Biochemistry Letters, 20(1) 2024, pages 26-44

Scientific Research & Studies Center-Faculty of Science- Zagazig University- Egypt

Biochemistry Letters

Investigating the Association Between Epstein-Barr Virus and Hepatocellular Carcinoma in Egyptian Patients

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ARTICLE INFO

Received : 4/1/2024
Accepted : 4/2/2024
Available online : 16/3/2024

Keywords:
Tumor marker, Epstein–Barr virus (EBV), HCC, liver cirrhosis.

ABSTRACT

Background: Hepatocellular carcinoma (HCC) is a prevalent kind of cancer that is widespread worldwide. Epstein-Barr virus (EBV). It can infect over 90% of the human population. EBV can integrate into the host genome in several tumor forms, thereby facilitating carcinogenesis. **Aim**: Study the association between EBV and HCC in Egyptian patients and evaluate Epstein-Barr virus among malignant patients (HCC patients) attending the oncology unit of Zagazig University Hospital. **Material and methods**: The study comprised 41 patients diagnosed with HCC, 15 patients with liver cirrhosis, and 15 healthy individuals serving as control subjects. The ELISA technique was used to assess serum EBV IgM and AFP levels. The distribution of biochemical parameters (ALT, AST, albumin, prothrombin, bilirubin, or ALP) was estimated among all the analyzed groups. **Results**: A substantial statistical difference was seen in the distribution of EBV IgM among all groups studied, with a p-value of less than 0.001. There was a direct link between AFP and EBV IgM, with a P value of 0.033. There was a statistically significant difference in EBV IgM between all HCC categories, with a P value of 0.022. A clear association was observed between EBV IgM and HCC groups, as the levels of EBV IgM rose in parallel with the progression of the disease. **Conclusion**: There exists a correlation between the presence of serum EBV IgM and the occurrence of liver disorders Further investigation is necessary to validate the detrimental impacts of EBV and determine the quantity of EBV present in various stages of HCC.

Introduction

Hepatocellular carcinoma (HCC) is one of the most common malignancies globally [1]. HCC is the most common type of liver cancer, accounting for 85-90 % of cases and having a high morbidity and death rate [2]. HCC the sixth most common disease worldwide and the fourth leading cause of cancer-related deaths each year, is one of the deadliest malignancies and has been on the rise for the past ten years [3]. Egypt has the

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highest prevalence of HCV in the world, with 14.7% of the general population infected studies Estimated Egypt has a high incidence of HCV infection, a large burden of nonviral liver disorders such as fatty liver disease and autoimmune liver diseases, and a moderately high prevalence of metabolic syndrome. Antiviral therapy has the potential to significantly alter the course and prognosis of chronic HBV and HCV liver diseases [4].

Because of its aggressive invasion, rapid progression, and dismal prognosis, HCC has had a tremendous influence on people's health. Patients do not have any identifiable symptoms or signs in the early stages of the illness. They are already in the late and middle phases after being identified. As a result, it is critical to develop a strategy for precise identification to reduce disease mortality and lengthen patients' lives.

Hepatobiliary system cancer can be categorized into three types of carcinomas, whose incidence and mortality continue to rise due to different risk factors, including some viruses, Epstein–Barr virus (EBV) also known as human herpesviruses 4 (HHV-4) belonging to the gamma-herpes virus family, which infects more than 90% of the human population [5]. B-lymphocytes are the most vulnerable to EBV, and after transmission, they begin to proliferate EBV is also present in T-cells and natural killer cells (NK). EBV infects B-lymphocytes leading to two outcomes concerning the physiological impact of antigen stimulation. Production of memory B-cells is the first outcome, the second leads to differentiation of B-cells into plasma cells that are programmed [6] . EBV plays a role in liver inflammation by impacting hepatocytes directly or indirectly through immune cells [7]. EBV is linked to a variety of cancers and is the first human tumour virus to be found. It is responsible for 1.8 percent of all cancer-related deaths worldwide [8].

In different types of tumors, EBV can integrate into the host genome to promote tumorigenesis [9]. The investigation of patients with EBV-infected tumors has provided a responsible degree of proof that EBV was present before neoplastic transformation highlighting the need for explaining the role of EBV in tumorigenesis [10]. A meta-analysis of 918 patients with hepatobiliary system cancer found a significant rate of EBV infection. The molecular mechanism of EBV infection in hepatobiliary system cancer is currently being investigated [11]. Epstein-Barr virus (EBV) and cytomegalovirus (CMV) are linked to a variety of liver disorders. EBV causes acute hepatitis, which can range in severity from asymptomatic, self-limited icteric hepatitis to abrupt liver failure. EBV infection has been linked to cholestasis, and acute and chronic hepatitis [12]. In the case of severe hepatitis or poor response to particular antiviral medicines, [7] advocated examining the potential effect of (EBV) using PCR determination of their DNA in patient blood samples or liver tissue biopsy. They also urged that more research be done with more patients to confirm the deleterious effects of EBV and viral hepatitis [7]. Tumor markers, on the other hand, are critical for detecting HCC early. In the current study, we aim to investigate the association between Epstein Barr Virus (EBV) and HCC in Egyptian patients.

Subjects and Methods

Ethics Approval:

The research protocol was approved by the Faculty of Medicine at Zagazig University. The study will adhere to the guidelines set forth by the Institutional Review Board (IRB) with the reference number Zu-IRB 9416/22-3-2022.

