Evaluation of Serum Level of Thioredoxin and its Gene Polymorphism in Diagnosis of Hepatocellular Carcinoma of HCV-Infected Patients

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Abstract:

Background: Thioredoxin (TXN) is an important regulator of redox balance in the cell and has been implicated as playing a role in cell survival in many conditions including cancer**. Aim:** To evaluate the role of TXN serum level and TXNDC5 gene polymorphisms in diagnosis of hepatocellular carcinoma (HCC) in cirrhotic Egyptian patients due to hepatitis C virus (HCV). **Subjects and Methods:** Thirty five cirrhotic patients with HCC patients, thirty five cirrhotic patients without HCC patients and 20 healthy volunteers were enrolled in this study. TXN serum level was quantitated using by human thioredoxin enzyme-linked immunoassay (ELISA) and molecular study of TXNDC5 (rs 1225943) polymorphism using real-time polymerase chain reaction by Taqman allele discrimination was done for all subjects. **Results:** Showed that TXNDC5 (rs 1225943) AA genotype was the most frequent genotype in HCC patients and the most frequent allele was A allele in HCC patients, without significant difference of TXNDC5 (rs 1225943) polymorphism in the studied groups and TXN serum level was significantly higher in HCC patients (mean 40.2±11.92) than in cirrhotic patients (mean 9.5±5.66) ($p < 0.001$) and normal controls (mean 7.1±1.67) ($p < 0.001$), and AFP ≥ 41 (ng/ml) and TXN serum level ≥14.3 (ng/ml) are diagnostic for HCC presence. **Conclusion:** Serum Thioredoxin level may be used as a molecular marker for HCC diagnosis, and TXNDC5 (rs 1225943) polymorphism was not associated with the risk of HCC in HCV-cirrhotic patients.

Key words: Thioredoxin , Thioredoxin domain containing 5, Hepatocellular carcinoma, Hepatitis C virus.

Introduction

Hepatocellular carcinoma (HCC) is the 5th most common cancer in men and represents the second leading cause of cancer death worldwide **(1)**. HCC is the main cause of death in patients with cirrhosis **(2)**.

Hepatocellular carcinoma (HCC) is the most common primary liver tumor and develops in most cases in patients with underlying chronic liver disease, usually cirrhosis. The main etiological factor is infection by the hepatitis B virus (HBV), chronic infection by the hepatitis C virus (HCV), and alcohol and, as recently reported, there is an increased incidence in patients with non-alcoholic fatty liver disease (NAFLD)/non-alcoholic steatohepatitis (NASH) **(3)**.

HCC is a major health problem especially in certain countries such as Egypt. In Egypt, HCC is the fourth most common cancer and is the second cause of cancer mortality in both sexes (**4).**

HCC is a unique malignancy in that, it can be diagnosed based on imaging findings alone, once a lesion is identified on non-contrast ultrasound measuring >1 cm, it is further evaluated with noninvasive diagnostic tests. For all guidelines, this involves multi-row detector CT or contrast-enhanced MRI using an extracellular contrast agent **(5).**

The detection of biomarkers associated with HCC in body fluids or tissues is the most promising approach to improve diagnostic accuracy and to overcome the disadvantages of current diagnostic strategies. Especially non-invasive techniques relying on blood or serum samples would be beneficial for both patients and clinicians **(6)**.

Thioredoxin is also a regulator of cellular functions in response to redox signals and stress, modulating various signaling pathways, transcription factors, and immunological responses **(7)**. TXN is an important regulator of redox balance in the cell and has been implicated as playing a role in cell survival in many conditions including cancer and neurodegenerative diseases **(8)**.

Increased levels of TXN have been reported in many pathological conditions associated with oxidative stress **(9*)***. Thioredoxin overexpression might represent a signature in cancer, and that over-activated thioredoxin signaling might be a prognostic marker in cancer, and might also serve as potential therapeutic targets in cancer therapy **(10)**.

Although accumulating evidence has demonstrated that TXNDC5 is important for cancer development and growth, there has been no enough report about TXNDC5 polymorphisms in cancer **(11)**

This study aimed to evaluate the role of TXN and TXNDC5 (rs 1225943) gene polymorphism in diagnosis of hepatocellular Carcinoma of HCV-infected Patients

Subjects and Methods:

This cross – sectional case/control study was conducted on 70 patients and 20 healthy volunteers, admitted to Hepatology, Gastroenterology and Infectious Diseases Department in Benha University Hospital during the period from March 2018 to November 2018 in cooperation with the Medical Biochemistry and Molecular Biology Department. The protocol of this study was approved by the Ethical Committee of the Faculty of Medicine, Benha University and informed consent was taken from each subject before participation in this study

The subjects were divided as the following:

- **Group (I) :** Included 20 apparently healthy subjects served as a control group.
- **Group (II):** included 35 cirrhotic patients due to chronic HCV infection without HCC.
- **Group (III): i**ncluded 35 cirrhotic patients due to chronic HCV infection with HCC.

