AQCH ANALYTICAL AND QUANTITATIVE CYTOPATHOLOGY AND HISTOPATHOLOGY®

An Official Periodical of **The International Academy of Cytology** *and the* **Italian Group of Uropathology**

Expression of AFP, P-sel, and MMP-9 in Cirrhosis with Portal Vein Thrombosis

Liping Hu, Ph.D., **Fenglei Tan,** Ph.D., **Jingping Xiong,** Ph.D., **Nulibiya Abudukeyoumu,** Ph.D., and **Yuexin Zhang,** Ph.D.

OBJECTIVE: To discuss and analyze the expression and clinical significance of serum alpha-fetoprotein (AFP), P-selectin (P-sel), and matrix metalloproteinase–9 (MMP-9) in hepatic sclerosis combined with portal vein thrombosis (PVT).

STUDY DESIGN: In this retrospective study, 38 patients with hepatic sclerosis combined with PVT diagnosed from August 2010 to February 2018 were chosen as the observation group. During the same period, 120 hepatic sclerosis patients without PVT were chosen as the control group. The serum AFP, P-sel, and MMP-9 expression levels of both groups were detected. Meanwhile, the expression levels of AFP, P-sel, and MMP-9 in liver tissue samples of the 2 groups were detected using the Elivision immunohistochemical method. The correlation and clinical significance of AFP, P-sel, and MMP-9 were evaluated.

RESULTS: In the observation group there were 9 cases with splenectomy (23.7%) and 13 with diabetes (34.2%). In the control group there were 3 cases with splenectomy (2.5%) and 14 with diabetes (11.7%). The comparison differences showed statistical significance ($p<0.05$). The levels of serum AFP, P-sel, and MMP-9 in the observation group were higher than those in the control group, and the difference was statistically significant ($p<0.05$). Among the 158 patients the best diagnostic thresholds for AFP, P-sel, and MMP-9 in the diagnosis of cirrhosis with PVT were 25.22 IU/mL, 292.22 ng/mL, and 372.43 ng/mL, respectively, and the AUC values were 0.779, 0.722, and 0.755, respectively. Multivariate logistic regression analysis showed that AFP (OR=2.244), P-sel (OR=3.179), and MMP-9 (OR=2.508) were the main risk factors for PVT ($p<0.05$). The expression level of AFP was positively correlated with the expression of P-sel and MMP-9 in hepatic sclerosis patients with PVT.

CONCLUSION: Serum AFP, P-sel, and MMP-9 present high expression in hepatic sclerosis combined with

From the Departments of Infectious Diseases, First Affiliated Hospital of Xinjiang Medical University and Second Affiliated Hospital of Xinjiang Medical University, Urumqi, China.

Liping Hu, Ph.D., and Fenglei Tan, Ph.D., contributed equally to the writing of this article.

Drs. Hu, Tan, Abudukeyoumu, and Zhang are Associate Professors, Department of Infectious Diseases, First Affiliated Hospital of Xinjiang Medical University.

Dr. Xiong is Associate Professor, Department of Infectious Diseases, Second Affiliated Hospital of Xinjiang Medical University.

Address correspondence to: Yuexin Zhang, Ph.D., Department of Infectious Diseases, First Affiliated Hospital of Xinjiang Medical University, Urumqi 830000, Urumqi, China (vpfrzh@sina.com).

Financial Disclosure: The authors have no connection to any companies or products mentioned in this article.

0884-6812/19/4105-0151/$18.00/0

PVT, with high diagnosis sensitivity and specificity, and may have synergistic effect on the pathogenesis leading to PVT. (Anal Quant Cytopathol Histpathol 2019;41:151–158)

Keywords: alpha-fetoprotein, hepatic sclerosis, liver diseases, liver dysfunction, liver sclerosis, matrix metalloproteinase-9, MMP-9 metalloproteinase, portal vein, portal vein thrombosis, P-selectin, risk factors, sclerosis, thrombosis, venous thrombosis.

