Global Journal of Science Frontier Research
Bio-Tech & Genetics
Volume 13 Issue 3 Version 1.0 Year 2013
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals Inc. (USA)
Online ISSN: 2249-4626 & Print ISSN: 0975-5896

Role of Hepatitis B Virus Surface Antigen Quantification in E Antigen Negative Chronic Hepatitis B Infection

By R. M. Mukherjee, Jyothi Arava, P. Balkumar Reddy, P. N. Rao, R. Gupta & D. N. Reddy

Asian Health Care Foundation, India

Abstract- *Background/Aim:* Hepatitis B surface antigen(HBsAg) is routinely detected qualitatively in Hepatitis B Virus(HBV) infection where its persistence beyond 6 months defines chronic hepatitis B(CHB) infection. Hepatitis B e antigen (HBeAg) usually indicates active HBV replication and risk of transmission of infection. In spite of the emerging use of chemiluminescence based quantitative HBsAg assays for therapeutic monitoring of the patients, we measured HBsAg concentration by sandwich ELISA as a cost effective alternative to ascertain the role of HBsAg levels as surrogate marker of untreated HBeAg negative subjects having CHB infection.

Keywords: *HBsAg, quantification, ELISA, viral load, HBeAg negative, CHB infection.*

GJSFR-G Classification : *FOR Code: 270899*

Strictly as per the compliance and regulations of :

Role of Hepatitis B Virus Surface Antigen Quantification in E Antigen Negative Chronic Hepatitis B Infection

R. M. Mukherjee [α], Jyothi Arava [σ], P. Balkumar Reddy [ρ], P. N. Rao [ω], R. Gupta [¥] & D. N. Reddy [§]

Abstract- *Background/Aim:* Hepatitis B surface antigen (HBsAg) is routinely detected qualitatively in Hepatitis B Virus (HBV) infection where its persistence beyond 6 months defines chronic hepatitis B(CHB) infection. Hepatitis B e antigen (HBeAg) usually indicates active HBV replication and risk of transmission of infection. In spite of the emerging use of chemiluminescence based quantitative HBsAg assays for therapeutic monitoring of the patients, we measured HBsAg concentration by sandwich ELISA as a cost effective alternative to ascertain the role of HBsAg levels as surrogate marker of untreated HBeAg negative subjects having CHB infection.

Methods : Sixty two subjects were evaluated for serum HBeAg,anti HBe and ALT status by standard ELISA and biochemical procedures.The amount of serum HBV DNA determined by real time TaqMan PCR assay(Roche Diagnostics,USA).Serum HBsAg level(ng/ml) was ascertained by a third generation sandwich ELISA kit(Alpha Diagnostics International,USA)and results expressed upon conversion to IU/ml.

*Results :*The median age of the subjects was 40.5 years(IQR=18;90% male) of which 92% were HBeAg negative and anti-HBe positive.Median ALT value was 35.5 IU/ml(IQR=37).Median viral load(Log copies/ml)and HBsAg(IU/ml) were 4.57(IQR=2.84)and 38002.3 (IQR=23736.8) respectively. When grouped on the basis of viral load(<2000 IU/ml>),the median HBsAg concentration seen is 35439.3 (IQR=43093.3) and 44712.7(IQR=25082.4) while median ALT values were 29(IQR=21.2)and 47(IQR=73.2) in lower(<2000 IU/ml) and higher(>2000 IU/ml)viral load groups respectively.

Conclusion : Serum HBsAg concentration showed no correlation with serum HBV DNA level in our study subjects which is at par with other studies on CHB patients. Based on the reported correlation between serum HBsAg level and intrahepatic ccc DNA, higher amount of HBsAg in subjects having lower viral load is indicative of the presence of higher amount of intrahepatic HBV DNA in these subjects which warrant further study. Quantitation of HBsAg by ELISA can be a cheaper alternative to chemiluminescence assay to be used as a surrogate marker differentiating certain phases of CHB infection.

Keywords: HBsAg, quantification, ELISA, viral load, HBeAg negative, CHB infection.

Author α: Institute of Basic and Translational Research, Asian Health Care Foundation India. e-mail: rathinmukh@gmail.com
Authors σ ρ: Asian Health Care Foundation.
Authors ω ¥ §: Asian Institute of Gastroenterology.

