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ORIGINAL RESEARCH ARTICLE

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Evaluation of serum thioredoxin as a hepatocellular carcinoma diagnostic marker

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Abstract

Background Hepatocellular carcinoma (HCC) is one of the most prevalent and fatal malignancies worldwide. Following an increase in reactive oxygen species (ROS), cancer cells enter an oxidative stress state. As a result, these cells experience an increase in antioxidant activity to counteract oxidative stress. The thioredoxin (TRX) system is a ubiquitous mammalian antioxidant system that neutralizes ROS and maintains intracellular reduction oxidation (redox) balance, which is essential for HCC growth. However, the role of TRX protein in HCC remains largely unknown. Hence, we aimed to assess the diagnostic utility of serum TRX in patients with HCC. A total of 50 patients were consecutively recruited in this observational study. They were classified into three groups: an HCC group (25 patients), a cirrhosis group (15 patients with liver cirrhosis on top of chronic HCV infection), and a control group (10 healthy individuals). Serum TRX levels were measured using ELISA.

Results Higher serum TRX levels were detected in the HCC group than in the cirrhosis and control groups (140.96 ± 12.70 *vs* 88.33 ± 10.34 *vs* 73.10 ± 13.22 ng/mL, respectively; $P < 0.001$). TRX was independently associated with the presence of HCC ($P < 0.001$). Regarding the detection of HCC, TRX at a cut-off value of 114 ng/mL had superior diagnostic performance to AFP with an AUC of 1.000, sensitivity of 100%, and specificity of 100%, whereas AFP at a cut-off value of 20.5 ng/mL had an AUC of 1.000, sensitivity of 100%, and specificity of 47%.

Conclusion Thioredoxin has the potential to be an HCC diagnostic marker. The clinical significance of thioredoxin in HCC requires further investigation.

Keywords Hepatocellular carcinoma, Thioredoxin, Redox signalling, Marker

Background

Hepatocellular carcinoma (HCC) is the fifth most common type of malignancy worldwide and the third most common cause of cancer-related mortality [1]. Metabolic reprogramming is currently recognized as a hallmark of cancer [2]. Therefore, elucidating the molecular pathogenesis of HCC is critical for identifying potential targets for diagnosing and treating HCC [3].

HCC is a complicated tumour influenced by numerous variables [4]. A common characteristic of hepatocarcinogenesis is that chronic hepatic inflammation, regardless of its aetiology, results in dysregulation in the hepatic reduction–oxidation (redox) homeostasis, causing oxidative stress, which promotes hepatocarcinogenesis by inducing DNA mutations and genetic instability [5]. However, an overabundance of reactive oxygen species (ROS) is harmful because it damages cellular components such as DNA, lipids, and proteins, resulting in cell cycle arrest and apoptosis [6]. Therefore, to lower ROS levels to a favourable range for tumour progression, cancer cells must actively upregulate several antioxidant mechanisms [7].

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The thioredoxin (TRX) system is one of the essential redox control systems. It consists of the small redox protein TRX, nicotinamide adenine dinucleotide phosphate in its reduced form (NADPH), and thioredoxin reductase (TRXR), a large homodimeric selenzoenzyme controlling the redox state of TRX [8]. This pathway begins with electron donation from NADPH. TRXR transfers the electron to TRX, which then transfers it for ROS scavenging [9].

Accumulating evidence shows that TRX is an essential modulator in HCC development [10]. Its upregulation stimulates hypoxia-inducible factor-1α, which increases the expression of vascular endothelial growth factor-A, promoting angiogenesis and tumour cell proliferation [11]. Additionally, positive correlations were found between TRX mRNA expression and the upregulation of the tumour-promoting genes, specifically mTORC1, E2F targets, and Myc targets [12].

Furthermore, TRX/TRXR overexpression has been reported in HCC and closely correlated with aggressive tumour phenotype, metastasis, poor patient survival, and resistance to chemotherapy [12–17]. Nevertheless, the expression of the thioredoxin-interacting protein (TRXIP), an endogenous inhibitor of TRX, was downregulated [12, 18].

In addition, the blockade of the TRX/TRXR system results in intracellular ROS accumulation, which promotes HCC cell apoptosis [19–21]. Additionally, sorafenib, a kinase inhibitor drug approved for the treatment of HCC, upregulates TRXIP while downregulating the TRX/TRXR pathway. In addition, in SNU475 cells treated with sorafenib, TRX downregulation has a notable synergistic pro-apoptotic effect on proteome rearrangement [15, 22]. Hence, the current study aims to assess the diagnostic utility of serum TRX in patients with HCC.

