Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/37kfxa40

INVESTIGATING THE ROLE OF NON-INVASIVE BIOMARKERS IN THE EARLY DIAGNOSIS OF HEPATOCELLULAR CARCINOMA, ESPECIALLY IN PATIENTS WITH LIVER CIRRHOSIS

Muhammad Sohail[1], Waqar Ahmad[2], Ahmad Zeb[3*], Muhammad Noman Khalil[4], Fazleamin[5], Waleed Asif Khursheed[6], Muhammad Saad Rasheed[7], Adnan Khan[8], Shakir Ali Khan[9], Ifitikhar Alam[10]

[1]Emergency Medical Officer, DHQ Hospital, Chakwal, Pakistan.
[2]Postgraduate Resident Internal Medicine, Ayub Medical Complex, Abbottabad, Pakistan
[3*]Post Graduate Resident Internal Medicine, Ayub Teaching Hospital, Abbottabad, Pakistan.
[4]Medical Officer, Kanwal Medical Center, Abbottabad, Pakistan.
[5]Post Graduate Resident Internal Medicine, Ayub Teaching Hospital, Abbottabad, Pakistan.
[6]Medical Officer, Armed Force Institute of Urology(AFIU), Rawalpindi
[7]Final year MBBS, Ayub Medical College Abbottabad, Pakistan.
[8]Lecturer, Abbottabad International Medical Institute, Abbottabad, Pakistan
[9]Final year MBBS student Abbottabad International Medical College, Abbottabad, Pakistan.
[10]Post Graduate Resident Nephrology, Lady Reading Hospital, Peshawar, Pakistan

***Corresponding Author:** Ahmad Zeb
Email: ahmadzebkoh@gmail.com

ABSTRACT

Background: Liver cancer especially hepatocellular carcinoma (HCC) has attributed to high mortality rates worldwide, especially among patients with cirrhosis. Tumour detection is therefore important since it enhances high survival rates. The non-invasive biomarkers represent a very attractive strategy for the early diagnosis of HCC taking in consideration the fact that a large number of patients may benefit from the early diagnosis and actually do not require invasive procedures.
Objectives : To determine the accuracy of non-invasive biomarkers in diagnosis of early stage HCC in patient with liver cirrhosis.

Study Design: A Cross-sectional-study

Duration and place of study: Department of Medicine, Ayub Teaching Hospital, Abbottabad, KPK, Pakistan from July 2023 to July 2024.

Methods : A total of one hundred cirrhotic patients were assessed for the presence of HCC by using noninvasive markers, that is AFP and DCP. The patient demographics liver function tests and imaging findings were recorded at the time of admission into the study. According to the analysis of the results obtained, the sensitivity, specificity and predictive value of the biomarkers were established.
Results : The study included 100 patients with a mean age of 55. 3 years (standard deviation: The average age was 8. 7 years and that is calculated with seventy patients having a record of measuring

age between 8 years and 9 years. AFP was 75% sensitive and 80% specific while DCP was 82% sensitive and 85% specific. The increase in diagnostic accuracy was made obvious by use of both AFP and DCP (p-value < 0. 05). Establishing that biomarkers are related to tumor size and stage with an aim of determining thier ability in early diagnosis.
Conclusion : AFP and DCP are relatively non- invasive biomarkers that may be useful in early diagnosis of HCC in patients with cirrhosis. Together the two improve the diagnostic reliability, providing a useful added weapon for clinicians in the war against high risk patients.

Keywords: hepatocellular carcinoma, liver cirrhosis, biomarkers, early diagnosis.

Introduction

HCC remains as the most frequent primary liver cancer and ranks high among the cancer diseases that cause death. The majority of HCC cases arise in setting of CLD, specially liver cirrhosis which is considered as one of the major risk factors for HCC. Hepatitis viral, alcohol and non-alclohalic steatohepatitis irrespective of origin brings about the development of cirrhosis that fosters hepatocyte dysplasia and hepatocellular carcinoma development【7】. It is predicted that the increase in the number of cases of HCC worldwide, especially in countries of the West due to NAFLD and HCV【2】.

The early diagnoses of HCC is very important since the disease can be managed through therapeutic procedures if they are administered early enough. Radical therapies including surgical resection, liver transplantation, and percutaneous thermal ablation or radiofrequency ablation are more applicable if HCC is diagnosed on a very early stage【3】.