  -HCC was diagnosed by the abdominal US and confirmed by triphasic CT scan with contrast **(12)**. The historical, clinical, and biochemical data of the patients were obtained, including age, gender, alcohol intakes, Hepatitis C Virus (HCV) and Hepatitis B Virus (HBV), liver function tests, and AFP levels. Assessment of liver cirrhosis was done using by modified Child-Pugh score (**13**), and MELD (Model for End-stage Liver Disease) score (**14)**. Tumor characteristics were identified by abdominal US and the triphasic CT scan (tumor size, number, site, halo sign and neovascularization) Tumor staging was done using Okuda staging system **(15)**.

  -Patients with other neoplasm, Patients with diabetes, Patients with chronic kidney disease, Patients with severe burn and Patients with cardiovascular diseases were excluded from this study.

Sample collection:

Peripheral venous blood sample (5 ml) was obtained from each subject under complete aseptic conditions. The blood sample was divided into 3 parts: the first part (1ml) was put into sterile vacutainer EDTA tube; 0.5 ml for CBC, and 0.5 ml for genotyping. The second part (0.9 ml) was withdrawn into a tube containing tri-sodium citrate (concentration 3.8%) solution in a ratio of 9:1 for determination of PT concentration, activity and INR. The third part (~ 3 ml) were left to clot and serum was separated for determination of thioredoxin protein by ELISA for other serological and biochemical investigations.

- **Laboratory investigations were done as follow:**

  a. **Complete blood picture (CBC)** was performed by automated hematology analyzer Sysmex XS-1000i **(Sysmex, Japan)**, Hb% (g/dl), WBC (c/mm^3), platelet count (c/mm^3) **(16).**

  b. **ESR** (ml/hour): **(17).**

  c. **Random blood glucose** (mg/dl) **(18)**.

  d. **Kidney function tests:** serum creatinine (mg/dl) and blood urea (mg/dl) **(19).**

  e. **Liver profile tests including:**

  - Serum level of alanine transferase (ALT) and aspartate transferase (AST) (U/dl): **(20)**
  - Serum level of albumin (mg/dl): **(21).**
  - Serum bilirubin (total and direct) (mg/dl): **(22).**
  - Prothrombin time (PT) (sec) and concentration (PC) (%) using Behring Fibrin timer II from (Behring, Germany) **(23).**

  f. **Viral markers:** HCV Abs **(24)** and HBsAg **(25):** by third generation of enzyme linked immuno-sorbant assay (ELISA)

  g. **Serum alpha feto-protein level (AFP)** (ng/ml): **(26)** by ELISA.

  - Random blood glucose, creatinine, urea, ALT, AST and albumin tests were performed by Microtech spectrophotometer (Vital Scientific, Netherlands).
  - Serum viral markers and AFP were performed by Tecan Infinite spectrophotometer F50 ELISA Reader (Singapore).

  h. **Estimation of thioredoxin protein (pg/ml) by** human thioredoxin ELISA kit PicoKine™, Boster Biological Technology, CA, USA) (Sumida et al., 2000). Then this results were divided by 1000 to get ng/ml

The assay is a quantitative solid phase sandwich enzyme-linked immunoassay (ELISA)

i. **Molecular study of thioredoxin domain -containing 5 (TXNDC5) gene polymorphism (rs1225943) polymorphism:**

rs1225943 genotyping was performed by Taqman allele discrimination through the following steps:

1) **Genomic DNA extraction:**

DNA was extracted from 200µl blood sample; using Purelink® Genomic DNA minikit Catalog No. K1820-01 (Invitrogen, Life Technologies)

2) **Real-time PCR** for detection of thioredoxin domain - containing 5 (TXNDC5) gene polymorphism (rs1225943):

It was done by 5' Nuclease Taqman single nucleotide polymorphism (SNP) Genotyping Assay Technology. In 20µl reaction, genomic PCR amplification was done using Taqman 5′allele discrimination assay for the SNP (Applied Biosystem, Foster City, California, USA). The rs1225943 assay contained sequence specific primers for both alleles (A and C) and 2 Taqman probes; one probe labeled with VIC dye detects the A allele and the other labeled with FAM detects the C allele. Amplification was done in Stepone Real-Time PCR System (Applied Biosystem, Foster City, USA). The following thermal cycling conditions were run: Pre-PCR Read (60^{o}C for 30 sec.), Amplitaq Gold Enzyme activation (95^{o}C for 10 min.) and 40 cycles (denaturation; 92^{o}C for 15 sec. and anneal/extend; 60^{o}C) and Post-PCR Read (60 ^{o}C for 30 sec.). Two non-template controls (NTCs) using nuclease-free water were essentially run to enable detection of DNA contamination. The success rate for this genotyping was 100%. The laboratory data for genotyping are manifested in **(figure 1)**.

