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Original Article

Fractional Clearance of Urate and Glomerular Filtration Rate in Leprosy Patients in a Nigerian Community

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Leprosy still remains a significant public health concern, particularly in developing countries including Nigeria. Hypouricemia in leprosy patients has been reported, but information on the role of the kidneys in the observed hypouricemia has not been sufficiently documented. Therefore, to provide additional information, we determined the Fractional Clearance of Urate (FCU) and Glomerular Filtration Rate (GFR) in leprosy patients. The study involved 61 leprosy patients on MDT; 58 leprosy patients relieved from therapy (RFT) and 50 healthy age and sex matched subjects which served as controls. Serum and spot urine urate were determined in all subjects from which GFR and FCU were derived. Serum creatinine and FCU were significantly increased in patients compared with controls. Urate and GFR were lower in leprosy patients on MDT than in RFT and control groups, with 5.7% having urate levels ≤ 3.0mg/dL and 58.1% having GFR values ≤ 80ml/min/1.73m^2. Urate level was significantly higher in RFT than those of MDT and of controls. We conclude that FCU is enhanced in leprosy patients who are on MDT resulting in reduced serum urate levels thus suggesting a role for renal tubules in the reported hypouricemia in leprosy patients on MDT.

Keywords : Leprosy, Urate, Urate clearance, Glomerular filtration rate, Multidrug therapy

Introduction

Leprosy remains an important health concern globally, particularly in developing countries such as Nigeria. Leprosy is a chronic debilitating infectious disease caused by *Mycobacterium leprae,* a rod like acid-fast bacillus. Though recent research suggests that individuals had been infected with *M. leprae* as early as 4000BC, the first known written reference to the disease was found in an Egyptian papyrus in about 1550BC (Rao et al 2012). *M. leprae* was first discovered by a Norwegian physician, Gerhard Hanrik Armuer Hansen in 1873, and it became the first microbe to be identified as a causative agent of human disease (Irgen 2002). Leprosy has long latent incubation period spanning 3-6 years in the paucibacilliary leprosy to 3-10 years in the multibacilliary form of the disease. WHO (1998a)

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has advocated the use of multidrug therapy (MDT) regimens for treatment of leprosy; the main drug components being Dapsone, Rifampicin and Clofazimine.

Renal involvement in leprosy was first reported by Mitsuda and Ogawa (1937) through autopsy findings. Since then a number of alterations in renal histological features have been described in leprosy. Renal assaults appear common in patients with erythema nodosum leprosum (ENL) (Nakayama et al 2001). Glomerular injuries have been demonstrated histologically in leprosy of which proliferative mensangial glomerulonephritis is the most common (Nakayama et al 2001); the prevalence of which has been reported as ranging from 6-50% (Klioze and Ramos-Caro 2002).

Alterations in renal functional status in patients with leprosy have been reported. These include glomerular and tubular alterations which have been found to occur even in the absence of demonstrable histological changes in patients kidneys (Aggarwal et al 2004 and Oliveira et al 2008). However, reports on serum urate levels in leprosy patients are very scanty. The few available reports are inconsistent. Osadolor and Okosun (2014) reported hypouricemia in leprosy patients on MDT while Aggarwal et al (2004) found no significant difference between serum uric acid levels in leprosy patients and in control.

The possible role of the kidneys in the reported hypouricemia in leprosy patients on MDT has not been sufficiently investigated. Furthermore, there appears to be a dearth of information on Fractional Clearance of Urate (FCU) and Glomerular Filtration Rate (GFR) in leprosy patients on MDT compared with those in leprosy patients relieved from therapy (RFT). This study therefore aims at evaluating renal tubular and glomerular functions in leprosy patients on MDT and their RFT counterparts with a view to providing information on the functional status of the kidney in these study groups especially in the handling of plasma urate. We are yet unaware of any such study in Nigeria or elsewhere.

Patients and Methods

Subjects : A total of 169 subjects were involved in this study. These consisted of 119 adult leprosy patients from Ossiomon Leprosarium Specialist Hospital, situated in Ogan, Edo State of Nigeria. Of these, 61 were on MDT while 58 were those who had been relieved from therapy (RFT) but now reside in and around the Leprosarium. The MDT group was classified according to WHO (1998b) criteria into 33 paucibacilliary (PB) leprosy patients and 28 multibacilliary (MB) form of the disease. The Control group consisted of 50 apparently healthy age and sex matched volunteers in similar socio-economic status and environmental conditions.

