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Serum total IgE as immunological marker in patients with chronic hepatitis B Virus infection, and hepatitis B related hepatocellular carcinoma

Mohammed Khalil[1], Abeer Sharaf Eldin[3], Ahmed Sadek[2], Bahaa M. Badr[1] and Ayman S. Yassin[1]

[1]Department of Medical Microbiology & Immunology, Faculty of Medicine, Al-Azhar University (Assiut branch), Assiut, Egypt.
[2]Department of Medical Microbiology & Immunology, Faculty of Medicine, Assiut University, Assiut, Egypt.
[3]Department of Tropical Medicine & Gastroenterology, Faculty of Medicine, Assiut University, Assiut, Egypt.

Corresponding author: Mohammed Khalil, Department of Medical Microbiology & Immunology, Faculty of Medicine, Al-Azhar University (Assiut branch), Assiut, Egypt.
Email: mhmd22ali_ali@yahoo.com.

Abstract

This study evaluated the efficacy of IgE in predicting disease progression in chronic hepatitis B virus (HBV) and HBV related hepatocellular carcinoma (HCC) compared to normal controls. The study included 60 HBV-infected patients. Of these, 30 patients with chronic hepatitis B but not related to HCC and 30 patients with related HCC. Serum level of IgE was measured by ELISA. Serum level of IgE was higher in HCC patients than non-HCC patients ($p< 0.005$). Significant correlations were detected between IgE, transaminases (ALT, AST), alpha-fetoprotein and severity scores in chronic hepatitis B (CHB) patients. The level of IgE was correlated with HB viral load. Stronger correlations were evident between IgE, prothrombin time and total bilirubin. In conclusion, IgE levels may be considered as non-invasive markers for monitoring liver disease progression in CHB.

Keywords: Hepatitis-B Virus, Hepatocellular carcinoma, Immunoglobulin-E.

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Introduction

Acute and chronic hepatitis, liver fibrosis, cirrhosis, and hepatocellular carcinoma (HCC) are the clinical sequences of hepatitis B virus (HBV) infection, the impact of noncytopathic consequence of HBV replication in hepatocytes is affected by host genetic and immunological factors,[1] the clinical sequelae of HBV infection is depending on the immunological and inflammatory effect of immune-inflammatory cells and its cytokines/chemokines secretion.[2]

Immunoglobulin E (IgE) is a type of antibody that originated in mammals only. IgE is produced by plasma cells. IgE is formed of two heavy chains and two light chains, it is involved in allergy, type I hypersensitivity, immune response against worm and parasitic infestations.[3] Immunoglobulins abnormalities is a common hallmark for acute hepatitis, some studies demonstrated increase IgE level in acute

hepatitis B and symptomatic chronic hepatitis. Patients with autoimmune hepatitis and alcoholic liver cirrhosis have higher level of IgE than normal individual. The level of IgE has relation with hepatitis B surface antigen.[31,6]

Hepatocellular carcinoma is reflecting the fifth most common cancer and sorted the second most common cause of death world widely.[5] In Egypt, it represents the fourth common cancer.[32] Risk factors for HCC development include hepatitis B virus (HBV) and hepatitis C virus infections, smoking, aflatoxin exposure, and excessive alcohol consumption. Despite of elaborations in prevention and treatment polices of cancers, HCC remains a major cause of death.[6] Some previous studies evaluated the role of IgE antibodies in hepatitis infection, demonstrated that IgE has a role in hepatitis C and B, but the effect was prominent in acute infection more than chronic infections.[8,21]

IgE antibodies mediate liver disease, cirrhosis, and carcinogenesis through production of inflammatory mediators, the most important is IL-6, interferon gamma and IL-17.[5] A previous study demonstrated the relation between IgE antibodies and chronic hepatitis B (CHB) under influences of interferon gamma which has regulatory and effector functions within the innate, acquired immunity and activation of nuclear factor κ, which has a prominent role in chronic liver disease.[11] Other studies evaluated the role of IL17A in production of IgE antibodies,[12] and their role in HCC development, progression, and bad prognosis.[9, 10] Significantly increased levels of IgE in the tumor tissues of patients with HCC was associated with overall mortality rate as well as tumor metastasis.[7]

Some studies validated the role of IgE in some cancers as breast, ovarian and GIT cancers.[28,5] Another study elucidated the association between allergy and risk of cancer by quantitated the IgE level.[27] Other studies evaluated the role of IgE in chronic hepatitis C viral infection. Some studies demonstrated the role of immunoglobulins G and M in chronic hepatitis B infection.[29, 31] This study aimed to determine whether IgE antibodies have a role in predicting disease progression in HBV and HBV related HCC.

