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# Prognostic Value of Soluble Programmed Death Ligand-1 (sPD-L1) in Hepatocellular Carcinoma

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## Abstract

*Background:* Hepatocellular carcinoma (HCC) is the fifth most commonly occurring cancer worldwide and the third cause of mortality due to cancer. Many clinical and laboratory findings used to assess prognosis of HCC. Different studies had been performed to investigate the prognostic value of sPD-L1 in HCC progression.

*Aim of Study:* To assess serum level of sPD-L1 in patients of HCC and to detect its role in HCC prognosis.

*Subjects and Methods:* This case-control study was conducted on 80 subjects divided into 2 groups (1 st group) 60 patients with HCC diagnosed by 2 imaging techniques and elevated alpha feto-protein and (2 nd group) include age and sex matched 20 healthy subjects. All the patients were evaluated by thorough full history taking, clinical examination, routine laboratory investigations including alpha feto-protein (AFP) and sPD-L1 measured by ELISA with calculation of MELD score and albumin/bilirubin score (ALBI), Imaging by abdominal ultrasound and triphasic CT scan, and assessment of HCC stage using Barcelona Clinic Liver Cancer (BCLC).

*Results:* There were highly statistically significant variations in sPD-L1 level according to the stage of HCC and vascular infiltration. Logistic regression analysis showed that sPD-L1 (odd ratio (OR) 1.01; 95% confidence interval (CI) was 1.003 to 1.03) and AFP (OR 1.01: 95% CI was 1 to 1.01) were significant independent predictors for occurrence of HCC (stage C & D versus stages A & B). sPD-L1 cut off value that diagnose the advanced HCC stages (C & D) was 586 (pg/ml) with sensitivity 80% and specificity 62.5% with area under ROC curve is 0.79, and at cut off level 1325 (pg/ml) sensitivity was 100%, specificity 94% and area under ROC curve was 0.97.

*Conclusion:* sPD-L1 has a good diagnostic and prognostic value in the diagnosis of HCC.

***Key Words:*** *Programmed death ligand-1 – hepatocellular carcinoma – Prognosis.*

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## Introduction

Hepatocellular carcinoma (HCC) accounts for 70-90% of primary liver cancer [1], and it is the sixth most commonly occurring cancer worldwide and the third cause of cancer related mortality [2]. Advancing age, alcohol, smoking and viral hepatitis are the most common risk factor of HCC independent of cirrhosis [3]. sPD-L1 is a 40 KDa type transmembrane protein that has been speculated to play a major role in suppressing the immune system during particular events such as pregnancy, tissue allograft, auto-immune disease and other states such as hepatitis [4]. sPD-L1 is over expressed by tumor cells, triggers T cell anergy or even death and helping the tumor cells escape the immune system [5]. The prognosis of HCC remains poor, with only one third of patients eligible for curative treatment and very limited survival benefits with the use of sorafenib [6]. Curative HCC treatment is only available in early stages, however, in intermediate or advanced stages there no curative treatment options. Immunotherapy in cancer is a recent and very promising approach namely inhibition of PD/programmed death ligand-1 axis [7].

Overexpression of programmed death ligand-1 was identified in peri-tumoral tissues, primarily in the liver portal region, the expression of sPD-L1 was observed to be significantly correlated with the large tumor size and poor differentiated tumors [8].

It has been shown that sPD-L1 can be detected in the serum of patients, which had a correlation to the amount of PD-L1 expressing cells, and also there was a negative correlation between PD-L1 expression and cancer prognosis [9].

The clinical trials targeting PD-L1 in HCC are ongoing, coming from the hypothesis that HCC is

proposed to be one of tumors susceptible to immunotherapies and from the PD-L 1 and HCC prognosis relationship, the prognostic value of circulating PD-L 1 level in HCC patients might has been shown **[10]**.

The aim of this study was to measure serum level of sPD-L1 in HCC patients and detect its role in HCC prognosis.

## Subjects and Methods

This case-control study according to the guidelines of the Helsinki declaration **[11]** and approved by the Research Ethics Committee of Benha University Hospitals. An informed consent was taken from all participates before enrollment in the study.

*This study was conducted on 80 participants; they were divided into 2 groups:*

a- HCC group: Including 60 HCC cases from outpatients' clinic of Benha University Hospital during the period from October 2017 to April 2018 diagnosed with 2 image techniques.

b- Control group: Including 20 age and sex matched apparently healthy subjects.