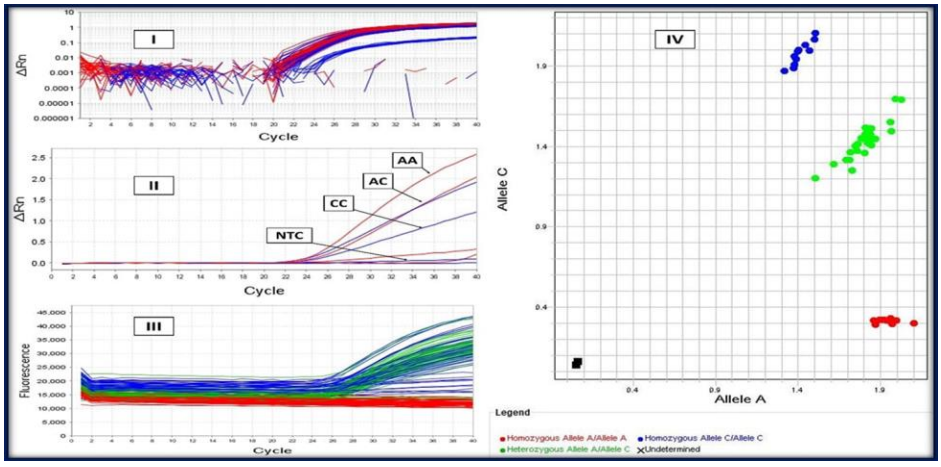

Fig 1 Real-time PCR for detection of thioredoxin domain - containing 5 (TXNDC5) gene polymorphism

Statistical analysis:

The SPSS 12.0 statistical software was used for statistical analysis (VER 21 SPSS Inc, Chicago, ILL Company). Categorical data were presented as number and percentages while quantitative data were expressed as mean ± standard deviation and range.

Chi square test (X^2) were used to analyze categorical variables, Odds ratios (OR) were calculated when applicable. Quantitative data were tested for normality using Shapiro-Wilks test, assuming normality at P>0.05.

Difference among 3 independent means was analyzed using analysis of variance (ANOVA) for parametric variables or Kruskal Wallis test (KW) for non-parametric ones. ROC curve was used to determine cutoff value of the studied markers with optimum sensitivity and specificity in early diagnosis of HCC.

Uni and multi variable logistic regression analysis were run to detect the significant predictors of HCC. The accepted level of significance in this work was stated at 0.05 (P <0.05 was considered significant). Genotype distributions in the studied groups were in Hardy-Weinberg equilibrium for gene polymorphisms

Results:

This study conducted on 90 subjects attending Department of Hepatology, Gastroentrology and Infectious Diseases in Benha University Hospital, in the period from March 2018 to November 2018. A total of 70 Egyptian subjects with chronic HCV (35 cirrhotic without HCC + 35 cirrhotic with HCC) and 20 healthy volunteers were enrolled in our study. Age and sex of cases and healthy volunteers are summarized in **(Table 1).**

No significant differences were found in age and gender between the cases and controls .Platelets (PLTs), ESR, AST, serum albumin, total bilirubin, INR and AFP levels were significantly different among the studied groups **(Table 2).**There was no statistically significant difference between the studied groups as regard Child-Pugh classification as shown in **(Figure 2).**

Regarding serum level of thioredoxin, there was highly statistical significant difference between controls and HCC patients and between cirrhotic and HCC patients (p<0.001 for all) **(Table 3).**

As regard TXNDC5 (rs1225943) genotype frequency and allele there was no statistically significant difference between HCC ,cirrhotic and control groups **(Table**

4), and the most frequent genotype in HCC group was AA (37,1%).