Hepatic sclerosis combined with portal vein thrombosis (PVT) is a type of deep vascular obstruction disease that develops in patients with hepatic sclerosis from a thrombus at the main portal vein, left and right branch, inferior mesenteric vein, splenic vein, and superior mesenteric vein.[1,2] The specific symptoms of this disease are closely related to the degree of portal vein vessel obstruction, degree of thrombosis urgency, and part of the thrombus affected. Mild symptoms include stomachache, abdominal distension, nausea, and vomiting, while severe symptoms include secondary peritonitis, septicemia, and intestinal tube necrosis.[3,4] Clinically, for most patients with hepatic sclerosis combined with PVT, the onset is hidden and the progress of the disease is slow. The disease mostly occurs in patients with basic liver disease, and it is easily masked by primary symptoms. Thus, there is a high rate of missed diagnoses and misdiagnoses clinically.[5,6] Ultrasound is the principal method of diagnosis for this disease. The sensitivity and specificity of color Doppler ultrasound in the diagnosis of hepatic sclerosis combined with PVT has reached 80%, but ultrasonic diagnosis also has drawbacks, such as diversity and uncertainty.[7] Seeking molecular markers or protein markers for hepatic sclerosis combined with PVT is a current research hot spot, and these markers would have the advantage of a low misdiagnosis rate, reduced pain, and low cost. In particular, specific markers can significantly improve the definitive diagnosis rate of the disease.[8,9] Alpha-fetoprotein (AFP) is a glycoprotein synthesized by fetal hepatocytes, intestinal cells, and embryonic yolk sac cells. Normal human hepatocytes cannot generate AFP protein. However, when liver damage occurs, AFP can be generated mainly in hepatocytes. However, the use of AFP has certain limitations in clinical application, with a certain rate of missed diagnoses and misdiagnoses. P-sel and MMP-9 are factors closely related to endothelial injury, coagulation, and inflammation.[10] P-sel can initiate and promote typical adhesion molecules of the inflammatory response and can also be used as an important indicator of platelet activation. As an important mediator of the degradation and remodeling of the extracellular matrix, MMP-9 is also involved in the occurrence and development of various inflammatory diseases.[11] In this study, the expression of serum AFP, P-sel, and MMP-9 in hepatic sclerosis combined with PVT and the influencing factors are discussed to provide potential references for early diagnosis.

Materials and Methods

Ethical Approval

The study was approved by the Institutional Ethics Committee of our hospitals, and written informed consent was obtained from all participants.

Study Subjects

In this retrospective study, 38 patients with hepatic sclerosis combined with PVT who were diagnosed at the First Affiliated Hospital of Xinjiang Medical University from August 2010 to February 2018 were chosen as the observation group. During the same period, 120 hepatic sclerosis patients without PVT were chosen as the control group. The inclusion criteria were as follows: inpatient; complete clinical data; age 20–70 years; hepatic sclerosis diagnosis referring to the diagnostic criteria of viral hepatitis and alcoholic liver disease; color ultrasound, computed tomography (CT) scan, and magnetic resonance imaging (MRI) imagological examinations verifying the existence of portal vein system thrombus and confirming the specific distribution of the thrombus; and approval by the Ethics Committee. The exclusion criteria were as follows: patients with myeloproliferative disease or congenital vascular malformation of the liver, lack of clinical data, patients with liver cancer or other tumors, and patients with Budd-Chiari syndrome.

Serum AFP, P-sel, and MMP-9 Examination

Peripheral venous blood (3–5 mL) was collected from all patients in the morning, was allowed to stand for 5–10 minutes at room temperature, and then was subjected to centrifugation for 10 minutes at 3,000 r/min. The supernatant was separated to gain serum samples that were kept at

−20°C. Chemiluminescence immunoassay was used to detect the level of serum AFP, P-sel, and MMP-9 using the Roche Cobas e601 electrochemistry luminescence immunity analyzer. The operation process was conducted in strict accordance with the operating manual and kit specifications. Normal reference values were as follows: AFP, <5.8 IU/mL; P-sel, <151 pg/mL; and MMP-9, <178 ng/L.

