Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/a9d21472

ASSOCIATION OF SERUM ALBUMIN LEVELS WITH AFP AND PIVA-II IN PATIENTS WITH HEPATOCELLULAR CARCINOMA

Muhammad Talha Asif[1], Shahzadi Javeed[2], Tayyab Khurshid [3,] Sadia Ahmed Zuberi[4], Qandeel Abbas Soomro[5], Amirullah[6], Qambar Abbas[7], Muhammad Rizwan[8*],Haseeb Khaliq[9]

[1]Faculty of Rehabilitation & Allied Health Sciences, Riphah International University, Islamabad
[2] Department of Biology, University of Okara
[3]Department of Pathology, University of Veterinary and Animal Sciences, Lahore.
[4]Faculty of Pharmacy, Salim Habib University, Karachi
[5]Assistant professor of pathology, Indus Medical College Tando Muhammad Khan, Sindh
[6]Department of Pharmacy, Abbottabad University of Science and Technology, Abbottabad
[7]Faculty of Biological, Pharmaceutical and Health Sciences, University of Balochistan
[8*]Allied Health professional, Government College University Faisalabad
[9]Department of Anatomy and Histology, Cholistan University of Veterinary and Animal Sciences, Bahawalpur

***Corresponding author:** Muhammad Rizwan
*E-mail: Dr.muhammadrizwan@gcuf.edu.pk

Abstract
Hepatocellular carcinoma (HCC) is one of the most common malignant tumors affecting the digestive tract, ranking fifth in morbidity and third in mortality among cancers globally, posing a severe threat to health and life. This study investigated whether serum albumin levels are associated with alpha-fetoprotein (AFP) and PIVKA-II in HCC patients. Conducted as an experimental study in the medical laboratory of Islamabad Diagnostic Center, Faisalabad, it involved the planned recruitment of 50 participants who provided written consent prior to sample collection. Patients were evaluated based on serum AFP, PIVKA-II, and other laboratory parameters. The findings from univariate logistic regression analysis revealed that the HCC group had significantly higher concentrations of AFP and PIVKA-II ($p < 0.01$). Additionally, a decrease in albumin levels ($p = 0.056$) was observed with increasing levels of AFP and PIVKA-II. The study concluded that there is a significant negative correlation between albumin levels and the levels of PIVKA-II and AFP in HCC patients, suggesting that lower albumin levels may indicate more severe liver disease and advanced disease progression. Furthermore, a positive correlation between AFP and PIVKA-II levels supports their combined use for diagnostic or therapeutic purposes.

Keywords: *Hepatocellular Carcinoma; AFP; PIVKA-II; Albumin; Prognosis*

1. Introduction

Hepatocellular carcinoma (HCC) is a primary malignancy of the liver and one of the most common malignant tumors affecting the digestive tract(Llovet et al., 2021). It ranks fifth globally in terms of morbidity (Case & Deaton, 2017)and is the third leading cause of cancer-related mortality(Gallagher & LeRoith, 2015), posing a severe threat to public health and life expectancy(Lindsay et al., 2014). The global burden of HCC continues to rise due to its strong association with chronic liver diseases,

including hepatitis B and C infections, alcohol abuse, and metabolic disorders such as non-alcoholic fatty liver disease (NAFLD)(Powell et al., 2021). Despite advancements in diagnostic techniques and therapeutic interventions, the prognosis for HCC patients(Ayuso et al., 2012) remains poor, primarily due to late-stage diagnosis and rapid disease progression(Mould, 2012).

Given the aggressive nature of HCC and its high mortality rate, early detection and accurate diagnosis are essential for improving patient outcomes. Biomarkers play a crucial role in this context by facilitating the identification of malignant liver conditions and guiding therapeutic decisions(Chen et al., 2023). Among the biomarkers commonly used in clinical practice, alpha-fetoprotein (AFP) (Terentiev & Moldogazieva, 2013)and protein induced by vitamin K absence or antagonist-II (PIVKA-II)(Wang et al., 2017) are well-recognized for their diagnostic and prognostic value in HCC management.

