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Original article

SERUM URIC ACID AND STANDARDIZED URINARY PROTEIN: RELIABLE BIOINDICATORS OF LEAD NEPHROPATHY IN NIGERIAN LEAD WORKERS

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The question as to whether lead causes renal damage still remains largely controversial. Eighty-five male lead workers and 51 control subjects who had never been occupationally exposed to lead were studied. They were also classified according to duration of exposure. The mean age of the lead workers was similar to that of control subjects. The mean duration of occupational exposure to lead was 16.7 ± 2.13 years. Blood lead level was significantly higher in *Pb* workers than in controls (P < 0.001). Serum creatinine level did not differ significantly between lead workers and controls. Urinary microalbumin level was elevated in lead workers compared with controls but this was not significant (P>0. 05). Serum uric acid level was significantly raised in lead workers than in controls (P<0. 001). In addition it was significantly correlated with blood lead level (r = 0.24, P < 0.0026). Standardized total urinary protein was also significantly raised in lead workers compared with control (P < 0. 001). Serum potassium level was equally significantly higher in lead workers than in controls (P < 0.01). In contrast serum total calcium level was significantly decreased in lead workers than in controls (P < 0.01), while serum phosphate level did not differ significantly. Serum uric acid level and standardized urinary protein determination may prove a readily available, reliable marker of lead nephropathy in Nigerians.

Keywords: Uric acid, lead nephrophaty, uric acid, serum, Nigerians

INTRODUCTION

The question as to whether lead (*Pb*) produces Kidney damage has been discussed for nearly a century and never answered satisfactorily (Radosevic Beritil, 1961). The problem is still relevant and probably more so now in the face of increased industrialization. Lead is one of commonest work place toxins. Inspite of abundant literature data, much still remains to be explained. There are controversial opinions not only on the type of renal lesions due to lead; lead nephropathy, but also whether lead affects the kidney at all (Radosevic and Beritic , 1961). Although the weight of evidence supports nephrothy currently (Cramer et a!, 1974; Weeden *et al*, 1975, Goyer, 1985, Khali-Manesh at al, Kim *et al*, 1996), there is the need to examine the possible reasons for the inconsistencies. The divergence of indicators employed by previous investigators. Few attempts if any have been made in this environment to investigate the nephrotoxicity of lead. This study was therefore designed to look into the possibility of arriving at more reliable yet simple indicators of this insidious problem which may culminate in preventable chronic renal failure (CRF) if detected early.

MATERIALS AND METHOD

Selection of subjects

In this study 85 male lead workers after excluding those with renal problems or potential renal patients and 51 control subjects who had never been occupationally exposed to lead were investigated. The lead workers comprised of battery workers (12), home painters (14), welders (31) panel beaters and auto-mechanics (20) plumbers, ceramic workers and printers (8). the mean duration of exposure of the lead workers was 16.71± 2.13 years. A questionnaire was administered to the subjects with entries as follows: age, occupational history, present and previous illness, exposure to nephrotoxic agents in leisure and ethnic origin, subjects who answer in the affirmative for the last but one entry were excluded from the study.

Specimen collection.

Venous blood and spot urine specimens were collected by the same laboratory personnel using standard procedures regarding contamination free handling, unlike hospital and experimental situations in the case of field surveys, like occupational studies, it is not feasible to collect reliable 24 hr urine specimen. Standardization with urinary creatinine was performed in a manner similar to that of Verschoor *et al* 1987. Creatinine is a metabolite excreted at constant rate in urine. Thus it is very useful in standardizing urinary studies.

Analytical Methods

Determination of blood lead was performed by flame atomic absorption by the modified method of Hessel (1968). Owing to the ubiquitous nature of lead, venous samples were collected into lead-free navy-blue top vacutainer tubes (Becton-Dickinson, Rutherford, NJ) containing sodium heparin.

As part of contamination control, all glassware was routinely washed and soaked in two successive dilute nitric acid bathes (0.8mg/l) then thoroughly rinsed in ultra pure (double distilled deionized Water) (ASL IITA). Additionally all reagent, glassware and sample collection devices were checked for contamination with lead. No contamination was found when randomly selected sample of tubes used to collect and store blood for lead assay were tested for lead. The tubes were washed with 10% nitric acid (HNO_3) and the effluent measured by AAS as described by Jacobson *et al* (1991) for low lead concentration.

