The Egyptian Journal of Hospital Medicine (October 2024) Vol. 97, Page 3788-3793

Value of Ascitic Fluid Lactoferrin in Diagnosis of Hepatocellular Carcinoma

Mustafa Elrefaei*[1], Rokiah Anwar[1], Hosam Abdel Tawab[2], Ahmed Saed[1]
Departments of [1]Internal Medicine (Gastroenterology and Hepatology),
[2]Clinical Pathology (Clinical Immunology Unit), Mansoura University, Egypt
***Corresponding Author**: Mustafa Foaad Saad Elrefaei, **Mobile**: (+20) 01096286810, **Email**: mustafaelrefaei1990@gmail.com

ABSTRACT

Background: Hepatocellular carcinoma (HCC) is a primary hepatic malignant tumor and represents about 82.5% of primary liver cancers. Lactoferrin level has been demonstrated to increase in both microbial infection and inflammation. As a result, an increased lactoferrin level could provide a talented biomarker for gastrointestinal (GI) disease. **Objective**: To evaluate the value of ascitic fluid lactoferrin (AFLAC) in diagnosis of HCC in cases with cirrhotic ascites. **Patients and Methods**: This case control study included 120 patients divided into two groups (Group A: 60 patients with cirrhotic ascites without HCC) and (Group B: 60 patients with cirrhotic ascites + HCC). Every participant was subjected to abdominal ultrasonography, triphasic CT abdomen, ascitic fluid analysis and assessment of AFLAC. **Results**: AFLAC level was statistically significantly higher in HCC group compared to non-HCC group. There was statistically significant higher ascitic fluid lactate dehydrogenase (LDH) in HCC group than in non-HCC group. In HCC group, there was a statistically significant positive correlation between AFLAC and ascitic fluid (AF) protein and ascitic fluid LDH. AFLAC could be used as an excellent predictor in differentiation between HCC and non-HCC groups. **Conclusion**: Increased AFLAC level in cirrhotic patients with HCC seems to be a talented diagnostic indicator, even following receiving systemic antibiotic therapy. HCC development is detected by accident in advanced stages in the majority of the cases when therapeutic measures cannot be provided, so rapid detection of high-risk cases by AFLAC could help to offer early and efficient management.

Keywords: Hepatocellular Carcinoma, AFLAC, Spontaneous Bacterial Peritonitis, Serum α-fetoprotein.

INTRODUCTION

Hepatocellular carcinoma (HCC) is a primary hepatic malignant tumor and represents about 82.5% of primary liver cancers, and mostly happens in cirrhotic cases [(1)]. The five-year survival rate differed by stages, with the rate of about 62.5% in the early stage and dropping to 3% in cases of HCC with distant metastases [(2)]. Hepatitis B/C virus infection, alcohols, aflatoxins, etc., were recognized as predisposing factors for HCC development. In clinical practice, HCC is often diagnosed in the late stages due to the lack of distinctive manifestations of the cancers. Only twenty percent of cases with HCC are suitable for curative management, which includes hepatic excision, transplant, or ablation, secondary to progressive tumor stage, hepatic impairment, or shortage of liver donors[(3)].

Serum alpha-fetoprotein (AFP) and ultrasonography are broadly used for initial identification of HCC [(4)]. On the other hand, with a moderate Sn and high Sp at a cutoff level of 20 ng/ml, Western nations have ruled out AFP for HCC diagnosis secondary to its absence of precision [(5)].

Lactoferrin is a 78 kDa iron-binding protein present in human breast milk as well as in bovine milk. In addition, it is present in body secretions, such as GI fluids, saliva, tears, semen, and nasal fluid [(6)].

