RESEARCH ARTICLE

Serum Biomarkers for Early Detection of Hepatocellular Carcinoma Associated with HCV Infection in Egyptian Patients

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Abstract

   <u>Background</u>: **Early detection of hepatocellular carcinoma using serological markers with better sensitivity and specificity than alpha fetoprotein (AFP) is needed. <u>Aims</u>: The aim of this study was to evaluate the diagnostic value of serum sICAM-1, β-catenin, IL-8, proteasome and sTNFR-II in early detection of HCC. <u>Materials and Methods</u>: Serum levels of IL-8, sICAM-1, sTNFR-II, proteasome and β-catenin were measured by ELISA assay in 479 serum samples from 192 patients with HCC, 96 patients with liver cirrhosis (LC), 96 patients with chronic hepatitis C (CHC) and 95 healthy controls. <u>Results</u>: Serum levels of proteasome, sICAM-1, β-catenin and αFP were significantly elevated in HCC group compared to other groups (P-value<0.001), where serum level of IL-8 was significantly elevated in the LC and HCC groups compared to CHC and control groups (P-value <0.001), while no significant difference was noticed in patients with HCC and LC (P-value=0.09). Serum level of sTNFR-II was significantly elevated in patients with LC compared to HCC, CHC and control groups (P-value <0.001); also it was significantly higher in HCC compared to CHC and control groups (P-value <0.001). ROC curve analysis of the studied markers between HCC and other groups revealed that the serum level of proteasome had sensitivity of 75.9% and specificity of 73.4% at a cut-off value of 0.32 µg/ml with AUC 0.803 sICAM-1 at cut off value of 778ng/ml, the sensitivity was 75.8% and the specificity was 71.8% with AUC 0.776. β-catenin had sensitivity and specificity of 70% and 68.6% respectively at a cut off value of 8.75ng/ml with an AUC of 0.729. sTNFR-II showed sensitivity of 86.3% and specificity of 51.8% at a cut off value of 6239.5pg/ml with an AUC of 0.722. IL-8 had sensitivity of 70.4% and specificity of 52.3% at a cut off value of 51.5pg/ml with AUC 0.631. <u>Conclusions</u>: Our data supported the role of proteasome, sICAM-1, sTNFR-II and β-catenin in early detection of HCC. Also, using this panel of serological markers in combination with αFP may offer improved diagnostic performance over αFP alone in the early detection of HCC.**

   Keywords: Hepatocellular carcinoma - αFP - sICAM-1 - proteasome - β-catenin - IL-8 - sTNFR-II

Introduction

   Hepatocellular carcinoma (HCC) is one of the most common primary malignancies of the liver which is not only increasing the global incidence but also is a major cause of cancer related mortality especially with a male predominance (Hiotis et al., 2012; Etsuji, 2013). Among the risk factors for developing HCC is the emergence of the hepatitis C virus (HCV), as it is considered to be the second most common cause of HCC and the leading etiology in Egypt, Japan and within the USA (Yamashita et al., 2011). Egypt has the highest prevalence of HCV in the world, estimated among the general population to be around 14% (El-Zanaty et al., 2009).

   The long term survival of postoperative HCC patients is unsatisfactory for the high incidence of recurrence. Moreover, HCC detection by serum αFP level is limited by its low sensitivity, so there is an urgent demand to identify new serological markers with high accuracy and feasibility for the early detection of HCC (Zhou et al., 2013). Serological markers could be divided into proteantigens, cytokines, enzymes and isoenzymes as well as transcripts of related genes (Zhou et al., 2013). In the current study, we aim to focus on the role of Interleukin 8 (IL8), soluble intercellular adhesion molecule -1 (sICAM-1), b etacatenin (β-catenin), soluble tumor necrosis factor receptor II (sTNFR-II) and proteasome as serological markers in the early detection of HCC.

