

Original Research

Received 03 July 2026

Revised 13 Aug 2026

Accepted 26 Aug 2026

Natural Resources for Human
Health 2026, Vol. 6 (18s): 266–273

KEYWORDS:

HCC, AFP-L3, CK19, early
detection, biomarkers, ELISA

Evaluation of Serum Alpha-fetoprotein-L3 and Cytokeratin 19 as Biomarkers for the Early Detection of Hepatocellular Carcinoma and Treatment Response in Iraqi Patients

Ban A. Jasim^{1,2*}, Firas A. Hassan¹

¹Department of Chemistry, College of Science, Al-Nahrain University, Baghdad, Iraq.

²Scientific Research Commission, Baghdad, Iraq

*Corresponding author E-mail: ban.abdulwahhab.sci25@ced.nahrainuniv.edu.iq

ABSTRACT: Background: Hepatocellular carcinoma (HCC) is the most predominant kind of primary liver cancer and the third most cause of cancer-related mortality globally. In Iraq, hepatocellular carcinoma is considered a major public health problem. Studies have indicated that the two main causes of the disease are chronic viral hepatitis (HBV and HCV), in addition to cirrhosis, with an approximate male-to-female ratio of 3:1, and that the disease is most prevalent among patients aged 41 to 60 years.

Objective: This study aimed to evaluate the efficacy of serum Alpha-fetoprotein-L3 (AFP-L3) and Cytokeratin 19 (CK19) as biomarkers for the early detection of HCC and assessment of treatment response in Iraqi patients. **Materials and methods:** This study was conducted among 150 participants aged between 35 and 65 years at Al-Yarmouk Teaching Hospital in Baghdad, Iraq, from 10 January 2026 to 30 June 2026. Three groups of participants were included: 50 healthy individuals (Group I), 50 untreated patients with HCC (Group II), and 50 treated HCC patients with transarterial chemoembolization or TACE (Group III). ELISA was used to measure serum levels of AFP-L3 and CK19.

Results: AFP-L3 levels were significantly higher in Group II (32.73 ± 7.16 ng/mL) than in Group I (0.68 ± 0.20 ng/mL), whereas AFP-L3 levels in Group III decreased significantly to 10.86 ± 1.73 ng/mL compared with Group II (32.73 ± 7.16 ng/mL) ($P < 0.0001$). Regarding CK19 levels, Group II showed significantly increased levels (24.94 ± 7.59 ng/mL) compared with Group I (3.87 ± 0.55 ng/mL). However, a marked reduction in CK19 levels was noted in Group III (8.12 ± 3.35 ng/mL) compared with Group II (24.94 ± 7.59 ng/mL) ($P < 0.0001$).

Conclusion: Both AFP-L3 and CK19 demonstrated excellent diagnostic performance as independent biomarkers for early detection of HCC and assessment of treatment response in Iraqi patients

INTRODUCTION

Hepatocellular carcinoma (HCC) is the most predominant kind of primary liver cancer and the third most common cause of cancer-related mortality globally [1]. The global burden of HCC is largely attributable to late-stage diagnosis, which markedly reduces the number of therapeutic choices available [2]. In Iraq, hepatocellular carcinoma is considered a major public health problem. Studies have indicated that the two main causes of the disease are chronic viral hepatitis (HBV and HCV), in addition to cirrhosis, with an approximate male-to-female ratio of 3:1, and that the disease is most prevalent among patients

aged 41 to 60 years [3]. Therefore, the urgent requirement to find reliable markers for early detection of HCC and assessment of treatment response is crucial to enhance patient outcomes and mitigate the impact of this malignancy [2,4].

In this context, more precise biomarkers, including alpha-fetoprotein-L3 (AFP-L3), have been developed to improve diagnostic accuracy. Recent studies have indicated that AFP-L3 is a promising biomarker for more reliable detection of HCC. As AFP-L3 is an isoform of AFP, this glycoprotein can be classified into three glycoforms: L1, L2 and L3, according to their interaction with Lens culinaris agglutinin (LCA). AFP-L1 is a non-LCA-binding glycoform and is found in individuals with nonmalignant hepatopathy, such as chronic hepatitis B virus infection and cirrhosis. AFP-L2 exhibits intermediate LCA-binding capacity and is primarily produced by yolk sac tumors. In contrast, AFP-L3 is the primary glycoform in individuals with HCC, is only generated from cancerous liver cells, and has strong affinity for LCA [5]. It is produced due to fucosylation by α -1,6-fucosyltransferase (FUT8), which is overexpressed in HCC, resulting in enhanced binding to LCA lectin, which in turn allows for serological detection of the fraction of AFP bound to LCA. This binding property is a hallmark of neoplastic hepatocytes in HCC, and its altered glycosylation pattern reflects the metabolic changes occurring in HCC cells. AFP-L3 demonstrates greater specificity than total AFP, particularly in discriminating malignant from benign hepatic conditions, establishing it as a targeted biomarker for differentiating HCC from other liver conditions with improved accuracy [4,6].