Clinical data:

The study included 71 participants, divided into two groups: a normal control

group of 15 patients and a sick group of 56 individuals. The patient group included 41 newly diagnosed patients with hepatocellular carcinoma (HCC) and 15 patients with liver cirrhosis. Participants were chosen from the internal medicine and oncology units of Hospital Zagazig University. The researchers received approval from Zagazig University's Faculty of Medicine. The research will follow the standards established by the Institutional Review Board (IRB) under the reference number Zu-IRB 9416/22-3-2022. In addition, signed informed consent will be obtained from all participating patients. The current study used a case-control design and included 71 participants from August 2022 to October 2022. Participants in this study were drawn from the oncology unit of Egypt's Zagazig University's Faculty of Medicine. The diagnosis of the patients was made using a combination of clinical evaluation, radiographic findings, and histological examination of tissue specimens. All of the aforementioned variables are incorporated into the patients' data profiles.

Inclusion criteria:

Participants (n=71) were divided into three groups: 15 healthy control subjects, 15 liver cirrhosis patients, and 41 HCC patients diagnosed through a history, CT scan, routine laboratory investigations (liver function tests), and alpha-fetoprotein.

Exclusion criteria:

Patients with a history of neoplasm treatment would make appropriate assessment of research factors such as the quantity of Epstein-Barrs (EBV) difficult.

Study design:

The 71 participants were separated into three groups:

• Group I: consisted of 15 healthy control participants.

• Group II: Patients with liver cirrhosis but no HCC (15 in total).

• Group III: Hepatocellular carcinoma patients (41 in total).

Based on the Barcelona Clinic liver cancer staging system (BCLC), the third group was divided into four separate stages. The BCLC staging method is widely used in both North America and Europe. The patients were initially classified using the BCLC classification, which required categorizing them into four unique categories labeled A, B, C, and D. Following that, a new stage 0 was created to exclusively identify patients with extremely early-stage hepatocellular carcinoma (HCC). The BCLC system takes into account a variety of criteria, including patient performance, tumour burden (including amount, size, vascular invasion, and metastases), and liver function. Individuals with Barcelona Clinic Liver Cancer (BCLC) stage 0 hepatocellular carcinoma (HCC), often known as an extremely early stage, have a solitary nodule less than 2 cm in diameter. Individuals with Barcelona Clinic Liver Cancer (BCLC) stage A have a single tumor of any size or up to three tumours, each measuring less than 3 cm in size. Patients diagnosed as having BCLC stage B have multinodular tumours that are larger than those seen in BCLC stage A. These tumours, however, show no evidence of vascular invasion or extrahepatic dissemination. Patients with advanced hepatocellular carcinoma (HCC) in stage C of the Barcelona Clinic Liver Cancer (BCLC) classification have many nodules in the liver, as well as evidence of vascular invasion and/or the spread of cancer cells beyond the liver to other organs or tissues [13].

According to the BCLC staging method, Group 3 was subdivided into four subgroups:

I. BCLC stage 0: 10 HCC patients in the BCLC-0 stage

II. BCLC stage A: 10 HCC patients in the BCLC-A stage

III. BCLC stage B: 10 HCC patients in the BCLC-B stage

IV. BCLC stage C: 11 HCC patients in the BCLC-C stage

Sample collection:

5 ml Fresh blood samples were taken from subjects with liver cirrhosis, HCC, and the control group. All blood specimens were obtained via venepuncture and split into two tubes: a plain tube for serum separation for biochemical parameter estimation and an EDTA tube for peripheral blood mononuclear cells (PBMCs) separation for EBV PCR estimation, both of which were stored at -30 °C until use. **Note:** All study participants had their blood samples tested for regular laboratory studies (liver function tests), alpha-fetoprotein, and EBV IgM.

Biochemical study:

•Serum biochemical parameters were determined

A Chemistry Analyzer semi-auto Photometer 5010 (Germany) was used to measure liver function assays by using different kits from a human diagnostic company (Stegelitzer Straße 339126, Magdeburg, Germany). The CoaData 504 device (a semi-automated 1-channel coagulation analyzer for fast and accurate determination of coagulation assays) (Germany) was used to determine prothrombin time by using prothrombin time (PT) (liquid reagent) kits (Cambridge, UK).

•Alpha-fetoprotein (AFP)

IMMULITE 2000, catalog number L2KAP2 (Siemens Healthcare GmbH) Henkestr. 12791052 Erlangen, Germany) was used to determine alpha-fetoprotein (AFP) in serum samples. The assay procedures were carried out according to the kit manual.

•Epstein-Barr virus (EBV) IgM determination.

EBV-VCA IgM ELISA TEST SYSTEM Manufactured by Monocent, Inc., 9025 Eton Ave. Ste C, Canoga Park, CA 91304, USA

Principle of the test

Purified antigen-coated wells are filled with diluted patient serum. If present, the IgM-specific antibody attaches itself to the antigen. The enzyme conjugate is added to the antibody-antigen combination if it is present after all unbound materials have been removed by washing. After washing off any leftover enzyme conjugate, the substrate is added. The plate is incubated to enable the enzyme to oxidize the substrate. The quantity of IgG-specific antibodies in the sample directly correlates with the color's intensity.

