Diagnostic Efficacy of Tumor Biomarkers AFP, CA19-9, and CEA in Hepatocellular Carcinoma Patients


Abstract
Background: Current diagnostic methods, including tumor markers such as alpha-fetoprotein (AFP), carbohydrate antigen 19-9 (CA19-9), and carcinoembryonic antigen (CEA), have limitations in sensitivity and specificity, particularly in differentiating Hepatocellular carcinoma (HCC) from chronic liver diseases. This study investigated the diagnostic efficacy of these markers, individually and in combination, using chemiluminescence assay (CLIA) in patients with and without HCC. Methods: A cross-sectional study design was employed, analyzing data from 800 patients at Ibn Sina Diagnostic and Imaging Center in Dhaka. Tumor marker levels were assessed using CLIA kits, and associations with HCC diagnosis, tumor differentiation, occupation, age, and tumor size were examined. Results: Descriptive statistics revealed higher tumor marker levels in poorly differentiated tumors compared to well-differentiated ones and controls. The combination of AFP, CA19-9, and CEA showed superior diagnostic accuracy for HCC, with a sensitivity of 86.5% and specificity of 92.3%. Occupation and age were found to correlate with tumor marker levels and HCC risk, with certain occupations and older age associated with larger tumor sizes. Regression analysis confirmed the associations between tumor markers and HCC. Conclusions: The study demonstrated the potential of AFP, CA19-9, and CEA as a panel of tumor markers for HCC detection and monitoring. CLIA emerged as a reliable diagnostic tool, offering high specificity and accuracy. Occupational factors and age were identified as relevant considerations in HCC risk assessment.

Keywords: Hepatocellular carcinoma; Tumor markers; Chemiluminescence immunoassay; Occupational exposure; Early diagnosis

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Introduction
Hepatocellular carcinoma (HCC) is a kind of primary liver cancer that begins in the liver’s main cell type, the hepatocyte (Ringelhan, et al., 2018; Herszenyi and Tulassay, 2010). About 750,000 people each year lose their lives to this disease (Fan and Farrell, 2009;
Williams, 2006), making it the third greatest cause of death due to cancer and the fifth most common cancer in the world. Due to rising rates of non-alcoholic fatty liver disease (NAFLD), hepatitis B virus (HBV), and HCV infections, the worldwide incidence of HCC has been on the increase in recent decades (Nordenstedt, 2010). Individuals having hepatitis B, hepatitis C, and NAFLD are at increased risk for developing this cancer (Galle et al., 2019). Tumor markers such alpha-fetoprotein (AFP), carbohydrate antigen 19-9 (CA19-9), and carcinoembryonic antigen (CEA) are commonly used in clinical practice for the diagnosis and follow-up of HCC (Bertino, et al., 2012; Bari, et al., 2023; Tufael et al., 2023). Surgical resection, transplantation of the liver, and radiofrequency ablation are all curative options for patients with early-stage HCC, making early detection of HCC of utmost importance for improving patient outcomes (Loosen, et al., 2017). However, due to the lack of specific signs and the limitations of present imaging modalities (Song et al., 2019; Wang et al., 2020), early diagnosis of HCC remains a problem. In patients with chronic liver illnesses such hepatitis B, hepatitis C, and NAFLD, AFP's low sensitivity and specificity become even more apparent. For instance, one study found only 41% sensitivity in hepatitis C patients and 29% sensitivity in non-alcoholic fatty liver disease patients (NAFLD) (Mirambo, et al., 2020; Sinha et al., 2022). Since this is the case, AFP can only be used in conjunction with other markers in the diagnosis and follow-up of HCC (Chatsirisupachai et al., 2022).

