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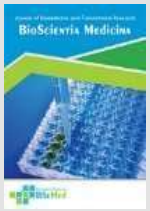

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Correlation of Soluble Programmed Death Ligand-1 (sPD-L1) with Alpha-Fetoprotein Levels as a Predictor of Prognosis in Hepatocellular Carcinoma Patients

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A B S T R A C T

Background: Hepatocellular carcinoma (HCC) is the most common primary liver malignancy and one of the leading causes of cancer death worldwide. Chronic hepatitis B virus infection is the most common etiology of HCC. Programmed death ligand-1 (PD-L1) is a protein that is overexpressed on the surface of tumor cells, which can be detected in serum (sPD-L1). Elevated sPD-L1 concentrations in the plasma of cancer patients are associated with poor prognosis in HCC patients, as indicated by increased AFP levels. There have been no previous studies examining the correlation of sPD-L1 levels with AFP in HCC patients. The aim of the study was to determine the correlation of sPD-L1 and AFP as predictors of prognosis in HCC patients. **Methods:** The research is an observational study with a cross-sectional design to see the correlation of sPD-L 1 with serum alpha-fetoprotein levels in HCC patients at Dr. Mohammad Hoesin General Hospital Palembang starts from April to July 2023. Measurement of sPD-L1 levels and serum AFP levels is carried out using ELISA examination. **Results:** Of the 28 subjects studied, HCC patients were more commonly found in men (89.29%) aged 18-60 years (75%). sPD-L1 and AFP values were found to be increased in the advanced stages of HCC. The sPD-L1 value is directly proportional to the serum AFP value. **Conclusion:** There is a strong positive correlation between sPD-L1 levels and serum AFP levels caused by hepatitis B in HCC patients, so the higher the sPD-L1, the higher the serum AFP levels.

1. Introduction

Hepatitis B virus (HBV) is an etiological factor in the development of HCC (hepatocellular carcinoma), where chronic HBV is associated with more than 50% of HCC cases. The lifetime risk of patients with chronic HBV developing HCC is 10 to 25-fold greater compared with the uninfected population. Chronic HBV-related HCC patients also had more presentations compared with cases of alcohol- and HCV-related HCC. The hepatitis B virus is thought to be able to integrate its DNA into liver cells so that it can cause mutagenesis at the integration site. This mutagenesis can trigger transformation in liver cells that causes HCC. The pathogenesis of carcinoma may involve changes in immunological cascades. Microsatellite instability mediated through activation of various signaling pathways occurs in 48% of liver carcinoma cases. Programmed death ligand-1 (PD-L1) is a protein that is universally expressed both inside and outside cells (including cancer cells). However, in HCC patients, PD-L1 is overexpressed on the surface of malignant tumor cells and binds to PD-1 substrates to inhibit T-cell proliferation and activation.[1-3]

PD-L1 can be secreted as a form of substrate called soluble PD-L1 (sPD-L1). Increased sPD-L1 levels can be detected through serum and plasma examination

and can be used to evaluate the prognostic value in HCC patients. The concentration of sPD-L1 is increased in the plasma of cancer patients, and this is associated with a poor prognosis, which can be assessed from the clinical stage and overall survival in HCC patients. Apart from that, sPD-L1 also has a relationship with CRP, hepatitis B viral load, and HCC risk, which can increase the predictive value for HCC prognosis. In HCC patients, alpha-fetoprotein (AFP) is also the most common and routinely used serum biomarker. AFP can be used for screening, diagnosis, and evaluation of HCC therapy. AFP is associated with proliferation, invasion, and migration in HCC cells, which can increase serum PD-L1 expression. AFP was also found to increase PD-L1 expression in HCC patients, which can trigger the development, metastasis, and recurrence of HCC tumors.[4-6] This study aims to determine the correlation of sPD-L1 and AFP as predictors of prognosis in HCC patients.

