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Assessment of cytotoxic T-lymphocyte antigen-4 (CD152) Levels Associated with HBV in Patients with Diabetes Mellitus

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ABSTRACT

Background: Diabetes mellitus is a chronic disease characterized by an imbalance in glucose homeostasis. The hepatitis B virus is a liver-attacking virus that can cause viral hepatitis, cirrhosis, and liver cancer. A cytotoxic T-lymphocyte antigen-4 called CTLA-4 is an immune checkpoint protein that is necessary for T cells to control immune responses and stop the HBV infection from spreading. It does this by acting as an inhibitory receptor, limiting liver damage during an acute infection, and making it easier for the infection to stay in the body during a chronic case.

Objective: The study aims to assess CTLA-4 levels in relation to HBV infection in diabetes mellitus patients.

Subjects and methods: A cross-sectional study was conducted from July to October 2023. The serum was obtained from 200 diabetic patients who were Iraqis. All of the patients were tested using an ELISA technique for CTLA-4 and HBc IgG. They were tested using a five-panel kit for HBsAb, HBsAg, HBcAb, HBeAg, and HBeAb and measured blood sugar levels. The statistical analysis approach was conducted using SPSS version 26.

Results: Serum CTLA-4 levels were correlated with serum HBc IgG positivity (P = 0.000), total HBc Ab positivity (P = 0.000), and HBs Ab positivity (P = 0.000), and CTLA-4 level was correlated with diabetes mellitus (P = 0.034).

Conclusions: The study concluded that elevated serum CTLA-4 levels in diabetic patients with HBV infection and type I diabetes compared to type II diabetes. This suggests that diabetes affects the immune system's response to HBV.

KEYWORDS: Anti-HBc IgG, CD152, CTLA-4, Cytotoxic T-lymphocyte antigen-4, Diabetes mellitus, HBV

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INTRODUCTION

Diabetes mellitus (DM) is a chronic disease characterized by an imbalance in glucose homeostasis, and it is becoming a worldwide epidemic (1). The two main kinds of diabetes mellitus are type 1 and type 2. The particular type of diabetes mellitus known as type 1 (T1DM) is a form of diabetes which is characterized by the destruction of pancreatic beta cells that make up 5%–8% of all patients who are diagnosed with diabetes mellitus. T2DM, often referred to as Type 2 Diabetes Mellitus, is the most common type of DM characterized by a deterioration of insulin sensitivity in a broad range of target tissues such as the liver, skeletal muscles, and adipose tissue (2). The most recent study shows that CTLA-4, the costimulatory molecule that boosts T cells, is among the potential risk signs that relate to the increased DM susceptibility (3). Human hepatitis B virus (HBV) is a contagious disease that most commonly affects the liver. It is generally harmful and causes a variety of liver illnesses (4). Hepatitis B is still among the well-known global health problems, as lots of cases of chronic infections are reported annually, which results in high mortality and morbidity. An estimated 820,000 deaths due to hepatitis B-related cirrhosis and cancer of the liver occur annually (5). An exploration of anti-HBc antibodies and their role in the identification of occult HBV forms has significantly accelerated the development of research on the reactivation of HBV (6). Patients who are seropositive for occult hepatitis B infection (OBI) have antibodies in their blood against the core antigen

