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Evaluation of the sensitivity and specificity of monoclonal antibodies for detecting the thioredoxin antigen in hepatocellular carcinoma patients

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Abstract: The objective of this study was to evaluate the sensitivity and specificity of the monoclonal antibody of thioredoxin (mAb-TXN) for detecting the thioredoxin (TXN) antigen in serum samples from patients with hepatocellular carcinoma (HCC). To achieve this, the mAb-TXN, generated from hybridoma cells, was utilized to optimize the conditions for an indirect ELISA immunoassay. The sensitivity, specificity, positive predictive value, and negative predictive value of the assay were evaluated on the basis of stored patient medical records. The results showed that indirect ELISA using mAb-TXN exhibited a sensitivity of 93.61%, a specificity of 96%, a positive predictive value of 97.77%, and a negative predictive value of 88%. In conclusion, mAb-TXN is a valuable tool for detecting TXN antigens in serum samples from HCC patients, offering high sensitivity and specificity for clinical application.

Keywords: *Hepatocellular carcinoma, Indirect ELISA, Monoclonal antibodies, Sensitivity, Specificity.*

1. Introduction

Hepatocellular carcinoma is the sixth most common cancer worldwide and the third leading cause of cancer-related mortality [1]. In 2022, there were over 866,000 new cases globally, with the highest incidence rates observed in East Asia, Southeast Asia and North Africa. The primary risk factors for HCC include viral hepatitis (hepatitis B virus (HBV) and hepatitis C virus (HCV)), a history of alcohol consumption, and aflatoxin exposure [1]. Globally, the burden of liver cancer is projected to increase by 55% by 2040, emphasizing the need for effective measures to improve region-specific prevention and treatment strategies [2]. Vietnam ranks seventh globally in terms of HCC incidence, with approximately 24,500 new cases reported in 2022. This cancer is particularly prevalent and concerning in men, who exhibit the highest age-specific incidence rates worldwide [2].

Imaging modalities such as ultrasound, computed tomography (CT), and magnetic resonance imaging (MRI) have long been regarded as essential tools for detecting HCC. These techniques provide detailed information about tumor size, location, and invasiveness [3]. However, imaging methods are often costly, limited in detecting small tumors, incapable of characterizing early-stage lesions, and require highly skilled operators to manage advanced equipment. Biomarkers such as alpha-fetoprotein (AFP), AFP-L3 and Des-gamma-carboxy prothrombin (DCP) play critical roles and are widely used in diagnosing HCC. Among these, AFP is the most commonly used biomarker, although its sensitivity is limited, particularly for early-stage HCC. Combining AFP with AFP-L3, which is associated with malignancy, and DCP, which is linked to coagulation abnormalities in HCC, enhances diagnostic accuracy [4].

In recent years, TXN has emerged as a potential biomarker for diagnosing HCC. Serum TXN levels are often elevated in HCC patients, suggesting its utility in distinguishing HCC from benign liver diseases and healthy conditions [5]. Compared with AFP alone, combining TXN with traditional

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biomarkers such as AFP may improve early-stage diagnostic accuracy, enhancing both sensitivity and specificity. TXN offers several advantages over AFP for early HCC detection, including its ability to reflect oxidative stress and other physiological changes, higher sensitivity for small tumors, and its applicability in patients with underlying liver conditions such as cirrhosis or chronic hepatitis. In contrast, AFP typically exhibits high sensitivity only in advanced HCC stages and has low specificity in chronic liver diseases [5].

2. Materials and Methods

2.1. Study Subjects, Materials and Equipment

The study subjects included serum samples collected from patients diagnosed with HCC at the 108 Military Central Hospital. The samples were preserved at 4°C and promptly processed within 48 hours for immunoassays. A total of 72 serum samples were divided into three groups on the basis of medical records: (1) HCC group: 8 samples from patients with early-stage HCC and 39 samples from patients with intermediate-, advanced-, or terminal-stage HCC, collectively referred to as late-stage HCC. (2) Liver disease group: This group included 10 serum samples from individuals diagnosed with liver-related conditions (e.g., positive for HBV or HCV or exhibiting cirrhosis) but without confirmed HCC. (3) Healthy control group: This group consisted of 15 serum samples from healthy individuals confirmed to have no liver-related diseases.