I. Introduction

The prevalence of Hepatitis B virus (HBV) infection in terms of the prevalence of Hepatitis B surface antigen (HBsAg) in the general population of Asia, Africa, Southern Europe and South America ranges from 2 to 20%.(1).While the persistence of HBsAg for more than 6 months defines chronic HBV state,its clearance from serum ,is considered the nearest-to-cure outcome of HBV infection. Hepatitis B e antigen (HBeAg) usually indicates active HBV replication and risk of transmission of infection. Although seroconversion from HBeAg to anti-HBe is usually associated with remission of liver disease, a certain proportion of anti-HBe-positive patients being harboring precore/core promoter mutations, continue to have viral replication with ongoing progression of the disease. Thus, studies regarding the prevalence and clinical significance of HBeAg-negative chronic hepatitis B (CHB) have been increasingly gained importance. Despite the usefulness of the routine qualitative detection of HBsAg as the first serological marker of the disease, recent studies indicate the importance of quantitative kinetic measurement of HBsAg for therapeutic monitoring particularly for the patients who are negative for HBeAg (2,3). HBsAg quantification is easy, cheap and may provide a mean to establish the prognosis of antiviral therapy in the future. moreover, HBsAg quantification appears to be a surrogate marker of the amount of covalently closed circular DNA (cccDNA), the persistent intrahepatic form of HBV DNA in the hepatocytes and a predictor of a sustained virological response to antiviral treatment off therapy(4,5). Despite the added clinical importance of HBsAg clearance, this has not been included as a primary endpoint in treatment trials due to the low frequency of its occurrence. The majority of nucleoside analogue studies, even with prolonged therapy, have demonstrated rates of HBsAg clearance comparable to those observed naturally i.e. between 1%-2% and 0.5%-1% annually for HBV carriers in western and Asian countries respectively (6, 7).Based on the above fact, one can envision scenarios where measuring HBsAg concentration could be an important, additive tool to currently used HBV DNA testing. The development of commercial assay systems particularly

the assay based on chemiluminescence (Architect, Abbott Diagnostics, USA) which has been used so far to quantify HBsAg concentration, provided an opportunity to compare the kinetics of HBsAg decline during interferon and nucleoside analogue therapy(2,3,5).In the present study, in order to ascertain the role of quantitative measurement of serum HBsAg for use as surrogate marker to viral load and ALT levels ,we attempted to evaluate HBsAg level in treatment naïve CHB patients with special reference to HBeAg negative anti HBe subjects. Due to non availability of a chemiluminescence platform (Architect, Abbott Diagnostics) for the said assay, we used a third generation quantitative sandwich ELISA kit(Alpha Diagnostics International, USA) as a cheaper alternative to measure HBsAg concentrations in our study subjects representing inactive HBsAg carrier state and CHB infections (8).

II. Materials and Methods

a) Subjects

A total of 62 subjects who attended the out patient's department of the Asian Institute of Gastroenterology, Hyderabad, India and were positive for HBsAg for more than six months were included in this study. Apart from the recording of demographic patterns and respective clinical investigations, five milliliter of blood was taken from each study subject to assess the various biochemical, serological anad virological parameters. Informed consent was taken from each study subjects prior commencement of the study.

b) Serological and Biochemical parameters

Serum HBeAg and anti HBe and status of the study subjects were determined by commercial ELISA kits(Amar-EASE, Taiwan) as per manufacturer's instructions. ALT value was ascertained by automated biochemistry analysis system (RandoxUSA).

c) Quantitation of serum HBV DNA

Extraction of viral DNA from patient's sera was brought out using High Pure System Viral Nucleic Acid kit(Roche Molecular Systems, NJ, USA) as per the manufacturer's instructions. Amplification and subsequent quantification of extracted DNA were performed by the COBAS® TaqMan® HBV Test using a COBAS® TaqMan® 48 Analyzer(Roche Molecular Systems, NJ, USA) which has a lower limit detection of 6 IU(35 copies) of HBV DNA/ml.

d) Quantitation of serum HBsAg

Serum concentration of HbsAg was measured by a sandwich ELISA kit(Alpha Diagnostic International, USA)as per manufacturer's instructions. The aforesaid kit meets the requirements for a 3rd generation test upon testing against the FDA reference panel. Performance of this kit compared with a commercial test kit (Abbot Auszyme II) and supposed to detect all possible combinations of HBsAg subtypes(adw, adr, ayw, ayr)having a lower limit of sensitivity of 0.3 ng/ml as per manufacturer's declarations..For convenience, HBsAg concentrations measured in ng/ml was converted to IU/ml considering 0.15ng/ml of HBsAg is equivalent to 350IU/ml(9).

e) Statitical analysis

Descriptive statistics (mean,median,standard deviations and interquartile ranges), Student's t test and Fisher's exact tests were performed as and where applicable using SPSS software ,version 13.0 (SPSS, Inc., Chicago, IL). A value of $P<0.05$ was considered statistically significant.

