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RESEARCH ARTICLE

Soluble CD25 and Hepatocellular carcinoma

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Abstract

Hepatocellular carcinoma (HCC) is the fifth most common cancer with relatively poor overall survival all over the world. The most commonly used tools for diagnosis are ultrasonography and measurement of α fetoprotein (AFP) in the blood. However there are limitations in this marker's sensitivity and specificity that necessitates the need for a biomarker that is able to detect HCC at an early stage.

Soluble CD25 (sCD25) is significantly elevated in a small series of HCC patients compared to controls also a significant positive correlation between the level of sCD25 in HCC patients and the tumor stages. This work aimed to evaluate sCD25 as a marker for HCC detection and whether it correlate with tumor stage. **Subjects &methods:** To achieve this aim, 134 hepatic patients were recruited from tropical department and Hepatology unit, Specialized Medical Hospital, Mansoura Faculty of medicine and divided in to two groups, **HCC group** which comprised 90 patients with mean age 52 y (range 30 – 69 y) and **Cirrhotic group** which comprised 44 patients with mean age 48.5 y (range 37 – 69 y). Thirty five healthy subjects with matched age and sex were included in this study as control group. Alpha fetoprotein was measured by ELISA using (R & D systems Inc., USA). While sCD25 was assayed using (Cell Science, Inc, Bldg Canton, MA). **Results:** There was significant increase in sCD25 level in HCC versus control and cirrhotic groups while no significant difference in its level in cirrhotic group versus control**.** Also a significant increase in sCD25 was observed in stage C and stage B in comparison to stage A**.** By using a cut off value > 1425 pg/ml, sCD25 had a sensitivity of 64.44% and Specificity of 96.15% (AUC: 0.7959). As regard AFP, at a cut off value > 21.45 ng/ml, the sensitivity was 73.33% and the specificity was 86.36% (AUC: 0.7933). **Conclusion:** sCD25 distinguish HCC from appropriate controls and this marker identify the presence of HCC more effectively than AFP in patients with early tumors. The high specificity of sCD25 suggests it holds a promise as a marker for early HCC detection which is an area of unmet need. To further characterize the utility of sCD25 in detecting early stages of HCC tumor development, larger longitudinal and validation studies are planned.

INTRODUCTION

HCC is the fifth most common cancer with relatively poor overall survival all over the world ***(Elserag,2011).*** HCC is unique compared with other human cancers as the majority of HCC occurs in patients with

chronic liver diseases, especially viral hepatitis (ie, hepatitis C and hepatitis B) and liver cirrhosis whatever the etiology ***(Elserag and rudolph,2007).*** There is latent period usually of 10 to 30 years from the original disease to the development of HCC; this long window of time provides an opportunity for investigating cancer development and designing strategies for intervention.

The current screening methods of ultrasonography and measurement of AFP in the blood have limitations from limitations in this marker's sensitivity and specificity. ***(Elserag et al.,2011)***. AFP is not elevated in a significant number of HCC ***(*Sherman,2011*)*** The poor sensitivity of AFP explains its absence from the AASLD practice guide-lines as a test recommended for screening of HCC ***(*Bruix and Sherman , 2005**). This substandard sensitivity necessitates the need for a biomarker that has the ability to detect HCC at an early stage.

Interleukin-2 (IL-2), an important cytokine in vivo, that is able to induce T cell proliferation and activation after binding to its IL-2 receptor (IL-2R) and then enhance the immune response in vivo. sCD25, is the free form of IL-2Rα subunit, can competitively bind to IL-2 with IL-2R, and inhibit the proliferation of lymphocyte and down regulate the activity of NK cells, lowering the immune function. So, circulating sCD25 level is considered as an indicator of the degree of immune inhibition ***(*Goto et al., 2005*). (*Bien and Balcerska 2008*)*** suggested that in most patients with haematological malignancies, tumor cells continuously producing a large number of sCD25 which is the main reason for the upregulation of sCD25.

sCD25 is significantly elevated in a small series of HCC patients compared to controls and a significant positive correlation between the level of sCD25 in the serum of HCC patients and the tumor stage of patients with HCC. ***(Roniel Cabrena et al.,2012)***

Aim of Work:

This work aimed to evaluate sCD25 as a marker for detection of HCC and whether it correlate with tumor stage