Methods

In this observational study, 50 patients were consecutively recruited from the Internal Medicine and Hepatology inpatient wards and outpatient clinics at Ain Shams University Hospitals from June 2021 to February 2022. The patients were classified into three groups: an HCC group (25 patients), a cirrhosis group (15 patients with liver cirrhosis on top of chronic HCV infection), and a control group (10 healthy individuals).

Patients with cirrhosis due to causes other than chronic HCV infection were excluded. Additionally, individuals with medical conditions which could alter serum TRX levels including diabetes, previous/concomitant neoplasm, chronic kidney disease, inflammatory conditions, severe burn injuries, and cardiovascular diseases, were excluded [23–26].

Diagnosis of HCC and cirrhosis

Clinical signs, laboratory parameters, and/or histological criteria were used to diagnose cirrhosis [27]. According to the practice guidelines, HCC was identified by contrast-enhanced imaging and/or histological criteria [28].

Serum human TRX measurement

Serum TRX level was measured using ELISA according to the manufacturer's instructions (Immuno-Biological Laboratories Co., Ltd, Gunma, Japan). The measurement range was 3.91–250 ng/mL and sensitivity was 0.43 ng/mL. The coefficients of variation for the intra- and interassays were 7.2–10% and 6.0–9.1%.

Alpha-fetoprotein (AFP) measurement

The AFP was measured using ELISA (Monobind Inc., Lake Forest, CA 92630, USA) with a sensitivity of 0.01 ng/mL.

The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki and its appendices and was approved by the ethics committee of the Faculty of Medicine, Ain Shams University (FMASU MSO 38/2021/2022-FWA 000017585). Written informed consent was obtained from all participants.

Statistical analysis

The data were analyzed using IBM SPSS Statistics for Windows (version 20.0; IBM Corp., Armonk, NY, USA). They were then presented as mean ± standard deviation (SD) for quantitative data and frequency and distribution for qualitative data.

Statistical significance was set at $P < 0.05$ in the statistical comparison between the different groups. The significance of difference was tested using one of the following:

1- Student's *t*-test: to compare the means of two groups of quantitative data
2- ANOVA and Tukey's post hoc test: to compare the means of more than two sets of quantitative data
3- Chi-square test and Fisher's exact test: to compare categorical data between groups
4- Pearson's correlation coefficient: to determine the relationships between variables
5- Receiver operating characteristic (ROC) curve with the estimation of Youden's index: to assess the diagnostic performance of TRX and AFP

Results

The current study included 25 patients with HCC, 15 patients with cirrhosis, and 10 healthy controls. They were 42 (84%) males and eight (16%) females with a mean age of 44.18 ± 10.1 years. Regarding age and sex, insignificant differences were observed between the groups

($P \geq 0.05$). In HCC group, the mean tumour foci size was 6.1 ± 2.5 cm. Multifocal HCC and portal vein thrombosis were detected in 19 (76%) and 3 (12%) patients, respectively. In cirrhosis group, 3 (20%), 4 (26.6%), and 8 (53.3%) patients were classified into Child–Pugh class A, B, and C, respectively. Study participant characteristics are shown in Table 1.

Significant differences were observed between the groups, with TRX and AFP being highest in the HCC group (Table 1 and Fig. 1). There was no difference in serum TRX levels between patients with solitary and multifocal HCC (139.947 ± 12.747 *vs* 144.167 ± 13.152 ng/mL, respectively, $P = 0.4898$). In addition, in the cirrhosis group, serum TRX levels were significantly higher in Child–Pugh class B and C patients as compared to class A patients (81.33 ± 15.17 *vs* 97 ± 6.27 *vs* 97 ± 5.80 ng/mL, $P = 0.03$). Both TRX and AFP were independently correlated with the presence of HCC (Table 2).

A significant negative correlation was observed between TRX and aspartate aminotransferase (AST) among the HCC group and between TRX and alanine

Table 1 Comparison of the laboratory test results between the HCC, cirrhosis, and control groups

