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Alpha-Fetoprotein and Neutrophil-to-Lymphocyte Ratio: Comparative Analysis in Hepatocellular Carcinoma Diagnosis

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Abstract

Aim: Assessing the diagnostic efficacy of combining alpha-fetoprotein (AFP) with the neutrophil-to-lymphocyte (NLR) ratio in comparison to using AFP alone for detecting hepatocellular carcinoma (HCC).

Method: Sixty patients new with HCC diagnosed as per EASL guidelines and 60 controls with chronic liver disease were studied. Serum AFP levels and the NLR of study participants were determined. The diagnostic values of these variables were assessed and compared by ROC curves.

Results: For diagnosing HCC, The AFP's C-statistic stood at 0.941 (p=0.000), demonstrating significance. With an optimal cut-off of 8.4, it showed a sensitivity of 93.3% and a specificity of 83%. Conversely, the NLR's C-statistic was 0.623 (p=0.101), lacking significance. Utilizing a cut-off of 4.4, its sensitivity and specificity for diagnosing hepatocellular carcinoma (HCC) were 63.3% and 60%, respectively. Notably, the difference in C-statistics between AFP and NLR was significant (p=0.0007), whereas the combination of AFP and NLR didn't yield a significant difference compared to AFP alone (p=0.54).

Conclusion: NLR was not useful for an early diagnosis of HCC. AFP was a reliable diagnostic marker for HCC but AFP in combination with the NLR demonstrated the highest diagnostic accuracy. Thus, this combination can be used for early diagnosis of HCC, ensuring a better outcome.

Keywords: Alpha fetoprotein (AFP); Neutrophil-to-lymphocyte ratio (NLR); Hepatoma; Diagnostic marker, Liver cancer, Risk factors.

INTRODUCTION

Liver cancer holds the sixth position among all cancer types globally and stands as the 4th leading cause of mortality related to cancer. A wide variety of histologically different malignant tumors having unfavorable prognoses are part of liver cancer [1,2]. Persistent alcohol consumption, diabetes, nonalcoholic steatohepatitis (NASH) linked to obesity, and infections from hepatitis B virus (HBV) or hepatitis C virus (HCV) are significant risk factors for hepatoma (HCC). [3]. HCC patients usually do not develop any symptoms. Vague complaints of pain in the epigastrium (50%), bloating (10%), reduction of body mass (10%), appetite loss (5%), jaundice, and malaise can be present [4]. So, making a diagnosis at an initial stage is difficult. The incubation period is long, and it has a rapid course of development. HCC patients have a high mortality rate [5]. The overall survival rate of HCC for an infant is not more than 15%. With the

availability of treatment modalities like surgery and transplantation for HCC at an early stage, the outcome has improved to more than 70% [6]. Therefore, for better clinical outcomes, diagnosing HCC at an early stage is important. AFP is a very widely used biomarker for HCC worldwide. HCC size, differentiation, invasion, and metastasis are all linked to serum AFP [7]. Values more than 400 ng/ml, although not observed in all cases, are considered diagnostic for HCC [8]. Serum AFP is not always reliable because it is often not above the value used for diagnosing HCC in some of the early-stage or late-stage patients with HCC [9]. Normal AFP levels are seen in 15-30% of cases of advanced-stage HCC. It has been seen that if a lower cut-off is used. Also, AFP levels are increased in benign diseases of the liver like hepatitis or cirrhosis [10]. Therefore, new markers are desirable for early detection of HCC. AFP is only used as a reference biomarker in the early stages of HCC diagnosis when no other clinical information is available.

The progression of tumor is closely linked to inflammatory factors. Inflammatory signaling pathways and tumor microenvironment changes are linked to hepatitis, cirrhosis, and HCC staging [11]. There are several inflammatory factors that reflect that a standard inflammatory response is present. And easily obtained at a low-cost examination done routinely [5]. NLR has gained attention because of its medical application in several diseases e.g. liver fibrosis, colorectal cancer, and cervical carcinoma [11]. Only a few studies have explored the potential of the neutrophil-to-lymphocyte ratio (NLR) for the early detection of hepatocellular carcinoma (HCC), suggesting its potential utility in this context. This observation sought to assess the diagnostic efficacy of combining alpha-fetoprotein (AFP) with NLR in comparison to AFP alone for detecting HCC.