of hepatitis B virus (anti-HBc) and/or the surface antigen of hepatitis B virus (anti-HBs). This form of OBI constitutes slightly 80% of all OBI cases (7). Alternatively, the use of anti-HBc assessments can be used as an alternative indicator for OBI determination (7, 8). A lot of IgM and then IgG anti-HBc are made because the hepatitis B core antigen is the part of HBV that makes people's immune systems react the strongest. Anti-HBc, typically assessed as a combination of IgG and IgM antibodies, is widely regarded as a highly sensitive and dependable marker for exposure to the hepatitis B virus. The levels of anti-HBc IgM rise fast after acute infection. Because of this, anti-HBc IgM levels are part of the first diagnostic tests for HBsAg-positive patients with high ALT levels, even if there isn't a liver disorder (9). There is HBV in the form of covalently closed circular DNA (cccDNA), and core antigen is made at low levels (10). This diminished immune response makes the host's immune system incapable of eradicating HBV, resulting in a persistent infection (11). After a long-term HBV infection, the host's immune system frequently exhibits deficiencies or lacks the reactivity of T-cells specific to the virus. T-cell exhaustion refers to a state in which T cells exhibit diminished immune responses characterized by cytotoxicity, reduced cytokine synthesis, and enhanced expression of several inhibitory molecules, including cytotoxic lymphocyte-associated antigen-4 (CTLA-4), programmed cell death-1 (PD-1), and lymphocyte activation gene-3 (12). One of the co-inhibitory receptors found on T cells is CTLA-4 (11). CTLA-4 is composed of three introns and four exons and is situated on the elongated arm of chromosome 2q33. CTLA-4, which is similar to CD28, is also a member of the immunoglobulin superfamily. Still, compared to CD28, it can bind that B7 molecule much more tightly, which is present on antigen-presenting cells. In this case, it potentiates T cell suppression (13). High level expression of inhibitory receptors on CD8 T lymphocytes in HBV patients limits their ability to defend, which ultimately results in an exhausted phenotype (14). Immunological checkpoint proteins play a crucial role in the advancement of HBV disease. They tend to minimize the liver damage during an acute stage of hepatitis and may facilitate the creation of chronic HBV cases (15). At present, there is very little data that investigates the link between the quantity of serum CTLA-4 and HBV's various clinical parameters. This is the first study to investigate the connection between levels of CTLA-4 in the blood serum and clinical parameters of HBV among Iraqi patients with diabetes mellitus. The study intends to estimate CTLA-4 immune checkpoint levels in correlation with HBV infection in diabetes mellitus patients.

METHODS

Study design and setting

A cross-sectional study is the subject of the current research project from July to October 2023, executed in Najaf governorate. The research included only Iraqi participants who were medical patients in the specialized endocrinology and diabetes center at the Al-Sader Teaching Hospital in Najaf city. It was carried out with the participation of 200 people.

Sample selection

All patients were diagnosed with diabetes mellitus (either type 1 or type 2 only) based on a clinical diagnosis by an endocrinologist and serological tests. The age range of the patients varied from 18 to 80 years, meeting the criteria for inclusion. Patients who fulfilled the criteria for exclusion included those with a medical history of autoimmune disorders, cancer, and other viral infections such as the hepatitis C virus (HCV), COVID-19, and HIV, as well as individuals who had received the HBV vaccine.

Data collection and outcome measurements

The patients' data were obtained through the implementation of a questionnaire and the collection of a blood sample. The specimens were obtained by extracting approximately 10 mL of venous blood from every participant. Blood samples were placed into a gel tube and allowed to coagulate at room temperature for thirty minutes. The serum was extracted using centrifugation and thereafter divided into 1.5-ml Eppendorf tubes. A part of the serum was immediately utilized for a fasting blood glucose test. The other part was then preserved in a refrigerator at a temperature of -80°C for immunological investigation until further examination. Enzyme-linked immunosorbent assays (ELISA) and rapid diagnostic tests are the main methods used in clinical laboratories to find HBV serological markers. A fraction of the serum was used for a human hepatitis B virus panel test (five panel kit): HBc Ab, HBs Ag, HBs Ab, HBe Ag, HBe Ab (Eugene Biotech/China), anti-HCV rapid test, One Step Cassette Style HIV Rapid Test (Wondfo/China), After that, an ELISA method (Sun Long Biotech, China) was used to find a cytotoxic T lymphocyte antigen (CTLA)-4 and a qualitative HBc IgG. The measurement of fasting plasma glucose (FPG) was conducted using an enzymatic colorimetric method and a kit available commercially from Spinreact, a company in Spain. The procedures for all tests were conducted according to the instructions outlined in the kit's manual. Before enrolling in the research study, all patients received a comprehensive briefing on the study's aims and objectives and were then given their informed consent to participate.

Ethics Committee approval

The ethics council of the University of Kufa's Faculty of Medicine gave its approval before starting this research project. All participants gave their consent, and the Al-Sader Teaching Hospital in the province of Najaf gave its approval.

