Original Research Article

Serum Thioredoxin Level for Assessment of Response after Ablation Therapy for Hepatocellular Carcinoma in Egyptian Patients

Abstract:

Background: Hepatocellular Carcinoma(HCC) is a major health problem worldwide and its diagnosis is still challenging. Thioredoxin (TRX) is a class of small redoxproteinsknown to be present in all organisms. It plays a role in many important biological processes, including redox signaling.

Aims: The main aim of this study was to study the serum thioredoxin level for assessment of response after ablation therapy for hepatocellular carcinoma in Egyptian patients.

Patients and Methods: This prospective case study was carried out in Department of Tropical Medicine and Infectious Diseases, Tanta University Hospital. The duration of study was from April 2019 till April 2020.

Results: Significant differences were found between the four groups regarding TRX as group B and C showed significant elevation in TRX level compared to group A & D.TRX was significantly decreased after 6 months compared to 3 months and preoperative. TRX at cutoff 100 ng/ml candifferentiate between early HCC and liver cirrhosis with the sensitivity, specificity, PPV and NPV was 95%, 85%, 86% and 94% respectively. There were positive significant correlations between TRX andfocal lesion size. There were also positive significant correlations between TRX and aspartate transaminase (AST), Total bilirubin, direct bilirubin and international normalization ratio (INR). In group C after therapeutic intervention, there was a significant decrease in TRX level among patients showing complete response compared to those with partial response or progressive course.

Conclusions: TRX could serve as a potential diagnostic biomarker for HCC. TRX could be used as a predictive marker of therapeutic ablation outcome in hepatocellular carcinoma patients.

Keyword: Serum Thioredoxin, Hepatocellular Carcinoma

Introduction:

"Hepatocellular carcinoma (HCC) is the most common type of primary liver cancer in adults and is the most common cause of death in people with cirrhosis"[(1)].

"It occurs in the setting of chronic liver inflammation and is most closely linked to chronic viral hepatitis infection (hepatitis B or C) or exposure to toxins such as alcohol or aflatoxin. Certain diseases, such as hemochromatosis and alpha 1-antitrypsin deficiency, markedly increase the risk of developing HCC"[(2)].

"As with any cancer, the treatment and prognosis of HCC vary depending on the specifics of tumor histology, size, how far the cancer has spread, and overall health"[(2)].

"Although hepatic resection is still the first line treatment for early-stage HCC patients with well-conserved liver function, thermal ablative therapies have emerged as a well-accepted alternative during recent decades"[(3)].

"Among various thermal ablative techniques, radiofrequency ablation (RFA) is the most commonly used one and has emerged as a curative treatment for early-stage HCC beyond hepatic resection and liver transplantation"[(4)].

"Microwave ablation (MWA), another thermal ablative technique destroys tumors by direct hyperthermia injury similar to RFA"[5].
"The recurrence rate of HCC after curative resection is still high due to lack of an effective method for timely diagnosis"[6].
"Alpha-fetoprotein (AFP) has been widely used as a reference biomarker to diagnosis of HCC. However, normal physiological levels of AFP are observed in approximately one third of HCC cases. Furthermore, a number of HCC positive patients have AFP levels less than the threshold value of 400 ng/mL. In addition, serum level of AFP increases in chronic liver diseases including chronic hepatitis and cirrhosis. These factors make an AFP-based diagnosis of HCC far from reliable"[7].
"The thioredoxin system comprises the small redox protein thioredoxin (TRX), nicotinamide adenine dinucleotide phosphate, in its reduced form (NADPH), and thioredoxin reductase (ThioredoxinR), a large homodimeric selenzoenzyme controlling the redox state of thioredoxin is an important antioxidant system"[8].
"There are two isoforms of Trx, the mainly cytosolic Trx1, which can be translocated into the nucleus and secreted out of the cell under certain circumstances, and Trx2, which is the mitochondrial isoform"[9].
"TRX performs many biological functions, including reactive oxygen species (ROS) scavenging, thus exerting protective effects against oxidative stress; moreover, serum and plasma levels of TRX are good indicators of oxidative stress and are important biomarkers of different diseases"[10].
"Plasma levels of TRX in normal individuals vary between (10 and 80 ng/mL). This level elevated in certain human diseases including HIV infection and cancer as hepatocellular carcinoma, pancreatic, lung, gastric, colorectal cancer, Adult T cell Leukemia, myeloma and non-Hodgkin lymphoma"[11].

Patients and Methods:

This prospective case study was carried out in Department of Tropical Medicine and Infectious Diseases, Tanta University Hospital.
The study was performed on 80 subjects from the outpatient clinic and inpatient of Tropical Medicine and Infectious Diseases Department at Tanta University Hospitalsfrom April 2019 till April 2020.

The subjects were classified into 4 groups:

1. Group A: Included 20 cirrhotic patients without HCC.
2. Group B: Included 20 HCC patients without treatment.
3. Group C: Included 20 HCC patients treated with ablation therapy (radiofrequency- microwave) and follow up after 3 and 6 months.
4. Group D: control group included 20 cases.