Several studies have examined the diagnostic efficacy of individual markers, such as AFP, CA19-9, and CEA, and combinations of these markers, for the diagnosis and follow-up of HCC (Kwak et al., 2019). According to a meta-analysis conducted by Bari et al. (2023), combining AFP and CA19-9 increased diagnostic accuracy for HCC from 68% to 89%. Combining AFP and CEA increased sensitivity to 47% and specificity to 89%, according to a separate meta-analysis by Zhou and Luo (2006). These indicators can be helpful in diagnosing liver disease, although their use varies by patient demographic and etiology. Contradictory results persist regarding the sensitivity and specificity (Motlagh et al., 2021) of CA19-9 and CEA in combination with AFP in hepatitis B-associated HCC. Similarly, AFP has poor diagnostic accuracy for detecting HCC in hepatitis C patients, especially in those with severe liver fibrosis (Huang et al., 2019). CA19-9 and CEA have been recommended as supplemental indicators for hepatitis C-associated HCC, although it is unclear how well they work. Nonalcoholic fatty liver disease (NAFLD) has been identified as a major risk factor for hepatocellular carcinoma (Zhang et al., 2020). It has been shown that the diagnostic accuracy of AFP in identifying HCC in individuals with NAFLD is poorer than in instances of hepatitis B or C (Hadi et al., 2022). In addition, there has not been much research on CA19-9 and CEA’s diagnostic use in NAFLD-related HCC.

Chemiluminescence assay (CLIA) has proven to be a reliable approach for detecting AFP, CA19-9, and CEA, among other tumor markers. Compared to other approaches, such as immunofluorescence assay (IFA) and radioimmunoassay (RIA), CLIA has been shown in previous research to have higher sensitivity and specificity (Parra et al., 2023). These results confirm the validity of CLIA as a tumor marker diagnostic tool, encompassing AFP, CA19-9, and CEA.

The major focus of this investigation is to determine the efficacy of biopsy in establishing diagnoses of hepatocellular carcinoma. It also aims to investigate the importance of biological markers including AFP, CA19-9, and CEA in differentiating between infected and uninfected patients. CLIA will be used to compare the levels of these biomarkers in infected and uninfected people. Since CLIA has higher sensitivity and specificity than alternative procedures like IFA and RIA, this research will also prove the case for implementing it. The ultimate objective of this study is to facilitate early diagnosis of hepatocellular carcinoma, which will allow for more effective treatment and a better chance of survival. In conclusion, our research hopes to advance hepatology by proving CLIA’s superiority as a diagnostic tool and expanding our knowledge of the diagnostic and prognostic usefulness of these biomarkers.

Material and Methods

Study design

The purpose of this cross-sectional study was to examine the correlation between hepatitis B, hepatitis C, and non-alcoholic fatty liver disease (NAFL) and three tumor markers: alpha-fetoprotein (AFP), carbohydrate antigen 19-9 (CA-19,9), as well as carcinoembryonic antigen (CEA). Ibn Sina Diagnostic and Imaging center in Dhaka collected and analyzed the data. Patients were selected at random, and their blood was tested using readily accessible Chemiluminescent immunoassays (CLIA) kits for the three tumor markers (AFP, CA-19,9, and CEA).

Study population

Patients were separated into those with and without a previous diagnosis of hepatocellular carcinoma (HCC) by biopsy. In the second group, HCC was verified by a hepatologist. There were at least 800 people, both male and female, in the study’s sample population. Patients were interviewed to collect data for demographics, fibroscopy findings, liver biopsy data, and laboratory results from the most recent tests. This data included demographics such as age, gender, and BMI.

Selection criteria

Patients with NAFL verified by FibroScan, FIB-4, and APRI and with no prior history of antiviral medication were included. Hepatitis C virus and hepatitis B virus were diagnosed with Qiagen and confirmed by real-time polymerase chain reaction (RT-PCR). Patients on hepatitis medication, those who have persistent renal
illness, those with heart disease, and smokers were all disqualified from participation. Before any medicine was given, a full blood count (CBC), serum bilirubin, ALT, AST, and ALP levels, and hepatitis B, C, and NAFL tests were all performed.

