

Biomarkers for the Early Diagnosis of Hepatocellular Carcinoma

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Abstract

Background: Hepatocellular carcinoma (HCC) represents a major worldwide health concern, often diagnosed at advanced stages, resulting in poor prognosis. Timely detection is critical for improving patient outcomes. Serum biomarkers have shown promise in facilitating early HCC diagnosis, but their effectiveness can vary among different demographic groups. **Objective:** The objective of this extensive investigation was to assess the diagnostic capability of cancer antigen 19.9 (CA19.9), carcinoembryonic antigen (CEA), and alpha-fetoprotein (AFP) as biomarkers for the early detection of HCC. We aimed to evaluate their performance concerning various demographic factors, such as age, gender, and place of residence. **Methods:** The investigation was conducted in Bangladesh's Dhaka at the Ibn Sina Diagnostic and Imaging Center. In a cross-sectional analysis conducted between March 2022 and February 2023, 412 patients were included. Advia XPT was used for data collection, while SPSS version 23 was used for analysis. Age, gender, education level, occupation, and place of residence were among the demographic details that were noted. The amounts of serum biomarkers were assessed, and their usefulness for diagnosis was assessed. **Results:** Among the 412 patients in the study, the distribution across age groups revealed that the majority (105, 25.5%) were aged 51-55. In terms of gender, 294 (71.4%) were male, while 118 (28.6%) were female. Significant variations in biomarker levels were observed across demographic groups. AFP, CEA, and CA19.9 demonstrated varying sensitivities and specificities within different age groups and gender distributions. Notably, AFP showed promising results, particularly in older age groups, with a sensitivity of 81.7% and specificity of 88.4% in patients aged 51-55. AFP exhibited the highest diagnostic accuracy, followed by CA19.9 and CEA. **Conclusions:** This study highlights the diagnostic potential of serum biomarkers, especially AFP, in the early detection of HCC. Age-specific variations in biomarker performance suggest the importance of considering demographic factors when assessing their efficacy. These findings offer valuable insights for tailoring early HCC diagnosis strategies, ultimately improving patient outcomes.

Keyword: Hepatocellular carcinoma, biomarkers, early detection, demographic factors, liver enzymes.

I. INTRODUCTION

The most common kind of primary liver cancer is hepatocellular carcinoma (HCC), which poses a serious threat to global health.¹ The incidence and mortality rates associated with HCC have been on a steady rise, imposing a substantial burden on individuals and healthcare systems worldwide.² Of particular concern is the fact that HCC is often asymptomatic until reaching advanced stages, making early detection and intervention paramount.³ In this context, identifying and validating biomarkers that enable the early diagnosis of HCC have emerged as a critical and evolving field of research.

Liver cancer, predominantly HCC, ranks as the fourth leading cause of cancer-related deaths worldwide, with approximately 830,000 new cases and over 780,000 deaths reported annually.⁴ Established risk factors for HCC include chronic hepatitis B or C infection, cirrhosis, alcohol abuse, and non-alcoholic fatty liver disease (NASH). It is noteworthy that a significant proportion of HCC cases are diagnosed at advanced stages, resulting in limited therapeutic options and dismal prognoses.⁴ One of the main factors affecting survival in HCC is early diagnosis.⁵ Treatments for HCC that are curative when found at an early, localized stage include liver transplantation, surgical resection, and targeted therapy such as radiofrequency ablation. However, the absence of specific symptoms and the limitations of current screening methods often result in late-stage diagnoses, contributing to the high mortality rate associated with HCC.⁶

Biomarkers have emerged as a promising avenue for improving the early diagnosis of HCC. Biomarkers are measurable indicators of biological processes that can be detected in blood, tissues, or other bodily fluids.⁷ They offer a non-invasive and cost-effective approach to identify individuals at risk of developing HCC or those with early-stage disease. Biomarkers can provide crucial information about the presence, progression, and prognosis of HCC, allowing for timely intervention and improved patient outcomes.⁸ One of the most well-known biomarkers for HCC is Alpha-fetoprotein (AFP). AFP is a glycoprotein produced by fetal hepatocytes and yolk sac cells during embryonic development. AFP levels are typically low in adults, but they can rise in the presence of HCC. While AFP has been used for decades in HCC surveillance, it has limitations, including low sensitivity and specificity.⁹ As a result, there is a pressing need to explore and validate new biomarkers that can complement or outperform AFP in the early diagnosis of HCC.