Parameter	HCC group	Cirrhosis group	Control group	*P* value	Post-hoc analysis
Hemoglobin (g/dl)	10.08 ± 1.51	9.97 ± 1.04	11.9 ± 0.88	< 0.001	P1 = 0.799
					P2 = 0.002
					P3 = 0.042
White blood cells (10^9/L)	6.94 ± 2.72	5.73 ± 2.07	6.29 ± 0.48	0.096	
Platelets (10^9/L)	102.44 ± 39.97	115.33 ± 33.69	271.2 ± 22.75	< 0.001	P1 = 0.303
					P2 < 0.001
					P3 = 0.020
Alanine aminotransferase (IU/L)	76.0 ± 59.36	51.73 ± 23.37	22.1 ± 3.41	0.007	P1 = 0.140
					P2 = 0.034
					P3 = 0.025
Aspartate aminotransferase (IU/L)	79.84 ± 44.96	88.6 ± 52.56	21.5 ± 3.92	< 0.001	P1 = 0.579
					P2 = 0.042
					P3 = 0.027
Alkaline phosphatase (IU/L)	231.6 ± 127.36	264.73 ± 131.14	89.8 ± 17.66	0.002	P1 = 0.436
					P2 = 0.024
					P3 = 0.012
Gamma Glutamyl transferase (IU/L)	296.04 ± 159.8	79.07 ± 60.29	32.8 ± 9.16	< 0.001	P1 < 0.001
					P2 = 0.027
					P3 = 0.012
Total bilirubin (mg/dL)	2.85 ± 1.86	1.94 ± 0.89	0.8 ± 0.27	< 0.001	P1 = 0.085
					P2 = 0.032
					P3 = 0.026
Direct bilirubin (mg/dL)	1.38 ± 1.08	0.85 ± 0.58	0.16 ± 0.11	< 0.001	P1 = 0.087
					P2 = 0.021
					P3 = 0.035
Serum albumin (g/dL)	2.78 ± 0.75	3.1 ± 0.61	4.4 ± 0.64	< 0.001	P1 = 0.172
					P2 < 0.001
					P3 = 0.053
Prothrombin time (sec)	17.76 ± 5.84	17.03 ± 2.99	13.9 ± 1.85	0.085	
Alpha fetoprotein (ng/mL)	234.32 ± 108.06	24.20 ± 10.54	6.35 ± 1.24	< 0.001	P1 < 0.001
					P2 < 0.001
					P3 > 0.050
Thioredoxin (ng/mL)	140.96 ± 12.70	88.33 ± 10.34	73.10 ± 13.22	< 0.001	P1 < 0.001
					P2 < 0.001
					P3 < 0.050

Data are presented in mean ± SD. P1: HCC group vs cirrhosis group; P2: HCC group vs control group; P3: cirrhosis group vs control group

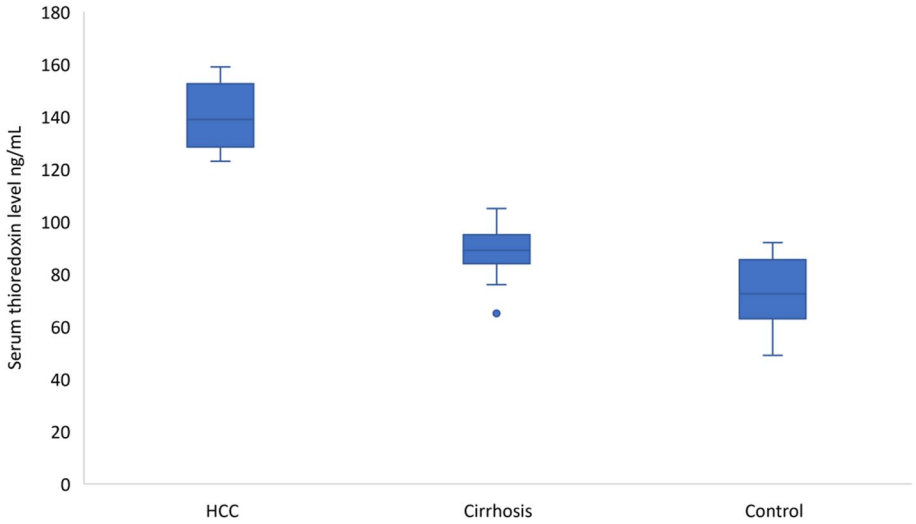

Fig. 1 Serum thioredoxin levels in all groups

Table 2 Candidate blood markers independently associated with the existence of HCC

Marker	Standard Error	Beta	t	*P* value	95% Confidence Interval	
					Lower Bound	Upper Bound
Alpha-fetoprotein	0.000	0.343	5.105	≤ 0.001	0.001	0.002
Thioredoxin	0.001	0.670	9.972	≤ 0.001	0.008	0.013

Table 3 Correlation between thioredoxin and other variables among HCC and cirrhosis groups