**Laboratory investigations**

All patients’ blood was drawn before therapy began, and the serum was centrifuged off and kept at -20°C in the meanwhile. The Sysmex XN-2000 was used to do a complete blood count (CBC). The Advia 1800 Chemistry Analyzer was used to check bilirubin, alanine aminotransferase, asparagine aminotransferase, and alkaline phosphatase concentrations in the blood. Hepatitis B, C, and NAFL were detected in the serum samples. PCR sample extraction by Qiagen and PCR run by Rotor-Gene Q. Histological findings were used to divide patients into three groups: individuals with well-differentiated disease (AFP values between 20 and 199), those with moderately-differentiated disease (AFP values between 200 and 399), and those with poorly-differentiated disease (AFP values exceeding 400).

**Ethical considerations**

The research followed the ethical guidelines outlined in the Declaration of Helsinki. All patients were fully briefed on the nature, goals, and methods of the study before consenting to participate. All contributors voluntarily provided written informed consent. The study’s protocol received approval from the ethics committee.

**Statistical analysis**

The accuracy, reliability, and completeness of the data were all evaluated. The data analysis was performed using IBM’s SPSS 23 program. The data was summarized using descriptive statistics including frequency, percentage, mean, and standard deviation. To extrapolate from the sample to the whole population, we used inferential statistics such as T-tests, Chi-square tests, correlation analysis, regression analysis, and analysis of variance. Statistical analyses were performed at the p 0.05 level of significance.

**Results and Discussion**

**Descriptive statistics**

The objective of this study was to assess the diagnostic performance of AFP, CA19-9, and CEA as a combined panel of tumor markers in patients with hepatitis B, hepatitis C, and non-alcoholic fatty liver disease (NAFLD), with the potential to enhance the precision and dependability of hepatocellular carcinoma (HCC) diagnosis and monitoring. The study evaluated the correlation between the markers’ diagnostic performance and tumor differentiation status (well, moderately, and poorly differentiated) in the control group.

Alpha-fetoprotein (AFP), cancer antigen 19-9 (CA 19.9), carcinoembryonic antigen (CEA), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) had mean values of 3.16, 13.27, 1.64, 25.12, 26.77, and 88.79, respectively, in the control group, as determined by descriptive analysis.

The significant results show a steady increase in total bilirubin levels from the well differentiated over the poorly differentiated group, indicating a possible association among higher total bilirubin levels with poorer tumor differentiation. On the other hand, the median tumor marker values in poorly differentiated tumors were substantially higher (AFP: 16489.01, CA 19.9: 3284.56, CEA: 645.15, ALT: 1174.53, AST: 721.59, ALP: 486.02) (Figure 1).

The differences between well-differentiated tumors and the control group were less, but the marker levels were greater. The control group’s respective mean AFP, CA19-9, and CEA values in this study were 3.16, 13.27, and 1.64. These numbers are in line with earlier research that found few tumor markers in healthy subjects (Song et al., 2011). The AFP averaged 16,489. The CA19-9 averaged 3,284.56. The CEA averaged 645.15. In contrast, the poorly differentiated group had much higher levels of these markers. The poorly differentiated group also had significantly higher levels of bilirubin and the liver enzymes ALT, AST, ALP, and T. Tumor marker standard deviations showed a wide range, from -1485.08 to +40,022.73. The average levels of AFP (a kind of apolipoprotein), CA 19.9 (carcinoembryonic antigen), and CEA in well-differentiated tumors were 69.75, 71.14, and 33.39, respectively, but liver enzyme levels were lower (Figure 2).

Alpha Fetoprotein (AFP), CA 19.9, and CEA descriptive analysis of the tumor markers showing mean, median, mode, and standard deviation values for the three groups of poorly differentiated, moderately differentiated, and well differentiated tumors. These results are in line with earlier research (Singal, 2010). These markers were expressed at intermediate levels in the well-differentiated group, which raises the possibility that tumor differentiation contributes to their expression. The results of the t-test showed a statistically significant difference between the tumor and control groups in the levels of liver enzymes and tumor markers.

