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Immunologic Aspects of Leprosy with Reference to Extravascular Immunoglobulins: Their Excretion Profile in Urine[1]

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Different types of lesions in the kidney have been described in patients with leprosy ([5, 11]). A significant number of deaths among patients with leprosy results from eventual renal failure, though the pathogenesis of the renal lesions is not clear ([6]). High incidence of secondary amyloidosis affecting kidney has been reported among the patients of Western origin, but such lesions are rare in patients of Indian origin ([16, 21]). A recent study of renal biopsies by Mittal *et al* ([11]) has shown that focal interstitial nephritis, glomerular hypercellularization and tubular degeneration are the most frequent renal lesions found in lepromatous leprosy, but no specific renal lesions can be attributed to the course of leprosy. Some authors have carried out studies of the functions of the kidney during reactive phases of lepromatous leprosy and observed significant impairment in the creatinine clearance rate but no change in blood urea and blood creatinine level ([17]). In the present report, attempts are made to assess kidney damage from a different angle. Since the molecular size of the excreted protein molecules in proteinuria throws some light on the nature of renal damage ([14]), we have studied the urinary protein excretion profile in patients with leprosy. These results have been compared with those found in the normal population and in nephrotic patients.

MATERIALS AND METHODS

Collection of serum and urine. Twenty-five biopsy proven leprosy patients formed the basis of the present study. Histopathologic classification was based on the criteria described by Ridley and Jopling ([15]). Borderline cases were grouped in the respective polar forms. Blood and 24-hour urine from leprous inpatients of the Leprosy Home, Delhi were collected under sterile conditions. Serum was separated from the blood-formed elements and stored at -20°C. Twenty biopsy proven nephrotic patients were available in the nephrology clinic of Irwin Hospital, New Delhi.

Preparation of urine samples. Aliquots of 24-hour urine in a dialysis bag were concentrated 10-15 times against solid sucrose in the cold, and then kept at -20°C. Serum samples were diluted 5-10 times in saline depending upon the concentration of the plasma constituents to be determined.

Detection and quantization of plasma proteins. Urinary proteins were detected in the agar-gel by the method of Ouchterlony *et al* ([13]). They were then estimated by single radial immunodiffusion method of Mancini *et al* ([8]), using nonspecific antihuman antisera. A 10 lambda Hamilton syringe was used to deliver the samples. All monospecific antihuman antisera, e.g., anti-albumin, anti-IgG, anti-IgA, anti-IgM, anti-transferrin, etc., and reference standards were obtained from Meloy Laboratories, U.S.A.

RESULTS

Table 1 shows the prevalence of albumin, transferrin, alpha-2-macroglobulin, C3, lambda-chain, kappa-chain and immunoglobulins such as IgM, IgG, IgA, IgD in normal subjects, nephrotic patients, and in lepromatous and tuberculoid patients. Out of 16 normal individuals, transferrin and alpha-2-macroglobulin were found only in one normal, but albumin and IgG were found in two persons. The person who excreted alpha-2-macroglobulin in the urine had renal calculi in the interstitial region. Others did not have any clinical history of kidney diseases. Incidence of albumin, transferrin, IgG, IgA and lambda-chain in the urine of nephrotic patients was found to be as high

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TABLE 1. *Incidence of plasma immunoglobulins and other plasma proteins in the urine of normal individuals and patients suffering from nephrotic syndrome and from leprosy.*

	Albumin	Trans-ferrin	a_2-macro-globulin	IgM	IgG	IgA	IgD	C3	Lambda-chain	Kappa-chain
Normal subjects (n = 16)	2 (12%)	1 (6%)	1 (6%)	N	2 (12%)	N	N	N	N	N
Nephrotic syndrome (n = 20)	20 (100%)	20 (100%)	N	2[b] (10%)	18 (90%)	10[c] (100%)	N	4 (20%)	8[c] (80%)	N
Lepromatous leprosy (n = 20)	13 (65%)	3 (15%)	N	N	14 (70%)	1[d] (5%)	N	N	11 (55%)	1 (5%)
Tuberculoid leprosy (n = 5)	N	N	N	N	N	N	N	N	N	N

[a] N = None.
[b] IgM content was 1 mg per 100 ml of urine.
[c] Only ten patients' sera were examined for IgA and lambda-chain protein. The IgA concentration in the urine varied from 0.5 mg to 1.1 mg per 100 ml.
[d] The concentration of IgA in urine was 0.6 mg per 100 ml.

as 80-100%, whereas that of IgM and C3 was as low as 10-20%. When the urinary protein excretion profile of normal subjects and nephrotic patients was compared with tuberculoid and lepromatous patients some marked differences appeared. No plasma protein could be detected in the urine of tuberculoid patients. In lepromatous subjects the prevalence of albumin and lambda-chain protein was as high as 55-65%, and that of IgG was about 70%. These levels were lower than those of the nephrotic group but higher than those of normal individuals. Excretion of transferrin, IgA and kappa-chain protein was found only in two, three, and one patient respectively out of twenty lepromatous subjects.

