J. clin. Path., 1972, **25**, 789-792

Serum protein levels in primary biliary cirrhosis

R. N. M. MacSWEEN, C. H. W. HORNE, ANNE J. MOFFAT, AND HAZEL M. HUGHES

From the University Departments of Pathology, Western and Royal Infirmaries, and the Centre for Rheumatic Diseases, Baird Street, Glasgow

SYNOPSIS Serum levels of albumin, transferrin, α_2-macroglobulin, β_1C/β_1A, IgA, IgG, and IgM have been determined in 73 patients with primary biliary cirrhosis and in age- and sex-matched controls. A highly significant fall in albumin was demonstrated, and there were highly significant increases in α_2-macroglobulin and all three immunoglobulin levels. Transferrin and $\beta_1/C\beta_1A$ levels were unchanged. No significant correlations were found between the titre of antimitochondrial antibody, the duration of symptoms, and any of the serum proteins estimated. A highly significant positive correlation was present between serum albumin and transferrin levels in both patient and control groups.

The presence of non-organ-specific antimitochondrial antibodies has been demonstrated in the serum of the majority of patients with primary biliary cirrhosis (Walker, Doniach, Roitt, and Sherlock, 1965; Doniach, Roitt, Walker, and Sherlock 1966; Goudie, MacSween, and Goldberg, 1966b; Paronetto, Schaffner, and Popper, 1967). Raised immunoglobulin levels, in particular of IgM, are found in these patients (Paronetto, Schaffner, and Popper, 1964; McKelvey and Fahey, 1965; Hobbs, 1967; Feizi, 1968), but no correlation has been demonstrated between the immunoglobulin levels and the presence of antimitochondrial antibodies, nor do either of these immunological parameters correlate with duration of symptoms, degree of jaundice, serum alkaline phosphatase level, or extent of the histological changes typically seen in liver biopsy material (Doniach *et al*, 1966; Feizi, 1968; Hadziyannis, Scheuer, Feizi, Naccarato, Doniach, and Sherlock, 1970). However, there do not appear to be any studies in which an attempt has been made to correlate antimitochondrial antibody titre and duration of symptoms with the levels of those serum proteins known to be produced in the liver.

In the present study the serum levels of albumin, transferrin, α_2-macroglobulin, β_1C/β_1A, and the immunoglobulins A, G, and M have been measured in a group of 73 patients with primary biliary cirrhosis, and in age- and sex-matched controls. Correlations between the serum protein levels, antimitochondrial antibody titre, and duration of symptoms have been sought.

Received for publication 31 May 1972.

Materials and Methods

PATIENTS AND CONTROLS

Specimens of serum sent to the regional diagnostic immunopathology laboratory over the period 1967-70 inclusive were available from 67 female and six male patients with a diagnosis of primary biliary cirrhosis. In all patients the clinical, biochemical, and serological studies were consistent with this diagnosis (Goudie *et al*, 1966; Scheuer, 1967; Sherlock, 1971) and confirmatory histological evidence from liver biopsy material was available from 27 of these cases. The mean age was 57·8 ± 10·4 years, with a range of 41 to 79.

Serum from age- and sex-matched control patients had been similarly referred to the diagnostic laboratory, and in matching a test and a control serum care was taken to ensure that the pair had been stored at −20°C for a similar period ± two months. The clinical diagnoses in the control group are detailed in Table I. In none of the control sera had any auto-antibodies been demonstrated, and diseases with a recognized immunological disturbance were specifically excluded.

SEROLOGY

Protein estimations

These were carried out using a radial immunodiffusion technique (Mancini, Carbonara, and Heremans, 1965; Fahey and McKelvey, 1966). Specific antisera to transferrin, α_2-macroglobulin, IgA, and IgM were prepared by the method of Goudie, Horne, and Wilkinson (1966a). Rabbit

Diagnosis	*No.*
Non-specific joint disease—osteoarthritis, psoriatic arthropathy	31
Non-toxic goitre	21
Miscellaneous skin diseases	5
Neurological and/or muscular disorders	5
Diabetes mellitus	4
Miscellaneous group	7
Total	73

Table I *Clinical diagnosis in age- and sex-matched controls*

antisera to albumin and IgG were prepared using purified human serum albumin (Behringwerke), and IgG eluted from diethylaminoethyl cellulose columns with 0·01 M sodium phosphate buffer *p*H 8·0. Antiserum to β_1C/β_1A was raised in rabbits using zymosan-complement complexes as described by Mardiney and Müller-Eberhard (1965).