ROC curve analysis showed that the area under the curve of TXN was higher than that of AFP **(Figure 3)**. The diagnostic validity and the optimal cut off values of TXN and AFP for HCC are listed in **(Table 5).**Cut off value of TXN ≥ 14.3 (ng/ml) recorded an AUC of 0,990 with sensitivity of 97.1 % and specificity of 96.4%. While cut off value of AFP ≥ 41 ng/mL showed an AUC of 0,892 with a sensitivity of 82.9% and specificity of 80%

Factors possibly associated with the development of HCC were assessed by univariable regression analysis compared with non HCC groups**.** These factors included loss of weight, serum albumin level < 2.5 (g/dl), ESR > 80(ml/h), AFP ≥ 41 (ng/ml) and serum level of TXN ≥ 14.3 (ng/ml) **(Table 6).**

Multivariable binary logistic regression analysis for prediction of HCC only AFP ≥ 41 (ng/ml), serum level of TXN ≥ 14.3 (ng/ml) are significant independent predictors of HCV- related HCC **(Table7).**

Table (1): Demographic Features of the studied groups:

Variable		**Group I (n=20)**		**Group II (n=35)**		**Group III (n=35)**		**ANOVA (P)**	**P of multiple comparisons**
Age (ys)	**Mean±SD**	57.2±8.9		57.9±9.1		62.0±8.7		2.6	P_1=1.0
	Range	45-73		40-80		45-80		(0.079)	P_2=0.17
								NS	P_3=0.17
		No.	%	No.	%	No.	%	χ^2/ Fisher's test	
Sex	**Male**	9	45.0	13	37.5	20	57.1	0.32	P_1=0.56
	Female	11	55.0	22	62.9	15	42.9	0.75	P_2=0.38
								2.80	P_3=0.094

SD: Standard deviation

Group I→ Control group, Group II→ Cirrhotic without HCC, Group III→ Cirrhotic with HCC

P1: between group I and II, P2: between group I and III, P3: between group II and III

Table (2): Comparison between studied groups as regard laboratory findings.

Variables	Group I (n=20)		Group II (n=35)		Group III (n=35)		ANOVA & P	P of multiple comparisons
	Mean	±SD	Mean	±SD	Mean	±SD		
PLTs(c/mm^3)	272.7	79.1	109.7	71.8	132.1	92.9	27.1 &<0.001 (HS)	P_1<0.001 (HS) P_2<0.001 (HS) P_3 =0.77
ESR (mm/hour)	13.5	7.96	51.4	34.0	81.0	38.4	43.5† & <0.001 (HS)	P1<0.001 (HS) P2<0.001 (HS) P3=0.001 (HS)
AST(IU/dl)	32.5	12.3	47.9	22.5	54.5	39.9	11.2 &0.004 (S)	P1=0.19 P2=0.026 (S) P3= 1.0
T. bilirubin (mg/dl)	0.99	0.23	3.2	4.54	3.45	3.1	31.8 & <0.001 (HS)	P1=0.0046 (S) P2=0.004(S) P3=1.0
D. bilirubin (mg/dl)	0.33	0.10	1.40	3.20	1.63	2.5	22.5 &<0.001 (HS)	P1=0.03 (S) P2=0.21 P3=1.0
S. albumin (g/dl)	4.22	.48	2.70	0.61	2.63	0.55	58.6‡ &<0.001 (HS)	P1<0.001 (HS) P2<0.001 (HS) P3=1.0
INR	1.03	0.09	1.44	0.39	1.85	2.31	31.3 &<0.001 (HS)	P1=0.016 (S) P2=0.002 (S) P3=0.07
AFP(ng/ml)	1.74	1.48	36.3	32.5	238.7	232.19	58.0 & <0.001 (HS)	P1=0.032 (S) P2<0.001 (HS) P3<0.001 (HS)

SD: Standard deviation; (s): significant P<0.05; (HS:) highly significant P≤ 0.001

PLTs; platelets; ESR: erythrocyte sedimentation rate; AST: aspartate aminotransferase; INR: international normalized ratio; AFP: Alpha-fetoprotein

P1: between group I and II, P2: between group I and III, P3: between group II and III

† Kruskal Wallis test (KW test) was used

‡ ANOVA was used

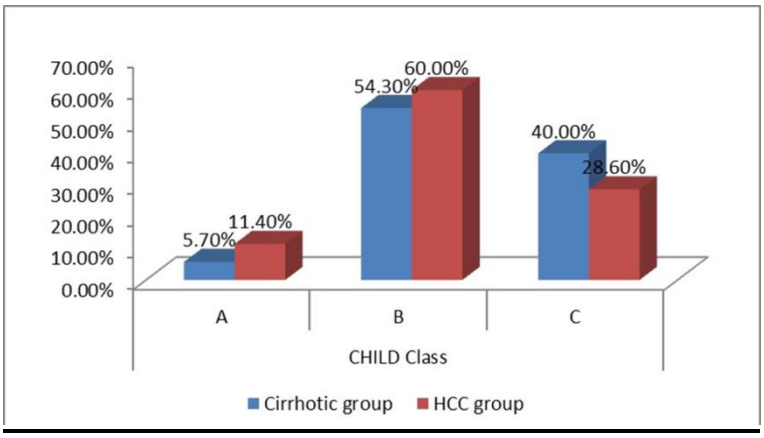

Fig 2: comparison between controls and HCC patients and between cirrhotic and HCC patients

