RESEARCH ARTICLE

# Evaluation of Diagnostic Utility of Protein Induced by Vitamin K Absence–II (PIVKA-II) in Hepatocellular Carcinoma

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## Abstract

Cancer is one of the leading causes of death in both economically developed and developing countries. Among all the cancers, hepatocellular carcinoma (HCC) was a significant contributor being the fifth most prevalent and third leading global cause of deaths related to cancer. The most common biomarker use in its detection is Alpha-fetoprotein (AFP), but it has low sensitivity and specificity. Many other biomarkers have been evaluated for HCC detection, of which Protein Induced by Vitamin K Absence-II (PIVKA-II) is one, that showed elevated levels in these patients. So, we tried to evaluate the role of PIVKA-II in diagnosing HCC and its usefulness in differentiating HCC and Hepatic Cirrhosis (HC).

The study group consisted of 70 patients with liver disease- 35 with HCC, 35 with cirrhosis; and 20 healthy study subjects who were age and gender matched. All patients and healthy subjects were evaluated for serum levels of both PIVKA-II and AFP.

The median serum concentration of PIVKA-II in HCC, HC patients and healthy subjects were found to be 40.37 (23.3–79.38) ng/mL, 2.33 (1.03–3.72) ng/mL and 2.27 (0.12–12.87) ng/mL, respectively. To assess diagnostic utility, Receiver operating characteristic (ROC) curves plotted for both PIVKA-II and AFP. At a cut-off level of 6.715 ng/mL, PIVKA-II showed 85.71% sensitivity and 95.0% specificity, whereas AFP at a cut-off level of 11.8 ng/mL showed 77.14% sensitivity and 95% specificity. When both combined, their sensitivity increased to 94.29%. Also, there was a positive correlation of PIVKA-II levels with tumor size ($p = 0.043$), while no such significant association was found with AFP.

Therefore, our study concludes that PIVKA-II is more sensitive than AFP in diagnosing HCC and differentiating it from Hepatic Cirrhosis; and when both combined, the sensitivity increased. Also, the positive correlation of Tumour size with PIVKA-II levels indicates that it may be useful in monitoring patients for progression of HCC.

**Keywords:** Protein induced by vitamin K absence or antagonist II (PIVKA-II); Alpha-fetoprotein (AFP); Hepatocellular carcinoma (HCC); Hepatic Cirrhosis (HC); Receiver operating characteristic curve (ROC); Area Under Curve (AUC)

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## Introduction

Hepatocellular carcinoma (HCC) belongs to the group of epithelial cancers and is the most common primary liver cancer. It is one of the major contributors to cancer burden worldwide.[1] While the prevalence is high, distribution is highly variable due to the differential prevalence of etiological factors in various geographic areas. Its prevalence in India varies from 0.2–1.6%,[2,3] the most significant risk factors being chronic infections with HBV, HCV, and cirrhosis.

Physicians monitor the individuals at risk for HCC with serial hepatobiliary ultrasounds, CT scans, and serial measurements of serum alpha feto-protein (AFP).

AFP is an oncofetal glycoprotein that frequently reappears in HCC. But, slightly elevated levels of it are also seen in patients with chronic hepatitis and hepatic cirrhosis (HC),[4] decreasing its specificity for HCC. Also, not all HCC patients express high levels of AFP, thus showing a low sensitivity for HCC detection.

As hepatic cirrhosis is an important pre-malignant lesion of HCC, a more specific and sensitive marker is needed for early detection of HCC, for differentiating it from premalignant lesions and for monitoring these patients. Another such marker that was seen elevated in HCC patients is Protein Induced by vitamin K Absence or Antagonist–II (PIVKA-II).[5-8]

## Aims and Objectives

The aim of the study is to evaluate the role of PIVKA-II in the diagnosis of HCC and to evaluate its usefulness in distinguishing Hepatic Cirrhosis from HCC.

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**Conflict of interest:** None

**Ethical Approval:** All Procedures performed in the study were in accordance with the ethical standards of the Institution.