The effects of interplate variation (Thompson, Horne, Steele, and Goudie, 1969) were minimized by testing in duplicate each test serum and its control on the same assay plate. The 'absolute values' of the serum proteins were determined from calibration curve unit solutions of a freeze-dried reconstituted pooled human serum containing 3, 6, 12, and 18 g protein per 100 ml, and standardized with reference to a serum (Behringwerke) containing a specified amount of the particular protein.

Antimitochondrial antibody

Antimitochondrial antibodies were demonstrated as described by Goudie *et al* (1966a) using commercially available fluorescein-conjugated rabbit antihuman immunoglobulin (Fraburg Ltd).

Student's t test was used for statistical analysis.

Results

The mean serum protein levels in the primary biliary

Protein	*Test*	*Control*	*P*
Albumin	3043 ± 936	4240 ± 1086	< 0·0005
Transferrin	252 ± 99	264 ± 97	NS
α_2-macro-globulin	225 ± 74	194 ± 70	< 0·0025
β_1C/β_1A	101 ± 38	100 ± 33	NS
IgM	258 ± 98	94 ± 75	< 0·0005
IgG	2106 ± 671	1337 ± 564	< 0·0005
IgA	294 ± 143	223 ± 127	< 0·0005

Table II *Serum protein levels in 73 patients with primary biliary cirrhosis and in age- and sex-matched controls*[1]

[1]Mean ± SD in mg/100 ml

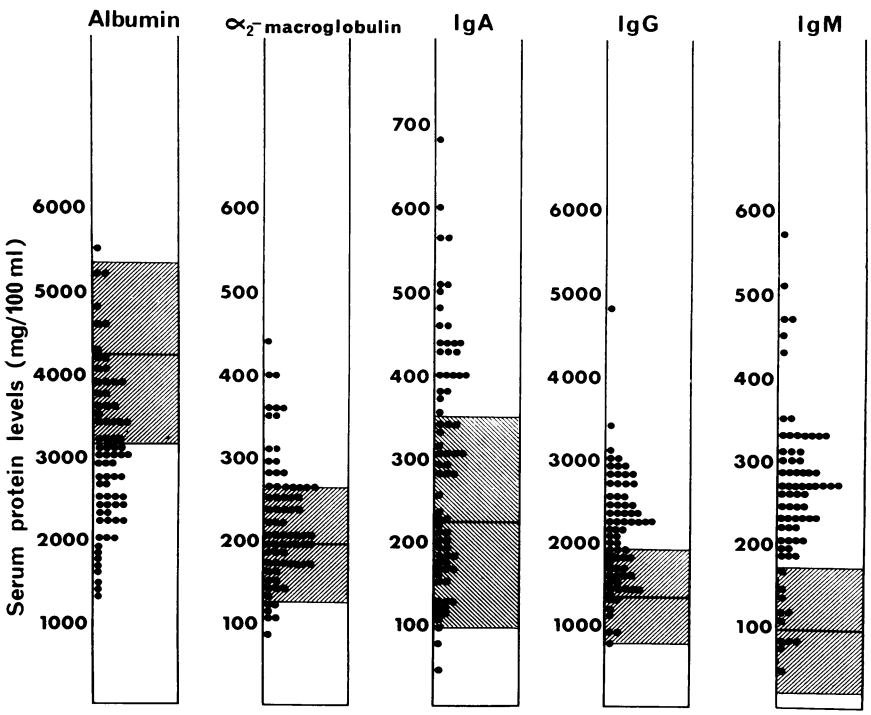

Fig. *Serum albumin, α_2-macroglobulin, IgA, IgG, and IgM in primary biliary cirrhosis patients: shaded area shows control series (mean ± 1 SD).*

cirrhosis patients and the control group are given in Table II. Statistically significant increases in α_2-macroglobulin (16%), IgM (174%), IgG (58%), and IgA (32%) were found in primary biliary cirrhosis, and in addition there was a significant fall (28%) in mean serum albumin.

A serum protein level more than 1 standard deviation (SD) above the mean for the control population was found in 32% of the patients and 18% of the controls for α_2-macroglobulin, in 82 and 5% for IgM, 56 and 7% for IgG, and 36 and 9% for IgA. In 59% the level of serum albumin was less than 1 SD below the mean for the controls, the corresponding figure for the controls being 16%. The distribution of the individual protein levels for the patients is shown in the Figure.