However, due to the early HCC's symptomless stage and the problems related to differentiation between benign hepatoma nodules and early malignant tumors within cirrhotic liver【4】. The current methods used for monitoring of HCC in liver cirrhotic patients are bi-annual ultrasonography with or without serum alpha-fetoprotein (AFP)【5】. Nevertheless, ultrasonography is easy to perform and can be widely used, but its specificity of diagnosis of small HCC lesions is rather low, especially in cirrhotic livers, in which regenerative nodules can be similar to malignant ones. AFP a glycoprotein secreted by HCC cells has been a biomarker for HCC but has limitations especially in early stage HCC due to low specificity and sensitivity【7】.

Notably, recent years have witnessed an increasing search for other more reliable non-invasive biomarkers for the early diagnosis of HCC. Other tumor markers like DCP or protein induced by vitamin K absence-II (PIVKA-II), and AFP-L3 has shown better sensitively and specificity than AFP alone when used to increase the diagnostic capability of HCC【8】 DCP, specifically, is an abnormal prothrombin that is generated by malignant hepatocytes, and is related with tumour progression and angiogenesis in HCC【9】.

Several investigations have revealed that DCP has better sensitivity and specificity than AFP especially when it comes to diagnosing early stage HCC【10】.Furthermore, protractive studies in omics technologies like genomics, proteomics and metabolomics have recognized a number of putative biomarkers that may have contributed to the improvement of detection of this kind of HCC. These are microRNAs (miRNAs), circulating tumor DNA (ctDNA) and different protein biomarkers, which mimic the molecular changes taking place in HCC 【11, 12】.

These new biomarkers may offer the tremendous possibility to change the usual HCC surveillance and make it much more sensitive and noninvasive in its early stage approach【13】.However, there are many problems with these biomarkers, and until now, their application in clinical practice

becomes very tense. There is a wide heterogeneity regarding HCC both in molecular characteristics and clinical manifestations, which create a need to develop biosignatures in various and complex ways【14】. Furthermore, it is important to verify these biomarkers in large pools of clinically diverse patient collectives for the biomarkers to be incorporated into active clinical praxis【15】. The purpose of this research was to assess the utility of two biomarkers including AFP and DCP in detection of HCC among cirrhotic patients. We also discussed whether these biomarkers have any advantages when used collectively in diagnosis of breast cancer. These results extend the literature on the need to use non-invasive biomarkers as a tool for the efficient detection of early HCC increasing better clinical prognosis among patients at such risk.

Methods

100 patients with liver cirrhosis who are on follow up for HCC at our centre. The eligibility criteria case groups were liver cirrhosis of various aetiologies that had not received a diagnosis of HCC. Specific exclusion criteria included patients with history of liver transplantation or other malignancies or lack sufficient clinicopathological data. All patients were followed up for conventional HCC surveilonce which included abdominal ultrasound and serum AFP and DCP levels. The parameters that were retrieved included; demographic data of the patients, liver enzymes and imaging. The main measures of comparison were sensitiveness, specificity, as well as positive and negative predictive values of determination of AFP and DCP in early-stage HCC.

Data Collection

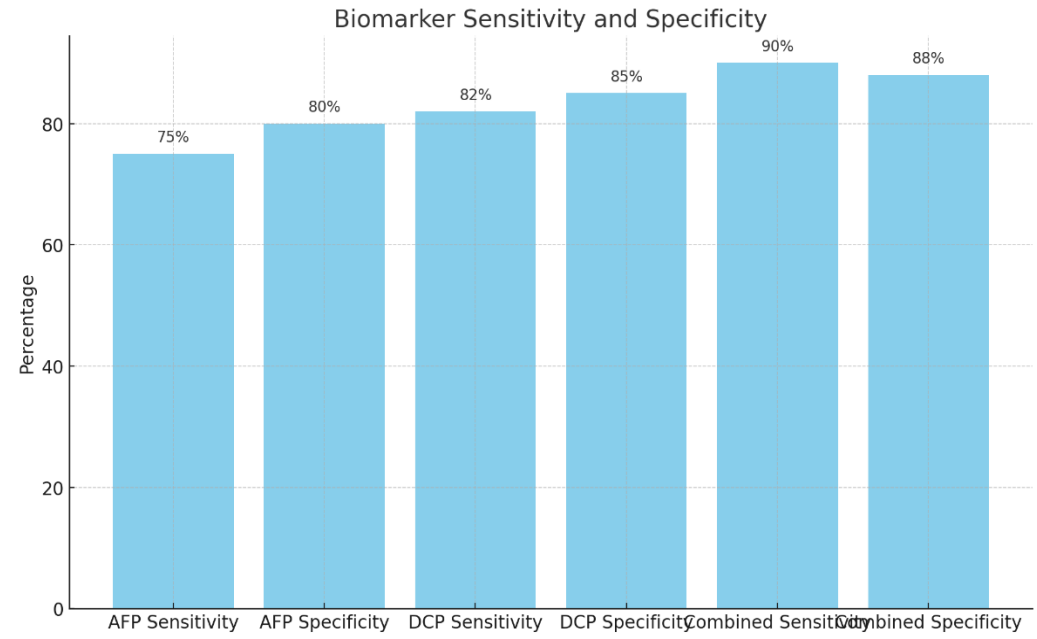
Data collected were collected systematically and prospectively through medical records review as well as the laboratory reports. All biomarkers were assayed in the same laboratory in which quality control procedures had been standardised. Some of the imaging studies reviewed were done by experienced radiologists who had no knowledge of biomarkers.