Inclusion criteria:

- Adult cirrhotic patients.
- Patients with Hepatocellular carcinoma (HCC) fit for ablation therapy.

Exclusion criteria:

- Primary hepatic tumor with metastasis.
- Secondary metastatic hepatic tumor.
- Chronic kidney disease.
- Coronary disease.
- Diabetes.
- Sever burn injury.
- Other malignancies.

- Patient unfit for ablation therapy.

Methods:

The patients will subject to the following:

1. Full history taking.
2. Clinical examination (general and local).
3. Investigations:

a- Laboratory:

- Liver function tests including:(Alanine Transaminase(ALT),Aspartate Aminotransferase (AST), serum albumin,total and direct bilirubin, prothrombin time and activity,INR, alkaline phosphatase (ALP).
- Viral marker including: (HCV Ab, HBs Ag, HBc Ab).
- Complete blood picture (RBC, WBC, platelet count)
- ESR.
- Fasting blood glucose level.
- Blood urea and serum creatinine.
- Serum alfa fetoprotein (AFP).
- Serum thioredoxin (TXN) by ELISA.

b-Radiological:

- Abdominal ultrasonography.
- Triphasic CT liver.

Statistical Analysis

Statistical presentation and analysis of the present study was conducted, using the mean, standard deviation, student t- test, Chi-square, Linear Correlation Coefficient and Analysis of variance [ANOVA] tests by SPSS V17.

Results:

This table shows comparison between the studied groups regarding TRX. As group B and C showed significant elevation in TRX level compared to group A & D (P=0.001) (table 1).

Table 1: Comparison between the studied groups regarding TRX

TRX (ng/ml)	Group A	Group B	Group C	Group D
Range	41.7 –102.3	102.9 – 393.09	94 – 133.2	7.6 – 15.1
Mean±SD	68.53 ± 11.85	178.42 ± 80.43	105.80 ± 8.54	11.14 ± 2.77
F. test	57.819			
p. value	0.001*			

**P value< 0.05 is significant, P value< 0.01 is highly significant SD: Standard deviation,*

There were positive significant correlations between TRX and FL size (r = 0.509, P=0.001), AST (r=0.279, P=0.031), total bilirubin (r=0.031, p=0.001), Direct bilirubin (r=0.372, P=0.003) and INR (r=0.003, P=0.001). There were insignificant correlations between TRX and each of AFP, Hb, TLC, platelet, RBCs, prothrombin activity, serum creatinine, serum albumin, Serum Urea, ESR (P > 0.05) (table 2).

Table 2: Correlation between TRX and the studied parameters

	TRX	
	r	p
AFP	0.001	0.993
ALT(U/L)	0.142	0.280
AST(U/L)	0.279	0.031*

Total bilirubin (mg/dl)	0.449	0.001*
Direct bilirubin (mg/dl)	0.372	0.003*
S. Albumin(g/dl)	0.054	0.683
Hb (gm/dl)	0.219	0.093
RBCs×(10^6/cmm)	0.035	0.790
TLC/(cmm)	0.174	0.184
Platelets× (10^3/cmm)	0.050	0.706
INR	0.435	0.001*
Prothrombin Activity%	0.093	0.460
S. Cr. (mg/dl)	0.093	0.479
S. Urea(mg/dl)	0.220	0.091
ESR 1(mm)	0.150	0.253
ESR 2(mm)	0.079	0.547
FL SIZE (cm)	0.509	0.001*

AFP: alfafetoprotein TLC: total leucocytic count, Hb: hemoglobin, ALT: Alanine aminotransferase, AST: aspartate aminotransferase, INR: international randomized ratio, ESR: erythrocyte sedimentation rate

This table shows that TRX was significantly decreased after 6 months compared to at 3 months and preoperative (P=0.001) (table 3).

Table 3: Follow up as regard TRX after 3, 6 months in group C

TRX ng/ml	Pre	After 3m	After 6m
Range	94 – 133.2	61.7 – 135.6	61.6 – 101
Mean ± S. D	105.80 ± 8.54	90.15 ± 19.30	77.78 ± 10.54
F. test	21.252		
p. value	0.001*		

**P value< 0.05 is significant, P value< 0.01 is highly significant SD: Standard deviation, X2: Chi-Square Test*

Receiver operating characteristic (ROC) analysis was performed to determine diagnostic value of TRX. TRX at cutoff 100 ng/ml can differentiate between group C and group A with the sensitivity, specificity, PPV and NPV was 95%, 85%, 86% and 94% respectively (table 4).

Table 4: Diagnostic performance of TRX for early HCC detection

	Cutoff	AUC	Sensitivity	Specificity	PPV	NPV	Accuracy
TRX ng/ml	100	0.978	95	85	86	94	90

PPV= Positive Predictive Value, NPV= Negative Predictive Value, AUC= Area Under Curve

Discussion and Conclusions:

"Hepatocellular carcinoma (HCC) is one of the most frequent malignant tumors and is the most common type of primary liver cancer in adults and is the most common cause of death in people with cirrhosis. The 5-year survival rate of all HCC is < 5%, placing it among the cancers with the worst prognosis"[(12)].