III. Results

Baseline features of the 62 study subjects are depicted in Table 1. The median age of the subjects was 40.5 years(IQR=18).Majority(90%)were male and most of them(92%)were already seroconverted from HBeAg positive to HBeAg negative status with concomittant anti-HBe positivity. The median ALT value noted was 35.5 IU/ml(IQR=37) while the median viral load(Log copies/ml) and HBsAg(IU/ml) concentrations were 4.57(IQR=2.84)and 38002.3 (IQR=23736.8) respectively. When grouped on the basis of viral load (<2000 IU/ml>),the median HBsAg concentration seen is 35439.3 (Range 815.5-111141;IQR=43093.3) and 44712.7(Range 723-111141; IQR=25082.4) while median ALT values were 29 (Range 10-184;IQR=21.2) and 47(Range 12-372;IQR=73.2) in lower (Group 1≤2000 IU/ml,n=32) and higher viral load (Group 2 ≥2000 IU/ml,,n=30) groups respectively(Fig.1).Higher ALT values observed with increasing viral load as evident from significant differences(p=0.017) of ALT values between the higher and lower viral load groups which was not reflected by the corresponding levels of HBsAg between these groups. In group 1(≤ 2000 IU/ml viral load),21.8% subjects had elevated ALT values (≥ 40 IU/ml, median=65.0) having median HBsAg level of 31757.9(log 4.50) IU/ml while in group 2(≥2000 IU/ml viral load), 36.6% subjects showed lower ALT values (≤ 40 IU/ml; median=32.0) with a median HBsAg concentration of 36441.2(log 4.56) IU/ml thus showing serum HBsAg concentration is independent of serum HBV DNA and ALT levels in these subjects.

IV. Discussion

Apart from its use as a routine diagnostic marker for HBV induced liver disease,the predominantly spherical pleomorphic particles of HBsAg are suggested to be more prevalent than complete virions of HBV(10) and this excess surface protein might have implications towards chronicity of the disease(11).The