Statistical analysis

The data was inputted using Excel 2016 for Microsoft Windows, while the statistical analysis was performed using the Statistical Package for Social Sciences (SPSS-26). The distribution of the study group according to different parameters was assessed using frequency and percentage. The variables were reported as the mean and standard deviation for normally distributed (parametric) data and the median and interquartile range (IQR) for non-parametric data. The Shapiro test was used to evaluate the normality of the data distribution, whether parametric or non-parametric, when evaluating the lab. The non-parametric Mann-Whitney U test was employed to demonstrate the relationship between categorical and numerical data. With a p-value of less than 0.05, it was possible to determine the statistical significance of the results. To assess the difference in the medians, the Kruskal-Wallis test was utilized as an alternative to the one-way ANOVA. The presentation of the results is done using MS Word and Excel 2016, with tables and figures along with an explanation paragraph.

RESULTS

The present study included a sample of 200 patients who were diagnosed with diabetes mellitus, specifically type 1 or type 2, while excluding other types. Out of the total sample size, 136 participants were female and 64 were male. Based on the findings, it was observed that 56 patients (28%) were diagnosed with type 1 DM, whereas 144 patients (72%) were diagnosed with type 2 DM, ranging in age from 18 to 80 years old. The participants' median age was 50 years, and the mean age ± standard deviation was 49.71 ± 13.656.

Table 1 shows the serum CTLA-4 values for the participants. A statistically significant increase (p-value < 0.05) in the mean serum CTLA-4 levels was seen in patients with type 1 diabetes compared to patients with type 2 diabetes. Males had a significantly higher mean level of CTLA-4 than females. At the same time, the disparity did not exhibit statistical significance (P = 0.370).

Table 1: Comparative evaluation of CTLA-4 levels between type 1, type 2 diabetes mellitus patients and sex

	Variables		**N**	**Mean Rank**	**P value**
Serum CTLA-4 (pg/ml)	Types of DM	Type 1 DM	56	114.38	P=0.034* Z=-2.114-
		Type 2 DM	144	95.10	
	Sex	Male	64	105.85	P=0.370 Z=-0.897-
		Female	136	97.98	
P values were calculated by the Mann-Whitney U test, * Significant difference (P < 0.05) between groups, DM: Diabetes mellitus.					

Data in Table 2 demonstrated a statistically significant higher (p-value<0.05) in the median serum CTLA-4 levels in patients within the age group of 20–39 years than in patients with the age groups of < 20, 40–59, and 60–79 years. While a statistically significant higher (p-value < 0.05) in the median serum CTLA-4 levels was seen in patients with a duration of diabetes mellitus less than 5 years than in patients with a duration of diabetes mellitus from 5 to 15 years or more than 15 years.

Table 2: The relation between CTLA-4 and age group and duration of diabetes in diabetic patients

	Variables		**N**	**Median(IQR)**	**P value**
Serum CTLA-4 (pg/ml)	Age group / Year	< 20	4	9.3700(5.50)	P=0.016*
		20-39	31	11.0200(7.87)	
		40-59	107	8.4700(6.89)	
		60-79	58	9.0900(6.55)	
	Duration of DM / year	less than 5 years	51	10.6700(7.4)	P=0.010*
		From 5 to 15 years	116	9.1000(7.64)	
		More than 15 year	33	8.0000(7.23)	
P values were calculated by the Kruskal-Wallis test, * level of significance at <0.05, IQR: interquartile range, DM: Diabetes mellitus.					

In this study, among the 200 diabetes mellitus patients, 151 (75.5%) had HBc IgG +, 154 (77%) had HBc Ab +, 21 (10.5%) had HBs Ab +, and 33 (16.5%) had HBe Ab +. There was a negative result for HBs Ag, HBe Ag, HCV, and HIV. As shown in Fig. 1,.

