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RESEARCH ARTICLE

THE VALUE OF HBSAG MONITARIZATION IN CHRONIC HEPATITIS B PATIENTS TREATED WITH ORAL ANTIVIRAL THERAPY.

Muammer Bilici, Muhammet EminKutu, Yasin Ozturk and Yucel Ustundag.
Department of Internal Medicine, BEU,Turkey.

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Abstract

Background: Monitarization of serum HBsAg levels during treatment of chronic hepatitis B (CHB) with pegylated interferon was popular. However, the value of HBsAg kinetics under the treatment of CHB with oral antiviral agents is not well-known. In this study we aimed to evaluate the levels of HBsAg and HBV DNA during the treatment with oral antivirals in CHB patients.

Material and Method: A total of consecutive 50 CHB patients (mean age: 42.62±12.74 years and male/female: 31/19) were enrolled in this study. The serum HBsAg and HBV DNA levels at 0, 3rd and 6th month follow up in CHB patients iniated on oral antiviral therapy (lamivudine 100 mg daily, telbuvidine 600 mg daily, entecavire 0.5 or 1.0 mg daily and tenofovire 300 mg daily) were evaluated. Histologic activity index and fibrosis score were determined by Modified Knodall Classification (Ishak) in assessment of CHB biopsy specimens. Univariate and multivariate statistical methods were used by using SPSS packet programme.

Results: The patients were grouped according to antiviral agents used. There was no statistically significant difference between the groups regarding age and gender (p=0.520 and p=0.816, respectively). The initial qHBSAg (log10 IU/mL) and HBV DNA (log10 IU/mL) levels were higher in HBeAg positive patients (p=0.002 and p<0.001 respectively). There was a positive correlation between the basal HBV DNA and HBsAg levels among CHB patients (r=0,398; p=0,004). At 6- month follow up, the serum HBsAg decline was significantly higher in patients treated with tenofovire (85%) and entecavire (82%) compared to those treated with telbuvidine (35%) and lamivudine (26%) (p<0.05).

Conclusion: The value of the HBsAgmonitarization in CHB patients treated with antiviral therapy has not been clarified yet.

Introduction:-

Hepatitis B virus (HBV) infection remains a major health problem, especially in developing countries affecting 2 billion people worldwide with 400 million chronic carriers of this virus. Each year 500, 000 to 1.2 million people die

Corresponding Author:-Muammer Bilici.
Address:- Department of Internal Medicine, BEU,Turkey.

due to HBV related complications such as cirrhosis and hepatocellular carcinoma (HCC). HBV is spread mainly through contaminated blood and blood products, sexual contact and contaminated needles (1-8).

However HBV does not induce direct cytopathic effects on infected hepatocytes under normal infection conditions. It has been revealed that there is an association between the continuation of the virus replication and liver damage. Viral replication can be suppressed by potent antiviral agents. Clearance of hepatitis B surface antigen (HBsAg) is an important goal for all HBV antiviral therapies. Besides rapid reduction of viral replication can be achieved by treating CBH with oral antiviral agents. Loss of HBsAg is rarely observed (9).

The antiviral agents including lamivudine, entecavire, tenofovire and telbuvidine are used for suppressing viral replication. These drugs are well tolerated and side effect profiles are almost similar to placebo. Although there are risks regarding the emergence of resistance (8,10).

The levels of HBV DNA during follow-up of CBH patients remains best indicator of viral replication. PCR (polymerase chain reaction) method is used to detect the level of HBV DNA (11). Recently, there are some reports about Quantitative measurement of serum HBsAg based on ELISA, as a potential marker for monitoring therapeutic responses (12,13). In this study we aimed to evaluate the levels of HBsAg and HBV DNA during the treatment with nucleotide or nucleoside analogues in CHB patients .

Material and Methods:-

A total of 50 consecutive naive patients with CBH who were receiving oral antiviral agents (nucleotide or nucleoside analogues) were enrolled in the study. CHB diagnosis was made by combination of serological, biochemical, virological and histological markers according to AASLD Guidelines for Treatment of Chronic Hepatitis B (14). The patients who previously receieved antiviral therapy or taking interferon treatment were excluded from the study. Biochemical, serological and virological markers were evaluated in CBH patients treated with nucleotide or nucleoside analogues (lamivudine 100 mg daily, telbuvidine 600 mg daily, entecavire 0.5 or 1.0 mg daily and tenofovire 300 mg daily). Data of patients were collected from hospital information system retrospectively.

