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Research Article

Determination of Alpha-fetoprotein, Golgi protein-73 and Glypican-3 Levels in Chronic Viral Hepatitis B Patients with Fibrosis in Baghdad Gastroenterology and Hepatology Hospital

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Abstracts

Background: Chronic hepatitis B (CHB) infects the liver and is considered the leading cause of fibrosis and hepatocellular carcinoma (HCC). As liver biopsy is invasive, biomarkers that predict CHB noninvasively could affect the overall complication of this dreaded disease. ***Objective***: The aim of this study was to evaluate the use of alpha-fetoprotein (AFP), Golgi protein-73 (GP73), and glypican-3 levels as new predictive biomarkers for assessing hepatitis B infection patients with fibrosis. ***Method***: This study included 60 participants and classified 30 chronic hepatitis B patients with fibrosis and 30 apparently healthy subjects who served as controls. The liver function test was performed for all participants, in addition to serum levels of AFP, GP73, and GPC3 level by using the sandwich ELISA technique. Liver fibrosis was assessed using FibroScan. ***Results***: The current study showed that GP73 and GPC3 levels were significantly elevated in the chronic hepatitis B with fibrosis group; furthermore, AFP levels were significantly different in the chronic hepatitis B with fibrosis group compared to the control group. ***Conclusions***: The current study demonstrated that GP73 alone or in combination with GPC3 level can be considered as an effective biomarker for assessing fibrosis among patients with CHB.

Keywords: AFP, GP73, GPC3, Hepatitis B, Liver fibrosis.

تحديد مستويات الفا فيتو بروتين، كولجي بروتين 73 وبروتين الكلاييكان-3 في مرضى التهاب لكبد الفيروسي نوع (ب) المزمن وتليف الكبد

الخلاصة

الخلفية : يصيب التهاب الكبد المزمن نوع (B) الكبد ويعتبر عامل سبب لاصابة خلايا الكبد بالسرطان وتلف خلايا الكبد. بعض المعلمات الحيوية تعتبر طريقة غير جراحية أمنة لتشخيص الكبد التي يمكن ان تسبب الطرق الجراحية مظاعفات للكبد. **الهدف:** الدراسة الحالية تهدف الى أهمية تشخيص المعلمات الحيوية (الفافيتو بروتين، كولجي بروتين 73 وكلاييكان-3) لدراسة حساسية وخصوصيه هذه المعلمات الحيوية الحديثة تساهم في التنبوء عن التهاب الكبد المزمن نوع (B) وتليف الكبد كنتيجة لاتهاب الكبد الفايروسي نوع (B). **الطريقة:**هذه الدراسة تضمنت (60) حالة. (30) مريض مصاب بالتهاب الكبد الفيروسي نوع (ب) يتضمن (15 مريض مصاب بالتهاب الكبد الفيروسي فقط و 15 مريض مصاب بتليف خلايا الكبد كنتيجة لالتهاب الكبد الفيروسي نوع B) و 30 شخص اصحاء لايعانون من مرض لمقارنة نتائج الدراسة .**النتائج:** لوحظ أن غالبية المرضى المصابين بالتهاب الكبد المزمن ب والتليف الكبدي في الفئة العمرية (50-60) سنة والتي تشكل (80%). أظهرت الدراسة الحالية أن مستويات GP73 و GPC3 كانت مرتفعة في مجموعة التهاب الكبد المزمن B والتليف الكبدي (p <0.001)، علاوة على ذلك كانت مستويات AFP مختلفة بشكل كبير في مجموعة التهاب الكبد المزمن B والتليف الكبدي مقارنة بمجموعة التحكم(p-value= 0.01). **الاستنتاج:** أظهرت الدراسة الحالية أن GP73 بمفرده أو بالاشتراك مع مستوى GPC3 يمكن اعتباره بمثابة علامة حيوية فعالة جديدة لتشخيص التهاب الكبد المزمن B وتليف الكبد المرتبط بالتهاب الكبد المزمن B.

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INTRODUCTION

Hepatitis B virus (HBV) infection is considered a significant health problem worldwide, with more than 350 million human beings infected with the virus [1]. Despite the availability of an effective vaccination, many people are still at risk of getting HBV, primarily through perinatal transmission or horizontal transfer during childhood [2]. Chronic hepatitis B infection (CHB) is regarded as the main cause of hepatocellular carcinoma (HCC) and liver fibrosis, with a mortality rate of nearly one million per year [3]. As the progression of CHB, most of the patients develop liver fibrosis after getting chronic hepatitis and then HCC [4]. A crucial predictor for selecting the right antiviral medication and the prognosis for disease progression is the grade of liver fibrosis caused by CHB [5]. Therefore, biomarkers for noninvasive CHB prediction may impact the disease's overall complications. While it has been a long-standing fact that the standard practice to evaluate the level of liver fibrosis is through obtaining a liver biopsy [6]. Though many limitations have been recognized with liver biopsy [7]. These limitations included being invasive, non-dynamic evaluation, and