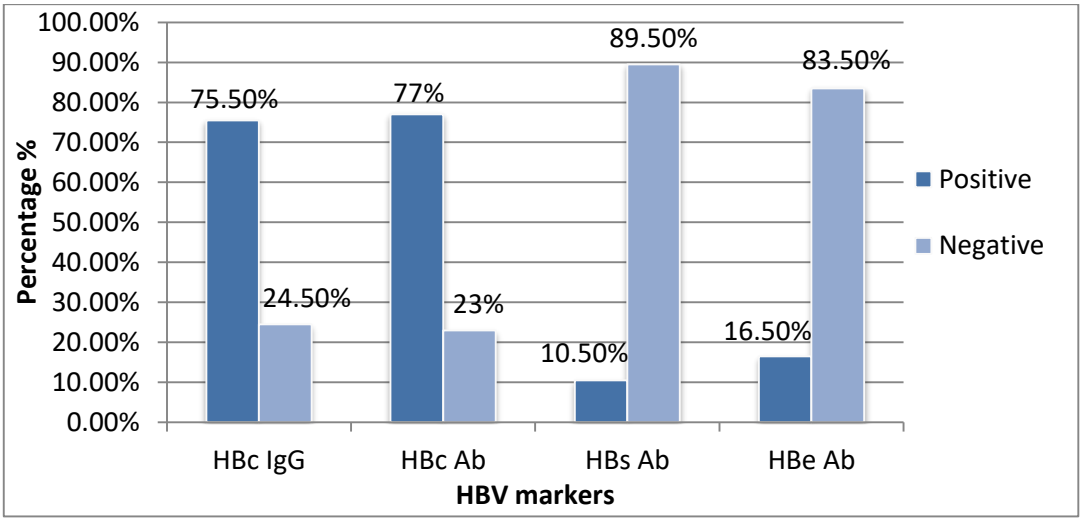

Figure 1: HBV biomarkers in patients with diabetes mellitus

Table 3 presents the serum CTLA 4 measurements for the participants. The mean serum CTLA-4 levels went up significantly (p-value < 0.05) in people who tested positive for HBc IgG, HBc Ab, and HBs Ab. Additionally, the difference did not exhibit statistical significance (P = 0.181) in the mean serum CTLA-4 levels in patients who tested positive for HBe Ab.

Table 3: Comparison of the levels of CTLA-4 in HBV markers HBc IgG, HBc AB, HBs AB, and HBe antibodies

	HBV markers	**positive**		**Negative**		**Test statistics**
		N	Mean Rank	N	Mean Rank	
Serum CTLA-4 (Pg/ml), Mean Rank	HBc IgG	151	120.94	49	37.50	P= 0.000* Z=-8.770-
	HBc Ab	154	119.05	46	38.41	P=0.000* Z=-8.292-
	HBs AB	21	161.14	179	93.39	P=0.000* Z=-5.076-
	HBe Ab	33	112.82	167	98.07	P=0.181 Z=-1.338-
* Level of significant variance (P < 0.05) , P values were computed via the Mann-Whitney test						

Individuals with HBsAg (-)/HBc IgG (+)/HBs Ab (+) (N = 21, M = 161.14) demonstrated a significant correlation with higher levels of CTLA-4 production compared to those with negative results (N = 179, M = 93.39) (p value <0.05, Z = -5.076-). As demonstrated in Table 4,

Table 4: The relation between CTLA-4 and the HBs Ag (-) / HBc Ab (+)/ HBs Ab (+) groups by Mann-Whitney test

		HBs Ag(-) / HBC Ab (+) /HBs AB(+) group		**Test statistics**
		Positive	Negative	
Serum CTLA-4 (Pg/ml), Mean Rank	N	21	179	P=0.000*
	Mean Rank	161.14	93.39	Z=-5.076-
* Level of significant variance P < 0.05.				

DISCUSSION

Previous research has shown that high levels of CTLA-4 in the blood are linked to autoimmune disease. However, this study shows that the levels of CTLA-4 in the blood are statistically significantly higher (p-value < 0.05) in people with type 1 diabetes compared to people with type 2 diabetes. The present outcome is in line with previous Sato et al. (2004) findings that indicate people with various autoimmune disorders, like type 1 diabetes, have high amounts of sCTLA-4 in their serum (16). The outcome reached in this investigation is inconsistent with Ryden et al.'s 2012 study, which revealed a substantial fall in CTLA-4 expression levels in patients with T1DM (17). The rationale for such variances in CTLA-4 concentrations may be related to differences in the technique utilized, changes in sensitivity levels of the method used, and differences in individuals' genetics and environmental factors. Alshareef et al.'s (2019) studies have indicated a strong association between CTLA4 polymorphisms and the prevalence of T1DM in diverse ethnic communities, like Japanese, Chinese, Egyptians, and Sudanese (18). Clark et al., (2017) showed that genes encoding cytotoxic T lymphocyte-associated antigen 4 are potential candidate genes for susceptibility to type 1 DM (19). New studies have shown that there are numerous significant genetic regions that carry a high risk for the emergence of diabetes, and the gene CTLA-4 has been recognized as one of these regions that increases susceptibility to type 1 diabetes mellitus (13, 3). CTLA-4 may serve as a potential susceptibility gene for type 2 diabetes mellitus. Patients with type 2 diabetes mellitus who possess the T allele at the CTLA4-318 C/T locus are more susceptible to developing diabetic ketosis (20).

