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ASSESSMENT OF PROGNOSTIC AND DIAGNOSTIC VALUE OF SOME BIOMARKERS IN HEPATOCELLULAR CARCINOMA

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Background: **Hepatocellular carcinoma (HCC) is an increasing problem worldwide. Determining a prognosis is important for the management of HCC.** ***Aim:*** **We aimed to investigate the impact of interleukin (IL)-29, galectin-3, leptin, fibronectin and protease-activated receptor-1 on the prognosis and diagnosis of patients with HCC.** ***Materials and Methods:*** **60 HCC patients (75% male) and 20 healthy volunteers (70% male) were enrolled in this prospective study. Serum samples were obtained during the first admission before any adjuvant or metastatic treatments were administered. Serum biomarkers were determined using ELISA kits.** ***Results:*** **All patients had cirrhosis, and the Child — Pugh stages were as follows: 61.5% Child — Pugh A, 35.9% Child — Pugh B and 2.6% Child — Pugh C (61.7% hepatitis B virus, 11.7% hepatitis C virus, 6.7% hepatitis B virus + hepatitis C virus, 11.7% alcoholic and 8.3% cryptogenic). Fifty-three percent of the HCC patients died within a median of 7.5 months. The mean serum level of IL-29 in patients with HCC was higher than that in the control group (32.55 pg/ml *vs* 11.46 pg/ml, $p < 0.015$). Galectin-3 levels were significantly higher in the HCC group (6.7 ng/ml *vs* 1.38 ng/ml, $p < 0.001$). Fibronectin levels were higher in the control group than in the HCC group (260 635 ng/ml *vs* 257 353 ng/ml). However, the mean protease-activated receptor-1 and leptin levels were similar between the two groups ($p > 0.05$). The biomarkers were divided into two groups according to their median level. In the log rank analysis, biomarkers had no effect on survival ($p > 0.05$).** ***Conclusions:*** **IL-29 and galectin-3 levels were significantly higher in HCC patients. Although IL-29 and galectin-3 can be used as diagnostic markers for HCC, they had no prognostic value in HCC patients.**

Key Words: **hepatocellular carcinoma, prognosis, biomarkers.**

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Hepatocellular carcinoma (HCC) is the most common primary liver neoplasm and causes the death of more than one million people annually. HCC is the second most frequent cause of cancer-related death according to the World Health Organization [1, 2]. Although there are geographical variations, chronic hepatitis B and C are the most frequently encountered factors in the etiology of HCC worldwide in addition to alcohol consumption, exposure to aflatoxin, and non-alcoholic fatty liver disease [3, 4].

Biomarkers can be used to predict early HCC diagnosis thereby increasing the potential for curative treatment and increasing survival rates with appropriate treatment modalities. Thus, many studies on novel biomarkers have been performed to determine both the diagnosis and prognosis [5]. Alpha-fetoprotein (AFP), a glycoprotein first described in the 1960s, is a primary marker of HCC that is most commonly used in the determination of HCC diagnosis and prognosis worldwide and is among the fundamental biomarkers included in the Barcelona Clinic Liver Cancer (BCLC) staging system and other staging systems [5, 6]. In addition, golgi protein 73, glypican 3, annexin A2, and dysregulated microRNAs are frequently studied biomarkers that can be used in HCC [5, 7].

In the regulation of the tumor microenvironment, cytokines lead to tumor growth, invasion, metastasis and angiogenesis and participate in tumor growth signaling pathways, therefore serving as the main points of emphasis in cancer studies [8, 9]. Three cytokines in the type III IFN (IFN-λ) family, interleukin (IL)-29 (IFN-λ1), IL-28A (IFN-λ2), and IL-28B (IFN-λ3), which were thought to be interrelated, were identified [10, 11]. Interferons, which constitute a large family, are shown to have many biological activities, such as the inhibition of cell growth, activation of the cytotoxic effects of natural killer cells, regulation of type I helper T cells and inhibition of angiogenesis [12, 13].

Galectins of the beta-galactosidase-binding protein family are responsible for the modulation of cell-cell and cell-matrix interactions. A total of 15 galectins have been identified in mammals, and galectin-3 is the most common type and has unique structural properties. Galectin-3 is a multi-functional protein responsible for various biological functions, such as metastasis, cancer progression, angiogenesis, tumor cell proliferation, differentiation and adhesion [14]. Galectin-3 is present in many tissues but not in normal hepatocytes, whereas it is abundant in HCC cells [15].

Leptin, a non-glycosylated protein of 167 amino acids encoded by the *LEP* gene, is correlated with body fat ratio and adipocyte size and is responsible for the proliferation, migration and angiogenesis in HCC [16].

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Abbreviations used: AFP – alpha-fetoprotein; BCLC staging system – Barcelona Clinic Liver Cancer staging system; HCC – hepatocellular carcinoma; IL – interleukin; MELD – Model for End-Stage Liver Disease; PAR – protease-activated receptor.

