Evaluation of Serum Soluble Interleukin -2Receptor (IL –2R) and α-Fetoprotein Levels in Patients with Liver Cirrhosis and Hepatocellular Carcinoma

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Abstract: Hepatocellular carcinoma (HCC) develops during the natural history of cirrhosis. HCC lesion of one cm in diameter with high or low echogenicity can be detected by ultrasonography and confirmed by needle biopsy. However, it is still very difficult to detect small isoechogenic HCC lesion, especially when AFP is normal. The serum level of IL-2R has been proposed as a marker of HCC. The aim of our study was to evaluate the serum soluble (IL-2R) andα-fetoprotein levels in patients with liver cirrhosis and HCC. All patients were subjected to full history taking, clinical examination, laboratory investigations, abdominal ultrasonography and ultrasonography guided percutaneous fine needle aspiration biopsy. To evaluate the role of serum IL2R in the diagnosis of hepatocellular carcinoma (HCC), we simultaneously studied both IL2R activity and alpha-fetoprotein (AFP) levels in 40 patients with cirrhosis, 40 patients with HCC and 40 healthy subjects. Serum soluble IL -2R activity in patients with HCC (573 ± 210 nmol/ml/hr) and cirrhosis (285 ± 143 nmol/ml/hr) was significantly higher than controls (216 ± 117 nmol/ml/hr, $p < 0.001$). With 450 nmol/ml/hr (mean value of controls plus 2 standard deviations) considered as the cut-off point, IL-2R was more sensitive (76 vs 65.4%) but less specific (90.9 vs 95.5%) than AFP at a level of > 400 ng/ml as a tumor marker of HCC. We concluded that IL-2R is a useful marker, in conjunction with AFP and ultrasonography, for detecting HCC.

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1. Introduction

Hepatocellular carcinoma (HCC) is one of the most common malignant neoplasms in cirrhotic patients. About 76% of patients with HCC is associated with cirrhosis (Chen, 2005).

Computed tomography and ultrasonography are the major imaging methods used to detect HCC.

Ultrasonogrophy is the most practical approach for the early recognition of HCC (Okuda, 2008).

Several surveillance programs have attempted to detect HCC at an early stage by ultrasonography and measurement of the serum α- fetoprotein (AFP) level, an HCC lesion of one cm in diameter with hight or low echogenicity can be detected by ultrasonography and confirmed by needle biopsy, however, it is still very difficult to detect small isoechogenic HCC lesions. Serum AFP level of more than 200 ng/ml is a marker of HCC, but not all of these tumors secrete AFP and this marker may also be increased in patients with chronic hepatitis and cirrhosis. Because serum AFP level is dependent on tumors size it may be normal in up to 40% of patients with early HCC and in 15% to 20% of patients with advanced HCC, thus this marker is not very sensitive for detecting.a small HCC (Maringhini *et al.,* 2010).

Serum soluble interleukin –2receptor (IL-2R) is a heterotrimeric protein that bind and responds to cytokine, it has been proposed as marker of HCC (stuber *et al*, 2010).

Up to now the measurement of IL-2R level in the follow up of cirrhotic patients for the early detection of HCC has not been reported, therefore the clinical usefulness of this new marker is still unknown.

Objective of the work:

The aim of the present study is the evaluation of serum levels of IL-2R and α- fetoprotein in patients with liver cirrhosis and HCC.

2. Subjects and methods:

The present study was conducted on 120 persons of both sexes (70 males and 50 females) attended to AL-Hussein and Bab AL-Sharia University Hospitals, AL-Azhar university during the period from july 2009 to September 2011. Those patients were classified into three groups.

Group I (liver cirrhosis):

Included 40 cases (21 males and 14 females) means ages 39 ± 7.2 years with liver cirrhosis.

Group II (hepatocellular carcinoma):

Included 40 cases (25 males and 15 females) mean ages ± 7.5 years with HCC.

Group III (controls):

Included 40 healthy subjects (24 males and 16 females) mean ages 38 ± 6.5 years.

The subjects were submitted to complete history taking with emphasis on symptoms of chronic liver disease, general and abdominal examination with routine and specific investigations.

The diagnosis of cirrhosis was established by clinical, biochemical and ultrasonographic findings.