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ICAM-1 is a proteantigen cell surface glycoprotein of the immunoglobulin super family. The expression of ICAM-1 mediates adhesion-dependent cell-cell interactions and facilitates the movement (or retention) of cells through the extracellular matrix which plays an important role in cells differentiation, movement and immunity (Lawson et al., 2009). In malignances the expression of ICAM-1 is up regulated due to its secretion by tumor cells, which may be one mechanism of tumor cells evading the body's immunity (Bernier et al., 2011). Therefore, it is of interest to elucidate the role of serum sICAM-1as a serological tumor marker in HCC patients.

Beta-catenin (proteantigen) is as a protein associated with the intracellular domain of E-cadherin, a component of the adherents junction (Aberle et al., 1996) .β-catenin is an essential component of the Wnt/Wingless (Wg) signaling pathway which is involved in a large variety of developmental processes, including cell fate regulation, proliferation and self-renewal of stem and progenitor cells (Clevers, 2006). Mutation affecting Wnt/ β-catenin pathway appears to be the most frequent event in HCC, and have been found in 20-40% of these tumors (Laurent-Puig et al., 2006). Therefore, it is of interest to illustrate the role of serum β-catenin in early detection of HCC.

The Proteasome is the main non-lysosomal proteolytic enzyme complex responsible for degrading proteins tagged by the ubiquitin conjugation system. It plays a vital role in degradation of short half-life proteins and peptides that are involved in basic cellular processes, such as cell-cycle regulation and apoptosis, transcriptional regulation, or antigen processing (Sorokin et al., 2009). The Proteasome most exclusively used in mammals is the cytosolic 26S Proteasome, which containing one 20S protein subunit and two 19S regulatory cap subunits (Sorokin et al., 2009). It has been reported that Proteasomes are often overexpressed in cancer e.g Human leukemia (Kumatori et al., 1990), renal cancer (Kanayama et al., 1991) and hepatocellular carcinoma (Henry et al., 2009). Therefore, it is of interest to elucidate the role of serum Proteasome as prognostic marker in early detection of HCC.

Interleukin-8 (IL-8), alternatively known as CXCL8, is a pro inflammatory CXC chemokine associated with the promotion of neutrophil chemotaxis (Waugh et al., 2008). IL-8 is produced by a wide variety of cell types, including macrophages, neutrophils, fibroblasts, and endothelial cells (Adler et al., 1994). It has direct effects on tumor and vascular endothelial cell proliferation, angiogenesis, and tumor migration (McConnell et al., 2009). Recent researches have indicated that IL-8 regulates tumor cell growth and metastasis in liver (Ren et al., 2003). Therefore, it is of interest to elucidate the role of serum IL-8 as a biological tumor marker in HCC patients.

Tumor necrosis factor-alpha (TNF-α) is a proinflammatory cytokine with a major role in both acute and chronic responses to viral, bacterial, fungal and parasitic infections (Bradley, 2008). TNF mediates various cellular responses by binding to two structurally distinct receptors: TNF receptor-1 (TNFR-I) and TNF receptor-II (TNFR-II), members of the TNFR superfamily (Rothe et al., 1992). Soluble TNF alpha receptors (sTNFR- I and II) are useful tools to investigate TNF alpha release,

which itself has a short half-life (Diez-Ruiz et al., 1995). It was previously reported that serum level of sTNFR-II was upregulated in hepatocellular carcinoma (Zekri et al., 2010). Therefore, it is of interest to elucidate the role of serum IL-8 as a biological tumor marker in HCC patients.

This current study was conducted as a confirmatory study of our previously published data by (Zekri et al., 2009), (Zekri et al., 2010) and (Zekri et al., 2011) in order to address the potential role of sICAM-1, β-catenin, Proteasome, IL-8 and sTNFR-II in early detection of HCC.

Materials and Methods

Study design

It was a case-control study conducted on 384 adult patients with HCV related chronic diseases divided into three groups: group 1 (HCC; n=192) attending the multidisciplinary HCC clinic, Tropical Medicine Department, Faculty of Medicine and National cancer institute (NCI), Cairo University; group 2 (Cirrhotic; n=96) attending Endemic Medicine Department ,Faculty of Medicine, Cairo University and group 3 (CHC; n=96) who were recruited from Kasr El Aini Viral Hepatitis Center, Faculty of Medicine, Cairo University, in addition to 95 healthy subjects who were enrolled as the control group during the period from May 2012 to April 2013. The study was approved by the Investigation and Ethics Committee of the hospital and a written consent was obtained from all the persons involved.