Among other important biomarkers, Cytokeratins (CKs) are the major intermediate filament proteins in the liver, and any damage to hepatocyte membrane integrity leads to their release into the bloodstream [7]. In terms of classification, they represent a polygenic protein family classified according to molecular weight and chemical structures into two principal subtypes: type II keratins (CK1-CK8), which are basic or neutral and of high molecular weight, and type I keratins (CK9-CK20), which are acidic and of low molecular weight [8]. CK19, also known as keratin 19 (KRT19), is a kind of acidic keratin protein that has a molecular weight of 40 kDa that is produced by the KRT19 gene at 17q12-q21 [9]. CK19 is a cytoskeletal protein predominantly expressed in epithelial cells, including hepatocytes, biliary epithelial cells and intrahepatic bile duct cells. Recognized as a cancer stem cell marker, CK19 plays a fundamental role in carcinogenesis, metastasis, and recurrence, and elevated CK19 expression has been significantly correlated with reduced survival and early recurrence of the tumor in patients with HCC [10,11]. Similar to all Cytokeratins, CK19 is composed of three distinct domains: a head, a central rod and a tail domain. The human CK19 protein consists of 400 amino acids (aa), inclusive of the initiating methionine, as determined by sequencing analyses, with a non-helical N-terminal head domain spanning 79 aa (1-79), an alpha-helical central rod domain encompassing 307 aa (80-387), and a short alpha-helical C-terminal tail domain consisting of 13 aa (388-400) [12].

Both AFP-L3 and CK19 are considered promising biomarkers for hepatocellular carcinoma [13,14]. Nevertheless, their efficacy for the early detection of HCC and the assessment of treatment response remains insufficiently evaluated among Iraqi patients. Therefore, the aim of this study was to evaluate the efficacy of these two biomarkers for the early detection of HCC and the assessment of treatment response.

MATERIALS AND METHODS

Study design

The research was performed among 150 participants aged between 35 and 65 years at Al-Yarmouk Teaching Hospital in the Department of Hepatology, Gastroenterology, and Oncology in Baghdad, Iraq, from 10 January 2026 to 30 June 2026. Three groups of participants were included: Group I (50 healthy controls) and Group II (50 untreated HCC patients), and Group III (50 treated HCC patients receiving transarterial chemoembolization, or TACE).

Exclusion Criteria:

Patients who suffered from any chronic diseases (kidney disease, diabetes, hypertension, and heart disease) and were pregnant or breastfeeding women; had a history of other malignancies; had previously received any treatment for liver cancer; had a history of alcohol abuse were excluded from this study.

Blood sample collection procedure

The participants in this research were 150 Iraqi individuals, including patients with HCC and healthy controls. HCC patients were diagnosed based on clinical assessment and serum biomarkers. A blood sample of about 5 mL was drawn from each individual. The samples were transferred directly into gel tubes and allowed to stand for at least 20 min at room temperature for clot formation. Subsequently, the clotted specimens were centrifuged at 3000 rpm for 10 min to extract the serum. The

separated serum was transferred with care into clean Eppendorf tubes using a micropipette, and all tubes were kept at -20°C until analysis.

ELISA Assay Procedure

2.3.1 Human Alpha-Fetoprotein Lens Culinaris Agglutinin 3 (AFP-L3) ELISA Kit

Human AFP-L3 levels were determined using a sandwich ELISA kit (AFG Scientific, USA). Reagents were equilibrated to room temperature, and 580 mL of deionized water was used to dilute 20 mL of concentrate to produce the wash water. The standard curve was established by preparing a series of concentrations (40, 20, 10, 5, 2.5, 1.25, 0.63, and 0 ng/mL as a blank) through serial dilutions of the stock standard (40 ng/mL). The assay was performed by adding 50 μL of duplicate standards to the well plate. Then, 10 μL of duplicate serum samples were introduced to each sample well after 40 μL of sample diluent. After a 40-minute incubation period at 37°C , the plate was rinsed 5 times with 300 μL of wash buffer in each well. Subsequently, all wells except the blank were filled with 50 μL of HRP conjugate, incubated for 40 minutes at 37°C , and rinsed five more times. To allow the color to develop, 50 μL each of Chromogen A and B were introduced and then maintained for 20 minutes at 37°C . Finally, the optical density (OD) was measured at 450 nm immediately after 50 μL of stop solution was introduced. The standard curve was used to calculate the AFP-L3 concentrations, which were then multiplied by a dilution factor of five.

