Frequency and Role of Hepatitis-C Virus and Type II Cryoglobulinemia in Membranoproliferative Glomerulonephritis

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Abstract
Background: Many studies have claimed a major role of chronic hepatitis-C virus (HCV) infection in immune-mediated diseases such as membranoproliferative glomerulonephritis (MPGN). Chronic HCV infection is also known to produce essential mixed cryoglobulinemia (EMC), which in turn may manifest as vasculitis and cryoglobulinemic MPGN.

Objective: The aim of the study therefore, was to determine frequency of association and pathogenetic role of HCV infection as well as that of EMC in MPGN patients.

Methods: Fifty-three adult patients of MPGN were studied for HCV, HBsAg, EMC, C3, anti-nuclear antibody (ANA), rheumatoid factor serologically. Histopathology, immunofluorescence (IF) were conducted in all patients and electron microscopy (EM) in those who were found HCV positive. Simultaneously, 37 follow-up patients of HCV associated chronic hepatitis were investigated for EMC, renal functions and urinalysis done for evidence of glomerulonephritis (GN).

Results: Thirteen percent MPGN patients were HCV positive, however, no viral particle could be seen in electron microscopy in glomeruli of these patients. There was no serologic evidence of HCV induced immune complex GN. None of the MPGN patients showed cryoglobulinaemia. Similarly none from HCV associated chronic hepatitis group had EMC nor showed evidences of glumerulonephritis.

Conclusion: Thirteen percent of adult MPGN patients in north India were seropositive for HCV, indicating significant association. However, clear evidence in favour of its pathogenetic role was lacking in our study. Secondly, this study reveals that MPGN is non-cryoglobulinemic and HCV is not a major cause in our population compared to what is reported from other countries. These observations need confirmation by a larger study.

INTRODUCTION
Association of chronic hepatitis C virus infection with immune mediated diseases was first suggested in 1990 when Pascual reported two patients with chronic HCV infection and EMC.1 Since then a number of such syndromes have been reported. However, more frequently and closely associated manifestations with HCV are MPGN and EMC.2,3 It is not clear if HCV with or without EMC plays any role in causation of MPGN, although in higher percentage of patients, HCV RNA and antibodies have been detected in cryoglobulin deposits.3,5 Furthermore electron microscopy has revealed features of cryoglobulins in sub-endothelial granular, finely fibrillar deposits or immunotactoid features.3

Importance of identifying HCV as a causative agent of MPGN lies in treatment of the disease by interferon alpha. Besides, there is limited information on frequency of HCV associated GN and EMC in South East Asia. We, therefore, decided to study the association between HCV and cryoglobulinemia in patients of MPGN and that between MPGN and EMC in HCV induced chronic hepatitis in adults.

MATERIAL AND METHODS
In the first part clinically diagnosed and biopsy confirmed 53 adult cases of MPGN and fifty age and sex matched healthy...
control subjects were taken up for comparison. Patients with history of blood transfusions and intravenous drug abuse were excluded. Urine analysis, renal function tests, blood glucose, lipid profile and liver function tests were done. Patients were screened for presence of antinuclear antibodies, C3, type II cryoglobulin and markers of HCV and HBV infection. IgM rheumatoid factor was measured by latex fixation technique (Hoeschst Pharma). Cryoglobulins were screened by standard technique of Grey and Kohler. The protein content of cryoprecipitate was measured by Lowry's methods. Further analysis of cryoprecipitate was done by gel filtration on Sephadex G-200 column and separate fractions analysed for IgM, IgG, IgA and light chains. Redissolved cryoprecipitate was screened for rheumatoid factors, HCV antibodies and HCV-RNA. HCV antibodies were estimated by quantitative 2nd generation enzyme immunoassay. Presence of HCV antigen was screened by 2nd generation ELISA and confirmed by polymerase chain reaction (PCR) technique. All patients had renal biopsy. Tissue obtained was processed for light microscopy and immunofluorescence. Electron-microscope examination for detection of viral particle and confirmation of MPGN were conducted in HCV positive patients. Simultaneously 37 chronic hepatitis patients associated with HCV were investigated for evidence of glomerulonephritis by urinalysis on three occasions over two weeks. They were screened for EMC and rheumatoid factor. Data were collected by d-Base and subjected to statistical analysis by chi-square test. P value < 0.05 was considered significant.

