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**Original Research Article**

# Serum Alfa Feto Protein and Protein Induced By Vitamin K Absence-II (PIVKA-II) Combined Strategy for a Better Diagnosis of Hepatocellular Carcinoma

**Dr. Sushrutha Chikkanayakanahalli Suresh[1], Dr. Balakrishna Nanjundappa Setty[2], Dr. Sathish Obalanarasimhaiah[3], Dr. Sagar Haridasanahalli Chandrashekar[4]**

[1]Associate Professor, Department of Surgical Gastroenterology and Liver Transplant, Institute of Gastroenterology Sciences and Organ Transplant, Victoria Hospital Campus, Bengaluru, Karnataka, India.
[2]Professor, Department of Surgical Gastroenterology and Liver Transplant, Institute of Gastroenterology Sciences and Organ Transplant, Victoria Hospital Campus, Bengaluru, Karnataka, India.
[3]Associate Professor, Department of Surgical Gastroenterology and Liver Transplant, Institute of Gastroenterology Sciences and Organ Transplant, Victoria Hospital Campus, Bengaluru, Karnataka, India.
[4]Assistant Professor, Department of Surgical Gastroenterology and Liver Transplant, Institute of gastroenterology Sciences and Organ Transplant, Victoria Hospital Campus, Bengaluru, Karnataka, India.

**Corresponding Acceptance**
Dr. Sagar Haridasanahalli Chandrashekar, Assistant Professor, Department of Surgical Gastroenterology and Liver Transplant, Institute of Gastroenterology Sciences and Organ Transplant, Victoria Hospital Campus, Bengaluru, Karnataka, India.

## ABSTRACT

**Background**
Hepatocellular Carcinoma (HCC) is conventionally diagnosed using serum alfa-fetoprotein (AFP). Prothrombin induced by vitamin K deficiency or antagonist- II (PIVKA-II) is another potential biomarker described in many studies. Neither of the biomarkers have been found to be efficient. The possibility of augmenting the diagnosis of HCC when both these tumour markers measured simultaneously needs to be evaluated.

**Methods**
Patients with biopsy proven HCC were taken for the study. Serum AFP and PIVKA II measurements were recorded in these patients. Radiology examination with CT Scan or MRI scan was grouped as typical or Atypical features of HCC.

**Results**
32 patients had biopsy proven HCC. AFP was elevated in 22 (68.8%) patients. PIVKA-II was elevated in 21 (65.6%) patients. Combined AFP and PIVKA-II was elevated in (90.6%).

**Conclusions**
Combined efficacy of doing both S.AFP & S. PVIKA-II is better than doing either S.AFP or S. PVIKA-II.

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**Keywords:** Hepatocellular carcinoma (HCC), Alfa-Fetoprotein (AFP), Prothrombin induced by vitamin K absence or antagonist-II (PIVKA-II).

## BACKGROUND

HCC is one of the most common malignancies of the gastrointestinal system with high morbidity and mortality. HCC has high rates of morbidity and mortality due to delay in diagnosis. Early diagnosis is critical for timely treatment and improved survival. To increase the reliability of an early HCC diagnosis, it is critical to look for new methods.[1,2]

Contrast CT scan abdomen, Ultrasound abdomen, magnetic resonance imaging (MRI), and other imaging modalities have greatly improved the diagnosis of HCC. The drawbacks of these imaging modalities are that they are operator dependent, high cost, insensitivity to small tumours. Biopsy is not done routinely in the patients who have classic findings on imaging modality.[3] Biopsy is a confirmatory test for diagnosing HCC. Biopsy of the tumour is done only when there is ambiguity in the radiological findings of the HCC. The invasiveness of the biopsy test and bleeding tendencies of the cirrhotic liver limits its application.[4,5]

Therefore, there is a need for a convenient, inexpensive, non-invasive and reproducible serum biomarker which shall assist in the early diagnosis of HCC. Alpha-feto protein (AFP) is a conventionally used biomarker for the diagnosis of HCC. However, diagnostic accuracy is limited due to high false-negative rates. In addition, AFP can also be elevated in some benign liver diseases, such as Chronic hepatitis and cirrhosis without HCC. Currently, the application of AFP for early detection of HCC is controversial as it has got no direct correlation to the tumour.[6,7]

PIVKA-II was significantly increased in the HCC patients and may serve as a new serum marker for HCC. Some investigators believed that PIVKA-II was superior to AFP and may replace AFP in the diagnosis of HCC. However, most studies have not reached to this conclusion. Many investigators have proposed that the combined detection of PIVKA-II and AFP may improve HCC diagnosis compared to using either biomarker alone. Therefore, the diagnostic value of PIVKA-II remains controversial as a stand-alone test for HCC or a combined modality along with AFP in HCC diagnosis.[8,9,10] There is a scope to explore new HCC-related biomarkers or achieve combined detection of multiple indicators that improve the accuracy of early HCC diagnosis.

