Membranous Nephropathy Associated With Indigenous Indian Medications Containing Heavy Metals

M. Naveen Kumar[1], P.S. Priyamvada[1], Anand Chellappan[1], Kappat Valiyapeediyekkal Sunooj[2], Bheemanathi Hanuman Srinivas[3], Rajesh Nachiappa Ganesh[3], Ezhilnilavan Sampath[4] and Sreejith Parameswaran[1]

[1]Department of Nephrology, Jawaharlal Institute of Postgraduate Medical Education and Research, Puducherry, India; [2]Department of Food Science and Technology, Pondicherry University, Puducherry, India; [3]Department of Pathology, Jawaharlal Institute of Postgraduate Medical Education and Research, Puducherry, India; and [4]Department of Nephrology, Sri Narayani Hospital & Research Centre, Vellore, Tamilnadu, India

Correspondence: P.S. Priyamvada, Department of Nephrology, Jawaharlal Institute of Postgraduate Medical Education and Research, Dhanvantri Nagar, Puducherry, India 605006. E-mail: priyamvadaps@gmail.com

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Membranous nephropathy (MN) is the most common cause of nephrotic syndrome in adults. About 80% of MN cases are primary, whereas the rest result from multiple secondary causes.[1] In the early days of modern medicine, mercurial organic compounds were used as diuretics, laxatives, and anti-infective agents. The use of mercury as a pharmacologic agent was abandoned by modern medicine owing to the toxic effects. However, various indigenous medical systems continue to use multiple forms of mercury. Chronic mercury exposure has been reported to be associated with a variety of renal manifestations, including acute tubular necrosis and tubulointerstitial nephritis. Glomerular diseases owing to minimal change disease and MN have also been reported.[2] MN secondary to mercury intake is reported in the context of usage of Chinese medications and skin-lightening ointments.[3] Reports of indigenous Indian medication intake as a cause of MN are scarce.[4] In this study, we report our experience with MN resulting from exposure to indigenous Indian medications containing mercury.

RESULTS

During the 2014–2019 period, a total number of 143 cases were diagnosed with MN. Serum and urine Mercury levels were measured in 11 patients with a history of indigenous medicine intake. Three patients had taken indigenous medications for a duration of 3 to 7 days after symptoms suggestive of nephrotic syndrome developed. The serum mercury levels were less than 5 μg/l in these 3 patients. A total number of 8 cases (5.5%) of MN had urine and serum mercury levels exceeding the limits defined in the inclusion criteria. The majority of patients were females ($n = 6$); all had taken indigenous medications for a variable period ranging from 1 month to 1 year, after which edema and proteinuria developed. Drugs were taken mostly for asthma, upper respiratory tract infections, and allergic symptoms. Three patients bought the indigenous formulations—namely, Swasa Kalpa syrup (Maruthi Pharma, Hyderabad, India) and Rasagandhi Mezhugu (SKM Siddha & Ayurveda, Erode, India). The patients noticed edema while taking the medications, after which they were referred to nephrology services. The baseline clinical and biochemical characteristics are provided in Table 1.

None of the patients had any other identifiable secondary causes to account for MN. Age-appropriate screening for malignancy was performed in all patients. Antinuclear antibody testing and serologic testing for hepatitis B, hepatitis C, and HIV were performed in all patients. Results of serum phospholipase A_2 receptor antibody and tissue staining for phospholipase A_2 receptor antigen were negative in all patients. Serum and urine mercury levels were obtained by inductively coupled plasma mass spectrometry. The serum mercury level ranged from 16.44 to 73.39 μg/l (normal levels <10 μg/l, without occupational exposure). Urinary mercury levels ranged from 18.9 to 86.72 μg/d (normal <10 μg/d, without occupational

Table 1. Baseline clinical and biochemical characteristics

Parameter	Value (Median, interquartile range)
Age, yr	36.5 (32.8, 56.5)
Serum creatinine, mg/dl	0.82 (0.62, 1.08)
24-h urine protein, g/d	8.9 (3.7, 14.1)
Cholesterol, mg/dl	287 (242, 534)
Serum albumin, gm/dl	2.1 (1.9, 2.6)
Serum mercury, μg/l	27.2 (19.1, 63.1)
24-h urine mercury, μg/d	30.2 (19.8, 59)

exposure). The serum and urine mercury showed a good correlation ($r = 0.957$; $P < 0.001$). There was no other evidence of systemic mercury toxicity in any of the patients. None of them had a history of having dental amalgams containing mercury or occupational exposure. The drugs were stopped in all patients at the time of nephrology review.

