Effects of statins on proinflammatory/prothrombotic biomarkers and on disease activity scores in SLE patients: data from LUMINA (LXXVI), a multi-ethnic US cohort

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Abstract

Objective

We sought to determine the effect of statin therapy on the levels of proinflammatory /prothrombotic markers and disease activity scores in patients with SLE in a multi-ethnic, multi-centre cohort (LUMINA).

Methods

Plasma/serum samples from SLE patients placed on statins (n=21) therapy taken before and after at least 6 months of treatment were tested. Disease activity was assessed using SLAM-R scores. Interleukin (IL)-1β, IL-6, IL-8, tumour necrosis factor (TNF)-α, vascular endothelial growth factor (VEGF) and soluble CD40 ligand (sCD40L) levels were determined by a multiplex immunoassay. Soluble intercellular cell adhesion molecule (ICAM)-1, vascular cell adhesion molecule (VCAM)-1 and anticardiolipin (aCL) antibodies were evaluated using ELISA assays while high sensitivity C-reactive protein (hsCRP) was assessed by nephelometry. Plasma/serum samples from frequency- matched healthy donors were used as controls.

Results

Levels of IL-6, VEGF, sCD40L and TNF-α were significantly elevated in SLE patients versus controls. Statin therapy resulted in a significant decrease in SLAM-R scores (p=0.0199) but no significant changes in biomarker levels were observed. There was no significant association of biomarkers with SLAM-R scores.

Conclusion

Statin therapy resulted in significant clinical improvement in SLE patients, underscoring the use of statins in the treatment of SLE.

Key words

lupus, statins, biomarkers of inflammation, biomarkers of thrombosis.

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Introduction

Systemic lupus erythematosus (SLE) is a systemic autoimmune disease characterised by autoantibodies against mainly intranuclear antigens that effect the abnormal activation of several immune pathways resulting in a heterogenous array of clinical manifestations (1). Abnormal biological activity of cytokines in SLE patients has been highlighted in several studies, in particular the association of proinflammatory cytokines with disease activity and specific clinical manifestations (2). Proinflammatory cytokines that have been shown to be correlated with disease activity in SLE patients include tumour necrosis factor α (TNF-α), soluble CD40 ligand (sCD40L) and interleukin-6 (IL-6) (3-10). Antiphospholipid antibodies (aPL), including anticardiolipin (aCL) and anti-β_2 glycoprotein I (anti-β_2 GPI) antibodies, are also seen in approximately 30–40% of patients with SLE and approximately 50% of those patients develop antiphospholipid syndrome (APS) (11). Vascular endothelial growth factor (VEGF) has been shown to be upregulated in endothelial cells and monocytes of patients with APS and they appear to be associated with the prothrombotic phenotype observed in these patients. (12). Similarly, IL-1, IL-6 and IL-8 have been shown to be upregulated by endothelial cells treated with aPL antibodies *in vitro* and TNF-α to be a cytokine in aPL-mediated pregnancy morbidity in mouse models (13, 14).

Statins have been shown to have numerous pleiotropic effects in addition to reducing cholesterol levels, all these effects contributing to the observed benefit in primary and secondary prevention of coronary heart disease and ischaemic stroke (15). The direct effect that statins have on endothelial expression of adhesion molecules, plaque formation and thromboxane synthesis may account for their beneficial cardiovascular effects with reduction in disease activity in SLE and APS patients and concordantly their increased use (16-18). There are limited and controversial data highlighting the effect this drug may have on disease activity scores (19). Furthermore, in a prior study from the LUMINA cohort an association between statin use and changes in disease activity could not be demonstrated (20). However, capturing subtle changes across the spectrum of SLE-related manifestations measured by the SLAM-R alone may be difficult. As such, we sought to determine the proinflammatory biomarker profile in patients from the LUMINA (LUpus in MInorties, NAture *vs*. nurture) cohort, a longitudinal study of outcome in SLE patients, and its relationship with disease activity using the actual differences in the scores rather than a predefined difference as previously done (13), to determine if changes in disease activity have occurred.