Table (3): Comparison between the studied groups regarding Serum level of thioredoxin

Variable	Group I (n=20)		Group II (n=35)		Group III (n=35)		KW test & P	P of multiple comparisons
	Mean	±SD	Mean	±SD	Mean	±SD		
Serum thioredoxin level (ng/ml)	7.1	1.67	9.5	5.66	40.2	11.92	63.3 & <0.001 (HS)	P_1=0.89 P_2<0.001 (HS) P_3<0.001 (HS)

P1: between group I and II, P2: between group I and III, P3: between group II and III

SD: Standard deviation; *HS: highly significant P≤ 0.001* *† Kruskal Wallis test (KW test) was used*

Table (4) Comparison between the studied groups as regard TXNDC5(rs 1225943) genotype and allele.

Variable		Controls (n=20)		Group II (n=35)		OR (95%CI)	P	Group III (n=35)		OR (95%CI)	P
		No.	%	No.	%			No	%		
Genotypes	**AA**	4	20.0	7	20.0	1.1 (0.2-5.2)	0.89 (NS)	13	37.1	2.27 (0.5-9.9)	0.27 (NS)
	AC	9	45.0	17	48.6	1.2 (0.3-4.2)	0.77 (NS)	12	34.3	0.93 (0.25-3.4)	0.91 (NS)
	CC	7	35.0	11	31.4	Ref.		10	28.6	R	
Allele	**A**	17	42.5	31	44.3	1.07 (0.49-2.3)	0.85 (NS)	38	54.3	1.6 (0.7-3.5)	0.23 (NS)
	C	23	57.5	39	55.7			32	45.7		

(NS): non-significant P value >0.05

Table (5): ROC curve analysis for the performance of AFP, serum thioredoxin in the prediction of HCC.

Variable	Cut off	Sens%	Spec%	PPV%	NPV%	AUC	95%CI	P
AFP (ng/ml)	≥41	82.9%	80%	72.5%	88%	0.892	0.82-0.96	<0.001 (HS)
Serum thioredoxin (ng/ml)	≥14.3	97.1%	96.4%	94.4%	98.1%	0.990	0.97-1.0	<0.001 (HS)

AFP: Alpha-fetoprotein; Sens: sensitivity ;PPV=positive predictive value; NPV=negative predictive value; AUC=area under curve; *(HS:) highly significant P≤ 0.001*

