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A study of some physiological and immunological criteria in patients with hepatocellular carcinoma at the diwaniya Teaching Hospital and the Middle Euphrates Oncology Center

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Abstract

The aim of the current research is to find out the physiological and immunological changes that accompany the development of hepatocellular carcinoma . The search results of some hematological parameters of the patient group illustrate a significant decrease in (erythrocytes, hemoglobin, platelets and lymphocytes) , while both white blood cells, neutrophils and the ratio of neutrophils – lymphocytes recorded a significant increased . With regard to the lipid profile ,the results indicated that the HDL ,LDL , Ch, TG were significantly lower. While liver function, increased in the level of each of (AST, ALT, ALP , PT ,TSB,DSB), respectively, while albumin recorded a significant moral decrease in patient compared to the control. Immunological study, it was found that the IL-2 recorded a significant moral increase in the patient group compared to the results of the control group .

Keyword : HCC, lipid profile ,liver function , IL-2, ROC ,

1. Introduction

The liver, which weighs between 1.2 and 1.5 kg and is located in the right upper quadrant of the abdomen under the lung, is the largest gland in the human body. It aids in the digestion and removal of toxins and harmful substances, stores sugars, and dissolves fats. The liver is also thought of as

a chemical laboratory where thousands of chemical processes occur effortlessly[1]. The most prevalent type of malignant liver cancer is hepatocellular carcinoma, which is also one of the other types [2]. HCC, which is ranked third in the world among cancer-related deaths, is responsible for 85–90 Percentage of liver cancer cases [3]. Liver enzymes, including alkaline phosphatase (ALP), aspartate transferase (AST), and alanine aminotransferase (ALT), are significant markers in the evaluation of liver function [4] ,[5] , Moreover, direct bilirubin (DBIL), total bilirubin (TBIL), and which can indicate the body's nutritional and immunological condition [6, 7, 8]. The immune system is impacted in pleiotropic ways by the tiny (15 kDa) cytokine IL-2. Minimal concentrations of IL-2 exhibit a preference for binding to the trimeric IL-2 receptor (IL-2R), which is mostly expressed on immunosuppressive regulatory T (Treg) cells and consists of IL-2Rα (CD25), IL-2Rβ (CD122), and the common gamma chain (CD132). Because of their about 10-100-fold greater affinity for IL-2 than dimeric IL-2Rs, trimeric IL-2Rs are also known as high-affinity IL-2Rs [9]. Decades ago, the US Food and medicine Administration (FDA) approved IL-2 as the first cancer immunotherapy medicine to treat metastatic melanoma and renal cell carcinoma [10], [11].

2. Materials and Methods

2.1. Division of groups and Patient data collection

In the current study, 44 patients from the Middle Euphrates Cancer Center in Najaf and the Diwaniya Teaching Hospital / Oncology Center had their hepatocellular carcinoma confirmed. The patients targeted in this study were of both sexes (male and female) and ranged in age from 47 to 84 years. The healthy group in the control group was evenly split into 22 males and 22 females, with ages ranging from 38 to 58. Samples for the group were obtained from the blood bank connected to the Diwaniyah health department. The study's patient data, which included age, height, weight, sex, smoking, family history, and chronic conditions, was gathered from the special patient records housed at the statistics unit of the oncology centers of the participating hospitals.

2.2. Blood samples Collection

A 7 ml blood sample was drawn from the patient and control groups using a 10 ml syringe. Following that, the (1 ml) blood was promptly placed in dipotassium-EDTA Vacutainer® tubes for the purpose of measuring the Complete Blood Count (CBC). About 4 milliliters of the remaining blood were transferred to anticoagulant-free tubes (Gel tubes) and allowed to clot for (2 min) at 37°. The serum was separated by centrifugation at 3000 rpm for 10 min. 2 milliliters blood were transfer to sodium citrate tubes to be used in measuring coagulation parameters (PT). Using eppendorf tubes, the separated serum was divided into portions and storage at -80˚C for qPCR, biochemical, and immunological analyses.

2.3.Biochemical parameters

Complete blood count (CBC) , Liver function , lipid profile and prothrombin time (PT) were measured using full automated analyzers with standard techniques.

2.4. ELISA technique

2.4.1. Human Interleukin-2 ELISA Kit

Human Interleukin 2 (IL-2) ELISA was utilized in this investigation to quantify IL-2 in patient and control serum samples, and it was carried out in accordance with company instructions (BT-LAB).