METHODS

Between July 2021 and June 2022, a case-control study was conducted at a tertiary care hospital in Dehradun, Uttarakhand, India. The study received approval from the ethics committee of Swami Rama Himalayan University in Dehradun, India (SRHU/HIMS/ETHICS/2022/339, dated 25/05/2021), and adhered to the guidelines outlined in the Declaration of Helsinki. Written informed consent was obtained from all participants involved in the study. The study enrolled sixty HCC patients aged eighteen and above who had recently been diagnosed and had not yet

initiated treatment [2]. Patients with concomitant sepsis or other coexisting malignancies were excluded from the study. Controls comprised an equal number of age- and sex-matched individuals with chronic liver disease but without hepatocellular carcinoma (HCC).

Demographic data, including age, sex, place of residence, and socioeconomic status, were collected for each enrolled patient. Detailed medical histories, including alcohol consumption habits, were obtained. Comprehensive clinical assessments were conducted. Both cases and controls underwent a battery of tests and assessments for viral markers. Imaging studies for HCC diagnosis, such as whole-abdominal ultrasonography and contrast-enhanced computed tomography scans (CECT), were performed on all patients. Serum alpha-fetoprotein (AFP) levels, neutrophil and lymphocyte counts, along with other pertinent parameters, were assessed. The neutrophil-to-lymphocyte ratio (NLR) was computed by dividing the neutrophil count by the lymphocyte count.

Data analysis was conducted using SPSS version 22.0. The Kolmogorov-Smirnov test was employed to assess the normality of quantitative data. Normally distributed quantitative data were presented as mean (±SD), while non-normally distributed data were reported as median and range. The Student's t-test was used for normally distributed quantitative data, and Fisher's exact test (chi-square test) was used for qualitative data comparison. The diagnostic performance of AFP, NLR, and their combination for HCC diagnosis was evaluated using the concordance (C-) statistic, akin to the area under the receiver operating characteristic curve (AUC). Receiver operating characteristic (ROC) curves were utilized to determine accuracy, sensitivity, specificity, and cut-off values for NLR and AFP. Statistical significance was determined with p-values below 0.05.

RESULTS

Demographic selection of patients with HCC, as well as chronic liver disease (CLD), are depicted in Table 1. The mean age of cases was 57.2±11.54 years and of controls 57.01±12.13 years. Both among cases and controls, 44 subjects were less than sixty years of age. While 42 subjects were male, and 18 subjects were female. HCV was found to be the most frequently associated with HCC in the current study, with a 53.3% positivity rate. 13.3% of the participants had positive hepatitis B surface antigen (HBsAg) results.

Table 1: Demographic characteristics of patients with hepatocellular carcinoma (HCC) and controls

Characteristics	No. of patients with HCC (n=60)	No. of controls (n=60)	p-value
Age (years)			
≤60	44 (73.33)	44 (73.33)	1.0
>60	16 (26.67)	16 (26.67)	
Gender			
Male	42 (70.00)	42 (70.00)	1.0
Female	18 (30.00)	18 (30.00)	
Residence			
Rural	22 (36.67)	28 (46.67)	0.60
Semi-urban	8 (13.33)	14 (23.33)	0.51
Urban	30 (50.00)	18 (30.00)	0.18
Socio-economic status			
Upper	6 (10.00)	4 (6.67)	1.0
Middle	34 (56.67)	34 (56.67)	1.0
Lower	20 (33.33)	22 (36.67)	1.0
Dietary habits			
Mixed diet	38 (63.33)	36 (60.00)	1.0
Vegetarian	22 (36.67)	24 (40.00)	1.0
Risk factors			
Alcohol	24 (40.00)	30 (50.00)	0.60
Smoking	16 (26.67)	16 (26.67)	1.0
Tobacco chewing	28 (46.67)	32 (53.33)	0.80
Hepatitis B	8 (13.33)	4 (6.67)	0.67
Hepatitis C	32 (53.33)	12 (20)	0.01

Hepatitis B + C	2 (3.33)	4 (6.67)	1.0

The most common symptom among patients with HCC was pain abdomen (86.7%), followed by diminished appetite (80%). Other common symptoms were nausea and vomiting (73.3%), altered bowel habits (66.7%), abdominal distension (53.3%), yellow discoloration of eyes (46.7%), and weight loss (33.3%). Less common symptoms were fever, upper gastrointestinal (UGI) bleeding, and altered sensorium. On general physical examination, the most common signs were pallor (43.3%), followed by icterus (40%) and nail changes (26.7%). On systemic examination, the most common findings were abdominal tenderness (70%), hepatomegaly (67.7%), and ascites (53.3%). Other findings were hepatic bruit (43.3%), splenomegaly (23.3%), encephalopathy (13.3%), and abnormal chest findings (20%) (Table 2).