## Materials and Methods

### Patients

This study was conducted after obtaining Institutional Ethics Committee approval. It was a cross-sectional study that included a total of 70 patients diagnosed with liver disease (35 HCC and 35 hepatic cirrhosis cases) who attended outpatient facility in Department of Medical Gastroenterology from March 2017 to August 2017 at Nizam's Institute of Medical Sciences, Hyderabad, India. They were compared with 20 age and gender-matched healthy volunteers. All patients were naïve to treatment and did not receive antiviral therapy for hepatitis B or C viral infection, neither

underwent HCC-directed therapies like TACE, PEI, nor resection before inclusion into the study.

### Inclusion Criteria

- Diagnosis of HCC was confirmed by histological confirmation or by imaging studies with findings typical of HCC, like a high-density mass in the arterial phase in dynamic computed tomography (CT) or magnetic resonance imaging (MRI).
- Cirrhosis of liver was diagnosed based on clinical, biochemical and radiological evidence suggestive of cirrhosis.

### Exclusion Criteria

- Patients with viral infections other than HBV and HCV, metastatic hepatic tumors, malignancies other than HCC, pregnancy and those on warfarin treatment were excluded from the study.

### Sample Collection

After taking informed consent from the study population, blood was collected from each patient and healthy volunteers in serum and plasma tubes, centrifuged, serum and plasma separated. Serum was analyzed for liver function tests—AST(IFCC Kinetic UV Assay without P5P),[9] ALT (IFCC Kinetic UV Assay without P5P),[10] ALP (PNPP method),[11] Bilirubin (DPD method),[12,13] Total protein (Biuret method),[14] Albumin (BCG method),[15,16] on Cobas C501 (Roche Diagnostic India Pvt Ltd) and AFP was analyzed (Chemiluminescence Immunoassay) on Advia Centaur (Siemen's Diagnostics Pvt Ltd.). Plasma was analyzed for Prothrombin Time (PT) and INR on Elite Pro (IL). Serum aliquoted and stored at –20°C, and later analyzed for PIVKA-II using ELISA kit (Sandwich) from Elabscience Ltd. (Human APT ELISA Kit, Catalog No: E-EL H0485, 96T)

All the patient groups were screened for HBV and HCV positivity. Patients negative for HBV, HCV were labeled as Non-HepB Non-HepC.

### Imaging

- USG and CT or MRI were done for all patients with HCC to study tumor characteristics like size, number, and associated portal vein invasion.
- USG was performed for all Cirrhosis patients to rule out the presence of liver tumors.

### Statistical Analysis

Statistical analysis is done using MedCalc 17.1 software.

Normality of data checked using the Shapiro–Wilk test. Normal data is expressed in terms of mean and standard deviation, while Skewed data is expressed as median and interquartile range. Normal Data of three groups compared using ANOVA and skewed data compared using the Kruskal–Wallis test.

Receiver operating characteristics (ROC) curves were constructed for both the biomarkers, and the AUROCs were analyzed to compare their performance and also to set optimal cut-off values. Sensitivity, specificity, negative predictive value (NPV), positive predictive value (PPV), and diagnostic accuracy of both the markers for diagnosing HCC were assessed. The association between different variables like AFP, PIVKA-II, and tumor size was analyzed using Pearson's or Spearman Rho Correlation according to the data distribution.

Results were considered statistically significant at $p < 0.05$.

## Results

Out of 35 HCC patients with mean age 52.5 ±10.9 years (M:F = 25:10), 13 (37.1%) were HBV positive and 11 (31.4%) were positive for HCV, 2 of them were positive for both HBV and HCV and 8 (22.8%) negative for both the viral markers. The results are presented in Table 1. Tumor was single in 32 (31.4%) patients and multiple in 3 (8.6%) patients.

The size of the tumour was found to be<3 cm in 10 (28.6%), 3–5 cm in 8 (22.8%), >5 cm in 17 (48.6%) patients respectively. The portal vein was thrombosed in 62.8% (n = 22) of cases with HCC.

Of the 35 patients with cirrhosis, mean age was 52.26 ± 12.28 years, ( M:F–26:9), 13 were HBV positive, 10 were HCV positive and 12 patients negative for both HBV and HCV. All these patients had undergone CT scan, to rule out the presence of any tumors in the liver. The results are presented in Table 2.