Methods

Patients

Patients were selected for inclusion in the study from the LUMINA cohort. LUMINA is a longitudinal study of outcome of multi-ethnic [Hispanic (Mexican/ Central American and Puerto Rican), African American and Caucasian] SLE patients enrolled within 5 years of fulfillment of the American College of Rheumatology (ACR) criteria at participating institutions in Alabama, Houston, Galveston and Puerto Rico (21, 22). Patients had clinical and laboratory evaluations which included blood samples being drawn and disease activity assessments using the Systemic Lupus Activity Measure-Revised (SLAM-R) performed at 6 month intervals for the first year of enrolment and annually thereafter. Patients placed on statin therapy at any time during follow-up were included in this study if serum or plasma samples that were taken before and after the commencement of therapy, at least 6 months apart and stored at -20°C, were available for testing. Since this study was not a clinical trial, the patients used whatever statin was prescribed by their treating physicians (and the dose that they felt necessary). Exclusion criteria included concurrent use of immunosuppressive drugs such as azathioprine, cyclophosphamide, mycophenolate mofetil, methotrexate, cyclosporine, rituximab or prednisone at doses greater than 10mg/day and hydroxychloroquine (HCQ) at baseline and at the follow-up visit. None

of the 21 patients were on angiotensin converting enzyme inhibitors or angiotensin II receptor blocker therapy. Serum samples taken from 32 frequency-matched controls with no evidence of autoimmune or inflammatory disease (85% females, age range 18–65) were identified from a databank of healthy persons at UTMB and those served as a comparison to baseline results only in SLE patients. These control subjects were identified from a databank of healthy persons at the University of Texas Medical Branch (UTMB).

The LUMINA study had been conducted following the declaration of Helsinki guidelines for inclusion of humans in research. All subjects had provided informed consent.

Antiphospholipid and biomarker testing

An enzyme-linked immunosorbent assay (ELISA) method was used to measure anticardiolipin (aCL) antibodies, IgG and IgM isotypes, as previously described (23) using a commercial kit (Louisville APL Diagnostics, Inc. Seabrook, TX, USA). Anti-β_2 GPI antibodies of the IgG and IgM isotypes were determined using a commercial assay (INOVA Diagnostics, Inc. San Diego, CA). Intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1) serum or plasma levels were assessed using a commercial ELISA kit (R&D systems, Minneapolis, MN). High sensitivity C-reactive protein (hsCRP) was determined using nephelometry (IMMAGE Immunochemistry System, Beckman Coulter).

The MILLIPLEXMAP human cytokine/chemokine panel assay (Millipore, Billerica, MA) which utilises Luminex xMAP technology was used to determine serum/ plasma levels of the cytokines IL-1β, IL-6, IL-8, TNF-α, VEGF and sCD40L. Briefly, 25μL of patient serum or plasma was incubated with color-coded bead sets, each set having a distinct internal fluorescent dye and a distinct coat of capture antibodies specific for one of the analytes being tested. A biotinylated detection antibody was then introduced followed by incubation with streptavidin-phycoerythrin which acted as the reporter molecule on the surface of each microsphere. Distinct lasers were used to excite the internal dyes marking each microsphere set and the dye in the reporter molecule followed by high speed digital signal processing to quantify the reporter signals from each bead set.

The normal ranges of the biomarkers that were not stated in the package insert of the kits (cytokines, tissue factor, cellular adhesion molecules and chemokines) were determined as the 95th percentile of 32 healthy controls for each assay.

Statistical analyses

The Kruskal-Wallis test was used to compare cytokine levels in SLE patients with those in controls. A signed rank test was used to calculate the effect of drug treatment on cytokine and disease activity levels. Spearman correlation was used to compare changes in levels of biomarkers with changes in SLAM-R. All *p*-values less than 0.05 were considered to be significant.

Results

Patient demographics

After application of selection criteria 21 patients were included in this study [86% women, their mean (range) age was 44.6 (16–67) years]. Caucasians (9/21, 43%) and African Americans (8/21, 38%) accounted for the majority of patients, the remaining patients being Puerto Rican (4/21, 19%). There were no Mexican or Central American Hispanic patients in this analysis group.

