Suez Canal University Medical Journal Vol. 21 (2),2018
Pages 99-106

Assessment of the Level of Soluble CD25 as a Marker for the Detection of Hepatocellular Carcinoma in Chronic Hepatitis C virus (HCV) Infected Patients

Shereen Z. Wagrees[1], Hanaa F. Abdelaziz[1], Basma Badr EL-Dein[2*] and Seham A. Omar[3]

Departments of [1]Clinical Pathology, Suez Canal University, [2]Clinical Pathology, Port Said University, and [3]Internal Medicine, Suez Canal University, Egypt

Abstract

***Background*: Hepatitis C virus (HCV) is one of the most potential pathogens all over the world. Egypt has the highest HCV prevalence in the world. The hepatocellular carcinoma (HCC) is among cancers with the poorest outlook. The detection of HCC improves the outcome. The poor sensitivity of AFP underlines the need for a biomarker that can detect HCC. It was found that the CD25 is increased in patients with HCC. *Aim*: To assess the performance of serum soluble CD25 (sCD25) in the prediction of early HCC and compare it to α-fetoprotein (AFP); the classical biomarker of HCC. *Patients and Methods*: This study was a descriptive study. Patients were recruited from the Hepatology and Gastro-enterology department at Suez Canal University hospital. The study included 60 subjects, normal healthy individuals (n=20), cirrhotic patients (n=20) and HCC patients (n=20). 2 blood samples were collected from each patient one for liver profile and second stored for sCD25. Liver function tests, AFP and sCD25 were done to all the participants. *Results*: Our results show a highly significant increased levels of sCD25 in patients with cirrhotic liver and HCC compared to normal controls, (*p*=0.001). No difference was found in sCD25 levels in HCC patients compared to liver cirrhosis patients (*p*=0.862). *Conclusion*: sCD25 can differentiate HCC patients from normal healthy persons but not from cirrhotic patients. Thus, sCD25 cannot be used as an accurate diagnostic marker for HCC.**

***Key words*:** sCD25, HCC, Liver cirrhosis, Hepatitis C

Introduction

Hepatitis C virus has been considered one of the most potential pathogens that have hindered the medical community all over the world. Indeed, since its discovery in 1989, hepatitis C virus (HCV) has been recognized as a major cause of chronic liver disease worldwide[(1)]. The data reported by WHO estimated that the prevalence of HCV infection is 2.2%, and more than one million new cases were reported annually. Furthermore, an estimated 27% of cirrhosis and 25% of hepato-cellular carcinomas (HCC)

*Corresponding Author: basmabdr@yahoo.com

worldwide occur in HCV-infected people[1,2]. Egypt has by far the highest HCV prevalence in the world[3]. The estimated percentage of the Egyptian population in the 15–59 years age group who are positive for HCV antibody is 14.7 %[4]. Over 80% of HCV infections in the Egyptian population are among individuals aged 30 years and above[4]. The HCV incidence appears to be driven by exposures within the health-care system[4-8]. Chronic HCV has many harmful sequels. One of these sequels is the hepatocellular carcinoma that is among cancers with the poorest outlook, with fewer than 12% of all patients surviving at five years[9]. The detection of hepatocellular carcinoma improves the outcome[10]. The poor sensitivity of AFP explains its absence from the American Association for the Study of Liver Disease (AASLD) practice Guidelines as a test recommended for screening of HCC. This substandard sensitivity underlines the need for a biomarker that is able to detect HCC[3,11]. The need for new marker for early detection of hepatocellular carcinoma was mandatory. Some studies found that soluble CD25 is increased in patients with hepatocellular carcinoma[12-17]. So, our study was aiming to measure serum level of sCD25 to provide a clue for early diagnosis of HCC.