Alpha-fetoprotein (AFP) is a glycoprotein produced primarily during fetal development(Adigun et al., 2024). In adults, elevated AFP levels are often indicative of liver pathology, including HCC. However, the sensitivity and specificity of AFP as a standalone biomarker are limited, necessitating the exploration of additional markers to enhance diagnostic accuracy(Hanif et al., 2022). PIVKA-II, also known as des-γ-carboxy prothrombin(Zhang et al., 2014), is an abnormal form of prothrombin produced in the absence of vitamin K or in the presence of vitamin K antagonists. Elevated PIVKA-II levels have been found to correlate with liver cancer progression, making it a valuable adjunct marker alongside AFP.

Another critical parameter in assessing liver function and disease severity is serum albumin(Wang et al., 2017). Albumin is the most abundant plasma protein synthesized by the liver and plays a vital role in maintaining oncotic pressure and transporting various substances in the bloodstream. In liver diseases, including HCC, serum albumin levels often decrease due to impaired liver synthetic function. Hypoalbuminemia is associated with poor prognosis and advanced disease stages in HCC patients(Núñez et al., 2022).

This study investigates the potential association between serum albumin levels and the concentrations of AFP and PIVKA-II in HCC patients. By examining the interplay between these biomarkers, the research aims to provide insights into the utility of albumin as a predictive factor for disease severity and progression. Understanding these correlations could pave the way for improved diagnostic strategies and patient management.

This study highlights the significance of serum albumin as a valuable biomarker in conjunction with AFP and PIVKA-II for evaluating disease severity and progression in HCC patients. The observed negative correlation between albumin levels and these biomarkers underscores the importance of comprehensive biomarker assessment in clinical practice. By advancing our understanding of the complex interactions between these markers, this research offers valuable insights that may inform future diagnostic and therapeutic strategies for HCC management.

2. Methodology

2.1. Sample Collection

This experimental study was approved by the Board of Advanced Studies and Research (BASR) and the Research Ethics Committee of Riphah International University, Lahore, Pakistan. A total of 50 pre-diagnosed Hepatocellular Carcinoma (HCC) patients visiting the Islamabad Diagnostic Center, Faisalabad, were enrolled between September 10, 2023, and June 10, 2024. Patients aged 18 years and above with chronic liver disease and confirmed radiological diagnosis were included, while pediatric patients, pregnant women, and those with a history of other cancers were excluded. Clinical and demographic information was obtained from the laboratory's medical records. All procedures followed the Declaration of Helsinki guidelines, and written informed consent was obtained from each participant.

2.2. Biomarker Estimations

2.2.1. Albumin Estimation: The albumin levels were measured using the Cobas c-311 analyzer, which forms a complex between albumin and BCP reagent. Changes in absorbance at 600 nm are

directly proportional to the albumin concentration.

2.2.2. Liver Profile Estimation: The Cobas c-311 analyzer was used to process blood samples, following strict loading guidelines to avoid cross-contamination. The system aspirated and analyzed small sample volumes for liver function assessment.

2.2.3. AFP Estimation: AFP levels were determined using the Alinity (Abbott) system. The principle involves agglutination caused by AFP binding to anti-AFP antibodies coated on latex particles. The degree of turbidity correlates with the AFP concentration.

2.2.4. PIVKA-II Estimation: The HISCL PIVKA-II assay, based on a two-step sandwich method with MU-3 and prothrombin monoclonal antibodies, was conducted using the Alinity (Abbott) system. The CLEIA detection technique quantified PIVKA-II levels.

2.3. Statistical Analysis

Descriptive statistics were used for categorical variables such as age and gender. Numerical data were analyzed using the Mann-Whitney U test and Spearman's correlation. All analyses were performed using JMP Pro 17.0, with correlations presented as Spearman's correlation coefficients (r). Results were interpreted at a 95% confidence interval.

3. RESULTS

This was an experimental study of 50 patients having Hepatocellular Carcinoma. A total of 50 samples were tested for assays. Among those, the mean patient age was 61.86 years.

Table 01: Percentage/Frequency of Male Female; showing 32 patients were male (64%) and 18 females (36 %).

MALE/FEMALE	FREQUENCY	PERCENTAGE
MALE	32	64%
FEMALE	18	36%

Table 02: Percentage of HBV, HCV, HCC Subjects; showing frequency and percentage of patients having hepatitis B and C and with nohepatitis. Among 50 patients 6 was with HBV positive and 22 individuals was HCV positive. While 22 patients were having HCC with other causes of liver diseases not HBV or HCV.