Table 2: Clinical profile of patients with hepatocellular carcinoma at the time of presentation

Clinical characteristics	**No. of patients (n = 60)**	**Percent**
Symptoms		
Pain abdomen	52	86.7
Diminished appetite	48	80.0
Nausea/ vomiting	44	73.3
Altered bowel habits	40	66.7
Abdominal distension	32	53.3
Yellow discoloration of eye	28	46.7
Weight loss	20	33.3
Generalized weakness	10	16.7
Upper gastrointestinal bleed	8	13.4
Altered sensorium	6	10.0
Fever	6	10.0

Signs		
Pallor	26	43.3
Icterus	24	40.0
Nail changes	16	26.7
Palmar erythema	12	20.0
Dupuytren's contracture	2	3.3
Spider naevi	8	13.3
Pedal edema	8	13.3
Abdominal tenderness	42	70.0
Ascites	32	53.3
Hepatomegaly	46	67.7
Hepatic bruit	26	43.3
Splenomegaly	14	23.3
Decreased bowel sound	10	16.7
Abnormal chest findings	12	20.0
Encephalopathy	8	13.3

Table 3: Laboratory profile of patients with hepatocellular carcinoma

Laboratory parameters	**Median**	**Range**
Haemoglobin (g/dL)	10.58	9.27-12.72
Mean corpuscular volume (fl.)	87	82.01-94.72
Mean corpuscular haemoglobin (pg)	28.25	26-32
Mean corpuscular haemoglobin concentration (g/dL)	33	31.2-33.77
Red blood cell count (million/cumm)	3.93	3.07-4.14
Platelet count (/cumm)	119500	75000-160000
Total white blood cell count (thousand/cumm)	7.75	6.07-9.69

Neutrophils (%)	74.93	61.90-80.10
Lymphocytes (%)	14	10.59-22.69
Neutrophil- to-lymphocyte ratio	5.1	3.06-7.20
Eosinophils (%)	1.95	0.57-5.25
Basophils (%)	0.30	0.01-0.62
Monocyte (%)	5.89	2.92-9.27
Red cell distribution width (%)	18.45	16.74-21.30
Serum total bilirubin (mg/dL)	1.98	1.03-3.06
Serum direct bilirubin (mg/dL)	0.81	0.45-1.55
Serum indirect bilirubin (mg/dL)	1.13	0.59-1.72
Serum Alanine aminotransferase (U/L)	48	33.5-80.25
Serum Aspartate aminotransferase (U/L)	88	66.75-143
Serum Alkaline phosphatase (U/L)	184.5	134-278.5
Serum total protein (g/dL)	7.01	5.99-7.42
Serum albumin (g/dL)	2.83	2.44-3.71
Serum globulin (g/dL)	3.62	4.57
Albumin/globulin Ratio	0.78	0.60-1
International Normalized Ratio	1.43	1.28-1.67
Erythrocyte sedimentation rate (mm)	42	21-65.25
Serum alpha-fetoprotein levels (ng/mL)	484	164-5732
Serum creatinine (mg/dL)	0.88	0.59-1.21
Blood urea nitrogen (mg/dL)	20	16.22-25.12
Serum sodium (mmol/L)	135	132-138
Serum potassium (mmol/L)	3.93	3.53-4.36

The C-statistic for NLR in diagnosing HCC was 0.623 (p=0.101, 95% CI 0.48-0.77), indicating no statistical significance. Using the optimal cut-off of 4.4, sensitivity and specificity for HCC diagnosis were 63.3% and 60%, respectively. The laboratory parameters of patients with HCC are listed in Table 3. In contrast, AFP demonstrated a statistically significant C-statistic of 0.941 (p=0.000, 95% CI 0.88-1.00), with sensitivity and specificity of 93.3% and 83%, respectively, at

the optimal cut-off of 8.4. When combined, AFP and NLR yielded a statistically significant C-statistic of 0.95 (p=0.000, 95% CI 0.89-1.00). For HCC diagnosis, sensitivity and specificity were 100% and 86.7%, respectively (Fig. 1).