Proinflammatory cytokines in SLE patients and controls

The levels of proinflammatory cytokines were strikingly different in our SLE patients at baseline compared to controls. Median levels of IL-6 (9.84 *vs*. 0.00), VEGF (261.19 *vs*. 88.29), sCD40L (1737.41 *vs*. 16.35) and TNF-α (7.19 *vs*. 0.00) were significantly elevated in SLE patients at baseline versus controls ($p<0.0001$–0.0002). Mean levels of IL-6 (114.92 *vs*. 0.70), VEGF (453.99 *vs*. 113.58), sCD40L (28342.33 *vs*. 24.66), and TNF-α (43.50 *vs*. 0.46) were also significantly elevated in SLE patients at baseline versus controls ($p<0.0001$–0.0002). Although not statistically significant, mean levels of IL8 (69.80 *vs*. 40.80, $p=0.5552$),and IL1 β (32.84 *vs*. 0.33, $p=0.10676$) were elevated in SLE patients at baseline when compared to controls. To determine the number/percentage of SLE patient samples elevated for each biomarker, we considered a cut-off value, above which samples were considered elevated, to be the 95th percentile of the levels obtained in the 32 control patients. The proportion of SLE patients' samples with elevated levels for each biomarker was: sCD40L (91%), VEGF (77%), IL6 (57%), TNF-α (55%), IL8 (32%), and IL1 β (32%). High sensitive CRP was elevated in 50% of the patients at baseline, when compared to controls. Likewise, aCL IgG, aCL IgM and anti-β_2 GPI IgG and IgM were elevated in 64%, 13%, 65 % 45% of the SLE subjects respectively. There was no correlation of any of the biomarkers at baseline with SLAM-R scores.

Effect of statin therapy

The effect of statin therapy on biomarker levels and SLAM-R are shown in Table I. Treatment with statins produced a significant decrease in median SLAM-R scores $p=0.0199$). Fourteen patients (66.66%) had decreased SLAM-R scores in response to statin therapy, 4 patients (19.05%) had increased scores and 3 patients (14.29%) had no change. Median levels of sCD40L, VCAM-1 and VEGF decreased with statin therapy by 46.5%, 17.9% and 13.8%, respectively. All other biomarkers and antiphospholipid antibodies either remained the same or had increased levels following treatment. However, none of these changes in biomarkers or antibodies were statistically significant. There were no significant correlations observed between changes in biomarker levels and SLAM-R scores.

Discussion

We provide evidence confirming the presence of elevated pro-inflammatory cytokines in SLE patients and we also demonstrated that statin therapy may result in decreased disease activity levels in SLE patients as measured by SLAM-R scores. Although there is no agreement on a single disease activity

Table I. Effect of statins therapy in SLE patients on biomarker levels and on disease activity scores.

Biomarker	Before Rx/median	After Rx/medan	*p*-value
IL6 (pg/ml)	9.81	23.93	0.3484
IL8 (pg/ml)	32.10	50.70	0.6812
VEGF (pg/ml)	350.17	301.95	0.7372
sCD40L (pg/ml)	1089.58	583.45	0.9199
IL1 β (pg/ml)	0.00	0.00	0.7646
TNF-α (pg/ml)	5.49	7.40	0.7756
hsCRP (mg/L)	0.63	0.64	0.6094
ICAM1 (pg/ml)	9.62	11.41	0.2688
VCAM1 (pg/ml)	48.36	39.71	0.4684
aCL IgG (GPL)	10.98	9.15	0.1232
aCL IgM (MPL)	6.10	5.84	0.1336
aCL IgA (APL)	0.67	0.50	0.7337
SLAM-R	6	3	0.0199

index to be used universally, the SLAM-R score has been validated as a reliable and sensitive indicator of change in disease activity in SLE patients (24).

