Recurrent bacteriuria and primary biliary cirrhosis: ABO blood group, P₁ blood group, and secretor status

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SUMMARY Patients with primary biliary cirrhosis have an abnormally high incidence of urinary tract infection (35%). Susceptibility to urinary infection and other infectious diseases has been linked with certain blood group antigens and secretor status. We have therefore studied these characteristics in patients with primary biliary cirrhosis. We were unable to show any abnormal distribution in blood groups or secretor status in patients with primary biliary cirrhosis (compared with a normal population) which might reflect their predisposition to urinary infection. The distribution of blood groups and secretor status in patients with primary biliary cirrhosis with a history of urinary infections was not significantly different from patients without such a history.

Escherichia coli strains isolated from patients with primary biliary cirrhosis did not bind in any greater numbers to the uroepithelial cells of primary biliary cirrhosis patients than to the cells of a normal healthy control.

We therefore conclude that blood group distribution, abnormal secretor status, and epithelial cell type are not important factors in the predisposition of primary biliary cirrhosis patients to urinary infections.

We have recently shown that women with primary biliary cirrhosis have a high prevalence (19%) and incidence (35%) of significant bacteriuria compared with women with other types of chronic liver disease (7%) or rheumatoid arthritis (8%).¹ The prevalence and incidence are also significantly higher than those in age matched normal healthy women.² Factors which predispose patients with primary biliary cirrhosis (who are predominantly female) to recurrent bacteriuria are as yet unknown.

A relation between ABO blood group or secretor status and susceptibility to certain infectious diseases has been previously documented.³⁻⁵ Other workers^{6,7} have shown that women of blood groups B and AB who are non-secretors of blood group substances have a significantly higher risk of developing urinary tract infection compared with women of other blood groups (or with those who are secretors).

It has recently been suggested⁸ that individuals who do not possess the P₁ blood group run a lower

risk of suffering recurrent urinary tract infections than P₁ positive individuals. Some strains of *Escherichia coli* possess pili with a chemical and structural configuration similar to that of the P₁ antigen (a glycosphingolipid). This is expressed on the surfaces of epithelial cells (as well as erythrocytes) of P₁ positive individuals.⁹ *E coli* strains with P₁-like pili composition can bind more effectively to the epithelial cells of the urinary tract of P₁ positive individuals and hence an infection is more readily established.

Although predisposing factors to bacteriuria in patients with primary biliary cirrhosis are likely to be multifactorial, we considered it worthwhile to determine the distributions of ABO and P₁ groups and the secretor status in these patients and to study the binding of *E coli* strains to their uroepithelial cells.

Patients and methods

PATIENTS

ABO blood group, P₁ blood group, and secretor

status were determined in 110 Caucasian patients (99 female, 11 male) with primary biliary cirrhosis attending the outpatient department at the Royal Free Hospital, London. Patients were aged between 33 and 80 (mean age 57). Forty nine of these patients had one or more previously documented (between November 1980 and December 1983) urinary tract infections. ABO blood grouping and details of urinary tract infections of a further 75 Caucasian patients with primary biliary cirrhosis (including 46 deceased patients) were obtained from patient records.

Uroepithelial cells were obtained from eight women with primary biliary cirrhosis. All patients were diagnosed using standard criteria described elsewhere in detail.¹⁰

CONTROL GROUPS

The ABO blood groups from 185 women with chronic active hepatitis and alcoholic liver disease attending the Royal Free Hospital were used as controls. Further control data for distribution of blood groups and secretor status were obtained from published work.¹¹

BLOOD GROUPING

ABO blood grouping was performed using conventional serological techniques on microtitre plates. Suspensions of patients' cells (3%) were tested against anti-A, anti-B, anti-A + B, and reverse grouping using patients' sera against 3% A1, A2, B, and O cells. The patients' own cells and sera were used as controls.

P₁ grouping was performed using two sources of potent anti-P₁ tested in precipitin tubes and read microscopically. Known P₁ weak positives were used as a positive control and P₁ negatives as a negative control.

