AN IMMUNOFLUORESCENCE STUDY OF RENAL LESIONS IN DIABETES MELLITUS

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ABSTRACT

Percutaneous renal biopsies were performed in 30 diabetic patients who pfesented with proteinuria. Tissues were studied for evidence of an immune lesion using immunofluorescence techniques. No consistent pattern of binding of FITC labelled polyvalent, monospecific lgG, IgA, IgM and labelled bovine insulin antisera in various diabetic renal lesions could be demonstrated. The binding of labelled insulin was observed even in biopsies of patients who had never received exogenous insulin therapy. It was therefore concluded that there is no evidence to support an immune pathogenesis in the morphogenesis of the diabetic renal lesions, nor do these lesions occur as a result of insulin therapy (JPMA 42: 34, 1992).

INTRODUCTION

The characteristic renal morphological lesions in diabetic nephropathy are well described but their pathogenesis remains controversial. A number of investigators have postulated that the majority of renal lesions are secondary to an immune mechanism[1,2]. Multiple serum proteins are demonstrable along the thickened glomerular basement membranes (GBM) by immunofluorescence techniques. Fixation of teterologous complement has, however, not been demonstrated[3]. The most consistent lesion comprises of linear deposition of IgG along the GBM, analogous to anti-GBM nephritis[1]. Other findings include deposition of various gamma globulins along capillary basement membranes[3] in the mesangium[4] and Kimmelstiel-Wilson (K.W.) nodules[2]. All investigators have failed to establish any consistent pattern of protein uptake which currently is thought to be a non-specific entrapment due to dysfunction of glomerular filtration or mesangial clearance[4]. Various other serum proteins such as complement, betalipoprotein, albumin, fibrinogen and fibrin derivatives have also been demonstrated in the hyaline arteriolar lesions, Bowman's capsule and tubular basement membrane (TBM). Exudative lesions, where the fibrin cap lesion has been shown to consistently demonstrate IgM and heterologous complement fixation are however, uniquely suggestive of a complement mediated immunological injury as their etiology[2]. The incidence of renal lesions has increased after the discovery of insulin. This could be the result of longer patient survival or of insulin itself In fact, exogenous insulin has been proposed to have a role as an antigen in immune complex deposition in diabetic renal lesions[5]. Studies showing a nonspecific binding of FITC labelled insulin, however, argue against a pathogenetic role for the hormone[1]. The present study was done to see the presence of various plasma proteins in diabetic renal lesions and to determine whether insulin is the antigen responsible for the lesions.

MATERIAL AND METHODS

Patients attending the outdoor clinic of the Diabetic Research Centre of Sir Ganga Ram Hospital, Lahore were evaluated for evidence of diabetic nephropathy. Urine samples collected routinely were tested for albuminuria using albustix (Almes). A total of 30 cases having 1 + or more albuminuria were

selected for renal biopsy. The ages of these patients ranged between 35 and 70 years; duration of diabetes ranged between 1-25 years; 14 patients were male and 16 female. The group included two insulin dependent diabetics whereas the rest were non-insulin dependent. Prior to biopsy, a battery of tests were performed to assess renal functional status including serum urea and creatinine levels, total urinary protein excretion/24 hours as well as urinary protein electrophoresis.

Morphological studies

Percutaneous renal biopsies were performed on the selected cases using disposable Tru-cut biopsy needles. A portion of the biopsy specimen was fixed in 10% formal saline and processed for paraffin sectioning. Sections were stained with H&E, PAS and methanamine silver staining techniques to study the morphological patterns of the renal lesions.

Immunofluorescent studies

A portion of the biopsy sample was utilized unfixed for preparation of frozen sections. 3-4u thick sections were fixed in 4% formal phosphate buffered saline for 2-3 min, rinsed in phosphate buffered saline (PH 7.4) and then undiluted labelled antiseras were layered over these. For this purpose FITC conjugated monoalent antiseras against human IgG, 1gM, IgA and polyvalent antiseras were obtained commercially (raised in goat or rabbit). FITC labelled insulin was also used (DAKO). The treated sections were left in a humid chamber for 30 min, washed with phosphate buffered saline for 15 min and studied by direct immunofluorescence technique on a Leitz Ortholux 11 microscope using transmitted light; BG 12 and EG 38 excitation filters and K530 and K510 barrier filters. Appropriate blocking studies with unconjugated immunoglobulins (IgG, IgM, polyvalent sera) and absorption of conjugated anti-IgG with normal human serum samples were used as control to confirm the specificity of reaction.

RESULTS

Light microscopy findings

The histological sections revealed a whole spectrum of characteristic lesions classically attributed to diabetic nephropathy. The severity of diffuse and nodular glomerulosclerosis (GS) was graded in a semi-quantitative manner according to their distribution within the glomeruli. Of 30 cases evaluated, 26 revealed the changes of diffuse GS and concomitant changes of nodular GS were observed in 13 cases (Table I).

TABLE I. Analysis of nature and severity of glomerular lesions in 30 renal biopsies in diabetic nephropathy.

