Analysis of rheumatoid factor according to various hepatitis B virus infectious statuses

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Abstract

Objective

Rheumatoid factor (RF) can be seen in hepatitis B virus (HBV) infection. We investigated RF positive rates according to various HBV infectious statuses and vaccination, and the relationship between RF titers and serum HBV DNA levels.

Methods

We examined 13,670 individuals who visited the Severance Hospital in Seoul, Korea, for a routine health check-up, and obtained serum samples from all individuals.

Results

RF was positive in 3.5% of all subjects, and HBsAg was positive in 4.3%. HBsAg was positive in 21.7% of all RF positive subjects. RF was positive in 17.5% of the HBsAg positive group, while it was positive in 2.9% of the HBsAg negative group ($p<0.001$). The RF positive rate was increased in positive HBsAg, female sex, and older age. The RF positive rate was lower in those who had anti-HBs after HBV vaccination than in HBsAg positive subjects (2.7% vs. 17.5%, $p<0.001$). Among the RF positive patients, the RF titer in HBsAg positive patients were higher than that in HBsAg negative patients (159.7±217.1IU/mL vs. 83.0±179.2 IU/mL, $p=0.001$). The load of HBV DNA may be closely correlated with RF titer in patients with chronic hepatitis B ($r=0.508$, $p=0.005$).

Conclusion

Persistent HBV infection is an important cause for the positive RF in HBV endemic areas. Hepatitis B viral load is associated with RF titer. HBV vaccination may reduce the risk of RF formation.

Key words

rheumatoid factor, hepatitis B virus, HBsAg, HBV DNA, HBV vaccination

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Introduction

Rheumatoid factor (RF) is an autoantibody that binds to the Fc portion of IgG. RF is commonly present in patients with rheumatoid arthritis (RA), and it has been used in the diagnosis of RA (1). However, RF positivity can be seen in several diseases other than RA, such as, Sjögren syndrome, systemic lupus erythematosus and infections as well as in normal individuals (2).

RF forms complexes with autologous IgG which is present in the synovium of RA patients. These complexes can subsequently cause inflammation by activating complements or through cytokine release following ligation of Fcγ receptors on macrophages (3). In normal immune response, RF can physiologically enhance elimination of immune complexes by macrophages and improve cytotoxicity of antiviral antibodies (4). Several ideas have been suggested to account for RF production. RF can be produced by antigen specific B cells, with help from T cells, as a result of binding and processing of immune complexes in which IgG functions as an antigen (5). Cross reactivity between epitopes of foreign antigen or autoantigen and IgG Fc can be another mechanism of RF formation (6). Polyclonal B cell activation, which is activated by the mitogenic effects of infectious agents or through bystander effects during specific responses, can be another cause of RF formation (6).

It has also been reported that RF was present in hepatitis B virus (HBV) infection, however, only few studies have reported on the RF positive rates in patients with HBV (7-9). A hypothesis states that the HBeAg-antibody complex may play a role in the formation of RF in HBV infection (7), however, the mechanism of RF formation in HBV infection is still unclear. Furthermore, obscurity exists in the types of antigens and antibodies that play important roles in the development of RF and the relation of hepatitis B viral load with RF production. In this study, we investigated the RF positive rates and titers of RF according to various HBV infectious statuses and vaccination, and the relationship between RF titer and serum HBV DNA levels in HBV endemic areas.

Patients and methods

The subjects included 13,670 individuals who visited the Severance Hospital Health Promotion Center in Seoul, Korea, for routine health check-up from January 2004 to December 2004. We obtained serum samples from all individuals. The study was approved by the Institutional Review Board of Severance Hospital, Yonsei University Health System, and informed consent was waived. The serum samples were tested for RF (IgM type) and HBV infection by screening for the presence of HBsAg, anti-HBs (IgG type), and anti-HBc (IgG type). HBeAg, anti-HBe (IgG type), and HBV DNA were analyzed in subjects positive for HBsAg. The flow diagram of this study is illustrated in Figure 1.