This study found that the CTLA-4 level in males is slightly higher than in females. However, this difference is not statistically significant (P = 0.370) and does not suggest any difference between the sexes. The variations can be related to the greater number of female participants enrolled in this investigation. This study's results don't match up with those of Schott et al. (2007), who found that CTLA4 haplotypes were different in men with self-limiting and ongoing infections but not in women (p = 0.043) (21).

In the present study, serum CTLA-4 levels were higher in patients within the age group of 20–39 years than in patients with the age groups of <20, 40–59, and 60–79 years. The results don't match up with the study by Canaday et al. (2013), which found that older people had higher levels of CTLA-4-expressing cells than younger people (88% vs. 79%, p < 0.002) among the participants (22). The elevated expression of CTLA-4 in this age group is attributed to genetics and immune system activity.The immune system's most active stage during this age group results in enhanced CTLA-4 protein synthesis, which helps regulate the immune response and inhibits immune cell activity.

In this study, serum CTLA-4 levels were seen in patients with a duration of diabetes mellitus less than 5 years higher than in patients with a duration of diabetes mellitus from 5 to 15 years or more than 15 years. This result does not align with a previous study conducted in Saudi Arabia that measured serum levels of sCTLA-4 and found no significant correlations with length of disease (p = 0.09) (23). There is still no clear reason for the higher level of CTLA-4 in people with diabetes who were diagnosed five years ago compared to those who were diagnosed 10 to 20 years ago. However, this study suggests that there may be reasons for this. For example, diabetes can get worse over time, which can change the immune system. Also, the medicines used to control diabetes may have an effect on CTLA-4 production.

Hepatitis B virus produces a variety of antigens, such as hepatitis B core, surface, and envelope antigens. These antigens possess strong immunogenicity and can induce an immune reaction (24). The study realized by Arrelias et al. (2018) in Brazil demonstrated that no cases of reactive HBsAg were detected. It aligns with the results of this research (25). As demonstrated by Caviglia et al.'s (2020) findings, absence of HBsAg in the blood may not signify the absence of HBV infection (26). As shown in the study conducted by Zheng et al. (2022), HBV genome has a high rate of mutation because no proofreading function is performed at the replication stage (27). People with a seropositive occult hepatitis B infection (OBI) have antibodies in their serum against the core antigen of HBV (anti-HBc) and/or antibodies against the surface antigen of HBV (anti-HBs) (7). The present study shows anti-HBs (+)/anti-HBc (+) (10.5%, 21/200). Pollicino et al. (2021) study suggested that the HBsAg- negative/anti-HBc-positive condition is a stage of occult (OBI) in the course of the disease (28). Regard the HBsAg negative/ HBc-Ab positive state like an OBI stage in the HBV infection's natural course. More than 90% of individuals who have anti-HBc positive are seemingly carriers of the OBI. The "alternative" antiHBc test is considered the most acceptable and practicable marker for occult hepatitis B infection diagnosis (29).

The study found that the greatest rate of positivity for seromarkers related to HBV infection was 77% for HBc Ab and 75.5% for HBc IgG among the diabetes participants examined. Compared to a study conducted in China, which reported HBc Ab positive rates of 62.3% among diabetic patients (30). Patients who have recovered from the infection will have a lifelong presence of anti-HBc antibodies. IgM anti-HBc indicates acute infection, but IgG anti-HBc indicates prior infection (31, 32). The current study's results contradicted the findings of Madhavan et al. (2021), which indicated a prevalence of anti-HBc at 14.6% (33). Variations in HBV exposure risk, sample size across different regions can be responsible for the differences in HBV infection rates worldwide. The variations in antibody frequencies can originate from variations in the assay, sensitivity levels of the

technique, sample size, or study methodology. There are variations in vaccination status. Due to the serologic window during the incubation period after infection, other studies have not been able to identify infected patients, and this HBs Ab positive result is from an infection that was not vaccinated.