1200ng/L	Standard No.5	120ul Original standard + 120ul Standard diluent
600ng/L	Standard No.4	120ul Standard No.5 + 120ul Standard diluent
300ng/L	Standard No.3	120ul Standard No.4 + 120ul Standard diluent
150ng/L	Standard No.2	120ul Standard No.3 + 120ul Standard diluent
75ng/L	Standard No.1	120ul Standard No.2 + 120ul Standard diluent

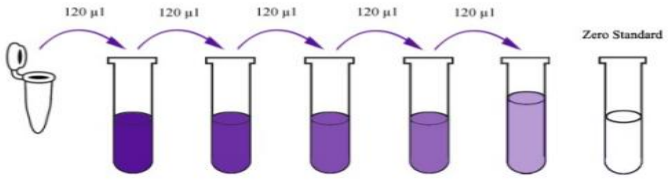

Figure 1:The standard pr of the human human Interleukin-2 ELISA Kit

2.5 . Statistical analysis :

The data was collected, assembled, analyzed, and presented using Microsoft Office Excel 2010 and the Statistical Programme for Social Sciences (SPSS) version 26. After establishing which variables were normally distributed and which were not, and performing the Kolmogorov-Smirnov normality test, The numerical data were presented as mean and standard deviation. The mean differences were investigated using the independent sample t-test, any two groups, assuming that the variable was normal. If the variable is normally distributed, A one-way The ANOVA test was used to compare mean differences among more than two groups. The chi-square test can be used to determine the relationship between any two categorical variables.To quantify risk, the odds ratio and 95% confidence interval were determined. The threshold value for predicting a positive discovery was determined using ROC curve analysis, which included the area under the curve (AUC), accuracy level, sensitivity, specificity, and degree of significance (P). In order to measure risk, the odds ratio and confidence interval (95% CI) were calculated , P-value of less than 0.05 was used to establish level of significance, with a P-value of 0.01 or less being considered highly significant [12].

3.Results

3.1. Characteristics of the study population

In this study, 44 hepatocellular carcinoma patients (30 males and 14 females) and 44 healthy control participants (22 males and 22 females) were enrolled. In terms of lipid profile, the current findings reveal that the mean levels of triglycerides in patients with HCC and the control group were, respectively, 65.39 ± 8.33 and 132.0 ± 7.00; the difference between the patient group's level and that of the healthy control group was statistically significant (P= 0.001). Additionally, the mean cholesterol levels in patients with HCC and control were 90.26 ± 4.83 and 208.50 ± 5.68, respectively; the difference between the patient group's level and the healthy control subject's level was extremely significant (P < 0.001). Furthermore, when comparing patients with HCC to healthy control subjects, all other lipid profile parameters decreased, and the difference was statistically significant (P < 0.001).In contrast, the mean level of Aspartate aminotransferase (AST) in patients with HCC was significantly greater than that of the healthy control subject, measuring 191.04 ± 16.94 against 24.10 ± 4.62, respectively, with a P < 0.001 for liver function parameters. Additionally, the current data demonstrate that the mean ALT count in HCC patients was significantly higher than the mean neutrophil count in the healthy control group (115.30± 9.49 versus 29.60± 6.62, respectively; P < 0.001). Additionally, the mean ALP levels in HCC patients were significantly higher than the control, at 445.65 ± 31.55 versus 183.70 ± 44.33, respectively (P < 0.001).

Table (1): Characteristics of patients with hepatocellular carcinoma and healthy control

Characteristic	Patients (n=44)	Healthy Control (n=44)	*P*
Sex			
Male	30 (68.2%)	22 (50.0 %)	0.083
Female	14 (31.8%)	22 (50.0%)	
BMI kg/m2	18.19 ± 1.52	21.81 ± 2.67	< 0.001
Triglyceride mg/dL	65.39 ± 8.33	132.0± 7.00	< 0.001
Cholesterol mg/dL	90.26 ± 4.83	208.50 ± 5.68	< 0.001
HDL mg/dL	23.60 ± 3.03	80.30 ± 9.61	< 0.001
LDL mg/dL	74.87 ± 7.76	118.10 ± 10.67	< 0.001
AST U/L	191.04 ± 16.94	24.10 ± 4.62	< 0.001
ALT U/L	115.30± 9.49	29.60 ± 6.62	< 0.001
ALP U/L	445. 65 ± 31.55	183.70 ±44.33	< 0.001
Albumin g/dL	1.97 ± 0.42	4.56 ±0.56	< 0.001
Total Bilirubin (TSB) mg/d	3.90 ± 0.75	0.51 ±0.144	< 0.001
Direct Bilirubin mg/dL	2.40 ± 0.43	0.31 ±0.13	< 0.001

3.2. Serum Interleukin-2 levels measured in HCC patients and control group

Figure (1) presents the findings of a comparison of the blood Interleukin-2 level between patients with HCC and a healthy control group. According to the current findings, patients with HCC had mean serum Interleukin-2 levels that were substantially higher than those of healthy control subjects (1363.60±151.68 vs. 512.21±79.38, respectively; P<0.001).