**RESULTS**

Thirty-six (68%) patients had normal renal function (serum creatinine < 1.6 mg%) rest 17 (32%) had renal failure of varying degree. Control subjects had normal renal function, and were negative for all immunological and viral markers. In the 53 MPGN patients three (5.6%) were positive for IgM rheumatoid factor (p > 0.05), seven (13.2%) were anti HCV antibody positive (p < 0.05); however none had cryoglobulinemia. Serum was also negative for ANA with normal complement (C3) level. Histology showed typical lobular pattern in the glomeruli with mesangial proliferation and thickening of basement membrane with complete splits suggesting membranoproliferative glomerulonephritis (MPGN). Immunofluorescence study of kidney biopsy was positive for IgG, C3 and variable IgM. Occasional IgA deposits were seen in three cases.

Electron microscopic examination of renal tissue in seven HCV positive patients failed to localize any viral particle in glomeruli.

Out of 37 HCV associated chronic hepatitis patients, none had proteinuria, hematuria, or casts to suggest glomerulonephritis. They had normal blood urea, creatinine and serum complement. Antinuclear antibody, cryoglobulin and IgM rheumatoid factor were negative in them.

**DISCUSSION**

As HCV serologic testing became available in early 1990s there have been several publications claiming strong association between HCV infection and MPGN with or without cryoglobulinemia. In this we found 13.2% HCV positivity in 53 MPGN patients. It may be mentioned here that incidence of MPGN constitutes 2.8% of 7560 biopsied patients of glomerulonephritis at our center and frequency of HCV seropositivity in non-MPGN group was 0.9% in screening of 426 patients. Therefore 13.2% HCV positivity as observed in MPNGN patients is much higher than non-MPGN although number of patients in the two groups is not comparable. It is at present unclear if this is higher than what is prevalent in Indian population due to lack of systematic prevalence studies. According to two Indian reports among blood donors, the incidences found were 1.5 and 1.8 percent respectively meaning our observation of 13.2% is significant. In contrast in a recent Japanese publication authors have reported 60% association between HCV infection and MPGN suggesting it to be a major cause in that country. Similar higher frequency have been reported between HCV infection, MPGN and EMC in US and Europe. The source of acquisition of HCV infection in these patients remains unclear and both transfusion and non-transfusion associated routes of transmission may be involved although such patients were excluded from our study. Even then it is difficult to rule out injection acquired infection, as these are chronic patients who visit several hospitals and receive treatment including injections. Poor hygiene, inadequate medical facilities, practice of tattooing may be suspected as routes of transmission in our patients.

Investigation in this study did not demonstrate virus particle in glomerular capillaries or in the mesangium of seven seropositive HCV patients, as EM was negative for the same. Serum C3 values were normal and autoantibodies tested negative in them. Thus we are inclined to think HCV positivity in seven patients was coincidence and unlikely to have pathogenetic role. However, identification of virus in the glomeruli has remained elusive in previous studies from other centers. More effective methods for detecting HCV antigens and RNA in tissue may provide evidence as they become available.

This study also did not find cryoglobulins in any of 53 MPGN (including seven HCV positive) patients. More importantly serum from 37 patients of HCV associated chronic hepatitis tested EMC-negative. This is in contrast to studies from Italy, USA, Japan and France where high incidence (40-90%) of EMC in HCV positive patients with or without MPGN have been observed. Authors in USA study demonstrated 10-100 fold circulating HCV-RNA concentrated in cryoprecipitate. It also contained IgG antibodies and IgM rheumatoid factor deposited in glomeruli resulting in MPGN. Despite the findings of a higher association between cryoglobulinemic immune complex MPGN and HCV infection,
noncryoglobulinemic MPGN in HCV infection are well documented.\textsuperscript{18,19} 

Majority (87\%) of our patients did not have HCV infection and all were negative for cryoglobulinemia. It is apparent that immune complexes composed of HCV antigens and antibodies may occur in absence of cryoglobulinemia and get deposited directly. Alternatively chronic HCV infection can lead to autoantibody formation, which may then react with native renal antigens accounting for the renal pathology. Chronic liver disease may contribute to the pathogenesis of MPGN directly as reduced reticuloendothelial cell function would enhance systemic circulation of immune complexes in which IgA is the dominant component. IgA was not observed in the glomeruli on immunofluorescence in this study. These hypotheses have been advanced to explain HCV induced MPGN. However, we have no evidence in the present study to support them. Therefore, we believe MPGN in northern Indian adult population is mostly non-cryoglobulinemic in which HCV infection is not a major cause.

\textbf{REFERENCES}


\textbf{Announcement}

8th Mayo Clinic Endocrine Course on February 27 - March 5, 2005. The Hapuna Beach Prince Hotel, Kohala Coast, Big Island of Hawaii, USA.

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