RF was analysed by the nephelometric method (Beckman-Coulter, Fullerton, CA, USA) and the normal value of this assay was below 20 IU/mL. Viral markers of HBV were detected by the Enzyme-Linked Immunosorbent Assay method (HBsAg, Anti-HBs, Anti-HBc, HBeAg, Anti-HBe: Enzygnost; Dade Behring, Marburg, Germany). Serum HBV DNA was quantified using real time polymerase chain reaction (PCR) assay (Artus HBV LC PCR Kit, Roche Diagnositcs, lower limit of quantification, 140 copies/mL).

The positive rates of RF were evaluated based on the presence of each HBV viral marker by the Chi-square test or Fisher's exact test. Results were presented as prevalence ratio (PR) with 95% confidence intervals (95% CI). Student's *t*-test or Mann-Whitney-U test was used to compare the titers of RF according to HBV antigen and antibody status. All measurements are expressed as mean ± standard deviation (SD). The correlation between RF titers and serum HBV DNA levels was assessed using Pearson's correlation test. Multiple logistic regression analyses were performed using age, sex, HBsAg, anti-HBs, and anti-HBc to determine the factors that affect the RF positivity. For all statistical evaluations of the results, p-values <0.05 were considered significant. All statistical analyses were conducted using the SPSS package for Windows version 13.0 (SPSS Inc., Chicago, Illinois, USA).

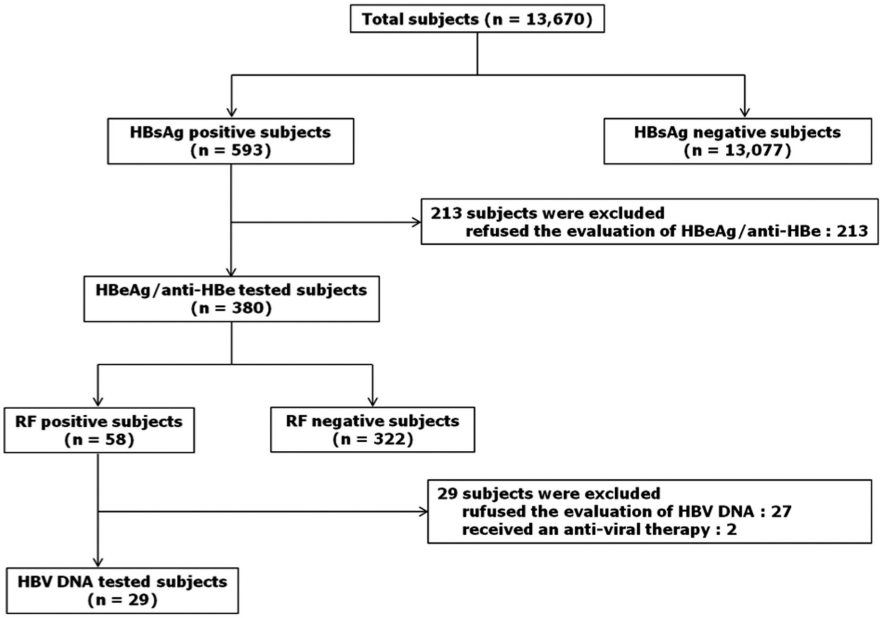

Fig. 1. Schematic diagram of the study design. RF, rheumatoid factor.

Table I. Rheumatoid factor positive rate according to the status of HBV antigen or antibody.