The materials and equipment used in this study included a purified recombinant antigen of TXN (rAg-TXN, 0.85 mg/mL) and a specific monoclonal antibody of TXN (mAb-TXN, 1.2 mg/mL), both of which were supplied by the Vietnam–Germany cooperative scientific research project (Code: NĐT.102.GER/21) and developed by the Microbial Genomics Laboratory, Institute of Genome Research, Vietnam Academy of Science and Technology. The secondary goat anti-mouse IgG (H+L) HRP-conjugated antibody was purchased from Invitrogen (USA). Various solutions were used, including 0.2 M bicarbonate buffer at pH 9.4 (Thermo Fisher, USA), blocking buffer (TBST containing 5% BSA; Thermo Fisher, USA), dilution buffer (TBST containing 1% BSA; Thermo Fisher, USA), washing buffer (TBST; Thermo Fisher, USA), and 1 M HCl stop solution (Sigma Aldrich, USA). Additional reagents included bovine serum albumin (BSA, Merck, USA) and TMB substrate solution (Thermo Fisher, USA). ELISAs were conducted in 96-well plates (Costar Assay Plate, Corning, USA), and the results were measured with a Biotek ELX808 plate reader (Biotek, USA). Pipettes (single-channel and multichannel) and various types of pipette tips were employed during the experiments. Data analysis was performed via GraphPad Prism 9 software (GraphPad Software Inc., La Jolla, CA).

2.2. Research Methods

The indirect ELISA was optimized for detecting the TXN antigen in serum samples. Each component of the reaction was systematically optimized. The positive control consisted of recombinant rAg-TXN antigen diluted in bicarbonate buffer at serial dilutions of 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, and 1:128. The monoclonal antibody mAb-TXN was diluted in dilution buffer at ratios of 1:8, 1:16, 1:32, 1:64, 1:128, 1:256 and 1:512. The HRP-conjugated goat anti-mouse IgG (H+L) secondary antibody was diluted according to the manufacturer's recommendations at ratios of 1:5000, 1:10000, 1:15000, 1:20000 and 1:25000.

The procedure for detecting the TXN antigen in the serum was conducted as follows. Patient serum was diluted in 0.2 M bicarbonate buffer at pH 9.4 at a ratio of 1:1. A total of 100 µL of diluted serum was added to each well, and the plate was sealed with specialized adhesive film and incubated at room temperature for 2 hours or overnight at 4°C. The antigen was aspirated, and the wells were washed three times with 300 µL of washing buffer per well. Next, 200 µL of blocking buffer was added to each well and incubated at 37°C for 1 hour. The blocking buffer was then removed, and the wells were washed three times with 300 µL of washing buffer per well. Subsequently, 100 µL of the specific monoclonal antibody mAb-TXN was added to each well and incubated at 37°C for 1 hour. After incubation, the antibody was aspirated, and the wells were washed three times with 300 µL of washing buffer per well.

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Next, 100 µL of appropriately diluted HRP-conjugated secondary antibody was added to each well and incubated at 37°C for 1 hour. The secondary antibody was then aspirated, and the wells were washed three times with 300 µL of washing buffer per well. Next, 100 µL of TMB substrate solution was added to each well, and the plate was incubated in the dark for 30 minutes. The reaction was stopped by adding 100 µL of 1 M HCl to each well.

The results were read using an ELISA plate reader at a wavelength of 450 nm. The cutoff value was determined on the basis of the OD450 values of 15 serum samples from healthy individuals without liver-related diseases and was calculated via Lardeux's formula [6].

$$Cutoff\ value = \bar{X} + 3.SD.$$

Serum samples with OD450 nm values exceeding the determined cutoff were considered positive, whereas those below the cutoff were deemed negative. The sensitivity and specificity of the immunoassay for detecting the TXN antigen in serum samples via monoclonal antibodies were evaluated on the basis of the definitive diagnosis recorded in patient medical files. Calculations of sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were performed via standard diagnostic test formulas.

