

Membranous glomerulonephritis

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SUMMARY In a clinicopathological survey of idiopathic membranous glomerulonephritis (MGN) in 85 patients the predominance of the condition among men could be at least partly explained by an increased risk of exposure to organic solvents and heavy metals in the course of work. This may have implications for the advice given to these patients after MGN has been diagnosed. The selectivity of proteinuria could predict the likely outcome of the disease.

Membranous glomerulonephritis (MGN) is the underlying pathology of about 25% of adults presenting with nephrotic syndrome. The disease predominantly affects men¹ and has a peak incidence between 30 and 50 years of age, with a low incidence in children.²

Histopathological diagnosis is based on light microscopic appearances and examination by electron and immunofluorescence microscopy. IgG is commonly seen in a granular pattern on glomerular capillary walls; IgM and C3 are present less often.³ Similar histopathological lesions have been produced in rabbits by continuous low dose antigen challenge, leading to the appearance of small immune complexes on the glomerular capillary wall.⁴ The same pathogenetic mechanism is indicated in man by the association between MGN and infectious diseases,⁵⁻⁷ neoplasia with tumour antigen release,⁸ and hypersensitivity to d-penicillamine.⁹ About a quarter of cases pass spontaneously to remission, with regression of the renal lesion.¹⁰ The remainder usually have a gradual progression towards renal insufficiency and renal failure.

This study assessed the pathology, aetiology, and natural history of 85 patients who presented with MGN in the Edinburgh region over 20 years. In no patient was there evidence of systemic lupus erythematosus, tumour, or ingestion of drugs such as gold or penicillamine, all of which could have been associated with the pathogenesis. Diagnostic methods were evaluated and aetiological factors analysed, and the course of the glomerular lesion, including clinical correlates, assessed.

Material and methods

CLINICAL DATA

Data from casenotes were summarised and tabulated for 73 patients. Recent studies have shown an associ-

ation between glomerulonephritis and exposure to organic solvents or heavy metals such as lead or mercury.¹¹⁻¹⁶ For this study we divided our patients into two groups according to their occupations, as listed in the 1971 census for the region. The first group comprised patients whose occupations exposed them to metals and organic solvents such as workers in paint manufacture or in foundries; the second group comprised patients whose occupations were thought to have a low probability of exposure to these substances such as housewives, clerical, and professional workers (Table 1). Using figures from the census, the incidence of cases per 100 000 population over the 20 year period studied was calculated. Statistical analysis (χ^2 test) was performed with the null hypothesis that there was no significant difference in the incidence of MGN between the high exposure and low exposure groups.

Remission was defined as proteinuria of less than 0.3 g/day with no impairment of renal function. To compare patients treated with and without steroids who had only minimal functional renal impairment a case with proteinuria of less than 2.5 g/day, with stable renal function and little or no residual oedema, was designated as being in partial remission.

HISTOLOGICAL DATA

Light Microscopy Tissue was obtained from 150 biopsies by the percutaneous needle method and fixed in 4% buffered formalin. Paraffin sections were cut at 1.5-2.0 μm .

1 General features were examined on sections stained with haematoxylin and eosin, periodic acid Schiff reagent and Picro-Mallory or Martius scarlet blue. Glomerular cell counts were performed on sections stained with periodic and Schiff.

2 The contour of the glomerular basement membrane (GMB) was examined by the silver methenamine impregnation technique, which was similar to

Table 1 Incidence of MGN with respect to potential environmental hazards for both men and women (cases per 100 000 per 20 years)

	Men		Women	
	No Healthy subjects employed	No with MGN	No Healthy subjects employed	No with MGN
Group 1 High exposure occupations	82 470	26*	21 420	1*
Group 2 Low exposure occupations	703 290	23‡	939 350	14§

$\chi^2 = 189$; $p < 0.01$ *Incidence = 31.5; †incidence = 4.67; ‡incidence = 3.3; §incidence = 1.49.

||Figures obtained from 1971 census for Edinburgh subregion.

Group 1: Foundry workers, engineering workers, miners, paint manufacturing workers, industrial solvents production workers, woodworkers, seamen, chemical workers, rubber manufacturing workers, textile fibre production workers.

Group 2: Clerical workers, salespeople, labourers, agricultural workers, building labourers, students, glass makers, leatherworkers, clothiers, caterers, paper production workers, transport workers, shop workers, managers, professionals, HM Forces, housewives, unemployed, athletes.

that described by Jones in 1957¹⁷ or by 0.5 μ plastic embedded sections stained with Toluidine blue.¹⁸

3 Immunofluorescence studies for IgG, IgM, IgA, C3, and fibrin were performed on 33 cases for which material was available. Tissue was snap frozen in dry ice, and cryostat sections were fixed in 95% alcohol for five minutes. After washing in phosphate buffered saline, fluorescein labelled rabbit and antihuman antiserum (Dakopatts, Mercia-Brocades) was added. Sections were rinsed in phosphate buffered saline after 30 minutes and mounted in phosphate buffered saline and glycerol solution at pH 7.2. Examination was at a magnification of $\times 40$ to $\times 1000$ using a Leitz Incident light ultra violet microscope with HBO 200 lamp and LIP and KP500 filters and a K510 barrier filter.