Serum albumin, total protein, total bilirubin, creatinine were measured by colorimetric method and AST, ALT levels were measured by enzymatic method on an AU 2700 analyser (Beckman Coulter, Tokyo, Japan), according to the manufacturer's specifications and using proprietary reagents. Measurements of the prothrombin time (PT) was performed on an Instrumentation Laboratory (Lexington, MA, USA) ACL TOP 500 coagulometer operated at 37°C. Haematological parameters (Haemoglobin and platelet) were obtained from the automated complete blood count using a Coulter LH780 HaematologyAnalyser (Beckman Coulter Inc. Brea., CA, and USA). The HBV markers (HBsAg, HBsAb, HBeAg, HBeAb, HBcAb, and anti-HBcAbIgM) were measured using the macro enzyme-linked immunosor-bent assay (ELISA) kits (Roche cobas 6000 system ,e601 module). Serum HBV DNA load in patients with CHB was measured by PCR metod (Taqman, Roche Diagnostic Systems). Histologic activity index (HAI) and fibrosis score were determined by Modified Knodall Classification (Ishak) in assessment of CHB biopsy specimens (15). The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a priori approval by our institution's human research committee. Informed consent was obtained from all individuals.

Statistical Analysis:-

Statistical analyses are conducted with SPSS 18.0 software. Descriptive statistics for continuous variables are presented with average and standard deviation while categorical variables are presented with frequency and percentage. Parametric values are evaluated by using student t-test and non-parametric values are evaluated by using Anova. For the significance between the groups Chi-Square or Fisher test is used. Paired t test was used to compare dependent variables. Relation between variables are evaluated with multiple-regression analysis. Statistical significance level was accepted as $p<0.05$.

Results:-

The patients were grouped into 4 groups according to oral antiviral agents used (lamivudine, telbuvidine, entecavire and tenofovire) for the treatement of CHB. The demographic, serological and virological features of the patients are presented in Table 1. There was no significant difference between groups in terms of age and gender ($p=0.520$ and $p=0.816$, respectively). HBeAg positive patients had higher baseline HBsAg (log10 IU/mL) and HBV DNA (log10

IU/mL) levels than HBeAg negative patients statistically (p=0.002 and p<0.001 respectively). There was a positive correlation between the baseline levels of HBV DNA and HBsAg among patients with CBH (r=0,398; p=0,004). Although no correlation was observed between the serum HBsAg and HBV DNA levels at 3rd and 6th month follow up in CHB patients.

Table 1:-Characteristics of chronic HBV patientstreatedwithoral antivirals.

Variable	Telbivudine (n=18) mean ± sd	Lamivudine (n=12) mean ± sd	Tenofovir (n=10) mean ± sd	Entecavir (n=10) mean ± sd	p
Age (year)	39.56±9.47	45.25±12.66	43.9±15.14	43.7±16.06	0.520
Male/Female	10/8	7/5	7/3	7/3	0.816
Hgb (gr/dl)	14.42±1.24	13.33±1.95	14.19±2.01	13.93±1.16	0.346
PLT(10^3/µL)	209.6±69.5	238.7±87.9	226±101.4	188.9±82	0.642
Albumin (g/dl)	4.4±0.38	4.8±0.32	4.6±0.46	4.5±0.49	0.065
ALT(U/L)	55.94±51.03	25.67±12.72	86.5±124.32	135.6±237.78	0.014
AST(U/L)	38.78±37.35	22.75±5.06	85.8±139.2	56.9±33.01	0.003
Totalbilirubin(mg/dL)	0.64±0.25	0.66±0.2	0.71±0.12	1.11±0.81	0.541
HBV DNA (log10 copies/mL)	4.5±1.04	3.71±0.73	6.19±1.87	5.18±1.36	0.005
HBeAgpositive(%)	11.1	0	60	30	
qHBsAg (log10 IU/mL)					
Baseline	10665±11652	4389±8452	18489±20045	24677±27190	0.011
3rdmonth	7647±8793	3237±6925	7848±7687	5941±5487	0.061
6th month	5449±7447	1404±1476	4156±4354	2801±2997	0.005
HAI (%)					
1-4	16.7	16.7	0	0	
5-9	66.7	83.3	90	60	
10-18	16.7	0	10	40	
Fibrosisstage (%)					
1	0	0	0	10	
2	33.3	83.3	30	20	
3	50	16.7	60	70	
4	16.7	0	10	0	

Abbreviations: PLT: platelet, Hgb:Hemoglobin, HBV: hepatitis B virüs, ALT: alanineaminotransferase, AST: aspartateaminotransferase, HAI: Histologicactivityindex, sd:standarddeviation.