Exclusion criteria for all study groups were hypertension (systolic $\geq$ 140mm/Hg; diastolic $\geq$ 90mm/Hg); urinary tract infection, renal or history of previous renal disease, tuberculosis, diabetes mellitus and obesity. Also excluded were patients with present or previous episodes of ENL. This study was guided in terms of ethics by the 2008 Helsinki Declaration (Bonsness and Taussky 1945) and ethical clearance for the work was obtained from the Ethical Committee of the Ministry of Health, Edo State of Nigeria.

Sample Collection : Venous blood and spot urine samples were obtained from all subjects using standard procedures between 8.00 - 10.00 hours. Serum was harvested from the clotted blood samples after centrifugation at 3000g for 5 minutes and stored along with the urine samples at -20^{0}C before analysis which was done within 72 hours.

Biochemical Analysis : All samples were allowed to attain ambient temperature before analysis.

Creatinine was assayed by the alkaline picrate method of Jaffe as modified by Bonsness and Taussky (1945) while uric acid was determined by enzymatic colorimetric method of Fossati, Prencipe and Berti (1980). Test kits, products of Randox Laboratories, UK, were used for the assay and the manufacturers' instructions were strictly adhered to. eGFR was estimated by the simplified MDRD (National Kidney Foundation, (2002) formula with four variables. Values ≤ 80ml/min/ 1.73m^2 were considered to be in the declined GRF range. Fractional Clearance of Urate (FCU), a spot urine urate clearance corrected for GFR as measured by endogenous creatinine clearance, was calculated from the equation below proposed by Indraratna et al (2010) and recently validated by Kannangara et al (2012).

$$FCU = \frac{[\text{urinary urate x serum creatinine}]}{[\text{serum urate x urinary creatinine}]} \times 100$$

These authors had concluded that FCU is a convenient, valid, and reliable indicator of the efficiency of the kidney in removing urate from the blood and thus from tissues and that Spot-FCU determinations may provide useful correlates in studies investigating molecular mechanisms underpinning the observed range of efficiencies of the kidneys in clearing urate from the blood. FCU has the practical advantage of eliminating the need for the commonly unreliable timed collection of urine needed for the direct measures of renal clearance.

Statistics : The group means ± SD was calculated for each analyte and significant differences between means evaluated using the student t-test, with P≤ 0.05 considered as statistically significant. Statistical Packages for Social Science (SPSS), version 16.0 soft ware (SPSS Inc. Chicago, IL, USA) for windows was used.

Results

Table 1 shows the demographic and clinical characteristics of the study population. The age and sex distributions were similar in study population. The only significant variable in their clinical spectrum was leprosy.

Serum creatinine level was significantly increased in leprosy patients on MDT than in their RFT counterparts and in controls (P ≤ 0.05). The mean GFR value in leprosy patients on MDT was decreased significantly compared with RFT and control groups (Table 2). Serum Uric acid levels were lower in MDT patients than in RFT and control groups (P ≤ 0.001). FCU was significantly raised (P ≤ 0.001) in MDT patients than in RFT and

Table 1 : Demographic and clinical characteristics of the study population

	MDT	PB	MB	RFT	Controls
	(n=61)	(n=33)	(n=28)	(n=58)	(n=50)
Age (yrs)	38±12	36±10	38±11	37±10	35±8
Males	45	20	18	40	34
Females	16	13	10	18	16
BMI	22.6±2.1	21.9±1.8	22.4±2.2	23.6± 2.4	23.2±1.3
SBP	118±18	116±11	119±16	117±18	117±10
DBP	79±11	75±10	78±8	79±12	77±8

Data are shown as Mean ± SD. BMI = Body mass index; SBP = Systolic blood pressure; DBP = Diastolic blood pressure; MDT = Patients on Multiple Drug Therapy; PB = Paucibacilliary; MB = Multibacilliary leprosy patients and RFT = Patients relieved from therapy.