Clinical evidence of liver disease was based on his presence of hepatosplenomegaly, ascites, jaundice, palmar erythema, and spider angioma Ultrasonographic findings for cirrhosis were liver coarse echopattern, nodular surface, enlarged caudate lobe and evidence of portal hypertension. The patients with HCC were diagnosed by presence of hepatic focal lesion detected by ultrasonography and confirmed by needle biopsy.

Aspartate trasferase (AST), Alanine transferase (ALT), Gamma glutamyl transferase (GGT), alkaline phosphatase and total bilirubin were measured autoanalyzer (Hitachi 917 Automate; Mannheim, Germany) and Roche Diagnostic reagent (Mannheim, Germany).

Albumin was assessed by bromocresol green method (Doumas *et al.,* 1971). Prothrombin activity was performed according to the method of Hull *et al.,* 1982.

Regarding to the causes of cirrhosis and HCC, hepatitis C virus antibody (HCV-Ab) was done by ELISA format according to the method of Roynard *et al.,* 1998.

Hepatitis B surface antigen (HBsAg) was detected according to the method of Tin and Tilles (1983).

IL-2R and α- fetoprotein determination:

IL-2R assayed, in sera stored at 20ºC,within 30 days after samples collection. Enzyme activity was expressed as nanomoles of P-nitroph-enyl- soluble I-L cytokinas cleaved per millitier per hour at 37 ºC (Giadina et al., millitier (2010).

AFP eas measured by an enzyme immunoassay (EIA) according to method of Trape *et al.* (2010)

Statistical analysis:

Student's test and Wilco-xon's test were used to compare data. All results were expressed as the mean ± SD Cutoff values of IL-2R and AFP for diagnosis of HCC were selected from experimental data as the values that maximized the sum of sensitivity and specificity In a clinical situation where false-negative classifications could be considered as harmful as false-positive classifications.

3. Results

As regards to both age and sex there is no significant difference between group I,II and III(P>0.05)Table (1).

Table (2) demonstrates comparison of laboratory findings in the different studied groups. There were significant differences between group I and II when compared to group III. " control group " P-value <0.05, also there were significant differences between groups I,II compared with group III "control group" as regards ultrasonographic findings P- value < 0.05 Table (3).

Table (4) shows the etiology of liver cirrhosis and HCC in the studied groups. HBV represented 40% (32 out of 80) while, HCV represented 60% (48 out of 80) of patients.

The mean serum level of IL-2R in HCC patients was significantly higher than controls (573 ± 210 nmoe /mL/hr vs 216 ± 117 nmol/ml/hr, P<0.001). Also IL-2R was significantly higher in patients with liver cirrhosis when compared to the control group (285 ± 143 nmol/ml/hr vs 216 ± 117 nmol/m-l/hr) and p<0.001. Also AFP was significantly high-er in HCC patients (220 ± 950 ng/ml vs 9.5 ± 8.7 ng/ml) and patients with liver cirrhosis (40 ± 33 ng/ml vs 9.5 ± 8.7) than controls (p<0.001)(Table5).

Table (6) shows the cutoff values, sensitivity and specificity of IL-2R & AFP. IL-2R at cutoff value of 450 nmol/mL/hr, mean plus double standard deviations, the patients in group I had low sensitivity

And specificity, 13% and 25%, respectively. While patients in group II had 76% sensitivity and 90.9% specificity. On the other hand the AFP at cutoff value of 400 ng/ml, which was the cut off value considered most effective in our study, group I had sensitivity of 40% and specificity of 54%, while group II had sensitivity and specificity of 65.4% and 95.5%, respectively

Table (1): Comparison of the age and sex of the studied groups

Item	Group I n=40	Group II N=40	Group III no=40
Age (by years)			
Mean ± S.D	39 ± 7.2	49 ± 7.5	38 ± 6.5
Sex			
Males	21(52.5%)	25(62.5%)	24(60%)
Females	19(47.5%)	15(37.5%)	16(40%)

Table (1): Comparison of laboratory findings in the different studied groups

Item	Group I n=40	Group II N=40	Group III no=40
Bilirubin mg/dl	1.8 ± 1.2*	2.3 ± 1.7**	0.8 ± 0.2
Albumin	2.5 ± 0.7**	2.5 ± 0.9**	4.2 ± 0.5
ALT U/L	45 ± 17**	73 ± 25**	27 ± 13
AST U/L	55 ± 18**	60 ± 19**	25 ± 15
GGT U/L	59 ± 12**	61 ± 14.3**	29 ± 18
Alkaline Phosphatase	105 ± 30*	210 ± 50**	70 ± 19.4
Prothrombin Conc. %	58 ± 10*	49 ± 12**	101 ± 10

* Significant (p<0.05) as compared to control group.
** Highly significant (p<0.001) as compared control group.