estimation of HBsAg concentration in sera of HBV infected subjects started long back(12) and prior to the advent of nucleic acid testing, quantitation of HBsAg was found to be a significant tool for predicting the outcome of an acute HBV infection(13). Despite its reported usefulness in predicting therapeutic response to interferon(14),the labour intensive procedures of such quantitative measurements(15) hampered the wide spread application of such assays. Quantitative measurement of serum HBsAg based on ELISA and chemiluminescence microparticle assay(CIMA)has been used (16,17) which recently gained importance providing level of HBsAg as a potential marker for monitoring therapeutic responses(2,3).Furthermore, the role of serum HBsAg quantification in distinguishing inactive carriers from the subjects having active form of the disease along with observations on significant differences in median baseline serum HBsAg titres across the different phases of CHB has also been implicated (18,19). The serum level of HBV DNA is thus being revisited as a dynamic parameter in chronic HBV and now becoming a vital part of the pre-treatment evaluation and assessment of the efficacy of antiviral treatment. Measurement of serum HBV DNA is the common tool to monitor treatment response. While the level of circulating HBV DNA testing has been shown to be the strongest predictor of the development of cirrhosis and hepatocellular carcinoma (HCC) (20,21), non association of serum HBV DNA with the histologic activity in patients with HBV-related decompensated cirrhosis has also been reported(22). In spite of the conflicting reports (5,16,17) regarding the correlation between serum HBV DNA and serum HBsAg concentrations, we tried to evaluate the same in treatment naïve CHB patients. In our study,correlation between serum HBV DNA and serum concentration of HBsAg was not evident, a similar observation noted in other studies(16,17) while such correlation(canonical correlation)in small number of subjects being reported by others(5).Since the majority(90%)of our study subjects already seroconverted from HBeAg positive to negative status with associated anti HBe positivity, the differentiating level of HBsAg between HBeAg positive and negative group of patients could not be ascertained in this study. Genotype related variation of HBsAg levels have been documented where median values of 4.11(log10) IU/ml and 3.85(log10)IU/ml measured by CIMA were shown to be present in patients infected with genotypes A and D respectively(2). In our study, the median HBsAg concentration measured by ELISA was 4.63(log10) IU/ml in a setting where genotype D is predominant(23).Interestingly, quantitative measurement of serum HBsAg gained importance as a surrogate marker for viral covalently closed circular DNA (cccDNA) and intrahepatic HBV DNA which are belived to maintain the replicative form of HBV DNA hence maintain the chronic form of the disease. The occurrence of viral reactivation observed in a significant proportion of patients who appear negative for serum HBV DNA after combined treatment course of peginterferon and lamivudine(24) seem to reflect the inadequacy of serum HBV DNA to represent the level of covalently closed circular DNA (cccDNA) and total intrahepatic HBV DNA as ultimate predictor of sustained virological response (25). Changes in ccc DNA were correlated with a similar reduction in serum HBsAg titre measured by ELISA during adefovir treatment (26). Furthermore, serum HBsAg levels shown to correlate well with the cccDNA and intrahepatic HBV DNA where patients with lower baseline cccDNA, intrahepatic HBV DNA, and HBsAg level but not serum HBV DNA level tend to develop sustained virologic response to peginterferon and lamivudine combination therapy(4).In light of the above mentioned fact,and based on our data of HBsAg levels in lower and higher viral load groups,it can be stated that the subjects in our study, having higher levels of HBsAg though differ significantly by their serum HBV DNA concentrations but might harbor similar amount of ccc DNA/intrahepatic HBV DNA pool in their liver. Considering a serum viral load of > 10000 copies/mL (2000 IU/mL) is a strong risk predictor of HCC, independent of HBeAg status, ALT level and liver cirrhosis (21,27),monitoring of our patients having higher level of HBsAg but comparatively lesser amount of serum HBV DNA is warranted. Our data showed a number of patients having higher levels of serum HBsAg and trend of elevated ALT but lesser amount of serum HBV DNA, thus, support a potential role of quantitative estimation of serum HBsAg in differentiating consecutive phases of CHB (18) that reflect the natural course of HBV infection. Finally, in corroboration to our previous report (28),our study demonstrated that serum HBsAg quantification assay is unable to substitute serum HBV DNA estimation in treatment naïve CHB patients but can be used as a surrogate marker of disease manifestations. Furthermore, in comparison to the chemiluminescence based assays, this ELISA based quantitation of serum HBsAg can be used as a cheaper alternative to monitor the therapeutic responses in developing countries like India.

References Références Referencias

1. Kao, J.H & Chen, D.S.(2002). Global control of hepatitis B virus infection. Lancet Inectf. Dis, 2, 395-403.
2. Brunetto, M.R., Moriconi, F., Bonino, F., Lau, G.K., Farci, P., Yurdaydin, C., Piratvisuth, T., Luo, K., Wang, Y., Hadziyannis, S., Wolf, E., McCloud, P., Batrla, R., Marcellin, P.(2009). Hepatitis B virus surface antigen levels: a guide to sustained response to peginterferon alfa-2a in HBeAg

negative chronic hepatitis B. Hepatology, 49,1141-50.