There were no relationships between HBsAg levels (log10 IU/mL) and both HAI and fibrosis score in CHB patients. There was a positive correlation between HBV DNA (log10 IU/mL) and fibrosis phase (r=0.406; p=0.003) as well as no correlation was observed between HAI and HBV DNA levels (r=0.146; p=0.313). The baseline serum HBsAg levels in Tenofovire and entacavire groups were significantly higher than the levels in lamivudine group (p=0.008 and p=0.007, respectively). Nevertheless there were no statistically significant differences among the others groups (p>0.05). Baseline HBV DNA (log10 IU/mL) levels in tenofovire group were higher than telbuvidine and lamivudine groups but not entecavire group statistically (p=0.008, p=0.007 and p>0.05, respectively).

The percentage decline of serum HBsAg levels at 0, 3rd and 6th month follow-up in different groups of CHB patients was shown in figure 1. With regard to the percentage decline of serum HBsAg levels in the groups, no significant difference was observed at 3- month follow up whereas it differed significantly at 6- month follow up (p=0,061 and p=0,005 respectively). At 6- month follow up, the percentage declines of serum HBsAg levels in the groups treated with tenofovire, entacavire, telbuvidine or lamivudine were 85%, 82%, 35% and 26% respectively (Figure 2). There were no statistically significant differences between the tenofovire and entacavire groups and also telbuvidine and lamivudine groups (p=0.912 and p=0.723, respectively). It was notable that the percentage declines of serum HBsAg levels in the tenofovire and entacavire groups were significantly higher than telbuvidine and

lamivudine groups (p<0.05). Moreover, HBeAg positive patients had significantly higher percentage decline of serum HBsAg levels at 6- month follow up (p=0.003).

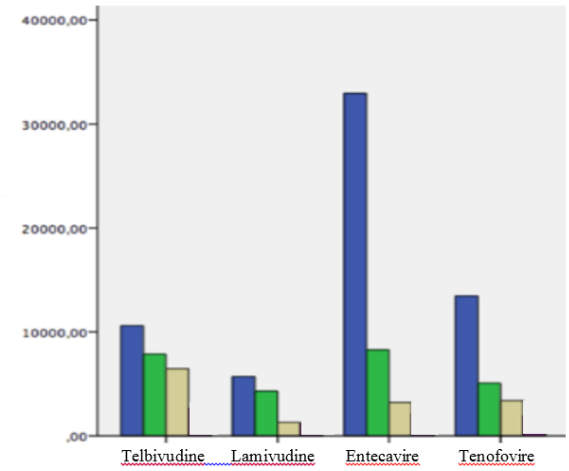

Figure 1. Serum qHbs Ag levels at 0, 3rdand 6th months of OAV therapy are seen.

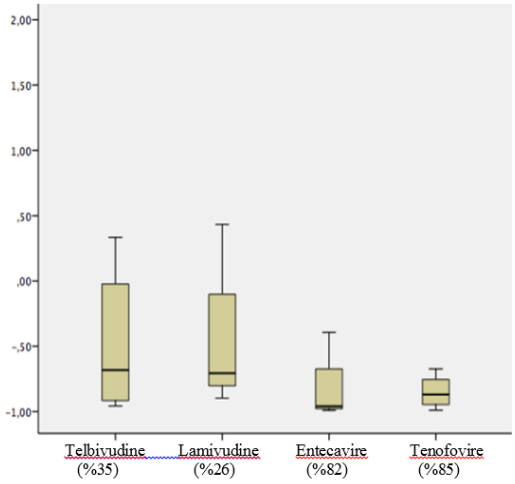

Figure 2. At the end of 6 months of OAV therapy, the rate of qHbsAg changes in all treatment groups is shown

Discussion:-

HBV DNA level is used to evaluate the response to treatment of CHB infection (16). While clearance of HBsAg is an important goal for all HBV antiviral therapies (14). Recently, predictive value of HBsAg levels in the follow-up CHB is investigated in assessment of response to treatment (17).

In our study, on the 6th month of the treatment with nucleotide or nucleoside analogues,various percentages of serum HBsAg decline were observed among the groups of CHB patients and there was practically no parallel between the serum HBsAg decline and achievement of undetectable HBV DNA levels. Although, the basal HBV DNA and HBsAg levels in patients with CHB showed a positive correlation. This is because the nucleoside or nucleotide analogues have no effect on cccDNA and HBsAg, even though preventing DNA synthesis via blocking viral reverse transcriptase by these drugs results in suppressing viral load in the early stage. At 6- month follow up, the percentage declines of serum HBsAg levels in the groups treated with tenofovire, entacavire, telbuvidine or lamivudine were 80%, 79%, 46% and 38% respectively. It is notable that significantly higher serum HBsAg decline rates occured in patients treated with tenofovire or entecavire which are more potent drugs compared to lamivudine and telbuvidine. However, HBsAg is not a marker of viral replication, but rather reflects a reduction in translation of mRNAs produced from transcriptionally active cccDNA and integrated HBV sequences (18). The mechanisms underlying HBsAg clearance during antiviral therapy remain unknown.