No significant correlations were found between the duration of clinical symptoms and the level of any of the serum proteins estimated. Antimitochondrial antibodies were present in all patients, the mean titre being 1/512, with a range of 1/32 to 1/2048. The antibody titre showed no significant correlation either with the level of any of the serum proteins estimated or with the duration of clinical disease.

Discussion

Four proteins known to be synthesized in the liver, namely, albumin, transferrin, α_2-macroglobulin, and the third component of complement (β_1C/β_1A), have been measured in a series of 73 patients with primary biliary cirrhosis. The serum levels of these proteins showed no statistically significant correlation with the duration of clinical disease or with the titre of antimitochondrial antibody.

Albumin showed a significant fall in these patients as compared with controls, a finding which is not surprising in that it is well known that hypoalbuminaemia is a feature of most acute and chronic liver diseases. The transferrin levels were unremarkable. However, as we have previously reported (Horne and MacSween, 1971), a highly significant correlation was observed between the albumin and transferrin levels, both in the patient and in the control group.

The serum α_2-macroglobulin showed a significant elevation in the primary biliary cirrhosis group. A significant increase in this protein has previously been reported in patients with haemochromatosis (Amin, Clarke, Freeman, Murray-Lyon, Smith, and Williams, 1970), and in the discussion of their results these authors predicted that in other diseases with primary liver damage similar high concentrations would be found. The present study supports their surmise, but does not indicate whether the elevated serum levels reflect increased synthesis or decreased catabolism. The physiological role of α_2-macroglobulin has not been defined and so the significance of the observed increase in serum levels in primary liver disease is obscure.

Amin *et al* (1970) found a significant increase in β_1C/β_1A levels in their patients with haemochromatosis. More recently Fox, Dudley, and Sherlock (1971) found normal concentrations of β_1C/β_1A in the majority of 150 patients with chronic liver disease, and in particular, normal levels were found in 30 patients with primary biliary cirrhosis, an observation consistent with our present results. On the basis of the high incidence of autoantibodies in primary biliary cirrhosis (Walker *et al*, 1965; Goudie *et al*, 1966b; Doniach *et al*, 1966), it has been suggested that disturbed immunity is of aetiological significance in this disease. As distinct from the findings of other workers (Wright, McCollum, and Klatskin, 1969; Fox, Niazi, and Sherlock, 1969; Kaplan and Grady, 1971), Krohn, Finlayson, Jokelainen, Anderson, and Prince (1970) found evidence of Australia (Au) antigen and antibody in 11 of their 12 patients with primary biliary cirrhosis, and it would seem possible that Au antigen/antibody complex formation within the liver might result in hepatic damage. Antibody to Au antigen can fix complement (Shulman and Barker, 1969), and if such complexes were of significance in the pathogenesis of primary biliary cirrhosis then evidence of hypocomplementaemia might be expected.

All three immunoglobulins measured showed significant elevation in primary biliary cirrhosis. The 174% mean elevation in IgM level is particularly striking and is in keeping with the previous reports of Hobbs (1967), Feizi (1968), and of Hadziyannis *et al* (1970). In the present series an elevated IgM level was present in 60 patients (82%) which compares with an incidence in Feizi's series of 12 of 16 (75%) and in Hadziyannis' series of 14 of 20 (70%). Increased levels of IgA and IgG were also observed by Feizi (1968) and by Hadziyannis *et al* (1970). No significant correlations were established between individual immunoglobulin levels, duration of disease, and titre of antimitochondrial antibody, and this is in agreement with previous reports (Doniach *et al*, 1966; Feizi, 1968; Hadziyannis *et al*, 1970). Examination of those patients with the highest levels of IgM, IgG, and IgA, ie, more than 2 standard deviations above the mean for the control group, did not show any difference in their titre of antimitochondrial antibody as compared with the whole series. Hadziyannis *et al* (1970) observed that their three patients with raised IgA had high antimitochondrial antibody titres.

This work was supported by the research funds of Glasgow University, and one of us (C.H.W.H.) is in receipt of a research grant from the Secretary of State for Scotland.

References

Amin, A. H., Clarke, H. G., Freeman, T., Murray-Lyon, I. M., Smith, P. M., and Williams, R. (1970). Studies by quantitative immunoelectrophoresis on iron binding proteins in haemochromatosis. *Clin. Sci.*, **38**, 613-616.