In our selected SLE patients, IL-6, VEGF, sCD40L, TNF-α and IL-1β were elevated compared to levels in control patients. The cytokines and adhesion molecules VEGF, VCAM-1 and ICAM-1 have been associated with thrombosis in SLE and APS patients (5-10). It is interesting to note that over 2/3 of the selected patents were positive for aPL antibodies. Soluble CD40 ligand and IL-6, have been associated with increased disease activity in lupus patients (4).

Our results suggest that statin treatment may play a role in improving disease activity scores in SLE patients and potential explanatory mechanisms include reduction of proinflammatory cytokines, co-stimulatory and adhesion molecules and the inhibition of MHCII mediated T cell activation. Statins interfere with aPL-mediated thrombosis by preventing the expression of cell adhesion molecules and IL6 in aPL-treated endothelial cells (25-28). In our ongoing mechanistic study (ClinicalTrials.gov Identifier: NCT00674297), which examines the potential efficacy of fluvastatin in reducing elevated proinflammatory/prothrombotic markers in APS patients, preliminary analysis has shown a variable but significant decrease in VEGF, soluble tissue factor (sTF) and TNF-α as a result of treatment (29). In a separate cross-sectional study, mean levels of TNF-α, VEGF and sTF were significantly elevated in the sera of 93 APS patients when compared to 60 controls (30). Utilising a proteomic analysis, the authors also showed that the pattern of protein expression in monocytes from aPL-positive patients (25 with APS and 10 asymptomatic) significantly changes following one month of 20 mg daily fluvastatin (30).

The reductions in sCD40L, VCAM-1 and VEGF levels as a result of statin therapy in our patients were not statistically significant and were not associated with the decrease observed in disease activity scores. Interestingly, the cell adhesion molecule ICAM1 and hsCRP, a potential marker for cardiovascular risk in SLE, remained unchanged after statin therapy. The small sample size in our study may have been a contributing factor in the failure of the observed effects of statin therapy on several biomarkers to achieve statistical significance. It is also very possible that the observed beneficial effects of statins on disease activity levels may be significantly associated with changes in cytokines only when particular subgroups of SLE patients are examined. For example, VEGF is most strongly associated with disease activity in SLE patients that have evidence of microvascular changes and systemic organ involvement and hsCRP levels are significantly associated with independent markers of cardiovascular risk such as high body mass index (BMI), hypertension and lipoprotein levels in SLE patients (5, 31).

Indeed, there is still debate as to whether SLE is a single disease with myriad phenotypes or a collection of different diseases of diverse pathogenic mechanisms producing a similar phenotype (32). The heterogeneity of the disease underlies the need for the individualised approach to patient management and further study on the complex interplay of genetic, hormonal and environmental aspects in different subgroups is needed. This would allow for better classification of patients and in so doing help to define the role of various agents, including statins, in preventive and therapeutic approaches to patient care (32). Early classification and treatment of patients is of paramount importance since this results in reduced morbidity and mortality; although the risk for disease flare and damage development is still substantial in some of these patients (33). Unfortunately, the relatively small sample size in our study precluded us from performing sub-analyses relative to either specific disease manifestations or different cut-offs of the SLAM-R scores.

In trying to reconcile the results of the current study with those from a similar analysis on the same cohort data (20), important differences in the study design must be considered. In that study, a change in disease activity was defined as a decrease in the SLAM-R score of ≥4 points whereas in the current study, the absolute difference was used. It is possible that the measure of change used in the earlier study was only able to capture large changes whereas the statistically significant results in the current study reflect the fact that the majority of patients that had reductions in disease activity experienced only small changes.

The recent Atherosclerosis Prevention in Paediatric Lupus Erythematosus (APPLE) and Lupus Atherosclerosis Prevention Study (LAPS) trials both assessed the efficacy of atorvastatin in reducing subclinical and clinical measures of atherosclerosis in pediatric and adult SLE populations respectively. In contrast to our findings, the APPLE trial found that despite significant decreases in total cholesterol, low density lipoprotein (LDL) and hsCRP levels, atorvastatin had no effect on subclinical atherosclerosis progression while the LAPS study found no significant differences between treatment and placebo