None of the patients had the kidney function documented before the onset of the current illness. Two patients (3 and 8) had decreased estimated glomerular filtration rate (<60ml/min per 1.73 m^2) at presentation. Both patients had a longer duration of exposure and higher mercury levels in urine and serum compared with others. Patient 3 had significant chronic changes on kidney biopsy. Renal biopsy in these patients showed evidence of acute tubular necrosis, which might be secondary to the severe nephrotic state in these patients. The referring physician prescribed corticosteroids for patient 3. Patient 8 started taking steroids after a diagnosis of mercury-related MN was made. The initial steroid dose was 1 mg/kg of body weight for 4 weeks. She attained remission by 1 month, after which steroids were tapered over the next 2 weeks. Despite their relatively higher mercury exposure, both patients who received glucocorticoids attained early remission. Patient 1 had retaken the same drug after attaining complete remission, which led to the reappearance of proteinuria. The proteinuria resolved over the next 6 months after the drug was stopped. He has continued to be in complete remission for the past 4 years. Patient 2 had normal glomeruli by light microscopy, with subepithelial humps visible on electron microscopy (Figure 1). Patient 5 did not attain remission and was lost to follow-up after 6 months. None of the other patients had any relapses once they attained complete remission. All patients who attained remissions, except patient 3, had no evidence of chronic kidney disease on the last follow-up. The individual patient details and relevant investigations are provided in Table 2. The serum and urine mercury levels for patient 7 after attaining a complete remission were in the normal range (serum mercury: 8.75 μg/l; urinary mercury: 5.12 μg/d).

Chemical Analysis

The drug Rasagandhi Mezhugu was analyzed by wavelength-dispersive spectrometer x-ray fluorescence (Bruker AXS S4 Pioneer XRF spectrometer, Bruker AXS GmBH, Karlsruhe, Germany). The drug contained 9100 μg of mercury per gram of the drug. The pill was heated at 60 °C for 2 hours and was then reanalyzed. The sample failed to show the presence of mercury, thereby revealing a sublimable form of mercury present in the drug formulations. Two different commercially available brands of Rasagandhi Mezhugu were tested, which showed mercury levels of 10,100 and 13,160 μg of mercury per gram of the drug, respectively. The lead levels varied from 6400 to 9100 μg per gram of drug. Traces of other metals, such as copper, aluminum, and manganese, were also present, but the levels were much lower. We could not test other medicines as wavelength-dispersive spectrometer x-ray fluorescence could test only the powdered formulations.

DISCUSSION

MN is a common cause of nephrotic syndrome in adults. Primary MN is an immune-mediated disease, mediated by autoantibodies directed against phospholipase A_2 receptor antigen, thrombospondin type 1 domain-containing 7A, and neural epidermal growth factor–like 1 protein.[1] However, MN can also be caused by a variety of secondary origins, including but not limited to, autoimmune diseases, malignancies, infections, and exposure to drugs and heavy metals, including gold and mercury. Exposure to mercury is rare in the general population; it usually occurs in the context of long-term occupational exposure. The toxicity profile of mercury depends on the form (elemental, mercurial salts versus organic), duration of exposure, and the dosage. Elemental mercury and organic mercury can cross the blood-brain barrier and result in neurotoxicity, whereas inorganic mercury salts are toxic to kidney and gut.[5] The inorganic mercury compounds do not pass the blood-brain barrier. Even though all forms of mercury can have poisonous effects on the kidney, exposure to inorganic mercuric salts leads to severe forms of renal involvement. Acute exposure leads to a dose-related acute tubular injury, predominantly affecting the pars recta, whereas low-grade chronic exposure leads to chronic tubulointerstitial and glomerular diseases.[5]