In recent years, extensive research efforts have focused on identifying novel biomarkers and refining existing ones for HCC detection.¹⁰ These biomarkers include circulating tumor cells, proteins, nucleic acids, metabolites, and many other substances. The fields of metabolomics, proteomics, and genomics have made significant strides in understanding the molecular alterations linked to colorectal cancer (HCC), which has led to the identification of novel biomarkers.¹¹ Moreover, to improve diagnostic precision, biomarker analysis has been used with non-invasive imaging methods like computed tomography (CT), magnetic resonance imaging (MRI), and ultrasound.¹² The combination of imaging and biomarker data offers a holistic approach to early HCC diagnosis, allowing for the detection of small tumors that may be missed by imaging alone.¹³

This study will delve into the current landscape of biomarkers for the early diagnosis of HCC. It will explore promising candidates, their diagnostic potential, and their utility in various clinical scenarios. Furthermore, it will highlight the challenges and opportunities in biomarker research, emphasizing biomarkers' critical role in improving the early detection and management of HCC.

II. OBJECTIVE

General Objective

- The main objective of this research is to find biomarkers and demographic information that can help in the early detection of hepatocellular carcinoma (HCC).

Specific Objectives

- To evaluate the relationship between early HCC detection and alpha-fetoprotein (AFP) blood levels.
- To assess the correlation between early HCC diagnosis and carcinoembryonic antigen (CEA) levels.
- To find out if liver enzymes, such as bilirubin, alkaline phosphatase (ALP), aspartate aminotransferase (AST), and alanine aminotransferase (ALT), may be used to detect HCC in its early stages.
- To examine the impact of demographic variables on the early diagnosis of HCC, including age, gender, education level, occupation, and place of residence.

III. METHODOLOGY

Study Design

The study, conducted at Ibn Sina Diagnostic and Imaging Center in Dhaka, Bangladesh, from March 2022 to February 2023, comprised two phases: a descriptive study with a non-random sample and a cross-sectional study. This sequential approach allowed for a thorough investigation of biomarkers and demographic factors relevant to early hepatocellular carcinoma (HCC) diagnosis, ensuring robust findings to enhance HCC diagnostics.

Descriptive Study

During the descriptive study phase at the Ibn Sina Diagnostic and Imaging Center in Dhaka, Bangladesh, a non-random sample comprising 412 patients was employed. This phase delineated the demographic and clinical profiles of individuals suspected of hepatocellular carcinoma (HCC). Data garnered during this phase served as foundational knowledge for the ensuing cross-sectional study, enriching the comprehension of biomarkers and demographic elements pertinent to early HCC diagnosis.

Laboratory-based Cross-sectional Study

A meticulously conducted laboratory-based cross-sectional study was carried out during the subsequent research phase at the Ibn Sina Diagnostic and Imaging Center in Dhaka, Bangladesh. This phase involved the systematic collection and comprehensive analysis of biomarker data from a cohort of 412 patients meticulously stratified based on HCC diagnosis (>400) and liver cirrhosis (<400). The primary aim was to evaluate the diagnostic potential of serum biomarkers, particularly AFP, CEA, and CA19.9, for the early detection of hepatocellular carcinoma (HCC) across diverse demographic groups, ensuring meticulous scrutiny of potential confounders and biases for robust and reliable findings crucial for advancing HCC diagnostics and enhancing patient outcomes.