groups with respect to disease activity, measures of inflammation or endothelial cell activation in addition to measures of atherosclerosis progression (34, 35). A similar study assessing rosuvastatin use in SLE patients also found no effect on disease activity or measures of atherosclerosis progression but a subgroup analysis of patients with mild to moderate disease revealed significant reductions in hsCRP and thrombomodulin levels (35). It is interesting to note that although the APPLE trial did not demonstrate a statistically significant change in subclinical progression of disease with statin therapy, the results did suggest a trend in the direction of a positive effect. This is perhaps a further indication that only particular subsets of SLE patients will benefit from statin therapy, which is supported by the results of subgroup analysis in the rosuvastatin trial. Further work needs to be done in identifying those patients most likely to benefit from statin therapy, perhaps by assessment of independent risk factors for cardiac disease progression (36).

As stated previously, a potential limitation of the study is that strict inclusion/exclusion criteria as well as limited availability of stored samples resulted in the small number of patients selected and consequently may have prevented several of the observed comparisons from attaining statistical significance. Another contributing factor is the fact that treatment of SLE with statin drugs, although relatively common today, was not frequently used over 10 years ago limiting the potential number of patients to be selected. Further considerations include the fact that the observed elevations in the cytokines may represent isolated peaks rather than consistent elevations and that the measurement of some biomarkers such as VCAM1 and ICAM1 in serum may not have been representative of actual elevations at the level of the endothelium.

Despite these potential limitations, we have shown that statin therapy resulted in statistically significant clinical improvement in SLE patients as measured by reductions in SLAM-R scores and may be beneficial in the treatment of lupus patients.

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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).

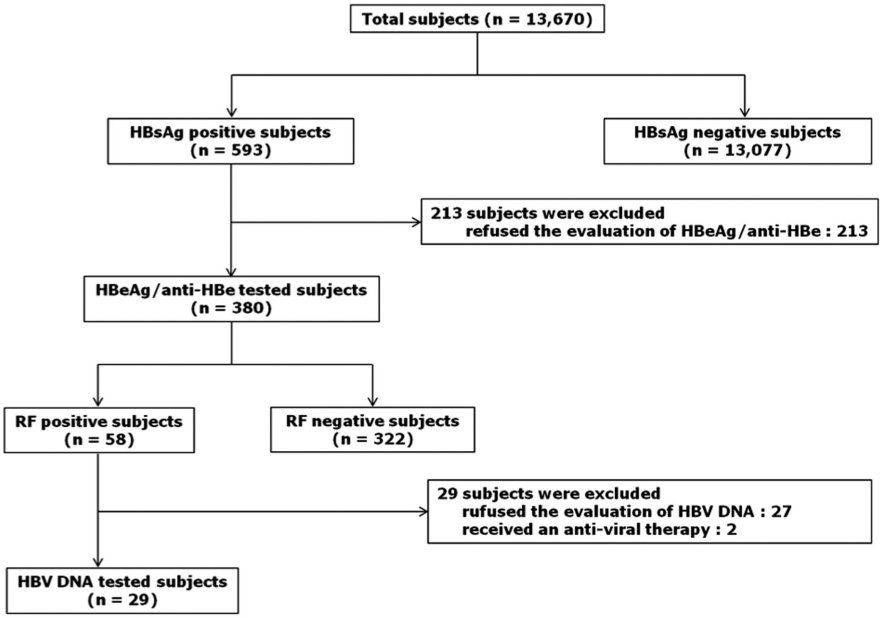

Fig. 1. Schematic diagram of the study design. RF, rheumatoid factor.

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.