Subjects and Methods

Study design

The study included 60 patients with hepatitis B related chronic liver diseases. They were classified equally into two groups. Group I included 30 patients positive for HBV infection and group II included 30 patients with chronic HBV infection related hepatocellular carcinoma (CHB-HCC). Patients infected with other types of virus hepatitis, autoimmune hepatitis, metabolic liver diseases were excluded from the study. The control group included 30 subjects with no prior history of hepatitis viral infection, no symptoms of liver disease and normal liver function tests.

This study was conducted from October 2019 to May 2020 at the Medical Microbiology and Immunology Department and recruited patients attending the Tropical Medicine and Gastroenterology Department, Assiut University Hospitals (AL-Ragehi Hospital). All studied patients were selected according to the guidelines of the European College of Hepatology [26]. Their age ranged from 35 to 70 years.

All patients were subjected to a detailed history taking and clinical examination, and liver disease stage was evaluated by liver function tests alanine aminotransferase (ALT), aspartate aminotransferase (AST), total and direct bilirubin, International Normalized Ratio (INR), complete blood count (CBC), alpha fetoprotein (AFP), viral load, and abdominal ultrasonography.

The severity of chronic HBV was graded matching to Child-Pugh and model for end stage liver disease (MELD) classifications [19]

Liver cirrhosis was diagnosed clinically and confirmed by ultrasonography (course liver, irregular surface ± reduced size, attenuated hepatic veins, and enlarged caudate lobe) and biochemically (low serum albumin and prolonged prothrombin time). Cases of HCC were diagnosed based on cytological findings, or an elevated serum AFP level (400 ng/ mL) combined with at least one positive liver image on computed tomography, magnetic resonance

imaging, or ultrasonography. All such data were obtained from hospital patient's records.

Laboratory diagnosis of HBV was based on, raised liver enzymes, detection of hepatitis B surface antigen (HBs antigen) by ELISA and HBV DNA load by real time PCR. All patients were confirmed to have a history of HBV infection of more than 6 months.

The study protocol was reviewed and approved by the Research Ethics Committee of the Faculty of Medicine, Al-Azhar University (May 2019). A written informed consent was obtained from each patient and control participant before enrolled in the study

Samples collection

A whole blood sample from each subject was collected under aseptic condition. An aliquot (2.5 ml) venous blood sample was withdrawn on plain tube from each subject, after clotting, the samples were centrifuged at 3000 xg for 5 minutes to separate serum then preserved in sterile Eppendorf tubes at -20 °C until used.

Laboratory tests included CBC, was determined by an automatic hematology analyzer (Symex XT 1800, Japan), liver function tests including serum albumin by a blood chemistry analyzer (Roche Integra 400 plus, Roche Diagnostic), AFP by an automated immunoassay analyzer (Hitachi cobas E 411) and prothrombin time by a coagulation analyzer (CoaDATA 504, GmbH, Germany).

Measurement of serum IgE

The IgE level was determined by a commercial enzyme-linked immunosorbent assay (ELISA) kit (Koma Biotech, Korea), based on a sandwich ELISA protocol,[4] according to the manufacturer's instruction. Briefly, 100 µl of standards and samples were added in duplicate to the antigen-affinity purified primary IgE antibody precoated microtiter wells and incubated at room temperature for 1 hour. The wells were aspirated and washed 4 times using 300 µl of washing solution per well. Biotinylated antibody was added (100 µl) to each test well and incubated for 1 hour. The wells were aspirated and washed for 4 times. TMB solution100 µl per well was added and incubated for 20 minutes. Finally, 100 µl of the stop solution was added to each well. Quantitative results were obtained by reading absorbance, measured at 450 nm using a microplate reader (Staat fax 2000, Korea).