Statistical methods

The data were subjected to analysis using IBM SPSS Advanced Statistics (Statistical Package for Social Sciences), version 28 (SPSS Inc., Chicago, IL). The numerical data were characterized using either the median and range or the mean and standard deviation, depending on the context. On the other hand, the qualitative data were presented in terms of the number of occurrences and the corresponding percentage. The Chi-square test (specifically Fisher's exact test) was employed to analyze the association between categorical variables, as deemed suitable. The normality of numerical variables was assessed through the utilization of the Kolmogorov-Smirnov test and the Shapiro-Wilk test. The

distribution of the variables deviated from normality, necessitating the use of non-parametric tests. Group comparisons were conducted using the Kruskal-Wallis test, followed by the Mann-Whitney U test. To account for multiple comparisons, the p-values were adjusted using the Bonferroni correction. The determination of sensitivity, specificity, positive predictive value, negative predictive value, and overall accuracy, along with their corresponding 95% confidence intervals, was conducted through the utilization of ROC (receiver operating characteristics) analysis and a logistic regression model. A p-value that is less than or equal to 0.05 was deemed to be statistically significant. The experiments conducted were two-tailed.

Distribution of sexes and ages within the research group

The sex distribution of the three groups does not differ statistically significantly, as shown by the P-value of 0.239 in Figure 2. A p-value of 0.001 indicates that we found a highly statistically significant variation in the age distribution between the three groups.

Examining the relationship between the various groups and liver function metrics.

The biochemical parameter distribution in the three groups (control, liver cirrhosis, and HCC) includes ALT, AST, albumin, total bilirubin, direct bilirubin, ALP, prothrombin time, and prothrombin concentration. As seen in Figure 2 and Table 1, there was a very statistically significant difference (p-value < 0.001) in the distribution of all biochemical parameters between the three groups.

The levels of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), albumin, Total bilirubin, direct bilirubin, Prothrombin time and Prothrombin Concentration in all studied groups were represented in **Table (1).** The mean value of alanine aminotransferase (ALT) activity was found to be 15.33 ±9.69 U/L in the control group. This value was significantly increased to 52.67 ±41.39 U/L in the liver cirrhosis group and 66.68 ±72.27 in the HCC group (p<0.0001).

The mean value of aspartate aminotransferase (AST) activity was found to be 11.87 ±6.56 U/L in the control group. This value was significantly increased to 74.19 ±63.25 U/L in the liver cirrhosis group and 118.43 ±109.96 in HCC group (p<0.0001).

The mean value of albumin was found to be 3.92 ±0.53 g/dl in the control group. This value was significantly increased to 2.47 ±0.76 g/dl in the liver cirrhosis group and 2.44 ±0.70 g/dl in the HCC group (p<0.0001).

The mean value of total bilirubin was found to be 0.58 ±0.15 g/dl in the control group. This value was significantly increased to 2.73 ±3.22 g/dl in the liver cirrhosis group and 5.67 ±4.88 g/dl in the HCC group (p<0.0001).

The mean value of direct bilirubin was found to be 0.20 ±0.06 g/dl in the control group. This value was significantly increased to 3.21 ±3.55 g/dl in the liver cirrhosis group and 4.59 ±3.96 g/dl in the HCC group (p<0.0001).

The mean value of Prothrombin time was found to be 12.18 ±0.69 sec. in the control group. This value was significantly increased to 19.42 ±5.34 sec. in the liver cirrhosis group and 19.63 ±4.90 sec. in the HCC group (p<0.0001).

The mean value of Prothrombin Concentration was found to be 101.87 ±5.70 % in the control group. This value was significantly increased to 50.17 ±18.09 % in the liver cirrhosis group and 52.08 ±22.08 % in the HCC group (p<0.0001).

Comparison between the examined groups regarding AFP and EBV IgM

In comparison to the control and cirrhotic groups, HCC is linked to extremely high median values of AFP, and the same is true for EBV IgM, with p values<0.001 for each. Furthermore, as seen in **Table 2 and Figure 3.**

The mean value of AFP was found to be 10.49 ±10.89 in the control group. This value was significantly increased to 5476.11 ±14469.59 in the liver cirrhosis group and 7783.16 ±12707.41 in the HCC group as (p<0.0001) **Table 2**.

The mean value of EBV IgM was found to be 2.09 ±0.61in the control group. This value was significantly increased to 5.06 ±2.38 in the liver cirrhosis group and 8.32 ±4.80 in the HCC group as (p<0.0001) **Table 2.**

There was a clear correlation between AFP and EBV IgM with statistically significant results (p-value = 0.033) in **Table 3.**

The area under the curve (AUC) is 0.789 in AFP The area under the curve (AUC) is 0.838 in EBV IgM, **Table 4**.

Investigating the relationship between various HCC subgroups (HCC0, HCC a, HCC b, and HCC c groups) and (AFP, EBV-IgM)

In terms of AFP and EBV-IgM, a statistically significant difference was discovered between all categories, with p-values of 0.035 and 0.022, respectively. A pairwise comparison showed that there was only a statistically significant difference between the HCC a and HCC c subgroups. There were no statistically significant differences in AFP levels or EBV-IgM levels between the other groups. As demonstrated in Table 5 and Figure 5, there is a direct relationship between EBV-IgM and HCC staging, with EBV-IgM increasing with the advanced stage.