The HCC group was diagnosed by abdominal ultrasonography, triphasic CT abdomen, serum αFP and confirmed histopathologically with no evidence of local invasion or distant metastasis; Cirrhotic group was diagnosed by abdominal ultrasonography and patients with CHC group with elevated ALT for more than six months. The control group had no clinical or biochemical evidence of liver disease or known medical illness at recruitment and with normal abdominal ultra-sonography. All controls were negative for HBV and HCV infection as evidenced by negative serological markers and negative PCR for HBV and HCV. Exclusion criteria were patients with HBV infection and patients with HCC who received previous treatment or antiviral therapy for HCV. A detailed history, clinical assessment, biochemical liver profile, abdominal ultrasonography were done to all study groups in addition to serological testing and abdominal ultrasonography with Doppler examination to identify focal lesions and Triphasic CT scan for patients with hepatic focal lesions.

Collection of clinical specimens: 5 ml of venous blood was left to coagulate, and then centrifuged at 5000 rpm for 10 minutes. Serum was collected after a second centrifugation and then stored at -800C until used.

Measurement of serum biomarkers

Serum levels of, sICAM-1, IL-8, sTNFR-II were measured by a commercially available ELISA kit from (R&D Systems, Inc., USA), Proteasome was measured by ELISA kit from (Enzo Life Sciences, Inc., Switzerland) and β-catenin was measured by ELISA kit from (Glory Science Co., Ltd, USA) according to the manufacturer's instructions.

Statistical analysis

The SPSS software package (version 15) was used. Continuous variables were expressed as mean ± SD, median and interquartile range. Comparisons between groups were analyzed by the χ2 test or Fisher's exact test for categorical variables, and by the Mann-Whitney test or Student's t-test when appropriate for continous variables. P-value was considered significant when P-value ≤0.05. Correlation between the variables is calculated using Pearson's product moment correlation coefficient. Receiver operating characteristic (ROC) curves are plotted in order to determine the best cut-off values of the studied markers with its sensitivity and specificity.

Results

Detailed clinical data of all studied groups are shown in Table (1). Smokers were significantly higher in HCC compared to other groups (P-value <0.001). The median value of αFP was significantly higher in HCC compared to other groups (P-value <0.001). The percentage of diabetic patients was significantly higher in HCC and LC groups compared to CHC and control groups (P-value <0.001). The percentage of ascites in LC group was significantly higher compared to other groups (P-value <0.001). The median value of AST was significantly higher in HCC group compared to other groups. The median value of albumin was significantly higher in normal group than the

other studied groups (P-value<0.001). The median of T-bilirubin was significantly higher in LC group compared to other groups (P-value <0.001). The median value of ALT was significantly higher in HCC, LC and CHC groups compared to normal controls (P-value <0.001). Also it was noticed that there were a trend of elder age with progression of liver disease from CHC through cirrhosis to HCC groups, as the mean value of age for HCC was significantly different from LC, CHC and control group also mean value of age for CHC group was significantly different from control group (P-value<0.001).The severity of liver disease in the HCC and cirrhotic groups was
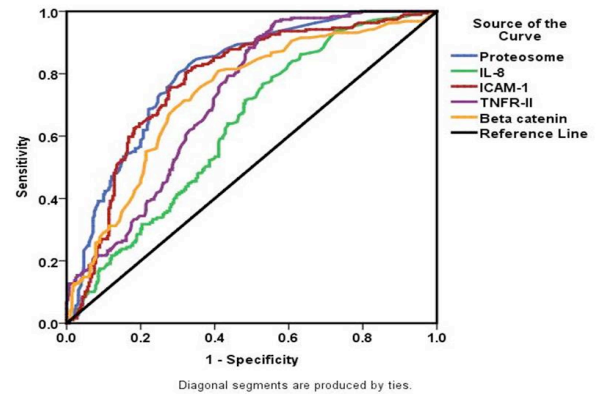

