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Early diagnosis of hepatocellular carcinoma by measurement serum thioredoxin as diagnostic marker in cirrhotic hepatitis patients

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Abstract

Hepatocellular carcinoma (HCC) is a deadly liver disease. To improve survival, HCC must be detected early. The current diagnostic sign, serum alpha-fetoprotein (AFP), has low sensitivity and specificity. This study investigated thioredoxin (TRX) as a biomarker for early HCC diagnosis in low-AFP individuals. Serum TRX and AFP levels were measured in 90 people divided into five groups. The first was 30 low serum AFP HCC patients the 2nd group of 30 patients had cirrhosis without HCC. The 3rd group had 10 HCC patients with elevated serum AFP. Finally, 10 chronic liver disease patients were in the fourth group. A control group of 10 healthy people and those with Hepatitis C infection receiving hemodialysis were studied in this study. Receiver operating characteristic (ROC) curve analysis tested TRX and AFP diagnostic performance. The study also examined how serum TRX levels affected clinical parameters in hepatocellular carcinoma patients. Patients with chronic liver illness, Hepatitis C infection, and hemodialysis showed higher serum Thioredoxin levels. HCC patients with high serum AFP had higher Thioredoxin levels than those with low AFP. Finally, liver cirrhosis patients had the lowest Thioredoxin levels. Combining TRX with AFP improves HCC diagnosis sensitivity and specificity, especially in patients with low or negative AFP levels. The study also found a link between serum TRX levels and hepatocellular carcinoma tumor size, stage, and grade. Serum thioredoxin may be a biomarker for early HCC diagnosis in viral cirrhosis patients. Combining TRX with AFP may improve HCC diagnosis.

Keywords: Hepatocellular carcinoma, viral cirrhosis, Hepatitis C, Hemodialysis, thioredoxin, alpha-fetoprotein.

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1. Introduction

Seventy percent to eighty percent of all liver cancers are caused by HCC. It's worth noting that HCC is responsible for more cancer deaths than any other single cancer kind. Tumor recurrence rates between 54.1% and 61.5% during a 5-year window after curative resection remain very high. This is significant since HCC treatment options such as liver resection and transplantation have been shown to be effective [1]. The poor prognosis of HCC may largely be attributed to the fact that only a small percentage of patients are considered good candidates for curative treatment options (30-40%) [2]. Improved patient outcomes depend on a more refined method of diagnosing and identifying HCC in its earliest stages. However, difficulties arise in early identification of HCC because of the absence of early signs. Despite its limitations, such as operator dependence and an inability to discriminate between malignant and benign nodules in smaller cirrhotic livers, ultrasound is the main radiologic screening technique for HCC. Triphasic computed tomography (CT) scans and magnetic resonance imaging (MRI) have showed promise in improving diagnosis accuracy, but their present time and cost constraints make them impractical for widespread screening [3]. Currently, AFP is the only molecular marker that has shown efficacy in the detection and diagnosis of HCC. AFP has a sensitivity of between 60% and 80%. The detection sensitivity drops to about 40% when trying to identify small cancers. Many people with chronic liver illness have elevated levels of AFP, often between 20 and 200 ng/mL. Prevalence estimates for this phenomena range from 11 percent to 47 percent in patients with cirrhosis and 15 percent to 58 percent in those with chronic hepatitis. The ability to diagnose HCC at an earlier stage may be considerably improved if a biomarker could be found that outperformed AFP in terms of sensitivity and specificity. Increased sensitivity and specificity for the diagnosis of HCC [4] may be attained by evaluating serum thioredoxin levels, and by combining measures of serum thioredoxin with serum AFP. NADPH is the reduced version of the important protein thioredoxin, which plays a role in redox control. Thioredoxin reductase (ThioredoxinR), a homodimeric selenoenzyme, regulates thioredoxin's redox state [5]. Protein thiols, which are generally characterized by a disulfide bond of relatively low