This discovery is in line with earlier research that found elevated liver enzyme levels in HCC patients (Kew et al., 1982). It is possible that tumor differentiation may play a significant role in the expression of these markers because the levels of tumor markers and liver enzymes were both significantly higher in the poorly differentiated group compared to the well-differentiated group (AlSalloom, 2016).

The combination of AFP, CA19-9, and CEA as a panel of tumor markers was discovered to have higher diagnostic accuracy for HCC than individual markers. This result is in line with earlier research (Song et al., 2011). The panel’s sensitivity and specificity were 86.5 and 92.3 percent, respectively, demonstrating its high diagnostic accuracy for HCC. The panel’s positive predictive value (PPV) and negative predictive value (NPV), which are 88.1% and 91.4%, respectively, show that it is a reliable diagnostic tool for...
Table 1. Baseline characteristics of the patients

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>AFP Median (Range)</th>
<th>CA 19.9 Median (Range)</th>
<th>CEA Median (Range)</th>
<th>ALT Median (Range)</th>
<th>AST Median (Range)</th>
<th>ALP Median (Range)</th>
<th>T.Bil Median (Range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poorly Differentiated</td>
<td>2918.9 (356491.19)</td>
<td>344.98 (62511.6)</td>
<td>84.34 (18400.34)</td>
<td>785 (7576)</td>
<td>609 (6225)</td>
<td>305 (2794)</td>
<td>7.83 (19.99)</td>
</tr>
<tr>
<td>Moderately Differentiated</td>
<td>283.67 (395.34)</td>
<td>88.21 (9978.21)</td>
<td>24.66 (3178.12)</td>
<td>252 (681)</td>
<td>93 (1426)</td>
<td>65 (3343)</td>
<td>3.24 (19.4)</td>
</tr>
<tr>
<td>Well Differentiated</td>
<td>71.14 (198.38)</td>
<td>53.04 (534.23)</td>
<td>12.805 (343.03)</td>
<td>52 (314)</td>
<td>51 (188)</td>
<td>89 (5427)</td>
<td>2.7 (5.4)</td>
</tr>
</tbody>
</table>

Table 2. Tumor marker levels and liver enzymes in poorly differentiated and well-differentiated groups.

<table>
<thead>
<tr>
<th>Marker/Enzyme</th>
<th>Poorly differentiated</th>
<th>Well differentiated</th>
</tr>
</thead>
<tbody>
<tr>
<td>AFP (ng/mL)</td>
<td>16489.01</td>
<td>69.75</td>
</tr>
<tr>
<td>CA19-9 (U/mL)</td>
<td>3284.56</td>
<td>71.14</td>
</tr>
<tr>
<td>CEA (ng/mL)</td>
<td>645.15</td>
<td>33.39</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>1174.53</td>
<td>62.65</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>721.59</td>
<td>56.38</td>
</tr>
<tr>
<td>ALP (U/L)</td>
<td>486.02</td>
<td>196.79</td>
</tr>
<tr>
<td>Total Bilirubin (mg/dL)</td>
<td>8.74</td>
<td>2.80</td>
</tr>
</tbody>
</table>

Table 3. Diagnostic performance of combined tumor marker panel.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>86.5%</td>
</tr>
<tr>
<td>Specificity</td>
<td>92.3%</td>
</tr>
<tr>
<td>Positive predictive value (PPV)</td>
<td>88.1%</td>
</tr>
<tr>
<td>Negative predictive value (NPV)</td>
<td>91.4%</td>
</tr>
</tbody>
</table>

Table 4. Relationship between occupation and HBsAg and HCV in poorly differentiated cancer patients

<table>
<thead>
<tr>
<th>Occupation</th>
<th>Observed frequency of positive HBsAg cases</th>
<th>Observed frequency of positive HCV cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Farmer</td>
<td>56</td>
<td>189</td>
</tr>
<tr>
<td>Housewife</td>
<td>38</td>
<td>101</td>
</tr>
<tr>
<td>Student</td>
<td>0</td>
<td>34</td>
</tr>
<tr>
<td>Other</td>
<td>2</td>
<td>19</td>
</tr>
<tr>
<td>Job</td>
<td>-</td>
<td>1</td>
</tr>
</tbody>
</table>

Table 5. Relationship between age and tumor size in differentiated groups.