Figure 1. ROC Curve Analysis for Different Studied Markers between HCC (Malignant Cases) and Other Studied Groups (Non-Malignant Cases)

Table 1. Clinical Data of the Studied Groups

		No	HCC n= 192	Cirrhotic n=96	Non-Cirrhotic n=96	Control n= 95
Age: mean ± SD (Range)			56.7±7.7a (29-80)	54.01±8.3b (27-66)	40.54±8.82c (22-61)	33.37±11d (19-62)
Gender	Male	317	151a (79%)	67 a (70%)	78 a (81%)	21b (22%)
	Female	161	40 a (21%)	29 a (30%)	18 a (19%)	74b (78%)
Smoker	Yes	123	74a (39%)	26ab (27%)	20b (21%)	3c (3%)
	No	354	116a (61%)	70ab (73%)	76b (79%)	92c (97%)
D.M.	Yes	73	43a (22.5%)	22a (23%)	8b (8%)	0c (0%)
	NO	405	148a (77.5%)	74a (77%)	88b (92%)	95c (100%)
HCV Ab	Present	351	165a (86%)	90a (94%)	96b (100%)	0c
	Absent	125	24a (14%)	6a (6%)	0b (0%)	95c (100%)
HBs Ag	Present	0	0 (0%)	0 (0%)	0 (0%)	0 (0%)
	Absent	478	191 (100%)	96 (100%)	96 (100%)	95 (100%)
Ascites	Yes	155	80a (42%)	75b (78%)	0c (0%)	0c (0%)
	No	314	102a (58%)	21b (22%)	96c (100%)	95c (100%)
Child score	A	81	69 (36%)	12 (12.5%)	0	0
	B	96	69 (36%)	27 (28%)		
	C	106	49 (28%)	57 (59.5%)		
ALT:	mean		64.79± 52.737	63.6± 42.54	63.41±44.22	21.84± 5.04
	(Range)		(5-395)	(6-290)	(10-223)	(11-33)
	Median		51a	60a	54a	22b
AST:	mean		94.39± 105.61	53.91±31.72	47.53± 29.04	26.68± 5.07
	(Range)		(16-1155)	(16-151)	(8-167)	(15-37)
	median		77a	46.5b	39.5b	27c
T-Bilirubin:	mean		2.4± 2.63	3.25± 2.51	0.88±0.49	0.79± 0.16
	(range)		(0.3-25.8)	(0.2-19.7)	(0.3-5)	(0.5-1.1)
	Median		1.7a	2.8b	0.84c	0.8c
Albumin:	mean		3.02 ± 0.63	2.55 ±0.55	4.28 ± 0.4	4.45 ± 0.34
	(Range)		(1.8-4.8)	(1.7-4.1)	(3.2-5.4)	(3.8-5.2)
	Median		3.1a	2.5b	4.2c	4.5d
αFP:	mean		3933.25± 16142	35.21±40.1	18.9± 29.62	0
	(Range)		(1.5-114170)	(1.7-196)	(0.65-112)	0d
	Median		152a	17.9b	4.6c	
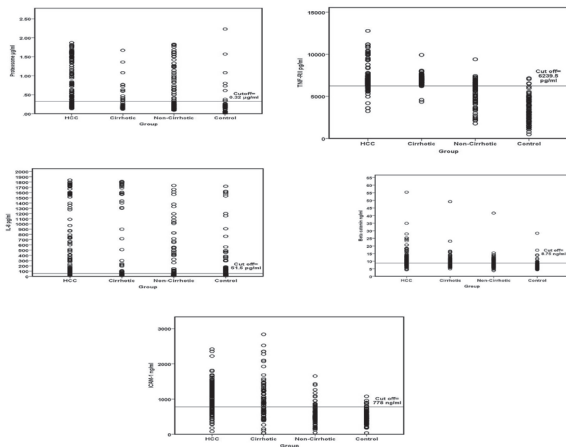
Figure 2. Scatter Plot of the Studied Markers in Different Investigated Groups Showing the Cutoff Values. Sample groups on x axis and protein concentrations on Y axis (A) Proteosome (B) TNFR-II (C) IL-8 (D) β-catenin (E) ICAM-1
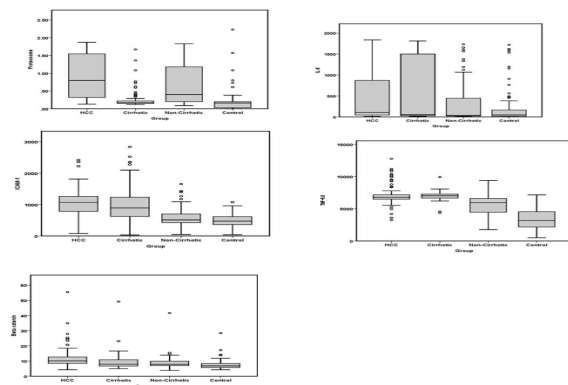
Figure 3. Box Plots Showing Distribution of Measured Serum Markers Levels with Median, Minimum and Maximum values in Different Investigated Groups and the Box Defines the Boundaries of the First and third Quartiles of Data. Sample groups on x axis and protein concentrations on Y axis (A) Proteosome (B) IL-8 (C) ICAM-1 (D) TNFR-II (E) β-catenin