Human Cytokeratin 19 (CK19) ELISA Kit

Serum human CK19 levels were measured using a commercial sandwich ELISA kit (Biomatik, Canada). All kit components and samples were equilibrated to room temperature ($18-25^{\circ}\text{C}$), and 20 mL of the concentrate was diluted with 580 mL of deionized water to yield the wash buffer. The standard curve was established by sequentially diluting the CK19 stock standard (40 ng/mL), yielding final standard concentrations of 40, 20, 10, 5, 2.5, 1.25, and 0.63 ng/mL in addition to a zero standard as a blank. Subsequently, the working solutions for Detection Reagent A and Detection Reagent B were prepared individually immediately before their addition to the assay wells by diluting each concentrate at a 1:100 ratio with its corresponding assay diluent. The assay procedure was performed by adding 100 μL of duplicate standards and serum samples into the appropriate wells, followed by incubation at 37°C for 1 hr. Then, the liquid in each well was removed without washing. Subsequently, each well received 100 μL of the Detection Reagent A working solution, which was then kept for one hour at 37°C . After aspirating the wells, 350 μL of wash buffer was used to rinse each well three times. After the rinse cycle, each well was filled with 100 μL of Detection Reagent B working solution, kept at 37°C for 30 minutes, and subjected to a five-fold wash sequence to remove any unbound conjugate. Each well received 90 μL of substrate solution to initiate color development and produced a blue color shift. The wells were then incubated at 37°C for 20 minutes. The enzymatic reaction was subsequently terminated by adding 50 μL of stop solution to each well, causing the liquid to turn yellow. A microplate reader was used to detect the optical density at 450 nm directly, and lastly, concentrations were calculated by interpolating the absorbance values against the standard curve.

Statistical Analysis

GraphPad Prism Software, version 9.0.2 (San Diego, California, USA), was utilized for data analysis. One-way analysis of variance (ANOVA) was employed to compare the mean values between the three groups. When a p-value less than 0.05 was obtained, the results were considered significant. Additionally, sensitivity, specificity, the best cutoff value, and area under the curve (AUC) of the AFP-L3 and CK19 markers were determined using the receiver operating characteristic (ROC) curve analysis.

RESULTS

The results of the analysis of AFP-L3 levels among the three groups demonstrated highly significant differences with a p-value less than 0.0001. The mean AFP-L3 values in untreated HCC patients (Group II) were significantly higher, at 32.73 ± 7.16 ng/mL, than the values in the control group (Group I) at 0.68 ± 0.20 ng/mL. However, after treatment the mean serum AFP-L3 values in (Group III) decreased significantly to 10.86 ± 1.73 ng/mL, compared with (Group II) at 32.73 ± 7.16 ng/mL, but remained higher than the control group, as demonstrated in Figure 1-a and Table 1.

Concerning the analysis of Cytokeratin 19 (CK19) levels, the mean CK19 values in the control group (Group I) were 3.87 ± 0.55 ng/mL. On the other hand, the untreated group (Group II) revealed a statistically significant difference ($P < 0.0001$), with a marked increase to 24.94 ± 7.59 ng/mL. However, as shown in Table 1 and Figure 1-b, CK19 values were significantly reduced in the treated group (Group III) post-treatment, with a mean of 8.12 ± 3.35 ng/mL, compared with the untreated group. But remained considerably elevated compared with the control group (Group I)

Table 1. Assessment of serum levels of AFP-L3 and CK19 in HCC patients and healthy individuals (control group)

Variable	Average value $\pm$ SD			P - value
	Control	Untreated	Treated	
AFPL3	0.68 ± 0.20 a	32.73 ± 7.16 b	10.86 ± 1.73 c	< 0.0001 **
CK19	3.87 ± 0.55 a	24.94 ± 7.59 b	8.12 ± 3.35 c	< 0.0001 **

Sig. * $P < 0.05$; high sig. ** $P < 0.001$; no sig. $p > 0.05$. a: Control vs. Untreated, b: Control vs. Treated, c: Untreated vs. Treated.

