Hepatitis B and C viruses are human pathogens of major significance. Their extrahepatic manifestations are global health problems. HBV is a well-known cause of membranous nephropathy, membranoproliferative GN and IgA nephropathy, frequently in Asian populations. Polyarteritis nodosa is a rare, but serious systemic complication of chronic HBV. Immunosuppressive therapy in HBV-related GN is not recommended. Interferon alpha treatment produces sustained remission of proteinuria, often associated with clearance of HBeAg and/or HBsAg, however, it has many side effects. Compared to interferon, nucleos(t)ide analogues offer some advantages. These antiviral agents suppress HBV replication through their inhibitory effect on viral DNA polymerase. They have convenient administration and high tolerability. Lamivudine is well tolerated and safe in long-term studies, but the resistance of HBV is an escalating problem. The resistance to newer polymerase inhibitors Entecavir and Tenofovir is significantly lower.

Hepatitis C virus causes cryoglobulinemia-mediated glomerulonephritis and other immune complex forms of GN. The renal manifestations are usually associated with long-lasting HCV infection. HCV glomerular disease is more frequent in adult males, and often leads to chronic renal insufficiency. The first line treatment in patients with mild to moderate clinical and histological kidney damage is the antiviral therapy with pegylated INF alpha and ribavirin. In case of severe HCV-associated cryoglobulinemic GN - nephrotic syndrome, nephritic syndrome and/or progressive renal failure, high activity score of glomerulonephritis on light microscopy, the initial treatment might consist of sequential administration of antiviral and immunosuppressive agents (corticosteroids, cyclophosphamide and plasma exchange), or rituximab. The treatment of HCV-related glomerular disease is still under debate and based on scant experimental evidence. Large randomized and controlled clinical trials are needed to establish guidelines for the treatment of HCV-related cryoglobulinemic glomerulonephritis. Acta Medica Medianae 2014;53(1):58-64.

Key words: hepatitis B virus, hepatitis C virus, glomerular disease, antiviral treatment

Hepatitis B virus (HBV) infection

Hepatitis B virus is a human pathogen of major significance. HBV infection and its complications are global health problems. HBV is estimated to have infected about 400 million people worldwide. The proportion of HBsAg positivity (chronic HBV carriers) in the general population varies from 1% in nonendemic areas to 10% in South East Asia. Every year, one million people in Europe are infected with HBV. Renal involvement is among its most common extra hepatic manifestations.

HBV immunopathogenesis

HBV is a small enveloped DNA virus belonging to the Hepadna family. It is composed of an outer envelope of host-derived lipid containing the surface proteins and an inner protein capsid that contains the genomic DNA (1). The capsid is composed of a single polypeptide chain, molecular weight 20 kDa, known as the core antigen, HBCAg (2).
Hepatitis B virus causes necroinflammatory liver disease of variable duration and severity, from asymptomatic HBsAg carrier state to chronic hepatitis with progression to cirrhosis, end-stage liver disease and hepatocellular carcinoma. HBV is not directly cytopathic. The host immune response to HBV-encoded antigens is responsible both for viral clearance and disease pathogenesis. HBV capsids are highly antigenic, eliciting a strong response with B cells, T helper cells and cytotoxic T cells.

The cellular immune response (especially the cytotoxic T lymphocytes) eliminates infected cells. HBV-specific cytotoxic T-lymphocytes can downregulate HBV gene expression and replication by INF gamma, TNF alpha and IL2. The cellular immune response determines the clinical course of viral infection: the T cell response to the virus is vigorous in acutely infected patients, who successfully clear the virus, but the response is relatively weak and more narrowly focused in chronically infected patients who do not, especially with neonatal exposure to HBV.

The humoral antibody response to viral envelope antigens contributes to the clearance of circulating viral particles. With persistent viral infection, the heightened immune response favors formation of circulating immune complexes. Circulating antigen-antibody complexes are important in the pathogenesis of extrahepatic disease manifestations (3, 4).