Parameter	Thioredoxin			
	HCC group		Cirrhosis group	
	r	*P* value	*r*	*P* value
Hemoglobin	0.128	0.541	-0.204	0.465
White blood cells	-0.101	0.632	-0.042	0.880
Platelets	-0.134	0.522	0.317	0.249
Alanine aminotransferase	-0.137	0.513	-0.673	0.006
Aspartate aminotransferase	-0.511	0.009	-0.074	0.794
Alkaline phosphatase	-0.002	0.990	0.162	0.563
Gamma Glutamyl transferase	0.205	0.326	-0.261	0.347
Total bilirubin	-0.217	0.298	-0.472	0.076
Direct bilirubin	-0.094	0.656	-0.458	0.086
Albumin	0.075	0.723	0.154	0.583
Prothrombin time	-0.116	0.582	0.303	0.273
Alpha fetoprotein	0.109	0.603	0.178	0.526
Age	-0.006	0.975	0.497	0.063

aminotransferase (ALT) among the cirrhosis group (Table 3).

To assess the diagnostic performance of serum TRX and AFP in identifying patients with HCC from those with liver cirrhosis, a ROC curve was plotted. TRX had an AUC of 1.000, sensitivity of 100%, and specificity of 100% at a cut-off value of 114 ng/mL, whereas AFP had an AUC of 1.000, sensitivity of 100%, and specificity of 47% at a cut-off value of 20.5 ng/mL (Table 4 and Fig. 2).

Discussion

Despite significant advancements in detecting and treating HCC, the majority of patients were diagnosed with the disease at advanced stages [29]. The most frequently utilized blood marker for diagnosing HCC to date is AFP [30]. The sensitivity of AFP ranges from 60 to 80% at a cut-off serum value of 20 ng/mL [31]. However, unless other diagnostic methods are used, up to 40% of advanced HCC and at least one-third of small HCC may go undetected [32]. Additionally, a significant increase in serum AFP levels (20–200 ng/mL) was detected in a large number of patients with chronic hepatitis and cirrhosis [33]. In an earlier study [34], AFP concentrations were increased in 11–58% of patients with cirrhosis and chronic hepatitis. Similarly,

Table 4 ROC curve analysis for the detection of HCC

Marker	Cut-off	AUC	Sensitivity	Specificity	*P* Value	Confidence Interval 95%	
						Lower Bound	Upper Bound
AFP	20.5 ng/mL	1.000	100%	47%	< 0.001	1.000	1.000
Thioredoxin	114 ng/mL	1.000	100%	100%	< 0.001	1.000	1.000

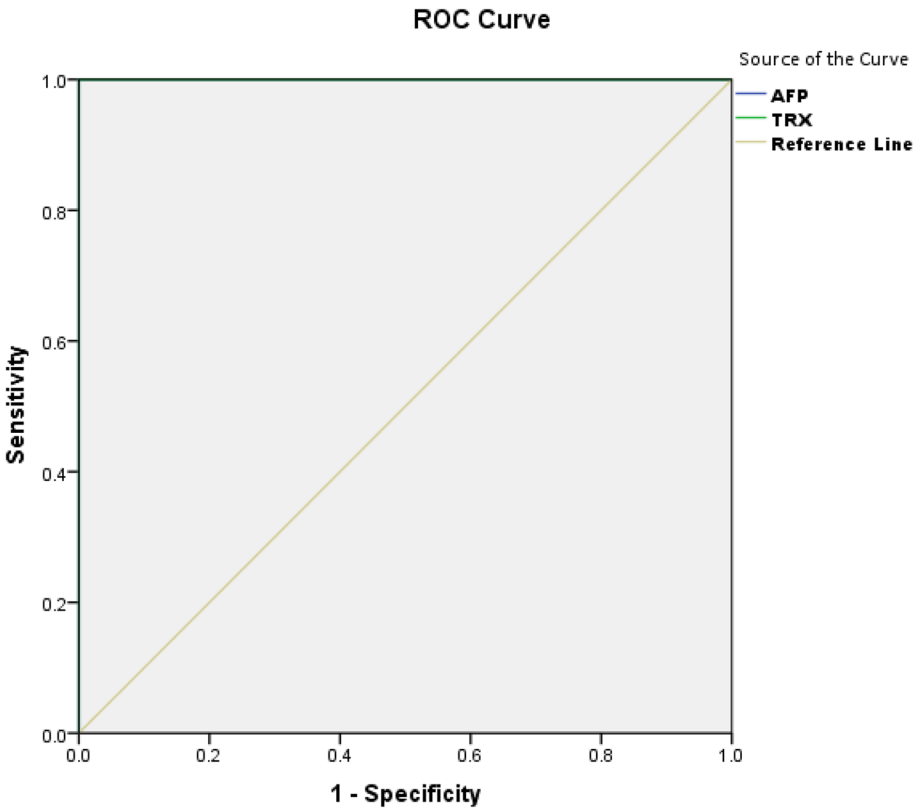

Fig. 2 ROC curve analysis for the diagnosis of hepatocellular carcinoma

in the current study, 53% of patients with cirrhosis had an AFP > 20 ng/mL. This necessitated the development of a reliable biomarker to diagnose HCC.