Parameters	Frequency	Percentage
HBV	6	12%
HCV	22	44%
HCC ONLY INDIVIDUALS	22	44%

Table 03 Clinical Characteristics of Study that shows number of males and females having hepatitis B & C positive along with HCC along with all parameters mean in HCC patients.

Parameters	Mean±SD (Range)
Age	61.42±10.12
Albumin (g/dl)	2.97±0.69
AFP (ng/ml)	15370
PIVKA-II (mAU/ml)	37074
Bili. T (mg/dl)	1.27±0.84
ALT(U/L)	95.5±140
AST (U/L)	117.96±81.49
ALP (U/L)	366.5±265
GGT (U/L)	170.86±121.9

Table 04: Clinical characteristics among HCC with Hepatitis that shows several males and females having hepatitis B & C positive along with HCC. The liver biomarkers AST, and ALP are much higher in HCC patients than in patients with HBV and HCV. While the mean value of serum albumin is high in HBV patients.

Parameters	HBV	HCV	HCC HCC individuals Only
Gender, n			
Male (n)	2	14	16
Female (n)	4	8	6
AGE Years (Mean)	60	63.07	63.25
ALT(U/L) (Mean)	65.66	98.77	99.88
AST(U/L) (Mean)	79.2	92.2	95.4
ALP(U/L) (Mean)	195.5	214.5	244.6
GGT(U/L) (Mean)	136	195.7	155.45
ALB(g/L) (Mean)	3.78	3.02	2.89

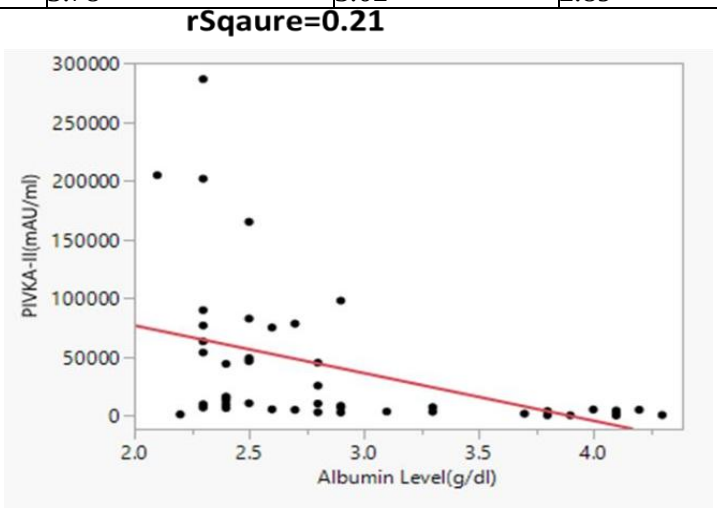

Fig 01. Association between Albumin and PIVKA-IL Levels. It indicates the relationship between albumin and PIVKA-II levels in HCC participants. A negative association is shown, meaning that PIVKA-II levels rise in response to a drop in albumin levels. This shows that PIVKA-II, which may be a sign of poorer liver function or more advanced disease states, may be greater in correlation with lower albumin levels.

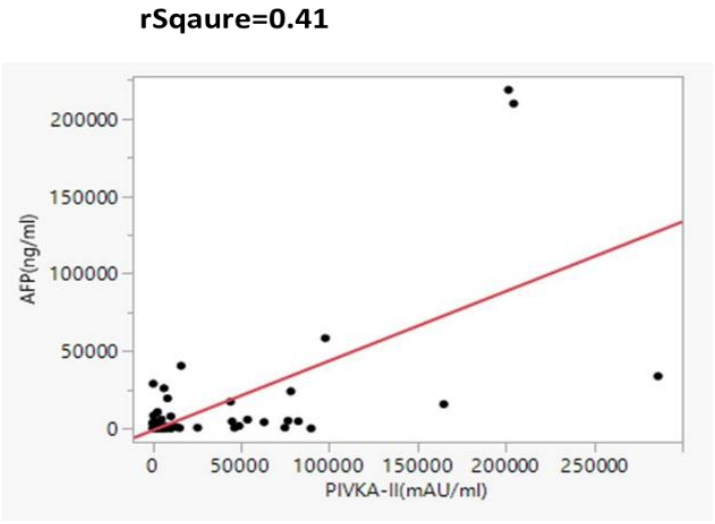

Fig 02: Association between Albumin and AFP Levels; indicates that greater AFP levels are correlated with lower albumin levels. HCC linked with the elevated AFP, and its correlation with declining albumin levels may indicate further disease progression.