Statistical analysis

Statistical analysis of data was conducted by SPSS software (version 28). Kruskal Wallis-Hand and One way ANOVA test were used to compare between groups. Spearman correlation test was used for r values calculation. The receiver operating characteristic curve (ROC) was used to determine the performance of IgE in predicting development of hepatocellular carcinoma in chronic hepatitis B patients. A *P* value of <0.05 was considered significant.

Results

Evaluation of biochemical data and disease progression among the study groups

Biochemical data and clinical characteristics of the HBV patients included in the study are shown in Table1.

Table 1. The biochemical data among the study groups.

Variable	GI (n=30) Mean	GII (n=30) Mean	*P* value
HB Viral Load (IU/ml)	1400024	136850	0.011
ALT (IU/L)	71.2	89.1	NS
AST(IU/L)	65.5	72	NS
ALP (IU/L)	55.07	77.9	NS
TBL (gm/dl)	3	4.9	0.01
INR	1.12	2.95	0.005
Albumin (g/dl)	3.37	2.26	0.002
AFP (ng/ml)	12.4	726.71	0.004

Table 1. Continued.

Variable	GI (n=30) Mean	GII (n=30) Mean	*p* value
RBCs (x10^6/µl)	3.4	3.6	NS
HG (g/dl)	12	11.3	<0.001
PLT (x10^5µL)	1.9	0.95	0.032
Tumor size		n (%)	
<5 cm		19 (63.3%)	
≥5 cm		11 (36.7%)	
Metastasis		5 (16.6%)	

Group I (Chronic hepatitis B), group II (Chronic hepatitis B related hepatocellular carcinoma), n= number, Kruskal Wallis-H test was used; $p > 0.05$ is not significant (NS). ALT alanine aminotransferase, AST aspartate aminotransferase, TBL total bilirubin, PT prothrombin time, HG hemoglobin level, PLT platelet count, HBV hepatitis B virus.

According to the Child- Pugh classification, 93.3% of cases in group I were grade A and 6.7% of cases grade B. In group II, 36.7 % of cases were grade A, 60 % of cases grade B and 3.3% grade C, and the difference between two groups was statistically significant (p =0.03). (Table 2)

Table 2. Child-Pugh classification among patient groups.

Child-Pugh classification	GI		GII		*p* value
	n	%	n	%	
Grade A	28	93.3	11	36.7	0.03
Grade B	2	6.7	18	60	
Grade C	0	0	1	3.3	

Group I (Chronic hepatitis B virus), Group II (Hepatitis B Virus related hepatocellular carcinoma). n=number. One way Anova test was used, *p-values are between the 2 patients' groups, significant if < 0.05.

Evaluation of IgE in the studied groups

The level of IgE was significantly higher in the two hepatitis B related chronic liver disease groups in comparison with the control group (p< 0.0001). Serum IgE levels were significantly different between the two patient groups (p < 0.0001). The highest mean IgE level was observed in Group II (237.9±36.4 pg/ml) (Table 3).

Table 3. The results of IgE among the two study patient groups.

Variable	G1	GII	Control	*P* value
IgE (pg/ml) Mean± SD	178.9±6.044	237.9±36.4	31.8±4.7	<0.0001

G groups; SD standard deviation; Group I (Chronic hepatitis B virus), group II (Hepatitis B Virus related hepatocellular carcinoma). Kruskal Wallis-H test was used, p-values are between the 2 patients' groups, significant if < 0.05.

Associations of the mean serum IgE level with biochemical results and hepatitis B viral load in both groups

Significant correlations were observed between the serum IgE level and the transaminases (ALT, AST), HBV load in both patient groups. Total bilirubin (TBL), Platelet count (PLT) and AFP were significantly correlated with IgE levels in group II (HBV-HCC) only (Table 4 and Figures 1, 2).

Table 4. Correlations between IgE and serum levels of liver function, platelet count and viral load.

Variable	G I (n=30)		G II (n=30)	
	p value	*r* value	*p* value	*r* value
AST (IU/L)	0.001	0.5	0.043	0.6
ALT (IU/L)	0.007	0.6	0.048	0.7
PT (Sec)	NS	0.2	NS	0.2
TBL (mg/dL)	NS	0.3	0.005	0.6
PLT (x10^3µL)	NS	0.3	0.001	0.7
AFP (ng/ml)	0.008	0.6	0.037	0.7
HB viral load (IU/ml)	0.04	0.5	0.001	0.6

P > 0.05 is not significant (NS); ALT alanine aminotransferase; AST aspartate aminotransferase; TBL total bilirubin; PT prothrombin time; HG hemoglobin level; PLT platelet count, HBV hepatitis B virus, r: correlation coefficient. Spearman correlation test was used.