In the present study, higher serum TRX levels were detected in the HCC group than in the cirrhosis and control groups. In agreement with the results, Li et al. [34] reported that TRX could be a diagnostic marker of HCC, with significantly higher serum TRX levels in patients with HCC than those in patients with liver cirrhosis, patients with chronic liver diseases, and healthy subjects (45.1 [28.2–56] *vs* 9 [6.1–11.9] *vs* 8.1 [5–10.2] *vs* 7.5 [6–9.2] ng/mL, respectively; $P < 0.0001$). In addition, although serum AFP levels were increased in the HCC group, as expected, significant increases were also observed in patients with liver cirrhosis and chronic liver diseases compared to the control group (142 [18–548] *vs* 15.4 [8.7–30.2] *vs* 13.6 [6.8–24.4] *vs* 6.6 [4.0–9.2] ng/mL, respectively; $P < 0.0001$).

Similar to the current findings, a previous study has reported significantly higher TRX levels in the HCC group than in the liver cirrhosis group (129.5 [112–135] *vs* 84.5 [37–126] ng/mL, respectively; $P < 0.001$). Additionally, the authors have found that the increase in serum AFP and TRX levels was significantly correlated to the presence of HCC ($P < 0.05$) [35].

In agreement with the current study, no influence of age, sex, ALT, AST, total bilirubin, prothrombin time, and AFP was detected on serum TRX levels in patients with HCC ($P > 0.05$) [34].

Our findings indicate that serum TRX complements AFP measurement in the detection of HCC. Similar to the present study, Li et al. [34] reported that TRX was superior to AFP in diagnosing HCC ($P < 0.001$). TRX at

a cut-off value of 20.5 ng/mL differentiated HCC from chronic liver diseases and cirrhosis with an AUC of 0.906, 95% CI = 0.870–0.925, sensitivity of 78.7%, and specificity of 87.8%, whereas AFP at a cut-off value of 20 ng/mL had an AUC of 0.840, 95% CI = 0.820–0.884, sensitivity of 74%, and specificity of 79.1%. In another study investigating the diagnosis of HCC [35], AFP at a cut-off value of 400 U/L only had an AUC of 0.69, 95% CI = 0.59–0.77, sensitivity of 29%, and specificity of 100% ($P < 0.0001$), whereas TRX at a cut-off value of 120 ng/mL had an AUC of 0.79, 95% CI = 0.69–0.89, sensitivity of 74%, and specificity of 71% ($P < 0.0001$). However, the diagnostic performance of TRX was better in the current study than in previous reports. This discrepancy in results may be attributed to the use of different cut-off values.

The current study is limited by the small sample size and lack of TRX assessment in histologic specimens in correlation with serum levels. Whether serum TRX levels reflect similar changes in the hepatic tissue remains uncertain. The relationship between serum and tissue TRX levels warrants further investigation. Additional research with a larger sample size is needed to validate TRX diagnostic value and determine the optimum cut-off value.

Conclusions

Thioredoxin has the potential to be a diagnostic marker of HCC. The clinical significance of thioredoxin in HCC remains to be comprehensively examined.

Abbreviations

AFP	Alpha fetoprotein
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
HCC	Hepatocellular carcinoma
NADPH	Nicotinamide adenine dinucleotide phosphate
ROS	Reactive oxygen species
TRX	Thioredoxin
TRXIP	Thioredoxin-interacting protein
TRXR	Thioredoxin reductase

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Not applicable.

Authors' contributions

KA, WI, SS designed the study; AE participated in the acquisition of data; KA, WI, SS, AE, GM participated in the analysis and interpretation of the data; KA, WI, SS, GM revised the article critically for important intellectual content; GM wrote the manuscript. All authors have read and approved the manuscript.

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Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki and its appendices and was approved by the ethics committee of the Faculty of Medicine, Ain Shams University (FMASU MSO 38/2021/2022-FWA 000017585). Informed written consent was obtained from each participant before enrollment in the study.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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