assessed using the child score where 36% of HCC group were child A, 36% were child B and 28% were child C compared to 12.5%, 28% and 59.5 % in cirrhotic group respectively (P-value <0.001) matches the conclusion that LC group was in more decompensated pattern than HCC group.

The levels of studied biomarkers in the different groups were expressed as scatter plot showing cut-off in Figure (2), as box plot showing biomarkers distribution in Figure (3) and as mean ±SD, median and range Table (2). The median concentration of Proteasome was significantly higher in HCC than LC, CHC and control groups (P-value<0.001) with mean values 0.91±0.6, 0.25±0.248, 0.68±0.58 and 0.19±0.31 respectively. It was also significantly higher in CHC group than cirrhotic and control groups (P-value <0.001). Also the median concentration of serum IL-8 was significantly elevated in the LC and HCC groups compared to CHC and control groups with mean values 520.14±657.42, 552.9±732.2, 283.8±442.69 and 238.8±431.6 pg/ml respectively (P-value <0.001). However, no significant difference was noticed in patients with HCC and LC (P-value=0.09). On the other hand ,the median concentration sICAM-1 significantly elevated in HCC group compared to LC, CHC and control groups with mean values 1034.68±373.72, 978.17±540.17, 569.99±286.99 and 493±188.06 ng/ ml respectively (P-value <0.001). However, there was no significant difference between CHC and control groups (P-value=0.07). On the other hand ,the median concentration sICAM-1 significantly elevated in HCC group compared to LC, CHC and control groups with mean values 1034.68±373.72, 978.17±540.17, 569.99±286.99 and 493±188.06 ng/ml respectively (P-value <0.001). However, there was no significant difference between CHC and control groups (P-value=0.07). Also the median concentration of sTNFR-II was significantly elevated in patients with LC compared to HCC, CHC and control groups (P-value< 0.001), also it was significantly