Subjects and Methods:

This study was carried out on 134 hepatic patients recruited from tropical department and hepatology unit, specialized medical hospital, Mansoura Faculty of medicine in 2013- 2014. Thirty five healthy subjects with mean age 48.5 y (range 33 - 65 y) and they were 25 males and 10 females were used as a control group. The patients were divided into two groups:

- ***HCC group:*** They comprised 90 patients with mean age 52 y (range 30 – 69 y). They were 67 males and 23 females. There were 28 patients with stage A and 26 patients with stage B and 27 patients with stage C. HCV was reported in 82 cases (91.1%) and HbsAg in 4 cases (4.44%).
- ***Cirrhotic group:*** they comprised 44 patients with mean age 489.5 y (range 37 – 69 y). They were 28 males and 16 females.

All patients were subjected to through history taking, physical examination, routine laboratory investigations (CBC, Liver profile including ALT, AST, total bilirubin and albumin, prothrombin time, RBS, creatinine), alpha feto-protein, sCD25 assay, US and CT.

This study was approved by the Ethical Committee of Mansoura University and all patients provided written informed consent prior to participation in any protocol-specific procedures.

Seven ml venous blood samples were withdrawn from each patient and divided as follow; 1ml into EDTA tube for CBC, 1.8ml into prothrombin tube for INR and the rest into plain tube, left to clot and serum were separated into two aliquots, one used for routine analysis and the other was stored at -20°C until sCD25 and alpha feto-protein assay.

CBC was analyzed using automated counter, Sysmex KX-21, USA. Routine laboratory investigations were analyzed using Dimension Xpand plus chemistry auoanalyzer, Siemens. Alpha fetoprotein was assayed by ELISA using (R & D systems Inc., USA). While sCD25 was assayed using (Cell Science, Inc, Bldg Canton, MA)

Statistical analysis.

Data for sCD25 and AFP levels are expressed as box plots with medians and range. Receiver operator characteristic (ROC) curves with respective points of maximal accuracy for sensitivity and specificity were generated to determine biomarker performance.. We used the Tukey's Multiple Comparison Test to assess the significance of group differences. Spearman's rank correlation coefficient was used to examine the correlation between the level of sCD25 and other laboratory parametrs. $P<0.05$ was considered to indicate a statistically significant result. Statistical data were analyzed using GraphPad Prism 5 version 5.01

Results*:*

Figure 1 represents the clinical presentation of HCC patients. Weight loss and dyspepsia were the most common presentations (26 cases each (28.9%), fatigue in 24 cases (26.7%) and right hypochondrial pain in 14 cases (15.6%). Splenomegaly were reported in 46 cases (51.1%)