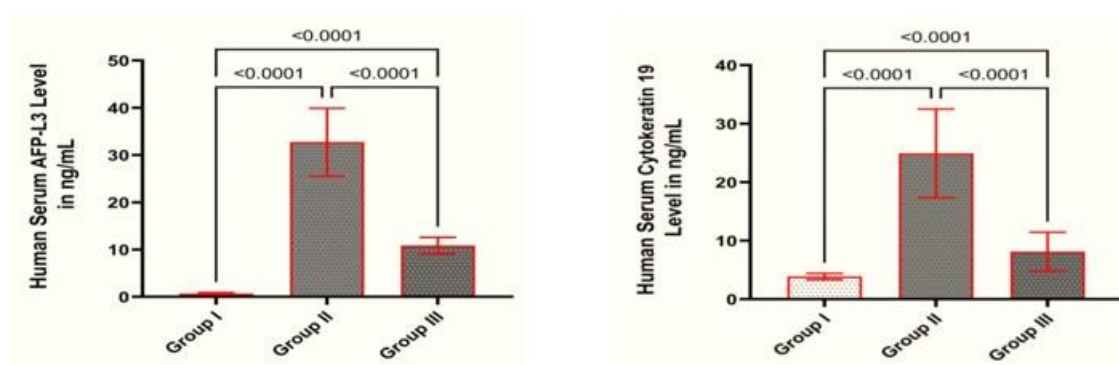


Figure 1 : The concentration of a - AFPL3 (ng/mL) and b- CK19 (ng/mL) in the control group (group I), untreated (group II) and, treated (group III).

3.1 Receiver Operating Characteristic Analysis

The ROC analysis demonstrated the diagnostic performance of AFP-L3 and CK19 biomarkers, as shown in Table 2. When comparing untreated HCC patients (Group II) with the control group (Group I), AFP-L3 showed excellent discrimination with 88% sensitivity and 82% specificity, at a cutoff > 0.7845 , and an AUC of 0.9236 ($P < 0.0001$). Furthermore, in the comparison between the control group (Group I) and treated patients (Group III), the area under the curve AUC of 0.9868 demonstrated excellent distinction between the two groups ($P < 0.0001$), with a cutoff of > 1.065 and both sensitivity and specificity values of 98%. Additionally, comparing Group II with Group III, AFP-L3 demonstrated 88% sensitivity, 80% specificity, an AUC of 0.9348 ($P < 0.0001$) and a cut off of < 22.29 , indicating excellent differentiation between untreated and treated patient groups.

Regarding CK19, the analysis demonstrated excellent discrimination ability when comparing Group I and Group II, with a sensitivity of 84 % and a specificity of 82 %, at a cut-off > 5.020 and AUC of 0.9072 ($P < 0.0001$). The comparison between the control group (Group I) and treated patients (Group III) showed excellent distinction with an AUC of 0.9636 ($P < 0.0001$).

0.0001), 92% sensitivity and 98% specificity at a cutoff > 5.020. Furthermore, good differentiation was found when Group III and Group II were compared at a cut-off < 12.85, with an AUC of 0.8939, 94% sensitivity, and 88% specificity.

Table 2: Assessment of ROC Analysis of AFP-L3 and CK19 Parameters Among HCC Patient groups and Control group showing AUC, P-value, Sensitivity, Specificity and Cut-off .

Parameters	Type of correlation	AUC	Sensitivity	Specificity	Cut-off	CI 95%	P-Value
AFPL3	Control vs. Untreated	0.9236	88 %	82 %	> 0.7845	0.872 - 0.98	< 0.0001
	Control vs. Treated	0.9868	98 %	98 %	> 1.065	0.9624 - 1.00	< 0.0001
	Untreated vs. Treated	0.9348	88 %	80 %	< 22.29	0.884 - 0.986	< 0.0001
CK19	Control vs. Untreated	0.9072	84 %	82 %	> 5.020	0.8863 - 0.98	< 0.0001
	Control vs. Treated	0.9636	92 %	98 %	> 5.020	0.9164 - 1.0	< 0.0001
	Untreated vs. Treated	0.8939	94 %	88 %	< 12.85	0.861 - 0.985	< 0.0001

Control (Group I) , Untreated (Group II), Treated(Group III)