Fig 03: Association between PIVKA-II and AFP Levels; Comparison of PIVKA-II as well as AFP levels and shows a positiveand significant association between elevated AFP levels and elevated PIVKA-II levels. The association between these indicators and their application in the diagnose and follow-up of HCC validates their joint use in clinical practice.

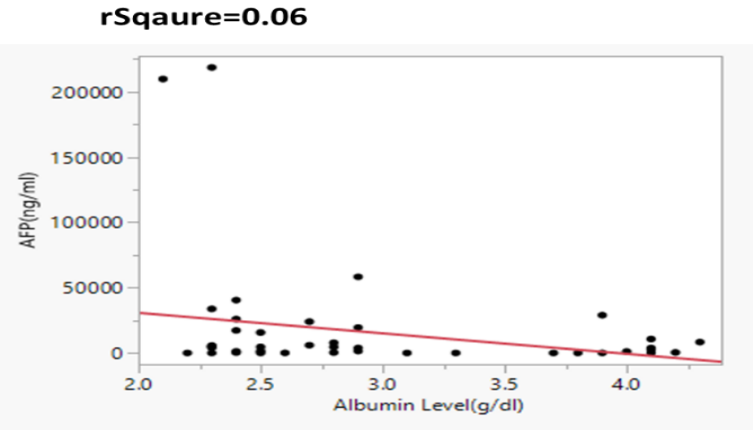

Table 05: Univariate logistic regression analysis in HCC group; showing univariate logistic regression analysis in HCC group to predict value of biomarkers of HCC. Gender, age, AFP and PIVKA-II higher values indicates significant predictor of HCC. As AFP and PIVKA-II level increases the albumin level goes down.

Parameters	**Univariate Analysis**	
	OR (95%CI)	**P value**
Age	1.042	<0.001
Gender	3.514	<0.001
AFP	7.652	<0.001
PVIKA-II	18.065	<0.001
ALB	0.923	0.056

Table 06: Spearman's correlation coefficient (r) analysis in HCC group; showing Spearman's correlation coefficient (r) analysis in HCC group to predict value of biomarkers of HCC. Albumin and PIVKA-II results are significant but negatively correlated toeach other. As AFP and PIVKA-II level increases the albumin level goes down. AFP and PIVKA-II higher values indicate significant predictor of HCC. AFP and PIVKA-II level also shows significant positive correlation with ALP and GGT but no significant correlation with Bilirubin Total and AST

Parameters compared	**Spearman's correlation coefficient r**	**p-value**
AFP and PIVKA-II	0.44	<0.05
Albumin and PIVKA-II	-0.696	<0.05
Albumin and AFP	-0.163	0.25
Bili. T and PIVKA-II	-0.18	0.20
Bili. T and AFP	0.011	0.938
ALT and PIVKA-II	-0.11	0.43
ALT and AFP	-0.08	0.57
AST and PIVKA-II	0.005	0.97
AST and AFP	0.096	0.63
ALP and PIVKA-II	0.6842	<0.05
ALP and AFP	0.307	<0.05
GGT and PIVKA-II	0.395	<0.05
GGT and AFP	0.579	<0.05