Lactoferrin is thought to have numerous relevant functions, which include anti-tumor, anti-inflammatory, and antioxidant effects, together with its role as an immunity regulator. Also, it protects against microbial contamination (such as bacteria, viruses, fungi, and parasites). It has been demonstrated that lactoferrin expression and level (from PMNs) are significantly increased after infection or inflammation [(7)]. As a result, an increased lactoferrin level could offer a talented and reliable biomarker for GI disease [(8)]. Of note, AFLAC is a reliable predictor for the presence of PMNs and detection of spontaneous bacterial peritonitis (SBP) in cases with liver cirrhosis [(9)]. **Lee *et al.*** found that AFLAC level in cases without SBP may be associated with hepatocellular carcinoma development [(10)]. So, we aimed to evaluate the value of AFLAC in diagnosis of HCC in cases with cirrhotic ascites.

PATIENTS AND METHODS

This single center case control study included 120 cases with cirrhotic ascites who were included in the study and were collected from specialized medical Hospital at Mansoura University of a period of one year from October 2022 to September 2023. The study subjects were classified into two groups, group A (non-HCC group) included 60 patients with cirrhotic ascites without HCC and group B (HCC group) included 60 patients with cirrhotic ascites and HCC.

Inclusion Criteria:

Any patient with liver cirrhosis and ascites with or without HCC and age above 18 years.

Exclusion Criteria:

Patients with non-cirrhotic ascites, which includes peritoneal carcinomatosis, pancreatitis, tuberculosis, hemorrhagic ascites, with SBP, with subsequent diagnosis of non-HCC malignancy, and with causes of secondary peritonitis (e.g. perforated acute appendicitis and perforated peptic ulcer…etc).

METHODS

Every participant was subjected to thorough history taking, complete clinical examination, laboratory analysis (such as complete blood count, liver and kidney function tests and AFP), abdominal

Received: 19/05/2024
Accepted: 17/07/2024

ultrasonography, triphasic CT abdomen, ascitic fluid analysis (including aerobic and anaerobic bacterial culture) and assessment of AFLAC using enzyme-linked immunosorbent assay (ELISA) (Human Lactoferrin ELISA Kit).

Ethical Considerations:

Our study was ethically approved by Mansoura University's Research Ethics. Informed consent was obtained from all contributed cases. The study adhered to the Helsinki Declaration throughout its execution.

Statistical Analysis

The collected data were revised, coded, and tabulated using SPSS Version 25.0. The Shapiro-Wilk test was done to test the normality of data distribution. Mean±SD was utilized for normally distributed numerical data. Median, range, and interquartile range were used for non-normally distributed numerical data. Student T-Test was utilized to evaluate the significance of the difference of parametric variable between two group means. U test was utilized to evaluate the significance of the difference of a non-parametric variable between two groups. The ROC Curve offered a helpful method to assess the Sn and Sp for quantitative measures which classifies cases into one of two groups. A p value was considered significant if <0.05.

RESULTS

Table **(1)** illustrates statistically significant higher mean hemoglobin level, platelet count, serum albumin (ALB) among non-HCC than HCC group. However, a statistically significant higher mean total leukocytic count, total bilirubin (TB), INR, serum creatinine (Ser Cr) and alpha fetoprotein was found among HCC than non-HCC group. A statistically significant higher mean ALT, AST and fasting blood glucose among HCC than non-HCC cases.

Table (1): Laboratory findings of the studied groups

	Non-HCC (N=60)	HCC (N=60)	Test of significance
Laboratory Findings			
Hemoglobin (gm/dl)	9.61±0.87	8.88±1.11	t=4.04 p=0.001*
TLC	3.38±0.82	4.44±1.10	t=3.88 p=0.001*
Platelet count	91.19±17.51	77.92±11.06	t=4.97 p=0.001*
Serum albumin (g/dl)	2.73±0.23	2.53±0.25	t=4.69 p=0.0001*
TB (mg/dl)	1.92±0.45	2.58±0.61	t=5.06 p=0.0001*
INR	1.58±0.16	1.86±0.13	t=10.15 p<0.001*
Ser Cr (mg/dl)	1.30±0.29	1.44±0.296	t=2.66 p=0.009*
AFP (ng/ml)	6.2(2.8-9.2)	870.5(170-21231)	Z=5.68 P<0.001*
ALT (U/L)	59.78±11.29	67.95±10.76	t=4.06 p<0.001*
AST (U/L)	56.78±10.44	63.95±11.09	t=3.64 p<0.001*
FBG (mg/dl)	109.68±26.56	134.92±31.98	t=3.06 p<0.001*