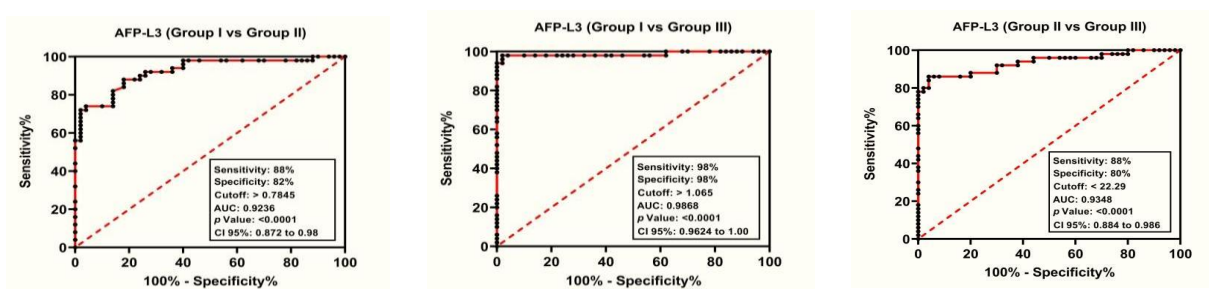


Figure 2: The ROC analysis of a- AFPL3 in Group I vs Group II untreated patients, b- AFPL3 in Group I vs Group III treated patients, and c- AFPL3 in Group II vs Group III, showing the AUC, p-value, sensitivity, specificity, and cut-off.

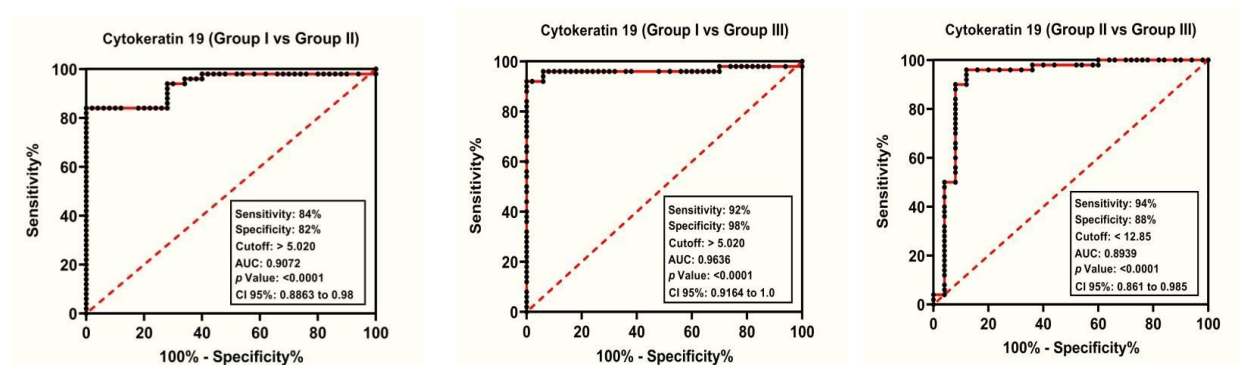


Figure 3: The ROC analysis of a- CK19 in Group I vs Group II untreated patients , b- CK19 in Group I vs Group III treated patients, and c- CK19 in Group II vs Group III , showing the AUC, p-value, sensitivity, specificity, and cut-off .

4- DISCUSSION

The current study, which was carried out on HCC patients, evaluated the performance of two biomarkers (AFP-L3 and CK19) individually for early detection of HCC and assessment of treatment response. A significant elevation in AFP-L3 levels

in the untreated HCC patient group relative to the control group indicated AFP-L3 potential as a reliable diagnostic marker for the early detection of hepatocellular carcinoma, as further supported by ROC curve analysis, with 88% sensitivity, 82% specificity, and an AUC of 0.9236. This demonstrated excellent discriminatory ability for AFP-L3 in distinguishing between untreated patients and healthy controls. The elevation can be explained by the fact that AFP-L3 promotes cancer cells proliferation by activating the Wnt- β -catenin pathway, as well as stimulating the invasion and spread of liver cancer cells by activating the TGF- β and VEGF pathways [15]. Our current results are in agreement with those of El-Halawany et al., who reported significantly elevated AFP-L3 levels in HCC patients (23.24 ± 4.29 ng/mL) compared with healthy individuals (4.53 ± 1.81 ng/mL) ($P < 0.0001$), with 80% sensitivity and 80% specificity. They confirmed the ability of AFP-L3 to differentiate HCC patients from healthy individuals [16]. Our results were aligned with the findings of Ibrahim et al., who demonstrated that serum AFP-L3 levels were considerably higher in HCC patients than in healthy individuals. These findings confirmed that AFP-L3 may act as a diagnostic marker for the early detection of HCC [17]. In this context, previous studies indicated that AFP-L3 is a form of AFP used as a biomarker for the detection of HCC, in addition to its ability to differentiate between hepatocellular carcinoma and benign liver diseases, early detection of small liver tumors, and prognosis prediction [18]. Whereas serum AFP-L3 levels in treated patients decreased compared to untreated patients. This indicated that the treatment contributed to reducing disease activity. This finding supported the use of AFP-L3 as a biomarker in the assessment of treatment response. Force et al. described the role of AFP-L3 in the assessment of response to transarterial chemoembolization (TACE) treatment in HCC patients, a decrease in AFP-L3 levels after treatment was linked to positive treatment response outcomes in HCC patients [19], which is consistent with our study's results.