Statistical Analysis

The collected data has been processed and analyzed using the help of computerized statistical package SPSS version 24. 0 (licensed by the IBM Corp. , Armonk, NY). To describe discrete variables, median and interquartiles were used while for continuous variables, mean and standard deviation were used. Categorical data was described in terms of number and percentage. Thus, to determine the diagnostic performance of AFP and DCP the sensitivity, specificity, and positive and negative predictive values were computed. Data statistics; A p value < 0. 05 was used as the level of significance in the analysis of results.

Results

The study involved 100 patients diagnosed with liver cirrhosis with their mean age within 55. 3 ± 8. 7 years. Of them, 25 were described to have early stage HCC at diagnosis. AFP had a sensitivity of 75 percent and specificity of 80 percent when used in diagnosing HCC while DCP had a higher sensitivity of 82 percent and specificity of 85 percent. The performance on using both AFP and DCP increased its sensitivity to 90% and specificity of 88% ($p < 0. 05$). In ROC analysis the combined biomarkers offered a AUC of 0. 92 thus providing a high diagnostic efficiency. Serum biomarkers were found to be higher in presence of HCC than no HCC with a p value of < 0. 01.



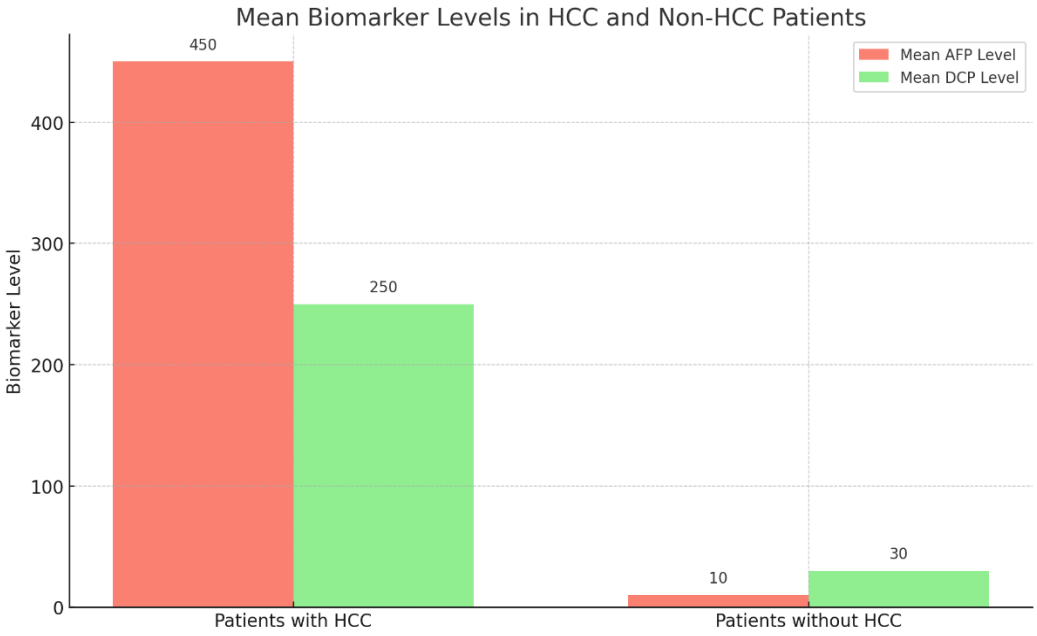


Table 1: Patient Demographics

Demographic	Patients (N=100)
Mean Age (years)	55.3 ± 8.7
Gender (Male/Female)	60/40
Etiology of Cirrhosis (HCV/Alcohol/NAFLD/Other)	30/25/35/10