Serum and urinary levels of albumin, transferrin and IgG in normal, nephrotic and lepromatous subjects are given in Table 2. These were quantized only in those urine samples which showed positive precipitin reactions. Transferrin and IgG content in normal urine was less than 1 mg%. Two albumin positive normal individuals were found to excrete 6 mg% and 55 mg% of albumin per 100 ml of urine, respectively. In the urine of nephrotic patients considerable amounts of albumin (162 mg%), transferrin (9.2 mg%), and IgG (37 mg%) were excreted, and as a result their serum levels of these substances were found to be depressed compared to normal. In the group of lepromatous subjects (n = 20), only three were transferrin positive, but the urine transferrin content was negligible and found to be less than 1 mg%. The albumin and IgG content in lepromatous urine was 18.0 mg% and 2.8 mg% respectively. This was lower than the corresponding values in the nephrotic subjects. The lepromatous serum IgG level was found to be increased by about 26% compared to normal, but the serum albumin and serum transferrin levels did not show any significant change.

DISCUSSION

The aim of the present study consists of assessing the characteristics of proteinuria, if any, in the leprosy patient. The presence of plasma immunoglobulins and other plasma proteins was studied in the 24-hour urine of the leprosy patients. Albumin, transferrin and other immunoglobulins could not be detected in the urine of tuberculoid patients. The urinary protein excretion profile in the case of lepromatous leprosy patients showed that the incidence of albumin, IgG and lambda-chain protein in the urine is more than 50%, and significant amounts of these substances are excreted in the urine (Table 2). The occurrence of transferrin in the urine of these cases is as low as 15%, and the amount excreted is less than 1 mg%. Further, alpha-

TABLE 2. *Serum and urinary levels of albumin, transferrin and IgG in patients suffering from leprosy and nephrotic syndrome.*

	Albumin		Transferrin		IgG	
	Serum (gm%)	Urine (mg%)	Serum (mg%)	Urine (mg%)	Serum (mg%)	Urine (mg%)
1. Normal subjects (n = 16)	3.86 ± 0.21 (3.5 – 5.6)	30.5[b] (6 – 55)	200 ± 33 (125 – 230)	Below 1 mg[c]	1076 ± 262 (250 – 1828)	Below 1 mg[d]
2. Lepromatous leprosy patients (n = 13)	4.33 ± 1.27 (1.0 – 5.6)	18 ± 8.2 (4 – 29)	222 ± 102 (120 – 390)	Below 1 mg[e]	1352 ± 290 (700 – 2000)	2.8 ± 2.7 (0.5 – 8.5)
3. Nephrotic syndrome (n = 15)	2.05 ± 0.87 (0.44 – 2.8)	162 ± 185 (2 – 600)	83 ± 33.6 (25 – 119)	9.2 ± 5.5 (2.3 – 23)	780 ± 349 (340 – 1650)	37 ± 44 (10 – 175)
Statistical Evaluation						
Differences between groups 1 and 2	t = 0.40 $p > 0.60$ not significant	ND	t = 0.73 $p > 0.40$ not significant	ND	t = 2.66 $p < 0.02$ significant	ND
Differences between groups 1 and 3	t = 7.8 $p < 0.001$ significant	ND	t = 8.7 $p < 0.001$ significant	ND	t = 2.6 $p < 0.02$ significant	ND
Differences between groups 2 and 3	t = 8.6 $p < 0.001$ significant	t = 2.9 $p < 0.01$ significant	t = 4.5 $p < 0.001$ significant	ND	t = 4.5 $p < 0.001$ significant	t = 2.7 $p < 0.02$ significant

[a] ND = Not done.
[b] Only two subjects had urinary albumin: 6 mg and 55 mg respectively.
[c] One subject excreted transferrin.
[d] Only two subjects excreted IgG.
[e] Only three patients excreted transferrin.