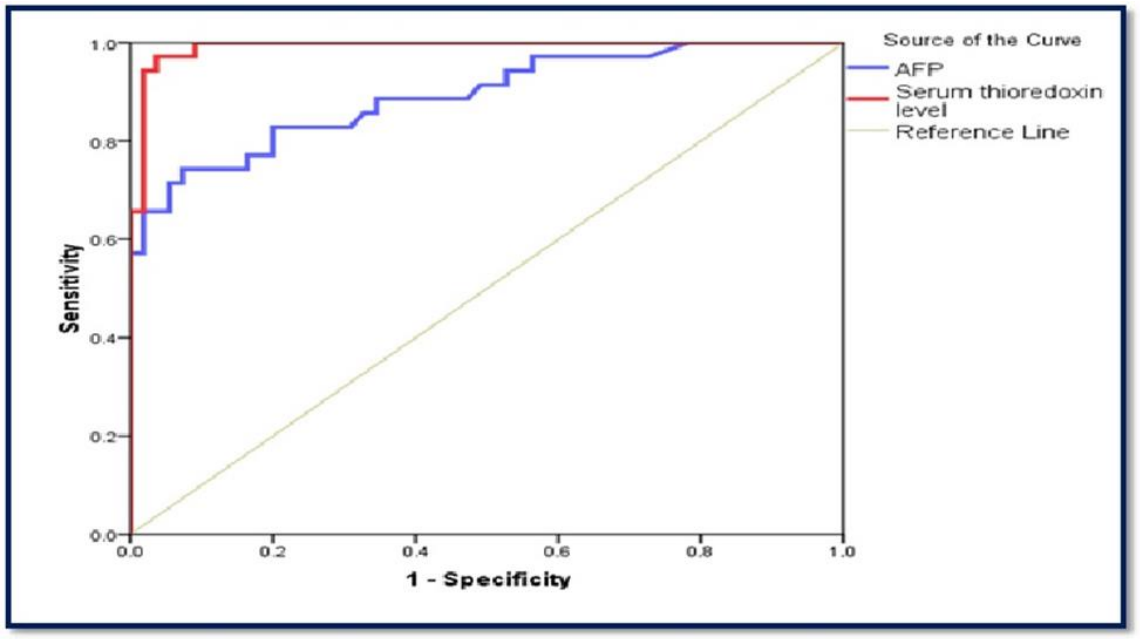

Fig 3: ROC curve analysis

Table (6): Univariable binary logistic regression analysis for the risk factors of HCC

Variable	Univraiate logistic regression			
	β	Crude OR	95%CI	P
Age >58 (yrs)	0.66	1.61	0.97-6.3	0.34
Sex (Male)	0.69	0.5	0.21-1.18	0.11
Smoking	0.256	1.29	0.49-3.3	0.60
H of HTN	-0.596	0.55	0.13-2.23	0.40
Loss of weight	2.91	18.3	4.8-70.0	<0.001 (HS)
H of abd pain	0.28	1.69	1.5-7.5	0.32
H of jaundice	-0.325	0.72	0.27-1.9	0.51
H of bleeding	-0.270	0.76	0.32-1.8	0.54
H of encephalopathy	0.93	2.05	0.97-7.1	0.15
H of blood transfusion	-0.170	0.84	0.32-2.19	0.72
PLTs < 130x10^3/ml	0.348	1.40	0.6-3.3	0.42
ALT>45 IU/dL	0.724	2.06	0.77-5.5	0.15
AST> 50 IU/dL	0.470	1.6	0.59-4.3	0.35
T.bilirubin >3 mg/dL	0.24	1.27	0.51-3.1	0.60
D. bilirubin >1.5	-0.596	0.55	0.14-2.2	0.40
S albumin< 2.5	1.02	2.77	1.12-6.8	0.026 (S)
ESR >80 mm/hr	2.45	9.44	3.0-29.3	<0.001 (HS)
INR>1.6	0.851	2.3	0.85-6.4	0.098
AFP ≥41ng/mL	2.96	19.3	6.4-58.0	<0.001 (HS)
TXN ≥14.3 ng/mL	5.2	91.2	0.38-993.7	<0.001 (HS)
AA genotype	0.860	2.36	0.91-6.1	0.077

H:history;abd:abdominal;T:total;D:direct;S:serum;HTN:hypertension;ALT:Alanine aminotransferase; PLTs; platelets; ESR: erythrocyte sedimentation rate ; AST: aspartate aminotransferase; INR: international normalized ratio ; AFP: Alpha-fetoprotein; TXN: thioredoxin

*(*s): significant *P<0.05*; *HS: highly significant P≤ 0.001*

Table (7): Multivariable binary logistic regression analysis for the predictors of HCC.

Variables	Multivariable logistic regression			
	β	Adjusted OR	95%CI	P
Loss of weight	13.7	6.8	0.41-46.8	0.91
S albumin< 2.5 g/dL	29.7	6.7	0.94-45.4	0.87
ESR >80 mm/hr	42.2	7.8	0.56-25.9	0.85
AFP ≥41ng/mL	149.9	32.3	6.9-67.8	0.008 (S)
thioredoxin ≥14.3 ng/mL	168.7	40.5	7.1-94.8	0.001 (HS)
Constant	-135.8			

Discussion:

Hepatocellular carcinoma (HCC) is the most common primary hepatic malignancy, constituting around 85–90% of primary liver cancers. The incidence of HCC is on the rise, and HCC is now the fastest-growing cause of cancer-related mortality in the United States **(27)**.HCC is the second leading cause of global cancer related deaths, especially in patients with liver cirrhosis **(28)**.

HCC mainly caused by hepatitis B virus (HBV), hepatitis C virus (HCV), alcohol abuse, and non-alcoholic fatty liver disease (NAFLD) **(29).**

Egypt has a high incidence of HCC about 21% in cirrhotic Egyptian patients. An active surveillance and secondary prevention programs for patients with chronic hepatitis are the most important steps to reduce the risk of HCC **(30).**

In Egypt HCV prevalence in the age group (15–59 years) was 14.7% in 2008 while it became 10% in 2015**(31)**. This decline in prevalence was related to aging of infected people receiving anti-schistosomal injections **(32)**.

The current study aimed to evaluate the role of TXN and gene polymorphism of TXNDC5 (rs 1225943) in diagnosis of hepatocellular carcinoma in chronic hepatitis C Egyptian patients.