The mean value of AFP was found to be 477.08 ±1123.53 in the HCC group. This value was significantly increased to 20320.51 ±17900.62 in the HCC c group as P value is 0.035 **Table 5.**

The mean value of EBV IgM was found to be 6.75 ±4.81 in HCC a group. This value was significantly increased to 11.44 ±5.53 in HCC c group as P value is 0.022 Table 5.

DISCUSSION:

Hepatocellular carcinoma (HCC) is frequently associated with a poor prognosis due to its proclivity to remain asymptomatic throughout the early stages when curative treatment options are most successful. As a result, by the time HCC is detected, it has frequently evolved to an advanced stage [14]. Non-invasive criteria have traditionally been used to diagnose HCC, and treatment options are determined by the total tumor burden and the degree of underlying liver disease. In North America and Europe, the BCLC staging approach is frequently used. The BCLC approach categorizes HCC phases based on patient performance status, tumor load (number, size, vascular invasion, and metastases), and metastases [13]. The ideal biomarker has several universal characteristics for routine clinical analysis, including sensitivity, specificity, low operator experience requirements, low cost, high reproducibility, rapid results, correlation

with tumor stages, and sample availability (such as blood or urine) without the need for pre-treatment [15]. EBV induces liver inflammation by exerting a direct or indirect influence on hepatocytes through immune cells [7]. EBV has been associated with multiple malignancies and was the initial human oncogenic virus identified. It constitutes 1.8% of all cancer-related deaths worldwide [8]. EBV can incorporate itself into the genetic material of the host, which contributes to the development of cancer in several types of tumors [9]. Researchers who looked at patients whose tumors were infected with EBV found a lot of evidence that EBV was present before the cancerous growth started. This shows how important it is to understand the role of EBV in the development of cancer[10]. Research combining data from multiple studies, involving 918 individuals with cancer in the hepatobiliary system, found that infection with the Epstein-Barr virus (EBV) was prevalent. Currently, researchers are investigating the precise molecular process by which EBV infects the hepatobiliary system in cancer patients[12]. Epstein-Barr virus (EBV) and cytomegalovirus (CMV) have been linked to several liver disorders. EBV induces acute hepatitis, which can range from asymptomatic, self-limiting icteric hepatitis to abrupt liver failure. EBV infection has been linked to cholestasis, acute hepatitis, and chronic hepatitis [7]. Yurlov et al. suggested testing for the possible effects of EBV by PCR on blood samples or liver tissue biopsies from people with acute hepatitis or who don't respond well to some antiviral drugs. They also advocated for more research with more patients to confirm the harmful consequences of EBV and viral hepatitis [8]. Early cancer detection can save lives and significantly reduce cancer mortality. So much time and effort has gone into developing new equipment that can detect the disease's early warning signs. Cancer biomarkers contain a wide range of biochemical components, such as entire tumor cells found in physiological fluids, nucleic acids, proteins, carbohydrates, trace metabolites, and cytogenetic and cytokinetic properties. They can be used to evaluate risks, make diagnoses, and forecast treatment effectiveness, toxicity, and recurrence [19]. AFP, on the other hand, has been widely studied and is frequently used as a biomarker for the diagnosis and prognosis of HCC [20]. The expression of AFP, which the fetus's liver primarily produces, has rapidly decreased to a very low level by the age of one. However, liver disease or cancer can induce a large increase in AFP levels in the blood. An elevated AFP level may be noticed six months before the diagnosis of HCC in a nested case-control study [21]. The principal criticisms leveled against the use of AFP at the moment center on its lackluster sensitivity and specificity for the early detection of HCC when used alone. Cirrhotic patients with active hepatitis-raised blood alanine aminotransferase (ALT), or non-HCC cancers may also have higher AFP levels. As of now, AFP detection alone is not recommended for HCC screening. Instead of AFP detection, the European Association for the Study of the Hepatic recommends using liver ultrasound for HCC surveillance [16]. We separated all samples in the current investigation into three groups (healthy controls, liver cirrhosis, and HCC patients). We began by estimating all routine blood parameters and collecting all patients' medical

histories, including diagnosis, duration, and pathology, if available. We discovered a very statistically significant variation in the distribution of all biochemical parameters across all groups when we analyzed liver function index tests (ALT, AST, albumin, prothrombin, bilirubin, and ALP), as shown in Figure 2 and Table 1. Alpha-fetoprotein (AFP) is extensively studied and commonly utilized as a biomarker for diagnosing and predicting the progression of HCC, as stated in the Marrero et al. study [17]. Nevertheless, liver illness or cancer can induce substantial elevations in AFP levels in the bloodstream. In a nested case-control study, the detection of an elevated AFP level may occur six months before the diagnosis of HCC. Our analysis revealed that AFP serves as a universal tumor marker across all groups. Figure 4 clearly illustrates a highly substantial statistical variance in the distribution of AFP among the three groups. An investigation combining data from multiple studies, known as a meta-analysis, involving 918 individuals diagnosed with cancer in the hepatobiliary system, revealed a substantial prevalence of EBV infection. The precise biological mechanism behind EBV infection in hepatobiliary system cancer is still not understood [11]. EBV and cytomegalovirus (CMV) are associated with various liver diseases. EBV commonly presents as acute hepatitis, varying in severity from asymptomatic, self-limiting icteric hepatitis to sudden liver failure. EBV infection has been associated with cholestasis as well as acute and chronic hepatitis [12]. Currently, in situations when there is severe hepatitis or a lack of positive results from specific antiviral medications, it is advised to investigate the potential impact of Epstein-Barr virus (EBV) by using PCR analysis to detect its DNA in patient blood samples or liver tissue biopsies. Furthermore, they emphasized the need for additional studies with a larger number of patients to validate the harmful effects of EBV and viral hepatitis. The subgroups of HCC about alpha-fetoprotein (AFP) and Epstein-Barr virus immunoglobulin M (EBV-IgM) are as follows [7]. In this study, the people who took part were split into three groups: healthy controls, people with liver cirrhosis, and people with HCC. We then used the ELISA technique to measure liver function parameters, EBV-Igm, and AFP in the serum of all three groups. At first, we approximated all routine lab parameters and obtained all patients' medical histories, including diagnosis, duration, and pathology. The liver function index tests we looked at were ALT, AST, albumin, prothrombin, bilirubin, and ALP. As shown in Figure 2 and Table 1, all biochemical parameters were spread out very differently across all groups (P value > 0.001).