4 Sixteen biopsies that were not examined by immunofluorescence were examined by an indirect immunoperoxidase technique. Sections previously fixed in formalin and embedded in wax were dewaxed through xylene and alcohol. After five minutes in Lugol's iodine sections were rinsed in sodium thio-sulphate. Acid haem was removed by rinsing in 5% hydrogen peroxide in methanol for 30 minutes. Endogenous peroxide was destroyed in 2.28% periodic acid after which sections were immersed in 0.02% potassium chlorohydrate for two minutes. Sections were rinsed in Tris-buffered saline and trypsinised for 20 minutes at 37°C in 0.1% trypsin in 0.1% calcium chloride solution at pH 7.8. Sections were rinsed again and immersed in normal swine serum and Tris-buffered saline (1/5) for 15 minutes. Antiserum at optimal dilution was added, and the excess washed away after 30 minutes. Swine antirabbit peroxidase (Dakopatts, Mercia-Brocades) 1/60 in Tris-buffered saline, at pH 7.6, was added for a further 30 minutes. After washing sections were treated with diaminobenzine tetrahydrochloride (1.0% in hydrogen peroxide and methanol) for five minutes. Sections were washed again, lightly counterstained with methyl

green, dehydrated, cleared, and mounted. Examination was at $\times 400$ and at $\times 1000$ under oil.

Electron microscopy Material was available from 103 biopsies from 85 patients.

1 Material was fixed in glutaraldehyde, then fixed with osmium and embedded in Araldite. Sections were stained with lead citrate and uranyl acetate and examined by a Corinth 275 electron microscope. General GBM morphology was observed, and particular note was made of the degree of spiking. The incidence, site, size, and character of deposits were recorded.

2 An attempt was made to determine the presence or absence of various metals in the glomerular deposits. The presence of lead, iron, mercury, and tin was sought by computerised x-ray microanalysis on an S180 electron microscope in transmission mode. Material was fixed in glutaraldehyde and then fixed with osmium but was not stained. Eighteen biopsies from seven patients with an aetiology thought to implicate exposure to metal were examined in this way and compared with other cases of membranous glomerulonephritis and with some biopsy specimens showing no observable renal lesion.

Results

CLINICAL DATA

Clinical data were available for 73 patients whose ages ranged from 9 to 75 years at onset (mean 45 years). Men composed 73% of those affected.

Fifty nine patients (81%) presented with nephrotic syndrome, and a further 11 (15%) subsequently developed the condition. Of three patients who did not have nephrotic syndrome, two remitted and one still had small amounts of protein in the urine after one year. None of these patients received treatment with steroids.

Frank haematuria occurred in two patients, but 28

patients (38%) had episodes of microhaematuria. No clinically important history of preceding infection was found. One patient had serum antibodies to hepatitis B surface antigen, but no patient had detectable titres of antigen.

STATISTICAL DATA

When patients were categorised by sex and occupation into high and low exposure groups there were significant differences in the number of cases for each group. The men in the high exposure group had a significantly higher incidence compared with patients from any other group ($p < 0.01$). The difference between the male and female low exposure groups was not significant ($p > 0.05$) (Table 1).

Treatment with steroids in this small group did not have an important effect on eventual outcome, although the number of remissions was higher than that in the untreated group (Table 2).

The total number of remissions was 17.5%, and that of partial remission, 16%, giving an overall remission rate of 33.5% (Table 2). Patients whose proteinuria had a selectivity greater than 2.0 had an increased remission rate that was significant at 5% confidence limits (Table 3).

HISTOLOGY AND ULTRASTRUCTURE

Although cellular proliferation was not an important feature of the glomerular lesion, fifteen patients showed minor mesangial or endothelial cell proliferation, or both. Patients with cellular proliferation tended to have deposits of C3 more often and a poorer outcome, but the differences were not significant.

Using silver methanamine impregnation, spiking of the GBM was seen in only 10.7% of the biopsies. Biopsy specimens showing spiking and also those shown by immunofluorescence to contain deposits of IgM tended to have a lower mean time from the onset of symptoms than those which showed neither spiking nor IgM (mean 1.9 years ν 3.3). Spikes, as well as lucent and granular regions of the GBM seen by electron microscopy, were also identified when the Araldite sections stained with Toluidine blue were examined, indicating that unlike silver spiking, plastic sections are useful for more than just early lesions of the disease.