*Inclusion criteria:*

Patients with hepatic focal lesion >2cm diagnosed by ultrasound and confirmed as HCC by triphasic CT scan.

*Exclusion criteria:*

- Patients with age <18 years.
- Other co-morbid illness.
- Patients with history of other malignancy within the past 5 years.

All individuals were subjected to full history especially (age, sex, occupation, special habits and symptoms of liver disease), full examination especially (signs of liver cell failure), laboratoty investigation including: (ALT, AST, albumin, bilirubin, CBC, prothrombin time, INR and creatinine) and imaging (ultrasound and Tri-phasic CT). HCC stage assessed by Barcelona-Clinic-Liver-Cancer staging and severity of liver disease assessed by Model for End-Stage Liver Disease (MELD score) and Albumin/Bilirubin ratio (ALBI). sPD-L1levels were measured by using an ELISA.

*Measurement of sPD-L1:*

The kit uses a double- antibody sandwich enzyme-linked immunosorbent technique (ELISA) to assay the level of soluble programmed death ligand-1.

Programmed death ligand-1 adopted to coat micro-titer plate, make solid phase antibody, then programmed death ligand-1 was added to wells, combined programmed death ligand-1 antibody with labeled HRP to antibody-antigen-enzyme antibody complex, after washed completely, TMB substrate solution was added, TMB substrate became-blue color at HRP enzyme-catalyzed reaction, reaction was terminated by addition of a stop solution and the color change was measured at a wavelength of 450nm. The concentration of programmed death ligand-1 in the sample was then determined by comparing the optical density of the samples to the standard curve.

*Statistical analysis:* Data was analyzed by SPSS V.23 (SPSS Inc. released 2015. IBM SPSS statistics for windows, version 23.0, NY: IBM Corp.). Paired t test was used to compare readings of normally distributed paired data, and Wilcoxon test was used to compare readings of not-normally distributed paired data. Student's *t*-test of significance used for comparison of quantitative variables between two groups of normally distributed data, while Mann Whitney's test was used for comparison of quantitative variables between two groups of not normally distributed data. Chi-square test ($\chi^2$) was used to study association between qualitative variables. Whenever any of the expected cells were less than five. Fischer's Exact test was used. Logistic regression was performed to ascertain the effect of the significant risk factor of responsiveness to treatment. Receiver operator characteristic curve (ROC) with respective points of maximal accuracy for sensitivity and specificity were generated to determine biomarker performance.

## Results

The baseline characteristics of the study groups, HCC group include 60 patients their mean of age was 66.18±8.35, they were 41 males (68.3%) and 19 females (31.7%) and control group include 20 healthy control of matched age and gender subjects with their mean of age was 62.19±8.31, they were 13 males (65%) and 7 females (35%). Most of the cases were post-hepatitis C cirrhosis (96.6%) and most of them stage C (BCLC) (50%). Tumor size ranged from 4 to 6cm with 2 cases with venous infiltration and no lymph node involvement. MELD score ranged from 8-20 with the estimated 3-months survival rate were 6% in most of cases (95%), Albumin/bilirubin score (ALBI) mean was (–0.3 ±0.41). The mean of AFP in HCC group 508.7± 231ng/ml. (Table 1).

There were significant positive correlations between sPD-L1 and tumor size, ALBI score, AFP and serum Albumin (Table 2).

There were statistically significant variations in sPD-L 1 level according to HCC stage, and also statistically significant variation in its level by venous infiltration. ROC curve analysis of the data showed that the best cut-off value of sPD-L 1 for stage (B) HCC was 572.5 (pg/ml), this value could predict stage B of HCC with sensitivity 53.85%, specificity 45.4%, PPV 53.8% and NPV 45.4%. the best cut-off value of sPD-L 1 for stage (C) HCC was 586 (pg/ml), this value could predict this stage with sensitivity 80% and specificity 62.5%, PPV 72.7% and NPV 71.4%. The best cut-off value of sPD-L1 in stage (D) HCC was 1325.5 (pg/ml), which predict this stage with sensitivity 100% and specificity 94.4%. (Table 3) (Fig. 1).

Logistic regression analysis shows odds ratios for the incidence of HCC stage C & D showed that sPD-L1 (OR 1.01; 95% confidence interval CI 1.003 to 1.03), AFP (OR 1.01; 95% CI 1.00 to 1.01) and serum creatinine (OR, 0.01; 95% CI 0.00 to 0.74) were significant independent predictors for occurrence of HCC (stage C/D versus stage A/B) (Table 4).