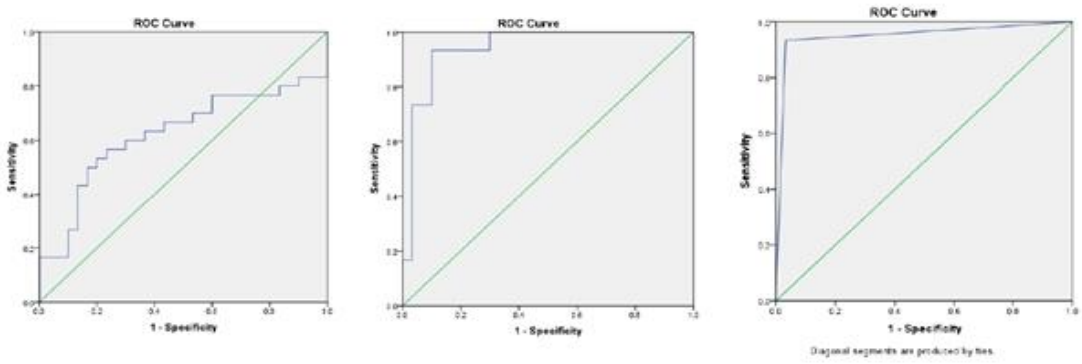

Fig. 1(a) Fig. 1(b) Fig. 1(c)

Figure 1. Receiver operating characteristic curves of diagnostic markers (a) Neutrophil-to-lymphocyte ratio (NLR) (b) Alpha-fetoprotein (AFP) (c) AFP in combination with NLR

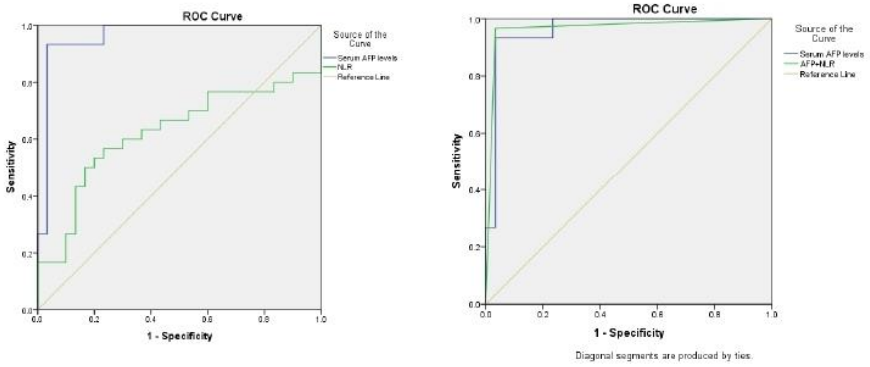

Fig. 2(a) Fig. 2(b)

Figure 2. Comparison of Receiver operating characteristic curves (a) Alpha-fetoprotein (AFP) and neutrophil-to-lymphocyte ratio (NLR) (b) Combination of AFP and NLR and AFP alone.

The disparity in C-statistics between AFP and NLR was statistically significant (p=0.0007). However, the contrast in C-statistics between the combination of AFP and NLR and AFP alone did not reach statistical significance (p=0.54) (Fig. 2).

DISCUSSION

The incubation period is long, and it has a rapid course of development. HCC patients have a high mortality rate [5]. With the availability of treatment modalities like surgery and transplantation for HCC at an initial stage, the outcome has improved [6]. Therefore, for better clinical outcomes, diagnosis is important.

In a study by Ding et al., patients with HCC had an average age of 47.3 years, with a standard deviation of 13.7 years. The study found that 75.8% of participants were male, while only 24.2% were female [12]. Petrick et al. noted that incidence rates among men are two to four times higher than among women [14]. Similarly, Kumar reported that in India, men are affected by HCC four times more frequently than women, with the typical age of presentation falling between 40 to 70 years old [15]. Consistently, our study also observed a male predominance (2.33:1) and a mean age of 57.2±11.54 years among patients diagnosed with HCC, aligning with previous research findings.

Alcohol is the common cause of CLD, which makes up 30% of HCC cases and HCC-associated deaths globally [16]. In epidemiological research, smoking is one of the highlighted risk factors for HCC [17]. In our study, among cases, 40% were habituated to alcohol, 26.7% had a history of smoking and 46.7% had a history of tobacco chewing.

However, in our study, HCV positivity was seen in more than half of the cases while among controls, two-thirds were non-reactive for viral markers. This difference was found to be statistically significant. HCV was found to be the most frequent cause of HCC in the current study, with a 53.3% positivity rate. 13.3% of the participants had positive HBV results [18].

