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RESEARCH ARTICLE

VIRAL LOAD AND T CELL SUBGROUP DYNAMICS OF PATIENTS TREATED FOR CHRONIC VIRAL HEPATITIS B.

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Abstract

Backround: T cell activation plays an important role in tissue injury and viral clearance during the antiviral immune response. In our study we aimed to assess the viral load and peripheral blood T lymphocyte subgroup dynamics in the first 6 months of treatment for patients with chronic viral hepatitis B.

Material and Method: A total of 31 naive patients without previous treatment were included in the study. For at least 6 months, 16 patients were given 300 mg/day telbivudine and 15 patients were given 100 mg/day lamivudine. Laboratory values were determined at the start of treatment and in the 1st and 6th months. Serum HBV DNA load was measured with PCR and peripheral lymphocytes subsets were measured with flow cytometry. During the treatment period, multistage analysis of the reduction in HBV DNA levels and peripheral blood lymphocyte dynamics was performed.

Results: The HBV DNA values in both groups fell to non-measurable levels in the 1st and 6th months. In both groups the CD4/CD8 ratio was similar and there was no significant difference in CD4/CD8 ratios during follow-up ($p>0.05$). The increase in CD8+CD28+ and CD8+CD38+ T cell levels was inverse to the reduction in viral load and the increase in both groups in the 1st month was noteworthy. However, though viral load continued at levels that could not be measured, the clear increase in CD8+CD28+ and CD8+CD38+ levels in the first month ended and formed a plateau leading to the consideration that this may be associated with viral load.

Conclusion: We believe focusing on immune mechanisms will increase the treatment success of new treatment modalities for chronic viral hepatitis B treatment.

Introduction:-

Chronic viral hepatitis B infection is a progressive disease affecting 350 million people globally and thought to cause over a million deaths annually linked to complications like liver cirrhosis and hepatocellular carcinoma. The disease vector of the hepatitis B virus (HBV) is a non-cytopathic virus (1-3). Chronic HBV infection causes liver cell damage due to immune mechanisms (4). Cellular immunity plays a critical role in determining the clinical outcome linked to the disease. Especially, the HBV-specific CD8+ T cell response to HBV is thought to have a determinative role in viral clearance and disease pathogenesis (5, 6).

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There are 2 signals required for T cell activation. One of these is linked to binding of the T cell receptor (TCR) to the antigen present on the cell surface of the MHC molecule. The interaction between TCR and the cell surface is not sufficient alone for optimal activation of naive and memory CD8+ T cells (7). Generally, a second signal (co-stimulus) is required for full activation and survival of T cells. CD28 is a co-stimulant molecule which activates T cells causing proliferation of naive CD8+ T cells and differentiation of cytotoxic T lymphocytes (CTL) and memory CD8+ T cells (7, 8). Apart from limiting infection, HBV-specific CTL are responsible for tissue injury to the liver. In chronic HBV infection, the CTL response in blood is weak, but actively continues in the liver (9).

For control of HBV, it is necessary for cellular and humoral immunity to effectively work together. If there are no CD4+ T cells, CD8+ activity and antibody response is disrupted; in the absence of virus specific CD8+ T cells, viremia continues and cannot be controlled by the antibody response alone. When HBV infection becomes chronic, a regression in acquired immunity is observed (10, 11).

There is no doubt that focusing on immune mechanisms will increase the treatment success of new treatment modalities for chronic HBV infection. In our study we aimed to show the dynamics of T lymphocyte subgroups in chronic viral hepatitis B patients undergoing antiviral treatment with telbivudine or lamivudine.

Material and methods:-

This study included 31 naive volunteer patients admitted to ZonguldakBülentEcevit University Faculty of Medicine Hospital Hepatology clinic receiving 300 mg/day telbivudine (n=16) or 100 mg/day lamivudine (n=15) for chronic hepatitis B diagnosis. Detailed medical history was obtained from all volunteers and typical physical examination was performed. Chronic hepatitis B diagnosis was placed based on the American Association for the Study of Liver Diseases (AASLD) hepatitis B treatment guide, Asia Pacific Association for Study of the Liver (APASL) and European Association for the Study of the Liver (EASL) guidelines by assessing biochemical, virologic and histological data of patients (12-14).

Patients receiving immunosuppressive or antiviral treatment within 1 year before treatment, patients with HCV, HDV, HIV infections, liver cirrhosis, or other liver diseases apart from HBV, pregnancy, malignancy, chronic renal failure or other serious medical disease that may be confused with these were excluded from the study.