errors during collecting biopsy samples, pain, and inter-observer variability. Sometimes the complications are considered life-threatening [8]. Given all these limitations and risks, it made sense to search to investigate a novel non-invasive method for assessing liver fibrosis [9]. These new techniques included biological strategies depending on serum biomarkers evaluating fibrosis in addition to physical strategies depending on the evaluation of liver stiffness utilizing transient electrography [10]. The ability to identify severe fibrosis or cirrhosis in people with chronic hepatitis B has been shown to be possible using a number of non-invasive techniques that just use standard blood indicators [11]. Alpha-fetoprotein (AFP) is identified as a fetal protein that is produced in the yolk sac and liver of the fetus in early developmental stages [11]. Serum AFP has been documented in previous studies as a tumor marker for HCC [12]. Regardless of being non-specific for HCC diagnosis, increased AFP levels are also detected in viral hepatitis and different liver diseases [13]. AFP levels > 400 ng/mL are considered very high and can be considered a strong predictor of HCC. AFP can also be used for indirect detection of fibrosis stage in chronic hepatitis C infection. However, data on AFP levels in CHB due to progressive cirrhosis are still scarce [14]. Golgi protein 73 (GP73) is often expressed in many epithelial cells (EP) of human tissues. The GP73 gene is located on chromosome 9. The hepatocyte expression of GP73 mRNA and protein was considerably elevated in severe viral liver infections, whether they were acute or chronic. Additionally, GP73 overexpression arises in activated hepatic stellate cells, which are responsible for the development of liver fibrosis. GP73 is recognized as an important diagnostic sign for fibrosis or liver failure. [15]. Glypican-3 (GPC-3) is a heparan sulfate proteoglycan generated from embryonic cancer that adheres to the plasma membrane through glycosylphosphatidylinositol. Usually, GPC-3 plays a critical role as a tumor cell suppressor and regulates cell survival and proliferation throughout embryogenesis [16]. Several studies have reported that GPC3 is absent in normal human tissues but highly expressed in fetal liver, HCC tissues, and most HCC cell lines [17]. Furthermore, there were only a few studies that investigated chronic hepatitis B patients and hepatic fibrosis through measuring the levels of AFP, GP73, and GPC3. Therefore, in the present study, it was aimed to examine the usefulness of these biomarkers as a tool to help in evaluating chronic hepatitis B patients and assessing liver fibrosis among this patients' group due to chronic hepatitis B.

METHODS

Study design

This case-control study was conducted on patients with CHB with fibrosis. Sixty participants were enrolled in the current study. Thirty of them had a viral hepatitis B infection with fibrosis and were admitted to the Gastroenterology and Hepatology Teaching Hospital in Medical City, Baghdad, during the period between December 2021 to March 2022. The other thirty participants are healthy subjects who served as controls. Liver function tests are performed in all participants in addition to the assessment of HBV DNA and other new markers such as serum levels of AFP, GP73, and GPC3. The degree of liver fibrosis was assessed using the FibroScan technique.

Inclusion criteria

All patients having a confirmed diagnosis of chronic hepatitis B virus infection based on serological testing for positive HBs Ag, presence of HBV DNA.

Exclusion criteria

Individuals with co-infection with other viral infections such as hepatitis C virus (HCV) or human immunodeficiency virus (HIV) and fatty liver disease and decompensated cirrhosis due to HBV, and those with hepatocellular carcinoma, are also excluded.

Outcome measurements

Serum AFP, GPC3, and GP73 levels were analyzed using sandwich ELISA (Bio sources, USA). The whole blood was used for measuring viral load by real-time PCR. Methods were conducted according to the instructions of the manufacturing company's leaflet.

Ethical approval

All subjects gave written informed consent prior to having their blood collected for this study. The Hepatology and Gastroenterology Teaching Hospital in Medical City's ethical committee approved the study protocol on May 12, 2021.

Statistical analysis

The Statistical Package of Social Science Software, version 20 for Windows, is recommended for statistical studies (SPSS). Statistical analysis was performed using the pre-coded private records that had been entered into the laptop. In this study, the CHB and fibrosis services to the management team were examined to establish the importance of the differences between variables using the Chi-square test. The data was formerly shown as mean standard error. Using the region under the receiver operating characteristic (AUROC) curve with a 95% confidence interval, the best cut-off values with the greatest degree of sensitivity and specificity have been identified (CI). P-values less than 0.05 were considered significant.

