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Anti-phospholipase A2 receptor antibody positive hepatitis B virus-associated membranous nephropathy remitted with entecavir after relapse with lamivudine

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ABSTRACT

Background: Phospholipase A2 receptor (PLA2R) is thought to be an intrinsic antigen of idiopathic membranous nephropathy (IMN), and has been widely used for the differentiation from secondary membranous nephropathies. However, the positive expression of PLA2R in the patients with hepatitis B virus associated membranous nephropathy (HBV-MN) is controversial in Asian countries, because co-localization of PLA2R and HBV antigens in glomeruli have been reported.

Case Presentation: We report a case of anti PLA2R antibody positive HBV-MN that was remitted with entecavir after a relapse during treatment with lamivudine. In a renal biopsy of the case, we could confirm the co-localized glomerular deposition of HBV surface antigen (HBs-Ag) and PLA2R using double staining of immunofluorescence. We also could observe the relapse of nephrotic syndrome correlated with the increased titer of HBs-Ag, and the remission with the decrease of HBs-Ag by the change of antiviral agents.

Conclusions: Our case demonstrated that the renal manifestation of HBV-MN clearly responded to antiviral agents. Furthermore, the co-localized glomerular depositions of PLA2R and HBs-Ag in HBV-MN may be concerned with the etiology of MN patients with chronic HBV infection.

Case Report

Implication for health policy/practice/research/medical education:
Our case demonstrated clear response of the renal manifestation of HBV infection to antiviral agents, and co-localized glomerular depositions of PLA2R and HBs-Ag that may be concerned with the etiology of HBV-associated membranous nephropathy such as possibilities of cross-antigenicity between PLA2R and HBs-Ag, or structural changes in PLA2R due to HBV infection.

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1. Background

Hepatitis B virus associated membranous nephropathy (HBV-MN) is thought to be caused by the immunoreaction to HBV associated antigens, and therefore can be effectively treated with antiviral agents (1). Phospholipase A2 receptor (PLA2R) is thought to be one of the major intrinsic antigens of idiopathic membranous nephropathy (IMN), and has been widely used for the differentiation from secondary membranous nephropathies such as lupus nephritis, MN associated with malignancy or HBV-MN (2,3). However, the role of PLA2R in patients with HBV-MN is still unclear in Asian countries. Frequent co-localization of PLA2R and HBV antigens are reported mainly from China (4). We report the case of PLA2R-positive HBV-MN that was remitted with entecavir after a relapse during the treatment with

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lamivudine. In the present case, we could observe the relapse and remission of proteinuria correlating with the titer of HBV surface antigen (HBs-Ag), and we also could confirm the co-localized granular depositions of HBs-Ag and PLA2R in the glomerular capillary walls using double staining of immunofluorescence. We will try to discuss the role of PLA2R in HBV-MN and its concern with the etiology of HBV-MN or not.

2. Case Presentation

A 57-year-old man was admitted for evaluation of a bipedal edema on February 2002. His history of illness started with proteinuria that was pointed out at annual health check-ups eight months prior to admission (June 2001). The patient noticed the bipedal edema two months after the health check-ups (August 2001). He visited a doctor due to severe bipedal edema following a sore throat and a fever for several days. The doctor referred the patient to our hospital for evaluation of massive proteinuria (February 2002). His past-medical history was remarkable for the inactive carrier of HBV due to unknown infectious route. He has no history of trauma, blood transfusion, homosexual intercourse or HBV-infected family. He had a smoking history of 30-pack-year. He consumed 500 mL of beer every night for 30 years.

On physical examination, his height was 170 cm, body weight was 72 kg, body mass index was 24.9 kg/m^2, and blood pressure was 146/86 mm Hg, respectively. His physical examination was unremarkable except for the bipedal pitting edema.