In contrast to studies that show leptin to be correlated with AFP in HCC patients, other studies show increased leptin levels in HCC patients, which correlate with a higher survival rate [16, 17]. Therefore, in our HCC patient group, we studied the role of leptin in survival.

Fibronectin, which functions as an acute phase reactant and plays a role in various cellular activities, such as wound healing, cell proliferation, adhesion, migration, and maintenance of normal cellular morphology, is a high-molecular weight protein. Its secretion increases in the presence of cytokines, such as IL-1a, IL6 and tumor necrosis factor-α [18, 19]. In the limited number of studies, the production of fibronectin, which plays a role in binding cellular and extracellular molecules, was found to be abundant in the ECM and increased in patients with HCC, but few studies on the correlation between fibronectin and survival in patients with HCC exist [19, 20].

The protease-activated receptor (PAR) family comprises PAR-1, PAR-2, PAR-3 and PAR-4 and constitutes one of the subgroups of G protein-coupled receptors [21]. Various studies have shown that PAR-1 and PAR-4 facilitate the thrombin-induced migration of HCC cells and contribute to HCC progression as a result of their interactions at the molecular and cellular level [21].

It is not possible to talk about a single molecule that affects the prognosis of HCC. Although the relationship between some inflammatory cytokines such as IL-6, anti-tumor necrosis factor, IL-1 and HCC prognosis has been previously investigated, its relationship with biomarkers such as IL-29, galectin-3, leptin, fibronectin and PAR-1 has less or never been evaluated. For this reason, these biomarkers, which we do not know much about their relationship between HCC and its prognosis or diagnosis, were used in our study in order to contribute to the literature.

Considering the etiopathogenesis of HCC, the roles of various cytokines, glycoproteins, hormones, and receptor molecules in the diagnosis and prediction of survival in this area need to be evaluated. In this regard, our aim was to investigate the role and significance of IL-29, galectin-3, leptin, fibronectin and PAR-1, which may act via various pathways in tumor pathogenesis and in HCC.

MATERIALS AND METHODS

Patients. Eighty people were included in this study. Of these individuals, 60 were HCC patients (75% were male), and 20 were healthy volunteers (70% were male). The demographic and clinical data were analyzed. This study was approved by the İstanbul University Ethical Committee (approval Number: 2556-24) and written informed consent was obtained from all patients.

Serum samples. Serum samples were obtained upon initial admission before any adjuvant or metastatic treatments were administered and before follow-up visits with the patients. Blood samples were obtained from the patients and healthy controls (n = 20) by venepuncture and clotted at room temperature. Sera were collected following centrifugation (10 min at 4000 rpm) at room temperature and frozen immediately at –20 °C until the time of analysis.

Analysis of biomarkers. Serum IL-29, leptin, fibronectin and PAR-1 were assessed by Galectin-3 ELISA kits (eBioscience, Bender Medsystems, Austria) using a double-antibody sandwich enzyme-linked immunosorbent assay to determine the level of human galectin-3 in the samples. Serum samples and standards were added to the wells, which were pre-coated with a human galectin-3 monoclonal antibody. Streptavidin-HRP was added to form the immune complex and allowed to incubate at room temperature for 1 h. The unbound material was washed away. The chromogen solution was added and incubated for 10 min (protected from light) for the conversion of the colorless solution into a blue solution, the intensity of which was proportional to the amount of galectin-3 in the sample. Due to the effect of the acidic stop solution, the color turned yellow. The colored reaction product was measured using an automated ELISA reader (ChroMate® 4300 microplate awareness technology) at 450 nm. The results are expressed in ng/ml.

Statistical analysis. To calculate the sample size of the control and experimental groups in our study, the power was set to at least 0.80, and type I errors were 0.05 for each variable. Descriptive statistics for the continuous (numeric) variables are presented as the median, mean, standard deviation, minimum and maximum values, whereas categorical variables are presented as numbers and percentages. The Kolmogorov — Smirnov test was used to analyze whether the continuous variables were normally distributed. Since the variables did not have a normal distribution, non-parametric tests were used. When comparing group means in terms of biomarkers and other characteristics, the Mann — Whitney U-test or Kruskal — Wallis H-test were used. Following the Kruskal — Wallis test, significance values were found, and the Bonferroni correction was used for post hoc (multiple) tests. Cut-off values of the biomarkers according to group were identified using ROC analysis as a diagnostic test. Similarly, to determine the effect of the HCC group and biomarkers on survival, survival analysis (Kaplan — Meier method) and log-rank tests were used. Spearman correlation coefficients were calculated to determine the correlation between the continuous variables. The level of statistical significance was set to (α) 5%, and SPSS (IBM SPSS for Windows, ver. 24) statistical package programs were used for the calculations.

RESULTS

The mean age of the sixty patients with HCC was 59.2 ± 10.2 years, while the mean age of the control group was 53.4 ± 8.2 years. Of the 60 patients with HCC, 17 (28%) were female, and of the 20 patients in the control group, 7 (35%) were female.

