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Original research article

REGULATORY T CELL (CD4) EXPRESSION IN CORRELATION WITH VIRAL LOAD IN HEPATITIS B VIRUS INFECTION

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ABSTRACT

Aim: The aim of the present study was to find out the correlation between regulatory T cell (CD4) expression and viral load in Hepatitis B infection.

Methods: The Hospital-based, observational study was conducted in the Department of Medicine, Assam Medical College for the period of one year (July 2018 to June 2019). The study was conducted on 63 patients fulfilling the inclusion criteria attending the outpatient Department or admitted in the Department of Medicine, Assam Medical College, and Hospital during the study period.

Results: In the present study, the maximum cases were in the age group between 30-39 years. Among the study population, 69.84% patient were male and 30.16% were female. The incidence of Hepatitis B infection was more common (80.95%) in married than the unmarried (19.05%). In the majority (90.48%) of patients, the onset of symptoms was insidious. A total of 34.95% of patients were incidentally detected with Hepatitis B infection. Out of 63 cases, 5 cases were acute hepatitis B (AHB) and the remaining 58 cases were in chronic hepatitis B (CHB). Among the chronic stage, 14 cases were HBeAg positive and 44 cases were HBeAg negative. Overall, a positive correlation was found between Hepatitis B DNA load and Regulatory T cell (Tregs) expression.

Conclusion: Regulatory T cells (Tregs) have attracted a great deal of attention over the past few years as a consequence of their ability to suppress CD4 and CD8 effector T-cell responses. The immune regulation of Tregs is complicated, and the mechanisms of suppression of antiviral immune responses are not yet very clear. It has been suggested that Tregs may have evolved to prevent immunopathological damage but also contribute to viral persistence. Our findings demonstrated that there is a positive correlation between Tregs (CD4+CD25+FXP3+) and

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Hepatitis B DNA load. This result indicates that modulation of CD4+ CD25+ Tregs might be one potential therapeutic strategy for the treatment of Chronic Hepatitis B infection.
Keywords: Hepatitis B infection, T cell (CD4) expression, viral load

INTRODUCTION

The hepatitis B virus (HBV) specifically infects hepatocytes, leading to acute or persistent liver infection. The majority of infected adults can resolve the infection, but 5–10% develop chronic hepatitis, which can lead to cirrhosis, hepatocellular carcinoma (HCC) and death.[1] Current therapy consists of suppression of viral replication by lifelong use of nucleos(t)ide analogues (NA) which reduces, but does not abrogate HCC risk. The viral HBV genome is formed as covalently closed circular DNA (cccDNA) which encodes for: three forms of the HBV surface antigen (HBsAg), the viral capsid-forming core (HBcAg) and its secreted form called the e antigen (HBeAg), viral polymerase and the non-structural X protein. In addition to complete viral particles, large amounts of HBeAg and HBsAg are secreted by infected hepatocytes, presumably as a decoy for the immune system.[2]

Chronic hepatitis B (CHB) is a common and serious infectious disease of the liver caused by the hepatitis B virus (HBV). About 350 million patients world-wide are chronically infected and become HBV carriers. Current antiviral therapy options include interferon (IFN)-c and nucleotide analogues, but the problems of drug resistance and severe side effects have not yet been resolved. It is therefore of great interest to identify and establish alternative approaches. During HBV infection, the host immune responses, particularly the cellular immune response, mediate clearance of HBV infection,[3] although the exact mechanisms remain unclear. Unfortunately, the patient often exhibits impairment of HBV-specific T-cell activity during chronic HBV infection.[4]

The Regulatory T cells (Tregs) formerly known as suppressor T cells are a subpopulation of T CELLS that modulate the immune system, maintains tolerance to self-antigen and prevent autoimmune disease. Tregs are immunosuppressive and generally suppress or downregulate the induction and proliferation of effector T cells.[5] Tregs express the biomarkers CD4, FOXP3, and CD25 and are thought to be derived from the same lineage as naïve CD4 CELLS.[6] CD4+ Foxp3+ CD25 (high) regulatory T cells have been called "naturally occurring" regulatory T cells[7] to distinguish them from "suppressor" T cell populations that are generated in vitro. Sakaguchi et al. were the first to identify a population of CD4+ T cells that showed high levels of expression of interleukin (IL)-2Ra (CD25) and that prevented autoimmunity in a murine model.[8]

The aim of the present study was to find out the correlation between regulatory T cell (CD4) expression and viral load in Hepatitis B infection.