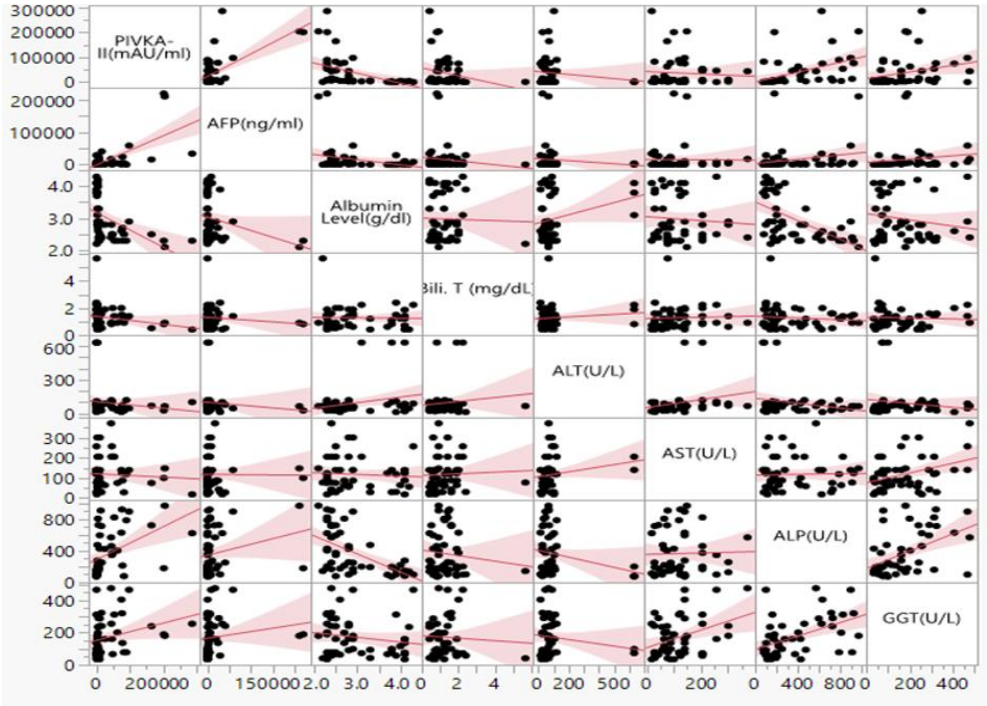

Figure 04: Spearman's Correlation between PIVKA-II, Albumin, AFP and other parameter.

4. Discussion

This study evaluated 50 patients diagnosed with hepatocellular carcinoma (HCC) to investigate the role of serum biomarkers in disease diagnosis and prognosis. Biomarkers from serum are considered more reliable and significant than other indicators for assessing disease severity and progression. By including potential serum indicators such as albumin, alpha-fetoprotein (AFP), and protein induced by vitamin K absence or antagonist-II (PIVKA-II), this research aimed to provide a practical approach for early HCC detection and prognosis.

The analysis revealed a statistically significant negative correlation between albumin levels and both PIVKA-II and AFP levels in HCC patients. Lower albumin levels were associated with higher concentrations of these biomarkers, suggesting more advanced liver disease and impaired liver function. Additionally, a positive correlation between PIVKA-II and AFP levels was observed, highlighting the potential of using these markers together for diagnosis and disease monitoring.

Approximately 30.51% of HCC patients in this study exhibited negative AFP results despite using a threshold of 30 ng/mL, consistent with previous research indicating that nearly 30% of liver cancer patients have negative serum AFP levels. In patients with hepatitis B virus (HBV) infections, AFP sensitivity for detecting HCC was 65.49%. In contrast, PIVKA-II showed superior specificity at 85.37% for HCC diagnosis when using a threshold of 50 ng/mL. When AFP and PIVKA-II were combined, specificity increased to 93.89%, demonstrating the advantage of a dual biomarker approach. These findings align with previous studies, such as those by Shu et al., which reported similar specificity improvements through combined biomarker usage.

Hepatocellular carcinoma develops through a multistage process involving significant alterations in liver function, including changes in enzyme activity, metabolism, and protein synthesis. Recent studies have highlighted the role of imbalanced hemostatic systems in promoting tumor invasion and metastasis. Prolonged prothrombin time, indicative of abnormal blood clotting, correlates with reduced survival and accelerated tumor growth in several cancers, including HCC. The present study's univariate regression analysis identified AFP and PIVKA-II as primary risk factors for HCC ($p < 0.001$).

Different stages and etiologies of HCC may affect biomarker levels. In this study, patients with HCC associated with hepatitis C virus (HCV) showed significantly higher concentrations of AFP and PIVKA-II compared to those with HBV-associated HCC. PIVKA-II was more effective than AFP

alone for diagnosing HCV-related HCC; however, combining both biomarkers further enhanced diagnostic accuracy.

Despite these promising findings, certain limitations must be acknowledged. The study did not account for clinical factors such as treatment history, tumor characteristics, and vascular involvement, which could influence outcomes. Additionally, the sample was limited to a single site, and the participant pool was relatively small. These constraints highlight the need for multicenter studies with larger sample sizes and extended follow-up periods to validate and expand upon these findings.

