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Original article

Role of several cytokines and adhesion molecules in the diagnosis and prediction of survival of hepatocellular carcinoma

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ABSTRACT

Background and study aims: There is still need for accurate markers for early diagnosis of hepatocellular carcinoma (HCC) and assessment of prognosis. The aim of this study is to investigate interleukin (IL)-32, IL-1 beta, IL-18, vascular cell adhesion molecule (VCAM)-1, and epithelial cell adhesion molecule (EpCAM) in the diagnosis and assessment of prognosis of HCC.

Patients and methods: Fifty patients with HCC and 15 healthy volunteers were enroled into this prospective study. Serum samples were obtained at the first admission before any treatment was given. Serum IL-32, IL-1 beta, IL-18, VCAM-1, and EpCAM levels were determined using ELISA kits.

Results: The mean age of the patient group and controls was 60 ± 9 years and 56 ± 8 years, respectively. The mean serum level of IL-32 was higher in patients with HCC than in the control subjects (65.1 vs. 14.1 pg/mL; $p < 0.001$). IL-18 levels were significantly higher in the HCC group (546.5 vs. 157.8 pg/mL; $p < 0.001$). EpCAM (20.3 vs. 1.5 pg/mL; $p < 0.001$) and VCAM (6.5 vs. 1.8 μg/mL; $p < 0.001$) levels were also higher in patients with HCC. The mean level of IL-1 beta in the HCC group was similar to that in the control subjects (1.9 vs. 1.9 pg/mL; $p = 0.97$). Fifty-eight per cent of the patients with HCC died at 7.3 months (median). Cytokine levels except EpCAM did not correlate with survival ($p > 0.05$). Alpha-foetoprotein, IL-32, IL-18, EpCAM, and VCAM had valuable cutoff levels to differentiate between patients with HCC and control group ($p < 0.001$).

Conclusions: Although cytokines can be a diagnostic marker for HCC, they did not have any significant prognostic value in patients with HCC. Only EpCAM may be used to determine the prognosis of HCC, thereby assisting with treatment management.

Introduction

Hepatocellular carcinoma (HCC) is one of the most common cancers worldwide and has a high mortality rate. Prognosis of HCC is closely associated with tumour stage and liver function tests. There are many HCC grading systems in use, but they are unable to predict pharmacologic responses to chemotherapeutic agents largely because of the lack of specific biomarkers. Some cytokines and growth factors have been investigated in HCC. Interleukin (IL)-6 and IL-10 levels were shown to be higher in patients with HCC [1]. However, a decreased level of IL-37 expression was reported in HCC specimens, and the levels of IL-37 were associated with the prognosis of HCC [2].

IL-32 is a cytokine produced by natural killer cells, T cells, epithelial cells, and monocytes [3]. Studies have shown an elevation of IL-32 levels in inflammatory diseases such as ulcerative colitis and cancers of stomach and lungs [4,5]. In a study on patients with HCC, IL-32 was shown to be overexpressed and associated with the progression of HCC [6]. IL-1 beta (β) is a proinflammatory cytokine with multiple effects, including the elimination of malignant cells in some cancer types [7]. A previous study showed a correlation between IL-1β levels and increased tumour burden during colitis [8]. Thus, IL-1β may favour the development and progression of tumour. In fact, a relationship between a polymorphism of the IL-1β gene and IL-1β levels in nonsmall cell lung cancer, colon cancer, and pancreatic carcinoma was previously shown [9–11]. IL-18 is also a proinflammatory cytokine that belongs to

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the IL-1 family and plays an important role in inflammation, anticancer immune response, and autoimmunity [12–14]. Vascular cell adhesion molecule (VCAM)-1 is a mediator of angiogenesis [15]. High serum levels of VCAM-1 are observed in various cancers such as colorectal and gastric cancers [16,17]. Epithelial cell adhesion molecule (EpCAM) is a cell adhesion molecule found in many epithelial cells. EpCAM is often expressed in epithelial tumours such as HCC [18]. Some studies have reported that elevated expression of EpCAM may be related to the poor prognosis of HCC [19,20]. However, data regarding the serum levels of EpCAM in patients with HCC is not available.

The present study aims to investigate IL-32, IL-1β, IL-18, VCAM-1, and EpCAM in the diagnosis and prognosis of HCC.

Patients and methods

Patients

This is a prospective study in which 50 patients with HCC and 15 healthy volunteers were enroled. All patients with HCC had cirrhosis. Patients were evaluated for the aetiology of cirrhosis. Viral [hepatitis B (HBV) and C (HCV)] and other aetiologies were documented. In addition, the patients' Child–Pugh and model for end-stage liver disease (MELD) scores were calculated. HCC was diagnosed using characteristic imaging findings in magnetic resonance images along with progressively elevated serum alpha-foetoprotein (AFP) levels, which was in accordance with the diagnostic criteria of the European Association for the Study of the Liver [21]. Informed consent was obtained from all the patients in this study. This study was approved by the Istanbul University Ethical Committee (approval number: 2556-24).

Assays for IL-32, IL-1β, IL-18, VCAM-1, and EpCAM

Serum samples were obtained when the patients were first admitted to the hospital, before any treatment was given, and frozen immediately at −20 °C until analysis. Serum IL-32 (USCN Life Science Inc., China), IL-1β (Invitrogen Corporation, Camarillo, California, USA), and EpCAM (USCN Life Science Inc., China) levels were determined using a solid-phase sandwich enzyme-linked immunosorbent assay (ELISA) according to the manufacturers' instructions. Serum IL-18 and VCAM-1 (eBioscience, Austria) levels were determined using a double-antibody sandwich ELISA. Assays were performed by the laboratory personnel who were unware of patients' clinical information.