However, the correlation between the proteins of HBV and the production of CTLA-4 after HBV infection is still unclear (34). The current investigation revealed a notable association between increased levels of CTLA-4 and markers of HBV in diabetic patients. The results were strongly related to those of Dharmadi (2023), who found a link between the amount of CTLA-4 in the blood serum and how quickly chronic hepatitis B got worse ($P< 0.001$). The serum amount of CTLA-4 was elevated in individuals with CHB, hepatocellular cancer, and liver cirrhosis. The level of CTLA-4 in the serum was found to have a positive correlation with the advancement of CHB. The importance of CTLA-4 levels in patients with HBV has significant clinical consequences. High levels of CTLA-4 may serve as a marker of more severe hepatic inflammation and worse outcomes. Additionally, using immunotherapy to target CTLA-4 appears to be a possible way to manage HBV infection (35). According to one study, CTLA-4 inhibition enhanced T cells specific to HBV and decreased hepatic inflammation in HBV individuals (36).These results are very similar to those reported by Tang et al. (2016), who found that CD4+ Th cells from people with chronic hepatitis B had higher levels of CD28 family receptors, such as PD-1 and CTLA-4, than CD4+ Th cells from healthy people (37). A recent study conducted by Peng et al. (2011) showed that the presence of HBeAg can enhance the production of CTLA-4 in CD8+ T cells. Furthermore, it has been noted that this condition is strongly associated with an elevated HBV DNA content. While these discoveries hold the possibility of major progress, the underlying mechanism of these events remains unknown (38).

LIMITATIONS OF THIS RESEARCH

Firstly, the study design (a cross-sectional study) cannot show cause and effect but can effectively validate or invalidate assumptions. Secondly, the individual's diabetes mellitus and HBV vaccination histories were obtained through self-reporting, posing a potential risk of biased recall. Third, the study was conducted at a single center with a narrow sample size.

CONCLUSIONS

The study concluded that increased levels of serum CTLA-4 in persons with type I diabetes compared to those with type II diabetes and the history of HBV or occult HBV infection can induce changes in the immune system's reactivity to the virus and the possible evolution of the disease. This suggests that diabetes affects the immune system's response to HBV. There was a strong link between the amount of CTLA-4 in the blood and proteins that are specific to the virus, such as HBs Ab, HBc Ab, and HBc IgG. Discovering these relationships will aid in deepening the understanding of immunological interplay in cases of dual illness with diabetes mellitus and hepatitis B virus. This finding could initiate a series of new studies that may use CTLA-4 as a treatment strategy for diabetic individuals suffering from HBV infection.

REFERENCES

I. Rachdaoui, N. Insulin: the friend and the foe in the development of type 2 diabetes mellitus. International journal of molecular sciences.2020; 21(5): 1–21.

II. Aghaei Zarch SM, Dehghan Tezerjani M, Talebi M, Vahidi Mehrjardi MY. Molecular biomarkers in diabetes mellitus (DM). Med J Islam Repub Iran. Apr, 2020,1;34:28.

III. Liu Z, Song Z, Sun J, Sun F, Li C, Sun J, Xu L. Association between CTLA-4 rs231775 polymorphism and hepatocellular carcinoma susceptibility. Int J Clin Exp Pathol. Nov, 2015, 1;8(11):15118-22.

IV. Zheng, J. R., Wang, Z. L., & Feng, B. Hepatitis B functional cure and immune response. Frontiers in Immunology.2022; 13, 1075916.

V. Lazarevic, I., Banko, A., Miljanovic, D., & Cupic, M. Clinical Utility of Quantitative HBV Core Antibodies for Solving Diagnostic Dilemmas. Viruses . 2023;15(2), 373.

VI. Cholongitas E, Haidich AB, Apostolidou-Kiouti F, Chalevas P, Papatheodoridis GV. Hepatitis B virus reactivation in HBsAg-negative, anti-HBc-positive patients receiving immunosuppressive therapy: a systematic review. Ann Gastroenterol. Jul-Aug, 2018; 31(4):480-490.

VII. Raimondo, G.; Locarnini, S.; Pollicino, T.; Levrero, M.; Zoulim, F.; Lok, A.S.;et al. Update of the statements on biology and clinical impact of occult hepatitis B virus infection. J. Hepatol. 2019, 71(2), 397–408.