	Subjects, n	RF positive, n (%)	*p*-value
HBs Ag (+)	593	104 (17.5)	<0.001
HBs Ag (-)	13.077	375 (2.9)	
Anti-HBs (+)	8.862	264 (3.0)	<0.001
Anti-HBs (-)	4.808	215 (4.6)	
Anti-HBc (+)	6.969	290 (4.2)	<0.001
Anti-HBc (-)	6.701	189 (2.8)	
HBs Ag (+)	593	104 (17.5)	<0.001
HBs Ag (-) anti-HBc (-) anti-HBs(+)*	3.808	103 (2.7)	
HBs Ag (+)	593	104 (17.5)	<0.001
HBs Ag (-) anti-HBc (+) anti-HBs (+)¥	5.021	151 (3.0)	

*Subjects who had anti-HBs after HBV vaccination; ¥Subjects who recovered from HBV infection; RF: rheumatoid factor.

Table II. Multiple logistic regression analysis of rheumatoid factor positive rate.

	Exp (beta)	95% C.I. for Exp (beta)		*p*-value
		Lower	Upper	
Constant	0.164			
Age	1.010	1.001	1.019	0.027
Female sex	1.214	1.007	1.461	0.042
HBsAg (+)	7.822	5.737	10.666	<0.001
Anti-HBs (+)	1.093	0.872	1.369	NS
Anti-HBc (+)	1.000	0.806	1.240	NS

95% C.I.: 95% confidence intervals; NS: not significant.

Results

We collected data from a total of 13,670 subjects comprised of 7,515 men (55.0%) and 6,155 (45.0%) women with the average age of 48.1±11.3 (range from 12 to 85) years. RF was present in 3.5% (479/13,670) of all subjects. HBsAg was present in 4.3% (593/13,670) of all subjects, and HBsAg was positive in 21.7% of RF positive subjects. The RF positive rate had tended to be higher in women (3.8% *vs*. 3.3 %, p=0.087) and in older age (p=0.142).

RF status and HBV serology

RF was positive in 17.5% (104/593) of the HBsAg positive group, while it was positive in 2.9% (375/13,077) of the HBsAg negative group (p<0.001). RF was positive in 3.0% (264/8,862) of anti-HBs positive subjects, whereas it was positive in 4.6% (215/4,808) of anti-HBs negative subjects (p<0.001). RF was positive in 4.2% (290/6,969) of the anti-HBc positive group, while it was positive in 2.8% (189/6,701) of the anti-HBc negative group (p<0.001). The RF-positive rate was lower in those who had anti-HBs after HBV vaccination (2.7%, 103/3,808) than in HBsAg positive subjects (17.5%, 104/593) (p<0.001) (Table I).

In multiple logistic regression analysis, the RF positive rate was increased in positive HBsAg (PR = 7.82, 95% CI 5.74 to 10.67, p<0.001), female sex (PR = 1.21, 95% CI 1.01 to 1.46, p=0.042) and older age (PR = 1.01, 95% CI 1.001 to 1.019, p=0.027), but not in the anti-HBs positive and anti-HBc positive groups (Table II).

RF status in subgroup analysis

Among HBsAg positive subjects, the RF positive rate in the anti-HBs positive group was higher than that in the anti-HBs negative group (p=0.047). However, there was no significant difference in the RF positive rate between the following groups: anti-HBc positive and negative, and HBeAg positive and negative (Table III).

Among HBsAg negative subjects, no significant difference in the RF positive rate was found between the anti-HBs positive and negative groups, or between the group positive for both anti-HBs and anti-HBc and the group positive for only anti-HBs. However, among anti-HBc positive subjects, the RF positive rate was higher in the HBsAg positive group (17.3%, 101/584) than in the HBsAg negative and anti-HBs positive groups (3.0%, 151/5,021) (p<0.001).

RF titers

The RF titers of all the RF positive patients were compared with their HBV antigen or antibody status. The mean titer of RF in the HBsAg positive group

Table III. Rheumatoid factor positive rate among HBsAg positive subjects.