MATERIALS AND METHODS

The Hospital-based, observational study was conducted in the Department of Medicine, Assam Medical College for the period of one year (July 2018 to June 2019). The study was conducted on 63 patients fulfilling the inclusion criteria attending the outpatient Department or admitted in

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the Department of Medicine, Assam Medical College, and Hospital during the study period. HBV infected patients, 13 years and above, attending Department of Medicine Assam Medical College and Hospital, during the period of one year were taken up for the study.

INCLUSION CRITERIA: HBsAg positive patients aged 13 years and above attending Out Patient Department of Medicine/and/or were admitted in Medicine ward, Assam Medical College and Hospital.

EXCLUSION CRITERIA:All patients who are HBsAg negative. All patients who refuse to give consent to be a part of the study population.

METHODS OF COLLECTION OF DATA:

Data were collected from patients attending the Department of Medicine, Assam Medical College & Hospital. Patients were selected according to inclusion and exclusion criteria mentioned above. For each patient, the following data were collected: Age, Sex, ultrasound whole abdomen, LFT, Complete Blood Counts, ESR, Prothrombin Time, International Normalised Ratio in a specially designed proforma.

METHODOLOGY:

All laboratory procedures were performed at Assam Medical College and ICMR, Dibrugarh. Patients were identified as the case by detection of HBsAg. After blood collection from each patient, the following tests including ALT, AST, Prothrombin time, bilirubin fraction, serum protein fraction and Complete Blood Count was carried out. Epidemiological data, clinical data (patient age, gender, body mass index [body weight in kg/height in meters]), and biochemical parameters were obtained.

Work Plan:

Data were abstracted from the medical records of participants regarding the date of diagnosis, the individual's HBV markers, including HBV surface antigen (HBsAg), IgM HBc, HBeAg, and the individual's sex and age at diagnosis. At the time of recruitment, each study subject was personally interviewed to ascertain the information on sociodemographic characteristics, lifestyle, and ethnicity, lifetime history alcohol consumption, dietary factors, and personal and family history of various chronic diseases followed by clinical examination.

STATISTICAL ANALYSIS:

The data recorded on predesigned and pretested proforma was tabulated and a master chart was prepared. All statistical data are presented in terms of proportions, percentages, and ratios. Spearman Rank Correlation was applied to correlate Regulatory T Cells (Tregs) value and HBV DNA viral titres. p-values less than 0.05 were considered statistically significant. HBV DNA LOAD value was converted into LOG value for better interpretation of results. Microsoft Word and Excel were used to generate graphs and tables.

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RESULTS

Table 1: Patient characteristics

AGE GROUP ***(in years)***	**NUMBER** ***(n)***	**PERCENTAGE** ***(%)***
<20	3	4.76
20–29	13	20.63
30–39	15	23.81
40–49	13	20.63
50–59	9	14.29
60–69	7	11.11
≥ 70	3	4.76
Mean ± S.D.	41.41 ± 15.46 *years*	
SEX		
Male	44	69.84
Female	19	30.16
MARITAL STATUS		
Married	51	80.95
Unmarried	12	19.05
MODE		
Insidious	57	90.48
Acute	6	9.52

In the present study, the maximum cases were in the age group between 30-39 years. Among the study population, 69.84% patient were male and 30.16% were female. The incidence of Hepatitis B infection was more common (80.95%) in married than the unmarried (19.05%). In the majority (90.48%) of patients, the onset of symptoms was insidious.

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Table 2: Incidental Finding

INCIDENTAL FINDING	**NUMBER** ***(n* = 63*)***	**PERCENTAGE** ***(%)***
Trauma	4	6.35
Diabetic Chronic Kidney Disease	4	6.35
Pregnancy	4	6.35
Chronic Obstructive Airway Disease	1	1.59
Mass in the Oral Cavity	1	1.59
Antral Polyp	1	1.59
Pleural Effusion	1	1.59
Routine Checkup	1	1.59
Carcinoma Ovary	1	1.59
Coronary Artery Disease	1	1.59
Pott's Spine	1	1.59
Disseminated Koch's	1	1.59
Thyroid Swelling	1	1.59
TOTAL	22	34.95

A total of 34.95% of patients were incidentally detected with Hepatitis B infection.