Median and range: Non-parametric test, *: Statistically significant, t: Student t test, Z: Mann- Whitny U test

Table **(2)** shows that there was statistically significant higher mean ascitic fluid total leukocytic, ascitic fluid protein, and ascitic fluid LDH, and median AFLAC in HCC cases compared to non-HCC.

Table (2): Comparison of ascitic fluid findings between studied groups

	Non-HCC (N=60)	HCC (N=60)	Test of significance
Ascitic fluid TLC	187.2±27.36	210.30±21.37	t=5.15, p<0.001*
Ascitic fluid protein (g/dl)	1.96±0.15	2.09±0.17	t=4.60, p<0.001*
Ascitic fluid LDH (U/L)	175.85±11.53	193.15±16.46	t=6.67, p<0.001*
Ascitic fluid glucose (mg/dl)	92.72±12.13	92.80±12.15	t=0.038. p=0.970
AFLAC (ng/ml)	31.1(6.9-103.3)	205(125-289.0)	Z=9.45, P<0.001*

Median and range: Non-parametric test, *: Statistically significant, t: Student t test, Z: Mann- Whitny U test

Table **(3)** demonstrates that area under curve for AFLAC was excellent in differentiation between HCC and non-HCC groups yielding the best detected cutoff point of 93.9 with sensitivity of 96.7%, specificity of 100% and overall accuracy of 98.3%.

Table (3): Validity of AFLAC in differentiation HCC from non-HCC

	AUC (95% CI)	P value	Cut off point	Sensitivity %	Specificity %	PPV%	NPV%	Accuracy %
AFLAC	1.0 (1.0-1.0)	<0.001*	93.9	96.7	100.0	100	96.8	98.3

AUC: Area under curve NPV: Negative predictive value, PPV: Positive predictive value

Table **(4)** demonstrates that area under curve for ascitic fluid LDH was good in differentiation between HCC and non-HCC groups yielding the best detected cut off point of 181.5 with Sn of 75%, Sp of 73.3% and overall accuracy of 74.2%.

Table (4): Validity of ascitic fluid LDH in differentiation HCC from non-HCC

	AUC (95% CI)	P value	Cut off point	Sensitivity %	Specificity %	PPV%	NPV%	Accuracy %
Ascitic fluid LDH	0.795 (0.715-0.874)	0.041*	181.5	75.0	73.3	73.8	74.6	74.2

AUC: Area under curve NPV: Negative predictive value, PPV: Positive predictive value

Table **(5)** illustrates no statistically significant correlation between AFLAC and all of the following; sex, occupation, smoking history, associated comorbidities for non-HCC group and for HCC group.

Table (5): Relation between AFLAC and demographic characteristics in non-HCC group and HCC group.

	AFLAC Median (min-max) (IQR)	**Test of significance**	**AFLAC** Median (min-max) (IQR)	**Test of significance**
	Non-HCC, N=60		**HCC, N=60**	
Sex				
Female	22.65(8.4-103.3) (17.13-51.78)	Z=0.659 P=0.510	209.0(126.0-289.0) (179.75-241.0)	Z=1.22 P=0.224
Male	33.35(6.9-83.7) (21.13-61.73)		198(125.0-270.0) (157.5-234.25)	
Occupation				
Unemployed	29.1(8.4-103.3) (16.4-53)	Z=0.712 P=0.476	203.0(125.0-270.0) (162.5-234.5)	Z=0.262 P=0.793
Employed	39.1(6.9-102) (22.1-59.15)		208.0(135-289) (172.5-237.0)	
Current smoker		KW=0.931		KW=1.54
Smoker	30.6(7.4-46.6) (12.4-45.1)	P=0.628	167.25 (152.5-182.0) (152.5-182.0)	P=0.462
Ex-smoker	33.9(6.9-83) (22.05-67.3)		217.0(125.0-252.0) (159.0-232.25)	
Non-smoker	26.85(8.4-103.3) (17.13-52)		204.5(126-289) (169.63-239.0)	
Associated comorbidities		Z=0.828		Z=1.66
YES	30.45(7.1-103.3) (13.68-47.2)	P=0.407	192(125.0-289.0) (155.13-226.25)	P=0.096
NO	32.2(6.9-102) (20.65-64.8)		217.0(135-252) (188.63-237.25)	