RESULTS

All the patients (30 cases) were positive for HBs-Ag. Table 1 showed that most cases of chronic hepatitis B (CHB) and fibrosis of the liver group occurred within the age group (50-60), which represented 8 (80%), and the least cases of the CHB and fibrosis group were recorded among patients within the age group 40-49

years, which comprised 6 (40%).

Table 1: Age-based distribution of study groups

Groups	Age range groups (Years)				Total
	(20-29)	(30-39)	(40-49)	(50-60)	
CHB and Fibrosis	7(41.2)	9(50)	6 (40)	8(80)	30(33.3)
Control	10(58.8)	9(50)	9(60)	2(20)	30(33.3)
Total	17(100)	18(100)	15(100)	10(100)	60(100)
p-value			0.001		

Values were expressed as frequency and percentage

These differences between the frequency and percentages of cases were highly significant at (p= 0.001) in comparison with the control group. Results of fibrosis stages in the patient group were documented in Table 2, which revealed that F4 was in 15 patients, which constituted 50%, while F3 was in 7 patients, which constituted 23.23%, and F2 and F1 constituted 5 (16.67%) and 3(10%), respectively.

Table 2: Fibrosis stage distribution of the participants

Fibrosis stage	n(%)
F1	3(10)
F2	5(16.67)
F3	7(23.33)
F4	15(50)
Total	30(100)

Table 3 showed that the levels of albumin (g/L) were normal in levels among both CHB with fibrosis and control groups (3.2-5 g/dl), which was indicated by measuring the means value (4.58±2.82 and 3.83±0.40), respectively; these differences between the groups were highly significant (p= 0.007).

Table 3: Biochemical markers of liver function among the studied groups

Markers	Groups	Value	*p*-value
Albumin (g/dl)	CHB with fibrosis	4.58±0.6	0.007
	Control	3.83±0.06	
ALT (U/L)	CHB with fibrosis	50.43±9.71	0.02
	Control	24.40±1.62	
AST (U/L)	CHB with fibrosis	47.53±9.11	0.02
	Control	26.96±1.39	
ALP (U/L)	CHB with fibrosis	110.02±12.08	<0.001
	Control group	77.96±5.63	
Total bilirubin (mg/dL)	CHB with fibrosis	0.93±0.17	<0.001
	Control group	0.16±0.02	

The results of this table also observed there are high levels of both ALT (50 < U/L) and AST (40 < U/L) among CHB with fibrosis in comparison with the control group (50.43±23.41 and 24.40±8.88), (47.53±24.75 and 26.96±8.55, respectively); statistically, these differences between the levels of both enzymes among groups were significant (p= 0.02) respectively. However, the levels of ALP (U/L) were found normal in the levels (40-300 U/L) among the patients group and control group (110.02±38.46 and 77.96±21.33, respectively); these differences between the levels statistically were highly significant (p<0.001). The results of these tables documented there are normal levels of total bilirubin (0.2-1.2 g/dL) among both CHB with fibrosis and control groups with means (0.93±0.51 and 0.16±0.1, respectively); these differences were highly statistically significant (p<0.001). Results of PCR for HBV DNA among study groups were listed in Table 4, which showed that HBV DNA was positive in 18 (60%) and negative in 12 (40%) among the patient group, while all the control group was negative for HBV DNA.

Table 4: Results of HBV DNA analyzed by PCR among the studied groups

Study groups	Results of PCR for HBV DNA n(%)		
	Positive	Negative	Total
CHB with fibrosis	18 (60)	12 (40)	30(100)
Control	0 (0.0)	30 (100)	30(100)

Results of AFP, GP73, and GPC3 levels among study groups were shown in Table 5, which revealed that concentrations of AFP (ng/ml) were found within normal level (≥0.05) among the CHB and fibrosis groups with a mean of 0.61±0.06, but GP73 and GPC3 levels were elevated more than normal values with a mean of 15.41±0.68 and 17.15±2.63, respectively, among CHB and fibrosis. Statistically, these differences in the concentration of AFL levels among studied groups were significant at p= 0.01 while highly significant with p<0.001 in GP73 and GPC3 levels.

Table 5: Detection of AFP, Gp73, GPC level among study groups

Parameters	Groups (n=30 each)	Value	*p-value*
AFP >6.05 ng/ml	CHB with fibrosis	0.61±0.06	0.01
	Control group	0.54±0.04	
GP73(2.3-13.9)	CHB with fibrosis	15.41±0.68	<0.0001
	Control group	4.52±0.21	
GPC3 (2.3-5.5)	CHB with fibrosis	17.15±2.63	<0.0001
	Control group	3.79±0.19	

Values were expressed as mean±SE. N= 30 cases in each group.