Extrahepatic manifestations in acute or chronic hepatitis B appear to be immune-mediated (4, 5):
- Transient serum sickness-like syndrome;
- Polyarteritis nodosa;
- Glomerulonephritis;
- Arthralgia;
- Papular acrodermatitis in children (Gianotti-Crosti syndrome);
- Palpable purpura.

No correlation between HBV genotypes and the presence of extrahepatic manifestations has been found.

Glomerulonephritis in association with HBV infection

Brzosko et al. (6) in 1974 were the first to suggest that HBV might be involved in the pathogenesis of high percentage of glomerulonephritis (GN) and found an incidence of 34.6% with various types of glomerular diseases. Subsequently similar incidence was reported by Nagy et al. (7). Over the last few years, various morphologic patterns of HBV related GN have been reported, with membranous nephropathy (MN) being the most commonly described. The importance of HBV in renal pathology is markedly higher in areas with endemic infection such as South East Asia than in Europe and USA.

Pure forms of glomerulonephritis or, occasionally, in overlapping forms have been seen in association with HBV infection:
- Membranous nephropathy (MN);
- Membranoproliferative glomerulonephritis (MPGN);
- Mesangioproliferative IgA nephropathy (IgAN);
- Focal and segmental glomerulosclerosis (FSGS).

Pathogenesis of GN associated with HBV

Both viral and host factors are involved. A prevalence of genotype A in patients with HBV-related nephropathy has been described. Also, an association with HLA genes has been reported. GN appears to be immune-mediated: deposition of circulating immune complexes, containing HBsAg or HBeAg in antigen excess; induction of local immune complex formation by viral antigens; viral-induced autoantibodies reacting with tissue antigens; direct viral reaction to kidney tissue sites (8).

HBx protein can up-regulate CDS9 expression in podocytes resulting in decreased complement activation, which may facilitate latent HBV infection in podocytes and play a role in development of hepatitis B virus associated GN.

In experimental studies HBsAg and HBCAg expressed in the cytoplasm of renal tubular epithelial cells without replication of the whole virus upregulated complement and coagulation pathways and acute phase response genes, and reduced the circulating C3 levels. Circulating HBV-DNA in HBV carriers was found to induce TGF beta, apoptosis of tubular epithelial cells and fibrosis (8,9). It has been reported that the sera of patients with chronic HBV infection could induce apoptosis in cultured cell line for the study of human proximal renal tubular epithelial cells, via up-regulation of Fas gene expression (10). Furthermore, the induction of apoptosis correlated with the level of circulating HBV DNA, and HBV carriers also showed a higher circulating level of TGF-beta, a growth factor implicated in the potentiation of apoptosis and renal fibrosis.

Autoimmune disorders have also been observed among certain susceptible vaccine recipients:
- Arthritis, including rheumatoid arthritis;
- Myelitis;
- Optic neuritis;
- Glomerulonephritis;
- Pancytopenia or thrombocytopenia;
- MS.

Membranous nephropathy (MN)

MN is the most commonly described glomerular disease in association with HBV. It occurs mainly in children, predominantly in males in HBV endemic areas (Asian populations) (11).

The reported prevalence of HBV-associated MN closely parallels the geographic patterns of prevalence of HBV. In South East Asia, where the proportion of chronic HBV carriers can exceed
10% in the general population, HBV is the underlying cause in 12% of patients with membranous nephropathy. The rarity of HBV-associated nephropathy in developed countries such as the USA and Europe probably reflects the rarity of HBV infection, particularly in children. In developed countries, HBV-associated nephropathy is frequently seen in adults who are high-risk groups such as intravenous drug abusers and in dialysis patients. Introduction of HBV immunization was one of the principle factors in lowering the incidence of HBV-associated nephropathy (12).