Table (1): Clinical and laboratory characteristics of HCC group.

| Variable | Cases (No.=60) | |
|---|---|---|
| | No. | % |
| *Hepatitis markers:* | | |
| HBV | 2 | 3.33 |
| HCV | 58 | 96.67 |
| *HCC stage:* | | |
| Stage A | 11 | 18.33 |
| Stage B | 13 | 21.67 |
| Stage C | 30 | 50.0 |
| Stage D | 6 | 10.0 |
| *Venous infiltration:* | | |
| Negative | 58 | 96.67 |
| Positive | 2 | 3.33 |
| *Tumour associated lymph nods:* | | |
| NAD | 60 | 100 |
| *Cirrhosis:* | | |
| Positive | 60 | 100 |
| *Estimated 3 months mortality (according to MELD score):* | | |
| 19.6% | 3 | 5 |
| 6% | 57 | 95 |
| | Mean ±SD | Range |
| Tumour size (cm) | 3.69± 1.08 | 2-6 |
| MELD score | 14.67±2.72 | 8-20 |
| Albumin/bilirubin score | –0.3±0.41 | –1.14- 0.6 |
| AFP (ng/ml) | 508.75±231.04 | 220-1200 |

Table (2): Correlation co-efficient ( *r*) between s. PD-L1 and quantitative data in hepatocellular carcinoma patients.

| Variable (No.=60) | Spearman correlation coefficient (rho) | *p* |
|---|---|---|
| Tumor size (cm) | 0.76 | <0.001 (HS) |
| Meld score | 0.05 | 0.68 |
| Albumin/bilirubin score | 0.39 | 0.002 (S) |
| AFP (ng/ml) | 0.69 | <0.001 (HS) |
| S. albumin (g/dl) | –0.40 | 0.001 (S) |
| S. total bilirubin (mg/dl) | 0.08 | 0.52 |
| Direct bilirubin (mg/dl) | 0.20 | 0.13 |
| S. creatinine (mg/dl) | 0.11 | 0.40 |
| Blood urea (mg/dl) | 0.003 | 0.98 |
| PT (seconds) | –0.07 | 0.59 |
| INR | –0.15 | 0.24 |
| ALT (IU/L) | 0.22 | 0.09 |
| AST (IU/L) | 0.19 | 0.15 |

Table (3): ROC curve analysis of s. PDL-1 Performance in prediction of hepatocellular carcinoma stage.

| HCC stage No.=60 | Best cut-off | Sensitivity (%) | Specificity (%) | PPV (%) | NPV (%) | AUC |
|---|---|---|---|---|---|---|
| Stage B | 572.5 (unit) | 53.85 | 45.45 | 53.85 | 45.45 | 0.465 |
| Stage C | 586 (unit) | 80.0 | 62.5 | 72.73 | 71.43 | 0.7903 |
| Stage D | 1325.5 (unit) | 100.0 | 94.44 | 66.67 | 100.0 | 0.966 |

Table (4): Logistic multivariate regression Analysis of Potential Prognostic Factors of hepatocellular carcinoma (stage C/D versus stages A/B).

| Variable | OR | 95% CI | *p* |
|---|---|---|---|
| S. PD-1 (pg/ml) | 1.01 | 1.003 to 1.03 | 0.016 |
| AFP (ng/ml) | 1.01 | 1.00 to 1.01 | 0.035 |
| S. creatinine (unit) | 0.01 | 0.00 to 0.74 | 0.035 |

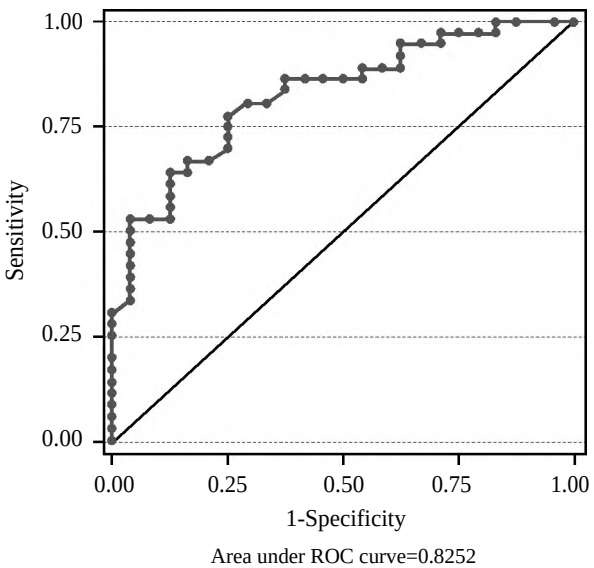


Fig. (1): ROC curve analysis of s.PD-L1 in stage C&D in all studied cases.