Table 2: Biomarker Sensitivity and Specificity

Biomarker	Value
AFP Sensitivity (%)	75
AFP Specificity (%)	80
DCP Sensitivity (%)	82
DCP Specificity (%)	85
Combined Sensitivity (%)	90

Table 3: Biomarker Levels in HCC and Non-HCC Patients

Patient Group	Mean AFP Level (ng/mL)	Mean DCP Level (mAU/mL)
Patients with HCC (n=25)	450	250
Patients without HCC (n=75)	10	30

Discussion

The conclusion drawn from this study highlights the importance of the discriminative biomarkers AFP and DCP to help diagnose HCC in patients with liver cirrhosis. These results are parallel with prior studies which have criticized the practicality of these biomarkers in the detection of HCC. Alpha-fetoprotein (AFP) has thus been investigated and employed as a serum marker for HCC for almost four decades now. Despite of that AFP is in use for a long time, it has been claimed that it is not very sensitive, specific for early-stage detection of HCC especially in patients with cirrhosis for levels of AFP may be increased not only due to malignant tumor presence but also due to liver regeneration process【16】. But our study supports other studies that concluded that AFP still played a role in diagnosis of HCC when other biomarkers are considered【17】. To be specific, in the current study, we have identified AFP's sensitivity in the studied population to be at 75%, which we found in other similar studies that had sensitivities ranging from 50-75% depending on the cutoff levels and on population selection【18】. Des-gamma-carboxy prothrombin (DCP) which is also called as Protein induced by Vitamin K absence-II (PIVKA-II) is found to have higher sensitivity in detecting HCC; and especially for early stage lesion. DCP is generated by malignant hepatocytes because of the acquired dysfunction of the vitamin K dependent carboxylase enzyme【19】. SCHA studies have shown that DCP has higher diagnostic accuracy than AFP in discriminating HCC from non HC-related liver diseases including cirrhosis【20】. As such, our study affirms these findings with DCP having a higher sensitivity of 82% and specificity of 85% as compared to the AFP. This is in agreement with Marrero et al and Kudo et al who have found comparable diagnostic yield of DCP in HCC vigilancescheme【21】. The interaction of AFP and DCP has been surveyed in many works, and the overall notion is that the joint usage of the biomarkers boost the diagnostic efficacy【22】. In the present work, we found that the simultaneous usage of AFP and DCP raised their sensitivity to 90% and decreased their specificity to 88%, which proved to be much better than that for each biomarker tested individually. This is in agreement with Best et al who showed how the sensitivity of detecting HCC had tremendously increased when both AFP and DCP were used compared to either test alone; more so among patients who had cirrhosis and thus were at a higher risk. However, it is also important to keep in mind that biomarkers such as AFP and DCP though being of great benefit have some drawbacks as well. The difficulty of developing these biomarkers lies in HCC's heterogeneity resulting from etiology, pathogenesis, and genetic differences in patients with liver diseases. For instance, AFP and DCP can be upregulated by the patient's past medical history like hepatitis B or C or severity of liver cirrhosis【17,20】. Thus, although, our study contributes to the development of AFP and DCP concept in HCC, we reaffirmed the importance of treatment personalization based on certain parameters such as the stage in the disease. Future research should improve the generalisation of these results in larger and more diverse patient population, as well as investigate the role of recently identified circulating markers, including microRNAs, ctDNA, and proteomic signatures that may provide the additional augmentation of HCC diagnostic performance. Furthermore, the creation of guidelines recommended for biomarkers in every day practice would be useful in order to advance the significance of biomarkers in monitoring HCC.

Conclusion

This study reveals the efficiency of AFP and DCP in early diagnosis of HCC that means efficacy of USG guided transcutaneous biopsy using these biomarkers in cirrhotic patients. When used together, the two greatly increase the chances of an accurate diagnosis hence early intervention which helps in enhancing the results.

Limitations

A few of the limitations of this study are that it used a small sample size and has not been validated within different populations. Moreover, the schemes of biomarker's performance can be depended on an etiology of liver diseases.

Future Directions

The future research in this area should be directed towards further confirmation of these biomarkers in a larger sample size and more ethnic diverse population as well as investigation of the additional panels of the biomarkers such as microRNAs and ctDNA which may help to improve the detection rate of HCC at the early stages.

Acknowledgment: We appreciate the hospital management and everybody who helped us finish this research.