Z: Mann Whitny U test, KW: Kruskal Wallis test, *: Statistically significant

Table **(6)** shows a statistically significant positive correlation between AFLAC and each of AF protein and ascitic fluid LDH in HCC group. In non-HCC group, there was a statistically significant negative correlation between AFLAC and each of TLC and serum albumin and AFLAC.

Table (6): Relation between AFLAC and age and laboratory findings of the non-HCC cases and HCC cases

AFLAC correlation with	**HCC (N=60)**		**Non-HCC (N=60)**	
	r	*P-value*	*r*	*P-value*
Age/ years	0.124	0.347	0.091	0.488
Hemoglobin (gm/dl)	-0.134	0.308	0.195	0.136
TLC	-0.02	0.878	-0.257	**0.047***
Platelet count	0.036	0.787	0.03	0.82
Serum albumin(g/dl)	0.029	0.824	-0.255	**0.049***
TB (mg/dl)	0.218	0.095	0.128	0.329
INR	-0.205	0.116	-0.03	0.823
Ser Cr (mg/dl)	-0.101	0.442	-0.160	0.221
AFP	0.026	0.844	-0.028	0.830
ascitic fluid TLC	-0.065	0.623	0.052	0.691
Ascitic fluid protein(g/dl)	0.379	**0.003***	-0.087	0.508
Ascitic fluid glucose(mg/dl)	-0.059	0.655	0.019	0.885
Ascitic fluid LDH (U/L)	0.270	**0.037***	0.202	0.121

r: Spearman correlation coefficient, *: Statistically significant

Table **(7)** demonstrates multivariate analysis for predictors of HCC, illustrates that presence of associated comorbidities increased HCC. Decrease hemoglobin level, decrease platelet count, decreased serum albumin and increased total leukocytic count were statistically significant predictor of HCC. Also increased ascitic fluid TLC, ascitic fluid protein and ascitic fluid LDH were statistically significant predictors of HCC. All of the previous factors can predict HCC correctly by 60%.

Table (7): Binary logistic regression for predictors of HCC cases

	β	**P value**	**Odds ratio (95% CI)**
Age/ years	0.079	0.06	1.08(0.998-1.17)
Sex Female(r) Male	-0.425	0.261	 1 0.654(0.312-1.37)
Occupation Unemployed(r) employed	-0.433	0.257	 0.649(0.307-1.37)
Current smoker Smoker Ex-smoker Non-smoker	 R 0.881 1.71	 0.301 0.07	 1 2.41(0.454-12.884) 5.54(1.06-28.93)
Associated co-morbidities Yes No (r)	0.815	0.03*	 2.26(1.08-4.69) 1
Hemoglobin (gm/dl)	-0.901	0.004*	0.406(0.219-0.753)
TLC	1.21	<0.001*	3.36(1.85-6.09)
Platelet count	-0.053	0.02*	0.948(0.905-0.993)
Serum albumin(g/dl)	-2.38	0.027*	0.092(0.01-0.765)
TB (mg/dl)	0.838	0.088	2.31(0.883-6.05)
Ser Cr (mg/dl)	0.047	0.962	1.05(0.155-7.07)
AFP (ng/ml)	0.181	0.972	1.19(0.002-34.58)
Ascitic fluid TLC	0.051	<0.001*	1.05(1.03-1.08)
Ascitic fluid protein(g/dl)	5.42	<0.001*	24.56(15.03-30.56)
Ascitic fluid LDH(u/l)	0.087	<0.001*	1.09(1.05-1.13)
Ascitic fluid glucose(mg/dl)	0.01	0.097	1.01(0.971-1.03)
AFLAC (ng/ml)	1.31	0.984	3.72(0.005-26.05)
Overall % predicted =60.0%			