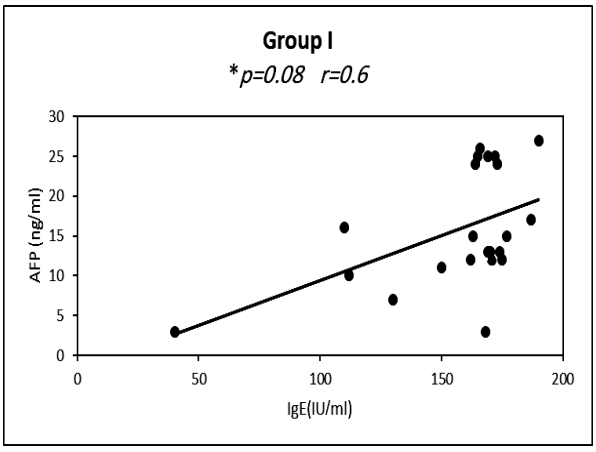

Figure 1. Correlation betwween serum level of IgE and AFP in group I.

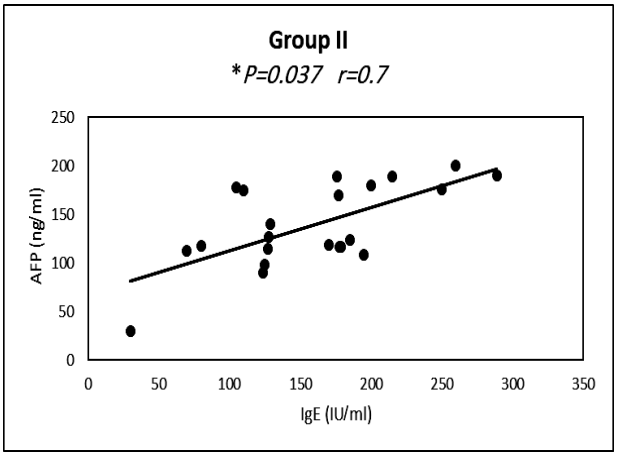

Figure 2. Correlation betwween serum level of IgE and AFP in group II.

Associations between Child-Pugh score, MELD score and serum level of IgE among the studied groups

Serum level of IgE was strongly correlated with MELD score (r= 0.7, *P* = 0.001) and mortality rate but, weakly correlated with Child-Pugh score (Table 5).

Relation between grades of fibrosis and levels of serum IgE

A higher level of IgE was observed in grade 4 fibrosis (F4 stage) than other fibrosis stages, however no differences observed between IgE and all other fibrosis stages (Table 6).

Table 5. Correlations between serum level of IgE with Child-Pugh score and MELD score among the 60 studied patients.

Parameters	IgE	
	p value	*r* value
Child-Pugh score	NS	0.1
MELD score	0.001	0.7

Spearman correlation test, *p* > 0.05 is not significant (NS), r correlation coefficient.

Table 6. The results of Fibroscan findings of the study patients.

Fibrosis stages	IgE (Mean±SD)
F0-F1	145.44±3.6
F2	114.15±4.77
F3	156.18±3.47
F4	186.35±2.32
p value	NS

F=fibrosis, SD=standard deviation,One way ANOVA test was used, $p > 0.05$ is not significant (NS).

A receiver operating characteristic curve (ROC) and cut off value for IgE

A receiver operating characteristic curve (ROC) was used to determine the performance of IgE in predicting development of hepatocellular carcinoma in chronic hepatitis B patients. Area under the curve (AUC) for IgE was 0.52. The value of cut off point for IgE in CHB patients was ≥ 245 with sensitivity 68.7, specificity 92.3, positive predictive value 93.6 and negative predictive value 64.3. (Table 7 and Figure 3).

Table 7. ROC curve analysis for determination of the cut-off value of IgE for prediction of HCC in CHB patients.