All 60 HCC patients included in our study had cirrhosis with different Child — Pugh scores. Of the HCC

patients, 61.5% were classified as Child — Pugh A, 35.9% were classified as Child — Pugh B, and 2.6% were classified as Child — Pugh C, and the patients were classified with liver cirrhosis due to hepatitis B virus (61.7%), hepatitis C virus (11.7%), hepatitis B virus and hepatitis C virus (6.7%), alcoholic (11.7%), and cryptogenic liver cirrhosis (8.3%). Twenty-five of the HCC patients had a Model for End-Stage Liver Disease (MELD) score of 11 or higher. Of the HCC patients, 27 (45%) had multiple tumor foci, and the mean tumor diameter in all patient groups was 46.4. Laboratory findings of the patients are summarized in Table 1.

In Table 2 and Fig. 1 below, the comparative results of the biomarkers in the HCC and control groups are provided. Regarding the PAR-1 and leptin measurements, there were no significant differences between the HCC and control groups ($p > 0.05$). However, there was a statistically significant difference between the groups in terms of fibronectin, IL-29 and galectin-3 levels ($p < 0.05$).

Fig. 2 provides the result of the evaluation of the sensitivity and specificity of fibronectin, IL-29, galectin-3, PAR-1 and leptin levels in distinguishing between HCC patients and healthy individuals using ROC curve analysis.

When the sensitivity and specificity of IL-29, galectin-3, leptin, fibronectin and PAR-1 were evaluated based on the ROC curve and the area under the curve, the capacities of galectin-3 and IL-29 were higher than those of the other biomarkers.

In the HCC patient group, which was divided into two groups according to the number of tumors present (i.e., one or multiple), IL-29 and PAR-1 levels were significantly higher in the group with multiple tumors ($p < 0.05$). The mean level of IL-29 was 24.29 pg/ml in the single tumor group and 43.08 pg/ml in the group with multipl tumors, representing a significant difference ($p < 0.032$). The mean level of PAR-1 was 0.74 ng/ml in the single tumor group and 1.41ng/ml in the multiple tumor group, also representing a significant difference ($p < 0.029$). In the single and multiple tumor groups, the mean levels of galectin-3 were 6.41 and 6.47 ng/ml, respectively ($p > 0.908$); the mean levels of leptin were 8.8662 and 8.5874 ng/ml, respectively ($p > 0.423$); and the mean levels of fibronectin were 263234.62 and

Table 1. Mean values of laboratory parameters in HCC patients

Laboratory parameters	Hemoglobin, g/dL	White blood cells	Platelets	Creatinine, mg/dl	International normalized ratio	Total bilirubin, mg/dl	Aspartate transaminase, IU/l	Alanine aminotransferase, IU/l	Alkaline phosphatase, IU/l	Gamma-glutamyl transferase, IU/l	Lactate dehydrogenase, IU/l	Albumin, g/dl	Alpha-fetoprotein, ng/ml
Mean value in HCC patients	12.3	6137	151650	1.09	1.32	1.69	52.8	60.07	236.75	86.18	523.96	3.3	957.3
Standard deviation	2.2	2863	112890	0.5	0.37	1.85	45	134	162	82	713	0.58	1782.71

Table 2. Mean values of IL, fibronectin, IL-29, galectin-3, PAR-1,and leptin levels in the HCC and control groups

	Fibronectin, ng/ml	IL-29, pg/ml	Galectin-3, ng/ml	PAR-1, ng/ml	Leptin, ng/ml
Control group (n = 20)	260 635 ± 60 735	11.46 ± 5.40	1.38 ± 0.79	0.73 ± 0.49	7.2 ± 3.2
HCC group (n = 60)	257 353 ± 63 986	32.55 ± 31.01	6.7 ± 4.2	1.10 ± 0.96	9.8 ± 10.5
p-value	0.015	0.015	0.001	0.197	0.731