Table 2. Levels of Studied Serological Markers in Different Studied Groups

	HCC n=192	Cirrhotic n=96	Non-Cirrhotic n=95	Control n=95	P value
Proteosome					
Median	0.8a	0.17b	0.4c	0.16d	<0.001
Mean±SD	0.91±0.6	0.25±0.25	0.68±0.58	0.18±0.3	
Range	0.13-1.87	0.12-1.67	0.09-1.83	0.01-2.22	
IL-8					
Median	107a	55a	36.5b	47b	<0.001
Mean±SD	518.19±656.23	552.9±732.2	283.76±442.7	238.8±431.6	
Range	14-1837	14-1811	8-1734	3-1719	
ICAM-1					
Median	1072.5a	892b	510.5c	473c	<0.001
Mean±SD	1034.73±372.7	978.17±540.17	570±287	493±188	
Range	79-2419	31-2838	46-1654	37-1078	
TNFR-II					
Median	6785a	7011.5b	5948.5c	3190.5d	
Mean±SD	7058.31±1338.78	6984.5±626.8	5456±1531.7	3400±1519	<0.001
Range	3250-12776	4351-9919	1772-9403	495-7157	
Beta catenin					
Median	10.1a	7.95b	7.85b	6.8c	<0.001
Mean±SD	11.26±5.49	9.34±5.13	8.81±4.07	7.5±3.2	
Range	4.3-55.4	5- 49.2	3.8-41.6	4.3-28.4	

*Groups bearing different initials are significantly different

Table 3. ROC Analysis Showing Cut-off, Sensitivity, Specificity, AUC, PPV and NPV of the Studied Markers

	Proteosome	IL-8	ICAM-1	TNFR-II	β-catenin
Sensitivity	75.9%	70.4%	75.8%	86.3%	70.7%
Specificity	73.4%	52.3%	71.8%	51.8%	68.6%
AUC	0.803	0.631	0.776	0.722	0.729
Accuracy	75.47%	59.74%	73.17%	66.95%	69.25%
PPV	67.13%	49.81%	63.72%	55.74%	59.73%
NPV	82.38%	72.68%	81.67%	85.8%	77.78%
Cut-off	0.32 μg/ml	51.5pg/ml	778 ng/ml	6239.5pg/ml	8.75 ng/ml

higher in HCC compared to CHC and control groups (P-value< 0.001) and with mean values 7059.35±1342.24, 6984.5±626.8, 5456±1531.66 and 3400±1519 pg/ml respectively. Median concentration of β-catenin was significantly elevated in HCC group compared to the other groups (P-value <0.001) with mean values 11.28±5.49, 9.34±5.13, 8.8±4.07 and 7.5±3.2 ng/ml respectively, whereas there was not any significant differences in its levels among LC and CHC groups (P= 0.76).

Further analysis of the data using Receiving Operating Characteristic (ROC) analysis curves and the corresponding area under the curve were attempted for the studied markers to investigate their diagnostic accuracy between HCC and other groups demonstrating cut off values with their specificity ,sensitivity, PPV(positive predictive values) and NPV(negative predictive values) as shown in Table (3) & Figure (1). It has been revealed that serum level of Proteasome had a sensitivity of 75.9% and specificity of 73.4% at cut off value of 0.32 μg/ml, PPV was 67.13% and NPV was 82.38% with AUC 0.803 and the overall accuracy was 75.47%. Serum level of IL-8 had sensitivity of 70.4% and specificity of 52.3% at cut off value of 51.5pg/ml, PPV was 49.81% and NPV was 72.68% with AUC 0.633 and the overall accuracy was 59.74%. While serum level of sICAM-1 had a sensitivity of 75.8% and the specificity of 71.8 at cut off value of 778 ng/ml, PPV was 63.72%, NPV was 81.67% with AUC 0.766 and the overall accuracy was 73.17%. Serum level of sTNFR-II showed a sensitivity of 86.3% and specificity of 51.8% at cut off value of 6239.5pg/ml, PPV was 55.74%, NPV was 85.8% with AUC 0.722 and the overall accuracy was 66.95%. Serum level of β-catenin had a sensitivity and specificity of 70% and 68.6% respectively at cut off value of 8.75ng/ml, PPV was 59.73%, NPV was 77.78% with AUC 0.729 and the overall accuracy was 69.25%.