Table (3): Comparison of ultrasonographic findings in the different studied groups

Item	Group I n=40	Group II N=40	Group III no=40
Coarse liver	100%**	100%**	0
Nodular surface of the liver	100%**	100%**	0
Enlarged caudate lobe	100%**	100%**	0
Focal lesion of Liver	0	100%**	0
Splenomegaly	100%**	100%**	0
Portal vein diameter (mm)	14 ± 3**	19 ± 7**	9 ± 3
Splenic vein diameter (mm)	9.9 ± 5	9.8 ± 7	5.2 ± 2.6
Collaterals	62.5%	75%	0
Ascites	52%	85%	0

* Highly significant (p<0.001) as compared to control group

Table (4): Comparison of etiology in the different studied group

Item	Group I n=40	Group II N=40	Group III no=40
HBs-Ag	(15/40)37.5 %	(17/40)42.5 %	0
HCV-Ab	(25/40)62.5 %	(23/40)57.5 %	0

Table (5): Serum IL-2R level and AFP levels in the different studied group.

IL-2R (nmol/ml/hr)			α- fetoprotein (ng/ml)		
Group I	Group II	Group III	Group I	Group II	Group III
285 ± 143**	573 ± 210**	216 ± 117	40 ± 33**	220 ± 950**	9.5 ± 8.7

** Highly significant (p<0.001) as compared to control group

Table (6): Cutoff values sensitivity and specificity of IL-2R & AFP in the group I and II.

IL-2R (cutoff 450 nmol/ml/hr)		AFP (cutoff 400ng/mL)	
Group I n=40	Group II n=40	Group I n=40	Group II n=40
Sensitivity 12.5% Specificity 25%	76% 90.9%	40% 54%	65.4% 95.5%

4. Discussion

AFP and ultrasonography have usually been used as diagnostic tools for HCC. (Giardina *et al.,* 2010). However, not all HCC secrete AFP and AFP levels may be normal in about 40% of patients with HCC. Ultrasonography is very effective in early diagnosis of HCC, however Ultrasonography can Detect only, at an early stage, about 76% of HCC patients (Zoli *et al.,* 2007). Therefore, more sensitive diagnostic tools for detecting HCC are desirable.

Serum soluble interleukin 2 receptor alpha is a Heaterotrimeric protein that binds and responds to acytokine (Stauber *et al.,* 2010).

The precise mechanism causing elevated level of this protein has not been determined. One possible explanation is the increased synthesis and secretion of proteins by tumor cells as occurs with AFP (Deugnier *et al.,* 2009). The usefulness of serum IL-2R activity for the diagnosis of HCC has been previously reported 2006. (Hutchinson *et al.,* 2006; smith 2006; Giardina *et al.,* 2010).

The serum IL-2R activity was not related to AFP or ALT levels: therefore, increased IL-2R levels in HCC patients are not related to liver regeneration or necrosis. Moreover, IL-2R may be more sensitive marker for HCC than AFP because IL-2R activity in HCC patients is not correlated with tumor size (Giardina *et al.,* 2010).

Hutchinson *et al.* (2006), reported that patients with high level of IL-2R actually have very early HCC that may be undetectable by ultrasonography and computed tomography.

However, significant lower level of IL-2R in HCC tissue compared to non tumoral liver tissues were obtained (Hutchinson, 2006; Cottone *et al.,* 2009).

Also, IL-2R level is known to Corresponding author Oli *et al.,* 2007 detects focal hepatic lesions in 76% of the studied patients. They also explained that, this difference may be due to selection of the patients, improved performance and difficulty to detect small isoechogenic hepatic focal lesions.

The current study revealed the prevalence of HCV in 25 patients with liver cirrhosis (62.5%) and 23of patients with HCC (57.5%). This is in agreement with Cottone *et al.* (1994) who reported that HCV Infection has been confirmed in our patients as the most significant factor in the development of cirrhosis and HCC.