Receiver operating curve (ROC) analysis is additionally employed to establish the ideal cut-off points for medical testing. Table 6 shows an area of cut-off between sensitivity and complement values of specificity, as well as significant levels for testing area parameters under fifty percent, with a 95% confidence interval concerning the AFP (ng/dl), GP37 (ng/dl), and GPC3 (ng/dl).

Table 6 : ROC curve analysis of studied groups

Parameters	Cutoff	AUC	*p*-value	95% C.I.		Sensitivity (%)	Specificity (%)
				L.B	U.B		
AFP	0.54	0.565	<0.0001	0.416	0.714	70	56.7
GP73	0.905	1.0	<0.0001	1.0	1.0	100	100
GPC3	5.82	0.895	<0.0001	0.797	0.993	76.7	100

Present the area under the curve (AUC) for ROC curve analysis for AFP (ng/dl), GP37 (ng/dl), and GPC3 (ng/dl) were 0.56, 1.0, and 0.89, respectively, among CHB and fibrosis patients**,** reflecting the great diagnostic power of tests. In addition to that, markers concerning the area under the ROC curve are accounted for as highly significant discrimination at *p*= 0.000, 0.000, and 0.001, respectively. Also, the present study documented that the sensitivity was 0.7, 1.0, and 0.76, respectively. While the specificity for all three tumor markers was 0.56, 1.0, and 1.0, respectively as shown in Figure 1.

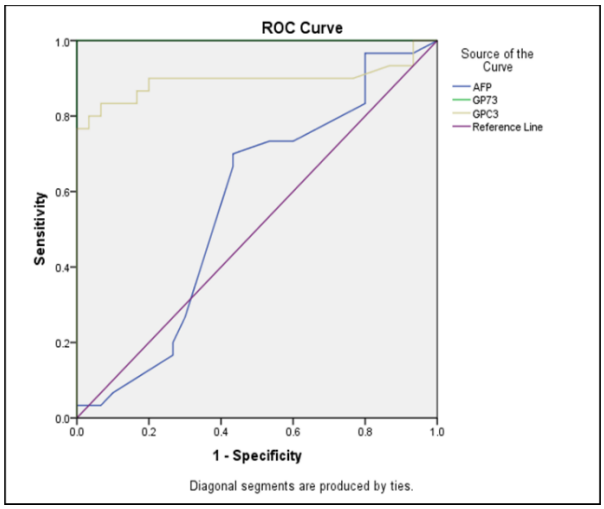

Figure 1: ROC chart for of the studied biomarkers among CHB patients with fibrosis.

DISCUSSION

The current study in Iraq revealed that relatively massive variations (*p*= 0.001) among CHB and fibrosis when comparing with the manipulation team according to age distribution are proven in Table 1. These results agree with the previous study [18]. In Japan [19], it was described that many persistent HBV sufferers had occurred in older age with a suggested age of 65 years. These outcomes are somewhat compatible with the present study, which observed that 80% of the patients with CHB with fibrosis were within the age group 50-60 years. The incidence of CHB was higher in the elderly patient's possible explanations for this include delayed screening and diagnosis, resulting in patients with HBV infection not being identified until later in life. Poor connections exist to care for CHB sufferers even in high-income countries [20,21]. Despite being used for years, alpha-fetoprotein (AFP) has sensitivity and specificity degrees of simply 40–65% and 76–96%, respectively, in the early diagnosis of hepatic carcinoma [22]. The current study showed that there is a significant difference in AFP level among CHB with fibrosis when comparing with the control group, these results are in line with another study [23]. Also, other studies showed that the AFP increases with the severity of fibrosis [24]. Also in the same table, the present study appeared to show that AFP concentration was within normal value (>6.05) among the CHB and fibrosis groups. These outcomes are dissimilar to the previous study [25]. Which demonstrated that high level of AFP among the CHB group. These variations might be connected to variations in sample sizes, methodology used to measure AFP, and cutoff points between studies. However, elevated serum AFP levels in hepatic disorders can result from persistent inflammation, abnormal hepatocyte-hepatocyte communication, or a breakdown of normal structural arrangements [26]. Patients with fibrotic CHB were assessed to have a risk of HCC ranging from 5% to 30%. The wide range of results in these calculations may be caused by the patients' enrolled for this research having varying degrees of fibrosis. [27]. For the early diagnosis of hepatocellular carcinoma, Golgi membrane glycoprotein 73 (GP73), a serum biomarker, is a viable candidate (HCC). People with chronic liver illness have particularly high levels of the molecule GP73, which is connected to liver fibrosis, inflammation, and several variables that contribute to the progression of liver disease. The blood level of GP73 may potentially serve as a helpful biomarker to detect severe fibrosis and cirrhosis in people with chronic liver disease, according to data from the last ten years [28,29]. The current study detected especially massive extension in GP73 level amongst CHB and fibrosis in evaluation with the control team (*p*>0.001) as shown in Table 2. These results resemble other trends [30-32]. In a previous study, it was demonstrated that there was a highly significant increase in GP73 among CHB and liver stiffness [33]; these findings are consistent with the present study. Gu *et al.* (2009) [34] published that GP73 concentrations in patients with liver disease have been threefold compared to healthy persons. In the other study, which reported that significant fibrosis could be accurately diagnosed by serum GP73 [35]. It has been suggested that activated stellate cells, which are thought to be the most important cell type involved in hepatic fibrogenesis, consistently express the gene GP73 and its mRNA in chronic liver disorders [36,37]. Inflammation, notably inflammatory cytokines like IL-6, might cause GP73 overexpression to start off, possibly in reaction to a persistent active viral infection [38]. The heparin sulfate proteoglycans family of oncofetal proteins includes the tumor suppressor glypican 3 (GPC3) [39]. The present study showed an increased level of GPC3 (17.15±2.63) among CHB and fibrosis in comparison with the control group (3.79±0.19). These findings are in concordance with another study, which explained how cirrhosis and chronic hepatitis raise the plasma GPC3 level [40]. In fact, several studies have discovered GPC3 in HCV samples, growing nodules, and even healthful liver tissues, proving that GPC3 ranges are accelerated for the duration of the early tiers of liver carcinogenesis or those non-malignant or pro-malignant liver cells may also be able to manufacture GPC3 [41,42]. Additionally, Jia *et al.* (2016) discovered no distinction in serum GPC3 levels between chronic HBV-infected patients and healthy controls [42]. These results are inconsistent with our study. These differences may be related to sample size, using different cut-off values for GPC-3, and different patients' characteristics. The current study's ROC curve analysis revealed an AUC of 0.565, at which point AFP exhibited 70% sensitivity and 56% specificity in predicting significant diagnoses of CHB and fibrosis in