	Subjects, n	RF positive, n(%)	*p*-value
Anti-HBs (+)*	33	10 (30.3)	0.047
Anti-HBs (-)	560	94 (16.8)	
Anti-HBc (+)	584	101 (17.3)	NS
Anti-HBc (-)	9	3 (33.3)	
HBe Ag (+)	119	16 (13.4)	NS
HBe Ag (-)	261	42 (16.1)	

*Subjects who mostly underwent subsequent HBV infection with different subtypes.
RF: rheumatoid factor; NS: not significant.

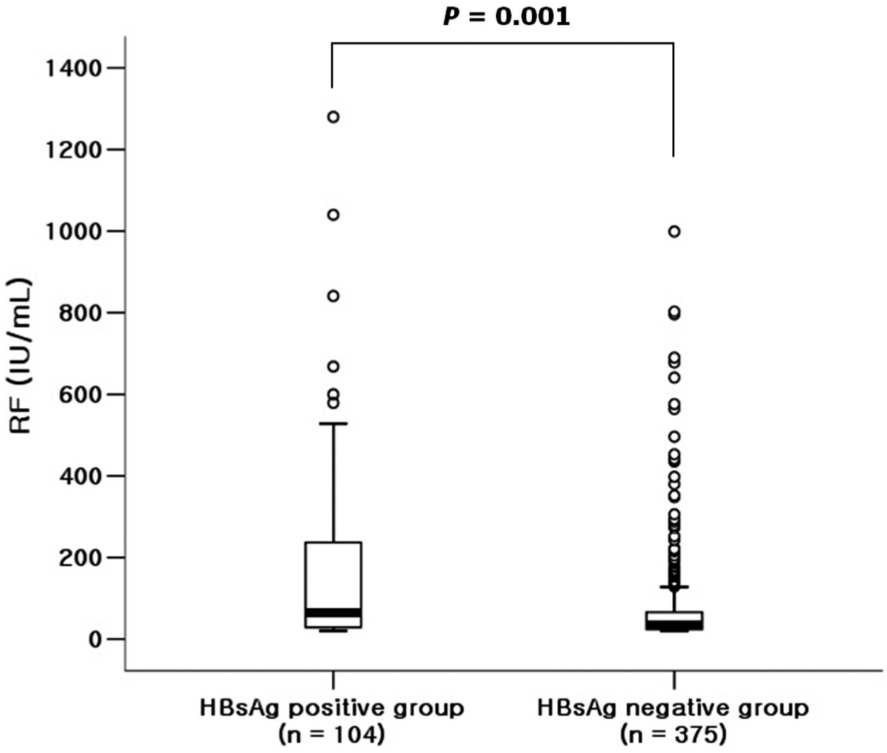

Fig. 2. The titers of rheumatoid factor (RF) according to HBsAg positive status. Titers of RF were significantly higher in the HBsAg positive group than in the HBsAg negative group for RF positive subjects.

was significantly higher than that in the HBsAg negative group (159.7±217.1 IU/mL vs 83.0±179.2 IU/mL, p=0.001) (Fig. 2). A lower RF titer was detected in the anti-HBs positive group than that in the anti-HBs negative group (82.0±141.2 IU/mL *vs*. 121.4±236.0 IU/mL, p=0.032), but no significant differences were observed between the anti-HBc positive and negative groups, and between the HBeAg positive and negative groups (110.2±219.7 IU/mL *vs*. 83.5±132.7 IU/mL, p=0.099; 143.1±224.6 IU/mL *vs*. 178.4±219.0 IU/mL, p=0.489). The HBV DNA levels were significantly correlated with the titers of RF in patients (r=0.508, p=0.005) (Fig. 3).

Discussion

In this study, the RF positive rate was 6-fold higher in HBsAg positive subjects than in HBsAg negative subjects, and HBsAg was positive in 21.7% of RF positive subjects (3.5% of population). Considering that the prevalence rate of RA is about 1% and the RF positive rate in RA patients is about 70-85% (10), the estimated numbers of RF positive subjects from HBV infection are similar to those from RA. Korea is known to be a HBV endemic country. Our results suggest that physicians should be aware that HBV infection is a common cause for the positivity of RF in HBV endemic areas.