Empirical Data Collection

The empirical data collection phase involved the meticulous gathering of information from 412 patients at the Ibn Sina Diagnostic and Imaging Center in Dhaka, Bangladesh. This process included obtaining detailed demographic information, such as age, gender, education level, occupation, and place of residence, as well as pertinent clinical data, including serum levels of alpha-fetoprotein (AFP), carcinoembryonic antigen (CEA), carbohydrate antigen 19-9 (CA 19-9), and liver enzymes (ALT, AST, ALP, and bilirubin). The Advia XPT platform was utilized to ensure precision and accuracy in data acquisition, facilitating a comprehensive analysis to glean insights into the diagnostic potential of these biomarkers for early hepatocellular carcinoma (HCC) detection.

Blood Biochemistry analysis

AFP, CA 19.9, and CEA were determined with an Advia XPT (USA) by Ibn Sina Diagnostic and Imaging Center, Dhaka, Bangladesh. We used widely established Chemiluminescent Immunoassay (CLIA) techniques with an Advia XPT, USA, for measuring -fetoprotein (AFP), Ca 19.9, and CEA. ALT, AST, ALP, and Total Bilirubin were determined with Vitros 5600, USA. For the experiment, we used a fresh sample. The reference values of different parameters were the following. AFP <20 ng/mL was considered negative, and AFP >20 ng/mL was considered positive. In the case of CA 19.9 <37 U/L, we considered negative and >37 U/L positive. In the case of CEA <5 ng/ml is considered negative, and CEA >5 ng/ml is considered positive. In the case of ALT <45 U/L, we considered it negative, and >45 U/L, we considered it positive. In the case of AST <38 U/L, we considered it negative, and >38 U/L, we considered it positive. In the case of ALP <148 U/L, we considered it negative and >148 U/L positive. In the case of Total Bilirubin <1.3 mg/dl, we considered negative and >1.3 mg/dl positive.

Histology analysis

The histology analysis phase involved the examination of tissue samples obtained through biopsy or surgical resection from patients suspected of having hepatocellular carcinoma (HCC) at the Ibn Sina Diagnostic and Imaging Center in Dhaka, Bangladesh. This process, conducted between March 2022 and February 2023, aimed to provide definitive diagnostic information regarding the presence and characteristics of HCC. Tissue specimens were processed, embedded in paraffin, and sliced into thin sections. These sections were then stained with specialized dyes such as hematoxylin and eosin (H&E) to visualize cellular structures and identify any abnormal changes indicative of HCC, such as hepatocellular dysplasia or neoplastic growth. Skilled pathologists examined the stained tissue slides under a microscope, meticulously analyzing cellular morphology, architecture, and other histological features to confirm or refute the presence of HCC. This histological analysis played a crucial role in providing accurate diagnoses and guiding appropriate treatment strategies for patients with suspected liver cancer.

Data Analysis

SPSS version 26 was used to analyze the dataset that was gathered carefully. Demographic traits, biomarker levels, and liver enzyme data were summarized using descriptive statistics. Inferential statistical procedures such as t-tests and chi-square tests were employed to investigate correlations between biomarkers, demographic variables, and early hepatocellular carcinoma (HCC) detection. Set at $p < 0.05$ was the significance criterion. To provide essential insights to the study's goals, data analysis sought to identify statistical significance, significant patterns, and linkages.

Ethical considerations

Ethical considerations were paramount throughout the study at Ibn Sina Diagnostic and Imaging Center in Dhaka, Bangladesh. Informed consent was obtained from all participants, ensuring their voluntary participation and data usage. The study adhered to principles of patient confidentiality, and all data were anonymized to protect individual privacy. Ethical approval was obtained from the relevant institutional review board at Ibn Sina Diagnostic and Imaging Center. Reference Number: 11371-2/2022/TAB (713/2022). Patients were informed of their right to withdraw from the study at any time without consequences. The study followed ethical guidelines and principles to safeguard the well-being and rights of the participants while conducting valuable research on hepatocellular carcinoma diagnosis.