2. Methods

This research is an observational study with a cross-sectional design. This research was conducted at Dr. Mohammad Hoesin General Hospital Palembang. Data collection was carried out from April 2023 – July 2023. A total of 28 research subjects took part in this study, and the research subjects met the inclusion criteria. The inclusion criteria for this study are being over 18 years old, being diagnosed with HCC in accordance with the diagnostic criteria for the cause of hepatitis B, and being willing to take part in the research by signing informed consent. The independent variable was serum AFP levels. The dependent variable is level soluble PD-L1. Confounding variables are cancer other than HCC, liver damage (due to anesthesia and history of treatment, epilepsy), alcohol consumption, pregnancy, and acute hepatitis. Research ethics have been approved by the ethics committee at the place where the research was conducted, namely at Dr. Mohammad Hoesin General Hospital Palembang with number DP.04.03/D.XVIII.6.11/ETIK/103/2023. Research subjects were given an explanation of the research objectives and procedures related to the research and signed an agreement to take part in the research.

5 ml of the subject's blood sample was taken using a 21 G syringe and stored in a glass tube. Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 minutes at 1,000×g at 2-8°C within 30 minutes after collection. Store samples in aliquots at -20°C or -80°C. Examination of sPD-L1 protein and AFP protein was carried out using the Enzyme-Linked Immunosorbent Assay (ELISA) method. The sPD-L1 protein level examination was carried out by referring to the ELISA Kit sPD-L1 manufacturer's manual book (Bioscience), and the AFP protein level examination was carried out by referring to the ELISA Kit AFP manufacturer's manual book (MyBiosource). All data obtained from research status is processed using the statistical package for the social sciences (SPSS) program version 26. Categorical variables are displayed in proportion form. The correlation test was carried out using the Spearman test. The correlation between the independent and dependent variables will be visualized with a scatter plot.

3. Results

In this study, it was discovered that the majority of research subjects with liver cell carcinoma were male and aged in the 18-60 year group with an average of 54.93 years, range 35-69 years (Table 1). Table 2 shows an overview of the laboratory values of the research subjects. Average hemoglobin was slightly below normal (12-15 g/dL). Average hematocrit was slightly below normal (36-48%). The average leukocyte is slightly above normal (4000-10000/µL). Average platelets are normal (150000-400000/µL). The average SGOT is above normal (5-40 IU/L). The average SGPT is above normal (5-40 IU/L). Average bilirubin was normal (0.3-1.2 mg/dL). The average INR is normal (0.9-1.1). Average albumin was normal (3.5-5.0 g/dL). Mean HBV DNA was high, indicating possible hepatitis B virus infection.

Table 1. General characteristics of research subjects.

Characteristics	Total	%	Mean±SD	Median (min-max)
Gender				
Male	25	89.29%	-	-
Female	3	10.71%		
Age				
18-60 years	21	75%	54.93 ± 8.04	56.00 (35.00 - 69.00)
> 60 years	7	25%		

Table 2. Characteristics of laboratory examinations.

Characteristics	Mean±SD	Median (min-max)
Hemoglobin	12.27 ± 2.30	11.90 (8.30 - 18.60)
Hematocrit	36.82 ± 7.30	36.50 (24.00 - 54.00)
Leukocytes	10562.86 ± 4036.92	10465.50 (2370.00 - 23200.00)
Platelets	273571.43 ± 140161.66	246500.00 (60000.00 - 756000.00)
SGOT	168.71 ± 124.35	139.00 (26.00 -544.00)
SGPT	63.86 ± 41.45	60.00 (11.00 - 167.00)
Bilirubin	1.52 ± 0.29	1.50 (1.10 - 2.10)
INR	1.40 ± 0.24	1.40 (1.00 - 1.90)
Albumin	2.87 ± .48	2.85 (2.00 - 4.00)
HBV DNA	1093435.01 ± 3652633.23	38100.00 (30.20 - 19000000.00)

Information: SGOT, serum glutamic oxaloacetic transaminase; SGPT, serum glutamic pyruvic transaminase, INR, international normalised ratio; HBV DNA, hepatitis B virus DNA.