Secretor status was determined by a well documented method,¹² which entails diluting saliva one in two with isotonic saline after boiling to remove enzyme activity. Saliva was added to dilutions of anti-H lectin (*Ulex europeus*), and inhibition was compared with a saline control using washed group O cells as an indicator. Anti-A and anti-B were also used in inhibition tests where appropriate using washed A2 and B cells as indicators. No weak or intermediate secretors were noted.

ADHESION TEST

The ability of strains of *E coli* to adhere to the uroepithelial cells of patients with primary biliary cirrhosis was investigated using a simple modification of the method of Svanborg Eden.¹³ Strains of *E coli* isolated from the infected urines of patients with primary biliary cirrhosis were subcultured twice

for 18 h in peptone water, harvested by centrifugation, and resuspended in phosphate buffered saline (PBS), pH 7.3, to a concentration of 10⁸ organisms/ml. Uroepithelial cells were obtained from early morning specimens of urine from eight patients with primary biliary cirrhosis and one healthy female control, washed twice in PBS, and adjusted in a counting chamber to 10⁵ cells/ml.

One millilitre of bacterial suspension and 1 ml of uroepithelial cells were incubated at 37°C for 1 h in an orbital incubator (60 rpm). Epithelial cells were recovered by spinning at low speeds and unattached bacteria removed by two washes in PBS. The drop of epithelial cell suspension remaining was air dried on a glass slide, heat fixed, and stained by Gram's method. This was preferable to counting unstained cells and bacteria used by other workers¹³ because stained adherent bacteria were easier to see. A control slide with no attached test bacteria was stained at the same time. The number of bacteria adhering to 40 cells was counted using a magnification of × 100.

STATISTICAL ANALYSIS

The distributions of ABO blood groups, secretor status, and P₁ groups in the patients with urinary tract infections were compared with those in the comparison population using the χ^2 test.

Results

ABO GROUP AND SECRETOR STATUS

The distribution of ABO blood groups in 185 patients with primary biliary cirrhosis (Table 1) was not significantly different from the distribution in a normal population or 185 patients with liver disease who were matched for sex and age.

There was no significant difference in the ratio of infected to non-infected patients within the group distribution in both the total group of 185 patients and in the prospective group of only 110 patients. Secretor status in the 110 patients tested also showed no significant disturbance in the ratio of infected to non-infected patients (Table 2).

The incidence of urinary infection in primary biliary cirrhosis patients with blood groups B and AB and non-secretor status was the same as the incidence of infection in the primary biliary cirrhosis group taken as a whole.

P₁ STATUS

Of the 115 patients tested, 82% were P₁ positive, which is similar to the figure of 79% found in the UK population.¹¹ There was no significant difference in the ratio of infected to non-infected patients within this group (Table 3).

Table 1 Distributions of blood groups in patients with primary biliary cirrhosis and two control groups

Group	No of subjects	Blood group		
		O	A	B and AB
Control group 1*	3459	1503 (43.4%)	1546 (44.7%)	410 (11.9%)
Control group 2†	185	76 (41.1%)	81 (43.8%)	28 (15.1%)
Primary biliary cirrhosis group	185	75 (40.5%)	90 (48.6%)	20 (10.8%)
Infected	84	37 (44.0%)	37 (44.0%)	10 (12.0%)
Men	3	1	0	2
Women	81	36	37	8
Non-infected	101	38 (37.6%)	53 (52.5%)	10 (9.9%)
Men	13	5	7	1
Women	88	33	46	9

*United Kingdom controls (Race and Sanger¹¹).

†Patient controls with liver diseases other than primary biliary cirrhosis matched for sex and age.

Table 2 Secretor status in patients with primary biliary cirrhosis

Group	No of subjects	Secretors	Non-secretors
Primary biliary cirrhosis group	110	74 (67.2%)	36 (32.7%)
Infected	49	35 (71.4%)	14 (28.6%)
Men	2	2	0
Women	47	33	14
Non-infected	61	39 (63.9%)	22 (36.1%)
Men	10	5	5
Women	51	34	17
Control group 1*	1118	864 (77.3%)	254 (22.7%)

*United Kingdom controls (Race and Sanger¹¹).Table 3 P_i status in patients with primary biliary cirrhosis

Group	No of subjects	P_i positive	P_i negative
Primary biliary cirrhosis group	115	96 (83.4%)	19 (16.5%)
Infected	49	42 (85.7%)	7 (14.3%)
Men	2	2	0
Women	47	40	7
Non-infected	66	54 (81.8%)	12 (18.2%)
Men	10	8	2
Women	56	46	10
Control group 1*	612	487 (79.5%)	125 (20.5%)

*United Kingdom controls (Race and Sanger¹¹).