Study location and duration: The study was conducted at the Microbial Genomics Laboratory, Institute of Genome Research, Vietnam Academy of Science and Technology. Serum samples were collected from the Department of Hepatobiliary and Pancreatic Medicine at the 108 Military Central Hospital between June 2023 and June 2024.

Research ethics: Serum samples were collected anonymously, and patient information was strictly confidential, adhering to international ethical guidelines. All samples were exclusively used for academic research purposes.

3. Results

3.1. Characteristics of the Study Groups

3.1.1. Characteristics of the HCC Group

The majority of patients with HCC were aged ≥60 years, accounting for 61.8% of the total patients. Among these patients, males predominated, with approximately seven times as many males as females in the study group. The most common etiological factor in the development of HCC was HBV infection, which was present in 81% of the patients. Coinfection with HCV was observed in a single patient, representing 2.1% of the cohort. Alcohol-induced cirrhosis was also a significant contributing factor, present in 6.3% of the patients.

With respect to the stage of diagnosis, only 8 patients (17%) were diagnosed at an early stage of HCC, whereas the remaining 39 patients (83%) were diagnosed at intermediate, advanced, or terminal stages. These later stages were collectively categorized as late-stage HCC, which represented a fivefold increase in comparison with early-stage diagnoses.

The liver disease group, consisting of 10 patients, included individuals aged between 38 and 75 years who were diagnosed with common liver conditions but did not have HCC. The nonliver disease group, on the other hand, comprised 15 individuals aged 38-79 years who were free from any liver-related conditions (Table 1).

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Table 1.
General characteristics of the HCC group.

Characteristics	**Hepatocellular carcinoma patients (n=47)**			
Gender	Male/Female			
	41/6			
Age	< 45		5	14.7 %
	45-59		8	23.5 %
	≥ 60		34	61.8 %
Total			n = 47	100 %
HBV	38			81 %
HCV	5			10.6 %
HBV and HCV co-infection	1			2.1 %
Alcohol-induced Cirrhosis	3			6.3 %
Total	n = 47			100 %
Stage	Early	Early	8	17 %
	Advanced	Intermediate	23	48.9 %
		Advanced	11	23.4 %
		Terminal	5	10.6 %
Total			n = 47	100 %

3.2. Biochemical Markers for Evaluating Liver Function in the Study Groups

The biochemical markers used to assess liver function in the study groups revealed distinct patterns (Table 2). In terms of AST and ALT, the liver disease group without malignancy presented the highest values for both markers, indicating the most severe liver cell damage. The large standard deviation in this group suggests significant variability in the degree of liver injury. Moreover, the AST and ALT values in the HCC group were greater than those in the healthy group, although they were lower than those observed in the liver disease group without malignancy. The nonliver disease group, on the other hand, presented the lowest and most stable levels of AST and ALT, which is consistent with the absence of liver damage.

Table 2.
Biochemical markers evaluating liver function.

	Group of patients with Hepatocellular Carcinoma (n=47)	**Group with liver disease but not malignant (n=10)**	**Group without liver disease (n=15)**	**P-value**
AST (U/L)	73.01 ± 47.09	190.93 ± 319.34	40.92 ± 27.31	< 0.001
ALT (U/L)	59.06 ± 36.97	312.2 ± 760.11	69.86 ± 91.47	< 0.001
Total Bilirubin (µmol/L)	15.94 ± 7.47	92.15 ± 96.31	22.32 ± 20.03	< 0.001
Albumin (g/L)	39.40 ± 4.66	29.88 ± 4.77	39.6 ± 2.5	< 0.001
PT-INR	1.08 ± 0.14	1.25 ± 0.24	0.997 ± 0.092	< 0.001

For total bilirubin, the liver disease group without malignancy also had the highest average value, reflecting clear metabolic disturbances and bile obstruction. The HCC group presented increased bilirubin levels, but these values were still lower than those in the liver disease group without malignancy. In contrast, the nonliver disease group maintained low bilirubin levels within the normal range, indicating healthy liver function.