Cut-off	Sensitivity	Specificity	PPV	NPV	AUC (95% CI)
≥245	68.7	92.3	93.6	64.3	0.52 (0.70-0.86)

AUC Area under the Curve, CI confidence interval, PPV positive predictive value, NPV negative predictive value.

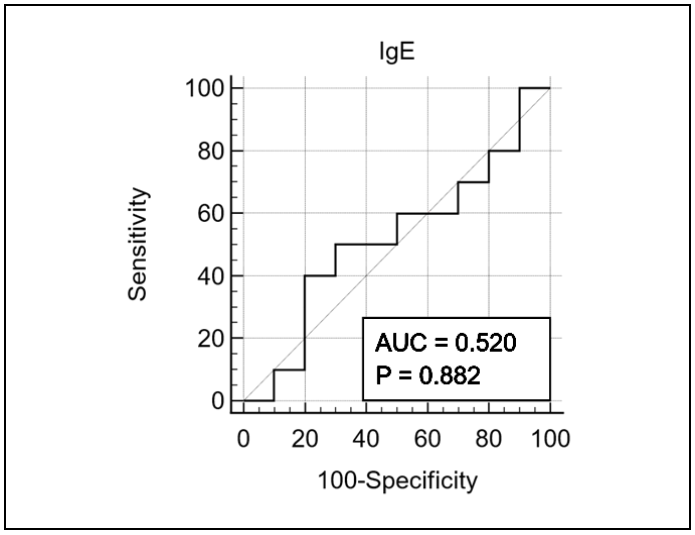

Figure 3. Receiver operating characteristic (ROC) curve to determine the accuracy of IgE for prediction of HCC in CHB patients.

Discussion

The current study intended to determine whether IgE antibodies have a role in predicting disease progression in HBV and HBV related HCC. The current study showed a significant elevation in serum IgE in HBV related to HCC than related to CHB. This observation agreed with that mentioned in a previous study,[20] who reported that the levels of IgE were higher in HBV related to HCC than related to CHB.

There were statistically significant differences between the two-study group of patients and the control group regarding the mean levels of IgE, the level of IgE was higher in CHB vs. control, and in HCC vs. control. Such

observation agreed with findings in previous studies[21, 22]. In the present study, IgE levels were proportionate with the degree of liver fibrosis (Table 6); however, these differences did not reach statistical significance.

The results of current study showed that there were significant correlations between the level of IgE and transaminases (ALT, AST) and between IgE and AFP in both study groups (group I and group II), which agreed with some previous studies [23,34]. However, these were in contradictory to findings of other studies [25,33], who reported negative association between IgE and transaminases. In addition, the Th17 cells were implicated in allergy and production of IgE.[24] Some authors proposed that IgE may have antitumor influence.[12,33]

The current study showed a significant elevation in serum IgE among CHB-HCC patients compared to CHB non -HCC. These data agreed with those reported by some other studies.[20,8,10] Analysis of ROC curve showed that IgE has good prognostic accuracy for predicting the development of liver cancer. In addition, the higher cut off observed in chronic hepatitis B patients group confirm that it is a marker for predicting liver disease progression in chronic hepatitis B.

In summary, the present study indicated that levels of IgE were higher in sera of HCC patients than in sera of CHB patients. Furthermore, levels of IgE in the patients' groups were higher than in control sera. Such observations support the possibility of using serum IgE as prognostic non-invasive marker for follow up the course of CHB. IgE in CHB related HCC was significantly correlated with AFP which is an important tumor marker.

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Author Contributions

AS; designed and approved the whole research protocol. BMB; contributed to the protocol design, revised laboratory work, and approved the final paper version to be published. monitored data collection process and the laboratory work, interpreted the data, and critically revised the paper. ASE; supervised sample collection according to inclusion criteria, revised clinical data, diagnosis, and patient classification. MK; collected the samples and patient's clinical data, carried out the laboratory work and analyzed it. ASY; carried out statistical analysis and drafted the paper. All authors read and approved the final manuscript.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Ethical approval

The study protocol was reviewed and approved by the Research Ethics Committee of the Faculty of Medicine, Al-Azhar University (May 2019).

Informed consent

A written informed consent was obtained from each patient and control participant before enrolled in the study.

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