Regarding the analysis of Cytokeratin 19 levels, elevated levels were revealed in untreated HCC patients relative to the control group, with 84% sensitivity, 82% specificity and an AUC of 0.9072. These findings demonstrated that CK19 has a high discriminatory ability between untreated patients and healthy individuals, supporting its role as a diagnostic marker for the early detection of HCC. This elevation may be attributed to the fact that CK19 is cleaved by caspase 3 into soluble fragments that are secreted into the circulation upon apoptosis and found in cancer patients; therefore, their levels correlate significantly with tumor mass and more especially with necrosis in the tumor [20]. Furthermore, the upregulation of CK19 may drive EMT, increasing tumor invasiveness and metastatic potential through loss of E-cadherin and gain of vimentin and N-cadherin [21]. Previous studies have demonstrated that several signaling pathways and transcription factors, including TGF- β , MAPK/JKK, MEK-ERK1/2, and noncoding RNAs associated with CK19 expression, play an essential role in the malignant properties of HCC [22]. Findings of our study are consistent with those of Al-Haddad et al., who reported significantly elevated CK19 levels in HCC patients (63.7 ± 3.6 ng/mL) compared with the healthy controls (25.3 ± 3.9 ng/mL) ($P < 0.0001$), with 94% sensitivity, 91% specificity and an AUC of 0.864. Those findings confirmed that CK19 represents a promising marker for the early detection of HCC [23]. However, Saad et al. reported no statistically significant difference in CK19 levels between healthy individuals and in HCC patients ($P > 0.05$), which contrasted with the findings of our current study. They attributed these discrepancies to differences in the number of patients [24]. Furthermore, serum CK19 levels decreased after TACE treatment, reflecting a positive tumor response to therapy. The significant decrease in CK19 levels may be attributed to the effectiveness of TACE therapy, which plays a role in stimulating tumor necrosis while maintaining enough liver function, as local drug concentrations in TACE are more than 10 times higher compared to systemic therapy [25]. Thus, CK19 considered a reliable marker for assessment of treatment response in HCC patients. In contrast, a previous study conducted by Zhu et al. on liver tissue using immunohistochemical staining demonstrated that CK19-positive patients exhibited poor response (resistance) to TACE therapy, while CK19-negative patients exhibited a positive therapeutic response [26]. This phenomenon can be explained by the fact that aggressive behavior and the expression of stemness markers are characteristic of CK19-positive hepatocellular carcinoma, a subtype of HCC. These stem cells possess the ability to differentiate, regenerate and form tumors. Moreover, the hypoxic microenvironment caused by TACE therapy promotes cancer stem cell growth by activating the HIF-1 α /HIF-2 α pathway, thereby conferring resistance to treatment in CK19-positive cells, as opposed to CK19-negative cells [27].

CONCLUSION

This study demonstrated that both AFP-L3 and CK19 represent reliable biomarkers with excellent diagnostic accuracy for the early detection of HCC in Iraqi patients. The results revealed a statistically significant elevation in both biomarker levels in untreated HCC patients compared with healthy controls, supporting their potential as diagnostic markers for the early detection of patients with HCC, as further confirmed by ROC curve analysis, which demonstrated excellent discriminatory

ability for both biomarkers in differentiating untreated HCC patients from healthy controls. Furthermore, treatment with transarterial chemoembolization (TACE) resulted in a significant reduction in AFP-L3 and CK19 levels among treated HCC patients compared to the untreated group. This indicates that these biomarkers are not limited to early detection but also serve as promising markers for assessment of treatment response, as confirmed by ROC analysis, which revealed excellent discriminatory performance in differentiating treated from untreated patients. Overall, these findings indicated that AFP-L3 and CK19 are promising biomarkers for the early detection of HCC and for assessment of treatment response.

Acknowledgements: We would like to thank Al-Yarmouk Teaching Hospital. Oncology and Gastrointestinal Centre, Al Baghdad Governorate, Iraq, for their invaluable collaboration in patient recruitment and sample collection. We are sincerely grateful to the patients who all participated in the current study.

Conflict of Interest: The authors declare that there is no conflict of interest.

Funding: No funding was received for this manuscript.