In the present study, the mean age of patients with HCC was (62.0±8.7) year

(ranging from 45-80 year) without significant difference between HCC and other groups **(Table 1) .** This result agreed with **El-Toukhy et al., (33)** reported that the mean age of HCC patients was 63.04 ± 7.486 years (ranging from 44 to 78 years)..Also agreed with **(34)** who reported that the age of the HCC patients (ranging from 24 to 83) years with the mean age of (62.73 ±10.59) years old , also agreed with **Park et al., (35)** who reported that, the mean age of HCC patients was (62.33±10.96) year.

On the other hand **El-Shahat et al., (36)** reported that the mean age of HCC patients (48.5±7.8)**.**Also **Tanaka et al., (37)** reported that the age of HCC incidence was higher in Japan (70–79 year).This difference may be partially attributed to the difference in the risk factors distribution among Japanese patients with HCC, which was highly variable, depending on geographic region, race or ethnic group.

In the present study, HCC presented more frequently in males than females with male to female ratio (1.33:1) with no significant difference between HCC and other groups**(Table1)**. This male predominance came in agreement with **Al-sheikh et al.,(38)** who reported that male/female ratio of HCC is (1.3:1) with no significant difference between HCC and other groups .

On the other hand **Omar et al., (39)** reported that There was highly statistically significant difference between studied groups as regard sex **(P < 0.001)** with male predominance in HCC group (male to female ratio was 4:1)

Factors may explain that males are more likely to be infected with HBV and HCV, in addition to cigarettes smoker, and alcohol consumer Testosterone rate has been shown to correlate with HCC indicating a probable role for the sex hormones in the development of HCC **(40)**.

Regarding laboratory investigations **(Table2)** there was statistically significant difference between HCC and control groups regarding platelet count **(P <0.001)**, this result was agreed with **Omar et al., (39** documented that there was statistically significant difference between cirrhotic with HCC, cirrhotic without HCC, chronic hepatitis C and healthy groups as regard platelet count **(P <0.001)**. As regard AST level, there was statistically significant difference between HCC group and control group **(P =0.026)** and was agreed with **Mohamed et al., (41)** reported statistically significant difference between HCC group

and other groups (control, LC groups) as regard AST **(P= 0.006).**

Serum albumin level was statistically significantly lower in HCC group compared with control (**p <0.001)** on the same hand **Mohamed et al.,(41)** reported statistically significant difference between HCC group and other groups (control, LC groups) for Albumin **(P =0.000)** Also with **Omar et al., (39)** who stated that there was statistically significantly lower in HCC group compared to control groups **(P < 0.001)**.

There was significant difference between HCC group and other groups (control, LC groups)as regards ESR **(P ≤ 0.001)**this came in agreement with **Youssef et al., (42)** reported statistically significant difference between HCC group and other groups (control, LC groups) as regards ESR **.**

In the present study, concerning INR level **(Table 2)**, there was statistically significant difference between HCC and control groups **(p =0.002).**This came in agreement with **Omar et al., (39)** reported that there was statistically significant difference between HCC and control group as regard INR level **(p <0.001)** also **Mohamed et al.,(41)** reported that , there was statistically significant difference between HCC and control groups as regard INR level **(p =0.000)**.

In the present study there was statistically significant difference in AFP level between HCC group in comparison with control and cirrhotic groups **(P < 0.001) (Table 2)**.These results were in agreement with **Hussein et al., (43)** reported that the alpha fetoprotein serum level showed a significant elevation in hepatocellular carcinoma patients also similarly **Li et al., (44)** reported, marked elevation of AFP level was observed in patients with HCC in comparison with healthy control subjects, patients with chronic liver disease and patients with LC **(P < 0.0001).**

Also **Omar et al., (39)** who stated that there was significant difference in AFP level between HCC group in comparison with cirrhotic, chronic hepatitis C and control groups **(P < 0.001)**.

In the present study, most HCC patients were Child B (60%), followed by Child C (28.6%) then Child A (11.4%) with no statistically significant difference **(figure 2)**Similar results were reported by **El-Sherbiny et al., (34)** who found that majority of HCC patients were Child B (46.25%)**.**

On the other hand **Abu El-Makarem et al., (45)** found that majority of HCC patients were Child C **also El-Toukhy et al., (33)** reported that 3.3% of HCC patients were

Child A, 26.7% of HCC patients were Child B and 70% were Child C without a significant difference