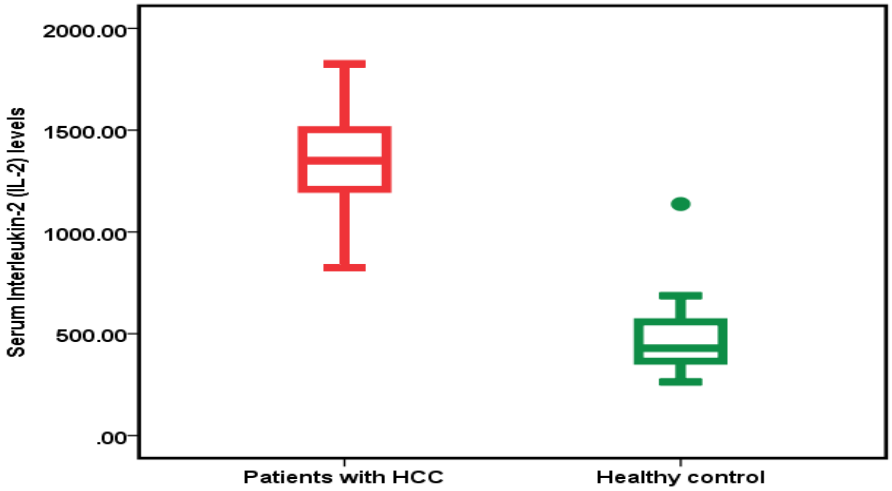

Figure 2: Serum Interleukin-2 level in patients with HCC and control . P < 0.05.

3.3. Diagnostic accuracy of Serum Interleukin-2

Serum Interleukin-2 was used to determine the diagnostic accuracy of separating HCC patients from control using ROC analysis. AUC value 0.979 (95% confidence interval [CI], 0.934-1.000, P < 0.001), sensitivity 97.7%, specificity 97.7%, PPV 97.7%, and NPV 97.7% were obtained with an ideal serum interleukin-2 cut-off value of > 921.65-fold.

Table (2): Roc curve of Serum Interleukin-2

Characteristic	Serum Interleukin-2
Cutoff value	> 921.65
P value	**< 0.001**
Sensitivity %	97.7 %
Specificity %	97.7%
PPV %	97.7 %
NPV %	97.7%
AUC (95% CI)	0.979 (0.934- 1.000)

CI: Confidence interval, AUC: Area under curve.

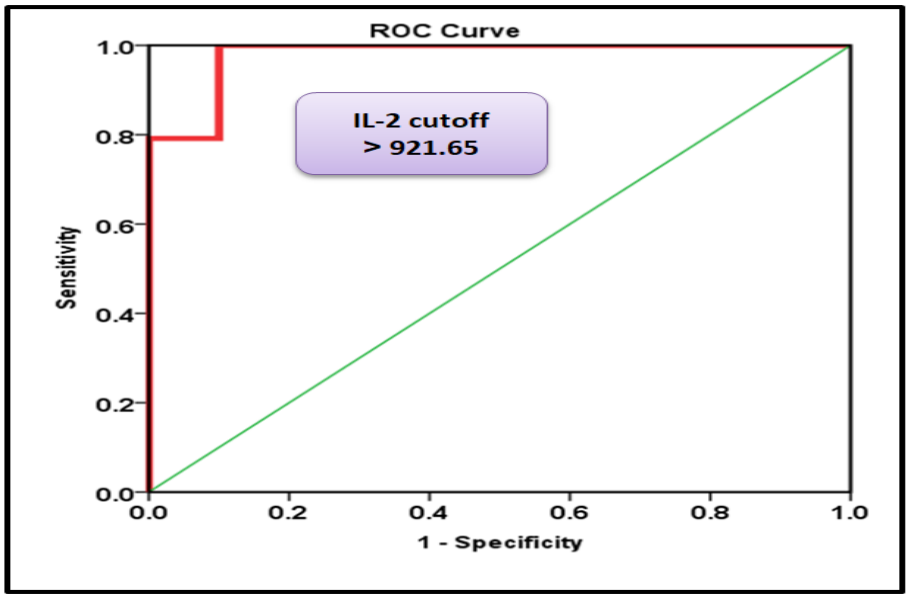

Figure (3): Analysis of the serum interleukin-2 receiver operator characteristic curve to determine a potential diagnostic cutoff value.

