionally, correlation studies also showed negative correlation between blood Pb levels and the urinary NAG estimations in the groups of subjects studied (Table 3)

Although increased urinary NAG activity has been reported to be associated with some measure of lead exposure in some studies (Wellwood *et al* (1975); Yuen *et al* (1984); Meyer *et al*, (1984), only a few have shown exposure-response correlation of NAG with blood lead. This generalized submission on the NAG activity in renal tubular disease is controversial. The few studies done so far on black Africans (including this) have shown that there was no linear or positive correlation between the observed blood lead and the urinary NAG levels in both the occupationally exposed and CRF subjects. The controversial linearity reported from experiments on Caucasians could probably be as a result of other co-founding toxicity like cadmium. Another possible reason for the observed disparity in Caucasians could also be due to the presence of higher cumulative body burden of lead in the blacks used in this experiment. This is most probable as there were little or no known regulatory measures for the control of occupational exposure to lead (or any other toxic metal) which could moderate the body pool of lead that greatly affects blood lead level. Also, the discrepancy observed in this study in comparison with those from Caucasians could be as a result of polymorphism in the NAG molecule. The different isoforms of the enzyme could exhibit different resistances and resilience in their reaction to toxic agents like lead. Thus, the isoform present/predominant in Caucasians might be lesser resistant to lead toxicity than those found in black Africans.

In conclusion, It is evident from this study that urinary GST was a better and more sensitive urinary enzyme than urinary NAG in detecting the advent/onset of renal tubular damage especially where most traditional methods have failed. This report has thus shown that in the group of subjects studied, urinary GST was more sensitive and specific in detecting early renal tubular damage than urinary NAG.

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