On the other hand, cancer and liver disease can greatly increase blood AFP levels. In a nested case-control study, an increased AFP level may be observed six months before the diagnosis of HCC. We identified AFP as a tumor marker for each group in the current investigation. As can be seen in Figure 3, we discovered that there was a highly statistically significant variation in the AFP distribution between the three groups. The P value is > 0.001. (16) Figure 3 and Table 2. We found there is a high statistical analysis between the three groups, with a p-value > 0.001. Figure 3: Table 2. EBV IgM increased from the

control group to the HCC group. There is a clear correlation between AFP and EBV IgM, with statistically significant results (p-value = 0.033). Table 3. We studied the association between AFP and EBV IgM, and we found there was a direct correlation between AFP and EBV IgM. EBV IgM has high specificity and sensitivity, as the area under the curve (AUC) is 0.838, as shown in Figure 4. We found that there was a direct relationship with EBV IgM, as EBV IgM increased with advanced stage (HCC c). The mean ± standard deviation is 6.75 ±4.81 in the HCC0 group, and the advanced stage HCC c is 11.44 ±5.53 **(Figure 5, Table 5).** There was a highly statistically significant difference regarding EBV IgM distribution between the HCC subgroups, with a P value of 0.022 (Figure 5, Table 5). There was a highly statistically significant difference regarding AFP distribution between the HCC subgroups, with a P value of 0.035 **(Figure 5, Table 5).** Next, we looked at EBV IgM levels in the HCC subgroup serum and found that they were higher in patients with a more advanced stage. We found EBV IgM levels between 6.75 and 13 ng/ml, which you can see in Table 5. It is very interesting to learn that the amount of EBV IgM in the blood of people with HCC is positively related to their clinical stage of the disease and the ROC analysis of people with HCC in different BCLC stages. We think the limitation of this study is the low number of included HCC patients, so further study with a larger number of serum samples is recommended.

Conclusion

There is a highly statistically significant difference regarding the EBV IgM distribution between the three groups (control, liver cirrhosis, and hepatocellular carcinoma), as the P value is <0.001. There is a highly statistically significant difference in EBV IgM between all HCC groups. There is a direct correlation between EBV IgM and HCC groups as EBV IgM increases with the advanced stage. Subsequent analysis of EBV IgM in HCC subgroup serum revealed that EBV IgM was upregulated with advanced stage and detected EBV IgM at an approximate concentration range of 6.75 to 13 ng/ml.

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Table (1): The association between the different studied groups (control, liver cirrhosis and HCC group) and biochemical parameters.

Group		Age (years)	ALT (U/L)	AST (U/L)	ALP (U/L)	Albumin (g/dl)	Total bilirubin (mg/dl)	direct bilirubin (mg/dl)	Prothrombin time (sec.)	Prothrombin Concentration %
Control (n=15)	Mean ± Standard Deviation	34.73 ±14.15	15.33 ±9.69	11.87 ±6.56	68.53 ±16.58	3.92 ±0.53	0.58 ±0.15	0.20 ±0.06	12.18 ±0.69	101.87 ±5.70
	Median (range)	27.00 (20.00-58.00) (b)	12.00 (2.00-34.00) (a)	12.00 (3.00-23.00) (a)	65.00 (46.00-91.00) (a)	3.89 (3.12-5.10) (a)	0.57 (0.32-0.85) (a)	0.19 (0.12-0.34) (a)	12.30 (11.00-13.40) (a)	101.00 (95.00-112.30) (a)
Cirrhosis (n=15)	Mean ± Standard Deviation	61.40 ±12.45	52.67 ±41.39	74.19 ±63.25	121.80 ±72.23	2.47 ±0.76	2.73 ±3.22	3.21 ±3.55	19.42 ±5.34	50.17 ±18.09
	Median (range)	60.00 (45.00-84.00) (b)	54.60 (6.90-124.30) (b)	63.50 (14.20-205.70) (b)	90.00 (50.00-275.00) (b)	2.31 (1.55-4.43) (b)	1.30 (0.16-10.00) (b)	0.75 (0.05-8.89) (b)	17.50 (11.90-26.80) (b)	50.70 (28.00-102.90) (b)
HCC (n=41)	Mean ± Standard Deviation	56.90 ±13.18	66.68 ±72.27	118.43 ±109.96	188.83 ±136.20	2.44 ±0.70	5.67 ±4.88	4.59 ±3.96	19.63 ±4.90	52.08 ±22.08
	Median (range)	58.00 (20.00-79.00) (a)	39.30 (10.10-421.00) (b)	73.40 (16.70-436.00) (b)	148.00 (44.00-784.00) (b)	2.37 (0.93-4.08) (b)	4.14 (0.35-15.80) (b)	4.10 (0.14-12.80) (b)	19.60 (11.00-26.80) (b)	50.30 (29.60-102.90) (b)
P value		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