Patients with HCC typically don't exhibit any symptoms. There may be sporadic symptoms of epigastric pain (50%), bloating (10%), loss of body mass (10%), appetite loss (5%), jaundice, and malaise. Clinical signs of HCC can consist of hepatomegaly and ascites along with the existence of other signs linked to paraneoplastic syndromes, such as hypercholesterolemia,

erythrocytosis, hypercalcemia, and hypoglycemia rarely [4,19]. However, in our study, 52 (86.7%) of the 60 cases exhibited abdominal pain, followed by diminished appetite (80%). Other common symptoms were nausea and vomiting (73.3%), altered bowel habits (66.7%), abdominal distension (53.3%), yellow discoloration of eyes (46.7%), and weight loss (33.3%). Among the symptoms, less common were fever, UGI bleeding, and altered sensorium.

The most prevalent signs on general physical examination were pallor (43.3%), icterus (40%), and nail changes (26.7%). On systemic examination, abdominal tenderness was present in 70%, hepatomegaly in 67.7%, and ascites in 53.3% of patients. Other findings were hepatic bruit (43.3%), splenomegaly (23.3%), encephalopathy (13.3%), and abnormal chest findings (20%) [20-24].

Elevated baseline NLR often signifies systemic and localized inflammation, which promotes tumor invasion and metastasis [25]. NLR represents a systemic immunological state that favors tumor invasion while decreasing host immune surveillance, in addition to a microenvironment that is favorable to tumors [26]. Various studies show the impact of NLR on overall survival of HCC patients. The association between NLR and HCC was initially explained by Halazun et al., who showed that patients undergoing transplantation of liver for HCC were more likely to experience high recurrence and poor overall survival when their NLR was elevated (>5) [27]. Kayadibi et al (2014) studied whether a higher NLR is linked to tumor recurrence and suggested that though NLR is easily available, it would be better to use it in addition to other inflammatory markers for predicting tumor microenvironment [28]. These studies indicate that NLR may help in screening HCC. However, a limited number of studies have evaluated the value of NLR for early diagnosis of HCC and it has been seen that NLR may be useful in this regard.

In our evaluation of HCC diagnostic tools, we examined the utility of AFP and NLR, and compared the recorded alone value of serum AFP with that of serum AFP plus NLR combined. Our findings revealed that serum AFP displayed a commendable accuracy (AUC=0.941) in detecting HCC and exhibited significantly higher in patients diagnosed with HCC. When utilizing the certain cut-off of 8.4, the sensitivity and specificity for HCC diagnosis were 93.3% and 83%, respectively.

The C-statistic for NLR in diagnosing HCC was 0.623 (p=0.101, 95% CI 0.48-0.77). Utilizing the optimal cut-off of 4.4, the sensitivity and specificity for diagnosing HCC were 63.3% and

60%, respectively. While NLR showed a slight elevation in patients with HCC, it did not demonstrate significant accuracy in HCC detection. Additionally, AFP outperformed NLR in diagnosing HCC. These findings align with a study by Johnson et al., where they evaluated the impact of baseline NLR on the overall survival of HCC patients and compared NLR levels between patients with chronic liver disease (CLD) and those with HCC. Although NLR was higher in HCC patients compared to controls at baseline (2.79 vs 2), the difference in NLR values between the two groups was not clinically significant. Furthermore, the accompanying AUC calculation indicated that NLR had very weak diagnostic ability, with an AUC of 0.65 (95% CI 0.62, 0.69) [29].

Study conducted by Ding et al., which investigated the combined value of an inflammatory score and AFP for diagnosing HBV-related HCC, it was found that AFP in conjunction with ALT, AST, and NLR improved the diagnostic accuracy for HBV-HCC compared to individual biomarkers [12]. Hu et al. (2019) explored the significance of the platelet ratio (GPR) and neutrophil-to-lymphocyte ratio (NLR) in patients diagnosed with hepatocellular carcinoma (HCC). Their study revealed that pre-intervention hematologic parameters (NLR and GPR) were correlated with the Barcelona Clinic Liver Cancer (BCLC) stages. Integrating these parameters with AFP levels could aid in the early detection of HCC [30]. In our study, although not statistically significant, we observed that the combination of AFP and NLR showed a marginal improvement in identifying HCC compared to AFP alone. Consequently, NLR alone does not contribute significantly to HCC diagnosis, but when used alongside AFP, it may modestly improve diagnostic accuracy.

CONCLUSION

NLR, or Neutrophil-to-Lymphocyte Ratio, was not deemed effective for the early diagnosis of hepatocyte carcinoma (HCC). Conversely, Alpha-Fetoprotein (AFP) emerged as a dependable diagnostic marker for HCC. Nevertheless, when AFP was integrated with NLR, their synergistic effect resulted in the highest diagnostic accuracy. Consequently, this amalgamation presents a promising avenue for facilitating the early detection of HCC, thereby enabling timely interventions and potentially improving patient outcomes.

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