The RF positive rate was significantly higher in the group positive for both HBsAg and anti-HBs than in the group positive only for HBsAg. Both HBsAg and anti-HBs may be positive in the following two conditions. One is the process of seroconversion from HBsAg to anti-HBs, which is rare. The majority is when a patient forms anti-HBs in the course of seroconversion after the initial infection with one subtype of HBV, and is subsequently infected with another HBV with a different HBsAg (11, 12). This result therefore shows that the formation of RF is related to persistent HBV infection. This is supported by higher RF positive rates in the group positive for both anti-HBc and HBsAg than in the group positive for both anti-HBc and anti-HBs, which is the condition of seroconversion after HBV infection.

The RF positive rate was lower in the anti-HBs positive group than that in the negative group. The reason for this is that majority of subjects positive for HBsAg were included in the anti-HBs negative group. Moreover, the RF positive rate in both the HBsAg and anti-HBs negative groups showed no significant difference with that in the HBsAg negative and anti-HBs positive groups. Anti-HBs itself could not have prophylactic effects on RF formation. However, those who were immunised may have a lower risk of RF formation. This hypothesis is supported by the result which showed that the RF positive rate was lower in those who had anti-HBs after HBV vaccination than in HBsAg positive subjects.

The reason for the higher positive rate of RF in persistent HBV infected patients has not been clarified. Instead, several mechanisms have been suggested for the formation of RF by HBV: 1) by the cytokine effect induced by viral infection of the cell, 2) by formation of immune complexes of the viral antigen and host antibody, 3) by the virus induced specific immunological effecter mechanism (13).

A hypothesis suggested that RF was caused by the HBeAg-antibody complex (7). However, RF positive rates were not significantly different between the anti-HBe positive and negative groups in that study and in our

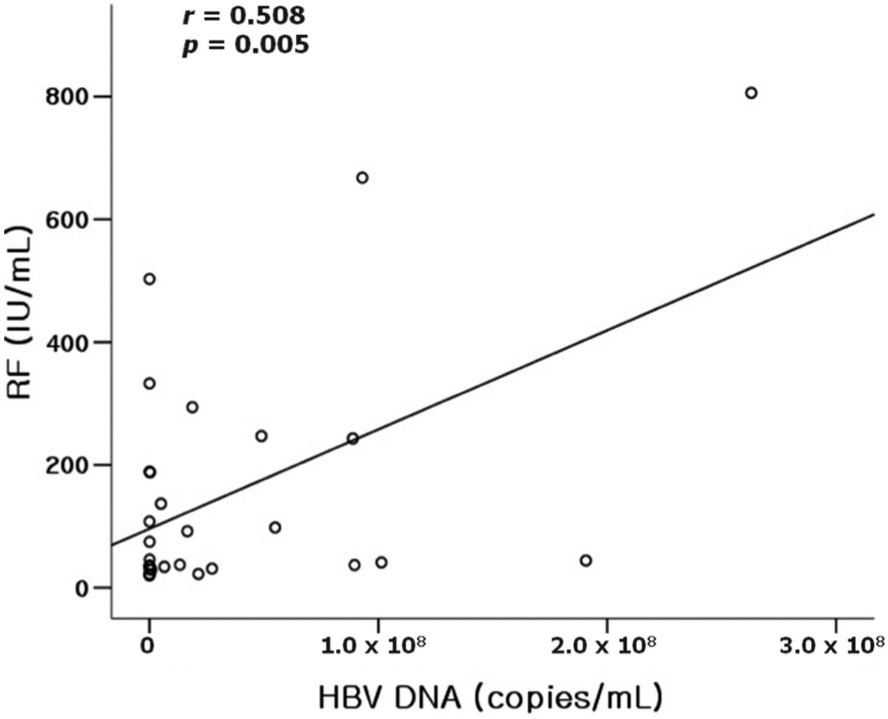

Fig. 3. Correlation between titers of RF and serum HBV DNA levels. Titers of RF were found to be correlated with circulating HBV DNA levels in HBsAg positive patients.

study (7). The titers of RF also showed no significant difference between these two groups in our study. Thus, the HBeAg-antibody complex does not seem to be a key factor in the formation of RF.