• Cells that are sharing same letters aren't statistically significant

Table (2): The relationship between AFP and EBV-IgM in the various study groups.

Group		AFP	EBV-IgM
Control (n=15)	Mean ± Standard Deviation	10.49 ±10.89	2.09 ±0.61
	Median (range)	6.10 (1.06-34.21) (a)	2.10 (1.20-3.40) (a)
Cirrhosis (n=15)	Mean ± Standard Deviation	5476.11 ±14469.59	5.06 ±2.38
	Median (range)	5.80 (1.70-44463.00) (b)	5.10 (2.30-8.50) (b)
HCC (n=41)	Mean ± Standard Deviation	7783.16 ±12707.41	8.32 ±4.80
	Median (range)	895.00 (1.50-36771.00) (b)	7.90 (2.10-17.30) (b)
P value		<0.001	<0.001

- Cells that are sharing same letters aren't statistically significant

Table (3): The relationship between AFP and EBV-IgM

Group			EBV-IgM		P -value
			<=3.5 (n=9)	>3.5 (n=32)	
AFP	<=11.10	no	4	4	0.033
		%	44.4%	12.5%	
	>11.10	no	5	28	
		%	55.6%	87.5%	

Table (4): Area Under the Curve regarding AFP and EBV-IgM

Area Under the Curve					
Test Result Variable(s)	AUC	Standard error	P value	95% Confidence Interval	
				Lower Bound	Upper Bound
AFP	0.789	0.060	<0.001	0.672	0.906
EBV-IgM	0.838	0.046	<0.001	0.747	0.929

Table (5): Classification of hepatocellular carcinoma (HCC) subgroups based on alpha-fetoprotein (AFP) levels and Epstein-Barr virus immunoglobulin M (EBV-IgM) status.

Diagnosis		Age (years)	AFP	EBV-IgM
HCC0	Mean ± Standard Deviation	53.33 ±10.80	477.08 ±1123.53	6.75 ±4.81
	Median (range)	52.50 (39.00-70.00)	11.90 (1.50-2770.00) (ab)	5.20 (3.10-15.70) (ab)
HCCa	Mean ± Standard Deviation	57.50 ±12.90	4134.22 ±9179.36	4.42 ±2.91
	Median (range)	58.00 (40.00-79.00)	3.85 (2.30-22801.00) (a)	2.80 (2.10-8.90) (a)
HCCb	Mean ± Standard Deviation	70.67 ±7.69	1185.08 ±2333.42	9.78 ±3.33
	Median (range)	71.00 (58.00-79.00)	190.00 (2.50-5926.00) (ab)	9.30 (4.80-14.30) (ab)
HCCc	Mean ± Standard Deviation	60.00 ±11.70	20320.51 ±17900.62	11.44 ±5.53
	Median (range)	59.00 (40.00-74.00)	30155.00 (7.60-36771.00) (b)	13.00 (3.70-17.30) (b)
P value		0.093	0.035	0.022

- Cells that are sharing same letters aren't statistically significant

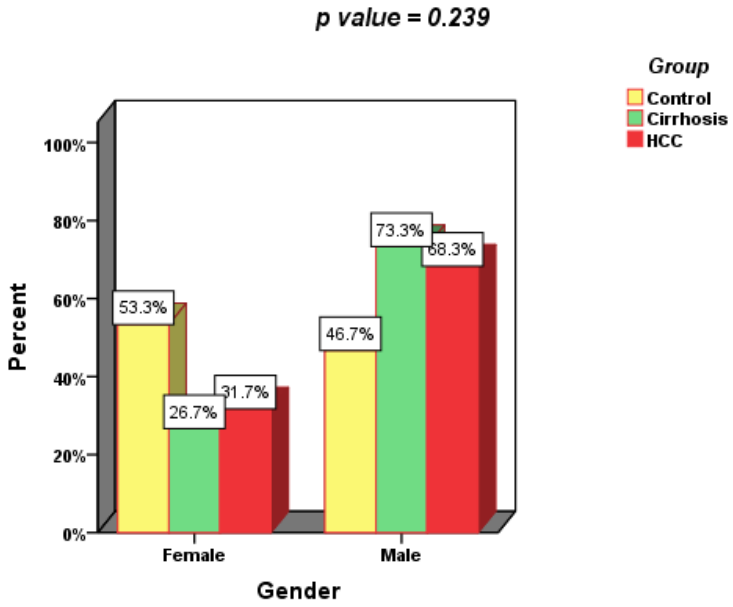

Figure 1. Gender distribution among the studied group

There is no statistically significant difference regarding the sex distribution between the three groups as the p-value is 0.239.