Doniach, D., Roitt, I. M., Walker, J. G., and Sherlock, S. (1966). Tissue antibodies in primary biliary cirrhosis, active chronic (lupoid) hepatitis, cryptogenic cirrhosis and other liver diseases and their clinical implications. *Clin. exp. Immunol.*, **1**, 237-262.

Fahey, J. L., and McKelvey, E. M. (1965). Quantitative determination of serum immunoglobulins in antibody-agar plates. *J. Immunol.*, **94**, 84-90.

Feizi, T. (1968). Immunoglobulins in chronic liver disease. *Gut*, **9**, 193-198.

Fox, R. A., Dudley, F. J., and Sherlock, S. (1971). The serum concentration of the third component of complement β_1C/β_1A in liver disease. *Gut*, **12**, 574-578.

Fox, R. A., Niazi, S. P., and Sherlock, S. (1969). Hepatitis-associated antigen in chronic liver disease. *Lancet*, **2**, 609-612.

Goudie, R. B., Horne, C. H. W., and Wilkinson, P. C. (1966a). A simple method for producing antibody specific to a single selected diffusible antigen. *Lancet*, **2**, 1224-1226.

Goudie, R. B., MacSween, R. N. M., and Goldberg, D. M. (1966b). Serological and histological diagnosis of primary biliary cirrhosis. *J. clin. Path.*, **19**, 527-538.

Hadziyannis, S., Scheuer, P. J., Feizi, T., Naccarato, R., Doniach, D., and Sherlock, S. (1970). Immunological and histological studies in primary biliary cirrhosis. *J. clin. Path.*, **23**, 95-98.

Hobbs, J. R. (1967). Serum proteins in liver disease. *Proc. roy. Soc. Med.*, **60**, 1250-1254.

Horne, C. H. W., and MacSween, R. N. M. (1971). Serum albumin and transferrin levels. (Letter.) *Lancet*, **2**, 1150.

Kaplan, M. M., and Grady, G. (1971). Serum-hepatitis antigen in chronic hepatitis and primary biliary cirrhosis. *Lancet*, **1**, 159-161.

Krohn, K., Finlayson, N. D. C., Jokelainen, P. T., Anderson, K. E., and Prince, A. M. (1970). Electron microscopical and immunological observations on the serum-hepatitis (S.H.) antigen in primary biliary cirrhosis. *Lancet*, **2**, 379-383.

Mancini, G., Carbonara, A. O., and Heremans, J. F. (1965). Immunochemical quantitation of antigens by single radial immunodiffusion. *Immunochemistry*, **2**, 235-254.

Mardiney, M. R., Jr., and Müller-Eberhard, H. J. (1965). Mouse β_1C-globulin: production of antiserum and characterization in the complement reaction. *J. Immunol.*, **94**, 877-882.

McKelvey, E. M., and Fahey, J. L. (1965). Immunoglobulin changes in disease: quantitation on the basis of heavy polypeptide chains, IgG (γG), IgA (γA) and IgM (γM) and of light polypeptide chains, type K (I) and type L (II). *J. clin. Invest.*, **44**, 1778-1887.

Paronetto, F., Schaffner, F., and Popper, H. (1964). Immunocytochemical and serologic observations in primary biliary cirrhosis. *New Engl. J. Med.*, **271**, 1123-1128.

Paronetto, F., Schaffner, F., and Popper, H. (1967). Antibodies to cytoplasmic antigens in primary biliary cirrhosis and chronic active hepatitis. *J. Lab. clin. Med.*, **69**, 979-988.

Scheuer, P. J. (1967). Primary biliary cirrhosis. *Proc. roy. Soc. Med.* **60**, 1257-1260.

Sherlock, S. (1971). *Diseases of the Liver and Biliary System*, 4th ed. (revised 3rd printing). Blackwell, Oxford.

Shulman, N. R., and Barker, L. F. (1969). Virus-like antigen, antibody, and antigen-antibody complexes in hepatitis measured by complement fixation. *Science*, **165**, 304-306.

Thompson, A., Horne, C. H. W., Steele, H., and Goudie, R. B. (1969). Concentration of serum α_2-macroglobulin in twins. *Nature (Lond.)*, **221**, 289-290.

Walker, J. G., Doniach, D., Roitt, I. M., and Sherlock, S. (1965). Serological tests in diagnosis of primary biliary cirrhosis. *Lancet*, **1**, 827-831.

Wright, R., McCollum, R. W., and Klatskin, G. (1969). Australia antigen in acute and chronic liver disease. *Lancet*, **2**, 117-121.