<table>
<thead>
<tr>
<th>Differentiation class</th>
<th>Coefficient for age</th>
<th>Coefficient for tumor size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poorly differentiated</td>
<td>4</td>
<td>Positive</td>
</tr>
<tr>
<td>Moderately differentiated</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>Well differentiated</td>
<td>Positive</td>
<td>Positive</td>
</tr>
</tbody>
</table>
Figure 1. Bar graph showing frequency distribution of ranges of tumor markers in both infected and non-infected cases of hepatocellular carcinoma (HC). The important findings imply a potential relationship between tumor marker levels and HCC infection since infected cases of HCC had greater frequencies in the raised range of tumor markers than uninfected ones.

Figure 2. Descriptive analysis of the tumor markers alpha fetoprotein (AFP), CA 19.9, CEA showing values of mean, median, mode and standard deviation in all the three categories poorly differentiated, moderately differentiated and well differentiated categories.

Figure 3. (a) effect of age on Tumor size with major line plot of predicted age. (b) Effect of Age on the HbsAg values with Predicted age line. (c) Effect of Age group on the chances for HCV to occur in a specific age group. (d) Age parameters effecting patients with Non Alcoholic Fatty Liver (NAFL). (d) Normal Probability Plot showing 100 percent Probability of the Effects of Age on different Parameters.
A. Figure 4. The t-test results confirming between infected and non-infected cases to identify the significance of the biological markers (AFP, CA 19.9 and CEA) as it helps to confirm the difference between infected and non-infected cases in terms of their total bilirubin (T.Bil) levels. The findings showed a statistically significant variance in T.Bil levels between the infected as well as non-infected subjects, indicating the possibility of these biomarkers in differentiating between the two groups.

B. Figure 5. Regression analysis showing no significant correlation between CA19-9 and HCC, as shown by the coefficient for CA19-9 being zero with a standard error of zero and a t-value of -0.1994 (p-value > 0.05).

A. Figure 6. (A) Rate of characterization (ROC) curve analysis between the true positives and the false positives. (B) The precision analysis curve of the effectiveness of the chemiluminescence assay in the diagnosis of HCC.
HCC. The correlation analysis found a significant correlation between tumor markers and liver function tests, indicating that liver function may be involved in the expression of these markers. This finding is in line with earlier studies that found a relationship between tumor markers and liver function tests in HCC patients (Zhu et al., 2018). A significant correlation between tumor markers and imaging results was also found by the correlation analysis, indicating that imaging may be a useful tool for tracking the progression of HCC. The conclusions from this study have substantial implications for the detection and monitoring of HCC.

As a panel of tumor markers, AFP, CA19-9, and CEA have the potential to increase the specificity of HCC diagnosis and promote earlier detection (He et al., 2013).

For bettering patient outcomes and lowering mortality rates, early detection is essential (Barrios, 2022). Liver function may play a significant role in the initiation and progression of HCC, according to the correlation between tumor markers and liver function tests (Ogunwobi, 2019). As a result, regular monitoring of liver function is likely a good way to catch HCC early.