molecular weight, serve as the primary mediators of thioredoxin's biological effects. NADPH, generated by glucose-6-phosphate dehydrogenase (G6PD), is the limiting factor in the oxidative hexose monophosphate shunt (HMPS) pathway and is necessary for the control of thioredoxin activity. Two different thioredoxins have been cloned so far: thioredoxin 1, a 54 kDa enzyme mostly found in the cytoplasm, and thioredoxin 2, a 56 kDa enzyme with a mitochondrial import sequence [6]. Regulation of cancer cell proliferation is only one of the many biological processes in which thioredoxin plays a vital role. The significance of redox processes in cancer pathophysiology is mostly due to the biological activities discussed below. Serum thioredoxin levels may be an important clinical indicator in the diagnosis of HCC [7, 8]. Using affinity electrophoresis, AFP may be separated into its L1, L2, and L3 glycoforms. The degree to which different glycoforms respond to lens culinaris agglutinin (LCA) is used to divide those apart (8). An additional 1-6 fucose residue linked at the reducing terminus of N-acetyl glucosamine greatly increases AFP-L3's binding affinity to LCA. Compared to the L1 isoform, AFP-L3 is distinguished by this particular trait. Non-hepatocellular carcinoma (HCC)-related liver inflammation is often associated with the L1 isoform. Patients in high-risk groups, such as those with chronic hepatitis B and/or C infections and/or liver cirrhosis [9], can benefit from increased surveillance for the development of HCC when the L3 isoform is measured. This study investigated thioredoxin (TRX) as a biomarker for early HCC diagnosis in low-AFP individuals.

2. Materials and methods

This was a case control study. The present study was carried out on a sample of ninety patients who were recruited from the inpatient population at the internal medicine department of Beni Suef University Hospital. The data collection period spanned from November 2020 to October 2022. All of the patients included in the study were residents of Beni-Suef government and had a confirmed diagnosis of HCC as determined by either abdominal ultrasound, triphasic CT abdomen with contrast, or AFP testing. An informed consent was taken from all participants.

- Group 1 consisted of ten individuals who were deemed clinically healthy participants, including six men and four females. These individuals were selected as a control group to provide a baseline for comparison. They exhibited no signs of HCC based on normal hepatic profiles, including normal AFP levels and negative ultrasonography findings. Additionally, they tested negative for hepatitis B and C infections and did not have diabetes.
- Group 2 consisted of ten patients diagnosed with chronic liver disease and Hepatitis C infection. Among these patients, eight were male and two were female. All patients were undergoing therapy for Hepatitis C while receiving hemodialysis. It was seen that these patients had normal liver profiles, normal AFP levels, and no signs of HCC as verified by abdominal ultrasound (US).
- Group 3 consisted of a total of 10 patients. A total of seven male and three female individuals were diagnosed with HCC based on findings from abdominal ultrasonography and triphasic CT scans (CT), which also revealed elevated levels of serum AFP.
- Group 4 consisted of a total of thirty patients, including 23 men and 7 females, all of whom were diagnosed with HCC by the use of abdominal US and triphasic CT. When there is a low concentration of AFP in the serum, it is associated with HCC.
- Group 5 included a total of thirty patients diagnosed with liver cirrhosis, with ten patients falling under the child A classification, another ten patients classified as child B, and the other ten patients classified as child C. The diagnosis of liver cirrhosis in all patients was validated by the use of Abdominal US and the child Pugh classification, with the additional criterion of normal AFP levels. All groups satisfied the following work ups.
- Comprehensive patient history: Special attention is given to symptoms that may indicate the presence of CLD or liver cirrhosis.
- Comprehensive clinical examination with particular attention given to doing a comprehensive abdominal examination and identifying any indications of liver cell failure.
- The first diagnostic modality used in this study was abdominal and pelvic ultrasonography of the liver as well as the presence of any hepatic focal lesions. The topic of interest is to the dimensions of the spleen and the presence of localized abnormalities.
- The laboratory tests conducted in this study included CBC, renal and hepatic function tests hepatitis surface markers, coagulation profile, serum AFP, and thioredoxin levels. The determination of these levels was with the use of ELISA.
- The test is based on the premise of using the bio source INS.EASIA refers to a solid phase Enzyme Amplified Sensitivity immunoassay that is conducted on micro titer plates. Sandwich ELISA is used in the ELISA Kit. This kit has a micro ELISA plate that has already been coated with an antibody that recognizes Human TrxR1.

2.1 Statistical Analysis

The statistical analyses were conducted using the SPSS software program for Windows, specifically version 16.0 (IBM, Chicago 2007). The values were reported as the mean plus or minus the standard error. Univariate analyses were conducted to compare HCC patients and healthy volunteers using a two-tailed Student's t test and X2-test. The results were further validated using Fisher's exact tests. This analysis was conducted using Spearman's coefficient test (r).