CHB patients. In the past, Güçlü *et al.* (2019) published results that were equivalent in their prediction of severe fibrosis in HBV patients [25]. Wang and his coworkers utilized AFP to identify liver fibrosis with 58% sensitivity and 87% specificity [43]. These results are lower in sensitivity when compared with our investigation. An explanation for this result may be related to the difference in the cutoff point used for AFP measurement between studies. Furthermore, our investigation in the same table identified an AUC of 1.0; at cutoff 9.0500, GP73 had 100% sensitivity and 100% specificity. GPC3 demonstrated 76% sensitivity and 100% specificity in determining a significant diagnosis of CHB and fibrosis in CHB patients, with an AUC of 0.895 at a cutoff of 5.8200. These findings are in line with those of Xia *et al.* (2017) [44]. However, the current investigation demonstrated that the GP73 biomarker had more sensitivity and specificity in CHB and fibrosis prediction diagnosis when compared with the AFP marker. Abdel-Azeez *et al.* (2020) discovered GP73 in serum from chronic HCV patients who had fibrosis, with 82% sensitivity and 80% specificity. The variances between these studies rely on their sensitivity and may be attributable to the unique characteristics of each patient, variations in specimen collection, reagent storage and use, and variations in detection methods [45], and so on. To accurately detect GP73 in liver illness and cancer, it is therefore important to increase the quantity of test samples, collect samples from diverse populations in the study, and develop more efficient and reliable detection techniques [44]. Consideration should also be paid to the limitations of the present study. In liver fibrosis caused by fatty liver disease, HCV infection, or other reasons, further investigations to detect the possibility of GP73 alone or with GPC3 as biomarkers are needed. It is still unknown how sensitive and specific GP73 is as a biomarker of liver fibrosis brought on by different etiologies. Moreover, because of the relatively small number of chronic hepatitis B patients and healthy volunteers in this study, we needed to confirm the results of this study in many cases to support the predictive value of GP73 and GPC3 for fibrosis occurrence in CHB patients.

Conclusion

The current study demonstrated that GP73 and GPC3 were significant effective biomarkers for diagnosing CHB and fibrosis and can be utilized as a serum marker in monitoring fibrosis progression. Therefore, we suggested conducting larger-scale research on a variety of liver diseases.

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Conflict of interests

No conflict of interest was declared by the authors.

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Data sharing statement

Supplementary data can be shared with the corresponding author upon reasonable request.