Table 3: Stages of Hepatitis B

STAGE	**NUMBER** ***(n* = 63*)***	**PERCENTAGE** ***(%)***
CHB HBeAg+	14	22.22
CHB HBeAg–	44	69.84
AHB	5	7.94

Out of 63 cases, 5 cases were acute hepatitis B (AHB) and the remaining 58 cases were in chronic hepatitis B (CHB). Among the chronic stage, 14 cases were HBeAg positive and 44 cases were HBeAg negative.

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Table 4: Correlation between HBV DNA load and Tregs Expression

	SPEARMAN RANK CORRELATION rho	*p-value*
Overall	0.732368	<0.001
CHB HBeAg+	0.34179	0.23167
CHB HBeAg–	0.73042	<0.001
AHB	0.1025978	0.869598

Overall, a positive correlation was found between Hepatitis B DNA load and Regulatory T cell (Tregs) expression.

DISCUSSION

Hepatitis B virus (HBV) infection leads to a wide variety of clinical manifestations ranging from acute self limited illness to different forms of chronic infection progressing to liver failure as well as hepatocellular carcinoma in some patients. India is known to be the largest pool of HBV carriers in the world next to China. There are 43 million estimated HBV carriers in India.[9] One-third of the patients with acute hepatitis and two-thirds of cases with chronic liver disease and hepatocellular carcinoma in India are due to HBV infection. HBV infection in India is a public health problem and its importance for morbidity and mortality has not been substantiated.[10] HBV has four genes: S, P, C, and X. The S gene codes for the major envelope protein (HBsAg). The largest gene is P. It codes for DNA polymerase. The C gene codes for HBeAg and HBcAg. The C gene has a core and a precore region. If the translation is initiated in the precore region, the protein product is HBeAg. If translation is initiated at the core region, HBcAg is the protein product. HBeAg is a marker of HBV replication and infectivity. The X gene codes for HBxAg, which may be involved in carcinogenesis.

In the present study, the maximum cases were in the age group between 30-39 years. In a study by Zhang et al[11], the median age was 49.±12.9 years. In a study by G. Peng et al[12], 63.3% study population were male and 36.7% were female. Among the study population, 69.84% patient were male and 30.16% were female. The incidence of Hepatitis B infection was more common (80.95%) in married than the unmarried (19.05%). In the majority (90.48%) of patients, the onset of symptoms was insidious. A total of 34.95% of patients were incidentally detected with Hepatitis B infection.

Among chronic Hepatitis B patients, the correlation was significant for HBeAg negative but it was insignificant for HBeAg positive patients. This result was contrary to the finding by Peng et

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al.[12] This may be due to the very small number of HBeAg positive patients in this study and that's too were on antiviral therapy which downregulates the Tregs expression during chronic Hepatitis B infection as shown in the study by N. Trehanpati and A.K Vyas[13] and also by Peng et al.[12] The statistically more significant correlation in Chronic Hepatitis B infection with HBeAg negative was might be due to large the proportion of the study population. Though, this group of study the population were HBeAg negative but their DNA load were high which might due to a mutation in the Hepatitis B virus.

Out of 63 cases, 5 cases were acute hepatitis B (AHB) and the remaining 58 cases were in chronic hepatitis B (CHB). Among the chronic stage, 14 cases were HBeAg positive and 44 cases were HBeAg negative. Overall, a positive correlation was found between Hepatitis B DNA load and Regulatory T cell (Tregs) expression. The regulation of CD4+ CD25+ Tregs is mostly nonspecific[14] while preferential inhibition of the HBV antigen- specific T-cell response has been reported in some cases.[15,16] In HCV- and HIV-infected subjects, Tregs may contribute to the persistence of infection by inhibiting HIV- or HCV-specific T-cell responses.[14,17]

CONCLUSION

Regulatory T cells (Tregs) have attracted a great deal of attention over the past few years as a consequence of their ability to suppress CD4 and CD8 effector T-cell responses. The immune regulation of Tregs is complicated, and the mechanisms of suppression of antiviral immune responses are not yet very clear. It has been suggested that Tregs may have evolved to prevent immunopathological damage but also contribute to viral persistence. Our findings demonstrated that there is a positive correlation between Tregs (CD4+CD25+FXP3+) and Hepatitis B DNA load. This result indicates that modulation of CD4+ CD25+ Tregs might be one potential therapeutic strategy for the treatment of Chronic Hepatitis B infection.

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