The nonparametric correlation test of Spearman's Rho was used to determine the correlation between sPDL-1 and AFP levels. The results of a strong and statistically significant positive correlation were found between the two variables (r = 0.648, p = <0.001) (Table 3, Figure 1). The higher the sPDL-1 level, the higher the AFP level, and vice versa.

Table 3. Correlation of sPDL-1 and AFP.

Variable		sPDL-1
AFP	*r*	0.648[a]
	n	28
	p	<0.001

[a]Spearman correlation test.

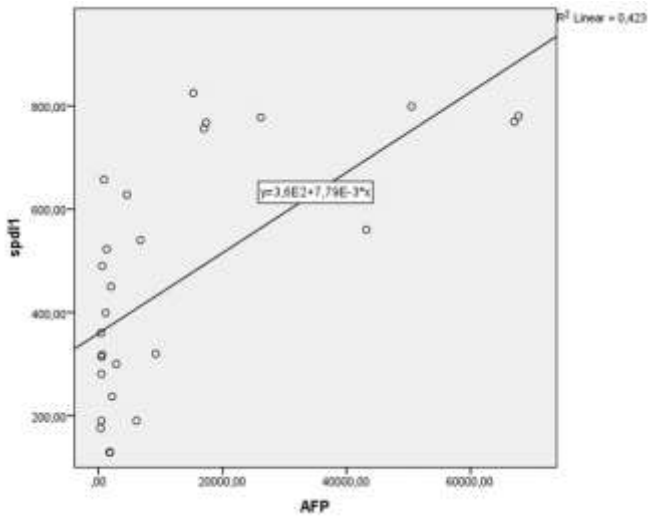

Figure 1. Correlation scatter graph of sPDL-1 and AFP.

4. Discussion

Based on the correlation test using Spearman's Rho test, it was found that there was a strong positive correlation between AFP levels and sPD-L1 significantly (r = 0.648, p = <0.001). This finding is also supported by research that demonstrated the correlation of sPD-L1 with other biomarkers, especially AFP, in HCC patients. The study found that high sPD-L1 levels in HCC patients were positively correlated with PD-L1 levels with a Spearman correlation coefficient of 0.4. In addition, high mPD-L1 expression was also found to correlate with high AFP levels, previous history of hepatitis, as well as poor tumor differentiation. This is supported by meta-analysis, which shows that there is a significant correlation between PD-L1 and AFP (OR = 1.46; 95% CI: 1.16–1.84; P = 0.001). The higher the AFP value, the higher the sPD-L1 level, and vice versa. AFP is routinely used as a tumor marker for screening, diagnosis, and treatment evaluation of HCC. Elevated serum AFP levels are often associated with HCC or other liver diseases. Studies have shown that AFP levels above 400 ng/mL can generally be considered diagnostic for HCC, so AFP is often used as a diagnostic tool for HCC.[7-12]

sPD-L1 has been used to determine the prognosis in the treatment of HCC with PD-1 inhibitors. Several studies validate that sPD-L1 can be a prognostic factor in overall survival (OS), disease-free survival (DFS), and relapse-free survival (RFS). A meta-analysis proved that high sPD-L1 expression was associated with poorer survival (hazard ratio [HR]: 2.39; 95% CI 2.20-3.91; p < 0.001). Program death 1 (PD-1) is a transmembrane receptor expressed by T cells, natural killers, B cells, and antigen-presenting cells. The binding between PD-L1 and the PD-1 receptor suppresses the migration, proliferation, and secretion of cytotoxic mediators of T cells, causing the condition of tumor cell immune escape. The PD-1/PD-L1 axis causes negative feedback on the immune response by blocking T cell receptors so that tumor cells often overexpress PD-L1 as a resistance mechanism to cause the PD-1/PD-L1 immunosuppressive pathway.[13-17]

5. Conclusion

There is a significant positive correlation with strong correlation strength between sPD-L1 levels and alpha-fetoprotein levels in patients with hepatocellular carcinoma caused by hepatitis B, so sPD-L1 can act as a predictor factor for prognosis in patients with hepatocellular carcinoma caused by hepatitis B.

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