**Occupational effects**

The research findings suggest there is a statistically significant relationship between occupation and tumor markers (HBsAg and HCV) in patients with poorly differentiated cancer. Farmers and homemakers have the highest observed frequency of positive HBsAg cases, followed by students, while the lowest observed frequency of positive HBsAg cases were in individuals with other occupations. Similarly, farmers have the highest observed frequency of positive HCV cases, followed by homemakers and individuals with other occupations, while students and other job categories have the lowest observed frequency of positive HCV cases. These results align with previous studies that have reported a higher incidence of HBV and HCV infections among farmers and homemakers due to their exposure to blood borne pathogens through their work (Oliveira et al., 2022; Sazzad et al., 2023). Furthermore, it has been proposed that farmers may be at an increased risk of developing liver cancer because of exposure to pesticides and other chemicals in agricultural settings (Kang et al., 2018; Hasan et al., 2023).

The findings indicate that occupation should be considered an important factor when assessing risk of viral infections and associated health outcomes. Targeted screening and preventive measures for at-risk occupational groups may help reduce the incidence of these infections and related health issues (Kang et al., 2018).

**Age and tumor size**

The correlation between tumor size and age was positive, showing that tumors tended to become larger with age. In the poorly differentiated class, the coefficient for tumor size and age is 4 (Table 6). This finding is consistent with previous studies that have reported age as a hazard factor for the development of various kinds of cancers (McGlynn et al., 2015; Ferdous et al., 2023). Moreover, the results also point that testing positive for HCV and NAFL is associated with larger tumor size in the poorly differentiated class (Figure 3). This finding is in line with previous research showing a positive association between HCV and NAFL and the risk of liver cancer (Liu et al., 2018; Mithun et al., 2023). In the moderately differentiated class, the results reveal a positive relationship between tumor size and age (Allahverdi, et al., 2021; Alam et al., 2023). Additionally, testing positive for HBsAg is linked to higher tumor size, but no significant relationship was found for HCV and NAFL.

These results are consistent with prior research that has suggested that HBsAg is a hazard factor for the development of hepatocellular carcinoma (Allahverdi, et al., 2021; Hossain et al., 2023). In the well-differentiated class, the results suggest a strong positive correlation between tumor size and the predictors (tumor size, HBsAg, HCV, and NAFL) (McGlynn et al., 2015). Furthermore, a unit increase in tumor size is associated with a 19.45 increase in tumor size of well-differentiated patients who test positive for HBsAg, HCV, and NAFL. The coefficient for HBsAg is also positive, indicating a positive effect on tumor size. However, the coefficient for HCV is not statistically significant, indicating that there is no significant relationship between HCV and tumor size. Similarly, the coefficient for NAFL is not statistically significant, suggesting no significant relationship between NAFL and tumor size (Figure 3).

**Inferential statistics**

Tumor markers and liver enzymes were shown to be significantly different between the tumor and control groups using chi-square testing. The chi-square test revealed a significant relationship between tumor differentiation and the risk of developing hepatocellular carcinoma (Figure 4). Tumors that were well-differentiated were simpler to spot and identify, whereas those that were not well-defined were at a more advanced stage of illness. Increased marker levels were indicative of more advanced illness in poorly differentiated tumors. Tumors that had undergone a lot of differentiation had average values. Changes in tumor markers and liver enzymes may occur from genetic abnormalities, the environment, dietary choices, and even other medical diseases. Total Bilirubin levels were shown to be significantly different between infected and non-infected patients, suggesting the potential of these biomarkers for discrimination.

**Regression analysis**

Blood tumor markers (AFP, CA19-9, CEA) were analyzed using multiple linear regression models to determine their association with hepatocellular carcinoma (Figure 5). There were substantial positive relationships between AFP and HCC and negative connections between CEA and HCC. As seen by the value of the coefficient for CA19-9 being zero with an average error of zero and
a non-significant t-value, there is no significant link between CA19-9 and HCC. However, a substantial coefficient for CEA suggests that there is a strong inverse association between CEA and HCC. However, CA19-9 was not shown to have a substantial association with HCC.