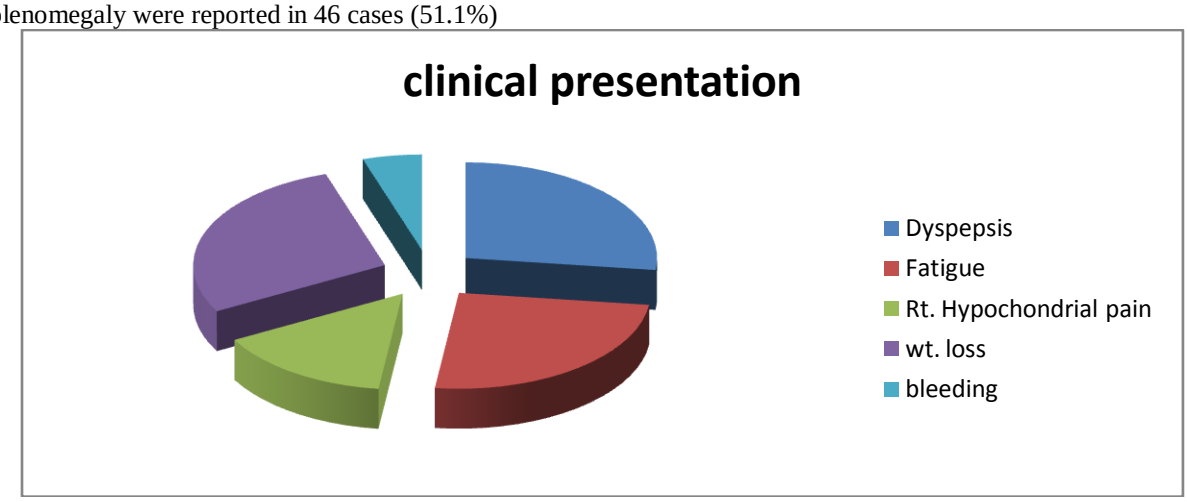


Table 1: Routine laboratory investigations in studied patient groups:
AST, bilirubin, albumin, INR, RBS and AFP were significantly higher in HCC group and liver cirrhosis group when compared to control group while no difference between HCC group and cirrhosis group. AFP, RBS and Creatinine showed no significant difference between liver cirrhosis group and control group. However, they were significantly higher in HCC group when compared to liver cirrhosis group or control group. ALT was significantly higher in all patient groups compared with each other.

	Control (n=25)	Cirrhotic (n=44)	HCC (n=90)	F	p-value	Tukey's Multiple Comparison Test	
						q	p-value
ALT (U/L):				**19.78**	**< 0.001**	**8.894**	**P1: < 0.05**
• **Median**	**20**	**51**	**45**			**6.222**	**P2: < 0.05**
• **Range**	**13 – 31**	**19 - 155**	**13 – 115**			**4.429**	**P3: < 0.05**
AST (U/L):				**18.4**	**< 0.001**	**7.683**	**P1: < 0.05**
• **Median**	**20**	**53**	**60**			**8.097**	**P2: < 0.05**
• **Range**	**12 - 30**	**26 - 180**	**21 – 200**			**0.530 6**	**P3: > 0.05**
Bilirubin (mg/dl):				**28.0**	**< 0.001**	**8.700**	**P1: < 0.05**
• **Median**	**0.8**	**2.65**	**2.4**			**10.40**	**P2: < 0.05**
• **Range**	**0.7 – 1.0**	**0.8 – 5.7**	**0.6 – 7.1**			**0.892**	**P3: > 0.05**
Albumin (gm/dl)				**71.6**	**0.0113**	**14.84**	**P1: < 0.05**
• **Median**	**4.8**	**3.0**	**3.0**			**16.19**	**P2: < 0.05**
• **Range**	**3.9 – 5.1**	**1.6 – 4.1**	**1.6 – 4.2**			**0.362**	**P3: > 0.05**
Creatinine (mg/dl)				**8.75**	**< 0.001**	**2.116**	**P1: > 0.05**
• **Median**	**0.6**	**0.8**	**0.9**			**5.434**	**P2: < 0.05**
• **Range**	**0.6 – 0.9**	**0.7 – 1.2**	**0.6 – 1.4**			**3.732**	**P3: < 0.05**
RBS (mg/dl)				**3.0**	**< 0.001**	**2.420**	**P1: > 0.05**
• **Median**	**84**	89	**110**			**6.801**	**P2: < 0.05**
• **Range**	**75 - 99**	72 - 196	**75 – 186**			**4.977**	**P3: < 0.05**
INR				**47.2**	**< 0.001**	**12.25**	**P1: < 0.05**
• **Median**	**1.1**	**1.5**	**1.5**			**13.02**	**P2: < 0.05**
• **Range**	**1.0 – 1.2**	**1.1 – 1.7**	**1.0 – 1.7**			**0.723**	**P3: > 0.05**

Hb (gm/dl)						**6.18**	**P1: < 0.05**
• **Median**	**13.5**	**10.6**	**11.6**	**9.55**	**<0.001**	**4.22**	**P2: < 0.05**
• **Range**	**10 – 14.2**	**7.1 – 14.7**	**6.3 – 15.4**			**3.2**	**P3: > 0.05**
WBCs (x10^3 /cmm)						**0.158**	**P1: > 0.05**
• **Median**	**5.2**	**4.2**	**4.9**	**5.75**	**0.003**	**3.54**	**P2: < 0.05**
• **Range**	**4.6 – 7.2**	**2.1 – 6.2**	**2.3 – 15.5**			**4.08**	**P3: < 0.05**
PLT (x10^3 /cmm)						**11.59**	**P1: < 0.05**
• **Median**	**292**	**143**	**105**	**61.1**	**< 0.001**	**15.61**	**P2: < 0.05**
• **Range**	**152 - 420**	**59 - 372**	**37 – 287**			**3.31**	**P3: > 0.05**
AFP (ng/ml)						**0.396**	**P1: > 0.05**
• **Median**	**3**	**12.35**	**87.3**	**14.46**	**<**	**5.720**	**P2: < 0.05**
• **Range**	**1.0 – 5.0**	**1.7 – 28.0**	**2.7 – 966**		**0.0001**	**6.387**	**P3: < 0.05**
P1: cirrhotic vs control		P2: HCC vs control			P3: cirrhotic vs HCC		