5. Conclusion

This study demonstrated that albumin levels are negatively correlated with AFP and PIVKA-II in HCC patients, while AFP and PIVKA-II showed a positive correlation. Combining AFP and PIVKA-II measurements enhances early HCC detection, making them valuable biomarkers for monitoring disease progression. Incorporating PIVKA-II alongside AFP in clinical practice may improve diagnostic accuracy and patient follow-up.

6. Limitations

The study faced certain limitations. First, the absence of clinical variables such as treatment methods, tumor differentiation, and vascular involvement may have introduced confounding factors. Second, the research was conducted at a single location with a limited participant pool. Future multicenter trials with larger sample sizes and long-term follow-up are necessary to validate these findings and provide more definitive conclusions.

7. References

1. Adigun, O. O., Yarrarapu, S. N. S., Zubair, M., & Khetarpal, S. (2024). Alpha-fetoprotein analysis. In *StatPearls [Internet]*. StatPearls Publishing.
2. Ayuso, C., Rimola, J., & García-Criado, Á. (2012). Imaging of HCC. *Abdominal imaging*, *37*, 215-230.
3. Case, A., & Deaton, A. (2017). Mortality and morbidity in the 21st century. *Brookings papers on economic activity, 2017*, 397.
4. Chen, J., Niu, C., Yang, N., Liu, C., Zou, S.-s., & Zhu, S. (2023). Biomarker discovery and application—an opportunity to resolve the challenge of liver cancer diagnosis and treatment. *Pharmacological Research, 189*, 106674.
5. Gallagher, E. J., & LeRoith, D. (2015). Obesity and diabetes: the increased risk of cancer and cancer-related mortality. *Physiological reviews*, *95*(3), 727-748.
6. Hanif, H., Ali, M. J., Susheela, A. T., Khan, I. W., Luna-Cuadros, M. A., Khan, M. M., & Lau, D. T.-Y. (2022). Update on the applications and limitations of alpha-fetoprotein for hepatocellular carcinoma. *World Journal of Gastroenterology*, *28*(2), 216.
7. Lindsay, G., Merrill, R., & Hedin, R. (2014). The contribution of public health and improved social conditions to increased life expectancy: An analysis of public awareness. *Journal of Community Medicine & Health Education*, *4*(5), 1-10.
8. Llovet, J. M., Kelley, R. K., Villanueva, A., Singal, A. G., Pikarsky, E., Roayaie, S., Lencioni, R., Koike, K., Zucman-Rossi, J., & Finn, R. S. (2021). Hepatocellular carcinoma (primer). *Nature Reviews: Disease Primers*, *7*(1), 6.
9. Mould, D. (2012). Models for disease progression: new approaches and uses. *Clinical Pharmacology & Therapeutics*, *92*(1), 125-131.
10. Núñez, K. G., Sandow, T., Patel, J., Hibino, M., Fort, D., Cohen, A. J., & Thevenot, P. (2022). Hypoalbuminemia is a hepatocellular carcinoma independent risk factor for tumor progression in low-risk bridge to transplant candidates. *Cancers*, *14*(7), 1684.
11. Powell, E. E., Wong, V. W.-S., & Rinella, M. (2021). Non-alcoholic fatty liver disease. *The Lancet*, *397*(10290), 2212-2224.
12. Terentiev, A., & Moldogazieva, N. (2013). Alpha-fetoprotein: a renaissance. *Tumor Biology*, *34*, 2075-2091.

13. Wang, X., Zhang, W., Liu, Y., Gong, W., Sun, P., Kong, X., Yang, M., & Wang, Z. (2017). Diagnostic value of prothrombin induced by the absence of vitamin K or antagonist-II (PIVKA-II) for early stage HBV related hepatocellular carcinoma. *Infectious agents and cancer*, *12*, 1-8.
14. Zhang, Y.-S., Chu, J.-H., Cui, S.-X., Song, Z.-Y., & Qu, X.-J. (2014). Des-γ-carboxy prothrombin (DCP) as a potential autologous growth factor for the development of hepatocellular carcinoma. *Cellular Physiology and Biochemistry*, *34*(3), 903-915.