REFERENCES

1. Yamada R, Hiramatsu N, Oze T, Morishita N, Harada N, Yakushijin T, et al. Impact of alpha-fetoprotein on hepatocellular carcinoma development during entecavir treatment of chronic hepatitis B virus infection. *J Gastroenterol*. 2015;50(7):785-794. doi: 10.1007/s00535-014-1010-7.
2. Seo SI, Kim HS, Kim WJ, Shin WG, Kim DJ, Kim KH, et al . Diagnostic value of PIVKA-II and alpha-fetoprotein in hepatitis B virus-associated hepatocellular carcinoma. *World J Gastroenterol.* 2015;21(13):3928. doi: 10.3748/wjg.v21.i13.3928.
3. Tong MJ, Huynh TT, Siripongsakun S, Chang PW, Tong LT, Ha YP, et al. Predicting clinical outcomes in patients with HBsAg-positive chronic hepatitis. *Hepatol Int*. 2015;9:567-577. doi: 10.1007/s12072-015-9651-z.
4. Su TH, Hu TH, Chen CY, Huang YH, Chuang WL, Lin CC, et al. Four-year entecavir therapy reduces hepatocellular carcinoma, cirrhotic events and mortality in chronic hepatitis B patients. *Liver Int*. 2016;36(12):1755-1764. doi: 10.1111/liv.13253.
5. Hadziyannis E, Laras A. Viral biomarkers in chronic HBeAg negative HBV infection. *Genes*. 2018;9(10):469. doi: 10.3390/genes9100469.
6. Yao M, Zhao J, Lu F. Alpha-fetoprotein still is a valuable diagnostic and prognosis predicting biomarker in hepatitis B virus infection-related hepatocellular carcinoma. *Oncotarget.* 2016;7(4):3702. doi: 10.18632/oncotarget.6913.
7. Ge T, Shen Q, Wang N, Zhang Y, Ge Z, Chu W, et al. Diagnostic values of alpha-fetoprotein, dickkopf-1, and osteopontin for hepatocellular carcinoma. *Med Oncology.* 2015;32:1-0. doi: 10.1007/s12032-014-0367-z.
8. Chien J, Liu J, Lee MH, Jen CL, Batrla-Utermann R, Lu SN, et al. Risk and predictors of hepatocellular carcinoma for chronic hepatitis B patients with newly developed cirrhosis. *Journal of gastroenterology and hepatology*. 2016 Dec;31(12):1971-7. doi: 10.1111/jgh.13422.
9. Liu YR, Lin BB, Zeng DW, Zhu YY, Chen J, Zheng Q, et al. Alpha-fetoprotein level as a biomarker of liver fibrosis status: a cross-sectional study of 619 consecutive patients with chronic hepatitis B. *BMC Gastroenterol*. 2014;14:1-8. doi: 10.1186/1471-230X-14-145.
10. Wu Y, Ma J, Wang Y, Zhang Y, Hou Y, Zhang C, et al. Development of an alpha-fetoprotein and Golgi protein 73 multiplex detection assay using xMAP technology. *Clinica Chimica Acta*. 2018;482:209-214. doi.org/10.1016/j.cca.2018.03.039.
11. Kim GA, Seock CH, Park JW, An J, Lee KS, Yang JE, et al. Reappraisal of serum alpha-foetoprotein as a surveillance test for hepatocellular carcinoma during entecavir treatment. *Liver Int*. 2015;35(1):232-239. doi: 10.1111/liv.12516.
12. Li S, Li X, Xu A, Zhang B, He X, Chen H, et al. Screening and clinical evaluation of dominant peptides of centromere protein F antigen for early diagnosis of hepatocellular carcinoma. *Mol Med Rep*. 2018;17(3):4720-4728. doi: 10.3892/mmr.2018.8372.
13. Lee JC, Cheng CH, Wang YC, Wu TH, Lee CF, Wu TJ, et al. Clinical relevance of alpha-fetoprotein in determining resection margin for hepatocellular carcinoma. *Medicine*. 2019;98(11):e14827. doi: 10.1097/MD.0000000000014827.
14. Tajiri H, Takano T, Tanaka H, Ushijima K, Inui A, Miyoshi Y, et al. Hepatocellular carcinoma in children and young patients with chronic HBV infection and the usefulness of alpha-fetoprotein assessment. *Cancer Med*. 2016;5(11):3102-3110. doi: 10.1002/cam4.917.
15. Riener MO, Stenner F, Liewen H, Soll C, Breitenstein S, Pestalozzi BC, et al. Golgi phosphoprotein 2 (GOLPH2) expression in liver tumors and its value as a serum marker in hepatocellular carcinomas. *Hepatology*. 2009;49(5):1602-1609. doi: 10.1002/hep.22843.
16. Huang TS, Shyu YC, Turner R, Chen HY, Chen PJ. Diagnostic performance of alpha-fetoprotein, lens culinaris agglutinin-reactive alpha-fetoprotein, des-gamma carboxyprothrombin, and glypican-3 for the detection of hepatocellular carcinoma: a