2-macroglobulin, IgM, IgA, IgD, C3 and kappa-chain proteins could not be detected in the urine of lepromatous subjects. The urinary proteins from patients having the nephrotic syndrome consisted of albumin, transferrin, IgA, IgG and lambda-chain proteins in considerable amounts as has been reported also by other authors ([3, 4]). No report dealing with the characteristics of proteinuria in leprosy subjects has been found to date. It has been shown that protein molecules of smaller size are excreted in the urine in disorders of kidney tubules, whereas the urinary proteins from patients with glomerular damage are found to be of larger size ([1, 3]). Our present observations raise the question as to how transferrin, having a molecular weight of 88,000 daltons, is not excreted in the urine of lepromatous patients; whereas IgG, a substance with a molecular weight of 150,000 daltons is found in significant amounts. Lepromatous proteinuria, as studied here, cannot be compared with that in patients with the nephrotic syndrome in terms of selectivity index ([4]) since urinary transferrin excretion in the above cases is insignificant. Further, the amount of IgG excreted in the urine of lepromatous subjects is about 30 times less than that in the urine of nephrotic subjects who have decreased serum IgG levels along with decreased serum albumin and transferrin levels ([4]). On the other hand, serum IgG level in lepromatous patients was found to be increased. Table 3 records the changes in serum and urinary levels of albumin, transferrin and IgG in the lepromatous and nephrotic groups as compared to normal.

Recent immunofluorescence studies have shown the presence of antibody-coated bacteria in the urine of the patients suffering from upper urinary tract infection, and the

TABLE 3. *Depiction of changes in the levels of serum albumin, transferrin and IgG in nephrotic and leprosy patients with reference to normal individuals.*

	Albumin		Transferrin		IgG	
	Serum	Urine	Serum	Urine	Serum	Urine
Nephrotic syndrome	↓	↑	↑	↑	↓	↑
Lepromatous leprosy	⟷	↑	⟷	⟷	↑	↑

High = ↑
Low = ↓
No change = ⟷

classes of immunoglobulin coating the bacteria were mostly IgG and IgA and in some cases IgM. Urinary immunoglobulin levels did not show any correlation with the presence of antibody-coated bacteria in the urine ([18, 19]). Levels of IgG and IgA in the urine of the lepromatous patients studied in the present report were in good agreement with those from patients with different urinary tract infections ([18]). Thus it appears likely that the excreted IgG in the urine of the leprosy patients are local antibody molecules synthesized somewhere in the urinary tract ([7]). This tract is infected with various pathogenic bacteria ([10]), probably along with *Mycobacterium leprae* ([9, 20]), and the kidney may be affected at the tubular level. However, studies by Masanti *et al* ([10]) showed that pyelonephritis is not more frequent in patients with leprosy than in the general population and, further, leprosy bacilli could not be detected in the urinary sediment of leprosy patients ([2, 10]). In our present study of 25 leprosy patients, two female patients with lepromatous leprosy having severe lepra reactions developed urinary tract infections. *E. coli* as well as *Klebsiella* were isolated from their urine. One was successfully treated with chloromycetin and the other developed fatal renal failure and terminal bronchopneumonia. Autopsy examination showed membranous glomerulosclerosis and acid-fast bacilli in the kidney. Urinary IgG excreted was found to be 8.5 mg and 5 mg per 100 ml of urine respectively. These were the highest recorded in the series. IgA was also found in the urine of the latter patient. It would have been interesting if the results of the kidney biopsy of the patients could be compared with the urinary protein excretion profile of each affected individual. However, the profile of protein excretion in the urine of the patients with lepromatous leprosy in the present study seems to have no correlation with the presence or absence of lepra reactions. During the reactive phase of the illness, deposits of immune complex take place in the kidney, causing renal damage ([12]), but IgG excretion was not found enhanced in patients with ENL in our series. Further studies to characterize the nature of urinary IgG in the lepromatous patients show that it gave two distinct lines with monospecific anti-IgG serum (Fig. 1). The difference in the diffusion rates of the two components might indicate a concentration effect. It appears (Fig. 2) that urinary IgG is a mixture of lambda-IgG and kappa-IgG, since it showed lines of identity with myeloma serum lambda-IgG and myeloma serum kappa-IgG. Further studies are in progress to characterize the nature of excreted IgG molecules in lepromatous urine.