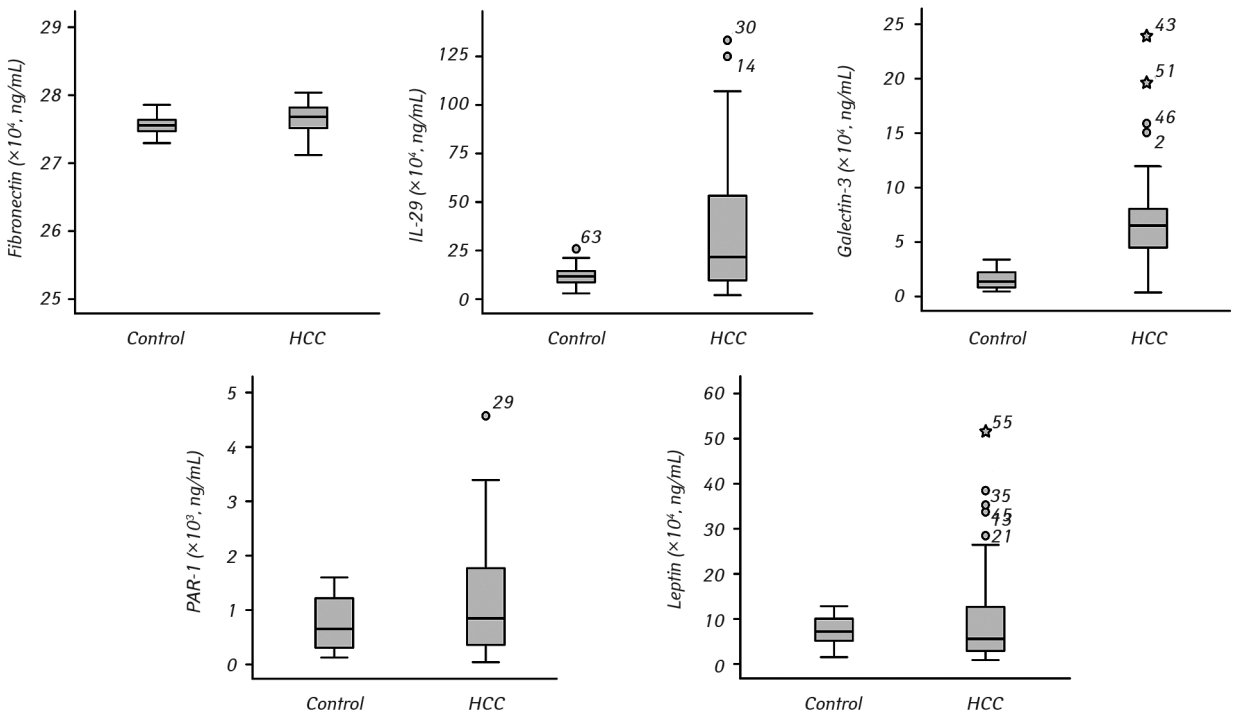

Fig. 1. IL, fibronectin, IL-29, galectin-3, PAR-1 and leptin levels in the HCC and control group

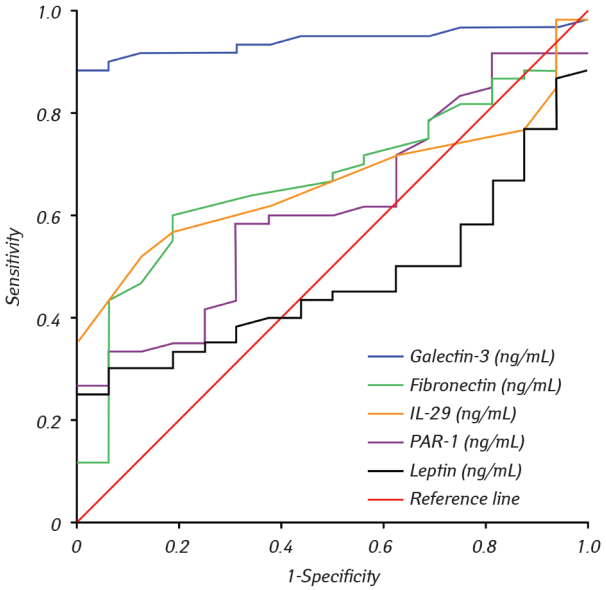

Fig. 2. ROC curve analysis

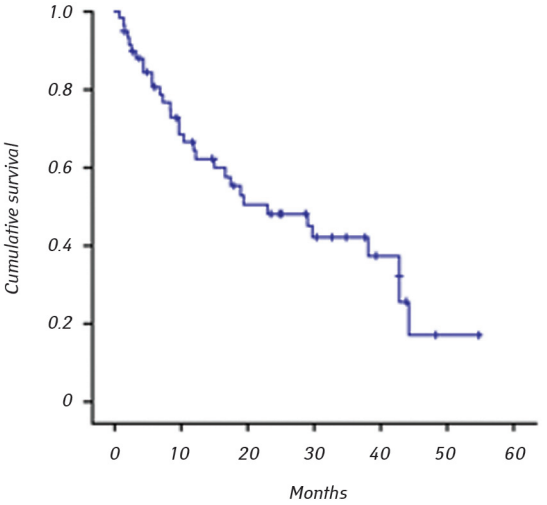

Fig. 3. Overall survival analysis results

255514.81 ng/ml ($p > 0.859$), respectively; the differences were not significant.

In the HCC patient group, when analyzed using Spearman's rho correlation coefficient (R), no significant correlation was detected among IL-29, galectin-3, PAR-1 and leptin ($p > 0.05$). Similarly, in the healthy control group, no significant correlation was detected between the biomarkers.

AFP levels were significantly higher (2400 ng/ml *vs* 343 ng/ml) in patients with extrahepatic spread ($p = 0.025$). No significant difference was detected between the biomarkers and AFP ($p > 0.05$).

Regarding patients mortality rates, 32 of 60 HCC patients died within a mean period of 7.5 months (min 1 month, max 50 months). No significant difference was detected between the two groups in terms of the role of biomarkers in survival ($p > 0.05$). In terms of survival, the levels in patients who died/survived are summarized in Table 3. The median value of the leptin level was 5.87 ng/ml, and survival increased at leptin levels above this point, although not significantly ($p > 0.978$).