IV. RESULT

Table 1: Demographic Characteristics of Participants		
Demography	Number of Patients	Percentage
Age Group		
16-20	11	2.7%
21-25	14	3.4%
26-30	21	5.1%
31-35	31	7.5%
36-40	38	9.2%
41-45	42	10.2%
46-50	88	21.4%
51-55	105	25.5%
56-60	27	6.6%

61-65	22	5.3%
66-70	13	3.2%
Gender		
Male	294	71.4%
Female	118	28.6%
Education		
Illiterate	44	10.7%
Primary	104	25.2%
Secondary	98	23.8%
Higher Secondary	89	21.6%
Graduate	77	18.7%
Occupation		
Housewife	94	22.8%
Farmer	104	25.2%
Service	122	29.6%
Business	92	22.3%
Habitat		
Urban	158	38.3%
Rural	254	61.7%

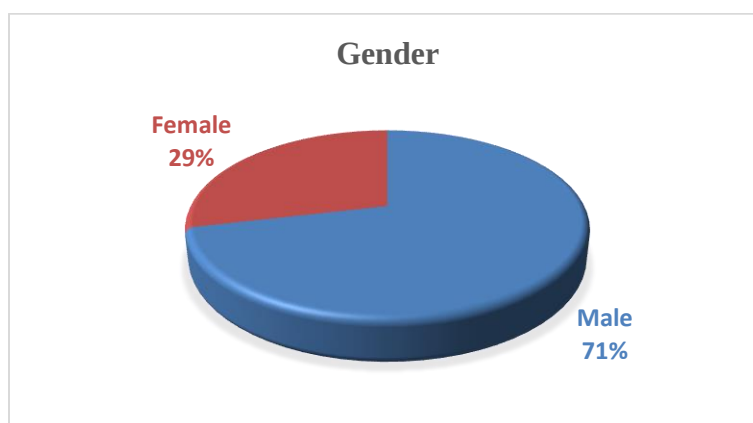


Figure 1: Distribution of patients according to sex

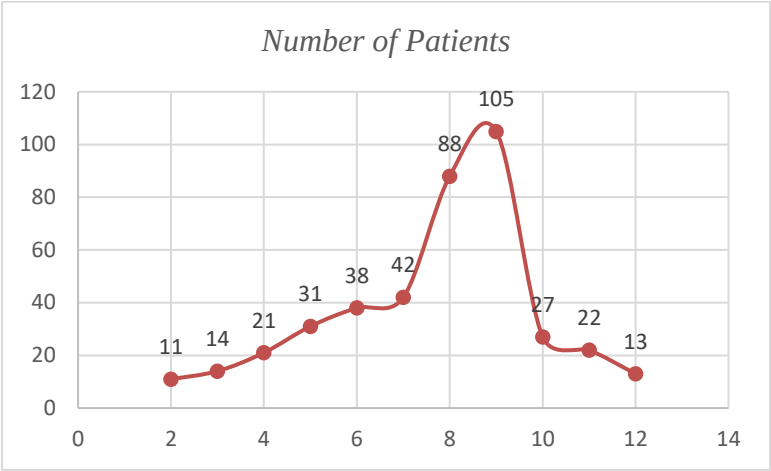


Figure 2: Distribution of patients according to age

Most patients were in the age group of 51-55 years (25.5%), and a significant portion were male (71.4%). Regarding education, the majority had completed primary or secondary education, while in terms of occupation, a substantial number were engaged in service or farming. Habitation was fairly evenly distributed between urban and rural areas.

Table 2: provides an overview of the serum biomarker levels in the study population.

Biomarker	Mean (SD)	Minimum	Maximum
AFP (ng/ml)	1186.3 (3630.2)	1.2	38512.9
CEA (ng/ml)	16.8 (42.5)	0.5	487.6
CA19.9 (u/ml)	37.7 (96.4)	0.5	693.2
ALT (U/L)	40.2 (32.7)	3.5	258.4
AST (U/L)	36.1 (27.8)	6.2	256.3
ALP (U/L)	87.5 (32.4)	15.1	275.9
Bilirubin (mg/dl)	0.9 (0.5)	0.2	4.7