HBV-related membranous nephropathy is characterized by thickened capillary wall and glomerular basement membrane on light microscopy. Mesangial abnormalities are more common compared to the idiopathic form, and deposits of membrane attack complex (C5b-9) in glomerular subepithelium are lower compared to idiopathic MN. Immunofluorescent staining and electron microscopy demonstrate subepithelial deposition of immune complexes containing HBsAg and HBeAg, IgG, C3 and IgM, extensive effacement of the podocyte foot processes, and in some cases viral particles in various locations within the glomerulus.

The clinical presentation of HBV-related MN is in most cases a nephrotic syndrome. In the asymptomatic cases proteinuria, impairment of renal function and hypertension have been found. In children it is usually self-limited, in 60-85% by 2 years, in 95% by 5-7 years. Only 5% are progression to renal failure is observed. Liver tests are frequently normal. In adults, the natural course of GN may be more relentless, slowly progressing to renal failure in about 30-50% of patients (4,12).

**HBV-associated membranoproliferative GN**

HBsAg has been detected with MPGN and IgAN patients. The pathogenesis of MPGN most likely involves deposition of circulating IC containing HBV antigens in glomeruli, due to persistent viral infection, in which the heightened immune response favors formation of circulating IC’s, with ultimate deposition into extrahepatic sites (13).

Histopathologically, MPGN is associated with mesangial and capillary wall deposits of HBsAg. Dense deposits bearing HBsAg immunoreactivity in a thickened glomerular basement membrane have been found. IgG, complement components, and IgM appear granular on immunofluorescent staining, located in the subendothelial, mesangial, and parame桑aligial areas.

Impaired renal function is more common in patients with membranoproliferative glomerulonephritis compared to membranous nephropathy.

**HBV-associated IgA nephropathy**

It is more frequent in adults, predominantly in Eastern Asia (up to 34% HBsAg positivity in patients with IgAN in China).

On immunofluorescence, mesangial IgA deposition in association with IgG subepithelial deposits, giving a combination of MGN and IgAN have been found (14). Immunohistochemically, HBeAg, HBsAg and HBCAg have been detected in glomeruli, tubular epithelial cells and infiltrating interstitial lymphocytes.

Electron microscopy frequently shows tubuloreticular inclusions, located within dilated cisternae of the endoplasmic reticulum belonging to the endothelial cell cytoplasm of glomerular and peritubular capillaries. They are the expression of endogenous virus-induced interferon production. Moreover, HBV-DNA has been detected by in situ hybridization and Southern blot analysis in the nuclei of mesangial and tubular epithelial cells. These facts lead to the suggestion that not only immune complex deposition play a role in the pathogenesis of IgA nephropathy associated with HBV, but also cellular mechanisms mediated by direct infection of renal tissue with HBV (15).

**Focal and segmental glomerulosclerosis (FSGS) in association with HBV infection**

Only a few cases have been reported till now. HBsAg was demonstrated in renal tissue of 2 cases. HBV-DNA has been detected in urinary podocytes by real-time PCR methods. They showed response to treatment with lamivudine, thus indicating a possible causal association between the viral infection and occurrence of nephrotic syndrome. After the anti-viral therapy FSGS improved, paralleling the decreased level of HBV-DNA in podocytes (16,17).

**Diagnostic criteria for HBV-associated GN**

It is difficult to assess the relation between HBV and GN because of the high frequency of HBV asymptomatic carriers in the general population. Diagnosis is based on the presence of persistent HBV antigenemia and tissue molecular analysis - detection of at least one HBV antigen in renal tissue by a monoclonal F(ab) antibody method. The spontaneous remission of glomerulonephritis after successful treatment of HBV infection favors an etiopathogenic link between HBV and GN. Spontaneous remission is usually associated with seroconversion of HBeAg (4).

**Treatment of HBV-associated GN**

The uncommon occurrence and small series, variability in renal histopathology, and heterogeneity in clinical course lead to uncertainty with regard to the optimal management of HBV-related GN. Much of the data on the treatment of HBV-related glomerular diseases came from patients with membranous nephropathy. The data on membranoproliferative glo-
The resistance of HBV to newer polymerase inhibitors Entecavir and Tenofovir is significantly lower.