3. Moucari, R., Mackiewicz, V., Lada, O., Ripault, M.P., Castelnau, C., Martinot-Peignoux, M., Dauvergne, A., Asselah, T., Boyer, N., Bedossa, P., Valla, D.,Vidaud, M., Nicolas-Chanoine, M.H., Marcellin, P. (2009).Early serum HBsAg drop: a strong predictor of sustained virological response to pegylated interferon alfa-2a in HBeAg-negative patients. Hepatology,49,1151-7.
4. Chan, H.L., Wong, V.W., Tse, A.M., Tse, C.H., Chim, A.M., Chan, H.Y.,Wong, G.L. et al. (2007).Serum hepatitis B surface antigen quantitation can reflect hepatitis B virus in the liver and predict treatment response. Clin Gastroenterol Hepatol, 5, 1462-1468.
5. Ozaras, R., Tabak, F., Tahan, V., Ozturk, R., Akin, H., Mert, A., Senturk, H.(2008). Correlation of quantitative assay of HBsAg and HBV DNA levels during chronic HBV treatment. Dig Dis Sci, 53,2995-2998.
6. Realdi, G., Fattovich, G., Hadziyannis, S., Schalm, S., Almasio, P., Sanchez-Tapiaas, J, et al.(1994).Survival and prognostic factors in 366 patients with compensated cirrhosis type B: A multicenter study. The Investigators of the European Concerted Action on Viral Hepatitis (EUROHEP). J Hepatol, 21, 656-666..
7. Chu, C.M & Liaw, Y.F. (2007).HBsAg seroclearance in asymptomatic carriers of high endemic areas: Appreciably high rates during a long-term follow up. Hepatology , 45, 1187-1192.
8. Lok, A.S.F & McMahon, B.J. (2009).AASLD Practice Guidelines. Chronic Hepatitis B: Update 2009. Hepatology, 50,1-36.
9. Biswas, R., Tabor, E., Hsia, C.C., Wright, D.J, Laycock, M.E., Fiebig, E.W., Peddada, L, et al. (2003).Comparative sensitivity of HBV NATs and HBsAg assays for detection of acute HBV infection. Transfusion, 43, 788-798.
10. Gerlich, W.H & Kann, M.(2005). Hepatitis B.in Topley Wilson's Microbiology and Microbial Infections,9th Edition. Edited by L. Collier, A. Ballows and M. Sussmann. London, UK. Edward Arnold Ltd.2005;pp:1226-1268.
11. Block, T.M., Mehta, A.S., Blumberg, B.S., Dwek ,R.A. (2006).Does. rapid oligomerization of hepatitis B envelope proteins play a role in resistance to proteasome degradation and enhance chronicity? DNA Cell Biol, 25,165-170.
12. Gerlich,W., Thomssen, R.(1975).Standardidised detection of hepatitis B surface antigen; Determination of its serum concentration in weight units per volume. Dev Biol Stand, 30, 78-87.
13. Joller-Jemelka, H.I., Pfister, H.F., Grob, P.J. (1985). The prognostic significance of quantitative HBsAG determination in acute hepatitis B. Schweiz Med Wochenschr, 115, 1249-1256.
14. Burczynska, B., Madalinski, K., Pawlawska, J. et al.(1994). The value of quantitative measurement of HBeAg and HBsAg before interferon-alpha treatment of chronic hepatitis b in children. J Hepatol, 21, 1097-1102.
15. Gerlich, W.H., Wend, U., Glebe, D. (2004).Quantitative assay of hepatitis B surface antigen in serum or plasma using laurel electrophoresis. Methods Mol Med, 95, 57-63.
16. Karakus, R., Aral, L.A., Basturk, B., Aybay, C.(2007). Development of a Highly Sensitive ELISA for Quantification of Hepatitis B Virus (HBV) Surface Antigen (HBsAg). Turk J Med Sci, 37, 87-92.
17. Wiegand, J., Wedemeyer, H., Finger, A., Heidrich, B., Rosenau, J., Michel, G., Bock, C.T., Manns, M.P., Tillmann, H.L. (2008). A decline in hepatitis B virus surface antigen (HBsAg)predicts clearance but does not correlate with quantitative HBeAg or HBV DNA levels. Antiviral Therapy, 13, 547-554.
18. Brunetto, M.R., Oliveri, F., Colombatto, P., Moriconi, F., Ciccorossi, P., Coco, B.,et al.(2010). Use of HBsAg serum levels help to distinguish active from inactive HBV genotype D carriers. Gastroenterology, 139, 483-90.
19. Nguyen, T., Thompson, A.J., Bowden, S., Croagh, C., Bell, S., Desmond, P.V., Levy, M., Locarnini, S.A. (2010). Hepatitis B surface antigen levels during the natural history of chronic hepatitis B: a perspective on Asia. J Hepatol, 52, 508-1
20. Chen, C.J., Yang, H.I., Su,J., Jen, C.L., You, S.L, Lu, S.N,et al.(2006). For the REVEAL-HBV Study Group. Risk of hepatocellular carcinoma across a biological gradient of serum hepatitis B virus DNA level.J Am Med Assoc, 295,65-73.
21. Iloeje, U.H., Yang, H.I., Su, J., Jen, C.L, You, S.L., Chen, C.J.(2006). Predicting cirrhosis risk based on the level of circulating hepatitis B viral load. Gastroenterology,130, 678-86.
22. Kim, J.D., Choi, J.Y., Bae, S.H., Yoon, S.K., Yang, J.M., Han, N.I,et al.(2010). Hepatitis B Virus Load in Serum Does Not Reflect Histologic Activity in Patients With Decompensated Cirrhosis. Clin Gastroenterol Hepatol, 8, 60-65.
23. Mukherjee, R.M., Balkumar Reddy, P., Sasikala, M, et al.(2009). Identification of genotype B among Hepatitis B Virus-infected patients in Hyderabad, India. Ann Hepatol, 8, 269-70.
24. Chan, H.L.Y., Hui, A.Y., Wong, V.W.S, et al. (2005).Long-term follow-up of peginterferon and lamivudine combination treatment in HBeAg positive chronic hepatitis B. Hepatology, 41,1357–1364.
25. Sung, J.J.Y., Wong, M.L., Bowden, S, et al.(2005). Intrahepatic hepatitis B virus covalently closed circular DNA can be a predictor of sustained