*: Statistically significant**.**

DISCUSSION

Hepatocellular carcinoma (HCC) is the commonest type of primary hepatic malignant tumors. HCC happens most often in subjects with chronic liver diseases [(11)]. So, we aimed to evaluate the value of AFLAC in the diagnosis of HCC by comparing its level in 60 HCC cases with matched 60 non-HCC cirrhotic patients.

Much research has reported that an increase in systemic inflammation is accompanied by poor survival in different types of malignant tumours. In cases with HCC, the systemic inflammatory response could be determined by traditional investigations, which include C-reactive protein [(12)].

Lactoferrin is a 78 kDa iron-binding protein present in human breast milk as well as in bovine milk. In addition, it is present in body secretions, such as GI fluids, saliva, tears, and semen [(6)]. Lactoferrin is thought to have numerous relevant functions, which involve anti-tumor, anti-inflammatory, and antioxidant effects, together with its role as an immunity regulator. Also, it protects against microbial contamination (such as bacteria, viruses, fungi, and parasites). It has been demonstrated that lactoferrin expression and level (from PMNs) are significantly increased after infection or inflammation [(7)].

The current study illustrated a statistically significant higher frequency of diabetes that was detected among cases with HCC than without HCC (38.3% versus 18.3%, respectively). Likewise, **Cho *et al.*** [(13)] displayed that the HCC group was significantly accompanied by greater incidence of co-morbidities such as DM, cardiovascular disorders and renal impairment, compared to the non-HCC group. **Kim *et al.*** [(14)] found that NAFLD and NASH due to type 2 DM have a role in HCC even without previous liver cirrhosis (NAFLD/NASH-derived HCC).

Several hypotheses have been proposed for the association between CKD and HCC development, including immunity dysregulation, impaired DNA repairing mechanisms, oxidative stress, and accumulation of carcinogenic agents mediated by a reduction of renal elimination [(15)].

Our study displayed a significant higher mean HB level, platelet count, serum albumin among non-HCC than HCC group, but it displayed a significant higher mean total leucocytic count, TB, INR, Ser Cr and AFP among HCC than non-HCC group. There was significant greater mean ALT, AST and FBG among HCC than non-HCC cases.

In the same line, **Hanafy *et al.*** [(16)] found statistically significant higher mean HB level and platelet count among non-HCC than HCC group, but a significant lower serum albumin, mean total leukocytic count and higher INR and serum creatinine among HCC than non-HCC group. There was a significant higher mean ALT, AST and FBG among HCC than non-HCC cases.

The current study showed statistically significant higher mean AF total leucocytic count among HCC cases compared to non-HCC ones. AF protein also was statistically significantly higher among HCC than non-HCC group. AF LDH was significantly increased in HCC cases compared to non-HCC ones. A statistically significant higher median AFLAC was determined among HCC than non-HCC (205 and 31.1, respectively).

In harmony with our findings, **Hanafy *et al.*** [(16)] analyzed ascitic fluid revealing a statistically significant difference concerning ascitic total protein and glucose values being higher in HCC group ($P<0.05$). But, in contrast to our findings, there was insignificant difference between HCC group and non-HCC group concerning ascitic albumin and LDH.

Our study demonstrated that AUC for ascitic fluid LDH was good in differentiation between HCC and non-HCC groups yielding the best detected cut off point, which was 181.5 with SN of 75%, SP of 73.3% and overall accuracy of 74.2%. The current study demonstrated that AUC for AFLAC was excellent in differentiation between HCC and non-HCC groups yielding the best detected cut off point of 93.9 with sensitivity of 96.7%, specificity of 100% and overall accuracy of 98.3%.