In Fig. 3 above, in the context of overall survival analysis, time (in months)-dependent survival rates are presented. When the patients were evaluated in a time-dependent manner, only 20% survived to the 55th month. This percentage also included censored patients (individuals who survived but could not be followed).

Estimated time (in months)-dependent survival rates were evaluated based on the median value calculated, including the effects of IL-29, galectin-3, leptin, fibronectin and PAR-1 (Fig. 4). When the median value of galectin-3 was set to below or above 5.18 ng/ml, galectin-3 levels were above the median value and the survival rate decreased, although not significantly ($p > 0.691$). When the median value of fibronectin was set to below or above 276 500 ng/ml, the survival rate increased, as the fibronectin levels increased above this value. When the overall survival analysis results were calculated, the median value of IL-29 was set to 13.90 pg/ml. If the levels were above the median value, the survival rate decreased ($p > 0.769$). The median value of the PAR-1 biomarker was calculated as 0.840 ng/ml, and the survival rate decreased above this median value, although not significantly ($p > 0.082$). The survival rate increased when the median leptin level was 5.87 ng/ml, but this difference was not significant ($p > 0.978$).

Table 3. In terms of survival, the levels of biomarkers in patients who died/survived were as follows

Variable	Patients who died (n = 32)	Patient who survived (n = 28)
Fibronectin, ng/ml	276 700	277 100
IL-29, pg/ml	21.16	19.19
PAR-1, ng/ml	1.23	0.96
Leptin, ng/ml	11.12	8.37
Galectin-3, ng/ml	6.47	7.11

DISCUSSION

Because HCC is a common cancer, several biomarkers used to determine its diagnosis, prognosis and aggressiveness have been studied, but a new marker that can be used in practice has not yet been found [22].

Because of their close association with carcinogenesis, cytokines are among the most frequently studied molecules in the field. In our study, we investigated the efficiency of IL-29, a cytokine of the type III IFN family (IFN-λ), in HCC patients alongside other biomarkers. We found that the IL-29 levels in the HCC group were significantly higher than those in the control group ($p < 0.015$). However, the results were not significant for prognosis. We found that although there are studies on the role of IFN-λ in the treatment of tumors, such as melanoma, pulmonary adenocarcinoma, colon cancer, lymphoma, and hepatoma, there are few studies on serum IFN-λ levels. This study is the first to evaluate the serum IL-29 levels in HCC patients. In a study by Naumnik *et al.* [12] the efficacy of IL-29 in clinical practice as a novel cytokine for the diagnosis of patients with lung cancer was evaluated and IL-29 levels in both serum and bronchoalveolar lavage fluid were measured in 45 non-small cell lung carcinoma patients and compared with those in 15 healthy patients, 8 patients with hypersensitivity pneumonia and 15 patients with