The lowest level of albumin was detected in the liver disease group without malignancy, which is indicative of impaired liver protein synthesis. The HCC group presented albumin levels comparable to those of the nonliver disease group, suggesting that liver synthetic function was not severely compromised in these patients.

Finally, for PT-INR, the liver disease group without malignancy presented the highest values, reflecting severe coagulopathy. Compared with the healthy group, the HCC group presented a slight increase in the PT-INR, but these levels were still within a manageable range. The nonliver disease group displayed stable PT-INR values, all within the normal limits, indicating no coagulopathy.

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These findings highlight the differences in liver function across the study groups, with the liver disease group without malignancy exhibiting the most severe disturbances, whereas the HCC and nonliver disease groups presented more moderate or stable liver function.

3.3. Optimization of Conditions for Indirect ELISA Immunoassay

To optimize the concentrations of the recombinant antigen and monoclonal antibody, it is essential to provide an adequate amount of monoclonal antibody to detect the antigen, ensuring that neither component is in excess, as this could result in a nonspecific background threshold. The optimal concentration of the monoclonal antibody mAb-TXN was achieved by diluting it 1:128, resulting in a final concentration of 9.10^{-3} mg/mL (Table 3). For the recombinant antigen rAg-TXN, which was used as the positive control, the optimal dilution was 1:8, resulting in a final concentration of 10^{-1} mg/mL (Table 3). The most suitable dilution for the conjugated antibody was 1:15,000, as demonstrated in Figure 1.

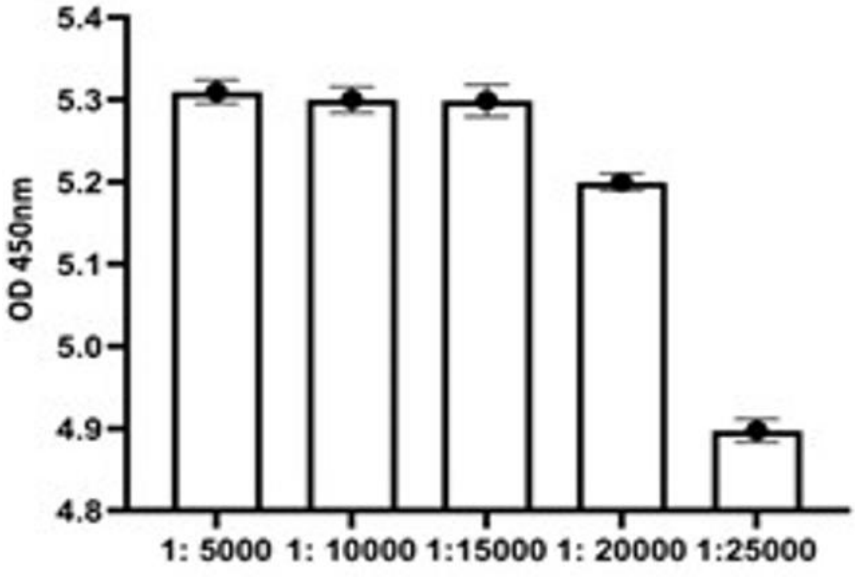

Figure 1.
Optimization of the dilution concentration of the conjugated antibody.

Table 3.
ELISA results for assessing the concentrations of the recombinant antigen and monoclonal antibody.

Dilution ratio		rAg							OD nm
		1:2	**1:4**	**1:8**	**1:16**	**1:32**	**1:64**	**0**	
mAb	1:8	1.275	1.169	1.018	1.143	0.981	0.782	0.124	450
	1:16	1.099	1.198	1.248	1.301	1.200	0.893	0.109	450
	1:32	1.490	1.344	1.198	1.296	1.209	0.955	0.113	450
	1:64	1.508	1.492	1.611	1.451	1.375	1.034	0.120	450
	1:128	1.899	1.961	**1.975**	1.503	1.115	1.012	0.189	450
	1:256	1.602	1.768	0.979	0.997	0.887	1.150	0.022	450
	1:512	1.532	1.806	0.928	0.910	0.825	0.766	0.176	450
	0	0.164	0.182	0.210	0.127	0.110	0.099	0.083	450

3.4. Evaluation of the Sensitivity and Specificity of the Test

The indirect ELISA test using the monoclonal antibody mab-TXN demonstrated a sensitivity of 95.74%, specificity of 84.00%, positive predictive value (PPV) of 91.83%, and negative predictive value (NPV) of 91.30% (Table 4).