Laboratory data (Table 1) was remarkable for hypoproteinemia, elevation of cholesterol and triglyceride, and remarkable proteinuria without hematuria. Serological markers on HBV infection were compatible with inactive HBV carrier (Table 2). HBV envelope antigen (HBe-Ag) was negative; anti-HBV envelope antibody (HBe-Ab) was positive. HBV-DNA was 2.8 log copies/mL. Serum tumor markers and hepatitis C virus antibody were all negative. The pre-treatment renal biopsy specimen included 12 glomeruli with one global sclerosis. All glomeruli showed mild thickening of basement membranes without proliferative changes. In Masson's trichrome stain, interstitial edema and age-matched arteriosclerotic change of interlobular artery were shown (Figure 1). In high magnification, granular sub-epithelial deposits were observed along the capillary walls in Masson's trichrome stain (not shown). Spike formations of the capillary walls in Periodic acid silver-methenamine-Hematoxylin-Eosin(PASM-HE) stain were seen, which were typical as membranous nephropathy (Figure 2). Electron microphotographs revealed sub-epithelial electron-dense deposits regularly,

Table 1. Laboratory data on admission

Blood count	
Hemoglobin (g/dL)	15.8
Hematocrit (%)	46.5
Leukocyte (/μL)	8,000
Platelet (× 10^3/μL)	304
Chemistry	
Sodium (mM)	142
Potassium (mM)	4.7
Chloride (mM)	107
Corrected calcium (mg/dL)	9.6
Phosphorus (mg/dL)	3.4
Total Protein (g/dL)	5.8
Albumin (g/dL)	2.7
Urea nitrogen (mg/dL)	9
Creatinine (mg/dL)	0.7
Uric acid (mg/dL)	5.5
Total bilirubin (mg/dL)	0.5
AST (IU/L)	12
ALT (IU/L)	10
LDH (IU/L)	328
ALP (IU/L)	78
GGT (IU/L)	16
Total cholesterol (mg/dL)	421
Triglyceride (mg/dL)	239
HDL-C (mg/dL)	37
Serology	
IgG (mg/dL)	609
IgA (mg/dL)	110
IgM (mg/dL)	62
C3 (mg/dL)	102
C4 (mg/dL)	29
Rheumatoid factor (IU/mL)	8
Anti-nuclear antibody	<40
M-protein	Negative
Urinalysis	
Protein	(+++)
Glucose	(-)
Occult blood	(-)
Urine sediment	
Leukocyte (/HPF)	3〜5
Erythrocyte (/HPF)	11〜15
Granular cast	(+)
Hyaline cast	(++)

Abbreviations: AST, aspartate aminotransferase; ALP, alkaline phosphatase; ALT, alanine aminotransferase; GGT, gamma-glutamyl transferase; HDL-C, high-density lipoprotein cholesterol; HPF, high-power field; IgA, immunoglobulin A; IgG, immunoglobulin G; IgM, immunoglobulin M; LDH, lactate dehydrogenase.

which were compatible with stage II of Ehrenreich and Churg classification (Figure 2).

Routine immunofluorescence study showed marked granular IgG deposition along the glomerular capillary walls. IgG subclasses were negative except for IgG4 (Figure 3). C3 was slightly positive in the capillary walls. Other immunoglobulins and compliments were negative. HBs-Ag was positive in the capillary walls (Figure 3).

Table 2. Serological data on infectious diseases

HBs antigen (COI)*	185.5
HBs antibody*	Negative
HBV-DNA (logcopies/mL)**	2.8
HBc antigen	Negative
HBc antibody	Positive
HBe antigen*	Negative
Hbe antibody (COI)*	100
Anti HCV antibody	Negative
STS	Negative
TPHA	Negative

Abbreviations: COI, cut off index; HBc, hepatitis B core; HBe, hepatitis B envelope; HBs hepatitis B surface; HCV, hepatitis C virus; STS, serum test for syphilis; TPHA, Treponema pallidum hemagglutination test.
*measure by radioimmunoassay. ** measured by polymerase chain reaction method.