## MATERIALS & METHODS

The study was conducted between January 2022 to April 2023. This was a retrospective study. All patients visiting the outpatient department with biopsy proven HCC were enrolled in the study. Patients who underwent surgery for HCC with post-operative histopathology were enrolled in the study. The patients who had atypical features on radiology were subjected to liver biopsy on day care basis. These set of patients who had biopsy features of HCC treated on outpatient basis were also enrolled in the study.

Inclusion criteria were Biopsy proven HCC, Typical or Atypical radiological features of HCC, Tumour markers with either AFP or PVIKA2. Patients with only radiology or serum markers were excluded from the study.

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Demographic features such as age, gender were recorded. Radiologic features were broadly classified as typical or atypical features. Tumour markers were recorded as elevated, not elevated or not done.

Patients with haemorrhagic or thrombotic disease taking anticoagulants, such as warfarin or related agents, or vitamin K were excluded from study. Serum AFP and PIVKA-II measurement was done using Chemiluminescence Microparticle Immunoassay (CMIA) method.

**Statistical Analysis**

Data was entered in excel spread sheet and analysed using SPSSV-20. The outcome variables were expressed by frequencies and percentages. The sensitivity analysis was carried out to find the sensitivity of the two biomarkers.

**RESULTS**

32 patients were enrolled in the study (n=32).

Elevated S.AFP was seen in 22 (68.8%) patients, PIVKA II was elevated in 21 (65.6%) patients.

| Biomarker | Outcome | Frequency | Percent |
|---|---|---|---|
| **AFP** | Elevated | 22 | 68.8 |
| | Not Elevated | 10 | 31.3 |
| **PIVKA_II** | Elevated | 21 | 65.6 |
| | Not Elevated | 6 | 18.8 |
| | Not Done | 5 | 15.6 |
| **AFP & PIVKA_II** | Elevated | 29 | 90.6 |
| | Not Elevated | 3 | 9.4 |

***Table 1: Outcomes of Serum ALFA Fetoprotein and PIVKA-11***

FIGURE-1. Shows the comparison of serum markers done alone and Combined efficacy of AFP and PIVKA for diagnosis, assuming Biopsy as a confirmatory test.

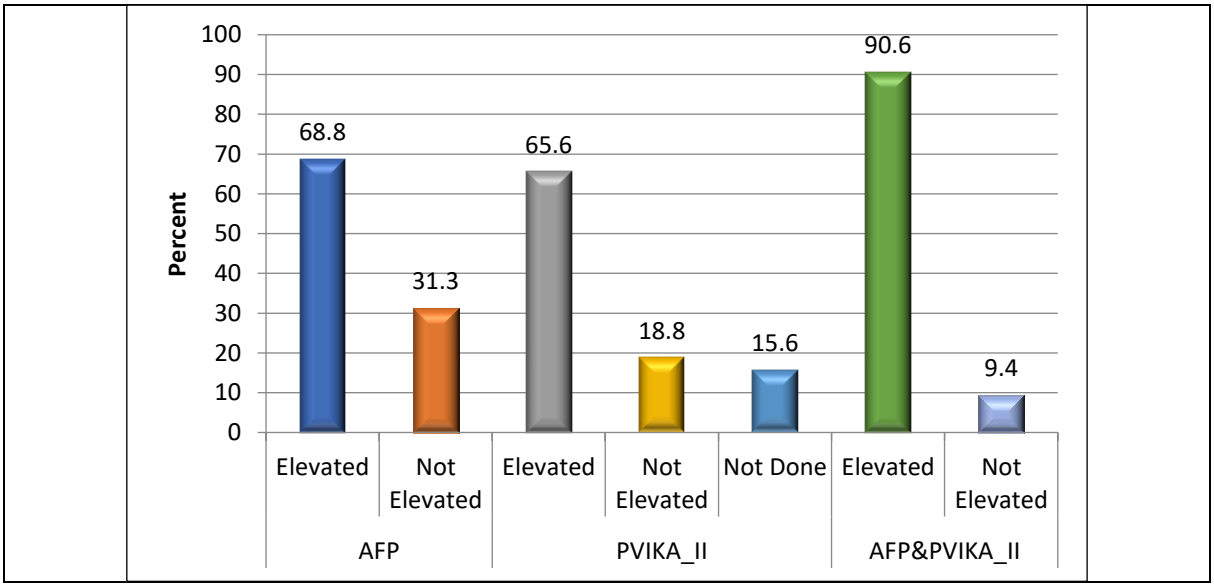

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***Figure 1.***

| Radiological_Features | Frequency | Percent |
|---|---|---|
| Typical | 24 | 75.0 |
| Atypical | 8 | 25.0 |
| Total | 32 | 100.0 |