**Hepatitis B virus-associated polyarteritis nodosa (PAN)**

PAN is a rare, but serious systemic complication of chronic HBV. Only 1% or less of the total population of patients who are HBsAg positive develops PAN. Analysis of the HBV genome revealed no mutations that could be associated with PAN (4).

Pathogenesis has been attributed to immune-complex deposition with antigen excess. Surface antigen-antibody complexes are deposited in vascular beds. Complement activation promotes inflammation mainly by production of pro-inflammatory substances, including prostaglandins, vasodilatory peptides, chemotactic substances, oxygen free radicals and lysosomal enzymes. Immune complexes also cause aggregation of platelets and activation of Hageman factor and initiate the formation of microthrombi (4,23).

Polyarteritis may occur at any time in patients who are HBsAg positive. It represents a typical form of classic PAN: systemic necrotizing vasculitis without glomerulonephritis. ANCA are not detected. Orchitis is more frequent in HBsAg positive patients with PAN. The major cause of death is gastrointestinal tract involvement.

Frequency of HBV-PAN has decreased significantly in relation to improved blood safety and vaccination campaigns (24).

**Treatment of HBV-associated PAN**

Antiviral therapy with interferon-alpha and lamivudine causing seroconversion leads to remission. Combining an antiviral drug with plasma exchange facilitates seroconversion and prevents the development of long-term hepatic complications of HBV infection. Clinical remission of PAN was observed in treated patients, but in none of the patients who were not receiving antiviral medication. Relapses are rare, and never occur once viral replication has stopped and seroconversion has been obtained (24).

**Hepatitis C virus (HCV) infection**

HCV is the major etiologic agent of chronic hepatitis and possible liver cirrhosis and hepatocarcinoma. It represents an enveloped RNA virus belonging to the Flaviviridae family. It possesses high genomic variability (6 genotypes, over 100 subtypes) and high dynamics.
Extrahepatic manifestation of HCV infection

Patients with chronic HCV infection are at risk of a great number of extrahepatic manifestations (EHMs) – up to 40-76% of patients infected with HCV develop at least one EHM during the course of the disease. EHMs are often the first and only clinical sign of chronic hepatitis C infection: mixed cryoglobulinemia, cryoglobulinaemic vasculitis, peripheral neuropathy, membranoproliferative glomerulonephritis, membranous nephropathy, rheumatoid arthralgia, cryoglobulinemia, autoimmune thyroiditis, insulin resistance, diabetes mellitus, lupus-like polyarthritis, autoimmune hepatitis, and cryoglobulinemia. Most studies suggest that the presence of mixed cryoglobulinemia, particular lymphoproliferative disorder, and cryoglobulinemia autoimmune phenomena constitute the major pathogenic factors (25).

There are two types of HCV-associated glomerular diseases:

- HCV-associated cryoglobulinemic glomerulonephritis;
- Immune-complex disease with HCV-containing immune complexes:
  - Membranoproliferative GN;
  - Membranous nephropathy.

Mixed cryoglobulinemia (MC) type II and III is the most known and studied syndrome associated with HCV infection. It is found in 19%-50% of patients with chronic HCV, but leads to clinical manifestations through vascular precipitation of immune complexes, in only 30% of them and causes systemic vasculitis of small and medium sized blood vessels that may involve the skin, kidney and nervous system. In 95% of cases with so-called “essential” MC anti-HCV Ab, HCV RNA in plasma and in cryoprecipitates are found.

Histopathologically, in membranoproliferative cryoglobulinemic glomerulonephritis glomeruli show accentuated lobulation, mesangial cellularity, mesangiolysis, capillary endothelial swelling and splitting of capillary basement membranes. There is a marked infiltration of glomerular capillaries by leukocytes. PAS stain highlights intracapillary aggregates of immune complexes involving cryoglobulins. Electron micrograph demonstrates subendothelial immune deposits, with some fibrillar organization (26, 27).