Chevaliez et al. assessed serum HBsAg kinetics in 30 patients with CBH who were treated with nucleotide or nucleoside analogues. The avarega length of a period of monitarizationHBsAg levels was 120 months. They revealed that the kinetics of serum HBV DNA and HBsAg levels were not parallel. Antiviral therapy was associated with a slow but consistent reduction in the level of HBsAg in most of the patients. Three distinct patterns of HBsAg level declines were identified according to the detectable or undetectable HBV DNA phases. In general, they showed that the HBsAg level decline was steeper during the HBV DNA detectable period and tended to slow when HBV DNA became undetectable. In this study, HBsAg loss was achieved in only one patient and the remaining patients had HBsAg detectable levels at the end of follow-up (19). Setoet. al. evaluated serum HBsAg kinetics during 5-year follow-up of 222 patients with CBH who were treated with entecavire. They showed that higher average HBsAg decline rate was observed, in particular,inHBeAg positive patients and those who have high baseline HBV DNA levels (≥ 8 log copies/mL or ≥ 7.3 log IU/mL). Although,cumulative HBV DNA undetectable rate for 5-year follow-up was 97.1%, long-term entecavire treatment achieved only a slow decline in serum HBsAg (20).

In our study ,HBeAg positive patients had higher percentage decline of serum HBsAg levels at 6-month follow-up. The highest percentage decline of serum HBsAg levels were in patients treated with entecavire and tenofovire. However, there was no correlation between HBV DNA levels and qHBSAg decline rate. Despite the fast and potent virologic suppression effects of the nucleotide or nucleoside analogues, reduction of HBsAg levels can vary and may be slow. HBsAg loss is rarely observed. Thus, long term nucleotide or nucleoside analogues treatment in CHB patients are required for providing HBsAgseroclearance (21).

Heathcote et al. followed patients with CBH for 3 years to determine the efficiency and reliability of tenofovire. After 144 weeks of treatment, HBV DNA levels were detected under 400 copies/ml in 72% of HBeAg-positive and 87% of HBeAg-negative patients. They reported that achievement rates of HBsAg loss in patients treated with tenofovire were 3%, 6% and 8% at the first, second and third year of therapy respectively (22). Gish et al. evaluated HBsAg kinetics in two groups of patients who treated with entecavire or lamivudine. After 120 week follow-up assessment, HBsAg loss in patients treated with entecavire versus lamivudine were 5% and 2.8% respectively. Additionally, they showed that 96% of patients lost HBsAg had HBV DNA levels <300 copies/ml (23). Zoutendijk et al. revealed that HBeAg loss was associated with HBsAg decline in HBeAg-positive patients treated with entecavir or tenofovir. In this study, it is pronounced that predicted median time to HBsAg loss was 36 years for HBeAg-positive and 39 years for HBeAg-negative patients. It seems that most patients treated with entecavir and tenofovir will probably need decades of therapy to achieve HBsAg loss (24). Consistent with previous studies (25-28), we found that HBeAg-positive patients had higher HBV DNA and HBsAg levels than HBeAg-negative patients. However the sample sizes of subgroups were not sufficient enough to make analyses to represent the general population.

Our study has several limitations, such as the low number of patients and no long-term outcomes of CBH patients regarding HBV DNA and HBsAg levels. Nevertheless, future comprehensive studies covering larger populations are needed to determine the predictive value of HBsAg levels for evaluating the response to treatment with nucleotide or nucleoside analogues in CBH patients. Another limitation of our study is the lack of HBV genotype data, even though HBV genotype is not an important indicator for HBsAg kinetics (29). According to several studies conducted in Turkey, genotype D is dominant genotype of hepatitis B virus (30-32).

Conclusion:-

Serum HBV DNA level as a measure of treatment response during follow-up patients with CBH is the most important marker of viral replication. Various results were reported in the studies assessing the availability of serum HBsAg levels in the follow-up patients with CBH. Long-term therapy with nucleoside/nucleotide analogues to achieve HBsAg loss in CHB patients is required. The findings of our study consistent with previous studies support that HBsAg kinetics can vary depending on nucleoside/nucleotide analogues used in the treatment of CHB.

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