## Discussion

Hepatocellular carcinoma (HCC) is the most common primary liver cancer. Its allover frequency in Egypt is 2.3% among other types of cancer [12]. Without pathologic confirmation, HCC can be diagnosed using combination of serum AFP level and imaging procedures, including ultrasonography, dynamic magnetic resonance imaging and triphasic computed tomography [13]. sPD-L1 can be detected in the serum of patients, which correlates with the amount of PD-L1 expressing cells. The expression of sPD-L 1 has been shown to negatively correlate with cancer prognosis [14]. In this study there was no statistically significant correlations of sPD-L1 with gender, age or hepatitis history, these findings go hand in hand with Finkelmeier et al., who found no correlation between gender or age with sPD-L1 [7]. Gu et al., negated any correlation between sPD-L1 with gender, age and hepatitis history [15]. The result of present study revealed that, there was highly statistically significant difference as regarding serum albumin which significantly decreased in HCC patients, while total and direct bilirubin, serum creatinine, prothrombin time, INR, aminotransferases increased significantly in HCC patients, these also noticed by Carr et al., Chan et al., and Schutte et al., who found significant increase in total bilirubin, AST, albumin and platelet count in HCC cases [16,17,18], while Yeh et al., found significant increase in serum creatinine and blood urea in HCC cases [19]. The current study revealed statistically significant correlation between tumor size and level of circulating sPD-L1, this finding is in agreement with Zeng et al., and Jung et al., who also found positive correlation between tumor size and level of sPD-L 1 [20,21], but this results are against the results from the study by Gu et al., who found no association between sPD-L1 and tumor size, but found significant correlation between high levels of sPD-L 1 and poor histological tumor differentiation even in small HCC [15].

The present study showed statistically significant correlation between ALBI score and sPD-L 1 which showed a significant increase in ALBI grade 3 more than ALBI grade 1. These findings are in agreement with Finkelmeier et al., who found sPD-L1 differed significantly between ALBI grade 1,2 and 3 patients [7] and Dai et al., found increased sPD-L 1 with low serum Albumin. Our results showed statistically significant negative correlation between serum albumin and level of sPD-L 1 as both indicate bad prognosis [22]. Carr and Guerra results showed that HCC patients with lower serum albumin levels had significantly large tumor diameter, multifocality, and higher AFP levels than HCC cases with higher serum albumin levels [23], while Bagirsakci et al., found that patients with lower albumin levels had significantly large tumor diameters, more portal vein thrombosis, more tumor multifocality, higher AFP levels and lower survival than HCC patients with higher Albumin levels. These results indicate that in addition of its role in HCC growth inhibition, either through its action on growth-controlling kinases [24]. The present study showed that abnormal bilirubin level occurred with aggressive HCC and coped with elevated sPD-L 1 and indicates poor prognosis. The present study showed high statistical significant correlation between the level of sPD-L 1 and elevated AFP level, these finding in agreement with studies done by Calderaro et al., Finkelmeier et al., and Gu et al., found that AFP is an important prognostic tumor marker for those patients with elevated AFP and had worst survival than those with low AFP level [6,7,15], but Younis et al., who noticed significant AFP elevation in patients eligible for intervention [25]. There were variations in sPD-L1 level with MELD score, indicating a relation between severity of cirrhosis and sPD-L1 level, these finding is similar to results of study done by Finkelmeier et al., and Dai et al., who revealed the same results [7,22].

The present study showed that the best cut-off value of sPD-L1 for stage (A) HCC was 550pg/ml, this value could predict stage (A) HCC with sensitivity 51.5%, specificity 43.44%, PPV 52.54% and NPV 44.45%. Cut-off value of sPD-L1 for stage (B) HCC was 572.5 (pg/ml), this value could predict stage (B) HCC with sensitivity 53.85%, specificity 45.4%, PPV 53.8% and NPV 45.4%. The best cut-off value of sPD-L1 for stage (C) HCC was 586 (pg/ml) this value could predict stage (C) HCC with sensitivity 80%, specificity 62.5%, PPV 72.7% and NPV 71.4%. The best cut-off value of sPD-L1 for stage (D) HCC was 1325.5 (pg/ml), this value could predict stage (D) HCC with sensitivity 100%, specificity 94.4%, PPV 66.7% and NPV 100%, these results come in agreement with Finkelmeier et al., and Mocan et al., who conclude that high levels of sPD-L1 is a good prognostic marker for a poor outcome [7,26].