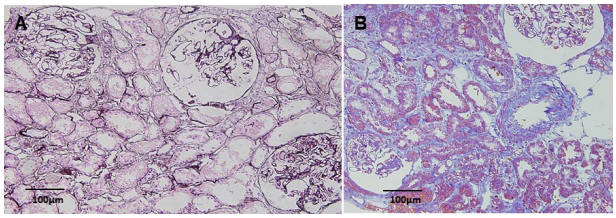

Figure 1. A; The biopsy specimen includes 12 glomeruli. Hypercellularity nor mesangial expansion are not observed except in one glomerulus with global sclerosis (PASM-HE stain; ×200). B; Interstitial edema is observed, and the arteriosclerotic change is mild, corresponding to his age by Masson's trichrome stain (×200).

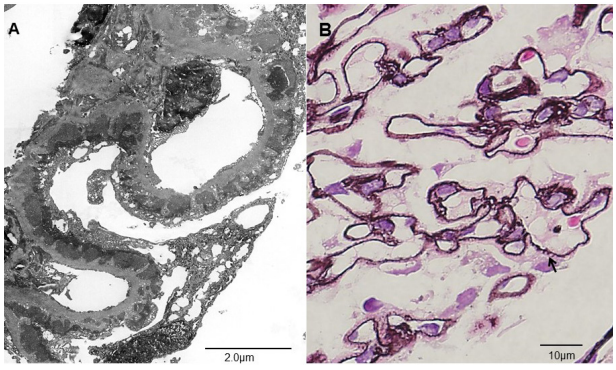

Figure 2. A; Electron microphotograph is remade from the paraffin section. Regrettably, no glomerulus was included in the specimen for electron microscopic study, so we made ultrathin sections from the paraffin sections for light microscopy. There are regularly arranged sub-epithelial electron dense deposits along the glomerular basement membranes, compatible with a diagnosis of membranous nephropathy stage II. Epithelial cells, endothelial cells and mesangial cells could not be estimated. B; The highest magnification of light microphotographs (×1000). Spike formations of the basement membrane are frequently seen in PASM-HE stain. The bubbling of the basement membranes is seldom seen.

According to these findings, we diagnosed the patient with HBV-MN.

We treated the patient with lamivudine 100mg/day, angiotensin II type1-receptor blockers (ARBs), statins, salt intake restriction, and a low-protein diet. Kinds and dosages of ARBs and statins were appropriately changed depending on blood pressure, amount of proteinuria, and serum low-density-lipoprotein cholesterol (LDL-C) level, respectively. We also performed LDL-C apheresis for nephrotic syndrome. HBV-DNA became negative soon after the initiation of lamivudine. Proteinuria and hypoalbuminemia also gradually improved parallel to HBs-Ag. We finally achieved partial remission for ten years. However, after the achievement of partial remission, the patient developed nephrotic syndrome again 10 years after the treatment initiation (2012, Figure 4). At first, we suspected a relapse of concurrent undiagnosed IMN because the patient was already treated with lamivudine. However, re-elevation of serum HBs-Ag levels correlated with exacerbation of proteinuria introduced us to the possibility of a relapse of HBV-MN due to acquired resistance to lamivudine. To distinguish relapse of IMN or HBV-MN, we performed immunostaining of PLA2R; we thought that negative PLA2R deposition would exclude the possibility of this. Contrary to our expectation, PLA2R was strongly granular positive in all glomerular capillary walls in the specimen (Figure 3F). To examine the association of HBs-Ag and PLA2R, we performed double immunofluorescence staining of HBs-Ag (green; Figure 3E) and PLA2R (red; Figure 3F). The merged microphotograph showed co-localized granular depositions of both HBs-Ag and PLA2R along the capillary walls (yellow; Figure 3G). Based on serum HBs antigen titer elevation correlating with proteinuria, we concluded that the patient developed relapse of HBV-MN due to acquired resistance to lamivudine, and changed the antiviral agent from lamivudine to entecavir 0.5mg/day. After the initiation of entecavir, proteinuria and hypoalbuminemia improved parallel to the decrease of serum HBs-Ag titer (Figure 4). As of now (August 2017), the patient has maintained good health status without any relapse or adverse events.