In the present study concerning serum level of thioredoxin, was statistically significantly higher in HCC group compared with cirrhotic and control groups **(P < 0.001) (Table 3).** This result was agreed with study of **Li et al., (44)** who reported significantly higher levels of thioredoxin, in HCC patients compared to the other groups **(P< 0.001)** also agreed with study reported by **Tamai et al., (46)**serum level of thioredoxin, was statistically significant higher in HCC group compared with CLD without HCC and controls **(p =0.04) (P < 0. 01)** respectively also was agreed with study of **Omran et al., (47)** who reported that Serum levels of TXN was significantly higher in HCC than in patients with liver cirrhosis **(P < 0.0001) .**

In the present study, there is no statistically significant difference between HCC ,cirrhotic and control group as regard TXNDC5 genotype frequency and allele **(Table 4)**, on the other hand , **Park et al.,(11)** stated that The genotypic frequency of TXNDC5(rs1225943) was associated with HCC in the co-dominant ,recessive, and log-additive models .This difference in results may be due to difference in ethnicity as the previous study was done on Korean patients, only males and large number of their sample size (160 male patients with HCC and 178 healthy male individuals) and due to difference in etiology of HCC patients, as they included chronically infected with HBV ,HCV and alcohol

The most frequent genotype in HCC group was AA (37,1%) followed by AC genotype(34.4%) then CC (28.6%), and that the most frequent allele of HCC group was A allele (54.3%) This result matched with study reported by **Park et al.,(11)** most frequent genotype in HCC group was AA (68.9%) followed by AC genotype(29.8%) then CC 1.3%, and that the most frequent allele of HCC group was A allele (83.8%).

In the present study, AFP sensitivity and specificity in the prediction of HCC were (82.9% and 80%) respectively with AUC of 0,892 **(Table 5).** Also **Li et al.,(44)** reported sensitivity and specificity of AFP for detection of HCC and were (78.4 % and 81.3 %, respectively).

Tamai et al., **(46)** reported much lower sensitivity and higher specificity of AFP in diagnosis of HCC were (33.3% and 97.1%) respectively, this difference in sensitivity and specificity may be due to difference in ethnicity as this study was conducted on Japanese patients.Also **Omran et al., (47)**

reported, AFP sensitivity and specificity in the prediction of HCC were (29 % and 100%) respectively with AUC 0. 0.69.This may be explained by difference in sample size as the study conducted on 122 patients (80 HCC and 42 LC).

In this study, the sensitivity and specificity of TXN were 97.1 and 96.4 respectively) with AUC of 0,990 **(Table 5)**. that was higher than **Li et al.,(44)** who stated that sensitivity and specificity of serum level of TXN in the diagnosis of HCC were 84.3 % and 91.8 %,) respectively, this difference in sensitivity and specificity may be due to different etiology, as his patients was of mixed etiology (HCV, HBV and alcoholic) this was excluded from our study and also may be due to different sample size , as he conducted his study on larger sample size (180 HCC, 120 LC ,120 CLD and 100 healthy volunteers). Also higher than **Omran et al., (47)** reported, the sensitivity and specificity of TXN were74% and 71% respectively) with an AUC of 0.79 to identify HCC patients, this may be explained by difference in sample size as this study conducted on 122 patients (80 HCC and 42 LC).

In the present study **(Table 6)**, Factors possibly associated with the development of HCC were assessed by unavailable regression analysis compared with non HCC groups**.** These factors included loss of weight, serum albumin level < 2.5 (g/dl), ESR > 80 (mm/h), AFP ≥ 41 (ng/ml) and serum level of TXN ≥ 14.3 (ng/ml). These result was agreed with, **Elgamal et al., (48)** who documented that, highest risk for development of HCC by binary logistic regression for prediction of HCC cases were hypoalbuminaemia and increase level of AFP.

Hedenstierna et al., (49) stated that decrease albumin levels remained significantly correlated with HCC development by univariate analysis.

In the present study **(Table7)**, by multivariable binary logistic regression analysis for prediction of HCC only AFP ≥ 41 (ng/ml), serum level of TXN ≥ 14.3 (ng/ml) are significant independent predictors of HCV- related HCC, on the same hand **Li et al., (44)** reported that by multivariable binary logistic regression analysis for prediction of HCC AFP ≥ 20 (ng/ml)and serum level of TXN ≥ 20.5 (ng/ml) are significant independent predictors of HCC

Tamai et al., (46) by multivariable binary logistic regression analysis found that AFP levels ≥ 40 ng/mL was independent risk

factors associated with poor prognosis in HCC patients

This study concluded that, there is significant association between serum level of TXN and HCC risk in Egyptian patients with chronic hepatitis C. Moreover, there was no significant association between TXNDC5 rs1225943 gene polymorphism and HCC risk.

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