Table 2 : Serum Creatinine and Urate; Fractional Clearance of Urate and eGFR in Leprosy patients and in Controls

Parameters	MDT (n=61)	FRT (n=58)	Controls (n=50)
SCr. (mg/dL)	0.91±0.15*	0.82±0.11	0.81±0.18
eGFR (mL/min/1.73m2)	96 ± 11*	100±14	103 ± 16
SUA (mg/dL)	3.5 ± 0.6**	6.4±1.1**	5.1 ± 0.4
FCU(%)	9.4 ± 0.21**	7.1±0.16	7.9 ± 0.18

Data shown as Mean ± SD. *P < 0.05; **P? 0.001 significant by Student t test. SCr = serum creatinine; eGFR = estimated glomerular filtration rate; SUA = serum uric acid; FCU = Fractional Clearance of Urate; MDT = multidrug therapy group; FRT = relieved from therapy group.

Table 3 : Comparison of FCU and eGFR in Leprosy types and in Controls

Leprosy Type	n	eGFR(mL/min/1,73m^2) Mean ± SD	FCU (%) Mean ± SD
All Patients	61	96 ± 11	9.4 ± 0.21
PB	33	100 ± 10	6.9 ± 0.18
MB	28	90 ± 17*	5.2 ± 0.16**
Controls	50	103 ± 16	7.9 ± 0.18
		*P < 0.05	**P < 0.001

Data shown as Mean ± SD. eGFR = glomerular filtration rate; FCU = Fractional Clearance of Urate.

control groups (Table 2). However, while other parameters in RFT group tended towards those in controls, their serum uric acid levels were significantly higher than in MDT and in control groups (P ≤ 0.001). Table 3 shows the analysis of leprosy patients according to their bacillary classification. Both PB and MB patients had similar GFR and FCU.

Discussion

The mean value of serum creatinine in leprosy patients on MDT in this study, though within reference range for humans, shows significant increase (P ≤ 0.05) compared with the control group, with 5.7% having values ≥ 1.5mg/dL. This observation is in accord with that reported by Oliveira et al (2008) who also observed serum creatinine ≥ 1.2mg/dL in 8.5% of their study cohort. The GFR of leprosy patients on MDT was significantly reduced compared with control and FRT groups (P< 0.05); with 58.1% having values in the declined GFR range (≤ 80ml/min/1.73m^2)[8]. This is a bit higher than the 50% reported by Oliveira et al (2008) but lower than the 62.9% reported by Nigam et al (1986) who investigated 71 Brazilian and 64 Indian leprosy patients. This seemingly differences may be due to genetic or socio-cultural variations or both. Though the mean GFR of the MB patients was lower than that of the PB patients, the difference was not significant. However, MB patients had lower GFR than controls which was significant at P < 0.05, and presented a higher frequency (35.3%) of GFR values in the declined range than PB patients

(28.6%); an observation similar to those of other workers (Aggarwal et al 2004 and Oliveira et al 2008).

The exclusion of patients with present or previous episodes of ELN and other confounders of reduced GRF from our study group suggests that immune complex deposition, hypertension or diabetes may not be plausible causes of the observed reduction of GFR in our study group. Therefore independent mechanism may play a role. Such mechanism may include among others bacterial load, duration of therapy or both which were not considered in this study.

Serum uric acid levels in leprosy patients on MDT in this study, show significant decrease compared with RFD and control values; with 5.7% having uric acid levels ≤ 3.0mg/dl. This is at variance with the findings of Aggarwal et al (2004) who observed no significant difference in uric acid levels in all forms of leprosy compared with controls but in agreement with Osadolor and Okosun (2014) who reported reduced serum uric acid in leprosy patient on MDT. They attributed this reduction to the presence of reactive oxygen species (ROS) generated by the therapeutic drugs which uric acid as antioxidant tries to scavenge thereby leading to its depletion. This is in line with the suggestion of Kong and Lilleh (1998) that overproduction of ROS following therapy tends to exhaust the production of adaptive oxidants defenses. With a pKa of 5.4, uric acid circulates as the anionic urate which ordinarily serves as antioxidant; a function thought to be related to its ability to reduce transition metals (Patterson et al 2003) and react with potent oxidants such as peroxynitrite (Santos et al 1999). Low serum uric acid had also been reported in patients with multiple sclerosis also (Chamarro et al 2002).