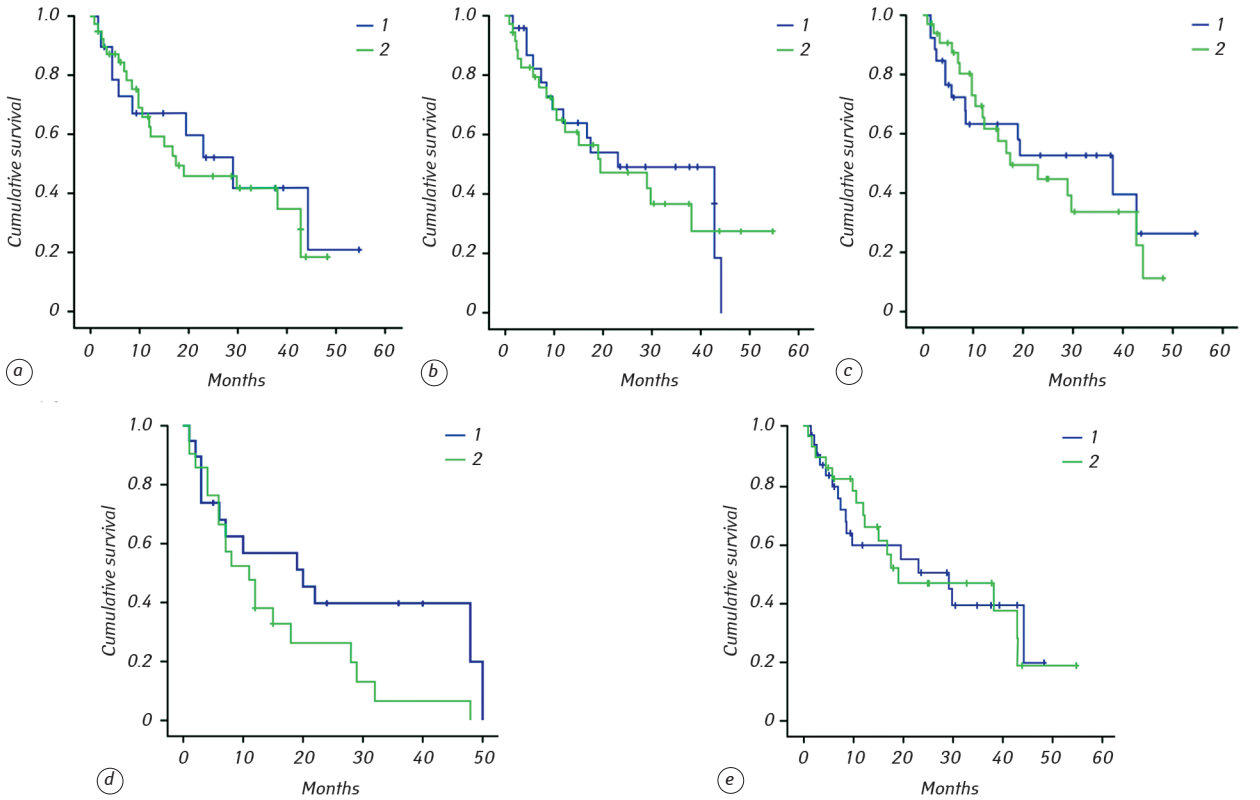

Fig. 4. Estimated time-dependent survival rates (log-rank analysis) based on the median value calculated when the effects of galectin-3 (*a*), fibronectin (*b*), IL-29 (c), PAR-1 (*d*), and leptin (*e*) were included; 1 — group with single tumor focus, 2 — group with multiple tumor foci

sarcoidosis. IL-29 expression in the non-small cell lung carcinoma patients was found to be significantly higher in both the serum and bronchoalveolar lavage fluids, but IL-29 expression was not related to prognostic factors [12]. When we evaluated the correlation between IL-29 and both multiple and single focus HCC patients, IL-29 expression was significantly higher (43.08 pg/ml) in multifocal HCC patients than in single foci HCC patients (24.30 pg/ml) ($p = 0.03$). When the correlation between patients with IL-29 and MELD scores was analyzed, no significant differences were observed between those with MELD scores lower or higher than 11. Fujie *et al.* [23] showed that type III IFN increases p21 gene expression in patients with non-small cell lung carcinoma, thereby inhibiting tumor growth. However, in the same study, the *in vivo* efficiency of IL-29 treatment in inhibiting tumor growth was correlated with the *in vitro* sensitivity of tumor cells to the growth inhibitory effect of IL-29 [23]. The higher serum levels of IL-29 observed in HCC patients as compared to the controls and the significantly higher levels of IL-29 observed in multifocal HCC patients as compared to single foci patients could be attributed to the host response aimed at inhibiting tumor growth. Furthermore, as in the study by Fujie *et al.* [23], to suppress the antiproliferative effect that occurs due to the differences in the sensitivity of HCC cells to IL-29, the production of more endogenous IL-29 may be required. More comprehensive studies on the diagnostic, prognostic, and even therapeutic effects of IL-29 in HCC patients are required.

Compared with those in the controls, levels of galectin-3, another biomarker that was evaluated in HCC patients, were found to be significantly higher in HCC patients (1.38 ± 0.79 ng/ml *vs* 6.7 ± 4.2 ng/ml; $p < 0.01$). In a study by Jiang *et al*. [14], in patients with HCC, galectin-3 levels were found to be high and correlated with a poor prognosis, similar to our finding. In our study, when we compared single and multifocal HCC patients in terms of galectin-3, we found that the galectin-3 levels were significant in multifocal HCC patients but non-significant in HCC patients with single tumors ($p = 0.537$). Similarly, in HCC patients with MELD scores equal to or greater than 11, galectin-3 levels (7.43 ng/ml) were higher than those in patients with MELD scores below 11 (6.31 ng/ml), but this difference was not significant ($p = 0.329$). To interpret the prognostic efficiency of galectin-3 in HCC patients with cirrhosis, an evaluation that comprises a larger number of patients is required. In a study by Hsu *et al*. [15], which evaluated galectin-3 expression in both cirrhotic patients and HCC patients using tumor biopsy samples, galectin-3 levels increased in both groups. They stated that the expression of galectin-3 by the regeneration nodules in cirrhotic patients correlated with rapid cellular proliferation [15]. While the galectin-3 levels in our study were significantly higher in HCC patients than in the healthy controls, galectin-3 levels were not found to be significant in terms of prognostic value. This result was attributed to the fact that all HCC patients in our study were in the cirrhotic stage based on the investigation by Hsu *et al.* [15]. To evaluate the significance of galectin-3 in HCC patients,

an evaluation in controlled studies involving both cirrhotic patients and patients with cirrhosis-independent HCC could be more informative. In our study, when galectin-3 was evaluated based on the area under the curve in ROC curve analysis, compared with other biomarkers, galectin-3 had a higher success rate of distinguishing between HCC patients and healthy individuals (94.1%).