Patients were divided into two groups as those receiving lamivudine or telbivudine treatment. In accordance with the AASLD, APASL and EASL guidelines, check-ups were performed at 0, 1 and 6 months. Biochemical investigations including serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and bilirubin levels were performed with routine automatic techniques. The enzyme immunofluorescence test was used to identify hepatitis B markers. Serum HBV DNA load was studied with the real-time polymerase chain reaction using a COBAS Taqman HBV test (Roche diagnostic, USA) system. In peripheral blood, CD19+ B lymphocytes, CD3+ T lymphocytes, CD4+ helper T lymphocytes, CD8+ T lymphocytes, CD8+CD38+ activated cytotoxic T lymphocytes and CD8+CD28+ cytotoxic T lymphocyte subgroups were analyzed with the immunofluorescence procedure recommended by Beckman Coulter. Histopathological assessment was recorded using the fibrosis-stage score based on the modified Knoddel system published by Ishak et al. in 1995 using liver biopsy material obtained at the start of treatment (15).

Statistical Analysis

The SPSS 19 (SPSS Inc., Chicago, IL, USA) program was used for statistical assessment. Normal distribution of numerical variables was investigated with the Shapiro-Wilk test. Descriptive statistics for numerical variables are given as mean±standard deviation and median (minimum-maximum), while categorical variables are stated as number and percentage. Comparisons of the groups in terms of categorical variables were performed with the chi-square test. Comparison of numerical variables between two groups used the significance test of the difference between two means if the parametric test assumptions were abided by, and the Mann-Whitney U test if not. Repeated measures were analyzed by the repeated measures variance analysis if parametric test assumptions were abided by and the Friedman test if not. After variance analysis of repeated measures, two-way comparison of subgroups was performed with the Bonferroni test and after the Friedman test two-way comparisons were performed with the Dunn test. P values <0.05 were accepted as significant.

Results:-

The mean age of the 31 patients included in the study was 42.8 ± 12.2 years. There was no significant difference between the patient groups receiving telbivudine and lamivudine treatment in terms of age and gender (p>0.05). Patients in both groups were determined to have Child score of A and MELD score of 6. When liver histopathology results for patients before treatment are assessed, both groups had no significant difference in terms of fibrosis stage scores (p>0.05). The characteristic features of patients are given in **Table 1**.

Table 1:-Characteristics of chronic HBV patientstreatedwithtelbivudineandlamivudine

Variable	Telbivudine (n=16) mean ± sd			Lamivudine (n=15) mean ± sd		
Age (year)	41.06 ± 10.5			44 ± 13.9		
Male/Female	9/7			7/8		
Hgb (gr/dl)	14.1 ± 1.2			13.5 ± 1.7		
Leukocyte x 109/L	6.5 ± 1.9			7.6 ± 1.4		
ALT(U/L)	43.5 ± 43.1			35 ± 24.7		
AST(U/L)	35.2 ± 39.8			24.3 ± 8.2		
FibrosisStage (%)						
1	6.2			-		
2	17.6			46.6		
3	56.2			40		
4	17.6			13.3		
Month	Baseline	1st month	6st month	Baseline	1st month	6st month
CD3+ T cells (%)	64.25±6.01	64.25±6.0	65.85±5.46	65.5±6.17	66.18±7.05	67.39±5.70
CD19+ B cells (%)	13.09±4.13	13.63±2.69	13.83±3.36	12.26±3.87	12.77±4.44	12.68±3.60
CD8+CD28+ T cells (%)	14.3 ± 7.37	15.41±8.12	13.73±3.97	10.65 ± 3.68	11.18±3.68	12.4±4.47
CD8+CD38+ T cells (%)	2.03±1.18	2.83±1.53	2.87±1.77	2.15±0.48	2.64±0.67	2.48±0.69
CD4+/CD8+	1.82±0.57	2.01±0.65	1.65±0.53	2.24±0.77	2.40±1.03	2.48±1.55
HBV DNA (IU/ml)	315595.44 ± 717090.98	366±1117.92	2.81±11.25	323155.20 ± 621544.86	726.47±2619.34	19.93±77.2
Abbreviations: HBV:hepatitis B virüs, ALT: alanineaminotransferase, AST: aspartateaminotransferase, sd:standarddeviation.						