Figure (2), AFP in different HCC stages. There is no significant differences between different stages of HCC

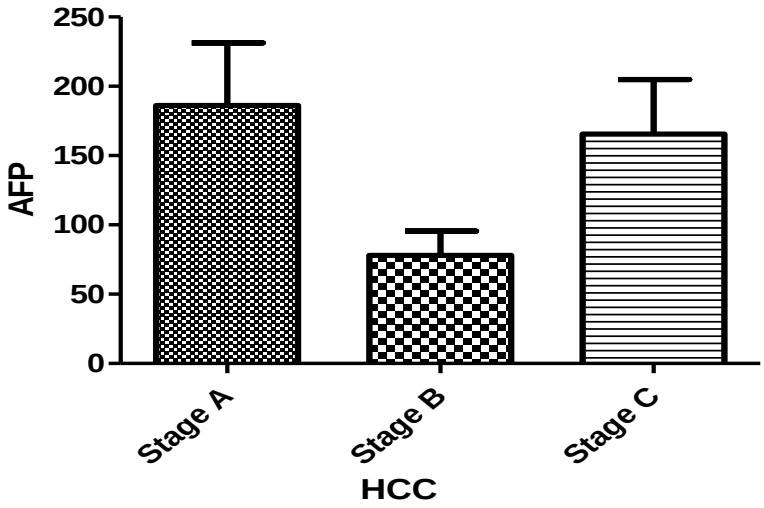


Figure (3): sCD25 in studied patient groups and in different stages of HCC: by comparing sCD25 levels in studied patient groups, there was significant increase in its level in HCC versus control and cirrhotic group while no significant difference in its level in cirrhotic group versus control**.** Also a significant increase in sCD25 was observed in stage C and stage B in comparison to stage

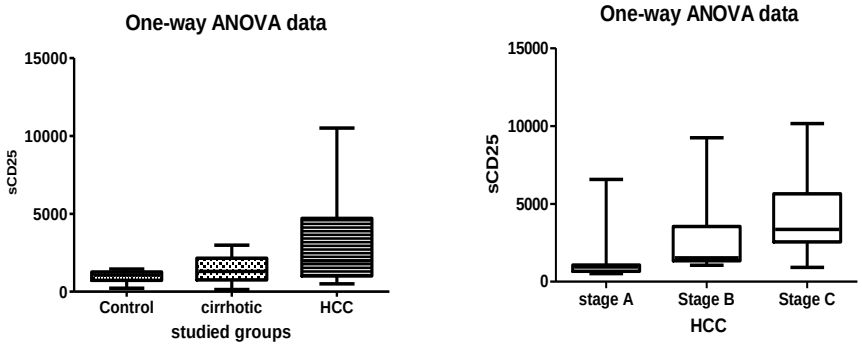


Figure (4): correlation coefficient between sCD25 and both albumin and INR: there was a negative correlation between sCD25 and albumin and significant positive correlation between sCD25 and INR. No significant correlation was found between sCD25 and other studied parameters (ALT, AST, bilirubin, creatinine, RBS and **AFP)**