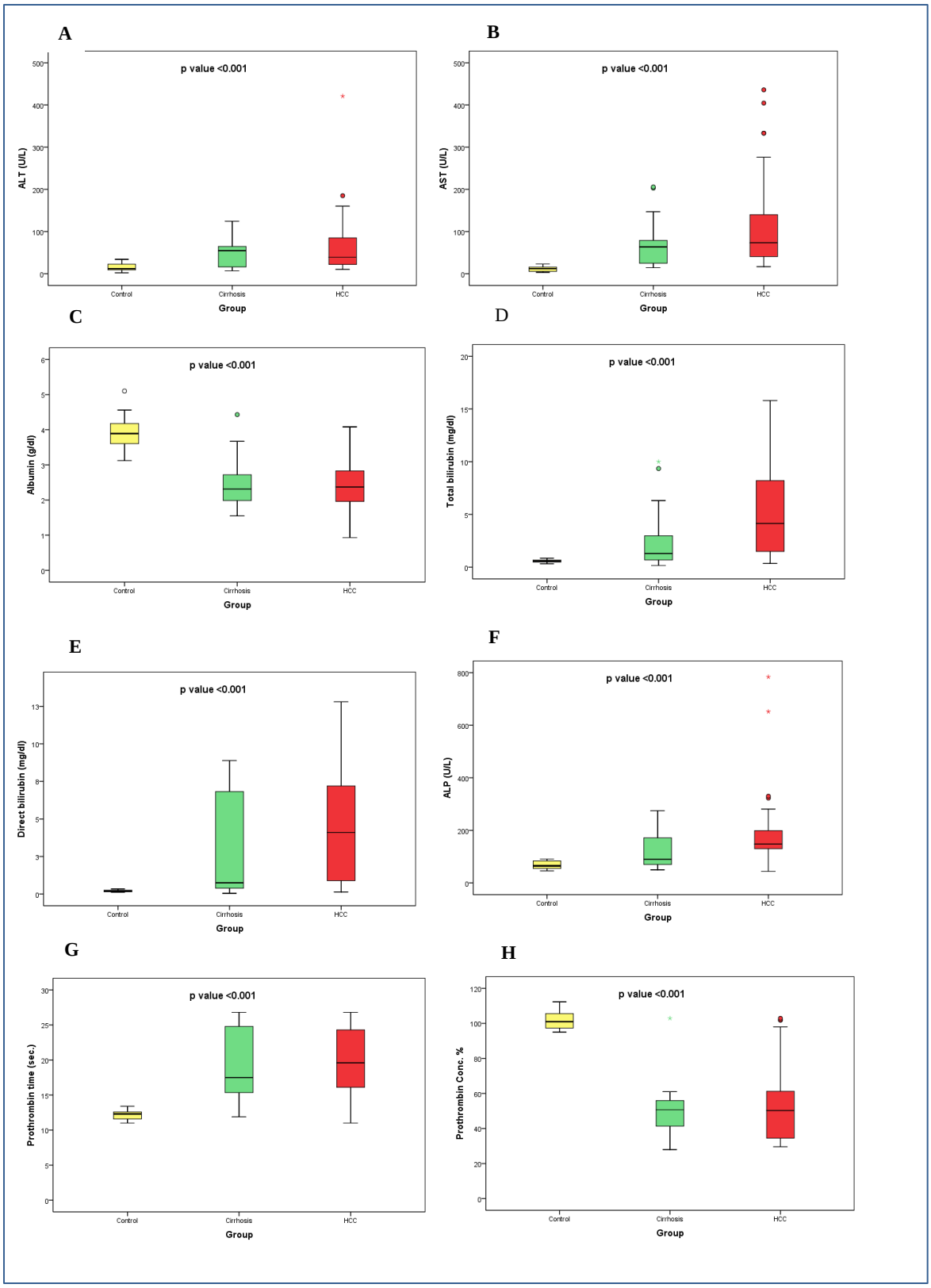

Figure 2. The distribution of biochemical parameters among all studied group

There is a highly statistically significant difference regarding all biochemical parameters' distribution between the three groups as the p-value is < 0.001.