There was no significant difference in the CD8+CD38+ and CD8+CD28+ levels between the telbivudine and lamivudine groups at 0, 1 and 6 months (**Figure 1**) (p=0.707, p=0.644 and p=0.491 and p=0.086, p=0.740 and p=0.390, respectively). When the groups are assessed separately, in both groups there was a significant difference in CD8+CD38+ and CD8+CD28+ levels with an increase from 0 to 1 month, with no significant differences identified between 0 and 6 month values and 1 and 6 month values (p=0.018, p=0.072 and p=1.000, with p=0.040, p=0.231, and p=1.000 for the telbivudine group respectively, p=0.004, p=0.098 and p=0,751 with p=0.035, p=0.259, and p=0.870 for the lamivudine group respectively).

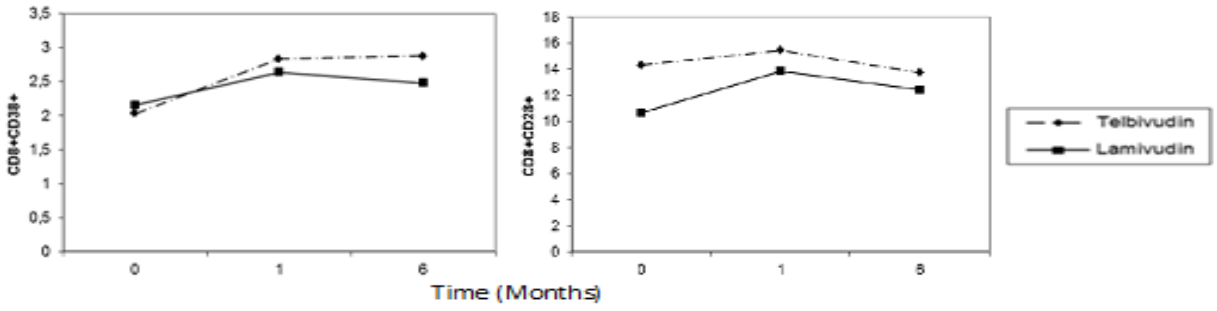

Figure 1: Changes in the proportion of CD8+CD38+ and CD28- CD8+ T cells in patients with HBV

There was no significant difference identified between the CD4/CD8 ratio values between the telbivudine and lamivudine groups at 0, 1 and 6 months (p=0.097, p=0.281 and p=0.650, respectively). When the variation over time is investigated, there was no difference between the two groups (**Figure 2**). For the telbivudine group p=0.097 and for the lamivudine group p=0.395.