In this study interleukin – 2 protein was significantly higher in both patients with liver cirrhosis and patients with HCC. However, IL-2R had low sensitivity (12.5%) and specificity (25%) in patients with liver cirrhosis. While in patients with HCC, at cutoff value of 450 nmol/mL/hr, IL-2R had a sensitivity of 76% and a specificity of 90.9%. in respect to α-fetoprotein, it was also significantly higher in both patients with liver cirrhosis and HCC. At cutoff value of 400 ng/ml, in patients with liver cirrhosis AFP had sensitivity and specificity of 40% and 54%, respectively.

While in patients with HCC, IL-2R had sensitivity and a specificity of 65.4% and 95.5%, respectively.

Similar results were obtained by Tangkijvanich *et al.* (2008), they reported that IL-2R was significantly higher in both patients with liver cirrhosis and hepatocellular carcinoma. At cutoff value of 870 nmol/mL/hr (mean of controls plus 3 standard deviations), IL-2R had 81.7% sensitivity while, the specificity was 39%. AFP, at cutoff value of 400 ng/ml had 70.7% sensitivity and 99.3% specificity.

Also Giardina *et al.* (2010) found that IL-2R in patients with HCC, at cutoff value of 443 nmol/m-L/hr, had a sensitivity of 76% and a specificity of 90.9%. Hutchinson *et al.* (2006) also recorded that IL-2R and AFP in patients with HCC had sensitivity, and specificity of 98% and 92.6%, respectively.

Giardina *et al.* (2010) and Oka *et al.* (2006) depend also on racial difference of IL-2R phenotype (Bukvfzer *et al.,* 1989; Deugnier *et al.,* 2009).

Ultrasonographic findings showed that coarse liver echopattern, nodular surface of the liver, enlarged caudate lobe of liver, splenomegaly and evidence of of portal hypertension are the most Ultrasonographic findings of liver cirrhosis. These findings are in agreement with Maringhini *et al.* (2010). They detected focal hepatic lesions in all patients (100%). While, Z-IL-2R is one of five tumor marker can be used combined for earlier diagnosis of HCC and can differentiate it from liver cirrhosis (Ma *et al.,* 2011).

In conclusion the serum IL-2R activity could be a useful marker for detection of HCC, especially of a small sized tumor, in addition to the assay of AFP and ultrasonography for the early detection of HCC in patients with cirrhosis.

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References

1. Bruix, J. (1997): Treatment of hepatocellular carcinoma. Hepatology, 25:259 – 62.
2. Bukvfzer,S.; Stass, P.; Kew, M.; and Berr, M. (1998). Soluble interleukin – 2 protein as a serum marker of hepatocellular carcinoma in Suthern Africans Blacks Br. J. Cancer, 59: 417 – 420.
3. Chen, D. (2005): Early diagnosis of primary liver cancer. Ital. J.Gastroenterol, 20: 287 – 290.
4. Colombo, M.; Franchis, R.; Del Ninno, E.; Sangivan-ni, A.; De Fazio, C. And Tommasint, M. (1991): He-patocellualar carcinoma in Italian patients with cirrh-osis. N. Eng. I. Med., 325:675-80.
5. Cottone, M.; Turri, M.; Caltagirone, M.; and Maranghini, A. (2009): Early detection of hepatocellular carcinoma associated with cirrhosis by ultrasound and alpha-fetoprotein: a prospective study. Hepatogastroentrology, 35:101 – 103.