Serum uric acid was determined by enzymatic method of Fôssati *et al* (1980), while serum inorganic phosphate level was determined by the method of Fiske and Subarrow (1925). Serum total calcium level was determined by the spectrophotometric method described by Baginsky *et al* (1973). Serum urea level was determined by the method of Jung *et al* (1985).

Serum creatinine level was determined by the method originally described by Benedict and Behie (1936) and reevaluated by Stevens *et al* (1983). Urinary creatinine was determined by standard Jaffe reaction as urine contains less concentration of non-creatinine chromogen, This was used to standardize urinary protein level, that is urinary protein was expressed as mg/100mg creatinine. Twenty four 24 hour urine collection was not feasible with these ambulant subjects. This method has also been previously employed by some previous investigators (Verschoor *et al*, 1987). Microalbumin was measured with the sclavo albumin screen kit (Sclavo, SPA, Siena Italy), modified by (Watts et a! 1988). This has been described as the most valid test for Microalbuminuria (Watts *et al* (1988). Photometric method (Ames Elkhart, Indiana).

Statistics

Statistical analyses of the' data were performed with the SAS software (SAS Institute Carry NC), using the unpaired 't' test. Correlation among data was performed with the Pearson's correlation coefficient. Results were expressed as Mean ± SEM. The statistical significance for the't' test was assessed with a 2-tailed probability level at $P \leq 0.05$.

RESULTS

Indices of Renal Function

The values of indices of renal function viz serum creatinine, urea, microalbumin and standardized total urinary protein are shown in table 1. Serum creatinine level did not differ significantly between lead workers and controls. Similarly, serum urea level did not differ significantly between lead workers and controls ($p > 0.05$). Urinary micro albumin level was elevated in lead workers compared with controls, but this did not reach statistical significance ($p < 0.001$).

Table 1

Serum creatinine, urea, microalbumin and standardized total urinary protein in Lead workers and controls

	Lead Workers (n = 85)	**Controls (n = 51)**	**t**	**p**
Creatinine	1.25 ±0.03	1.28 ± 003	0.29	>0.05
Urea (mg/dl)	25.5 ± 1.09	21 ± 2.43	1.09	>0.05
Urinary Protein (mg/dl)	7.50 ± 1.26	5.0 ± 0.78	4.9	<0.001
Urinary Microalbumin (mgldl)	22.52 ± 2.66	19.95 ± 1.7	0.82	>0.05

Values are Mean ± SEM

Table 2

Blood lead level, serum uric acid, total calcium, inorganic phosphate and potassium levels in lead workers and controls.

-	Lead Workers	Controls	t	p
Blood lead ugldl	56.30 ±0.95	30.47 ±1.4	18.91	<0.001
Uric acid (mg/dl)	5.22 ± 0.28	3.4 ± 0.19	5.28	<0.001
K^+ (mmol/1)	4.70 ±0.10	4.20 ±0.13	2.63	<0.01
Total Calcium (mg/dl)	8.86 ± 0.09	9.22 ± 0.08	2.6	<0.01
Inorganic Phosphate (mgldl)	3.67 ± 0.09	3.48 ± 0.09	1.5	>0.05

Correlation of uric acid Vs lead

R	p
0.24	0.026

[1]Values are Mean + SEM, 2. correlate significantly with lead; Sign and controls.

Blood lead and other biochemical variables

Table 2 shows the other biochemical variables of lead workers and controls. The blood lead level was very highly raised in lead workers than in controls. ($p < 0.001$). Serum uric acid level was also significantly higher in lead workers than in controls ($p < 0.001$). Additionally, serum uric acid was positively correlated with blood lead level ($r = 0.24$; $P < 0.026$).

Total serum calcium level in contrast to that of urate was significantly lower in lead workers than in controls ($p < 0.01$). Serum inorganic phosphate level however, did not differ. Classification of renal parameters according to duration of exposure did not reveal any difference between lead workers and control ($p>0.O5$) subjects.

DISCUSSION

Despite the observation over a decade ago (Goyer *et al*, 1989; Landrigan, 1990. Landrigan, 1991) that the most important research needed in the study of lead nephropathy is a reliable early biological indicator of renal damage, this important problem has received inadequate attention worldwide and very little or none from this environment. Where lead exposed individual are monitored at all (a highly infrequent practice) in this environment, only the indirect indices of glomerular filtration rate (GFR), creatinine and urea levels in serum are employed. This study and several others have shown than these are insufficiently sensitive to detect or exclude lead nephropathy. The blood lead level as expected was significantly raised in lead works than in controls ($p<0.001$).