systematic review and meta-analysis protocol. *Syst Rev*. 2013;2:1-8. doi: 10.1186/2046-4053-2-37.
17. Yamauchi N, Watanabe A, Hishinuma M, Ohashi KI, Midorikawa Y, Morishita Y, et al. The glypican 3 oncofetal protein is a promising diagnostic marker for hepatocellular carcinoma. *Modern Pathol*. 2005;18(12):1591-1598. doi: 10.1038/modpathol.3800436.
18. Tseng CH, Hsu YC, Ho HJ, Nguyen MH, Wu CY. Increasing age and non-liver comorbidities in patients with chronic hepatitis B in Taiwan: a nationwide population-based analysis. *Digest Dis*. 2021;39(3):266-274. doi: 10.1159/000511585.
19. Yotsuyanagi H, Kurosaki M, Yatsuhashi H, Lee IH. Brooks-Rooney C, Nguyen MH. Characteristics and healthcare costs in the aging hepatitis B population of Japan: a nationwide real-world analysis. *Digest Dis*. 2022;40(1):68-77. doi: 10.1159/000515854.
20. Ogawa E, Yeo YH, Dang N, Le MH, Jeong D, Tran S, et al. Diagnosis rates of chronic hepatitis B in privately insured patients in the United States. *JAMA Network Open*. 2020;3(4):e201844. doi:10.1001/jamanetworkopen.2020.1844.
21. Le MH, Yeo YH, Cheung R, Henry L, Lok AS, Nguyen MH. Chronic hepatitis B prevalence among foreign-born and U.S.-born adults in the United States, 1999-2016 *Hepatology*. 2020;71(2):431–443. doi: 10.1002/hep.30831.
22. Galle PR. Management of liver cancer. *Digest Dis*. 2016;34(4):438-439. doi: 10.1159/000444559.
23. Cheng WQ, Guan SH, Zhang H, Yang K, Chen ZD, Shao XX. Value of serum alpha-fetoprotein in predicting liver fibrosis in patients with chronic hepatitis B. *J Clin Hepatol*. 2018;34(5):1015-1019. doi: 10.3969/j.issn.1001-5256.2018.05.018.
24. Feng L, Sun K, Zhang J, Feng G, Zhao Y. A novel non-invasive index using AFP and APTT is associated with liver fibrosis in patients with chronic hepatitis B infection: a retrospective cohort study. *BMJ Open*. 2015;5(9):e008032. doi: 10.1136/bmjopen-2015-008032.
25. Güçlü E, Öğütlü A, Kösem M, Erkorkmaz Ü, Karabay O. Relationship between basic laboratory results and fibrosis in chronic hepatitis B patients. *Viral Hepat J*. 2019;25:45-49. doi: 10.4274/vhd.galenos.2019.2019.0010.
26. Demir NA, Kolgelier S, Ozcimen S, Gungor G, Sumer S, Demir LS, et al. Evaluation of the relation between hepatic fibrosis and basic laboratory parameters in patients with chronic hepatitis B fibrosis and basic laboratory parameters. *Hepatitis Monthly*. 2014;14(4). doi: 10.5812/hepatmon.16975.
27. Abdel-Aziz MM, Elshal MF, Abass AT, El-Kafrawy S, Ezzat S, Abdel-Wahab M. Comparison of AFP-L3 and p53 Antigen Concentration with Alpha-Fetoprotein as Serum Markers for Hepatocellular Carcinoma. *Clin Lab J*. 2016;62(6):1121-1129. doi: 10.7754/clin.lab.2015.151102.
28. Xu Z, Shen J, Pan X, Wei M, Liu L, Wei K, et al. Predictive value of serum Golgi protein 73 for prominent hepatic necroinflammation in chronic HBV infection. *J Med Virol*. 2018;90(6):1053-1062. doi: 10.1002/jmv.25045.
29. Yao M, Wang L, Leung PS, Li Y, Liu S, Wang L, et al . The clinical significance of GP73 in immunologically mediated chronic liver diseases: experimental data and literature review. *Clin Rev Allergy Immunol*. 2018;54:282-294. doi: 10.1007/s12016-017-8655-y.
30. Atilla A, Taşkın MH, Kazak Z, Aydın S, Kılıç SS. GP73 level in patients with chronic hepatitis B: relationship with liver biopsy, levels of ALT, AST and HBV DNA. *Indian J Pathol Microbiol*. 2022;65(1):55-58. doi: 10.4103/IJPM.IJPM_1149_20.
31. Zhou Q, Yan L, Du L, Tang X, Tang H. Combined serum Golgi membrane glycoprotein 73 improves the accuracy of transient elastography for significant fibrosis detection in patients with chronic HBV infections. *Hepatitis Monthly*. 2021;21(2). doi: 10.5812/hepatmon.102039.