***Table 2: Radiology features showing typical and atypical features of HCC***

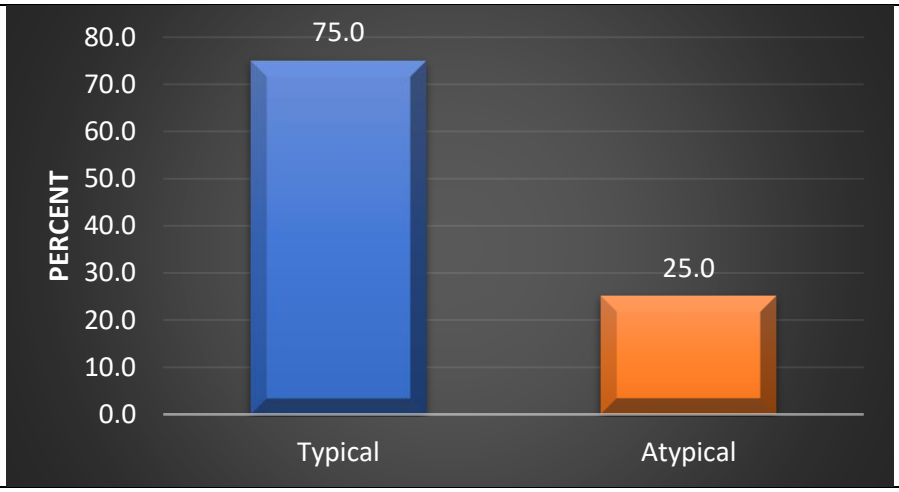


***Figure 2: Radiology features showing typical and atypical features of HCC***

| Radiological Features | AFP PIVKA II | | Total |
|---|---|---|---|
| | Elevated | Not Elevated | |
| **Typical** | 22 | 2 | 24 |
| **Atypical** | 7 | 1 | 8 |
| **Total** | 29 | 3 | 32 |

***Table 3: Comparison of the combined tumour markers with Radiological features***

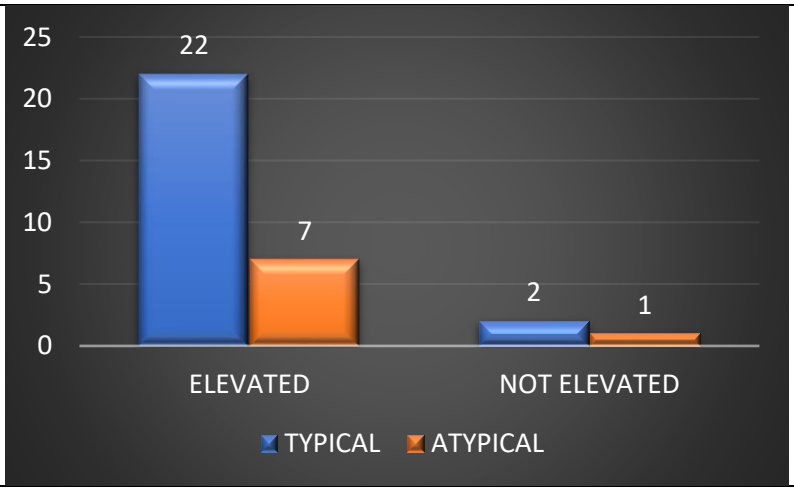


***Figure 3: Comparison of both the biomarkers with Radiological features***

| Biomarker | Frequency | Percent |
|---|---|---|
| AFP | 22 | 68.8 |

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| | | |
|---|---|---|
| PVIKA_II | 21 | 65.6 |
| AFP & PVIKA_II | 29 | 90.6 |