Discussion

Hepatocellular carcinoma (HCC) is a common problem worldwide which ranks the 5th and 7th most common cancer among men and women respectively (Goe et al., 2014). In Egypt, hepatocellular carcinoma (HCC) ranks as the second most common cancer in men and the 6th most common cancer in women (GLOBACAN, 2008). This rising incidence of HCC may be due to high prevalence of hepatitis C virus (HCV), estimated among the general population to be around 14% (El-Zanaty et al., 2009). The prognosis of HCC is very difficult which necessitates finding of new appropriate either single or panel serum markers that could be used for early detection of HCC in high risk patients. As the level of serum alpha-

fetoprotein (αFP), recommended marker, frequently was not elevated at a significant proportion in patients with early-stage, potentially curable, HCC (Zhou et al., 2013).

Our aim of this study is to evaluate serum Intercellular Adhesion Molecules (sICAM-1), Beta catenin (β-catenin), Interleukin 8 (IL-8), Proteasome and soluble Tumor Necrosis Factor Receptor II (sTNFR-II) in early detection of HCC.

The current study showed that serum level of αFP was significantly elevated in patients with HCC than other groups, a finding that came in agreement with previous studies of many authors (Gad et al., 2005; Mittal et al., 2011; Guan et al., 2012).

The serum level of sICAM-1 was significantly elevated in HCC group compared to LC, CHC and control groups (P-value <0.001). However, there was no significant difference between CHC and control groups (P-value =0.07). This data comes in agreement with previous reports by (Shimizu et al., 1995; Moriyama et al., 2006 and Mohamed et al., 2014), who reported that increasing level of sICAM-1 over time are significant risk factors for the occurrence of HCC in patients with HCV-associated CH or liver cirrhosis (LC). The same conclusion was detected at gene level by (Rizk et al., 2012). Against to our results, the study results by (Hyodo et al., 1993) who reported that there was no difference in serum levels of sICAM-1 between their patients with HCC and liver cirrhosis. This is may be due to difference in the sensitivity of ELISA kit and small population size. The elevated serum level of sICAM-1 in HCC group is due to the tumor antigens stimulate the expression of ICAM-1 by immune cells in addition to the expression of ICAM-1 by tumor cells themselves (Bernier et al., 2011). Serum level of sICAM-1 showed a sensitivity of 75.8% and a specificity of 71.8% at a cut off level 778ng/ml with AUC 0.776 between HCC and other groups. Thus, regular measurements of sICAM-1 concentrations would be of clinical significance and could be used as a prognostic marker for early detection of HCC with fair diagnostic accuracy as AUC was in the range of (0.7-0.79).

Serum level of β-catenin was significantly elevated in HCC group compared to the other groups (P-value <0.001), whereas there was not any significant difference in its level among LC and CHC groups (P-value= 0.76). This result comes in agreement with previous studies by (Zekri et al., 2011), where higher serum level of β-catenin was measured in patients with HCC compared with those in CH, ASC (chronic HCV with persistent normal alanine aminotransferase levels) and healthy controls patients. In concordance with our results, previous work by (Li et al., 2014; Guan et al., 2012) who reported higher nuclear

accumulation of β-catenin by immunohistochemistery in HCC tissue than in the corresponding para-carcinoma, cirrhotic and normal tissues. The elevated level and stability of the β-catenin and in HCC group is due to point mutation in its amino terminus that changes the serine and threonine residues, thereby blocking its phosphorylation and subsequent degradation (Morin et al., 1997; Rubinfeld et al., 1997). Also, other studies revealed that mutation in Axin-1 gene, an important regulator of β-catenin, leads to the stability of the β-catenin and subsequent its nuclear accumulation (Satoh et al., 2000). Hence, Wnt/β-catenin pathway is activated that is involved in many vital processes including cell proliferation (Clevers, 2006). Serum level of β-catenin had a sensitivity and specificity of 70% and 68.6% respectively at cut off value of 8.75ng/ml with AUC 0.729 between HCC and LC groups. This data suggested that serum level of β-catenin could be used as prognostic marker for early detection of HCC with fair diagnostic accuracy as AUC was in the range of (0.7-0.79).