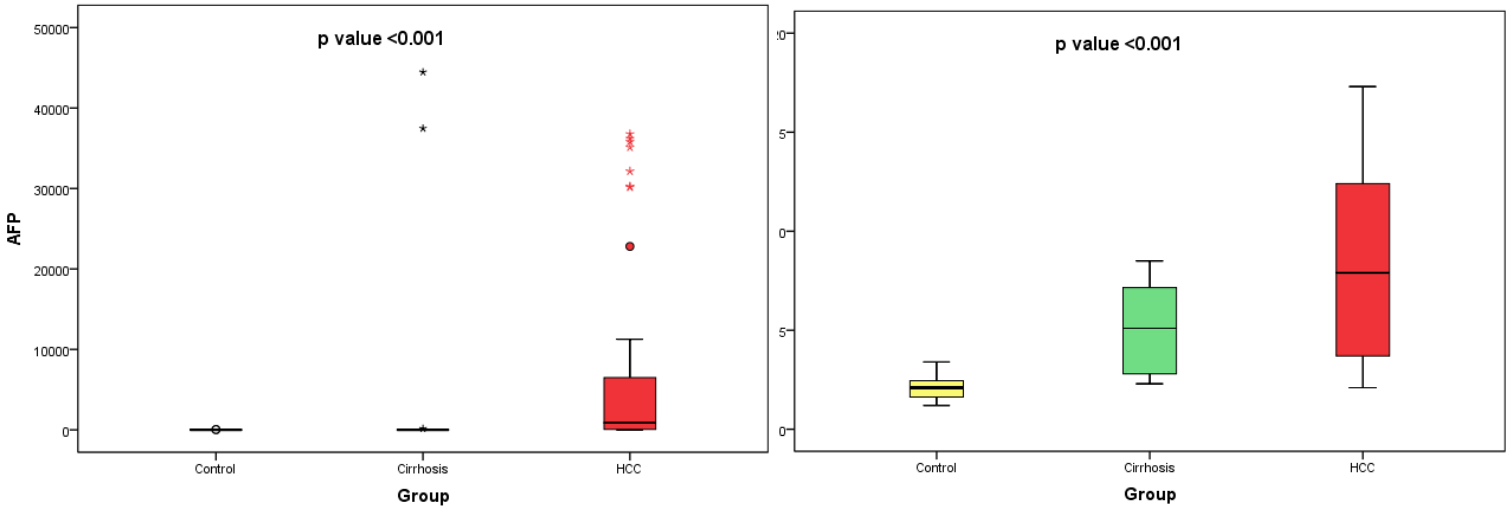

Figure 3. AFP and EBV IgM concentration distribution (ng/ml) among all studied groups

There is a highly statistically significant difference between the AFP and EBV IgM. The distribution between the three groups as p-value is < 0.001.

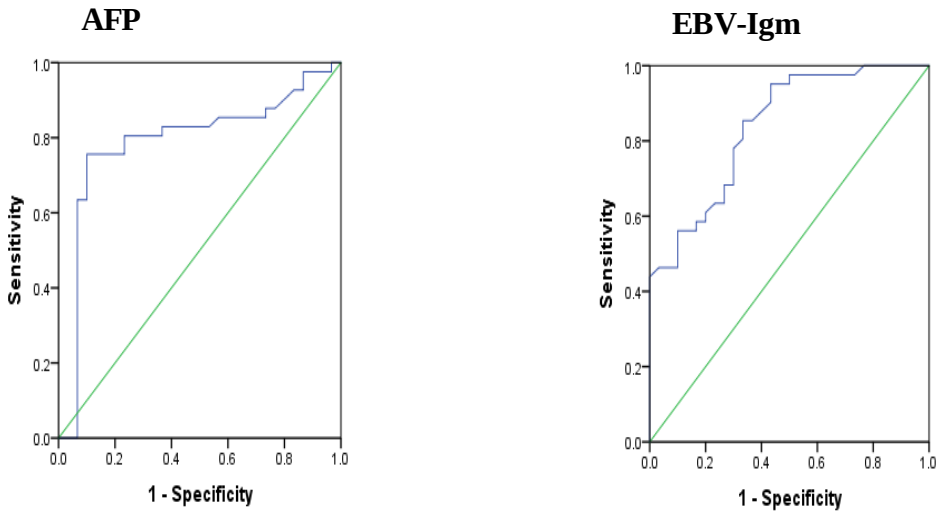

Figure 4. ROC curve of AFP and EBV-IgM

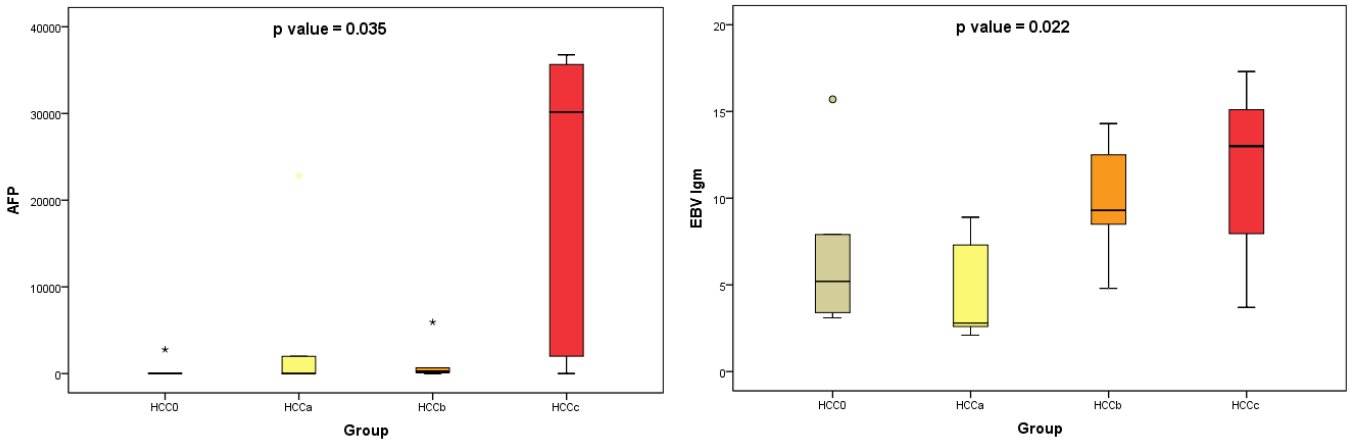

Figure 5. AFP and EBV IgM Concentration distribution among HCC subtypes