Ethical Approval: The current study obtained ethical approval from the Postgraduate Studies Ethical and Scientific Committee, College of Science, Al-Nahrain University, Baghdad, Iraq (Approval Code: 1/28-RECSNU). All patients and controls signed informed written consent after explanation of the aim of the study and the procedure.

REFERENCE

- [1] Park MK. Serum biomarkers for hepatocellular carcinoma: A comparative review of traditional and emerging markers. *J Dig Cancer Res.* 2025;13(3): 242–250. <https://doi.org/10.52927/jdcr.2025.13.3.242>
- [2] Kandil A, Ameen SG. Hepatocellular carcinoma detection in hepatitis c virus-related cirrhosis by Glypican-3. *Benha Medical Journal.* 2023;40(2):343–358. <https://doi.org/10.21608/BMFJ.2022.175414.1712>
- [3] Saadoon AA, Hayyawi MR, Hassan MF, Abdul Hassan YK. The incidence of hepatocellular carcinoma in Iraq population. *Thi-Qar Med J.* 2023;26(2):120-130. <https://doi.org/10.32792/TMJ.V26I2.472>
- [4] Wu ST, Zhu L, Feng XL, Wang HY, Li F. Strategies for discovering novel hepatocellular carcinoma biomarkers. *World J Hepatol.* 2025;17(2):101210. <https://doi.org/10.4254/wjh.v17.i2.101201>
- [5] Rauf A. Diagnostic value of alpha-fetoprotein and lens culinaris agglutinin-reactive Alpha-fetoprotein in liver cirrhosis and hepatocellular carcinoma patients in Pakistan. *Int J Pathol.* 2024; 22(1): 3–8. <https://doi.org/10.59736/IJP.22.01.880>
- [6] Norman JS, Mehta N. The role of AFP-L3 and DCP biomarkers in the diagnosis and management of hepatocellular carcinoma. *Curr Hepatol Rep.* 2025;24(1):16. <https://doi.org/10.1007/s11901-025-00686-7>
- [7] El Raziky M, Abdel Hafez H, Elsharkawy A, Moneer TA, El-Sheikh SM, Maher RM, et al. Serum level of cytokeratin 19 as a diagnostic and prognostic marker in patients with HCV-related hepatocellular carcinoma. *Egy Liver J.* 2021;11(1):57. <https://doi.org/10.1186/s43066-021-00125-4>
- [8] Mehrpouya M, Pourhashem Z, Yardehnavi N, Oladnabi M. Evaluation of cytokeratin 19 as a prognostic tumoral and metastatic marker with focus on improved detection methods. *J Cell Physiol.* 2019; 234(12): e21425. <https://doi.org/10.1002/jcp.28768>
- [9] Menz A, Bauer R, Kluth M, von Bargen CM, Gorbokon N, Viehweger F, et al. Diagnostic and prognostic impact of cytokeratin 19 expression analysis in human tumors: a tissue microarray study of 13,172 tumors. *Hum Pathol.* 2021;115:19–36. <https://doi.org/10.1016/j.humphath.2021.05.012>
- [10] Zhou L, Chen Y, Li Y, Wu C, Xue C, Wang X, et al. Diagnostic value of radiomics in predicting Ki-67 and cytokeratin 19 expression in hepatocellular carcinoma: a systematic review and meta-analysis. *Front Oncol.* 2024;13:1323534. <https://doi.org/10.3389/fonc.2023.1323534>
- [11] Kawai T, Yasuchika K, Ishii T, Katayama H, Yoshitoshi EY, Ogiso S, et al. Keratin 19, a cancer stem cell marker in human hepatocellular carcinoma. *Clin Cancer Res.* 2015;21(13):3081–3091. <https://doi.org/10.1158/1078-0432.CCR-14-1936>
- [12] Mujiyambere B, Jayaraj R, Suja S. Cytokeratin 19 (CK19) as a marker for epithelial differentiation and malignant transformation: Its clinical relevance in diagnosis, prognosis and treatment response monitoring. *Iconic Res Eng J.* 2018; 2(3): 51-61.