VIII. Raimondo, G., Allain, J. P., Brunetto, M. R., Buendia, M. A., Chen, D. S., Colombo, M., et al .Statements from the Taormina expert meeting on occult hepatitis B virus infection. Journal of hepatology, 2008, 49(4), 652-657.

IX. Park, J. J., Wong, D. K., Wahed, A. S., Lee, W. M., Feld, J. J., Terrault, N.,et al . Hepatitis B virus–specific and global T-cell dysfunction in chronic hepatitis B. Gastroenterology, 2016; 150 (3), 684-695.

X. Zoulim F, Durantel D. Antiviral therapies and prospects for a cure of chronic hepatitis B. Cold

Spring Harb Perspect Med. Apr, 2015, 1;5(4):a021501.

XI. Wang L, Li N, Fan X, Wang X, Zhang X, Zhang K,et al. Circulating CTLA-4 levels and CTLA4 polymorphism associate with disease condition and progession and hepatocellular carcinoma patients' survival in chronic hepatitis B virus infection. Int Immunopharmacol. 2020; 82:106377.

XII. Ye, B., Liu, X., Li, X. et al. T-cell exhaustion in chronic hepatitis B infection: current knowledge and clinical significance. Cell Death Dis (2015).6, e1694.

XIII. Wagner MJ, Othus M, Patel SP, Ryan, C., Sangal, A., Powers, et al. Multicenter phase II trial (SWOG S1609, cohort 51) of ipilimumab and nivolumab in metastatic or unresectable angiosarcoma: a substudy of dual anti-CTLA-4 and anti-PD-1 blockade in rare tumors (DART). Journal for Immunotherapy of Cance r. 2021; 9(8) , e002990.

XIV. Bertoletti, A., & Ferrari, C. Adaptive immunity in HBV infection. Journal of hepatology.2016; 64(1), S71-S83.

XV. Wykes, M. N., & Lewin, S. R. Immune checkpoint blockade in infectious diseases. Nature Reviews Immunology (2018); 18(2), 91-104.

XVI. Sato S, Fujimoto M, Hasegawa M, et al. Serum soluble CTLA-4 levels are increased in diffuse cutaneous systemic sclerosis. Rheumatology.2004 , 43(10):1261–1266.

XVII. Ryden A, Bolmeson C, Jonson CO, Cilio CM, Faresjo M. Low expression and secretion of circulating soluble CTLA-4 in peripheral blood mononuclear cells and sera from type 1 diabetic children. Diabetes Metab Res Rev.2012, 28:84–96.

XVIII. Alshareef SA, Omar SM, Hamdan HZ, and Adam I. Cytotoxic T lymphocyte antigen 4 +49A/G polymorphisms in Sudanese adults with type 1 diabetes and latent autoimmune diabetes. BMC Res Notes. 2019;12:769.

XIX. Clark M, Kroger CJ, Tisch RM.Type 1 diabetes: a chronic anti-Self-Inflammatory response. Front Immunol .2017, 8:1898.

XX. Ren, D., He, L., & Pang, X. Association of CLTA-4 Gene Polymorphisms with Diabetes Mellitus: A Study Based on the Han Population of Northern China. Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy.2022; 2705-2712.

XXI. Schott, E., Witt, H., Hinrichsen, H., Neumann, K., Weich, V., Bergk, A., Halangk, J., Müller, T., Tinjala, S., Puhl, G., Neuhaus, P., Wiedenmann, B., & Berg, T. Gender-dependent association of CTLA4 polymorphisms with resolution of hepatitis C virus infection. Journal of hepatology. 2007, 46(3), 372–380.

XXII. Canaday, D. H., Parker, K. E., Aung, H., Chen, H. E., Nunez-Medina, D., & Burant, C. J. Age-dependent changes in the expression of regulatory cell surface ligands in activated human T-cells. BMC immunology, (2013).14, 1-5.

XXIII. Al-Hakami A. Serum sCTLA-4 level is not associated with type 1 diabetes or the coexistence of autoantibodies in children and adolescent patients from the southern region of Saudi Arabia. Auto-immunity highlights. 2020; 11 (1), 19.