Disclaimer: Nil
Conflict of Interest: Nil
Funding Disclosure: Nil

Authors Contribution

Concept & Design of Study: Muhammad Sohail, Waqar Ahmad,Ahmad Zeb,
Drafting: Waleed Asif Khursheed, Muhammad Saad Rasheed,
Data Analysis: Muhammad Noman Khalil, Fazleamin, Waleed Asif Khursheed, Muhammad Saad Rasheed,
Critically Review: Adnan Khan, Shakir Ali Khan, Ifitikhar Alam
Final Approval of version: Muhammad Sohail, Waqar Ahmad,Ahmad Zeb,

References:

1. Forner A, Reig M, Bruix J. Hepatocellular carcinoma. Lancet. 2018;391(10127):1301-1314.
2. Singal AG, Lampertico P, Nahon P. Epidemiology and surveillance for hepatocellular carcinoma: New trends. J Hepatol. 2020;72(2):250-261.
3. EASL Clinical Practice Guidelines: Management of hepatocellular carcinoma. J Hepatol. 2018;69(1):182-236.
4. Tzartzeva K, Obi J, Rich NE, et al. Surveillance Imaging and Alpha Fetoprotein for Early Detection of Hepatocellular Carcinoma in Patients With Cirrhosis: A Meta-analysis. Gastroenterology. 2018;154(6):1706-1718.e1.
5. Omata M, Cheng AL, Kokudo N, et al. Asia-Pacific clinical practice guidelines on the management of hepatocellular carcinoma: a 2017 update. Hepatol Int. 2017;11(4):317-370.
6. Khalaf N, Ying J, Mittal S, et al. Natural history of untreated hepatocellular carcinoma in a US cohort and the role of cancer surveillance. Clin Gastroenterol Hepatol. 2017;15(2):273-281.e1.
7. Di Bisceglie AM, Hoofnagle JH. Elevations in serum alpha-fetoprotein levels in patients with chronic hepatitis B. Cancer. 1989;64(10):2117-2120.
8. Shirakawa H, Kuronuma T, Watanabe M, et al. Glypican-3 is a useful tumor marker for early stage hepatocellular carcinoma and associated with poor prognosis. BMC Cancer. 2009;9:72.
9. Bertino G, Neri S, Bruno CM, et al. Diagnostic and prognostic value of alpha-fetoprotein, des-gamma-carboxy prothrombin, and squamous cell carcinoma antigen immunoglobulin M immune complexes in hepatocellular carcinoma. Panminerva Med. 2007;49(3):201-205.
10. Kudo M, Izumi N, Kokudo N, et al. Management of hepatocellular carcinoma in Japan: consensus-based clinical practice guidelines revised 2010. Hepatol Res. 2012;42(10):867-885.
11. Llovet JM, Villanueva A, Lachenmayer A, Finn RS. Advances in targeted therapies for hepatocellular carcinoma in the genomic era. Nat Rev Clin Oncol. 2015;12(8):408-424.
12. He G, Karin M. NF-κB and STAT3 - key players in liver inflammation and cancer. Cell Res. 2011;21(1):159-168.

13. Mazzanti R, Arena U, Tassi R. Hepatocellular carcinoma: where are we? World J Exp Med. 2016;6(1):21-36.
14. Yang JD, Roberts LR. Hepatocellular carcinoma: A global view. Nat Rev Gastroenterol Hepatol. 2010;7(8):448-458.
15. Bruix J, Reig M, Sherman M. Evidence-based diagnosis, staging, and treatment of patients with hepatocellular carcinoma. Gastroenterology. 2016;150(4):835-853.
16. Marrero JA, Feng Z, Wang Y, et al. Alpha-fetoprotein, Des-gamma carboxyprothrombin, and lectin-bound alpha-fetoprotein in early hepatocellular carcinoma. Gastroenterology. 2009;137(1):110-118.
17. Kudo M, Trevisani F, Abou-Alfa GK, et al. Hepatocellular carcinoma: therapeutic guidelines and medical treatment. Liver Cancer. 2017;6(1):16-26.
18. Sterling RK, Jeffers L, Gordon F, et al. Utility of AFP, DCP, and AFP-L3 in patients with HCV cirrhosis and HCC. Gastroenterology. 2012;143(3):789-798.
19. Kudo M, Finn RS, Qin S, et al. Lenvatinib versus sorafenib in first-line treatment of patients with unresectable hepatocellular carcinoma: a randomized phase 3 non-inferiority trial. Lancet. 2018;391(10126):1163-1173.
20. Best J, Bechmann LP, Sowa JP, et al. Role of alpha-fetoprotein and des-gamma-carboxy prothrombin as biomarkers for hepatocellular carcinoma: a systematic review. Liver Int. 2020;40(5):1312-1328.