The indigenous Indian system of Siddha medicine uses heavy metals. As per the product inserts, the drug Rasagandhi Mezhugu contained 1.04% of elemental mercury and mercurous chloride. We found that the mercury content of Rasagandhi Mezhugu varied from

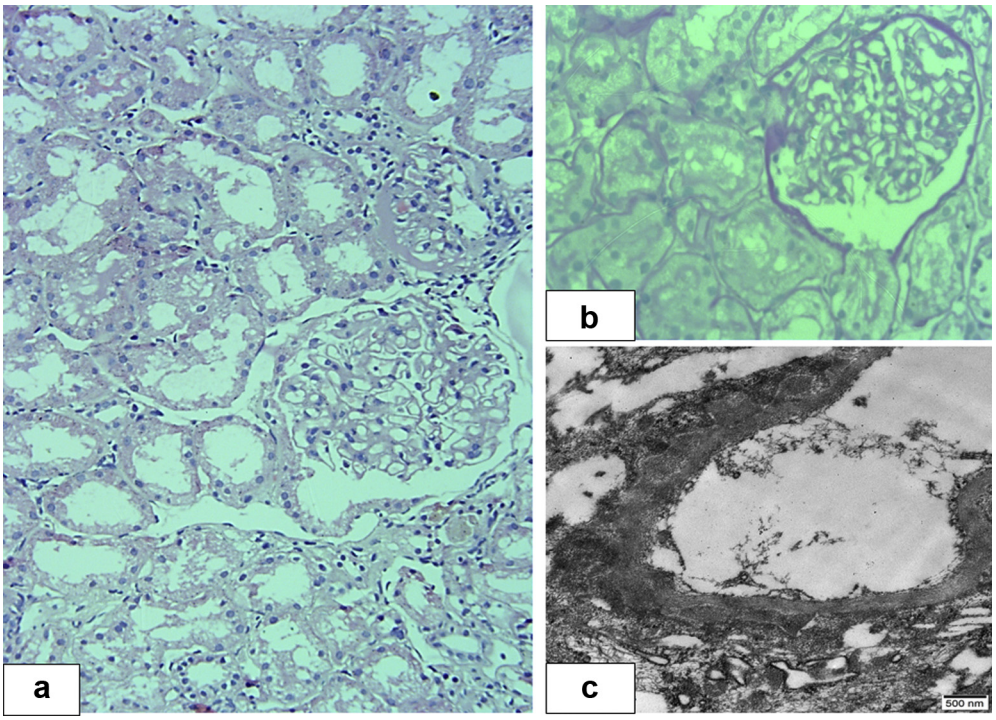

Figure 1. (a) Glomerulus showing mild stiffness of capillary basement membrane and mild acute tubular injury (hematoxylin and eosin stain, original magnification ×200). (b) Higher magnification of glomerulus with mild stiffness of capillary basement membrane (hematoxylin and eosin stain, original magnification ×400). (c) Ultrastructure of glomerular capillary basement membrane with prominent subepithelial and intramembranous electron deposits (uranyl acetate stain, original magnification ×6000).

0.9% to 1.31%. The capsules were available in 500-mg strengths. The usual dosage advised was a daily intake of 2 tablets, which would result in an ingestion of approximately 9 to 13.1 mg of mercury per day. This is much higher than the permissible upper limits of intake of mercury from all dietary sources, which is estimated to be approximately 0.003 mg/d.[5,S1,S2]

Despite widespread usage of indigenous medications by the general public in India, the literature on mercury exposure after indigenous medicine intake is scanty. The possible reasons include the lack of awareness and data on the toxicity profiles of these drugs. The medications sold by traditional medicine manufacturers are not often tested before marketing, and chemical compositions are not regulated. The commercially available preparations do not undergo routine postmarketing quality testing. A previous analysis of random samples of indigenous herbs and medicines revealed that 4.1% of analyzed samples contained mercury in quantities above the permissible levels. The products manufactured in India had much higher levels of mercury compared with products made in the United States. The estimated mercury content of some of the medications was as high as 20,800 μg per gram of the drug.[6] Moreover, these drugs are freely available over the Internet and in the conventional market, which might lead to unintentional heavy metal exposures with resultant long-term effects.