Then we can focus the HBsAg as a candidate factor for the RF formation in HBV infected patients. In the natural course of HBV infection, anti-HBs is generated, and free HBsAg in the blood, by way of IgG-bound HBsAg, forms an immune complex with HBsAg for subsequent removal of HBsAg. Some undergo seroconversion to become anti-HBs and some enter the chronic HBV infectious status (14). Some patients with chronic HBV infection may harbor a low level of anti-HBs and form the HBsAg-antibody immune complex (15). In this process, RF may develop in response to these HBsAg-antibody immune complexes. RF was found to bind to the HBsAg-antibody immune complexes (16). Considering that the RF positive rates were higher in patients with persistent HBsAg positive than in the patients who underwent seroconversion after HBV infection, and that RF titers were also higher in the HBsAg positive group than in negative group, we think that individuals with RF are those who did not succeed to clear the virus. Thus, RF appearance could be the signal of a less efficient immune system, and the HBsAg induced specific immunological effecter mechanism could be suggested as an important mechanism in RF formation. Serum HBV DNA levels had a positive correlation with RF titers in both HBsAg and RF positive patients. This result suggests that circulating HBV DNA itself may play an important role as a trigger in RF formation. DNA immunisation is able to raise a range of cell mediated immune response and humoral response, and elicit variable antibodies (17). On the contrary, a recent study reported that HBV developed several escape mechanisms to avoid Toll-like receptor (TLR) 9 activation in both plasmacytoid dendritic cells and B lymphocytes, which may contribute to the persistence of chronic infection (18). However, circulating HBV DNA may activate the innate immune system such as dendritic cells or macrophages through other TLR, consequently activate non-specific B cells. In this respect, we can deduce the reason why the HBsAg positive group had a higher RF titer than the HBsAg negative group. The quantity of HBsAg was known to be correlated with HBV DNA levels during chronic HBV hepatitis (19). Therefore, the RF titers in the HBsAg positive group, which contained some patients with active HBV hepatitis, could be elevated in connection with HBV DNA levels.

In multiple logistic regression analysis, the RF positive rate was increased in the HBsAg positive group as well as in females and older age. Old age may be an independent factor in that RF positivity can be a result from other infectious diseases such as tuberculosis and HCV infection (20, 21). It is interesting that female sex is related to RF formation. There might be a relationship between sex hormones and RF formation, considering that sex hormones are risk factors for RA onset (22). Further studies are needed to assess the potential relationship between sex hormones and RF formation.

Our study has several limitations. First, we could not have the information regarding the presence of RA or other rheumatic diseases in all the RF positive subjects, and HCV positivity in studied population. However, the large number of study subjects was the strength of our study. Second, the IgA type and the IgG type of RF were not measured in this study. Nonetheless, the fact that the IgM type of RF is commonly used in the clinical field, including diagnostic criteria of RA and Sjögren syndrome, was of significance of our study (1, 23). Third, we should exclude RF negative patients when investigating the possible associations between RF titers and other parameters, because the quantitative titers of these patients were not evaluated.

In conclusion, persistent HBV infection is an important cause for the positive RF in HBV endemic areas. The RF positive rate was 6-fold higher in HBsAg positive subjects than in HBsAg negative subjects, and HBsAg was positive in 21.7% of RF positive subjects. The presence of RF is related to HBsAg, female sex and old age, but not to HBeAg. Hepatitis B viral load is associated with RF titer. HBV vaccination may decrease the risk of RF formation.

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