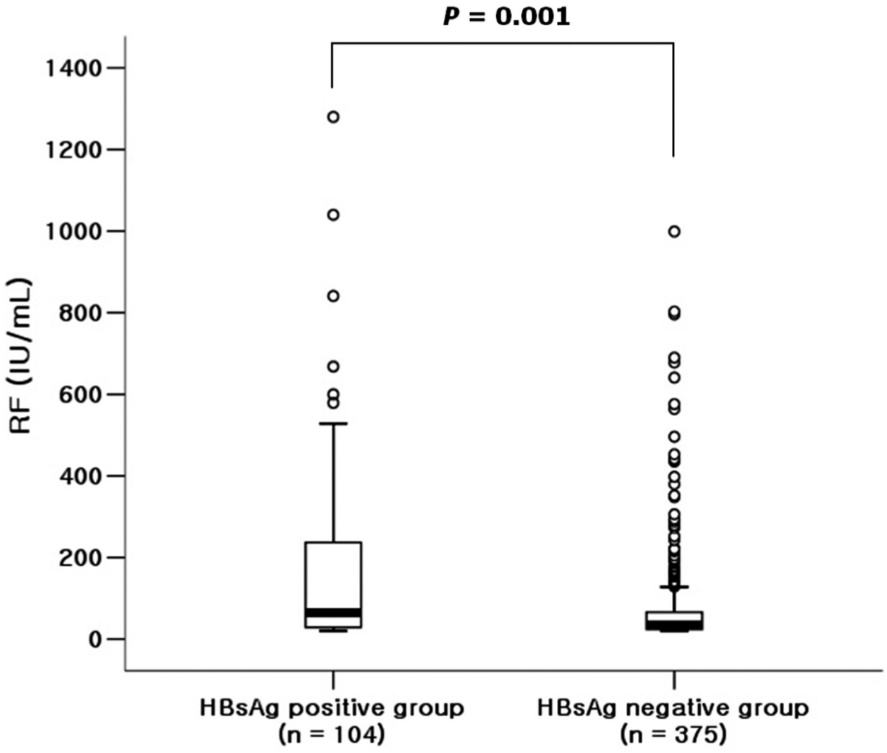

Fig. 2. The titers of rheumatoid factor (RF) according to HBsAg positive status. Titers of RF were significantly higher in the HBsAg positive group than in the HBsAg negative group for RF positive subjects.

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

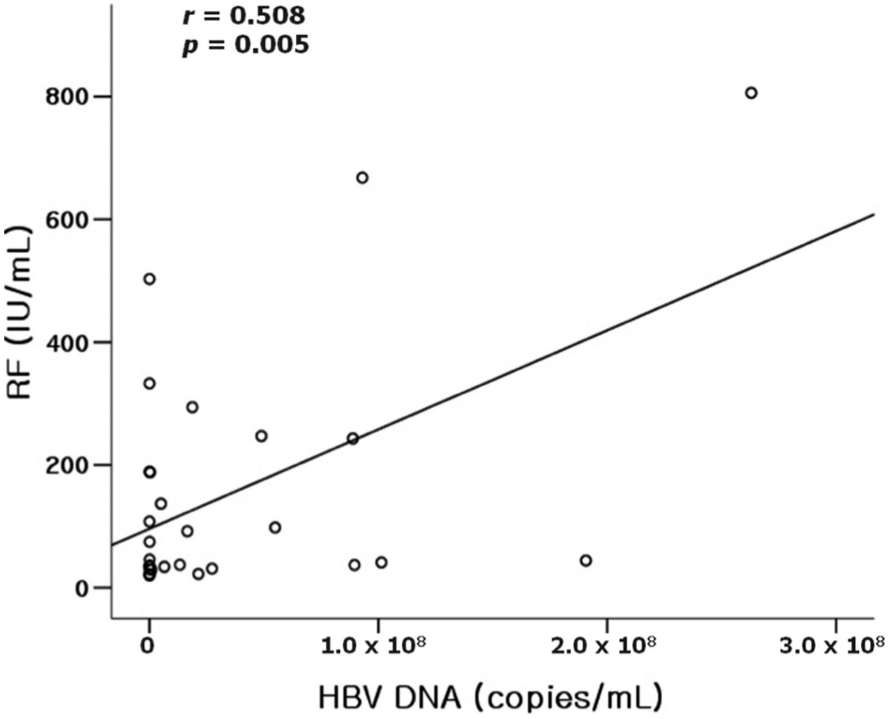

Fig. 3. Correlation between titers of RF and serum HBV DNA levels. Titers of RF were found to be correlated with circulating HBV DNA levels in HBsAg positive patients.

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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