A systematic review of literature by Miller *et al.* identified 26 cases of mercury-related glomerulonephritis reported from 1950 to 2010.[2] A total of 26 individuals underwent kidney biopsy. MN was documented in 15, minimal change disease in 4, and chronic proliferative glomerulonephritis in 1. The remaining patients had tubulointerstitial lesions predominantly. Another series from China identified minimal change disease as the predominant histology (60%, $n = 21$) followed by MN in 37 % ($n = 13$).[3] They also reported that patients with minimal change disease had a shorter duration of exposure and higher urinary mercury levels.[3] The potential sources included dermal absorption from skin-lightening agents and mercury-containing pills for rheumatologic disorders. Other causes include consumption of meat from animals that have consumed mercury-infested grains as well as long-term consumption freshwater fish from water bodies contaminated by methyl mercury.[2,3] Another series from China reported Chinese herbal medicine as the predominant source of mercury exposure.[3] Recently Doshi *et al.* reported 5 cases of mercury-related MN from India. Other causes include vapor inhalation and injection of mercury.[4]

Table 2. Individual patient characteristics

Parameter	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8
Age	65	35	52	58	35	32	38	30
Sex	Male	Female	Female	Male	Female	Female	Female	Female
Indigenous drug[a]	Siddha	Siddha	Siddha	Siddha	Siddha (Swasa Kalpa)	Siddha (Swasa Kalpa)	Ayurveda	Siddha (Rasaganthi mezhuhu)
Duration of indigenous medication intake, mo	3	2	12	1	2	2	3	11
Serum mercury, normal <10 μg/l	16.4	21.5	71.2	32.1	18.3	22.4	38.6	73.4
24-h urinary mercury, normal <10 μg/d	18.9	19.6	86.7	36.8	23.5	17.5	42.1	64.7
Creatinine, mg/dl	0.91	0.78	1.80	0.86	0.53	0.63	0.75	1.14
eGFR, ml/1.73 m^2	89.3	98.5	39.6	98.5	115.6	118.7	122.8	50.3
Urine protein, g/d	14.3	5.3	16.2	13.3	3.3	4.7	2.2	12.5
Albumin, g/dl	2.3	2.8	1.8	1.8	2	2.2	2.7	2
Hemoglobin, g/dl	13.3	11	11	12.4	14.3	11.1	11.8	13.5
Light microscopy	MN,5% tubular atrophy, mild interstitial inflammation	MN, mild ATN, interstitial inflammation in 10% (foamy histiocyte)	MN,20% glomerulosclerosis, 10% ATN,10% IFTA, interstitial inflammation in 20%	MN, ATN, 15% interstitial inflammation in 10%	MN	MN, mesangial proliferation in 20%	MN, focal interstitial infiltrates (lymphocytes and foamy macrophages)	Normal basement thickness by LM[b], 10% ATN
IF microscopy	IgG 4+ C3 1+ C1q1+	No tissue $C4d^+$ on IHC	IgG 3+, IgM 1+, C3 1+	IgG 2^+, C3 1^+	$IgG3^+$, IgA 2^+, C3 1^+	IgG 3^+, C3 1^+	IgG 4^+, C3 trace $C1q^+$	IgG 1^+, C3 2^+
Treatment	Conservative	Conservative	Steroids ×2 wk	Conservative	Conservative	Conservative	Conservative	Steroids ×6 wk
Outcome	CR	CR	CR	CR	Not in remission	PR	CR	CR
Time to reach CR, mo	15	17	01	18	Lost to follow-up at 6 mo	6	6	1
Follow-up duration after attaining remission, mo	48	18	20	3	—	11	9	24
eGFR on last follow-up, ml/1.73 m^2	74.8	103.5	41.1	95.6	123	121	121	82.4

ATN, acute tubular necrosis; CR, complete remission; eGFR, estimated glomerular filtration rate; IF, immunofluorescence; IFTA, interstitial fibrosis/tubular atrophy; IHC, immunohistochemistry, LM, light microscopy; MN, membranous nephropathy; PR, partial remission.
[a]The system of medicine, as stated by the patients. Positive identification of the drug was not possible in patients 1 through 4 and 7 as the drugs had no labels or a prescription was not available.
[b]Electron microscopy showed membrane thickening and subepithelial deposits.