XXIV. Cao, H., Zhang, R., & Zhang, W. CTLA‐4 interferes with the HBV‐specific T cell immune response. International Journal of Molecular Medicine.2018; 42(2), 703-712.

XXV. Arrelias CCA, Rodrigues FB,Torquato MTDCG, Teixeira CRS, Rodrigues FFL, Zanetti ML. Prevalence of serological markers for hepatitis and potential associated factors in patients with diabetes mellitus. Rev Lat Am Enfermagem. Nov, 2018, 29; 26:e3085.

XXVI. Caviglia, G. P., Olivero, A., Ciancio, A., Tandoi, F., Troshina, G., Rosso, C et al ., Analytical and clinical evaluation of a novel assay for anti-HBc IgG measurement in serum of subjects with overt and occult HBV infection. Diagnostic Microbiology and Infectious Disease (2020), 96(4), 114985.

XXVII. Yan L, Zhang H, Ma H, Liu D, Li W, Kang Y, et al. Deep sequencing of hepatitis B virus basal core promoter and precore mutants in HBeAg-positive chronic hepatitis B patients. Sci Rep. 2015;5:17950.

XXVIII. Pollicino, T., and Caminiti, G. HBV-integration studies in the clinic: role in the natural history of infection. Viruses (2021),13 (3), 368.

XXIX. Wang C, Xue R, Wang X, Xiao L and Xian J High-sensitivity HBV DNA test for the diagnosis of occult HBV infection: commonly used but not reliable. Front. Cell. Infect. Microbiol (2023),13:1186877.

XXX. Lu J, Hou X, Tu H, Tang Z, Xiang Y, Bao Y, et al. Chronic hepatitis B virus infection status is more prevalent in patients with type 2 diabetes. J Diabetes Investig (2017): 8(4):619–625.

XXXI. Ndako JA, Nwankiti OO, Olorundare JO, Ojo SKS, Okolie CE, Olatinsu O, Dojumo VT. Studies on the serological markers for hepatitis B virus infection among type 2 diabetic patients. J Clin Lab Anal. Jan; 2021: 35(1):e23464.

XXXII. Rehermann B, Ferrari C, Pasquinelli C, Chisari FV. The hepatitis B virus persists for decades after patient's recovery from acute viral hepatitis despite active maintenance of a cytotoxic T-lymphocyte response. Nat Med Journal, 1996: 2:1104-1108.

XXXIII. Madhava n A, Sachu A, Balakrishnan AK, Balakrishnan S, Vasudevapanicker J. Prevalence of Anti-HBc Antibodies among HBsAg Negative

Individuals and Its Association with Occult Hepatitis B. J Lab Physicians. Mar. 2021:13(1):1-5.

XXXIV. Liu Y, Cheng LS, Wu SD, Wang SQ, Li L, She WM, Li J, Wang JY and Jiang W.IL‐10‐producing regulatory B‐cells suppressed effector T‐cells but enhanced regulatory T‐cells in chronic HBV infection. Clin Sci (2016):130: 907‐919.

XXXV. Darmadi D, Lindarto D, Siregar J, Widyawati T, Rusda M, Amin MM, et al.Association Between Serum Cytotoxic T Lymphocyte Antigen (CTLA)-4 Level and Disease Progression in Patients With Chronic Hepatitis B. Med Arch. Apr; 2023,77(2):142-145.

XXXVI. Zhang, H., Wang, Z., Zhang, J., Zhang, X., Gui, Z., Sun, L. & Gu, M. The synergism of B and T lymphocyte attenuator (BTLA) and cytotoxic T lymphocyte associated antigen-4 (CTLA-4) attenuated acute T-cell mediated rejection and prolonged renal graft survival. Translational Andrology and Urology, (2020): 9(5), 1990.

XXXVII. Tang ZS, Hao YH, Zhang EJ, Xu CL, Zhou Y, Zheng X and Yang DL: CD28 family of receptors on T cells in chronic HBV infection: Expression characteristics, clinical significance and correlations with PD‐1 blockade. Mol Med Rep (2016) .14: 1107 ‐1116.

XXXVIII. Peng G, Luo B, Li J, Zhaod, Wu W, chen F;et al. Hepatitis B e‐antigen persistency is associated with the properties of HBV‐specific CD8 T cells in CHB patients. J Clin Immunol . 2011; 31, 195‐204.