In general, leptin secreted by adipose tissue triggers a cascade of cellular events after binding to the receptor and plays a role in the regulation of energy homeostasis in the body, and there are studies showing its fibrogenic effect on the liver [24]. Based on *in vitro* studies showing that leptin has an anti-apoptotic effect on HCC cells and increases neovascularization, various studies evaluating leptin levels in HCC patients have been performed. Wang *et al.* [24] investigated the role of leptin in HCC development in cirrhotic patients and found that elevated serum leptin levels were correlated with cirrhosis, but elevated serum leptin levels were not correlated with HCC development [24, 25]. In contrast, a study by Sadik *et al.* [26] showed that leptin levels were normal in cirrhotic patients without HCC and in controls, while leptin levels were higher in HCC patients [25]. In our study, the difference between leptin level in HCC patients and control group was not significant. There were also no correlations between leptin levels and MELD scores and between leptin levels and Child — Pugh scores.

Fibronectin is a large dimeric glycoprotein, and its soluble form is a component of plasma, while its insoluble form is a component of the ECM in almost all tissues [27]. In tissue culture studies, hepatocytes are among the basic cells that produce fibronectin found in plasma [28]. In a study by Kim *et al*. [29], serum fibronectin levels in HCC patients were significantly higher than those in patients with cirrhosis [28]. In our study, the serum fibronectin levels in the control group were higher than those in HCC patients (260 635 *vs* 257 353 ng/ml, $p > 0.015$). We attributed this difference to the probability that hepatocytes, the source of serum fibrinogens, can produce more fibronectin in healthy individuals.

The development and spread of HCC occurs because of various complex interactions at the cellular and molecular level, and the interactions throughout this process have not yet been revealed. PAR-1, one of the molecules studied in this field, is thought to be correlated with the progression and spread of HCC [21]. Mussbach *et al.* [21] showed that the cells increase transmembrane migration, which breaks the collagen barrier due to the induction of HCC cells by a peptide that selectively activates thrombin and PAR-1 [21]. Moreover, the effects of thrombin, which plays a fundamental role in tumor growth, metastasis and angiogenesis, particularly in solid cancers, are thought to be correlated with PAR [30]. In our study, serum PAR-1 levels were higher in HCC patients. While this result was not significant, when corroborating this correlation with the tumor progression shown in previous studies, PAR-1 levels were significantly higher in multifocal patients.

The inclusion of patients with cirrhosis without HCC, patients with HCC without cirrhosis alongside with a healthy control group, and a cirrhosis-dependent HCC patient group could increase our interpretative power when assessing biomarkers. In addition, larger group size could also have made this study more powerful. One of the restrictive aspect of the study is that we could not specify the BCLC score in the article because we could not measure the Child — Pugh score and tumor characteristics simultaneously to calculate the BCLC. Nevertheless, for the first time we attempted to evaluate multiple biomarkers, and serum IL-29 levels in HCC patients.

CONCLUSION

In this study, where in serum IL-29, galectin-3, leptin, fibronectin and PAR-1 levels in patients with HCC due to cirrhosis were compared to those in a healthy control group, IL-29 and galectin-3 levels were statistically significantly higher in the HCC group. However, no statistically significant correlation was detected between the biomarkers included in this study and survival. Our study showed that IL-29 and galectin-3 as well as the correlations between PAR-1 and IL-29 and tumor progression may be significant diagnostic markers in patients with HCC; however, no biomarkers studied were statistically significant in the context of survival.

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DECLARATION OF INTERESTS

The authors have no conflicts of interest to declare.

AUTHORS CONTRIBUTIONS

FA designed the research, BÇ wrote the paper. Rİ, ÜA, DD, MS, DT, SE were responsible for data collection and analysis. ÇK, KD, FB and SK were responsible for critical revision of the manuscript for important intellectual content. FA, mentor and primary investigator, was responsible for the study concept and design, critical revision of the manuscript for important intellectual content, and study supervision.

REFERENCES

1. **Dimitroulis D, Damaskos C, Valsami S, *et al.*** From diagnosis to treatment of hepatocellular carcinoma: an epidemic problem for both developed and developing world. World J Gastroenterol 2017; **23**: 5282–94.

2. **Heimbach JK, Kulik LM, Finn RS, *et al.*** AASLD guidelines for the treatment of hepatocellular carcinoma. Hepatology 2018; **67**: 358–80.

3. **Balogh J, Victor D, Asham EH, *et al.*** Hepatocellular carcinoma: a review. J Hepatocell Carcinoma 2016; **3**: 41–53.

4. European Association for the Study of the Liver. EASL-EORTC clinical practice guidelines: management of hepatocellular carcinoma. J Hepatol 2012; **56**: 908–43.

5. **Rich N, Singal AG.** Hepatocellular carcinoma tumour markers: current role and expectations. Best Pract Res Clin Gastroenterol 2014; **28**: 843–53.

6. **Ji J, Wang H, Li Y, *et al.*** Diagnostic evaluation of des-gamma-carboxy prothrombin versus α-Fetoprotein for hepatitis B virus-related hepatocellular carcinoma in China: a large-scale, multicentre study. PLoS One 2016; **11**: e0153227.