Subjects and Methods

The study was conducted in Suez Canal university hospitals (gastroenterology and Hepatology unit) after obtaining an informed consent and the protocol was approved by faculty of medicine review board and ethical committee. Sixty patients in 3 groups, 20 of them were normal control; the second 20 were patients with liver cirrhosis due to hepatitis C viral infection and the last 20 were patients with hepatocellular carcinoma complicating hepatitis C viral infection. Healthy controls had matched age and sex with HCC patients. All included cases of HCC were diagnosed based on the presence of typical vascular enhancement pattern of liver lesion (s) in contrast enhanced dynamic CT scan or MRI[18]. Diagnosis of cirrhosis was based on combined historical, clinical, laboratory and radiological findings. The staging of HCC was performed using the Barcelona Clinic Liver Cancer (BCLC) staging system. The following data were obtained for all patients: age, gender, ethnicity, etiology of HCC, BCLC stage. For the cirrhotic patients the following clinical data are obtained: age, gender, and ethnicity. All patients had complete laboratory profile including CBC, liver panel, creatinine as well as serum level of sCD25 and AFP. ELISA kit (R&D systems Inc., USA) was used to quantify blood level of AFP while ELISA kit (Cell Science, Inc, Bldg Canton, MA) was used to measure serum level of sCD25. Whole blood samples were collected in the clinic from the three groups. About 10 cm were collected from each patient; 1.8 mL for the citrated tube for prothrombin time, the rest is for serum sample. Samples are processed for serum isolation. This was done immediately after clotting by centrifugation at approximately 1000x g for 10 minutes. Then the serum was removed and separated in Eppendroff tubes for chemistry and sCD25 assay.

Statistical Analysis

SPSS, version 21 for windows *(Inc, Chicago, IL, USA)* was used for all statistical analyses. Qualitative data were presented as frequency and percentage. Chi square and Fisher's exact tests were used to compare groups. Quantitative data were presented as mean and standard deviation. For non-parametric data, student test and Mann-Whitney U test were used to compare

level difference of sCD25 between two groups while ANOVA and Kruskal Wallis were used to compare level difference of sCD25. Between more than two groups. Receiver operator characteristic (ROC) curve analysis was used to generate sensitivity and specificity at different cutoffs. The best cutoff was set at the value where sensitivity and specificity were maximal. Correlation between serum level of sCD25 and laboratory parameters was assessed by Spearman's correlation coefficient. The statistical significance was set at P-value of less than 0.05 for all tests.

Table 1. Distribution of participants according to their sex & age in different study groups

		Control (*n* =20)		Cirrhosis (*n* =20)		HCC (*n* =20)		Total	*p*-value
		No.	%	No.	%	No.	%		
Sex	Male	14	70. %	14	70%	14	70%	42	0.517
	Female	6	30. %	6	30%	6	30%	18	
Age (yrs)	Mean ±SD	57.1 ± 12.3		50.4 ± 9.7		58.4 ± 5.6		60	0.51

**. Statistically significant at *p* <0.01; ANOVA test; [a]. Bonferroni post hoc test was performed and revealed a statistically significant difference between every pair of the groups

Results

The studied populations were mostly males representing 70% in all studied groups. The mean age was 58.4 ± 5.6 years in HCC group while was 50.4 ± 9.7 and 57.1 ± 12.3 years in cirrhotic and healthy controls respectively. Hepatitis C virus (HCV) represented the underlying etiology of cirrhosis HCC group and cirrhotic group as shown in table 1. The mean sCD25 level was 11.1±3.9, 10.8 ±3.8 and 5.0±2.0 and 4.97±3.031 ng/ml in HCC, cirrhotic and healthy control groups respectively. There were statistically significant higher mean values of s CD25 in both HCC group and cirrhotic group than in control group (p<0.001). Difference between Cirrhotic group and HCC group was not significant (p=0.862) showed in table 3. The mean AFP level was 880.7±1557, 42.6±169.6 and 3.1±1.7 ng/ml in HCC, cirrhotic and healthy control groups respectively with significant statistical difference between HCC versus cirrhotic (P= 0.001). The rest of the laboratory data as well as their statistical differences between the studied groups are shown in Table 2. There were statistically significant higher mean values of ALT, AST, total bilirubin and direct bilirubin in HCC group than in cirrhotic group (p<0.001), and significant higher mean values of ALT, AST, total bilirubin and direct bilirubin in cirrhotic group than in control healthy group (p<0.001).