Another research conducted by **Ali *et al.*** [(17)] explained the clinical value of AFLAC as an indicator for SBP. Cases with SBP were accompanied by a significant increase in AFLAC level compared to the controls (180.8 versus 42.2 ng/ml) ($P=0.001$), with an AFLAC level of 88 ng/ml recognized as a cutoff on ROC analysis to differentiate between cases with SBP and SBP free ones.

Our study demonstrated no statistically significant association between AFLAC and all of the following, age, sex, occupation, smoking history, associated comorbidities, HB (gm/dl), TLC, platelet count, sSerum ALB, TB, INR, Ser Cr, AFP, ascitic fluid TLC, AF protein, ascitic fluid glucose and number of focal lesions among HCC cases ($p>0.05$). A significant relationship was detected between AF LDH and lactoferrin among HCC cases ($r=0.270$, $p=0.03$). For non-HCC group, our study illustrated a significant negative relationship between AFLAC and TLC ($r=-0.257$) and serum albumin ($r=-0.255$), but there was no significant relationship AFLAC and all of the following, sex, occupation, smoking history, associated comorbidities ($p>0.05$). Moreover, for HCC group, the current study showed a statistically significant positive relationship between AFLAC and ascitic fluid protein ($r=0.379$), number of focal lesions and ascitic fluid LDH.

Neutrophil reflux is correlated with the existence of AFLAC, and local inflammation may serve as a relevant marker for HCC development [(18)]. In **Lee *et al.*** [(10)] they found that AFLAC level in cases without SBP may be linked to HCC development.

The current study demonstrated multivariate analysis for predictors of HCC illustrating that presence of associated comorbidities increased HCC. Decrease hemoglobin level, decrease platelet count, decreased serum albumin and increased total leukocytic count were statistically significant predictor of HCC. Also increased ascitic fluid TLC, ascitic fluid protein and ascitic fluid LDH were statistically significant predictors of HCC. All of the previous factors could predict HCC correctly by 60%. In univariable analyses, **Ioannou *et al.* [19]** revealed that the most essential predictors of HCC among all hepatic pathological conditions were elderly, male gender, Hispanic ethnicity, increased AFP level, AST/ALT ratio, thrombocytopenia, and serum albumin level. The parameters that are still significant following the adjustment of the most essential potential confounders were age, sex, race, comorbidities (such as diabetes mellitus), body mass index, ALB, platelet count, and AST/pALT ratio.

The alterations in results could be clarified by several causes, which include changes in genetic background, the original cause of HCC, different populations, and selection of cases.

Despite, the promising outcome of the current study, the small sample size is considered the main limitation. Further major studies have to be conducted to accurately detect the cutoff level and the drop in the rate of AFLAC value following receiving systemic antibiotic therapy.

CONCLUSION

The current study concluded that increased AFLAC level in cirrhotic cases with HCC seems to be a talented diagnostic indicator, even following receiving systemic antibiotic therapy. HCC development is detected unintentionally in advanced stages in nearly all cases when treatment options cannot be provided, so rapid detection of high-risk cases by AFLAC could help to offer early and efficient management.

Fund: None declared.
Conflict of interest: None declared.