7. **Lou J, Zhang L, Lv S, *et al.*** Biomarkers for hepatocellular carcinoma. Biomark Cancer 2017; **9**: 1–9.

8. **Chen ZY, Wei W, Guo ZX, *et al.*** Using multiple cytokines to predict hepatocellular carcinoma recurrence in two patient cohorts. Br J Cancer 2014; **110**: 733–40.

9. **Uze G, Monneron D.** IL-28 and IL-29: newcomers to the interferon family. Biochimie 2007; **89**: 729–34.

10. **Cao Y, Zhang R, Zhang W, *et al.*** IL-27, a cytokine, and IFN-lambda1, a type III IFN, are coordinated to regulate virus replication through type I IFN. J Immunol 2014; **192**: 691–703.

11. **Yu Y, Gong R, Mu Y, *et al.*** Hepatitis B virus induces a novel inflammation network involving three inflammatory factors, IL-29, IL-8, and cyclooxygenase-2. J Immunol 2011; **187**: 4844–60.

12. **Naumnik W, Naumnik B, Niewiarowska K, *et al.*** Novel cytokines: IL-27, IL-29, IL-31 and IL-33. Can they be useful in clinical practice at the time diagnosis of lung cancer? Exp Oncol 2012; **34**: 348–53.

13. **Witte K, Witte E, Sabat R, *et al.*** IL-28A, IL-28B, and IL-29: promising cytokines with type I interferon-like properties. Cytokine Growth Factor Rev 2010; **21**: 237–51.

14. **Jiang SS, Weng DS, Wang QJ, *et al.*** Galectin-3 is associated with a poor prognosis in primary hepatocellular carcinoma. J Transl Med 2014; **12**: 273.

15. **Hsu DK, Dowling CA, Jeng KC, *et al.*** Galectin-3 expression is induced in cirrhotic liver and hepatocellular carcinoma. Int J Cancer 1999; **81**: 519–26.

16. **Jiang N, Sun R, Sun Q.** Leptin signaling molecular actions and drug target in hepatocellular carcinoma. Drug Des Devel Ther 2014; **8**: 2295–302.

17. **Shen J, Yeh CC, Wang Q, *et al.*** Plasma adiponectin and hepatocellular carcinoma survival among patients without liver transplantation. Anticancer Res 2016; **36**: 5307–14.

18. **Lynagh GR, Collins RA, Kaiser P.** Development and use of monoclonal antibodies to chicken fibronectin to show that the chicken hepatocellular carcinoma cell line, LMH, constitutively expresses fibronectin. Res Vet Sci 2000; **68**: 147–52.

19. **Kaspar M, Zardi L, Neri D.** Fibronectin as target for tumor therapy. Int J Cancer 2006; **118**: 1331–9.

20. **Gupta N, Kakkar N, Vasishta RK.** Pattern of fibronectin in hepatocellular carcinoma and its significance. Indian J Pathol Microbiol 2006; **49**: 362–4.

21. **Mussbach F, Henklein P, Westermann M, *et al.*** Proteinase-activated receptor 1- and 4-promoted migration of Hep3B hepatocellular carcinoma cells depends on ROS formation and RTK transactivation. J Cancer Res Clin Oncol 2015; **141**: 813–25.

22. **Scaggiante B, Kazemi M, Pozzato G, *et al.*** Novel hepatocellular carcinoma molecules with prognostic and therapeutic potentials. World J Gastroenterol 2014; **20**: 1268–88.

23. **Fujie H, Tanaka T, Tagawa M, *et al.*** Antitumor activity of type III interferon alone or in combination with type I interferon against human non-small cell lung cancer. Cancer Sci 2011; **102**: 1977–90.

24. **Wang SN, Lee KT, Ker CG.** Leptin in hepatocellular carcinoma. World J Gastroenterol 2010; **16**: 5801–9.

25. **Andrighetto LV, Poziomyck AK.** Serum leptin levens and hepatocellular carcinoma: review article. Arq Bras Cir Dig 2016; **29**: 276–8.

26. **Sadik NA, Ahmed A, Ahmed S.** The significance of serum levels of adiponectin, leptin, and hyaluronic acid in hepatocellular carcinoma of cirrhotic and noncirrhotic patients. Hum Exp Toxicol 2012; **31**: 311–21.

27. **Moriya K, Sakai K, Yan MH, Sakai T.** Fibronectin is essential for survival but is dispensable for proliferation of hepatocytes in acute liver injury in mice. Hepatology 2012; **56**: 311–21.

28. **Tamkun JW, Hynes RO.** Plasma fibronectin is synthesized and secreted by hepatocytes. J Biol Chem 1983; **258**: 4641–7.

29. **Kim H, Park J, Kim Y, *et al.*** Serum fibronectin distinguishes the early stages of hepatocellular carcinoma. Sci Rep 2017; **7**: 9449.

30. **Han N, Jin K, He K, *et al.*** Protease-activated receptors in cancer: a systematic review. Oncol Lett 2011; **2**: 599–608.