Table 2. Distribution of lab. Findings according to study groups

	Control (*n* =20)		Cirrhosis (*n* =20)		HCC (*n* =20)		*p*-value
	Mean	±SD	Mean	±SD	Mean	±SD	
ALT (U/L)	22.3	8.3	75.0	59.4	85.0	128.4	< 0.001**
AST (U/L)	25.5	10.1	85.6	55.9	118.2	133.5	< 0.001**
ALB (g/dl)	4.6	.3	2.8	1.0	3.0	1.0	< 0.001**
bilirubin(mg/dl)	0.7	0.1	2.6	1.6	4.8	6.9	< 0.001**
Bilirubin (mg/dl)	0.2	0.0	1.3	.9	3.6	6.4	< 0.001**
AFP (ng/ml)	3.1	1.7	42.6	169.6	880.7	1557.0	< 0.001**
Prothrombin (sec)	12.8	2.8	17.3	4.0	17.5	8.5	< 0.001**
INR	1.1	0.1	1.55	0.4	1.61	2.2	0.001**

**. Statistically significant at *p* <0.01; Kruskal Wallis test

Table 3: Distribution of Soluble CD25 according to study groups

		Control (*n* =20)	Cirrhosis (*n* =20)	HCC (*n* =20)	*p*-value
Soluble CD25 (pg/ml) (×10³)	Mean ± SD	5.0±2.0	10.8 ±3.8	11.1±3.9	< 0.001**[a] 0.862[b]
	Minimum	2.47	4.03	7.01	
	Maximum	11.08	16.72	21.79	

**Statistically significant difference at *p* <0.01; Mann-Whitney test; [a]. Difference was significant between control group and each other study groups (Cirrhosis & HCC); [b]. Difference between Cirrhosis and HCC was not significant.

As regards prothrombin time there were significantly higher mean values of prothrombin time in HCC and cirrhotic groups than in control group (p<0.001). On the other hand, there were statistically significant lower mean values of albumin levels in HCC and in cirrhotic groups than in control group (P<0.001). Correlation analyses between sCD25 and laboratory parameters among the studied groups are shown in Table 4. There was statistically significant direct correlation between the levels of s CD25 and levels of ALT, AST, total bilirubin, direct bilirubin, prothrombin time (p<0.001) and there is statistically significant inverse correlation between the levels of s CD25 and albumin levels (p<0.001). sCD25 performed well in predicting HCC presence among patients with cirrhosis; sensitivity and specificity were 65% and 65% respectively at a cut-off value of 10.22 ng/ml For prediction of HCC in patients with cirrhosis, while, sensitivity and specificity of AFP were 80% and 65% respectively at a cut-off value of 12.4 ng/ml in the same settings (Fig. 2, 3).

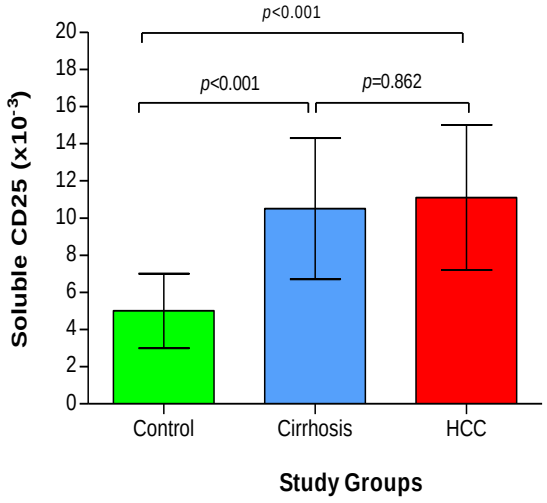

Figure 1: the relation between values of sCD25 and study groups

Discussion

Primary liver cancer often emerges as a complication of chronic liver disease, specifically cirrhosis. The most common cause of cirrhosis is chronic HCV infection[14]. Aproximately 75% of patients with HCC present with advanced unresectable disease with some element of hepatic dysfunction[16]. There is a pressing need for a biomarker that detects the presence of HCC at a better capacity than AFP.