Serum level of Proteasome was significantly higher in HCC than LC, CHC and control groups (P-value<0.001). This result comes in agreement with previous studies by (Henry et al., 2009) where plasma Proteasome level was significantly higher in patients with HCC compared to controls and LC patients. However, serum level of Proteasome was also significantly higher in CHC group than cirrhotic and control groups (P-value <0.001). The possible role of Proteasome in hepatocellular carcinoma was revealed from study by (Higashitsuji et al., 2005) who reported the over expression of the gankyrin protein by gene expression analysis. This protein was identified as component of the 19S Proteasomal regulatory particle and it can associate with protein Mdm2 (E3 ligase of p53) and promote its interaction with p53 (tumor suppressor gene) and subsequent degradation. P53 is implicated in a wide range of cell processes including apoptosis induction, stimulation of DNA reparation, cell cycle arrest, and regulation of basic metabolism; so its degradation leads to increasing cell proliferation and subsequently tumor formation (Chumakov, 2007). Also, the potential role of Proteasome in hepatocellular carcinoma was revealed from previous study by (Albekairy et al., 2013) who reported that application of Proteasome inhibitors represent a noval potential therapy in liver cancer. Serum level of Proteasome had a sensitivity of 75.9% and specificity of 73.4% at cut off value of 0.32 μg/ml with AUC 0.803 between HCC and other groups. This data suggested that serum level of Proteasome is a reliable marker for early detection of HCC with good diagnostic accuracy as AUC was in the range of (0.8-0.89).

The level of serum IL-8 was significantly elevated in the HCC and LC groups compared to CHC and control groups (P-value <0.001). This result comes in agreement with previous study by (Ren et al., 2003) as the level of serum IL-8 was significantly elevated in the HCC group compared to control group. Also, this result comes in concordance with previous study by (Zimmermann et al., 2011) as the level of serum IL-8 was significantly elevated in the LC group compared to CH and control groups. However, no significant difference was noticed between HCC and LC groups, also between CHC and

control group (P-value=0.09) in the current study. The high serum level of IL-8 in HCC group is due to its secretion by tumor cells. The high serum level of IL-8 in LC group is due significantly increased numbers of macrophages with progression of liver fibrosis\cirrhosis which also the main important target of IL-8 in liver cirrhosis (Zimmermann et al., 2011). Serum level of IL-8 had a sensitivity of 70.4% and specificity of 52.3% at cut off value of 51.5pg/ml with AUC 0.631 between HCC and other groups. This result suggested that diagnostic accuracy of IL-8 in the early detection of HCC was poor as AUC was in the range (0.60-0.69).

Serum level of sTNFR-II was significantly elevated in patients with HCC compared to CHC and control groups (P-value< 0.001). This result comes in agreement with previous work by (Zekri et al., 2010), where serum level of sTNFR-II was significantly elevated in patients with HCC compared to CLD (chronic liver disease associated with elevated liver enzyme levels), ASC and control groups. However, the serum level of sTNF-RII was significantly elevated in patients with LC compared to HCC in the currennt study (P-value< 0.001). The possible role of TNF-α receptor 2 signaling pathway was the activation of two important transcription factors NF-ϰB or AP-1 (Molenates et al., 2013). NF-ϰB pathway play a vital role in tumorgensis as it controlled many vital process e.g productions of cell regulator and anti-apoptotic proteins resulting in stimulation of cell proliferation and inhibition of apoptosis. Also, NF-kB pathway play a vital role in inflammation, largely based on the role of NF-kB in the expression of pro inflammatory genes including cytokines, chemokines, and adhesion molecules (Lawrence, 2009). Serum level of sTNFR-II showed a sensitivity of 86.3% and specificity of 51.8% at cut off value of 6239.5pg/ml with AUC 0.722 between HCC and other groups. This result suggested that sTNFR-II could be used as prognostic marker for early detection of HCC with fair diagnostic accuracy as AUC was in the range of (0.7-0.79).

In conclusion, These findings support the potential role of Proteasome, sICAM-1, sTNFR-II and β-catenin in early detection of HCC and discrimination of malignant cases from the other studied non-malignant cases. Also, using this panel of serological markers in combination of αFP may offer improved diagnostic performance over αFP alone in the early detection of HCC. .

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