6. Cottone, M.; Turri, M.; Caltagirone, M.; Parisi, P.; O-rlando, A. and Florentino, reported that serum IL-2R is very useful marker in the detection of HCC, in addition, because serum AFP level is dependent on tumor size it may be nor-mal in up to 40% of patients with early HCC and in 15% to 20% of patients with advanced HCC.
7. Wei et al. (2010) mentioned that IL-2R is a useful marker of primary liver cancer for diagnosis and post treatment monitoring.
8. Giardina, M.; Matarazzo, M.; Morante, R.;Lucariello, A.; Varriale, A.; Guardasole, V. and De Marco, G (2010): Serum IL-2R activity and early detection of hepatocellular carcinoma: a prospective study of patients with cirrhosis. Cancer, 83(12): 2468-74.
9. Giardina, M.; Matarazzo, M.; Varriale, A.; Morante, R.; Napoli, A. and Marino, R. (2010): Serum IL-2R A useful marker in the diagnosis of hepatocellular carcinoma. Cancer, 70:1044-8.
10. Hutchinson, W.; Johnson, P.; Du, M-Q. and Williams R. (2006): Serum and tissue IL-2R activity in the pr-e-clinical stages of hepatocellilar carcinoma. Clin. Sci., 81:177-182.
11. Hull, R.; Hirsh, H. and Jay, R. (1982): Different inte-nsities of oral anticoagulant therapy in the treatment of proximal vein thrombosis. N Engl J Med., 307:1676-81.
12. Ma, J.; Gong, Q.; Lin, M.; Xi, Y.; Wang, M.; Chen, Z.; Pei, Z.and Ma, W. (2011): combined five tumor Markers in detecting primary hepatic carcinoma. Chinese Journal of Surgery, 38(1):14-6.
13. Maringhini, A.; Cottone, M.; Sciarrino, E.; Marceno, M.; La Seta, F.; Fusco, G. and Rinaldi, F. (2010): Ultrasonongraphy and alpha fetoprotein in diagnosis of hepatocellular carcinoma in cirrhosis. Dig. Dis. Sci.,33: 47 – 51.
14. Oka, H.; Tamori, A.; Kuroki, T.; Kobayashi, K. and Yammamoto, S. (2006): Prospective study of α-feto-protein in cirrhotic patients monitored for developm-ent of hepatocellular carcinoma. Hepatology; 19:61-6.
15. Okuda, K. (2008): Early recognition of hepatpcellular carcinoma. Hepatology, 6: 729- G. (1994): Screening for hepatocellular carcinoma in patients with child's A c-irrhosis an 8-year prospective study by ultrasound and alpha fetoprotein. J. Hepatol., 21:1029-34.
16. Deugnier, Y.; David, V.; Leray, G. and Balyau, M. (2009): Les marquers biologiques du carcinoma. Ann Biol. Clin. (Paris), 46:85-88.
17. Doumas, B.; Watson, W. and Biggs, H (1971); Albu-min standards and the measurement of serum albumin with bromocersol green. Clin Chem Acta, 31:87 – 96.
18. Smith K A (2006): The interleukin 2 receptor, Annu Rev. Cell Boil. 5 (1): 397 – 425.
19. Stauber DJ, Debler EW, Wilson IA. (2010): crystal, structure of IL2R singnalling complex paradign for a heterotrimeric cytokine receptor. Proc. Natl. Acad. Sci U.S.A. 103 (8): 2788 – 93.
20. Tangkijvanich, P.; Tosukhowong, P.; Bunyongyod, P.; Lertmaharit, S.; Hanvivatvong,, O.; Kullavanijaya P. and poovorawan, Y. (2008) interleukin – 2 protein as a serum marker of hepatocellular carcinoma in Thailand. Southeast Asian Journal of Tropical Medicine & Public Health, 30(1); 110-4.
21. Tin, C and Tilles, G. (1983): Purification and biophy-sical characterization of hepatitis B surface antigen. J Med. Invest., 52: 1176-86.738.
22. Roynard, T.; Marcellin P.; Lee S. and Niederau C. (1998): Randomised trial of interferon α2b plus riba-virin for 48 weeks or for 24 weeks versus interferon α2b plus placebo for 48 weeks for treatment of chronic infection with hepatitis C virus. Lancet, 352(9138): 1426 – 32.
23. Shi, X.; Zhou, Y. and Xia, L. (1998): Clinical evalua-tion of several tumor markers in the diagnosis of primary hepatic cancer. Chinese Journal of Oncology, 20 (6): 437-9.
24. Trape, J.; Botragues, J.; Porta, F.; Ricos, C. and Badal, M. (2010): Reference change value for α-feto-protein and its application in early detection of hepat-ocellular carcinoma in patients with hepatic disease. Clin. Chem., 49(7): 1209-11.
25. Wei, X.; Wang, S.and Rui, J. (2010): The value of serum interleukin – 2 protein in the diagnosis of primary liver cancer. Chinese Journal of Oncology, 22(2): 148-50.
26. Zoli, M.; Magalotti, D.; Bianchi, G.; Gueli, C.; Marc-hesini, G. and Pisi, E. (2007): Efficacy of a surveilla-nce program for early detection of hepatocellular Carcinoma. Cancer, 78:977-85.

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