Table 4. Correlation between Soluble CD25 and lab. findings

	Soluble CD25 (×10³)	
	Spearman's Rank Correlation	*p*-value
ALT (U/L)	0.423**	0.001**
AST (U/L)	0.447**	<0.001**
ALB (g/dl)	-0.577**	<0.001**
T.bili (mg/dl)	0.441**	<0.001**
D.bili (mg/dl)	0.524**	<0.001**
AFP (ng/ml)	0.300*	0.020*
PT (sec)	0.496**	<0.001**
INR	0.422**	0.001**

**. Correlation is statistically significant at p<0.01 (two-tailed).
*. Correlation is statistically significant at p<0.05 (two-tailed).

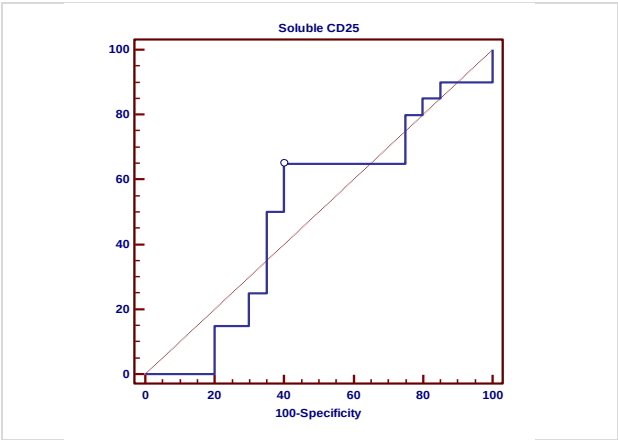

Figure 2: ROC curve for sCD25 in diagnosis of HCC among HCV-patients (n=20)

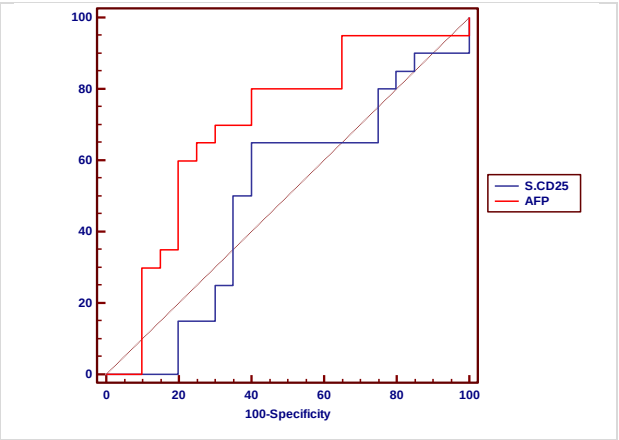

Figure 3: ROC curves of sCD25 and AFP in the diagnosis of HCC among HCV-patients (n=20)

In our study, we examined the relation between s CD25 and the presence of HCC in an attempt to find a reliable measurable predictor biomarker to detect the presence of HCC, we found that the mean age of the control group was 57.1±12.3 years, the mean age of the cirrhotic group is 50.4±9.7 years and the mean age of the HCC group is 58.4±5.6 years. This is in agreement with the study of Mohamed et al. (2013)[10] who reported that the frequent age category affected by HCC was between 51 and 60 years as the progress of the disease takes time from hepatitis to cirrhosis to HCC. In this study, HCC is more presented in males than in females this is in agreement with Abdel-Samee et al 2017 (2017)[16] who presented that men are two to three times higher than women, this is due to high prevalence of HCV in males than in females. The same results were reported in Egyptians series by El-zayadi et al. (2009)[3]. Similar results about higher prevalence in males than in females were found in a recent African study performed by Ayele and Gebre-Selassie. (2013)[11]. from a total of 120patients with chronic hepatitis C, 76 (63.3%) were males and 44 (36.7%) females (male to female ratio of 1.7: 1). In this study, there were statistically significant higher mean values of ALT and AST in patients with liver cirrhosis with the mean level was 75±59.4 (U/L) and 85.6±55.9 (U/L) respectively, And more higher levels of ALT and AST in patients with HCC with the mean level 85±128.4 (U/L) and 118.2±133.5 (U/L) respectively (*p*<0.001). This do not come in agreement with Lee et al. (2010) and Park et al.