The histology of mercury-induced MN is no different than any other cause of secondary MN. In addition to the typical thickening of the glomerular basement membrane, there may be mild mesangial hypercellularity. Acute tubular necrosis may be seen more often, resulting from mercury-induced tubular toxicity. C4 and C1q deposits were reported by Li *et al.* in addition to the typical IgG and C3 deposits.[3] In addition, the report documented the IgG1 subtype as the predominant IgG deposit as against the commonly observed IgG4 subtype in phospholipase A2 receptor–mediated MN.

The exact pathogenesis of mercury-induced MN in humans remains uncertain. However, in rat models, mercury chloride was shown to induce T-cell–mediated polyclonal B-cell activation, leading to the production of numerous autoantibodies in a dose-dependent manner.[S3] Successive subcutaneous injections of mercury chloride in rat experiments are reported to lead to subepithelial immune complex deposition.[S4] It was hypothesized that mercury binds to proteins on the epithelial side of the glomerular basement membrane and acts as a hapten, eliciting antibodies that bind the target antigens on the glomerular basement membrane and form immune complexes.[3,7]

The optimal treatment of mercury-induced MN remains unclear. It is imperative to withhold all medications suspected of containing mercury. The serum half-life of mercury is about 10 days, and a rapid clearance of mercury will occur through urine once the exposure is terminated. Low levels of mercury exposure may be managed conservatively, whereas

extremely high levels of exposure (serum mercury levels 100 μg/dl) would require chelation with sodium dimercaptopropane sulfonate.[3] As the mercury levels in our series were much lower, we did not consider chelation therapy. The role of glucocorticoids and immunosuppressive drugs in the management of mercury related to MN is not clearly defined. Theoretically, immunosuppression might induce remission by decreasing autoantibody production. Nevertheless, there are only limited data on the use of any forms of immunosuppression. Qin *et al*. reported their experience with combined immunosuppression and chelation with sodium dimercaptopropane sulfonate.[8] Those patients who received glucocorticoids and immunosuppression, along with chelation, had lower sodium dimercaptopropane sulfonate requirements. The median duration of remission with combined therapy was 2.5 months versus 4.5 months in patients who received chelation alone. They also observed that in patients who received glucocorticoids or immunosuppression.[8] In our series, patients who received steroids had obtained an early remission despite having relatively higher urine and serum mercury levels. Mercury may persist in tissues despite normal serum and urine mercury levels. Long-term exposure to mercury may result in a state of persistent immune activation even after clearance of mercury from the system. This might account for failure to achieve remission after chelation, and such patients might need immunosuppressive agents.[8]

Despite not receiving chelation, all patients eventually headed for a remission. Patient 3 did not recover renal function despite the reduction in proteinuria. The patient had underlying chronic kidney disease, as evidenced by glomerulosclerosis and tubulointerstitial fibrosis on biopsy. The patient had no other identifiable cause for chronic kidney disease. We observed that serum albumin levels normalized even before the complete remission of proteinuria. Of particular significance in this series is the distinct absence of other systemic features of mercury toxicity, including neurologic and hematologic manifestations. The absolute level of mercury exposure may not be adequate in such cases to result in systemic toxicity.

Our study has a few limitations. As it is a retrospective series, we do not have mercury levels in other glomerular diseases. It is possible that the disproportionate anemia in some patients might be secondary to lead toxicity. We do not have data on the presence of lead or other heavy metals in blood or urine, which could possibly account for the disproportionate anemia in the patients. We could characterize the chemical composition of the drug in only 1 patient.

CONCLUSIONS

MN secondary to mercury exposure is a clinical entity that is often overlooked. Although it causes significant morbidity, most patients eventually recover without chelation after stopping the drugs. Chelation may be needed in patients with severe exposure or signs and symptoms of other organ toxicities. Steroids may have an adjunctive role. Mercury exposure needs to be ruled out as a potential cause for glomerular disease in communities where indigenous medication use is rampant.

DISCLOSURE

All the authors declared no competing interests.

SUPPLEMENTARY MATERIAL

Supplementary File (PDF)

Supplementary Methods.

Supplementary References

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