Thirty eight biopsies (78%) were shown to have IgG present on the GBM IgM (16 biopsies, 32%), C3 (20 biopsies, 41%) was present less often. These findings agree with other reported series.³

X-RAY ANALYSIS

Results from the x-ray analysis failed to show any important deposits of heavy metals in the GBM or in the granular deposits themselves. The amount of osmium fixative detected by this analysis was uniform throughout the glomerulus and was not found to be higher in regions of GBM with granular deposits. This uniformity makes it unlikely that osmium had displaced heavy metal atoms previously present in granular deposits.

PATHOLOGICAL GRADING OF MGN

An attempt was made to grade cases according to the histopathological and ultrastructural appearance of the renal lesion.^{1,3} This was abandoned as most cases showed features of several grades, particularly when clinical relapse occurred.

Discussion

The role of organic solvents in the aetiology and pathogenesis of glomerulopathies has been documented,^{13-16,19} and improvement of glomerulonephritis after stopping exposure to solvents has also been reported.¹⁵ Association between heavy metals and MGN has been described in both animals¹¹ and man.¹² Based on the division of patients in this study into high and low exposure groups, it seems that the environmental precipitating factor for MGN may be very important.

The highest incidence of MGN was found in the male high exposure group, the difference between this group and the other being highly significant ($p < 0.01$). Finn and Harmer suggested that the difference in incidence of various renal diseases between the sexes may be at least partly determined by environment.²⁰ This agrees with our findings, as over half the excess of men fell into the high exposure group. Although this study is retrospective and allocation to exposure groups is therefore of limited accuracy, any errors introduced are likely to lessen the significance

Table 2 Effect of treatment with steroids on rate of remissions. (Figures in parentheses are numbers%)

Treatment group	Outcome		
	No remission	Partial remission	Full remission
Steroids	10 (50)	5 (25)	5 (25)
Diuretics and dietary control	32 (74)	5 (12)	6(14)
Combined groups	42 (66.5)	10 (16)	11 (17.5)

$\chi^2 = 3.7$. $p > 0.1$ for steroid treated versus untreated groups.

Table 3 Outcome according to selectivity of proteinuria

Selectivity of proteinuria	No remission	Remission
Greater than 2.0	6	5
Less than 2.0	29	2

$$\chi^2 = 8.89. p < 0.05.$$

of this difference in incidence. This is because groups such as labourers, HM Forces, and building workers, for whom some degree of exposure is likely, have been included in the low exposure group: we believed that this would represent the overall nature of this group more accurately. Failure to show metal in deposits by x-ray analysis does not necessarily conflict with the view that occupational exposure is important as processing may have removed any metal present; metal may have been present in undetectable quantities; or metals might have acted through an indirect mechanism. In a study of two cases of MGN associated with exposure to mercury Tubbs *et al* were unable to show glomerular mercury.²¹ A mechanism has been described in rats whereby metals were found to cause the release of small amounts of antigenic nuclear proteins, which led to the appearance of immune complexes on the GBM.²² Organic solvents may well act in a similar way, causing damage to the membranes and release of antigens.

Although cellular proliferation is not a major feature of MGN, cases of mesangial proliferation have been reported.²⁴⁻²⁶ The association of proliferation with C3 positivity was not significant, but a similar trend has been reported by Porth and Williams.²⁷ The number of remissions tended to be lower in the hypercellular group, and such an association between hypercellularity and outcome has already been reported by Gaffney and Panner²⁶ in a study of 41 cases of idiopathic MGN.

Treatment with steroids in our small study had no important effect on outcome, although it has been reported that steroids administered on alternate days may be beneficial, if started before deterioration of renal function.²⁸ Our full plus partial remission rate was 33%, which agrees with reported series; most series do not differentiate between full and partial remission.

The proteinuria of MGN is usually of low selectivity,²⁹ although cases of selective proteinuria have been described and may be associated with increased responsiveness to steroids in children.³⁰ A selective proteinuria indicates preferential excretion of smaller protein molecules and so may be the result of mild damage to the GBM permeability regulatory mechanism. This should be the case if hyperpermeability is the result of biochemical changes in GBM gly-

coproteins, as suggested by Misra and Berman.³¹ In this study cases of selectivity of 2 or greater and massive proteinuria (greater than 15 g/day) were recorded, thus showing that the variation in permeability is a qualitative, but not necessarily quantitative, phenomenon. Patients with a selective proteinuria throughout the course of their disease had a higher remission rate than those with proteinuria of low selectivity, regardless of the method of treatment. Regular assessment of proteinuria selectivity may therefore be a monitor of potentially permanent glomerular damage, of the continuing course of the disease, and of the eventual clinical outcome.

At least part of the sex difference in incidence of MGN is probably attributable to environmental causes. This raises the question of whether a patient in a high risk occupation should be advised to seek alternative employment when membranous glomerulonephritis has been diagnosed. The monitoring of proteinuria selectivity is advocated as an early prognostic indicator.

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