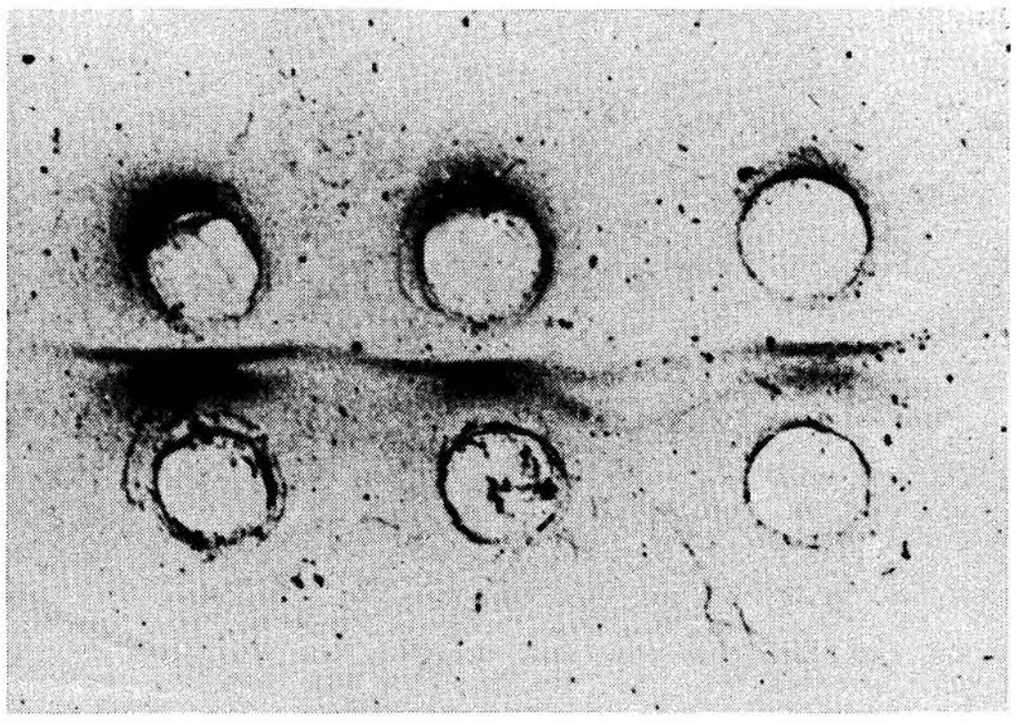

FIG. 1. Double gel diffusion study of urinary IgG from lepromatous patients. Upper wells contained monospecific anti-IgG antiserum, and the lower wells contained patient's urine in different dilutions.