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Table 4.
The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were evaluated when the monoclonal antibody TXN was used for predicting HCC.

ELISA TXN	Confirmed Diagnosis of HCC		Total
	(+)	(–)	
(+)	45	4	ELISA (+) = 49
(–)	2	21	ELISA (–) = 23
Total	47	25	72
Se (%)	95.74		
Sp (%)	84.00		
NPV (%)	91.30		
PPV (%)	91.83%		

According to the results of the indirect ELISA test, the optical density (OD) values of the early-stage HCC patient samples were very close to the cutoff threshold. Six patients tested positive for TXN, while two did not show any detectable TXN antigen. Among the samples at high risk of developing HCC, the majority fell below the cutoff threshold, with 4 patients testing positive for TXN and 6 testing negative. In contrast, the 39 late-stage HCC patients presented significantly higher OD values, well above the threshold, with all 39 patients testing positive for the TXN antigen in their serum (Figure 2).

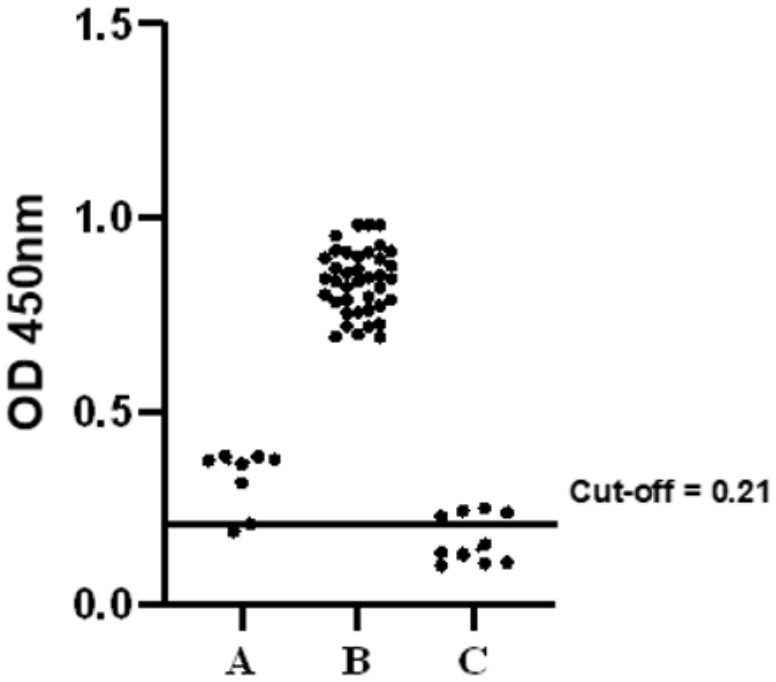

Figure 2.
ELISA results for identifying positive and negative samples in the three study groups and the cutoff value for healthy human serum samples without liver disease. Group A: 8 samples from early-stage HCC patients; Group B: 39 samples from late-stage HCC patients; Group C: 10 samples at high risk of HCC.

4. Discussion

4.1. General Characteristics of the Study Group

Hepatocellular carcinoma primarily affects men and older adults, with the risk increasing over time due to factors such as HBV/HCV infections and cirrhosis. Our study confirms that the incidence of HCC is strongly associated with these demographic and clinical factors, highlighting the importance of addressing these risks in prevention strategies.

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Among the risk factors, HBV infection was the most prevalent in the study population, which underscores the critical role of HBV vaccination and treatment in reducing the risk of HCC. Effective management of HBV could significantly lower the burden of liver cancer, particularly in regions with high prevalence rates of this viral infection.