This was not accompanied by significant elevation in creatinine and urea levels in serum traditionally employed as markers of lead nephropathy. The blood lead level of controls (unexposed or the general population, was at a level (3Oug/dl) which the Word Health Organization (WHO) (1980) believes is indicative of significant exposure. This suggests general environmental pollution probably arising from the high lead level in the petrol consumed in this environment (Arah, 1985, Okoye, 1994, Adeniyi and Anetor, 1999). In addition regardless of the gradual disappearance of lead-based paint in developed countries, lead exposure from paint is likely to be high in various brands of point owing to the property of lead to be corrosion resistant in environments with high humidity (Ward, 1999) such as ours. This may contribute to unrecognized low level (subclinical) renal impairment which may progress to clinical renal disease in the presence of other risk factors for renal damage. Staessin *et al* (1990) and Staessin *et al* (1991) have made this observation in a non- occupational population where the level of environmental pollution with heavy metals was high. It is probably time to consider heavy metal pollution as a slow but definite etiological agent of chronic renal disease in this environment. Thus it should be added to the list suggested recently by Kadiri (2001).

Microalbumin urea, an index or marker of glomerular disease (Kow *et al*,1990, Ruilope *et al*, 1992) though raised in lead workers was not significantly so. Thus it may probably not be a sufficiently reliable index of lead nephropathy. There have been very few studies relating microalbuminuria with *Pb* nephropathy. The absence of significance in creatinine and urea levels between lead workers and unexposed subjects may reflect the well known high functional and metabolic reserve of the kidney. The evolution of lead nephropathy is usually silent (Landrigan 1990b; 1991). Clinical manifestation of renal impairment consisting of elevations in serum creatinine and urea levels do ordinarily become evident until about 50 to 70% of the nephrons have been destroyed owing to the large functional and metabolic reserve of this organ. This suggests that these popular tests of renal function are not sensitive enough to rule out nephropathy when normal levels are obtained. Since serum creatinine and urea are commonly employed as indirect measures of GFR (Hare 1950; Lauson, 1951; Tietz 1987). These data may more specifically suggest that GFR was unaffected in lead workers. This was probably what led Buchet *et al* (1980) to suggest that “Moderate exposure to lead” (blood lead 62 ug/dl) did not alter renal function in industrially exposed lead workers employed for a mean of 13.2 years (range 3.1 - 29.84) Omae *et al* (1990) have made similar observations based on their inability to detect any lead related changes in serum creatinine concentration, 8-microglobulin and uric acid clearances.

This study however, shows that standardized urine protein determination may prove a reliable marker for lead nephropathy. It was markedly elevated in lead workers compared with controls ($P <0.001$). This is often underrated in evaluation of renal function in lead workers. This is inspite of earlier studies indicating correlation of urinary protein excretion with EDTA mobilization test (Batuman eta!, 1981).

Uric acid was apart from being significantly raised in lead workers ($p < 0.001$) was also significantly correlated with blood lead level ($r = 0.24$; $p < 0.026$. Lead has long been recognized as an etiological factor in both gout and nephropathy. In the study of Batuman *et al* (1981) patients with industrial lead exposure or consumption of Moonshine had markedly elevate mean serum uric acid level. Renal biopsies obtained from a segment of these patients showed interstitial nephritis and nephrosclerosis, suggesting an association between raised urate level and nephropathy.

Additionally severity of’ renal disease in the lead workers was correlated with lead burden as well as urinary protein excretion, thus supporting the finding in this study. This is also consistent with the classic epidemiological studies of

Henderson in Queensland, Australia in 1958 which established 'that early exposure of children to lead paint predisposes them to renal scarving in adult life (Epstein, 1982).

The usefulness, of uric acid in lead nephropathy has also been poorly recognized in this environment in spite of many earlier studies that 'are consistent with findings in this report. Before looking. at these earlier reports it is also important to note that uric acid is an endo antioxidant (Ames *et al*, 1981). Thus the raised urate level may in part be an antioxidant responses to protect against the prooxidant effect of lead; Evidence for the involvement of free radicals in the pathophysiology of lead poisoning is growing (Monteino *et al*, 1985; Bechara 1996).