Diagnostic accuracy
Across all three subgroups, AFP’s ROC analysis demonstrated moderate to good diagnostic accuracy in identifying liver cancer. The curve suggests that the test can accurately assess whether there is or absence of the disease since it has a moderate to excellent diagnostic accuracy (Figure 6-A). The true positive rate (sensitivity) provides a crucial indicator of how well the test can detect people who have the condition. The greatest true positive rate and AUC were seen in patients with tumors that were well-differentiated. In poorly differentiated instances of liver cancer, AFP demonstrated excellent specificity. The study found that the Chemiluminescent immunoassays (CLIA) test is successful in detecting hepatocellular carcinoma (HCC), with an outstanding specificity of 98.5%, as shown by the precision analysis curve in Figure 6-B. This shows that the test has a high degree of accuracy in accurately detecting HCC patients, reducing both false-positive and false-negative findings.

Limitations and Implications
Limitations of this study include reliance on cross-sectional data, which restricts the ability to establish causality or assess the temporal dynamics of the relationship between age and tumor size. Additionally, the study only focused on a limited number of tumor markers and differentiation categories, and did not consider other potential confounding factors that could influence the relationship between age and tumor size. For the course of future research, the study’s findings have a number of implications. First, additional research could look into the possibility of combining other tumor markers with AFP, CA19-9, and CEA to boost the accuracy of HCC diagnosis. Second, to improve the accuracy of HCC diagnosis and monitoring, future studies might investigate the use of machine learning algorithms to create predictive models that include clinical and laboratory parameters. Thirdly, research could also look into how these tumor markers are used to predict the prognosis and effectiveness of treatments for HCC. Future research could also look into the underlying mechanisms causing the notable variations in tumor markers and liver enzymes between the control and tumor groups, particularly in HCC with poor differentiation. A deeper comprehension of the pathophysiology of HCC might facilitate the creation of brand-new therapeutic approaches and biomarkers. Future investigations may also examine the potential value of these tumor markers in treating other liver conditions like primary biliary cirrhosis, autoimmune hepatitis, and alcoholic liver disease. This could offer insightful information about the diagnostic and prognostic utility of these tumor markers in various liver diseases and support the creation of personalized medicine strategies for these patients.

Conclusion
In conclusion, the early diagnosis of hepatocellular carcinoma is crucial for better patient outcomes and management. Our study using chemiluminescence assay demonstrated the effectiveness of AFP, CA19-9, and CEA as tumor markers for detecting HCC. Moreover, we found that certain occupations, such as those involving exposure to environmental toxins, were associated with a higher prevalence of HCC. Additionally, our results revealed a positive correlation between age and tumor size, indicating that age may be a risk factor for HCC. The CLIA platform was also found to be an effective tool for detecting tumor markers in HCC patients. Our study highlights the importance of early screening for high-risk populations and the use of advanced diagnostic techniques for accurate detection of HCC.

Author contributions
M.T., A.K., and M.H.R. conceptualized, designed, and conducted the research. They performed fieldwork, analyzed data, drafted the original manuscript, and reviewed and edited it. A.R.S. and A.R. designed the research, validated the methodology, conducted formal analysis, corrected data, visualized results, and reviewed and edited the manuscript. M.S.I. and M.A.H. validated the methodology, conducted formal analysis, investigated the data, visualized results, and reviewed, edited, and proofread the manuscript. H.H., H.D.M.C., M.S.U., and M.M.R. conceptualized the study, designed the research, validated the methodology, conducted formal analysis, investigated the data, visualized results, reviewed funding acquisition, supervised the project, and edited the manuscript. All authors have read and agreed to the published version of the manuscript.

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Ethics statement
This research was approved by the Scientific and Research Ethics Committee of the Ibn Sina Diagnostic and Imaging Center, Dhaka, Bangladesh. However, ethical assessment and approval were not required for the study involving human participants because it complied with local legislation and institutional rules. Patients who participated in the study provided written, informed consent to participate.
Competing financial interests
The authors have no conflict of interest.

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