In this study, we observed a significantly increased FCU in leprosy patients on MDT compared with controls ($P<0.001$). FCU was lower in PB than in MB patients though this was not significant. This appears to be the first time FCU in leprosy patients is being reported in Nigeria and these authors are also unaware of any such report elsewhere. However, FCU had been used to measure renal tubular function in homozygous sickle cell disease (Ceulaer et al 1981, Oforofuo, et al 1996) and they all reported a decreased FCU in their study cohorts.

FCU does not measure the volume of plasma cleared of urate per unit time nor the amount of urate excreted by the kidneys (Kannangara et al 2012) as does renal urate clearance; rather it expresses urate clearance as a function of creatinine clearance, thus it provides information about the efficiency of the renal tubular mechanisms of urate clearance by correcting for the effect of GFR as estimated by creatinine clearance. Being essentially independent of glomerular kidney function, FCU is proposed to be a better correlate than renal clearance of urate with which to identify subjects whose kidney tubules, inherently, are less efficient at clearing urate from the blood (Kannangara et al 2012).

Humans lack the ability to further degrade uric acid. About two-thirds of uric acid is excreted through the kidney, the rest being eliminated through the gastrointestinal tract. Uric acid is freely filtered in the glomerulus and almost completely reabsorbed in the proximal tubules. It is actively secreted at the distal proximal tubules and the majority of the secreted uric acid is reabsorbed at the distal convoluted tubules so that only about 12% of uric acid is excreted. Thus the great reabsorption capacity of uric acid in the proximal tubules makes secretion and post-secretion reabsorption the major determinants of its clearance. This is supported by the report of Enomoto et al (2002) who cloned URAT 1, the

anion exchanger responsible for uric acid reabsorption in the kidneys and demonstrated that inactivating mutants in SLC22 A12, the gene encoding URAT 1, were responsible for idiopathic renal hypouricemia. They also showed that uricosuric agents, such as probenecid, inhibited uric acid reabsorption by altering the function of URAT 1. This appears to explain the observed raised FCU in leprosy patients on MDT. Ceulaer et al (1981) and Oforofuo et al (1996) however, attribute their reported significant hyperuricemia with concomitant low FCU in sickle cell disease patients to the presence of homozygous S gene in their study cohort. They hypothesize that sickling process probably damages the medullary vasculature. This suggests a probable difference in the mechanisms of renal tubular handling of uric acid in sickle cell disease and in leprosy patients on MDT. From the concomitant hypouricemia, hyperuricosuria and enhanced FCU observed in MDT patients in this study, we hypothesize that renal tubular handling of UA is impaired in leprosy patients on MDT resulting in enhanced FCU, and thus explains, at least in parts, the hypouricemia reported in these patients.

We determined FCU and eGFR in leprosy patients relieved from therapy (RFT) in an attempt to define the role of therapy on these parameters. Serum uric acid was observed to be significantly higher in the FRT group than in the MDT and control groups; an observation previously reported elsewhere (Osadolor and Okosun 2014). This is curious because in this study FCU and GFR in the RFT group, show no significant difference from controls. This implies that this increase in serum urate in FRT patients is not likely to be due to impaired renal tubular reabsorption or glomerular filtration of urate. A more extensive longitudinal study to include more leprosy patients before, during and after therapy may elucidate the underlying mechanism.

Conclusion

Our findings in this study show that the renal handling of uric acid appears compromised in leprosy patients on MDT, especially in the MB group leading to enhanced urate clearance due possibly to the inhibition of renal tubular reabsorption of urate by the drug components of MDT which alter the function of URAT1. This renal tubular dysfunction may play a major role in the observed hypouricemia in leprosy patients. The observed increased uric acid in leprosy patients relieved from therapy (FRT group) in this study requires further investigations in order to elucidate the possible underlying mechanism.

Though serum creatinine and eGFR values in leprosy patients in this study are within the reference values for humans, the significant alterations in these parameters in these patients when compared with controls underscores the necessity to evaluate renal function in all leprosy patients, with or without reaction episodes or other confounders of renal dysfunction, for early diagnosis, treatment and management of complications.