The majority of patients in this study were diagnosed at an advanced stage of HCC, which limits the effectiveness of treatment and worsens the prognosis. This finding emphasizes the need for earlier detection and monitoring of individuals at high risk, as early intervention could significantly improve treatment outcomes and survival rates.

In terms of liver pathology, the nonmalignant liver disease group presented the most severe liver damage, reflected by elevated AST, ALT, and bilirubin levels, as well as reduced albumin and PT-INR values. These results suggest that nonmalignant conditions such as cirrhosis and severe hepatitis can cause substantial liver dysfunction, which is potentially even more severe than liver cancer at certain stages.

In contrast, the HCC group exhibited moderate liver damage, indicating that although liver function was impaired, the damage was not as severe as that observed in late-stage cirrhosis or other advanced liver diseases. This aligns with the characteristics of liver cancer in its intermediate stage, where liver function may still be somewhat preserved but begins to show signs of significant impairment.

Finally, the group without liver pathology maintained normal liver function indicators, reflecting the absence of any liver damage. These findings highlight the potential for early diagnosis and intervention to prevent the progression of liver diseases, including HCC.

4.2. Optimization of Conditions for Indirect ELISA

Das and White [7] used a sandwich ELISA method with polyclonal goat antibodies that reacted with human thioredoxin and were labeled with digoxigenin. This method was optimized for antibody concentration, blocking solution, plate type, incubation time, and reaction volume [7]. The results revealed that thioredoxin could be detected at a minimum concentration of 15 pg/mL in serum samples [7]. In the study by Lee, et al. [8] using HEHPA nanoparticles, a direct sandwich ELISA method was able to measure TXN-1 in the range from 10 femtomolar (fM) to 100 picomolar (pM), which was significantly more sensitive than the use of only monoclonal antibodies for detection [8]. Therefore, further studies are needed to assess the accuracy of the monoclonal antibody developed to evaluate the detection threshold of TXN.

4.3. Evaluation of the Sensitivity and Specificity of the Test

In this study, the indirect ELISA test using the monoclonal antibody mab-TXN had a sensitivity of 95.74%, specificity of 84.00%, positive predictive value (PPV) of 91.83%, and negative predictive value (NPV) of 91.30%. Currently, TXN is emerging as a promising biomarker for HCC diagnosis, with significantly higher TXN concentrations in the serum of HCC patients than in that of cirrhosis patients and those at high risk for HCC. According to Abdelwahab et al., at a threshold of 20.5 ng/mL, TXN achieved a sensitivity of 78.7% and specificity of 87.8%, outperforming AFP at the same threshold, with an AUC of 0.906 compared with 0.840 [9]. In another study by Lee, et al. [8] which was conducted on 25 HCC patients, the expected optimal threshold for TXN was determined to be 20.5 ng/mL, with an AUC of 0.946 (95% confidence interval: 0.923–0.969). Notably, the percentage of very early-stage HCC patients who tested positive for thioredoxin was significantly greater than that who tested positive for AFP (73.7% vs. 31.6%; $P < 0.0001$) [10]. A further study by Sheta, et al. [11] comparing the sensitivity of TXN and AFP in HCC patients with cirrhosis revealed that at a threshold of 23.28 ng/mL, TXN achieved a sensitivity of 85.4%, specificity of 89.6%, PPV of 89.1%, NPV of 86%, and an AUC of 0.841. In combination with AFP, the sensitivity reached 93.8%, the specificity reached 97.9%, and the AUC reached 0.99, demonstrating superior diagnostic ability to differentiate HCC from simple cirrhosis [11].

In our study, monoclonal antibodies effectively detected TXN antigens in late-stage HCC patient samples, but there was still some ambiguity in early-stage HCC and high-risk cases. Nevertheless, the

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potential of TXN to complement AFP in detecting and distinguishing HCC is well known for assessing disease status. Further studies with larger sample sizes are needed to refine these findings.

5. Conclusion

The results of this study demonstrate that the indirect ELISA test developed using the monoclonal antibody mAb-TXN has significant application value in detecting the TXN antigen in serum.