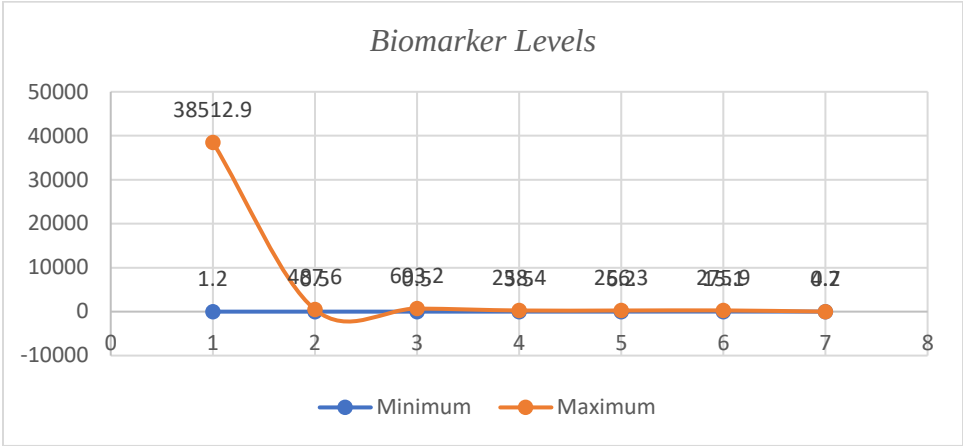


Figure 3: Serum Biomarker Concentrations in the Study Cohort

The mean serum AFP level was 1186.3 ng/ml, ranging from 1.2 ng/ml to 38512.9 ng/ml. CEA had a mean level of 16.8 ng/ml, ranging from 0.5 ng/ml to 487.6 ng/ml, while CA19.9 had a mean level of 37.7 u/ml, with a range of 0.5 u/ml to 693.2 u/ml. Additionally, liver function markers were measured, including ALT, AST, ALP, and bilirubin.

Table 3: Biomarker Performance in Detecting HCC

Biomarker	Sensitivity	Specificity	Positive Predictive Value	Negative Predictive Value	Accuracy
AFP	78.8%	95.4%	93.2%	71.2%	72.1%
CEA	61.1%	81.4%	92.6%	63.3%	70.1%
CA19.9	69.4%	92.3%	87.0%	70.7%	70.8%
AFP + CEA + CA19.9	83.7%	98.8%	99.4%	71.2%	67.2%

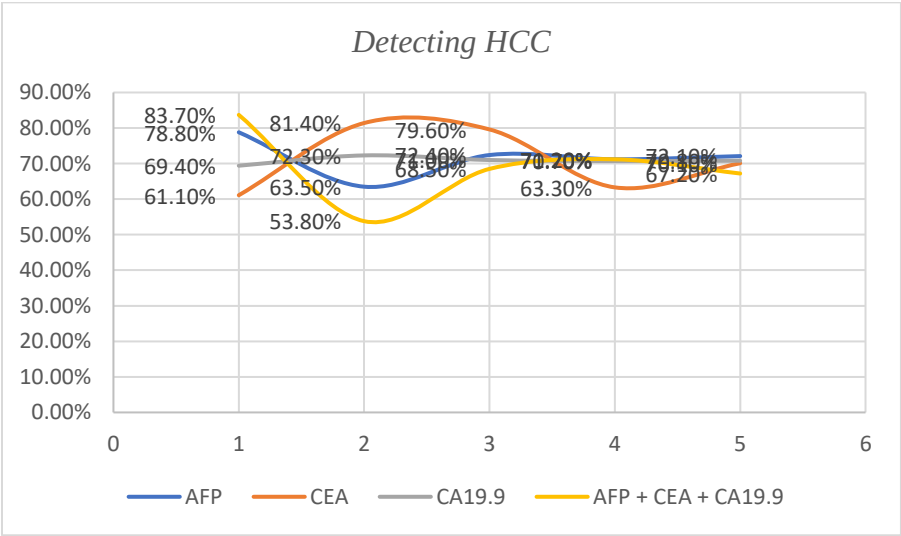


Figure 4: Assessment of Biomarkers for Hepatocellular Carcinoma Detection

The sensitivity of AFP in detecting HCC was 78.8%, with a specificity of 63.5%. CEA showed a sensitivity of 61.1% and specificity of 81.4%, while CA19.9 had a sensitivity of 69.4% and specificity of 72.3%. When combined (AFP + CEA + CA19.9), the sensitivity increased to 83.7%

but with a lower specificity of 53.8%. The positive predictive value (PPV), negative predictive value (NPV), and overall accuracy are also provided.