response to therapy. Gastroenterology, 128, 1890–1897.
26. Lapostolle, B.W., Bowden,S., Locarnini, S,et al.(2004). Persistence of cccDNA during the natural history of chronic hepatitis B and decline during adefovir dipivoxil therapy. Gastroenterology, 126, 1750-58.
27. Iloeje, U.H., Yang, H.I., Jen, C.L., Su, J., Wang, L.Y., You, S.L., Chen, C.J. (2007). Risk and predictors of mortality associated with chronic hepatitis B infection. Clin Gastroenterol Hepatol, 5, 921-931.
28. Mukherjee, R. M., Balkumar Reddy, P., Arava,J., Rao, P.N., Sasikala, M., Gupta, R., Reddy, D.N.(2010). Relationship between serum HBsAg level, HBV DNA level, and peripheral immune cells in patients with chronic hepatitis B virus infection. Hepatic Medicine: Evidence and Research, 2, 157–162

Table 1 : Baseline Feature of the Study Subjects

Charasteristics	All patients(n=62)
Age(years);Mean(Range)	38.2± 13.9(14-78)
Sex(% male)	90.3
Serum ALT(IU/ml);Mean±SD (Range)	57.41± 60.7(10-372)
Serum HBV DNA(Log Copies/ml);Mean±SD (Range)	4.45± 2.13(1.54-8.80)
HBeAg –Ve/anti HBe +Ve (%)	92.0
HBeAg +Ve/anti HBe –Ve (%)	8.0
Serum HBsAg(IU/ml);Mean±SD (Range)	43032.81±32848.02 (723-111141)

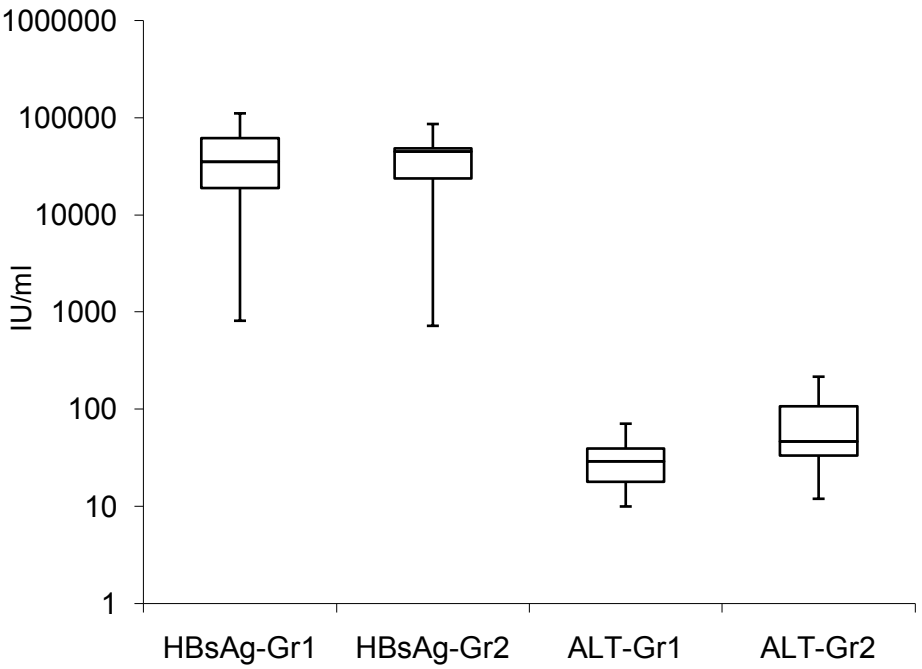

Figure 1 : HBsAg and ALT Values in Gr-1(n=32,HBV DNA ≤ 2000IU/ml) and Gr-2(n=30,HBV DNA ≥ 2000 IU/ml) Subjects(The boxes represent the interquartile ranges(IQR), the whiskers indicate the ranges and the center line in the box indicate median).