***Table 4: Sensitivity of Biomarkers***

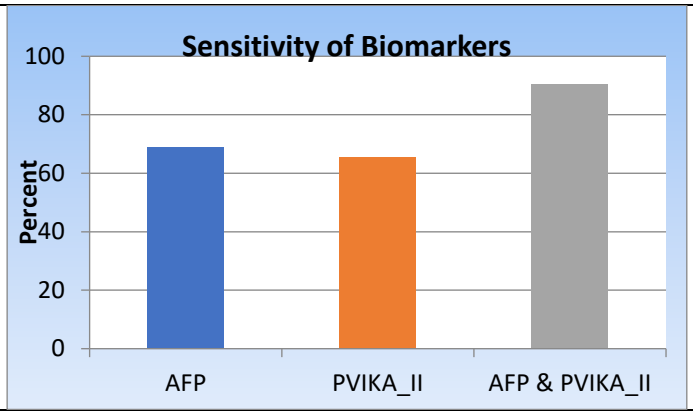


***Figure 4 Sensitivity of individual and combined tumour markers***

## DISCUSSION

HCC is a common malignancy of the gastrointestinal system. With the increasing incidence of cirrhosis, the incidence of HCC has also increased. The early symptoms are not obvious in many HCC patients, so they are diagnosed at an advanced stage. High-risk populations screening is necessary for early diagnosis especially in cirrhotic patients.[11,12] AFP is the most commonly used biomarker for HCC patients despite its poor sensitivity and specificity, especially in the early stages of the disease.[13] A novel and reliable biomarker is needed to improve HCC diagnosis. Many tumour markers have been evaluated in many studies, but none of them have been fool proof. Recently, the diagnostic role of PIVKA-II has been proposed by many authors.[14] PIVKA-II depends on the vitamin K depended clotting cycle. The mechanisms behind the functionality are not entirely clear. The researchers attributed them to abnormal enzymes related to vitamin K metabolism which is produced when malignant transformation of hepatocytes occurs.[15]

Over the years, numerous tumour markers have been for proposed to detect HCC namely, Golgi protein 73 (GP73), glypican-3 (GPC3), and cytokeratin 19 (CK-19).[9-11] GP73 is considered a potential marker for HCC, but GP73 was found to be inefficient in differentiating HCC from benign liver disease. Serum GPC3 levels were elevated in HCC patients, but GPC3 was less sensitive to distinguish between benign disease and early HCC. Studies have shown that CK-19 expression is associated with aggressive behaviour in HCC like Low degree of differentiation, metastasis and microvascular invasion. Hence CK-19 may be used as an indicator of survival and relapse in his HCC patients. The above described markers not considered effective enough for clinical use as indicator of HCC diagnosis.[16,17,18]

There is a need for a reliable tumour marker which shall help in early diagnosis of HCC. The available tumour marker as AFP and PIVKA 2 are time tested but lack accuracy. A debate weather a combined modality of the available tumour marker may help in increasing the accuracy of diagnosis.[19]

The diagnostic efficiency of PIVKA-II is superior to that of AFP in some studies. But the available literature does not suggest that PIVKA-II can completely replace AFP in the early detection of HCC. Serum AFP is mostly commonly used in clinical settings. It is a time

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tested tumour marker for the diagnosis of HCC. Serum AFP will not be raised in all patients with HCC Table 1 and Figure 1 Serum AFP quite often is used as a screening tool and post procedural surveillance, this monitoring can only be done in cases where the pre procedural serum AFP is elevated.[20]

Radiology findings of early arterial enhancement and early washout out is diagnostic of HCC. Further confirmatory test is seldom done when liver tumours have such radiological findings. Atypical radiological findings warrant further investigations as tumour markers or biopsy. In our study typical features was seen in 24 cases, atypical features was seen in 8 cases.(Figure 2, Table 2) A correlation between the radiology features (typical and atypical) and the predictivity of the tumour markers (AFP and PIVKA II) can be done, but this needs more number of cases to arrive at a statistically significant number.[21] In our study the predictivity was highest when we did combined tumour & when the radiological features had typical features of HCC (Table 3, Figure 3).

PIVKA-II levels were positively correlated with TNM stage and tumour size, suggesting a possible role for PIVKA-II in predicting disease severity. Higher concentrations of PIVKA-II were seen in larger tumour volume and higher clinical stage. AFP does not have any relation with the tumour size or the stage of the clinical disease. Patients with lymph node positivity and distant metastasis had significantly higher PIVKA-II levels than those without metastasis. Metastases imply a poor prognosis, high levels of PIVKA-II may therefore partly reflect the poor prognosis of HCC patients. The degree of differentiation and the serum level of AFP has no relation. Some studies postulated that PIVKA-II levels increased with decreasing HCC differentiation grade, suggesting that PIVKA-II may be associated with HCC malignancy and prognosis.[22,23]

The therapeutic index for evaluating the curative effect of liver cancer surgery is low in both PIVKA II and AFP. Surgery is the most effective way to treat HCC. Immediate Postoperative surveillance can be efficiently done by PIVKA-II as the half-life of serum PIVKA-II (40–72 hours) and that of his AFP (5–7 days). PIVKA-II may play an important adjunctive role to AFP.[24,25] The AFP & PIVKA II does not exist in the available literature but a weak co relation exist as studied by many authors.[26]

Each of the tumour markers have their own nuances. A typical gold standard tumour marker does not exist in the diagnosis of HCC. Several upcoming tumour markers need more evaluation before they are put into routine clinical use. Hence a combination of test was proposed for better diagnosis. In our study combined sensitivity of AFP and PIVKA-II has a sensitivity of 90.6%. (Table 4, Figure 4). Which was better when either was used alone.

## CONCLUSION

Early diagnosis of HCC is essential for the effective treatment. S.AFP and PIVKA-II are two well established serum tumour markers for the diagnosis of HCC. Combined efficacy of doing both S.AFP & S.PIVKA-II is better than doing either S.AFP or S.PIVKA-II.

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