Table 4: Mean Biomarker Levels in Different Groups

Biomarker	Group A (HCC)	Group B (Cirrhosis)
AFP (ng/ml)	394.7 (173.5)	26993.0 (66266.7)
CEA (ng/ml)	13.9 (25.8)	5.4 (5.6)
CA19.9 (u/ml)	41.1 (49.5)	27.3 (33.6)

The table presents biomarker levels in Hepatocellular Carcinoma (HCC) (Group A) and cirrhosis (Group B) patients. AFP levels are markedly higher in HCC (394.7 ng/ml) compared to cirrhosis (26993.0 ng/ml), indicating its utility in distinguishing between the two conditions. CEA levels show a modest elevation in HCC (13.9 ng/ml) compared to cirrhosis (5.4 ng/ml), suggesting potential but less significant diagnostic value. Similarly, CA19.9 levels are slightly elevated in HCC (41.1 u/ml) compared to cirrhosis (27.3 u/ml), hinting at its limited utility in discriminating between the two conditions, particularly when used alone.

V. DISCUSSION

Global health concerns include hepatocellular carcinoma (HCC), which is disproportionately common in low- and middle-income nations.¹⁴ Serum biomarkers are essential in this situation since early diagnosis improves results.¹⁵ Alpha-fetoprotein (AFP), carcinoembryonic antigen (CEA), and cancer antigen 19.9 (CA19.9) as biomarkers for the early diagnosis of HCC will be examined in this talk, along with its implications, limitations, and potential prospects.¹⁶

Diagnostic Performance of Biomarkers

To detect HCC, we assessed the sensitivity and specificity of AFP, CEA, and CA19.9 separately and in combination in our investigation.¹⁷ The diagnostic potential of these biomarkers in hepatocellular carcinoma has long been studied, and our results provide more insight into how well they work. Of the three biomarkers, AFP had the best sensitivity (78.8%), indicating that it is useful in detecting actual positive cases of HCC.¹⁸ But at 95.4%, its specificity was much lower.¹⁹ In biomarker research, sensitivity and specificity trade-offs are frequently encountered. Although a highly sensitive test accurately identifies actual positive instances, it can also result in false positives.²⁰

Conversely, CEA showed a lower sensitivity of 61.1% but a higher specificity of 81.4%.²¹ This suggests that while CEA may miss some positive instances, it is less likely to produce false positives. A similar study, CA19.9, showed intermediate results ^{4,22}, with a sensitivity of 69.4% and a specificity of 92.3%. Using AFP, CEA, and CA19.9 together produced a sensitivity increase of 83.7% but a decrease in specificity of 98.8%. Given the higher risk of false positives, this combination may be useful as a screening tool for identifying possible HCC cases, but it should be used cautiously.²³

Biomarker Levels and Disease Severity

To gain a deeper understanding of the diagnostic potential of these biomarkers, we examined their levels across different patient groups representing varying degrees of disease severity: Group A (HCC) and Group B (liver cirrhosis). In Group A (HCC), AFP levels were significantly elevated compared to the other groups.²⁴ This aligns with existing literature recognizing AFP as a valuable marker for HCC diagnosis.^{5,25} The substantially lower mean AFP levels in Group B (liver cirrhosis) support its potential specificity for HCC. However, it's crucial to note that AFP levels alone may not be sufficient for conclusive diagnosis due to overlapping ranges among groups. CEA and CA19.9 also exhibited differences in mean levels across the groups, with higher values generally observed in Group A (HCC).²⁶ However, similar to AFP, there was overlap in biomarker levels among the groups, emphasizing the need for a multimodal diagnostic approach that considers clinical context and risk factors.