(2012)[13,14],who reported that serum ALT can be elevated in persons with normal liver histology and can be normal in patients with advanced liver fibrosis. There were statistically significant low mean values of serum albumin in both liver cirrhosis and HCC groups being 2.8±1 (g/dl) and 3.0±1 (g/dl) respectively (p<0.001). This comes in agreement with Brian et al. (2014)[15], who reported that the incidence of hepatocellular carcinoma is high in patients with advanced cirrhosis consequently these HCC patients have marked hypoalbuminemia. There were also high levels of serum total bilirubin, direct bilirubin and prothrombin time. This comes in agreement with Brian et al. (2014) [15], who reported that HCC patients with elevated serum bilirubin have worse prognosis. There were statistically significant higher mean values of AFP in HCC group being 880.7±1557.0 (ng/ml) than in cirrhotic group with the mean being 42.6±169.6 (ng/ml) and in both groups (cirrhotic and HCC) than the control group with the mean value 3.1±1.7 (ng/ml) (*p*<0.001). Similarly Grizzi et al. (2007)[16] On the other hand, Cabrera et al. (2012)[17], reported that normal AFP levels are present in as many as 30% of patients with HCC at the time of diagnosis and usually remain low even with advanced HCC. In this study the sensitivity of AFP was 65.0% and the specificity was 80.0% at a cutoff value of >12.4 (ng/ml). In a previous study by Cabrera et al. (2012)[17], the sensitivity was 53.8% and the specificity was 86.8% at a cutoff value of 32.8 (ng/ml) and in a study by Rizk et al. (2015)[19], the sensitivity of AFP was 73.3% and the specificity was 86.6% at a cutoff value of 21.45 (ng/ml). These finding may indicate difference in analytical methods and statistical cutoff points. Our results show highly significant increased levels of sCD25 in patients with cirrhotic liver and HCC as compared to normal controls, the mean value of the control group is 5±2 (pg/ml), the mean value of the cirrhotic group is 10.8±3.8 (pg/ml) and the mean value of the HCC group is 11.1±3.9 (pg/ml)(*p*<0.001). This comes in agreement with cabrera et al. (2012)[17], who reported that sCD 25 was significantly increased in patients with cirrhosis than in healthy group. Also, our results show significant increased levels of s CD25 in patients with HCC as compared to healthy group. This also comes in agreement with Caberera et al (2012)[17] and Abdel-Samee et al 2017 (2017)[16]. In this present study, there was no significant increase in the level of s CD25 in patients with HCC than in pateints with liver cirrhosis (*p*=0.862). This was not in agreement with Caberera et al (2012)[17], who reported that the levels of s CD25 were significantly higher in patients with HCC than in normal group and than in patients with liver cirrhosis. A study done by Ararat et al. (2011)[20], Showed that the level of s CD25 in the serum of HCC patients was significantly higher (mean 12799 pg/ml) than that in the patients with cirrhosis (mean 3585 pg/ml). This analysis found that s CD25 possess a sensitivity of 65% at a cutoff value of >10.22 x 10^3 (pg/ml) for the presence of HCC and a specificity of 65.0% at the same cutoff value and warrants an additional investigation as a potential screening test. This do not come in agreement with Ararat et al (2011)[20] who reported that at a cutoff value of > 5.15 x10^3 (pg/ml), sCD25 has a sensitivity of 62.1% and a specificity of 83.3%, our results are lower this may be due to genetic difference, environmental factors and duration of HCC. When comparing s CD25 with AFP we found that the specificity of AFP (80.0%) was higher than that of s CD25 (65.0%). The positive predictive value of s CD25 in the present study was 65.0 at a cutoff value of > 10.22 x 10^3 (pg/ml) compared to AFP, which has

a positive predictive value of 69.6% at a cutoff value of >12.4ng/ml). The negative predictive value of s CD25 in the present study is 65.0% at a cutoff value of > 10.22 x 10^3 (pg/ml) as compared to the negative predictive value of AFP, which has a negative predictive value of 69.6% at a cutoff value of >12.4 (ng/ml). Soluble CD25 is correlated to the severity of liver disease in the form of negative correlation between s CD25 and albumin (p< 0.001) and significant positive correlation between s CD25 and INR (p< 0.001). This comes in agreement with Rizk et al (2015)[19] who reported the same results.