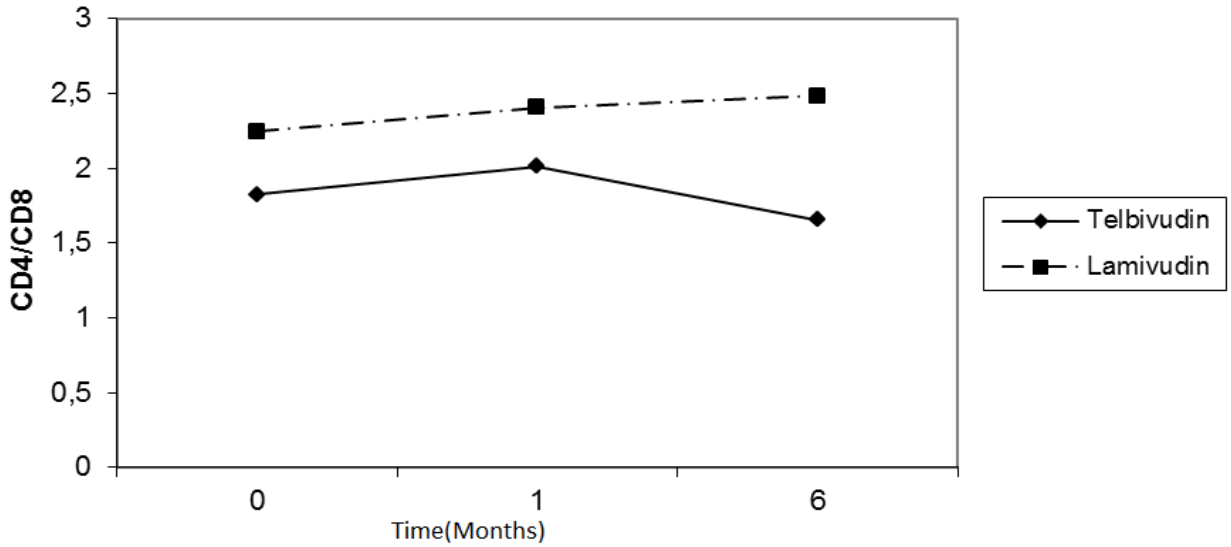

Figure 2 : Changes in CD4/CD8 ratio in patients with HBV

When the HBV DNA values are compared in the groups, at the start (0 months) there was no statistically significant difference identified (p=0.953). In the 1st and 6th months, HBV DNA fell to levels that could not be measured in both groups, with no significant difference between the values for the two groups in these months (**Figure 3**). These values were p=0.953, p=0.892 and p=0.984 for 0, 1 and 6 months, respectively. When the sequential variation in HBV DNA at 0, 1 and 6 months is examined within each group, significant differences were identified. For the telbivudine group these values were p<0.001 for 0 and 1 months, p<0.001 for 0 and 6 months and p=1.00 for 1 and 6 months. In the lamivudine group, these values were p<0.001 for 0 and 1 months, p<0.001 for 0 and 6 months and p=1.000 for 1 and 6 months.

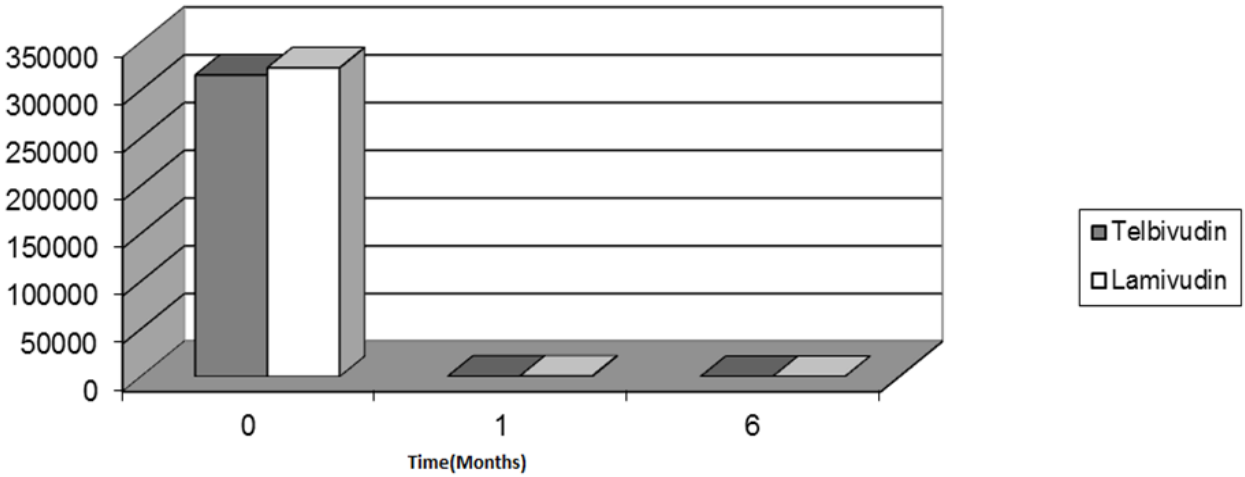

Figure 3 :Changes in the levels of HBV DNA in patients with HBV

Discussion:-

The result of activation occurring due to interaction between infected cells and T lymphocytes leads to changes in the T lymphocyte subgroups. The T lymphocyte subgroups reflect CD8+CD38+ cytotoxic T cell activation (16, 17). In this study in the 1st month of follow-up HBV DNA fell to levels that could not be measured in patients treated with telbivudine and lamivudine, and this continued until the 6th month. Inverse to this reduction in viral load, it is noteworthy that CD8+CD28+ and CD8+CD38+ T cell levels increased in both groups in the 1st month. However, though viral load continued at unmeasurable levels, the clear increase in CD8+CD28+ and CD8+CD38+ levels of the 1st month did not continue but formed a plateau. This situation is considered to be associated with viral load.