In non-cryoglobulinemic MPGN the pattern is similar, but mesangiolysis, leukocyte influx and intracapillary accumulation are less likely to be apparent. Both subendothelial and subepithelial immune complexes can be identified by electron microscopy (28).

The renal manifestations are usually associated with long-lasting HCV infection, longer than 10-year history. Most often, there are clinical and laboratory features of chronic active hepatitis and/or cirrhosis. HCV glomerular disease is more frequent in adult males, it is rare in children.

In mixed cryoglobulinaemia, patients usually have palpable purpura, arthralgia, neuropathy. Rarely, the presentation may include severe vasculitis with abdominal pain and other solid organ involvement. Renal manifestation includes nephritic or non-nephrotic proteinuria and microscopic hematuria. Renal insufficiency is commonly identified. Laboratory tests demonstrate viremia (circulating HCV DNA by PCR analysis), low C3 and C4, mixed cryoglobulinaemia and positive rheumatoid factor, ANCA negativity (25).

The clinical evolution is slow, in about 30% renal function is maintained for many years. In 10-15% clinical regression has been observed, recurrent episodes in 20% and end stage renal disease in 15%. Recurrence of MPGN in allografts has been suspected in a small number of patients, but this problem has not yet adequately studied.

Small series of membranous nephropathy have been reported in HCV-infected patients (29).

HIV-HCV co-infection may be seen nowadays, presenting with nephrotic syndrome and sometimes with immunotactoid deposits.

Treatment of HCV-associated cryoglobulinemic glomerulonephritis

Results of treatment of these patients with interferon alone have been disappointing, since relapse of the viraemia and subsequent relapse of the renal disease are major problems. Combination of interferon with ribavirin has been shown to increase the rate of sustained response.

The first line treatment in patients with mild to moderate clinical and histological kidney damage is the antiviral therapy with pegylated INF alpha and ribavirin for at least 48 weeks. A significant benefit has been reported with this regimen. Ribavirin doses should be adapted according to creatinine clearance in order to avoid its main side effect, i.e. hemolytic anemia. (25,30-32).

In patients on regular dialysis, monotherapy with conventional interferon without ribavirin is recommended, while after renal transplantation interferon must not be used except for dire circumstances.
In case of severe HCV-associated cryoglobulinemic GN - nephrotic syndrome, nephritic syndrome and/or progressive renal failure, high activity score of glomerulonephritis on light microscopy, the initial treatment might consist of sequential administration of antiviral and immunosuppressive agents or rituximab (30,32). Immunosuppressive therapy consists of plasma exchange (in acute manifestation), corticosteroids for a short period and mild dosage cyclophosphamide in life-threatening organ involvement when there is no response to steroids.

In refractory or intolerant patients the alternative to conventional therapy is B-cell depletion agent anti-CD20, rituximab. Drug-induced viral load elevation is of minor extent after this treatment.

The vaccination campaigns on large populations worldwide lead to a decrease in HBV infection and its extrahepatic consequences.

The problem of HCV infection remains unsolved, the prevalence of HCV seropositivity is stable and associated kidney disease is of potential magnitude. The precise pathogenic sequence of injury resulting in glomerulonephritis is not known. The treatment is still under debate and based on scant experimental evidence. No definitive data are yet available from the literature. Large randomized and controlled clinical trials are needed to establish guidelines for the treatment of HCV-related cryoglobulinemic glomerulonephritis.

Moreover, the management of HBV and HCV infections in CKD and kidney transplanted patients remains a challenge to nephrologists.

References


Glomerular diseases associated with HBV and HCV infection

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GLOMERULARNA OBOLJENJA POVEZANA SA HBV I HCV INFEKCIJAMA

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Ključne reči: Virus hepatitisa B, virus hepatitisa C, glomerularno oboljenje, antivirusa lečenje