REFERENCES

1. **Sun N, Zhang C, Lee Y *et al.* (2023):** HCC EV ECG score: An extracellular vesicle-based protein assay for detection of early-stage hepatocellular carcinoma. Hepatology, 77(3): 774-88.
2. **Siegel R, Miller K, Fuchs H *et al.* (2022):** Cancer statistics, 2022. CA Cancer J Clin., 72(1): 7-33.
3. **Colli A, Nadarevic T, Miletic D *et al.* (2021):** Abdominal ultrasound and alpha-foetoprotein for the diagnosis of hepatocellular carcinoma in adults with chronic liver disease. Cochrane Database Syst Rev., 4(4): Cd013346. doi:10.1002/14651858.CD013346.pub2
4. **Parikh N, Mehta A, Singal A *et al.* (2020):** Biomarkers for the early detection of hepatocellular carcinoma. Cancer Epidemiology, Biomarkers and Prevention, 29(12): 2495-503.
5. **Schlosser S, Tümen D, Volz B *et al.* (2022):** HCC biomarkers - state of the old and outlook to future promising biomarkers and their potential in everyday clinical practice. Front Oncol., 12: 1016952. doi:10.3389/fonc.2022.1016952
6. **Darmawan K, Karagiannis T, Hughes J *et al.* (2023):** Molecular modeling of lactoferrin for food and nutraceutical applications: insights from in silico techniques. Crit Rev Food Sci Nutr., 63(28): 9074-97.
7. **Coccolini C, Berselli E, Blanco-Llamero C *et al.* (2023):** Biomedical and nutritional applications of lactoferrin. International Journal of Peptide Research and Therapeutics, 29: s10989. doi:10.1007/s10989-023-10541-2
8. **Johnson L, White S, Schmidt R (2020):** Are calprotectin and lactoferrin equivalent screening tests for inflammatory bowel disease? Clin Chim Acta., 510: 191-5.
9. **Parsi M, Saadeh S, Zein N *et al.* (2008):** Ascitic fluid lactoferrin for diagnosis of spontaneous bacterial peritonitis. Gastroenterology, 135(3): 803-7.
10. **Lee S, Min H, Choi J *et al.* (2016):** Usefulness of ascitic fluid lactoferrin levels in patients with liver cirrhosis. BMC Gastroenterol., 16(1): 132. doi:10.1186/s12876-016-0546-9
11. **McGlynn K, Petrick J, El-Serag H (2021):** Epidemiology of Hepatocellular Carcinoma. Hepatology, 73(1): 4-13.
12. **Tan D, Ng C, Lin S *et al.* (2022):** Clinical characteristics, surveillance, treatment allocation, and outcomes of non-alcoholic fatty liver disease-related hepatocellular carcinoma: a systematic review and meta-analysis. Lancet Oncol., 23(4): 521-30.
13. **Cho Y, Cho E, Yoo J *et al.* (2021):** Association between lipid profiles and the incidence of hepatocellular carcinoma: A nationwide population-based study. Cancers (Basel), 13(7): 3390. doi:10.3390/cancers13071599
14. **Kim H, Lee D, An T *et al.* (2021):** Metabolic spectrum of liver failure in type 2 diabetes and obesity: From NAFLD to NASH to HCC. Int J Mol Sci., 22(9): 94495. doi:10.3390/ijms22094495
15. **Yeh H, Chiang C, Yen T (2021):** Hepatocellular carcinoma in patients with renal dysfunction: Pathophysiology, prognosis, and treatment challenges. World J Gastroenterol., 27(26): 4104-42.
16. **Hanafy A, Mohamed M, Alnagar A (2020):** Ascitic calprotectin as an early predictor of hepatocellular carcinoma in patients with cirrhotic ascites. J Cancer Res Clin Oncol., 146(12): 3207-14.
17. **Ali F, Shehata I, El-Ansary M (2013):** Diagnostic value of lactoferrin ascitic fluid levels in spontaneous bacterial peritonitis. Egyptian Liver Journal, 3(2): 54-61.
18. **Khalifa N, Abdel-Azzez A, Hassaneen A *et al.* (2013):** Ascitic fluid lactoferrin as a diagnostic marker for spontaneous bacterial peritonitis. Afro-Egyptian Journal of Infectious and Endemic Diseases, 3(2): 49-55.
19. **Ioannou G, Green P, Lowy E *et al.* (2018):** Differences in hepatocellular carcinoma risk, predictors and trends over time according to etiology of cirrhosis. PLoS One, 13(9): e0204412. doi:10.1371/journal.pone.0204412.