The present study showed that as regardcomparison between the studied groups regarding TRX. Group B and C showed significant elevation in TRX level compared to group A & D (P=0.001).

Our results were supported by **Li et al., 2015**[(13)] as they revealed that "the median levels of serum thioredoxin was 45.1 (IQR, 28.2–56.0) ng/ml, which was significantly higher than that of healthy subjects, patients with cirrhosis and chronic liver diseases

(P < 0.0001). Similarly, thioredoxin was also significantly higher as compared to controls in validation cohort".

Also, **Mollbrink et al., 2014**[14] showed that "Trx1 and Trx2 were upregulated in HCCs as compared to the respective surrounding liver". **Eun et al., 2019**[15] reported that HCC patients were shown to have the enhanced expression of TRX compared with that of controls.

The present study showed that there were positive significant correlations between TRX and FL size (r = 0.509, P=0.001). There were also positive significant correlations between TRX andAST (r=0.279, P=0.031), total bilirubin (r=0.031, P=0.001), Direct bilirubin (r=0.372, P=0.003) and INR (r=0.003, P=0.001).

There were insignificant correlations between TRX and each of AFP, platelet, Hb, RBCs, TLC, prothrombin activity, serum creatinine, serum albumin, Serum Urea, ESR (P > 0.05).

Li et al.,2015[13] showed that There was a significant correlation between thioredoxin concentrations and tumor size (r = 0.311, P < 0.0001), and there was no influence of, ALT, AST, total bilirubin, prothrombin time and AFP on thioredoxin in HCC patients (P > 0.05, respectively).

TRX was significantly decreased after 6 months compared to at 3 months and preoperative (p=0.001).

Our results showed that TRX at cutoff 100 ng/ml can differentiate between early HCC and liver cirrhosis with the sensitivity, specificity, PPV and NPV was 95%, 85%, 86% and 94% respectively. While AFP can detect patients at cutoff 30 ng/ml with the sensitivity, specificity, PPV and NPV was 75%, 70%, 71% and 74% respectively.

Our results were supported by study of **Li et al.,2015**[13] as they reported that "a ROC curve was plotted to define the optimal cut-off values, and to identify the sensitivity and specificity of serum thioredoxin and AFP levels in differentiating patients with HCC versus all other conditions. Based on the ROC curve, the optimal cutoff value of serum thioredoxin levels as an indicator for auxiliary diagnosis of HCC was projected to be 20.5 ng/mL, which yielded a sensitivity of 84.3% and a specificity of 91.8%, with the area under the curve at 0.946 (95% CI, 0.923–0.969)". The optimum cutoff value for AFP was 18.5 ng/mL (AUC 0.878, 95% CI: 0.841–0.914, sensitivity 78.4%, specificity of 81.3%). As the sensitivity and specificity were similar to those for the recommended clinical cutoff of 20 ng/mL (80.1% and 85.9%, respectively; P = 0.231), they chose 20 ng/mL as the cutoff value for AFP in this study. Thioredoxin had a better AUROC compared with AFP (P < 0.001), indicating both a higher sensitivity and specificity of thioredoxin compared with AFP in the diagnosis of HCC. When HCC patients were compared with CLD and LC patients, the AUC for thioredoxin was also larger than that for AFP (0.901, 0.875–0.923 vs. 0.842, 0.821–0.889, P = 0.002). Similarly, when HCC patients were compared with LC patients, the AUC for thioredoxin was also larger than that for AFP (0.874, 0.843–0.901 vs. 0.824, 0.801–0.853, P = 0.004).

Also, **Sheta, 2021**[16] revealed that "the ROC curve analysis demonstrated that TRX at a cut-off value of 198.19 (IU/ml) has 85.4% sensitivity and 89.6% specificity for differentiating HCC cases from cirrhotic cases with 89.1% PPV, 86% NPV, and AUC equal to 0.841".

Moreover, the previous study held by **Omran et al., 2020**[17] found that thioredoxin at (cut-off value 120 ng/ml and AUC 0.79) showed higher sensitivity (74% vs 29%) and accuracy (73% vs 53%) and lower specificity (71% vs 100%) when compared to AFP at (cutoff value 400U/L and AUC 0.69) for differentiating HCC from cirrhosis.

Recommendations:

- We can recommend the measurement of serum Thioredoxin as a diagnostic biomarker for HCC.
- Serum Thioredoxin could be beneficial as a biomarker for HCC ablation therapy outcome follow up.
- Further studies on larger scale should be conducted to confirm our findings.

Ethical Approval:

- The study protocol was approved by the ethical committee of Faculty of Medicine, Tanta University.
- The study had started after medical ethical committee approval. All included cases were informed about aim of our study, risk factors, possible complication and risk of failure.

Consent

As per international standard or university standard, patients' written consent has been collected and preserved by the author(s).

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