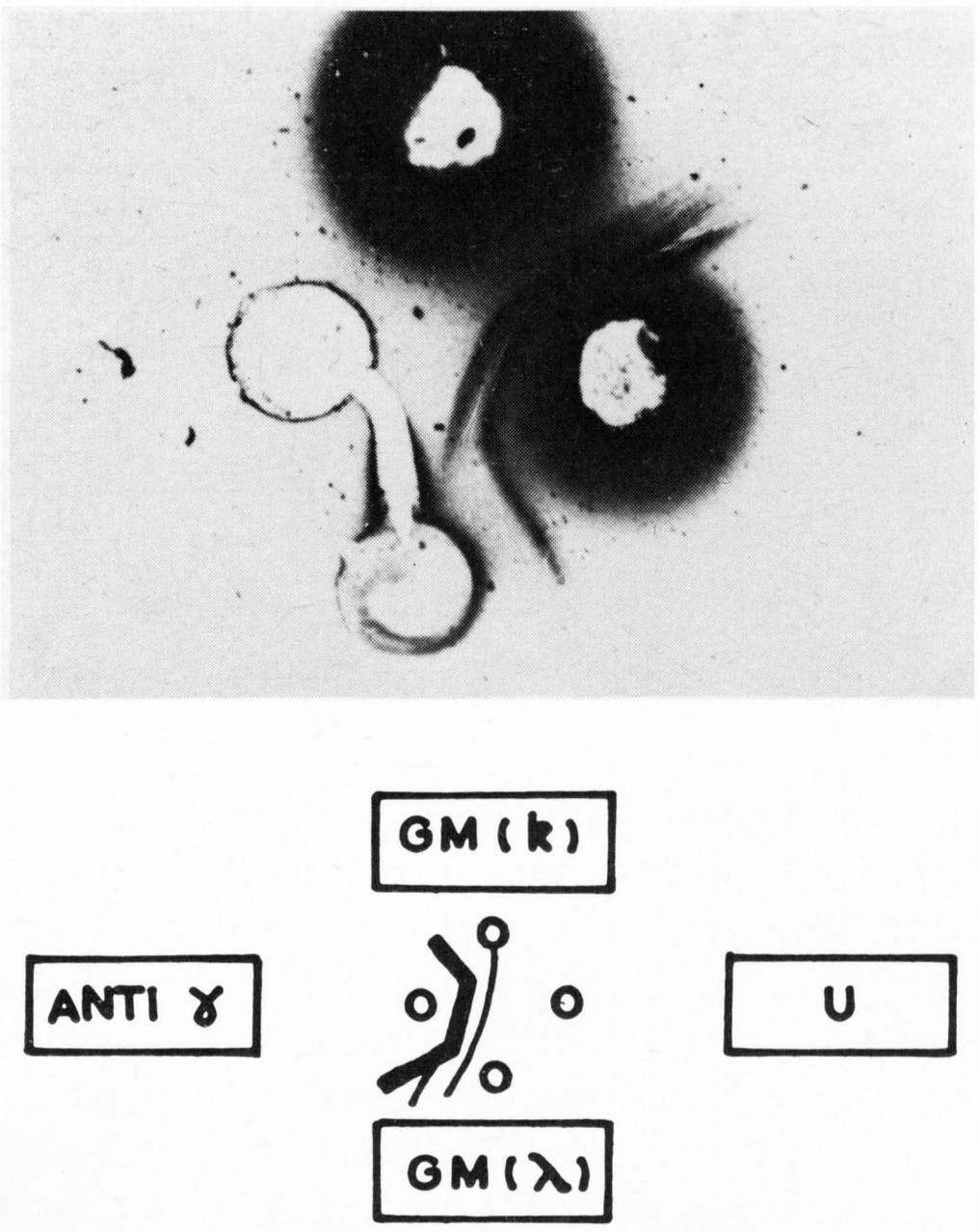

FIG. 2. Identity of urinary IgG. Both G myeloma (kappa) and G myeloma (lambda) formed lines of identity with the patient's urine sample against monospecific anti-IgG antisera.
U = Urine sample of leprosy patients.
G M(K) = G myeloma (kappa).
G M(λ) = G myeloma (lambda).
Anti- γ = Anti-heavy chain (gamma) antisera.

SUMMARY

An aliquot of 24-hour urine collected from leprosy patients was concentrated and examined for the presence of albumin, transferrin, IgG, IgA, IgM, IgD, C3, kappa and lambda light chains by the gel diffusion technic using respective monospecific antisera. Urinary protein excretion profile in lepromatous leprosy patients showed that while excretion of transferrin in the urine was negligible; that of IgG molecules, a substance of higher molecular weight, was significant. It is suggested that the immunoglobulins excreted in the urine may not be plasma-derived, but extravascular in origin. They are probably synthesized in the urinary tract.

In the present study, out of 25 leprosy patients, 2 female patients having severe lepra reactions developed urinary tract infections. *E. coli* and *Klebsiella* were isolated from their urine. The urinary IgG levels in those two cases were found to be the highest in the series.

RESUMEN

Se determinó la presencia de albúmina, transferrina, IgG, IgA, IgM, IgD, C3, cadenas ligeras kappa y lamnda, en la orina de pacientes con lepra colectada durante 24 horas y posteriormente concentrada. Se utilizaron, la técnica de inmunodifusión en gel y los antisueros monoespecíficos correspondientes. El perfil de excreción de proteínas en la orina de los pacientes con lepra lepromatosa reveló que mientras la excreción de transferrina fue insignificante, la excreción de IgG, una substancia de mayor peso molecular, fue claramente aparente. Se sugiere que las proteínas excretadas en la orina pudieran no ser derivadas del plasma sino tener un origen extravascular. Probablemente estas inmunoglobulinas se sintetizan en el mismo tracto urinario. En este estudio de 25 pacientes con lepra, dos pacientes del sexo femenino con reacciones leprosas severas desarrollaron infecciones del tracto urinario y, de su orina, se aislaron *E. coli* y *Klebsiella*. Estas dos pacientes tuvieron los niveles más altos de IgG urinaria.

RÉSUMÉ

On a concentré des spécimens d'urine de 24 heures recueillies chez des malades de la lèpre, qui ont ensuite été examinées pour mettre en évidence la présence éventuelle d'albumine, le transferrine, d'IgG, d'IgA, d'IgM, d'IgD, de C3, de chaínes courtes kappa et lambda, au moyen d'une méthode de diffusion sur gel utilisant les antisera monospécifiques respectifs. Les profils d'excrétion des protéines dans l'urine, chez des malades lépromateux, ont révélé que l'excrétion transferrine dans l'urine était négligeable. Par contre, l'excrétion de molécules d'IgG, et de substances de poids moléculaire élevé, était significative. On suggère que les immunoglobulines qui sont excrétées dans l'urine ne sont pas dérivées du plasma, mais ont une origine extra-vasculaire. Elles sont probablement synthétisée sans le système urinaire.

Dans cette étude, parmi 25 malades atteints de lèpre, 2 femmes avec réactions lépreuses graves ont développé une infection du système urinaire. On a pu isoler *E. coli* et *Klebsiella* de leur urine. Les niveaux de IgG urinaire dans ces deux cas se sont révélés être plus élevés que dans l'ensemble des malades.

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