3. Discussion

We would like to discuss to two remarkable points of the present case. First, we could observe the relapse and remission of proteinuria correlating with the serum titer of HBs-Ag with the change of antiviral agents by serial observation for more than 14 years. Second, we could confirm the co-localized depositions of HBs antigen and PLA2R using double staining of immunofluorescence.

Antiviral therapy against HBV with nucleoside/

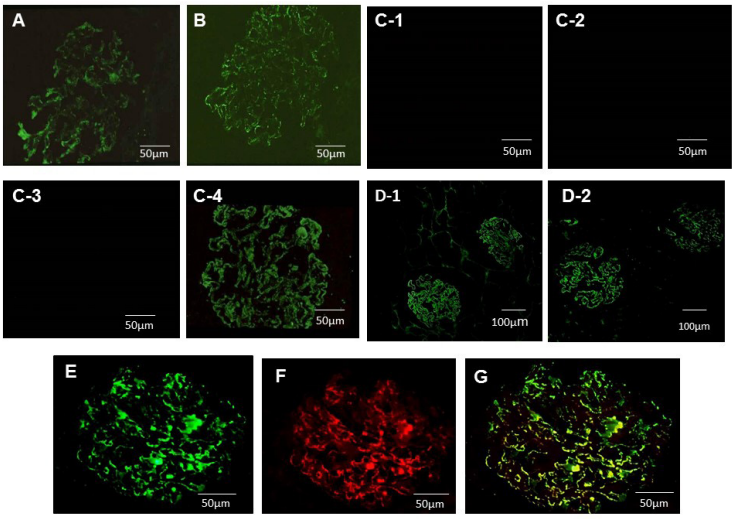

Figure 3. Routine direct immunofluorescent study using frozen sections show IgG (A) and C3 (B) granular positive expression along the capillary walls. Others are all negative. C-1-4; IgG subclass by frozen sections. Only IgG4 shows granular positive expression along the capillary walls (C-4). D-1, 2; indirect immunofluorescent study using paraffin sections. Kappa (D-1) and lambda (D-2) are expressed equally positive along the capillary walls. E, F, G shows double staining of HBs and PLA2R. E: HBs shows positive expression along the glomerular capillary walls, and the finding shows this membranous nephropathy to be HBV-related (green). F: PLA2R also shows apparently positive expression along the glomerular capillary walls (red). G; merging the two microphotographs shows that the positive expression is almost identical in the capillary walls (yellow).

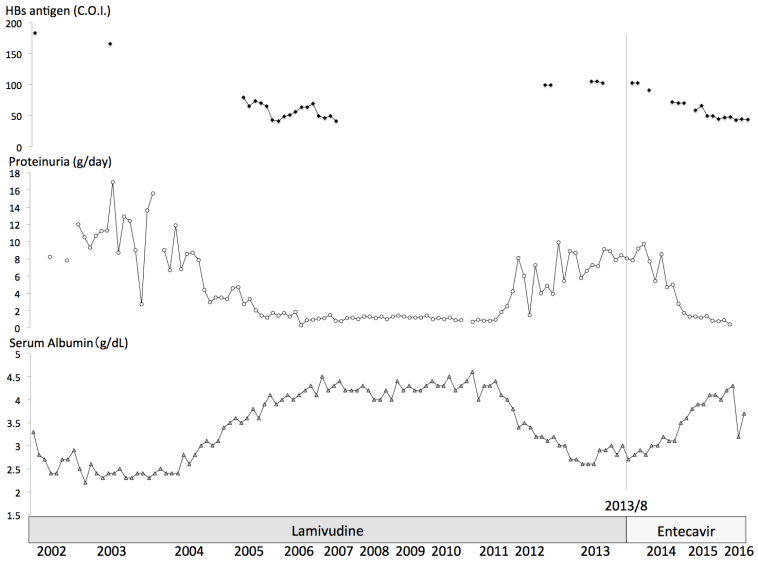

Figure 4. Trends of anti HBs antigen, proteinuria, and serum albumin during antiviral therapy. Proteinuria and hypoalbuminemia improved parallel to HBs-Ag after initiation of lamivudine. However, the patient developed nephrotic syndrome again 10 years after the initiation lamivudine. After the change of antiviral agent to entecavir, proteinuria and hypoalbuminemia improved again parallel to the decrease of serum HBs-Ag titer.