- [13] Yu J, Park R, Kim R. Promising novel biomarkers for hepatocellular carcinoma: Diagnostic and prognostic insights. *J Hepatocell Carcinoma*. 2023;10:1105-1127. <https://doi.org/10.2147/JHC.S341195>
- [14] Schlosser S, Tümen D, Volz B, Neumenyer K, Egler N, Kunst C, et al. HCC biomarkers-state of the old and outlook to future promising biomarkers and their potential in everyday clinical practice. *Front Oncol*. 2022;12:1016952. <https://doi.org/10.3389/fonc.2022.1016952>
- [15] Wang J, Wang F, Wang N, Zhang MY, Wang HY, Huang GL. Diagnostic and prognostic value of protein post-translational modifications in hepatocellular carcinoma. *J Clin Transl Hepatol*. 2023;11(5):1192–1200. <https://doi.org/10.14218/JCTH.2023.00006S>
- [16] El- Halawany F, Mahmoud S, Oun MR, Kadry M, Tarek H, Hanna R, et al. Detection of serum biomarkers in hepatocellular carcinoma patients. *Int J Adv Sci Res Eng*. 2019;5(1):123–130. <https://doi.org/10.31695/IJASRE.2019.32933>
- [17] Ibrahim HM, Elghannam MZ, Elkhawaga OAY, El-Sokkary AMA. Evaluation of serum alpha fetoprotein-L3 as an accuracy novel biomarker for the early diagnosis of hepatocellular carcinoma in Egyptian patients. *Saudi J Biol Sci*. 2021;28:5760–5764. <https://doi.org/10.1016/j.sjbs.2021.06.020>
- [18] Jia Z, Wang L, Liu C, Yu Z, Chai L, Zhao M. Evaluation of α -fetoprotein-L3 and Golgi protein 73 detection in diagnosis of hepatocellular carcinoma. *Contemp Oncol*. 2014;18(3):192–196. <https://doi.org/10.5114/wo.2014.43157>
- [19] Force M, Park G, Chalikonda D, Roth C, Cohen M, Halegoua-Demarzio D, et al. Alpha-Fetoprotein (AFP) and AFP-L3 is most useful in detection of recurrence of hepatocellular carcinoma in patients after tumor ablation and with low AFP level. *Viruses*. 2022;14:775. <https://doi.org/10.3390/v14040775>
- [20] Taher MY, Hassouna E, El Hadidi A, El-aassar O, Bakosh MF, Shater MS. Serum CYFRA 21-1 and CK19-2G2 as predictive biomarkers of response to transarterial chemoembolization in hepatitis c–related hepatocellular carcinoma among Egyptians: A prospective study. *J Clin Exp Hepatol*. 2025;15(1):102405. <https://doi.org/10.1016/j.jceh.2024.102405>
- [21] Khodabocus Z, Zhang H, Li D, Chen Z, Mu X, Wang X. Clinical significance of cytokeratin 19 expression in hepatocellular carcinoma. *J Cancer Metastasis Treat*. 2025;11:14. <https://dx.doi.org/10.20517/2394-4722.2025.35>
- [22] Zhu D, Yang W, Zhou HF, Liu S, Zhou WZ, Shao ZF, et al. Early recurrent hepatocellular carcinoma with CK19 positive receiving transarterial chemoembolization after surgical resection: a novel risk model study. *J Hepatocell Carcinoma*. 2026;13:563631. <https://doi.org/10.2147/JHC.S563631>
- [23] Al Haddad M, El-Mezayen HA, El-Kassas M, Metwally F, El-Sharkawy A. Clinical utility of cytokeratins for accurate diagnosis of hepatocellular carcinoma among hepatitis c virus high-risk patients. *Asian Pac J Cancer Prev*. 2024;25(4):1325–1332. <https://doi.org/10.31557/APJCP.2024.25.4.1325>
- [24] Saad AA, El Gamal SD, Hendawy SR, Saleh A. Influence of cytokeratin-19 level on pattern of hepatocellular carcinoma in patients with liver cirrhosis. *Egypt J of Hosp Med*. 2022;89:5444–5451. <https://doi.org/10.21608/ejhm.2022.264524>
- [25] Wu MS, Zhong JH, Chen K, Luo CP, Zhang J, Zhou YJ, et al. Association of CK19 expression with the efficacy of adjuvant transarterial chemoembolization after hepatic resection in hepatocellular carcinoma patients at high risk of recurrence. *J Clin Transl Res*. 2022;8(1):71–79. <https://doi.org/10.18053/jctres.08.202201.009>
- [26] Zhu D, Yang W, Zhou HF, Shi HB, Liu S, Shao ZF, Zhou WZ. Prognostic implications of CK19 positivity in patients with early recurrent hepatocellular carcinoma after hepatic resection undergoing transarterial chemoembolization. *BMC Gastroenterol*. 2024;24:347. <https://doi.org/10.1186/s12876-024-03417-2>
- [27] Rhee H, Kim H, Park YN. Clinico-radio-pathological and molecular features of hepatocellular carcinomas with keratin 19 expression. *Liver Cancer*. 2020;9:663–681. <https://doi.org/10.1159/000510522>