All controls with mean age 48.2 ± 10.3 years (M: F—14:6) were negative for HBV and HCV.

### PIVKA-II and AFP

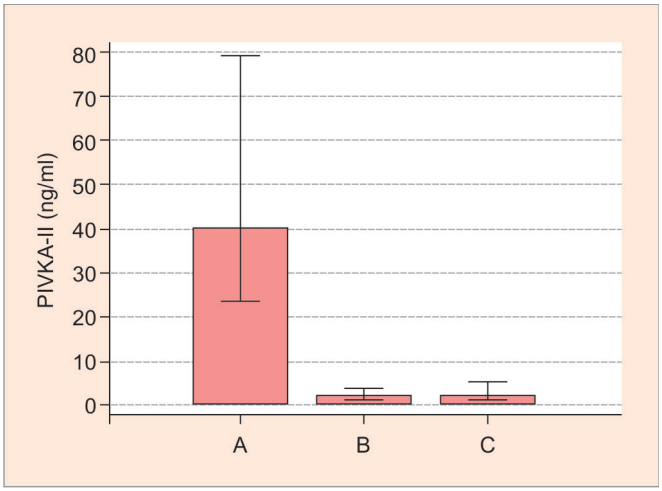
The median serum PIVKA-II levels in HCC, hepatic cirrhosis, and healthy subjects was 40.37 (IQR: 23.3-79.38) ng/ml; 2.33 (1.03-3.72) ng/mL and 1.27 (0.12-3.4) ng/mL respectively. (Graph 1).

**Table 1:** Demographic and Etiological data of study population

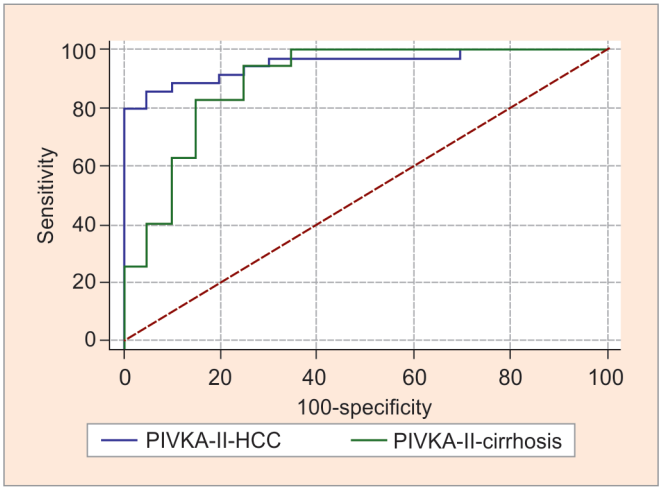
| *Parameter* | *HCC (n = 35) Mean ± SD n (%)* | *Cirrhosis (n = 35) Mean ± SD n (%)* | *Controls (n = 20) Mean ± SD n (%)* |
|---|---|---|---|
| Age | 52.35 ± 10.9 | 52.26 ± 12.28 | 48.2 ± 10.3 |
| Gender | 25 M (71.4%) | 20 M (88.67%) | 14 M (70%) |
| | 10 F (28.6%) | 9 F (11.4%) | 6 F (30%) |
| HBV Positive | 13 (37.1%) | 13 (37.1%) | – |
| HCV Positive | 11 (31.4% ) | 10 (28.6%) | – |
| Both HBV and HCV Positive | 2 (8.7%) | – | – |
| Non B, Non C | 8(22.8%) | 12 (34028%) | 20 (100%) |