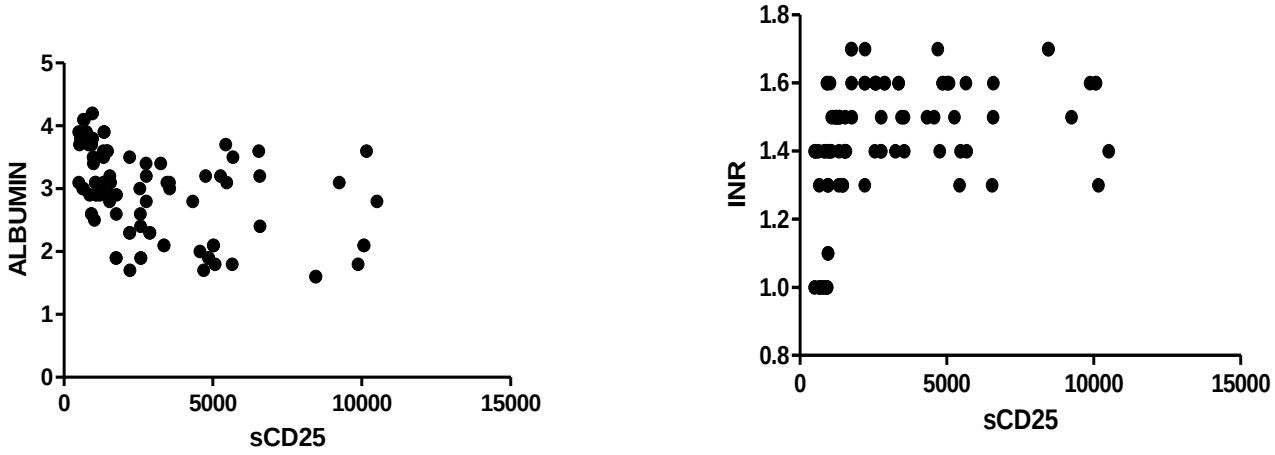
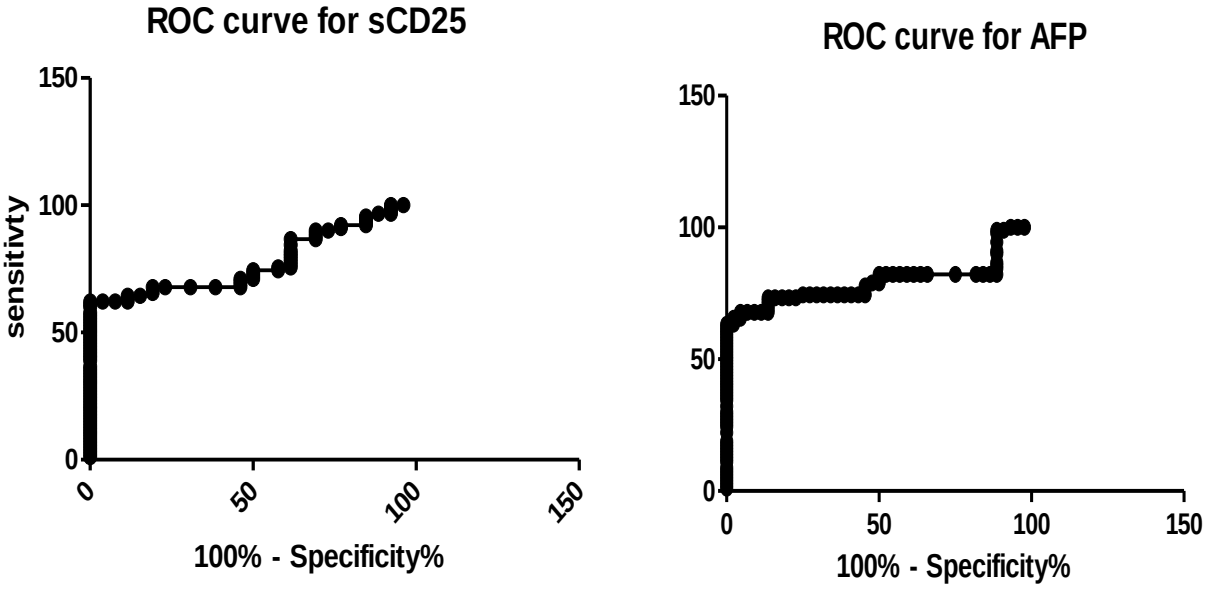


Figure (5): Roc curve for sensitivity and specificity of both sCD25 and AFP in detecting HCC. By using a cut off value > 1425 pg/ml, sCD25 had a sensitivity of a sensitivity of 64.44% and Specificity of 96.15% (AUC: 0.7959). As regard AFP, at a cut off value > 21.45 ng/ml, the sensitivity was 73.33% and the specificity was 86.36% (AUC: 0.7933)



Discussion*:*

This study demonstrated a male predominance of HCC (77.7%) and this is in accordance with the study conducted by***, (Roniel Cabrena et al.,2012)***. They reported that the majority of HCC patients were male (79%). The average age of the HCC patients was 52 years and this is in agreement with the study of***, (Mohamed et al.,2013)*** that reported the most frequent age category affected by HCC was between 51 and 60 years (45.7%).