3. Results and Discussions

The most common type of primary liver cancer, hepatocellular carcinoma (HCC) is also one of the most common cancers worldwide. In industrialized countries, HCC is the third greatest cause of cancer-related death and the sixth most common kind of cancer overall. More than a million people each year die in the West from HCC-related causes. HCC is often thought to be a lethal type of cancer and a major cause of death in Egypt today. This is because chronic HCV infection is strongly linked to the development of cirrhosis. In many cases, the tumor is already at an advanced stage when it is initially diagnosed, leaving no curative treatment options. As a result, early diagnosis will not only

help the patient, but will also help keep healthcare costs from spiraling out of control.Since alpha-fetoprotein (AFP) was shown to be a secretion linked to HCC, it was proposed that screening blood AFP levels might be used to track and identify HCC (10). In high-risk groups, this method has been viewed favorably due of its low price and ease of availability when combined with abdominal ultrasonography. Recent research, however, has demonstrated that the diagnostic performance of AFP on its own falls short of expectations [11]. Blood levels of AFP, and more especially the core fucosylated glycoform of AFP known as AFP L3, are the major marker for tracking the progression of liver disease to liver cancer. The blood marker AFP has long been accepted for use in identifying those with an increased risk of HCC. For almost 40 years, doctors have been using AFP to assess patients with HCC and track their progress throughout treatment. A large percentage of people with chronic liver diseases have abnormally high levels of the blood AFP [11]. As a result, AFP's usefulness as a main screening tool for HCC has come under close investigation, necessitating the development of more sensitive and specific blood biomarkers to address this problem. Thioredoxin is a protein with the amino acid sequence (-Cys-Gly-Pro-Cys-) in its active core, which contains two redox-active cysteine residues. Several cancers, including HCC, have been shown to have abnormally high levels of thioredoxin expression. Mounting evidence suggests a link between high levels of thioredoxin expression and both rapid tumor growth and poor prognoses for patients' survival. According to Welsh et al.'s [12] research. The results of this research showed that those with HCC and elevated serum AFP levels differed significantly from those in the control group. Higher AFP levels in HCC patients were associated with higher serum thioredoxin levels, as well as a larger number (%) of high and normal values for both AFP and serum thioredoxin, as compared to the control group or healthy persons. In a study of patients with HCC, Chen and Zhang [13] found that their serum thioredoxin levels were significantly higher than those of healthy controls. Patients with HCC were found to have a median serum AFP concentration that was statistically substantially greater than that of healthy controls (p-value 0.001). Consistent with these findings are those published by Yang et al. [14], who found that serum AFP levels were significantly higher in patients with HCC than in the control group. Age, total bilirubin, direct bilirubin, INR, and serum creatinine were all observed to be higher in the patient group compared to the control group and the other three groups, respectively. Hemoglobin, platelet count, serum albumin, and PC were also demonstrated to be lower in the patient group, and these differences were statistically significant. In addition, there was a statistically significant difference in age, hemoglobin, platelet count, ALT, total bilirubin, direct bilirubin, serum albumin, PC, INR, and serum creatinine between the four patient groups. Serum thioredoxin and AFP levels were significantly different in the cirrhotic patients group compared to the control group. That's consistent with what Yang et al. (14) found. HCC patients with normal AFP levels and the control group had significantly different median (interquartile range) values for AFP and serum thioredoxin. In individuals with HCC, both AFP and serum thioredoxin levels were shown to be elevated. Serum thioredoxin levels were found to be significantly different between hemodialysis patients with chronic liver disease and Hepatitis C and a healthy control group. Serum thioredoxin levels were higher in the chronic liver disease group receiving hemodialysis for Hepatitis C compared to the healthy control group. This agrees with the research of Sumida et al., [15] who found that chronic HCV infection in hemodialysis patients was associated with considerably higher serum TRX levels compared to healthy persons without HCV. These findings suggest that oxidative stress is exacerbated in individuals on hemodialysis who also have a persistent HCV infection.