A study by Cao et al. investigated the 72-week follow-up outcomes of T lymphocyte subgroups among 13 hepatitis B patients responding to adefovir treatment. When hepatitis B patients are compared with hepatitis B carriers and a control group, CD4+ and CD8+ T cells were found to be lower among chronic hepatitis B patients and HBV carriers compared to subjects in the control group. There were no significant differences in the T cell subgroups between the three groups. When CD8+CD38+ T cell levels are examined, there was elevation with statistical significance in the chronic hepatitis B patient group compared to the HBV carriers and healthy control group. After seventy-two weeks follow-up, in parallel with the reduction in viral load with adefovir treatment, the CD8+CD38+ T cells were observed to fall from 53.9±18.4% before treatment to 20.1±11.3%. This fall in CD8+CD38+ T cells, a parameter of T cell activation, was considered to be linked to the reduction in viral load and the interaction with adefovir treatment. Their study has shown CD8+CD28+ and CD8+CD38+ T cell levels are low in chronic hepatitis B and HBV carrier groups, with no significant difference compared to a control group. There was no significant difference in CD8+CD28+ and CD8+CD38+ T cell levels before and after adefovir treatment (18).

Tilling et al. in a study assessed the 96-week follow-up outcomes of 131 patients with human immunodeficiency virus (HIV) and began patients on zidovudine, lamivudine, abacavir and amprenavir as antiviral treatment. Before antiviral treatment CD8+CD38+ T cell values were clearly observed to fall in the first two weeks of treatment in parallel with the reduction in viral load. It was stated that CD8+CD38+ T cell levels in patients with viral load continuing below 52 copies/ml may be marker of residual viral replication in HIV infection (19). Additionally, a study by Lechner et al. observed the activation marker of CD8+CD38+ cells was high at the maximal elevation time for ALT showing hepatocyte injury in HCV infection (20). CD28+ is the best-known co-stimulus marker with a critical role in T cell modulated antiviral immune response activation. Li et al. in a study of 70 chronic hepatitis B patients identified no significant difference in CD8+CD28+ levels compared to a control group. When patients receiving effective antiviral treatment are monitored, there is an increase in CD8+CD28+ T cells in chronic hepatitis B patients when 52 week and initial levels are compared. When CD8+CD28+/CD8+CD38+ T cell ratios are examined, these ratios display an inverse correlation with HBV DNA levels during treatment. Abnormalities in CD8+ T cell CD28 expression and CD8+CD28+/CD8+CD28- ratios reflect disruption of T cell activation regulation and this situation is proposed to be associated with pathogenesis of chronic viral hepatitis B infection (21).

Cao et al. found CD8+CD28+ levels were higher in chronic hepatitis B patients compared to a healthy control group. When chronic hepatitis B patients were divided into two groups as HBeAg positive and HBeAg negative, there was no difference between the CD8+CD28+ levels in the two groups. There was no significant difference found between serum HBV DNA levels and CD8+CD28+ levels in chronic HBV patients. As abnormalities in the construction of so-stimulus molecules reflect regulation disorder in T cell activation, it was stated to be associated with the severity and pathogenesis of chronic HBV infection (22).

When the T lymphocyte subgroups, accepted as having the main role in tissue injury in chronic viral hepatitis B patients, are examined, though some researchers have shown an increase when CD8+ T lymphocyte rates are compared with CD4+ T lymphocytes leading to a reduction in CD4/CD8 ratio (23-25), some researchers have found no significant difference (26, 27).

In this study when the T lymphocyte subgroups of patients receiving telbivudine and lamivudine treatment are compared initially and at 1- and 6-month follow-up, both groups had similar CD4/CD8 ratios and there was no significant difference observed in CD4/CD8 ratios during follow-up. The important co-stimulant marker regulating T cell activation of CD8+CD28+ levels were not different between the two groups, but were found to be significantly increased in the 1st month of follow-up. There was no significant difference between the first- and sixth-month values in both groups. When the activated T lymphocyte marker of CD8+CD38+ levels are examined, there was no significant difference between the groups. Similarly, in both groups there was a significant degree of

increase in the 1st month, with no significant difference when compared with 6-month values. Similar T lymphocyte subgroup variations in both groups lead to the consideration that telbivudine and lamivudine antiviral treatment does not have an effect on T lymphocyte dynamics causing changes in CD4/CD8, CD8+CD28+ and CD8+CD38+. However, as in previous studies, the lack of a long duration follow-up is a significant factor limiting our study.

Conclusion:-

The results of our study support that co-stimulant (CD28+) positive, CD8+ cytotoxic T cells and activated T lymphocyte marker of CD8+CD38+ levels with critical role in the immune response of chronic viral hepatitis B patients may be associated with viral load. We believe focusing on immune mechanisms will increase the success of new treatment modalities for treatment of chronic viral hepatitis B.

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