*Conclusion:*

sPD-L1 has a good diagnostic and prognostic value in the diagnosis of HCC, sPD-L1 expression correlates with HCC stage and high levels of sPD-L1 indicate poor prognosis.

*Author contributions:*

All authors contributed to data analysis, drafting or revising the article, gave final approval of the

version to be published, and agreed to be accountable for all aspect of the work.

*Disclosure:*

The authors reported no conflicts of interest in this work.

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# أهمية تقيم ليجند الموت الذاتى-١
# فى المصل لتطور مرض سرطان الكبد

سرطان الكبد هو خامس أكثر أنواع السرطان شيوعاً فى جميع أنحاء العالم والسبب الثالث للوفاة بسبب السرطان. علاج سرطان الكبد لا يتوفر إلا فى مراحل المرض المبكرة ولكن فى المراحل المتوسطة أو المتقدمة لا يوجد خيارات علاجية فى المرضى الغير قابلين للعلاج الموضعى أو الذين يعانون من تشعب الورم فى الجسم، وقد أجريت العديد من الدراسات لتقييم ليجند الموت الذاتى وعلاقته بتطور الأورام المختلفة، وأجريت هذه الدراسة لتقييم المستوى المصلى لليجند الموت الذاتى-١ وعلاقته بتطور سرطان الكبد.

المرضى وطرق الدراسة: أجريت هذه الدراسة على عدد ٨٠ مشارك منهم عدد ٢٠ من الأصحاء كمجموعة ضابطة وعدد ٦٠ مريض مشخصين بسرطان الكبد وتم التو قيع على إقرار الموافقة على المشاركة بالبحث من كل المشاركين. وقد تم الموافقة على البحث من قبل الأخلاقيات المؤسسية لكلية الطب جا معة بنها.

المرضى سوف يخضعون لما يلى:

- التاريخ المرضى
- الفحص الإكلينيكى

تحديد مرحلة السرطان عن طريق MELD SCORE ونسبة الالبيومين إلى نسبة الصفراء ويحتوى على اسئلة تكشف المسبب الرئيسى لسرطان الكبد ومضاعفاته.

التحاليل وتشمل:

وظائف كبد: مثل نسبة الألبيومين والصفراء وأنزيمات الكبد والألفا فيتو بروتين فى المصل، وزمن البروثرومبين، وقياس المستوى المصلى، ووظائف الكلى نسبة الكرياتينين واليوريا، وقياس المستوى المصلى لليجند الموت الذاتى-١.

والأشعة وتشمل الموجات الصوتية على البطن والأشعة المقطعية ثلاثية المراحل على البطن.

التحليل الإحصائى للنتائج:

وقد تم جمع النتائج وكانت كما يلى:

لقد تم ملاحظة زيادة فى المستوى المصلى لليجند الموت الذاتى فى حالات سرطان الكبد مع وجود علاقة طردية مع زيادة حجم الورم وعلاقة طردية مع الحالة العامة للمريض ومرحلة الورم.

كما أثبتت النتائج أن نسبة ليجند الموت الذاتى-١ فى المصل تصاحبه زيادة شديدة الوضوح فى وظائف الكبد، الكلى، الصفراء، الألفا فيتو بروتين وسرعة التجلط وإنخفاض ملحوظ للغاية فى نسبة الا لبيومين.

لم تشير النتائج إلى وجود صلة واضحة لليجند الموت الذاتى فى المصل بعمر أو نوع المريض أو أى عادات خاصة بالمريض ذات تأثير صحى.

الخلاصة: ومن هنا فقد وجدنا أن زيادة ليجند الموت الذاتى-١ فى المصل له علاقة وثيقة بتطور الحالة لدى مرضى سرطان الكبد وحجم الورم.

التوصيات: نوصى بدراسات مستقبلية على نطاق أوسع لاستخدام الاجسام المضادة لليجند الموت الذاتى-١ كجزء من العلاج المناعى كوسيلة لدرء تدهور الجهاز المناعى لمريض سرطان الكبد.