Table 4 Adhesion to uroepithelial cells of primary biliary cirrhosis patients

Patient	Previous urinary tract infections	Blood group	Secretor status	Mean no bacteria/cell Average of 7 strains
1	Yes	B	Secretor	15
2	Yes	A	NT	4
3	Yes	A	Non-secretor	18
4	Yes	O	NT	1
5	No	O	Secretor	4
6	No	A	Non-secretor	10
7	No	A	Secretor	36
8	No	AB	Non-secretor	85
Healthy control	No	A	Non-secretor	15

NT = Not tested

ADHESION

Based on the findings of eight patients with primary biliary cirrhosis and one healthy control, strains of *E coli* did not seem to adhere in any greater numbers

to the uroepithelial cells of primary biliary cirrhosis patients than to those obtained from a healthy control with no history of urinary tract infection (Table 4).

Discussion

Primary biliary cirrhosis is a chronic progressive liver disease of unknown cause, characterised by destruction of the intrahepatic bile ducts, which results in chronic cholestasis, liver fibrosis, and ultimately cirrhosis.¹⁴ The prevalence of diagnosed primary biliary cirrhosis is only about 50 per million population,¹⁵ although there may be many more undiagnosed cases. Primary biliary cirrhosis remains the most common cause of cirrhosis in women. The Royal Free Hospital is a major national referral centre and has therefore collected a large group of patients with this disorder. We have found that these patients are particularly susceptible to recurrent urinary tract infections.¹ Factors associated with the high incidence in patients with primary biliary cirrhosis (35%) are as yet unclear. Previous studies indicate that women with B or AB and P₁ blood groups who are non-secretors are more at risk of repeated urinary tract infections than the rest of the female population.⁶⁻⁹ We have shown that the high prevalence of urinary tract infection in patients with primary biliary cirrhosis (45% in the group studied) is not associated with AB or B blood group, P₁ blood group, non-secretor status, or epithelial cell type. In view of the fact that the Royal Free Hospital is a national referral centre for patients with primary biliary cirrhosis, and patients with other liver diseases, it seems unlikely that racial stratification will occur in either group. Hence our blood grouping results will reflect the national figures, and test and control groups will be similar. Notwithstanding the diagnosis of primary biliary cirrhosis, our results also cast doubt on the suggestion that blood group factors are important predeterminants to urinary tract infection in otherwise normal healthy women. The P₁ status has, however, previously been studied only in otherwise healthy children developing acute pyelonephritis.⁸

Some workers have noted that adhesion of *E coli* to the epithelial cells from patients with recurrent infections is greater than to cells from individuals with no such history,¹⁶ which has in turn been linked with the possession of P₁ blood group.⁸ With our limited number of tests we have been unable to extend this finding to patients with primary biliary cirrhosis. Furthermore, Harber *et al*¹⁷ have doubted the importance of adhesion in general as being a decisive factor in the pathogenesis of urinary tract infection.

We have found that the adhesion test is itself inherently difficult to interpret (unpublished observations) and we believe that until a more satisfactory and reproducible test is developed, it will be difficult to assess the importance of adhesion in the initiation

of urinary tract infections.

Patients with primary biliary cirrhosis suffer from "dry gland" syndrome (dry mucosal surfaces, including vagina and urethra,¹⁷) but whether this has any bearing on their predisposition to urinary infections is as yet unclear. Further investigations are required to determine which factors are important in causing the high incidence of urinary infections in patients with primary biliary cirrhosis, since experimental results to date have failed to provide a satisfactory explanation.

IR is in receipt of a grant from the North East Thames Regional Health Authority.

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