**Table 2:** Radiological profile (based on ultrasound/CT-scan/ MRI findings) of HCC patients

| *Tumor characteristic* | *n (%)* |
|---|---|
| Portal vein thrombosis (*n* = 35) | |
| Present | 22 (62.85% ) |
| Absent | 13 (37.14%) |
| Localization (*n* = 35) | |
| Right lobe | 18 (51.4%) |
| Left lobe | 11 (31.4%) |
| Both lobes | 6 (17.14%) |
| Tumour number (*n* = 35) | |
| Single | 32 (91.4%) |
| Multiple | 3 (8.57%) |
| Tumour size (*n* = 35) | |
| < 3 cm | 10 (28.6%) |
| 3–5 cm | 8 (22.8%) |
| > 6 cm | 17 (48.6%) |
| Portal vein thrombosis (*n* = 35) | |
| Present | 22 (62.85%) |
| Absent | 13 (37.14) |




**Graph 1:** Mean serum levels of PIVKA-II in the 3 study

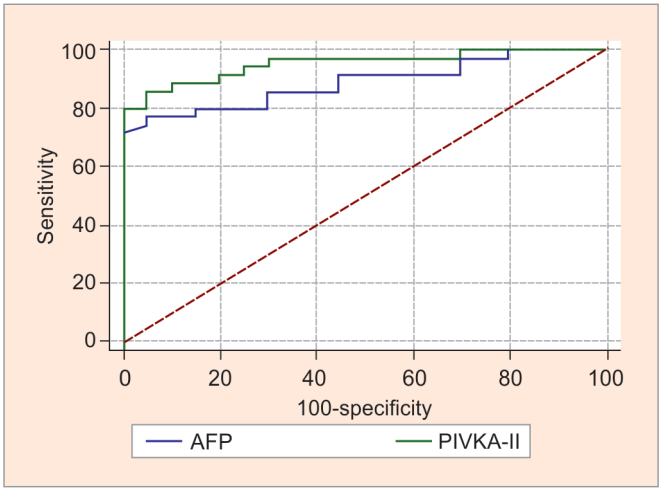



**Graph 2:** ROC curves comparing PIVKA-II in HCC and HC

**Table 3:** Serum markers of the study population

| *Parameter* | *HCC (n = 35)* *Median;IQR* | *Cirrhosis (n = 35)* *Median;IQR* | *Controls* *Median; IQR* | *p value* *(Kruskall-Wallis)* |
|---|---|---|---|---|
| AST (5–40 U/L) | 92.0 (38–496) | 79.0 (57.3–133.5) | 40.5 (34.5–54.5) | <0.000001* |
| ALT (5–40 U/L) | 84.0 (22–182) | 63.0 (40.25–95.75) | 37.0 (28–48.5) | 0.00003* |
| ALP (<370 U/L) | 161.0 (121.5–358.5) | 238.0 (144.25–341.5) | 92.0 (75.5–109.5) | <0.000001* |
| Bilirubin (0.2–0.8 mg/dl) | 2.1 (1.65–3.8) | 2.0(1.35–3.78) | 0.67 (0.57–0.81) | <0.000001* |
| Albumin (3.5–5.0 g/dL) | 3.2 (2.43–3.8) | 3.2 (2.72–3.58) | 3.92 (3.6–4.2) | 0.0023* |
| Prothrombin time (PT) | 16.0 (13.6–19.9) | 15.1(12.3–16.3) | 12.7 (11.6–13.9) | 0.0004* |
| INR | 1.5 (1.2–1.78) | 1.3 (1.1–1.47) | 1.2 (1.0–1.2) | 0.0002* |
| PIVKA –II (ng/mL) | 40.37 (23.3–79.38) | 2.33 (1.03–3.72) | 1.27 (0.12–3.4) | <0.000001* |
| AFP (ng/mL) | 181.0 (12.93–1051.75) | 5.0 (2.1–9.75) | 4.6 (1.85–7.5) | <0.000001* |

*highly significant p value




**Graph 3:** ROC curves comparing PIVKA-II and AFP in patients with HCC

The median serum concentrations of AFP in these study groups were found to be 181 (12.93–1051.75) ng/mL; 5.0 (2.1–9.75) ng/mL and 4.6 (1.85-7.5) ng/mL, respectively. The results are presented in Table 3.