Histological Features	Grades of damage				Total No. of abnormal	Percentage of total cases
	+	++	+++	++++		
Nodular glomerulosclerosis	4	5	4	-	13	43.33
Diffuse glomerulosclerosis	5	13	5	3	26	86.63
Exudative lesions	2	2	1	2	7	23.33
Capillary aneurysms	-	1	-	-	1	3.33
Capsular adhesions	6	1	2	3	12	40.00
Pericapsular fibrosis	6	3	1	1	11	36.60
Complete hyalinisation	2	1	7	2	12	40.00

Exudative lesions in the form of hyaline cap and capsular drop were noticed in seven cases (Table I).

Afferent hyaline arteriosclerosis was encountered in 26 cases and concomitant hyalinization of efferent arteriole in three cases only (Table II).

TABLE II. Analysis of nature and severity of arteriolar lesions in 30 renal biopsies.

Histological features	Grades of damage				Total No. of cases	Percentage of total cases
	+	++	+++	++++		
Hyalinisation of afferent arteriole	6	7	8	5	26	86.6
Hyalinisation of efferent arteriole	-	2	-	-	2	7.7
Sclerosis	10	2	-	-	12	60.0
Narrowing of lumen	5	5	-	-	10	33.3

Immunofluorescent findings

A total of 17 cases (56.7%) showed specific fluorescence with various antiseras. Three patients demonstrated the phenomenon of autofluorescence whereas nonspecific fluorescence was observed in ten cases when compared to controls. These latter 13 patients were therefore thought to demonstrate negative results. Using labelled polyvalent antisera, specific fluorescence was observed along the GBM in 12 cases, 10 of whom showed a linear pattern of distribution. Specific fluorescence was demonstrated in only two instances of exudative lesions and K.W. nodules each whereas the mesangium had specific binding in five cases. A similar pattern of distribution was seen along the tubular basement membrane (TBM) in 15 cases (Table III). Binding studies with labelled IgG sera showed fluorescence along the GBM in 12 patients, in exudative lesions in 7 patients, in 2 cases of K.W. nodules and mesangium respectively and along the TBM in 10 cases (Table III).

TABLE III. Analysis of specific immunofluorescence findings in 17 cases of diabetic nephropathy.

FITC labelled	Number of cases with specific immunofluorescence localised to					
	GBM		Exudative lesion	K.W. nodules	Tubular B.M.	Mesangium
	Linear	granular				
Polyvalent sera	10	2	2	2	15	5
IgG	11	1	1	2	10	2
IgA	3	-	-	1	3	-
IgM	4	-	-	-	4	-
Complement (C3)	-	-	-	-	-	-

Similarly, when treated with labelled anti-IgA, only 3 cases revealed fluorescence along the GBM, 1 demonstrated fluorescence in the K.W. nodule and 3 along the TBM. With labelled anti-IgM, specific fluorescence was observed along the GBM and tubular basement membranes in only 4 cases (Table III). Labelled bovine insulin was used in 15 of the 30 patients. Six of them demonstrated strongly

positive fluorescence and only four of these gave a history of treatment with insulin in the past (Table IV).

TABLE IV. Uptake of FITC labelled bovine insulin in 15 cases with diabetic nephropathy.

	Number of cases	Percentage of total cases	Number of cases +ve for insulin treatment
Strongly +ve fluorescence	6	40	4
Weekly +ve fluorescence	3	20	1
Nonspecific fluorescence	6	40	4

Weakly positive fluorescence was observed in 3 cases and nonspecific fluorescence in 6 cases. One in the former and 4 in the latter group were on insulin. None of the cases showed heterologous complement fixation.

DISCUSSION

Many investigators have shown the presence of various plasma proteins in the renal lesions associated with diabetes mellitus. Currently, these plasma proteins are best demonstrated by immunofiuorescence techniques. In the present study, three patients exhibited the phenomenon of autofluorescence, i.e., fluorescence was noted when the sections were examined after washing with buffered saline (and without the application of any FITC conjugate). This phenomenon has previously been reported and is regarded as an important confounding variable in the interpretation of specific fluorescence studies. Predictably, one-third of our patients also revealed diffuse, nonspecific fluorescence. Thus, specific fluorescence was seen in only 56.6% cases. Seven cases demonstrated positive fluorescence for gamma globulins in the mesangial area. Light microscopic examination in these cases revealed a diffuse mesangial expansion of advanced degree. These findings raise two important questions; Is the mesangial expansion and sclerosis primarily responsible for the accumulation of macromolecules in this area or is this a structural change secondary to some mesangial dysfunction and consequent macromolecular accumulation. Various studies point towards both processes playing a role. Ithas been suggestedthatbiochemicalalterations in the GBM with resulting increased permeability results in deposition of immune complexes or nonimmune aggregates in the mesangium[6,7]. This could act as a stimulus for mesangial matrix production. Consequent sclerotic changes in late stages would then cause a functional impairment of normal mesangial clearing leading to entrapment of macromolecules. Reflux of these macromolecules could even lead to their accumulation in the sub-endothelial space and glomerular basement membrane[3]. Deposition of gamma globulins along the GBM has been demonstrated by various investigators[1]. We were able to define a linear distribution of IgG in 64.7% cases, whereas a granular pattern was observed in only one case. Anti-IgA studies showed linear distribution along the GBM in 17% cases, which is similar to the 20% reported in other studies[1]. This thin linear staining along the GBM resembles the pattern observed in anti-GBM nephritis. Elution