Gittleman *et al*, (1994) have recently reported that uric acid may be a consistent and reliable biomark of significant exposure to lead. The pathophysiology by which lead exposure causes elevation in uric acid level is thought to be due to damage tubules which cause retention of uric act (Bal and Sorensen, 1969). Inhibition of guanase (guanine aminohydroxylase) by lead is also thought to be a factor (Farkas *et al*, 1978). This results in highly insoluble purine which damages the tubules. Elevation in uric acid is now considered a common manifestation of subclinical lead intoxication (Goyer and Rhyne 1973a; Campel *et al* 1978). Alteration in uric acid since it is predominantly excreted by the tubule as an index of tubular injury in lead workers. Sohler *et al* (1977) have suggested that even marginal elevation in blood lead level (*Pb*) if accompained by a high uric acid level may make the *Pb* level suspicious (Clinically significant), (Mahaffey *et al* (1981). The extreme usefulness of urate levels in lead toxicity has been extensively reviewed (Anetor, 1997). Thus it is recommend that uric acid because of its route of excretion could be a better indicator of lead rephropathy in this environment and other developing countries where technical cconstraints ma *Pb* determination impossible.

The significantly decreased serum total calcium level is most probably due to impaired vitamin D metabolism. The active form of this vitamin required for calcium metabolism is processed first in the liver (25-hydroxylation) and finally the proximal tubules the kidney. (1-hydroxylation) resulting in the fully active vitamin or hormone (1 ,25-dihydroxycholecalciferol, 1,25-DHCC or calcitriol). This is because the cells lining the proximal tubules appear to be the tissue in the kidney most sensitive to lead (Goyer and Rhyne 1973). At blood lead levels of about 25ug/dl (which is less than what obtains in the general population), lead inhibits the metabolic activation of vitamin D, a transformation which takes place in these cells (Rosen *et al*, 1980). This is closely followed by hyperuricaemia at *Pb* of about 4Oug/dl the mechanism of which has been previously discussed above. Thus the decreased calcium level confirms the elevated urate level as arising from renal damage and indirectly alluding to its concomitant usefulness as an index of renal tubular damage. The heavy nutritional influence over calcium limits its usefulness as an index of *Pb* nephropathy. The non significant difference in phosphate level in this report may suggest that parathyroid mechanisms are not involved in the calcium phosphate homeostasis in this study, hyperactivity or hypoactivity would result in hypophosphataemia and hyperphosphaturia respectively and associated calcium changes. The raised potassium level may suggest hyporeninaemic hypoaldosteronism which is associated with attendant hyperkalaqemia (Epstein, 1980). The hyperkalaemia, of chronic lead toxicity of which occupational exposure is the commonest form, arises as a consequence of progressive leadnephropathy in turn due to insidious interstitial nephritis which i]k=8probably has a depressive effect on the release of renin from the Juxta glomerular apparatus (JGA). This iriturn leads to a depressive effect on the release of aldosterone hence inhibition of renal tubular extrusion of K and subsequent elevation in K level. Thus it now appears that lead poisoning may, in some patients produce the syndrome of hyporeinaemic hypoaldosteronism with attendant hyperkalaemia. Though plasma renin activity (PRA) was not measured in these subjects the significantly raised potassium level suggests this possibility. This observation has inturn, raised the possibility that the hyperkalaemia observed infrequently in other forms of interstitial nephritis might arise from decreased renin and aldosterone secretion, rather than intrinsic renal tubular disease. Defonzo *et al* (1979) investigated this in sicklers and found that their renin-aldosterone axis, unlike patients with lead poisoning and hyperkalaemia were intact.

Though hyperkalaemia was found in this study and is consistent with some other studies (Gozalez et al, 1979) it has not been as consistently reported as elevated uric acid level. Its elevation in combination with that of uric acid probably helps to strengthen the presence of *Pb* induced nephropathy. It should also be borne in mind that lead may also cause impaired membrane metabolism by impairing Na^+ - K^+ ATPase which helps to maintain an asymmetric distribution of K' with a higher K' concentration intracellularly, (Jan and Jan 1994, Anetor, 1997).

Though urinary N-acetyl-B-D-glucocaminidase (NAG), a lysosomal enzyme throughout the entire

nephron (Wellwood et al 1975) has been suggested to be one of the most sensitive indicator for estimating renal dysfunction due to lead poisoning (Staessen et al, 1990, Staessen et a! 1992), It is not yet a routine test particularly in this environment. This study, however suggests that a standardized urine protein and uric acid determinations may prove a readily available, reliable marker of lead nephropathy. Others like calcium and K^+ may reinforce this combination.

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