ROC curves were constructed to identify optimal cut-off values that would better distinguish Carcinoma from Hepatic Cirrhosis cases (Graph 2). They showed optimal cut-off values for PIVKA-II and AFP as 6.715 ng/mL and 11.8 ng/mL, respectively. At these cut-off values, the sensitivity and specificity for PIVKA-II was 85.71% and 95.0%; and for AFP was 77.14% and 95.0%, respectively. The Area under the curve (AUC) indicated a better sensitivity and specificity for PIVKA-II compared to AFP in differentiating HCC from Hepatic Cirrhosis (0.953 vs. 0.888) (Graph 3). Table 4 shows a comparison of sensitivity, specificity, positive predictive value (PPV) and negative predictive value(NPV) for both the markers and also for a combination of these two markers.

Combined markers showed a sensitivity of 94.29% (95% CI: 80.84–99.3%) and specificity of 90.0% (95% CI: 68.3–98.77%). Thus when these two markers were combined, they showed an increase in sensitivity (94.29) and diagnostic accuracy (92.72%) compared to PIVKA-II and AFP individually.

Univariate analysis showed significant negative correlation of PIVKA II with albumin (p = 0.0024); significant positive correlation with AST ($p = 0.0032$), Bilirubin ($p = 0.0029$) and tumour size ($p = 0.043$). The results are presented in Table 5.

## Discussion

HCC is difficult-to-treat as most of the patients present in an advanced stage by the time of symptomatic presentation to the physician. And it is a very common complication in patients with chronic viral hepatitis and liver cirrhosis. Therefore, screening this high-risk population would benefit the early detection of HCC at a curable stage and improving survival rates.[17,18]

AFP, ultrasonography, CT/MRI is the most commonly used modalities for both screening and diagnosis of HCC by physicians from a very long time.[19-24] However, many studies conducted in recent times showed poor sensitivity and specificity of AFP for HCC

**Table 4:** PIVKA-II versus AFP in differentiation of patients with HCC from cirrhosis

| *Variable* | *TP* | *FP* | *TN* | *FN* | *PPV (%)* | *NPV (%)* | *Sensitivity [% (95% CI)]* | *Specificity [% (95% CI)]* | *Diagnostic Accuracy (%)* |
|---|---|---|---|---|---|---|---|---|---|
| PIVKA-II | 30 | 1 | 19 | 5 | 96.77 | 79.17 | 85.71 (69.70–95.21) | 95.00 (75.12-99.90) | 89.09 |
| AFP | 27 | 1 | 19 | 8 | 96.4 | 70.38 | 77.14 (59.90–89.57) | 95.00 (75.12–99.90) | 83.63 |
| PIVKAII + AFP | 33 | 2 | 18 | 2 | 94.29 | 90.00 | 94.29 (80.84–99.32) | 90.00 (68.31–98.77) | 92.72 |

**Table 5:** Correlation of PIVKA–II values in HCC patients with serum parameters and tumor size

| *Parameter* | *Correlation coefficient (Rho); (p value)* |
|---|---|
| AST (U/L) | 0.485 ($p = 0.0032$)* |
| ALT (U/L) | 0.305 ($p = 0.07$) |
| ALP (U/L) | 0.725 ($p < 0.0001$)* |
| Bilirubin (mg/dl) | 0.489 ($p = 0.003$)* |
| Albumin (g/dl) | 0.497 ($p = 0.002$)* |
| Prothrombin Time (sec) | 0.336 ($p = 0.048$)* |
| INR | 0.342 ($p = 0.045$)* |
| Tumour Size (cm) | 0.345 ($p = 0.043$)* |

*Statistically significant ($p < 0.05$)
AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; PIVKA-II, protein induced by vitamin K absence or antagonist-II; AFP, alpha fetoprotein; INR, international normalized ratio

and also that the likelihood of the disease being present is variable at different serum AFP concentrations.