studies in such cases, however, are reported to give negative results[1] suggesting a nonspecific trapping of globulins. It is proposed that the globulin molecules are bound to or get incorporated into an unknown structural unit of biochemically altered BM and thus assume a linear configuration[8]. The presence of gamma globulins in K.W. nodules as demonstrated by labelled anti-gamma globulin binding was seen in 29.4% cases. Specificity of binding was confirmed by using control sections treated with unlabelled anti-gamma globulins prior to application of labelled globulins. These findings suggest an immune mechanism to account for the presence of antibody in the nodules[1,9]. Other investigators, l3owever, either could not demonstrate binding of heterologous complement at this site[1] or obtained negative results with gamma globulins and complement. Thus the possibility of an immune pathogenesis remains unproved[3]. In our series exudative lesions demonstrated positive fluorescence with gamma globulins in only three cases but no fixation of complement could be demonstrated. This is in accordance with the studies which suggest trapping of simple filtration[10]. Thus the etiologic role of immune proteins in the vessel wall by complex deposition in diabetic nephropathy, as proposed by previous observers is unsupported. Diabetic nephropathy leading to renal failure has become more common since the introduction of insulin. This increased renal morbidity has been explained on the basis of increased longevity of diabetics following the advent of insulin therapy. An immunological mechanism has however been suggested for the renal disease with insulin acting as an antigen. The data initially supported the hypothesis that GS could be due to deposition of complexes of insulin and its antibody[5,11,12]. Later studies, however, could not demonstrate any insulin or insulin antibody binding to basement membrane'. Our study demonstrated a positive fluorescence with labelled insulin in 60% cases and of these only 33.3% gave a history of treatment with insulin. Fluorescence was noticed in the mesangial area, tubular basement and nodular lesions. No significant similarity between the distribution of insulin and gamma globulins could be demonstrated2. The demonstration of a positive reaction with insulin in a significant number of patients who did not give any history of treatment with insulin suggests that heterologous insulin cannot be held responsible for the formation of immune complexes. Moreover, circulating anti-insulin antibody can be demonstrated in a majority of the patients receiving insulin whereas only a small minority develops glomerulosclerosis. The possibility of endogenous insulin playing such a role, however, cannot be ruled out.

REFERENCES

1. Westberg, N.G. and Michael, A.F. Immunohistopathology of diabetic glomerulosclerosis. Diabetes, 1972; 21:163.
2. Burkholder, P.M. Immunohiatopathologic atudy of localised plasma proteins ant fixation of guinea-pig complement in renal lesions of diabetic glomeruloacleroaia Diabetes, 1965; 14:755.
3. Ainsworth, S.K., Hirsch, H.Z., Bracketc, NC. Jr., Brissie, R.M., Williams, A.V. Jr. ant Hennigar, OR. Diabetic glomerulonephropsthy; bistopashologic, immunofluorescen and ultraatructural studies of 16 cases. Hum. Pathol., 1982; 13:470,
4. Mauer, SM., Steffes, M.W., Cbern, M. and Brown, D. Messngial uptakesnd processini of macromolecules in rstswith disbetes mellisus. Lab. Invest., 1979; 41:401.
5. Bern, LW., Owens, C.T., Hints, Y. and Blumenthal, H.T.The pathogenesis of diabetic glomerulosclerosis II. A demonstration of insulin-binging cspscity of various his topsthological components of the disease by fluorescence microscopy. Diabetes, 1962:11:308.
6. Brenner, B.M., Hossetter, H.T snd Humes, H.D. Molecular basis of proteinuria o glomerular origin. N. Engl. 3. Med., 1978; 298:826.
7. Couser, W.O., Hoyer, Jr., Sulmant, M.M., Jermanovich, N.E. and Belok, S. The effecto sminoneucleoside nephrosis on immune complex locslisstion in sutologous immun complex

nephropsthy in rats.). Clin. Invest., 1978; 61:561.
8. Shsrms, H.M., Yum, M.N. and Kleit, S. Acuteglomerulonephritiswith diabetes mellituc Arch. Pathol., 1974; 97:152.
9. Freedman, P., Peters, J.H. and Kark, R.M. Localization of gamms- globulin in th diseased kidney. Arch. Intern. Med., 1960; 105:524.
10. Dsvies, M.J., Woolf, N. and Csrstairs, ICC. lmmunohistochemicsl studies in disbeti glomeruloscierosis. 3. Pathol., 1966; 92:44L
11. Blumenthal, H.T., Berns, LW., Owens, CT. and Hirsta, Y. The psthogenesisofdisbeti glomerulosclerosis. I. The significance of various histopathological components of th disease. Diabetes, 1962; 11:296.
12. Farrant, P.C and Shedden, WI. Observssions on the uptake of insulin conjugatedwit fluorescein isothiocysnase by diabetic kidney tissue. Diabetes, 1965; 14:274.