nucleotide analogs or interferon is the de facto standard management of HBV-MN with positive HBs-Ag or HBV-DNA. In cases with initially negative HBe-Ag, the therapeutic goal is to suppress viremia. Thus, nucleoside/nucleotide analogs may be required for life in most cases; discontinuation has a risk of a relapse and acquired resistance. Although researches on HBV-MN are still sparse, entecavir should be the first-line agent because of high antiviral efficacy and low risk of drug resistance (5). In our case, we initiated lamivudine after discussion with a hepatologist in our hospital and continued until the relapse. Given that entecavir was not approved at the time of diagnosis (February 2002), we regard our selection and duration of antiviral agent as appropriate (lamivudine and entecavir were approved in 2000 and 2006 in Japan, respectively). At the timing of relapse

(2012), entecavir had been approved and recommended as the first-line drug of chronic HBV hepatitis because of low risk of drug resistance, we changed lamivudine to entecavir. Eventually, the change of agent achieved the remission of HBV-MN. To the best of our knowledge, this is the first report of the observation of changes of HBs-Ag titer parallel to renal manifestation due to HBV-MN. Our experience clearly demonstrated the efficacy of antiviral therapy to renal manifestation of HBV-MN.

Glomerular depositions of PLA2R have been thought as specific findings of IMN. However, the significance of PLA2R for differentiation of IMN from HBV-MN is controversial because co-localized deposition with HBs-Ag has been reported from China (4). In the present case, we performed double immunofluorescence study with both of the antibodies against HBs-Ag and PLA2R, and found the co-localized deposition of them shown along the glomerular capillary walls. This finding suggests that presence of anti-PLA2R antibody as an intrinsic antigen in the cases of HBV-MN is the same as in cases with IMN.

PLA2R deposition can be caused by concurrent IMN. However, improvements of serum HBs-Ag titer and the amount of proteinuria after initiation of antiviral agents exclude the possibility of concurrent IMN in the patients with chronic HBV infection. Cross-reaction between the antigenicity of PLA2R and HBs-Ag may explain the co-localized glomerular deposition of these antigens. However, knowledge on the intrinsic antigens of IMN and HBV-MN are sparse to interpret the co-localization. Another hypothetical cause may exist of these co-localized antigens, HBV infection may induce structural changes in PLA2R of glomerular epithelial cells and the changed PLA2R acquire antigenicity to produce auto-antibody against PLA2R. Further studies are warranted.

4. Conclusions

We report a case of anti-PLA2R antibody positive hepatitis B virus-associated membranous nephropathy remitted with entecavir after relapse with lamivudine. In this case, we could observe the relapse and remission of nephrotic syndrome correlated with the titer of HBV surface antigen (HBs-Ag) with the change of antiviral agents, which clearly demonstrated the efficacy of antiviral therapy to renal manifestation of HBV-MN. We also observed the co-localized glomerular deposition of HBs antigen and PLA2R using double staining of immunofluorescence, which may be concerned with the etiology of HBV-MN such as possibilities of cross-reaction between the antigenicity of PLA2R and HBs-Ag, or structural changes in PLA2R due to HBV infection.

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Authors' contribution

YS wrote the manuscript. YN was the patient's treating physician and supervised the manuscript preparation, collected information on the patient, and supported the writing of the manuscript. TM, YA, and KS contributed histopathological interpretation. YU contributed by reviewing and revising the manuscript.

Conflicts of interest

There were no points of conflicts.

Ethical considerations

Ethical issues (including plagiarism, data fabrication, double publication) have been completely observed by the authors. The patient has given his informed consent regarding this case report.

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