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References

1. Aggarwal HK, Sharma P, Jaswal TS et al (2004). Evaluation of renal profile in patients of leprosy. *J Indian Acad Clin Med*. **5**: 316-321.
2. Bonsness RN, Taussky HH (1945). Creatinine estimation using Jaffe's method. *J Bio Chem.* **158**: 581-583.

3. Ceulaer KD, Morgan AG, Choo-Kang E (1981). Serum urate concentrations in homozygous sickle cell disease. *J Clin Pathol.* **34**: 965-969.
4. Chamarro A, Obach V, Cervera A et al (2002). Prognostic significance of uric acid serum concentration in patients with ischemic stroke. *Stroke*. **33**: 1048-1052.
5. Enomoto A, Kimura H, Chairoungdua A et al (2002). Molecular identification of a renal urate anion exchanger that regulates blood urate levels. *Nature.* **417**: 467-452.
6. Fossati P, Prencipe L, Berti G (1980). Use of 3, 5-dichloro-2-hydroxybenzenesulfonic acid/4-amino-phenazone chromogenic system in direct enzymic assay of uric acid in serum and urine. *Clin Chem.* **26**: 227-231.
7. Indrarantna PL, Stocker SL, Williams KM et al (2010). A proposal for identifying the low renal uric acid clearance phenotype. *Anthritis Res Ther.* **12**: 149.
8. Irgen LM (2002). The discovery of leprosy bacillus. *Tidsskr Nor Laegeforen.* **122**: 708-709.
9. Kannangara DRW, Ramasamy, SN, Indraratna, PL et al (2012). Fractional clearance of urate: validation of measurement in spot-urine samples in healthy subjects and gouty patients. *Anthritis Reseach & Therapy*. **14**: 187.
10. Klioze AM, Ramos-Caro FA. (2002). Visceral leprosy. *Int J Dermatol.* **39**: 461-658.
11. Kong Q, Lillehei KO (1998). Antioxidant inhibitors for cancer therapy. *Medical Hypothesis.* **51**: 405-409.
12. Mitsuda K, Ogawa M (1937). A study of 150 autopsies on cases of leprosy. *Int J Lepr*. **5:** 53-60.
13. Nakayama EE, Ura S, Fluery RN, et al (2001). Renal lesion in leprosy: retrospective study of 199 autopsies. *Am j Kidney Dis.* **38**: 26-30.
14. National Kidney Foundation: KDOQ1 (2002). Clinical Practice Guidelines for Chronic Kidney Disease: Evaluation, Classification and Stratification. *Am J Kidney Dis.* **39**: S1 266.
15. Nigam P, Pant KC, Kapoor KK et al (1986). Histofunctioal status of kidneys in leprosy. *Indian J Lepr.* **58**: 567-575.
16. Oforofuo IAO, Unuigbe EI, Halem NK (1996). Renal plasma clearance of uric acid and creatinine in young adult with homozygous sickle cell disease. *J Med Lab Sci.* **5**: 137-143.
17. Oliveira RA, Silva Jr GB, Souza CJ et al (2008) Evaluation of renal function in leprosy: a study of 59 consecutive patients. *Nephrol Dial Transplant.* **23**: 256-262.
18. Osadolor HB, Okosun RE. (2014). Non-enzymatic antioxidants status of leprosy patients in a leprosarium settlement in Nigeria. *Biokemistri*. **26**: 52-54.
19. Petterson RA, Horsley ET, Leake DS (2003). Prooxidant and antioxidant properties of human serum ultrafiltrates towards LDL: important role of uric acid. *J Lipid Res*. **44**: 512-521.
20. Rao AL, Prabhakar MC, Krupa DS et al (2012). Leprosy: disease prevailing from past to present. *Int J Res Pharm Chem*. **2**: 770-778.
21. Santos CX, Anjos EI, Augusto O (1999). Uric acid oxidation by peroxynitrite: multiple reactions, free radical formation and amplification of lipid oxidation. *Arch Biochem Biophys.* **372**: 285-294.
22. WHO (1998a). Model prescribing information: drugs used in leprosy. *WHO, Geneva* pp. 98-99.
23. WHO (1998b). Expert Committee on Leprosy: Technical report series, clinical aspects of leprosy related to control. 6th report. *WHO Geneva.* **768**: 13-19.

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