32. Hailin B, Qun X. Study on the application value of HBV DNA, AFP and GP73 in the diagnosis of chronic hepatitis B severity and liver fibrosis. *J Hainan Med Univ*. 2019;25(9):700-703. doi: 10.13210/j.cnki.jhmu.20190510.003.
33. Gatselis NK, Tornai T, Shums Z, Zachou K, Saitis A, Gabeta S, et al. Golgi protein-73: A biomarker for assessing cirrhosis and prognosis of liver disease patients. *World J Gastroenterol*. 2020;26(34):5130. doi: 10.3748/wjg.v26.i34.5130.
34. Gu Y, Chen W, Zhao Y, Chen L, Peng T. Quantitative analysis of elevated serum Golgi protein-73 expression in patients with liver diseases. *Ann Clin Biochem*. 2009;46(1):38-43. doi: 10.1258/acb.2008.008088
35. Cao Z, Li Z, Wang Y, Liu Y, MoR, Ren P, et al. Assessment of serum Golgi protein 73 as a biomarker for the diagnosis of significant fibrosis in patients with chronic HBV infection. *J Viral Hepat*. 2017;24(Suppl 1):57–65. doi: 10.1111/jvh.12786.
36. Yan LB, Zhang QB, Zhu X, He M, Tang H. Serum S100 calcium binding protein A4 improves the diagnostic accuracy of transient elastography for assessing liver fibrosis in hepatitis B. *Clinics Res Hepatol Gastroenterol*. 2018;42(1):64-71. doi: 10.1016/j.clinre.2017.05.013.
37. Carpino G, Morini S, Corradini SG, Franchitto A, Merli M, Gaudio E, et al. Activated hepatic stellate cells in liver cirrhosis. Alpha-SMA expression in hepatic stellate cells and quantitative analysis of hepatic fibrosis in cirrhosis and in recurrent chronic hepatitis after liver transplantation. *Dig Liver Dis*. 2005;37(5):349-356. doi:10.1016/j.dId.2004.11009.
38. Liang H, Block TM, Wang M, Nefsky B, Long R, Hafner J, et al. Interleukin-6 and oncostatin M are elevated in liver disease in conjunction with candidate hepatocellular carcinoma biomarker GP73. *Cancer Biomarkers*. 2012;11(4):161-171. doi: 10.3233/CBM-2012-00276.
39. Badr EA, Korah TE, Ghani AA, El-Sayed S, Badr S. Role of serum glypican-3 in the diagnosis and differentiation of small hepatocellular carcinoma from hepatitis-C virus cirrhosis. *Alexandria J Med*. 2014;50(3):221-226.doi: 10.1016/j.ajme.2014.01.002.
40. Shimizu Y, Mizuno S, Fujinami N, Suzuki T, Saito K, Konishi M, et al. Plasma and tumoral glypican-3 levels are correlated in patients with hepatitis C virus-related hepatocellular carcinoma. *Cancer Sci*. 2020;111:334–342. doi: 10.1111/cas.14251.
41. Guo M, Zhang H, Zheng J, Liu Y. Glypican-3: A new target for diagnosis and treatment of hepatocellular carcinoma. *J Cancer*. 2020;11(8):2008-2021. doi: 10.7150/jca.39972.
42. Jia X, Gao Y, Zhai D, Liu J, Cai J, Wang Y, et al. Assessment of the clinical utility of glypican 3 as a serum marker for the diagnosis of hepatocellular carcinoma. *Technol Cancer Res Treat*. 2016;15:780-786. doi: 10.1177/1533034615605248.
43. Wang J, Zhang P, Liao J, Zhu Y, Liu X, Tang H. Association of α-fetoprotein levels with liver stiffness measurement in outpatients with chronic hepatitis B. *Biosci Rep*. 2021;41(1):BSR20203048. doi: 10.1042/BSR20203048.
44. Xia Y, Li Q, Gong L, Xu H, Pang L, Li Z, et al. Detection of hepatocellular carcinoma biomarker Golgi Protein 73 based on magnetic enzyme-linked immunoassay. *J Nanosci Nanotechnol*. 2017;17(1):231-237. doi: 10.1166/jnn.2017.12456.
45. Abdel-Azeez HA, Sharaf SM, Elshamy EA, Fikry AA. Serum Golgi protein 73 as a biomarker for liver fibrosis in chronic hepatitis C patients. *Egypt J Hospit Med*. 2020;81(5):1993-2000. doi: 10.21608/ejhm.2020.124772.