3.4. Correlation between *Serum Interleukin-2*and other parameters.

Tables displaying the relationships between serum interleukin-2 and other markers in HCC patients (3). The current data indicate that there is no significant association between any of the measures and serum Interleukin-2.

Table (3): Correlation between Serum Interleukin-2 and other parameters.

Parameters	Serum Interleukin-2	
	R	***P***
TG	0.301	0.161
Chole	0.333	0.117
HDL	0.311	0.158
LDL	0.342	0.109
AST	-0.171	0.547
ALT	-0.313	0.151
ALP	-0.301	0.161
PT	-0.234	0.201
Albumin	-0.172	0.439
Bilirubin	-0.301	0.161

r: correlation coefficient.

4. Discussion

Per Petrick et al. (2020), the incidence rates of HCC in males are greater than in women in the United States by a factor of two to four. The disparity between the incidence rates for men and women is even more prominent in Europe. According to Yu et al. (2019), there were 891 (58·5%), 107 (7·0%), and 526 (34·5%) patients in the normal-weight, underweight, and overweight groups, respectively. All patients had a preoperative BMI of 23·5 (range: 14·3–29·9) kg/m2.Our findings were at odds with those of Zhang et al.'s (2024) study, which examined the connection between blood cholesterol levels and the risk of developing liver cancer.. According to their findings, the serum TC concentration's pooled HR (highest vs lowest) was 0.45. The HRs for TGs, HDL-C, and LDL-C were 0.67, 0.72, and 0.51, respectively. Consequently, the study's findings indicated that there is a negative correlation between the risk of liver cancer and serum of total levels of Ch, TG, and HDL-C, indicating that higher concentrations of these lipids are linked to a lower risk of liver cancer.

Nevertheless, there is no conclusive evidence linking LDL-C levels to the risk of liver cancer. Regarding the liver function test results, the preceding table indicates that all patients in the group with HCC had high ASTs, with rates ranging from 162.0 to 215.0. In contrast, the control group's members had low ASTs. The AST level ranged from 18.0 to 31.0, and the same was true for the ALT and ALP, with rates varying from 98.0 to 129.0, or 15.0 to 45.0, for the ALT and from 404.00 to 525.00, or 125.00 to 255.00, for the ALP, patients, and control group, respectively. Additionally, the HCC group's serum albumin level dramatically decreased, but their levels of total bilirubin, ALT, AST, and INR significantly increased (Hawash et al., 2024).

Xu et al. (2023) conducted an experiment to investigate the feasible prognostic laboratory parameters. A score model was established to estimate the individualized overall survival (OS) in HCC following resection. The criteria were total bilirubin (TB), direct bilirubin (DB), and albumin (ALB) of HCC. The findings were as follows: (TB: 14.7 μmol/L (11.60- 20.00), DB: 5.3 μmol/L (3.92-7.40), and ALB: 41.1 g/L (37.90–43.70) correspondingly. A study by Xu et al. (2023) looked into the practical prognostic laboratory parameters. To determine each patient's unique overall survival (OS) after resection in HCC, a score model was developed. The parameters were albumin (ALB) of HCC, direct bilirubin (DB), and total bilirubin (TB). The following were the conclusions: Accordingly, TB measured 14.7 μmol/L (11.60–20.00), DB measured 5.3 μmol/L (3.92–7.40), and ALB measured 41.1 g/L (37.90–43.70).

CONCLUSIONS

The efficacy of CBC measures such as PLR and NLR are cost-effective techniques with some diagnostic value; nevertheless, they cannot be utilized as a stand-alone predictor of malignancy and must be linked with other clinical, laboratory, and radiologic tests.. Results the present study provided important information concerning the relationship between the incidence of HCC and different blood parameters. Concerning the mean values of serum biochemical markers (Complete

blood count (CBC) , Liver function , lipid profile and prothrombin time (PT)), they were associated with Stage of the disease . The present results indicates IL-2 is considered as excellent diagnostic marker.

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