Conclusions

sCD25 sounds not to be a good marker for predicting early HCC. CD25 can differentiate HCC patients from normal healthy persons but not from cirrhotic patients.

References

1- Abbassy HA and Elbanna AA 2016. Noninvasive Markers for the Assessment of Liver Fibrosis and Cirrhosis in Egyptian Patients with Chronic Hepatitis C and Schistosomiasis Coinfection. J Medical science and clinical research 2016 vol.4(12);14773-14780.

2- Miller FD and Abu-Raddad LJ (2010) Evidence of intense ongoing endemic transmission of hepatitis C virus in Egypt. Proc Nat Acad Sci USA 107: 14757–14762.

3- El-Zanaty F and WayA (2009): Egypt Demographic and Health Survey (2008). Cairo: National Population Council, El-Zanaty and Associates, and ORC Macro.p 431

4- Saleh DA, Shebl FM, El-Kamary SS, et al (2010): Incidence and risk factors for community-acquired hepatitis C infection from birth to 5 years of age in rural Egyptian children. Trans R Soc Trop Med Hyg 104: 357–363.

5- Esmat G, Hashem M, El-Raziky M, et al (2011): Hepatocellular carcinoma. N Engl J Med. 365:1118–1127.

6- El-Serag HB (2011): Hepato-cellular carcinoma. N Engl J Med; 365: 1118–1127.

7- Cabrera R, Ararat M and Cao M (2010): Hepatocellular carcinoma: immunopathogenesis: clinical evidence for global T cell defects and an immunomodulatory role for soluble CD25 (sCD25). Dig Dis Sci 55: 484-495.

8- Mühlberger N, Schwarzer R, Lettmeier B, et al (2009): HCV-related burden of disease in Europe: a systematic assessment of incidence, prevalence, morbidity, and mortality. BMC Public Health.2009; 9: 34.

9- Sherman M (2011): current status of AFP testing. Gastroenterol Hepatol (NY) 7: 113-114.

10- Mohamed YA, Mumtaz RG, Riome S, Miller D and Abd-Raddad LJ (2013): the epidemiology of hepatitis C virus in Egypt;1471-2334.

11- Ayele AG and Gebre-Selassie S (2013): Prevalence and Risk Factors of Hepatitis B and Hepatitis C Virus Infections among Patients with Chronic Liver Diseases in Public Hospitals; org/10.1155/2013/56382.

12- Tatsuo S, Masahiko S, Kenji G, et al (2018). Prognostic impact of soluble intercellular adhesion molecule-1 in hepatocellular carcinoma: Oncology Letters 16: 6013-6018, 2018

13- Lee JK, Shim JH, Lee HC, et al (2010): Estimation of the healthy upper limits for serum alanine aminotransferase in Asian populations with normal liver histology. Hepatology; 51:1577–1583.

14- Park HN, Sinn DH, Gwak GY, et al (2012): Upper normal threshold of serum alanine aminotransferase in identifying individuals at risk for chronic liver disease. Liver Int; 32:937–944.

15- Brian I. C, Vito G, Giannini, et al (2014): association of abnormal plasma bilirubin with aggressive HCC phenotype; PLOS MED 41(2): 252-258.

16- Grizzi F, Franceschini B, Hamrick C, et al (2007): Usefulness of cancer-testis antigens as biomarkers for the diagnosis and treatment of hepatocellular carcinoma. J Transl Med; 5: 3.
17- Cabrera R, Fitian AI, Ararat M. et al (2012): serum level of s CD25 as a marker for HCC; Oncol Lett. 4(4): 840-846.
18- Abdel-Sameea E, Zakareya T, Metwaly KH, et al 2017. Serum soluble CD25 in hepatocellular carcinoma, shall we be able to change the natural history? 2017. J Cancer and Tumor Int, 5: 4 2017
19- Rizk E, Zakaria S and Farouk N (2015): soluble CD25 and hepatocellular carcinoma. Int J advanced Res; 3(5); 658-664.
20- Ararat M, Fitian AI, Brusko T, et al (2011): the soluble form of IL-2 (s CD25) as a novel biomarker for HCC. J clin oncology 29.