Hence a more specific and sensitive marker of HCC is desirable in monitoring such patients. PIVKA-II is another biomarker tested to be used for HCC diagnosis.[5-8]

Protein Induced by Vitamin K Absence or antagonist II (PIVKAII) is also known as Desgammacarboxy Prothrombin (DCP). It is a modified form of the normal coagulation protein, prothrombin. In an unaffected liver, prothrombin undergoes posttranslational modification where the N-terminal glutamic acid residues are carboxylated before they are released into the blood circulation. The carboxylation thus converts specific aminoterminal glutamic acid residues into gamma-carboxy glutamic acid form. This vitamin K dependent carboxylase enzyme is absent in many malignant hepatic cells, and hence abnormal prothrombin with all or some of non-carboxylated glutamic acid is secreted from the malignant hepatic cells. This abnormal prothrombin is non-functional as the 10 glutamic acid residues in the N-terminal portion of the molecule are not carboxylated. Hence this has been used as an HCC biomarker.[25]

Leibman et al. in 1984[26] reported significantly elevated PIVKA-II levels in HCC patients while its levels were undetectable healthy Controls. In A Western study done by Volk MI et al., showed higher sensitivity and specificity for PIVKA-II compared to AFP.[27] Based On the results of our study, the AUROC Of PIVKA-II showed high sensitivity compared to AFP (85.71% *vs.* 77.14%), But both showed same specificity (95% *vs.* 95%) in differentiating malignant liver disease from other chronic pre-malignant liver diseases.

In another recent study done by Beale et al.,[28] where they compared serum PIVKA-II levels in HCC and Hepatic Cirrhosis patients; they also claimed higher median PIVKA-II levels in HCC (42.74 ng/mL) compared to cirrhosis (7.8 ng/mL). Grosley et al. also showed similar results in their study.[29]

In our study, we obtained similar results. Median serum levels of PIVKA-II were high in the HCC group (40.37 ng/mL) in comparison to the Cirrhosis group (2.33 ng/mL). Based on AUROC, we obtained an optimal cut-off of the level of 6.715 ng/mL of PIVKA-II, for the diagnosis of HCC and its differentiation from Cirrhosis. At this cut-off level, its sensitivity is 85.71%, and specificity is 95%. Though PIVKA-II is more sensitive for HCC (85.71 % *vs.* 77.17%), specificity was same as AFP (95% *vs.* 95%).

PIVKA-II levels when compared with other clinicopathological variables by univariate analysis, it showed that tumour size ($p$=0.04), AST ($p = 0.003$) and Bilirubin ($p = 0.002$), PT ($p = 0.048$), INR ($p = 0.045$) showed a strong positive correlation, while Albumin ($p = 0.002$) had strong negative correlation with the PIVKA-II levels.

Other studies have also shown that larger tumours were associated with elevated PIVKA-II levels.[30,31] Our study results also suggested similar findings where PIVKA-II levels were closely associated with tumor size than with AFP (P = 0.04).

Many other studies have reported that PIVKA-II can also predict the severity of HCC and its progression, as the HCC patients with higher PIVKA-II levels had a significantly higher frequency of complications indicating severity like portal vein thrombosis.[32,33] However, we did not find any such relationship in our study between PIVKA-II levels and portal vein thrombosis, may be because many of the selected cases had small sized and single tumors.

Some other studies have also shown an association of PIVKA-II and AFP levels and aetiology of the liver disease.[26,34,35] A study by Collier et al. had shown that the performance of AFP for HCC diagnosis might depend on the underlying etiology of liver disease.[34] In another study done by Ohhira et al., they found higher levels of PIVKA-II in chronic liver disease with alcoholic aetiology than that of chronic viral hepatitis.[35] However, we could not find any such relationship between the liver disease aetiologies supporting these results, as the participants of our study population had mixed viral and alcoholic aetiologies.

## Conclusion

Our study results showed that PIVKA-II is more sensitive than AFP, in discriminating Hepatocellular Carcinoma from Hepatic Cirrhosis, at an optimum cut-off value 6.715 ng/ml. And the combined sensitivity of PIVKA-II and AFP was higher than their sensitivities.

Our study results also showed a positive correlation of tumour size with PIVKA-II levels indicating that it may be a useful tool in monitoring patients for progression of HCC.

As it is also suggested as a prognostic marker in patients with HCC, future multicentric studies using longitudinal PIVKA-II data analysis or studies regarding its effect on the survival rate of HCC patients after treatment are needed.

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