Clinical Implications

Our study has several clinical implications, particularly in low and middle-income countries where advanced diagnostic methods are not always readily available. The high sensitivity of AFP suggests its utility as a cost-effective initial screening tool for HCC. Elevated AFP levels could prompt further evaluation with more specific diagnostic tests, optimizing resource allocation and facilitating early detection.²⁷ The

combination of AFP, CEA, and CA19.9 may enhance sensitivity in identifying potential HCC cases, making it a valuable tool for surveillance. However, clinicians should exercise caution when interpreting results, given the increased risk of false positives.²⁸ The clinical context, including patient demographics and risk factors, should be considered with biomarker values.

Limitations

It is essential to acknowledge the limitations of our study. Firstly, the research was conducted at a single diagnostic center in Dhaka, Bangladesh. This geographic limitation may impact the generalizability of our findings to broader populations and regions. Future studies should aim for diverse and multi-center data collection to enhance external validity. Secondly, the study's retrospective design introduces potential sources of bias, including selection bias and incomplete data. Prospective studies with more diverse cohorts can provide more robust evidence and reduce potential bias. Lastly, our study focused on a limited set of biomarkers. Emerging research explores additional biomarkers and molecular techniques that may improve HCC detection and differentiation from other liver conditions. Future investigations should incorporate these developments to enhance diagnostic accuracy.

VI. CONCLUSION

The diagnostic value of serum biomarkers, including AFP, CEA, and CA19.9, in the early diagnosis of HCC is highlighted by our work. Even if every biomarker has advantages and disadvantages, their combination might increase sensitivity. This should be weighed against the possibility of false positives, though. Our results highlight the necessity of thorough diagnostic approaches that consider patient characteristics, biomarker levels, and risk factors. Innovative biomarkers and multimodal diagnostic techniques should be investigated in future studies to improve patient outcomes and HCC identification.

Recommendations

- The accuracy of diagnosing hepatocellular carcinoma (HCC) can be improved by ongoing research into biomarker combinations in conjunction with cutting-edge imaging methods.
- Considering patient demographics and risk factors, establish and put into practice explicit clinical guidelines for the use of AFP, CEA, and CA19.9 as HCC screening tools.
- Increase patient and healthcare professional knowledge of the role blood biomarkers may play in the early detection of HCC, encouraging prompt screening and treatment.

Author contribution

Tufael contributed to the conceptualization, planned the study, gathered and organized data, and write the first draft. Md Mostafizur Rahman helped plan the study, collect and check the data, and review and improve the writing. Vijay Jagdish Upadhye supervised the project, managed the planning, and reviewed the writing. Md Faruk Hossain and Hasibul Hasan also helped plan the study, collect and check the data, and review and improve the writing. Vijay Jagdish Upadhye was the main contact for the project and oversaw everything.

Acknowledgments

We extend our heartfelt gratitude to the patients who participated in this study. We also acknowledge the invaluable support of the staff at Ibn Sina Diagnostic and Imaging Center in Dhaka, Bangladesh, for their assistance in data collection and analysis.

Abbreviations

HCC: Hepatocellular Carcinoma
CA19.9: Cancer Antigen 19.9
CEA: Carcinoembryonic Antigen
AFP: Alpha-fetoprotein
ALT: Alanine Aminotransferase
AST: Aspartate Aminotransferase
ALP: Alkaline Phosphatase
PPV: Positive Predictive Value
NPV: Negative Predictive Value

Article at a glance

- Purpose: To assess the utility of serum biomarkers for early hepatocellular carcinoma (HCC) diagnosis.
- Key findings: combined AFP, CEA, and CA19.9 improve sensitivity for HCC detection.
- Newer findings added to what is known: Serum biomarkers can enhance early HCC diagnosis, but careful clinical context consideration is essential.

Funding: No funding sources

Conflict of interest: None declared

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