A near-equal distribution of patients was reported across all stages of HCC, with 28 patients having stage A disease, 27 patients being in the stage B and 26 patients having stage C cancer. This is in agreement with***, (Roniel Cabrena et al.,2012)*** who reported 48 patients having stage A disease (early HCC), 45 patients being in the stage B subset (intermediate HCC) and 50 patients having stage C cancer (advanced HCC).

Investigations in Egypt have shown the increasing importance of HCV infection in the etiology of liver cancer, so we reported that HCV Ab was positive in 82 cases (91.1%) and HbsAg in 4 cases (4.44%) and this in agreement with***, (Mohamed et al.,2013)*** who reported that HCV Ab was detected in 91.32% of the studied patients while HBV infection was reported in 2.51%.

Weight loss and dyspepsia were the most common presentations (26 cases each (28.9%), fatigue in 24 cases (26.7%) and right hypochondrial pain in 14 cases (15.6%). Splenomegaly were reported in 46 cases (51.1%). ***(Elserag et al.,2008)*** reported that patients with HCC present with one or more of several clinical features including right upper quadrant pain, weight loss, and/or worsening liver enzymes in a patient known to have cirrhosis.

AFP and liver US are the most widely used tools for HCC surveillance however the performance of US depends on the experience of the examiner, the technology used, the body habitus, the presence of cirrhosis, and the size of the tumor. A regard AFP we found significant increase in its level in HCC and cirrhosis groups versus control. It was normal (< 7 ng/ml) in 12 patients (13.3%). At a cut off value of 21.45 ng/ml, the sensitivity was 73.33% and the specificity was 86.36% (AUC: 0.7933)***, (Roniel Cabrena et al.,2012)*** found that α-fetoprotein (AFP) had a sensitivity of 53.8% and a specificity of 86.8% at a cut-off value of 32.8 ng/ml (AUC=0.755; P<0.0001)

sCD25 levels were significantly increase in HCC versus control and cirrhotic group and by using a cut off value of 1425 pg/ml, sCD25 had a sensitivity of 64% and Specificity of 96.15% for the presence of HCC this is in agreement with***, (Ararat et al.2011,)*** who reported that the level of sCD25 in the serum of HCC patients was significantly higher (mean+SE: 12,799 + 2,030 pg/mL) than in the control group having cirrhosis without HCC (3,585 + 293 pg/mL; p.0.00002). They also reported that at a cut-off of >5,150 pg/mL the serum level of sCD25 had a sensitivity of 62.1% and a specificity of 83.3% (AUC=0.740, p< 0.0001). however our levels are lower than their levels, this may be related to different genetic, environmental factors and duration of HCC.

sCD25 is correlated to severity of liver disease in the form of negative correlation between sCD25 and albumin and significant positive correlation between sCD25 and INR. We also observed a positive correlation between the level of sCD25 and the degree of tumor burden of HCC, with levels of sCD25 progressively increasing from early (stage A) to advanced stage (stage C) HCC while AFP show non-significant difference among different HCC stages. This positive correlation between serum levels of sCD25 and tumor stages suggest that the measurement of serum levels of the immune marker sCD25 may improve earlier detection of HCC and could potentially be a useful novel prognostic marker. This is in agreement with***, (Roniel Cabrena et al.,2012)*** who found a significant positive correlation between the level of sCD25 in the serum of HCC patients and the tumor stage of patients with HCC.

Summary and Conclusion:

We currently lack a reliable serum marker for the early detection of HCC. Our findings show that sCD25 distinguished HCC from appropriate controls and that this marker identified the presence of HCC more effectively than AFP in patients with early tumors. The high specificity of sCD25 suggests it holds promise as a marker for early HCC which is an area of unmet need. To further characterize the utility of sCD25 in detecting early stages of HCC tumor development, larger longitudinal and validation studies are planned.

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