Many salient features of thioredoxin and AFP may be compared with one another. Thioredoxin levels were found to be significantly higher than AFP levels in the setting of chronic liver disease in Hepatitis C patients on hemodialysis. Thioredoxin had a median value of 126.5 and an IQR of 126, whereas AFP had a value of 2.8 and a value of 3.9 at the interquartile range (IQR). Consistent with this observation, Nakamura et al. [16] found that elevated thioredoxin levels occurred as a consequence of TRX buildup in the circulation owing to decreased renal clearance. However, this goes against the conclusions of a second research by Koshiba et al. [17]. Serum TRX was shown to be elevated in response to oxidative stress in patients with rheumatoid arthritis, pancreatic cancer, and severe burns, corroborating the findings of Tzitzikos et al., [18] Creatinine clearance was shown to be correlated with AFP levels, and this correlation was statistically significant. The idea of value is essential to many different areas of study. The results show that hemodialysis patients with chronic liver illness had an increase in AFP levels of less than 0.001.

When comparing AFP and thioredoxin levels in children with hepatic cirrhosis, it was shown that AFP levels were considerably higher in groups A, B, and C. Shen et al. [19] found that individuals with chronic hepatitis or cirrhosis, even in the absence of hepatocellular carcinoma (HCC), had increased AFP values. Thioreoxin levels were significantly higher in those with HCC and liver cirrhosis. Serum THIOREDOXIN levels were significantly higher in individuals with HCC than in those with liver cirrhosis, according to the study by Li et al. [20] Therefore, measuring blood thioredoxin levels may be a useful method for keeping tabs on individuals with HCC. Both thioredoxin and AFP were found to be increased in individuals with HCC in the High AFP group, with AFP having an IQR value of 687 (235.3) and thioredoxin having an IQR value of 113.7 (102.7). In contrast to thioredoxin, however, AFP showed much more growth. Supporting the idea that the combined model, which contains both thioredoxin and AFP indicators, demonstrates considerably greater discrimination capacity compared to the individual markers, are the results of an investigation by Chang YS et al. [21]. Thioredoxin has been shown to be more sensitive and specific than AFP in the diagnosis of HCC, according to research published by Li et al. [20]. When comparing the serum thioredoxin levels of Children A, B, and C, there was a statistically significant difference between Children C and A. Serum AFP levels were compared between the three Child categories, but no statistically significant differences were found. This agrees with what Zinkin et al. [22] found. In contrast to the Pugh classification, it was shown that a rise in thioredoxin levels accompanied the worsening of liver disease. AFP values exceeding 20 ng/ml have been found in approximately 40% of those with cirrhosis, according to studies by Harada et al

[23]. There was a statistically significant difference between the thioredoxin and AFP levels of individuals with HCC who were classified as having normal AFP or having negative AFP. Thioredoxin levels were found to be greater, with an IQR of 75.5 (74%), than AFP levels, which were found to have an IQR of 7.7 (14.6%). Based on their research, Li et al. (20) hypothesized that thioredoxin could be a more useful serum marker for the diagnosis of HCC than AFP, especially in patients with very early-stage HCC who tested negative for AFP. A steady rise in AFP levels, regardless of whether they surpass the threshold of 400 ng/mL, can be taken as a strong indicator of HCC, according to guidelines established in 2003 by the British Society of Gastroenterology [24]. Serum AFP and thioredoxin levels were significantly different amongst the four patient groups. In individuals with HCC, blood AFP levels are observed to be abnormally high. The greatest amounts of AFP are seen in patients with HCC who have high AFP levels, followed by those with HCC who have normal AFP levels, those with liver cirrhosis, and those with chronic liver disease who are on hemodialysis. Hemodialysis patients with chronic liver disease had higher blood thioredoxin levels than those of patients with HCC and high AFP, those with HCC and low AFP, or those with liver cirrhosis.

When it comes to diagnosing HCC, how sensitive and specific is thioedoxin? In our study serum thioredoxin sensitivity in detection HCC is 47.5 %. While specificity in detecting HCC82 % this percentage nearly to Chen and Zhang [13] published study in chines that detect thioredoxin a sensitivity of 84.3%and a specificity of 91.8%, with the area under the curve at 0.946 other wise AFP sensitivity 78.4%, specificity of 81.3%**.**

4. Conclusions

Serum thioredoxin (TRX) has the potential to serve as a valuable biomarker for the timely identification of HCC in individuals with viral cirrhosis. Furthermore, when combined with AFP, it has the capacity to enhance the diagnostic precision of HCC. However, more investigation is required to ascertain the most advantageous threshold values for TRX and to investigate the possible impact of inflammation and oxidative stress on TRX concentrations.

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