Statistical methods

Descriptive values were expressed as mean/median and standard deviation. Categorical variables were expressed as the number of cases and percentage value. Continuous variables were analysed using Student's *t*-test and Mann–Whitney *U* test. Categorical variables were compared using chi-square and Fisher's exact tests. We used receiver operating characteristic (ROC) analysis to obtain the cutoff values of cytokines to differentiate between HCC and controls. In addition, we used Spearman's correlation analysis to determine the relationship between cytokines and liver function tests. Statistical significance was set as $p < 0.05$.

Results

The mean age of the HCC group and control group was 60 ± 9 years and 56 ± 8 years, respectively ($p = 0.09$). In the HCC and control groups, 82% (n = 41) and 60% (n = 9) were males, respectively ($p = 0.09$). When we evaluated the aetiology of cirrhosis, 86% (n = 43) of patients had viral hepatitis (HBV = 35 patients and HCV = 8 patients), 6% (n = 3) were alcohol related and 8% (n = 4) were cryptogenic. Of the patients with cirrhosis related to HBV, 80% (n = 28) were on antiviral treatment at the time of HCC diagnosis. For the remaining seven patients, HBV was not known prior to HCC diagnosis, and concurrent HBV and HCC were diagnosed. Seventy per cent (n = 35) of the patients with cirrhosis were of Child A, 28% (n = 14) of Child B, and 2% (n = 1) of Child C. The mean tumour diameter was 56 ± 28.8 mm (range, 10–120 mm). Twenty-nine (58%) patients in the HCC group died during follow-up, and the median survival in this group was 7.3 ± 1.4 months (range, 1–58 months).

The mean serum level of IL-32 was higher in patients with HCC than in control subjects (65.1 vs. 14.1 pg/mL; $p < 0.001$). IL-18 levels were significantly higher in the HCC group (546.5 vs. 157.8 pg/mL; $p < 0.001$). EpCAM (20.3 vs. 1.5 pg/mL; $p < 0.001$) and VCAM (6.5 vs. 1.8 μg/mL; $p < 0.001$) levels were also higher in patients with HCC. However, the mean IL-1β level in the HCC group was not different from that in the control group ($p = 0.97$; Table 1).

In patients with HCC, tumour diameter did not correlate with AFP levels ($r = 0.196$, $p = 0.181$). Furthermore, AFP levels, as a classical tumour marker for HCC, did not correlate with IL-32, EpCAM, VCAM, IL-1β, and IL-18 levels (for all parameters $p > 0.05$). However, VCAM levels correlated with serum IL-32 levels ($r = 0.333$, $p = 0.02$). In the correlation analysis of these cytokines, IL-32 and IL-18 levels showed positive correlation with MELD and Child–Pugh scores (MELD: $r = 0.315$, $p = 0.03$ and $r = 0.297$, $p = 0.04$; Child–Pugh: $r = 0.298$, $p = 0.04$ and $r = 0.291$, $p = 0.04$, respectively).

In the ROC analysis of tumour markers, AFP, IL-32, IL-18, EpCAM, and VCAM demonstrated valuable cutoff levels to differentiate between patients with HCC and control group ($p < 0.001$ for all parameters). All these tumour markers showed high sensitivity, specificity, accuracy, and positive predictive values that are detailed in Table 2 and Fig. 1. EpCAM with a cutoff of 3.40 seems to be the most valuable marker for the diagnosis of HCC (AUC = 0.981, $p < 0.001$). For IL-1β, a discriminative cutoff value for HCC diagnosis was not obtained ($p = 0.06$).

EpCAM levels were significantly higher in patients with HCC with subsequent mortality (22.9 vs. 16.9 pg/mL, $p = 0.02$). Other markers were not associated with overall mortality (Table 3). However, 3-month mortality was higher in patients with higher IL-1β levels (3.1 vs. 1.7 pg/mL, $p = 0.004$). In patients with mortality in the first 6 months, VCAM levels were lower than those in the survivors (5.2 vs. 7 pg/mL, $p = 0.03$). There was no correlation between the survival of patients with HCC and AFP levels.

Discussion

Serum AFP is the most important tumour marker for HCC; however, it is not specific and does not help in detecting early-stage HCC. Therefore, our study aimed to evaluate the diagnostic value of serum IL-32, IL-1β, IL-18, VCAM-1, and EpCAM levels in patients

Table 1
Characteristics of the groups.

Parameters, mean ± SD	HCC (n = 50)	Control (n = 15)	*p* value
Sex, Male (n/%)	41/82%	9/60%	0.09
Age (years)	60 ± 9	56 ± 8	0.09
IL-32, pg/mL	65.1 ± 63.9	14.1 ± 7.5	<0.001
IL-1β, pg/mL	1.9 ± 1.3	1.9 ± 0.5	0.97
IL-18, pg/mL	546.5 ± 414.5	157.8 ± 65.4	<0.001
EpCAM, pg/mL	20.3 ± 9.6	1.5 ± 3.8	<0.001
VCAM, μg/mL	6.5 ± 2.8	1.8 ± 0.7	<0.001