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Transparency:
The authors state that the manuscript is honest, truthful, and transparent, that no key aspects of the investigation have been omitted, and that any differences from the study as planned have been clarified. This study followed all writing ethics.

Competing Interests:
The authors declare that they have no competing interests.

Authors' Contributions:
All the authors contributed equally to the conception and design of the study. All the authors have read and agreed to the published version of the manuscript.

References

[1] D. Kim, "Changing etiology and epidemiology of hepatocellular carcinoma: Asia and worldwide," *Journal of Liver Cancer*, vol. 24, no. 1, pp. 62-70, 2024. https://doi.org/10.17998/jlc.2024.03.13

[2] J. H. Oh and D. W. Jun, "The latest global burden of liver cancer: A past and present threat," *Clinical and Molecular Hepatology*, vol. 29, no. 2, p. 355, 2023. https://doi.org/10.3350/cmh.2023.0070

[3] E. Chartampilas, V. Rafailidis, V. Georgopoulou, G. Kalarakis, A. Hatzidakis, and P. Prassopoulos, "Current imaging diagnosis of hepatocellular carcinoma," *Cancers*, vol. 14, no. 16, p. 3997, 2022. https://doi.org/10.3390/cancers14163997

[4] K. G. Núñez *et al.*, "Baseline alpha-fetoprotein, alpha-fetoprotein-l3, and des-gamma-carboxy prothrombin biomarker status in bridge to liver transplant outcomes for hepatocellular carcinoma," *Cancers*, vol. 13, no. 19, p. 4765, 2021. https://doi.org/10.3390/cancers13194765

[5] N. D. Parikh, A. S. Mehta, A. G. Singal, T. Block, J. A. Marrero, and A. S. Lok, "Biomarkers for the early detection of hepatocellular carcinoma," *Cancer Epidemiology, Biomarkers & Prevention*, vol. 29, no. 12, pp. 2495-2503, 2020. https://doi.org/10.69622/26763760

[6] F. Lardeux, G. Torrico, and C. Aliaga, "Calculation of the ELISA's cut-off based on the change-point analysis method for detection of Trypanosoma cruzi infection in Bolivian dogs in the absence of controls," *Memórias do Instituto Oswaldo Cruz*, vol. 111, no. 08, pp. 501-504, 2016. https://doi.org/10.1590/0074-02760160119

[7] K. C. Das and C. W. White, "Detection of thioredoxin in human serum and biological samples using a sensitive sandwich ELISA with digoxigenin-labeled antibody," *Journal of Immunological Methods*, vol. 211, no. 1-2, pp. 9-20, 1998. https://doi.org/10.1016/s0022-1759(97)00163-4

[8] M.-J. Lee, E.-S. Lee, T.-H. Kim, J.-W. Jeon, Y. Kim, and B.-K. Oh, "Detection of thioredoxin-1 using ultra-sensitive ELISA with enzyme-encapsulated human serum albumin nanoparticle," *Nano Convergence*, vol. 6, pp. 1-7, 2019. https://doi.org/10.1186/s40580-019-0210-5

[9] K. M. Abdelwahab, W. A. Ibrahim, S. A. B. Saleh, A. A. A. Elbarky, and G. A. Mohamed, "Evaluation of serum thioredoxin as a hepatocellular carcinoma diagnostic marker," *Egyptian Liver Journal*, vol. 14, no. 1, p. 3, 2024. https://doi.org/10.1186/s43066-024-00309-8

[10] J. Li *et al.*, "Serum thioredoxin is a diagnostic marker for hepatocellular carcinoma," *Oncotarget*, vol. 6, no. 11, p. 9551, 2015. https://doi.org/10.18632/oncotarget.3314

[11] T. Sheta, M. Selim, M. Sabry, and A. Saed, "Serum thioredoxin as a diagnostic marker for hepatocellular carcinoma in cirrhotic hepatitis C patients," *Medical Journal of Viral Hepatitis*, vol. 5, no. 2, pp. 9-15, 2021. https://doi.org/10.21608/mjvh.2021.158271

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