Immunohistochemical Detection

After obtaining valid consent from the patients, the biopsy specimens were sent for immunohistochemical examination. A total of 158 blocks (38 from patients with PVT and 120 without PVT) were sectioned and stained by Envision two-step kit (Agilent, California, USA) for AFP, P-sel, and MMP-9. Blocks without representative tissue or containing only superficial mucosa were eliminated. The specimens were routinely fixed, embedded in paraffin, and serially sectioned at 4 μm, and each specimen was cut into 3 sheets: 1 for HE staining and the other 2 for immunohistochemical staining. The antibodies used in the experiments were mouse anti-human monoclonal antibodies: AFP (Beijing Thermo Fisher; Cat. No. #MA1-19178) and P-sel (Beijing Thermo Fisher; Cat. No. #MA1-81809). The percentage of positive cells and staining intensity were observed under a light microscope. The staining of AFP, P-sel, and MMP-9 was localized in the cytoplasm, and all antigens were positive for brownish yellow staining. The staining results were assessed using the following criteria: positively stained cells >10%=positive; stained cells <10% or no staining=negative.

Clinical Data Survey

The demographics data of all patients were surveyed and registered and included gender, age, BMI, hospital stay, phone number, Child-Pugh grade, address, pathogenesis, imaging characteristics, clinical symptoms, and laboratory examination indicators.

Statistical Method

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS version 11.0). Measurement data conforming to a Gaussian distribution were expressed as means± standard deviation, while measurement data conforming to a skewed distribution were expressed using the interquartile range method. Either the *t* test or the rank-sum test was used for comparison. Enumeration data were expressed as a percentage, and χ^2 analysis was used for contrast. The Pearson correlation test was used for analyzing the correlation between AFP and P-sel and MMP-9. Receiver-operating characteristic (ROC) curves were obtained by using Excel to analyze the area under the curve (AUC), the sensitivity, and the specificity. A p value of <0.05 was considered statistically significant.

Results

Comparison of the General Data

The comparison differences of both groups in gender, age, Child-Pugh grade, BMI, course of disease, and pathogenesis showed no statistical significance ($p>0.05$) (Table I). In the observation group there were 9 cases with splenectomy (23.7%) and 13 cases with diabetes (34.2%). In the control group there were 3 cases with splenectomy (2.5%) and 14 cases with diabetes (11.7%). The comparison differences showed statistical significance ($p<0.05$).

Comparison of the Serum AFP, P-sel, and MMP-9 Contents

The serum AFP, P-sel, and MMP-9 contents of

Table I *Comparison of General Data*

Group	No.	Gender (male/female)	Age (yrs)	Child-Pugh score* (class A/B/C)	BMI (kg/m²)	Course of hepatic sclerosis (yrs)	Pathogenesis (virus/ethyl alcohol/autoimmunity)
Observation group	38	20/18	56.33±2.29	20/10/8	22.18±1.33	2.19±0.44	30/6/2
Control group	120	63/57	56.22±1.89	66/33/21	22.44±2.48	2.22±0.33	90/20/10
t or χ^2		0.000	0.156	0.243	0.298	0.078	0.431
p Value		1.000	0.744	0.886	0.614	0.899	0.806

*Child-Pugh classification.

Table II *Comparison of the Serum AFP, P-sel, and MMP-9 Contents Between the 2 Groups*

Group	No.	AFP (IU/mL)	P-sel (ng/mL)	MMP-9 (ng/mL)
Observation group	38	46.45±3.08	399.23±54.91	523.41±43.38
Control group	120	16.22±4.44	221.54±43.12	245.44±37.12
t test		24.583	10.534	22.563
p Value		0.000	0.001	0.000

Values shown as mean±standard deviation.

the observation group were higher than those of the control group, and the comparison difference showed statistical significance ($p<0.05$) (Table II).

Diagnostic Value

ROC curves for the AFP, P-sel, and MMP-9 diagnosis of hepatic sclerosis combined with PVT were drawn for 158 patients. The AUC of AFP was 0.779 (95% CI 0.733–0.812, $p<0.05$), and when the optimal critical value of AFP for diagnosis was 25.22 IU/mL, the sensitivity and specificity were 79.2% and 81.4%, respectively. The AUC of P-sel was 0.722 (95% CI 0.697–0.751, $p<0.05$), and when the optimal critical value of P-sel for diagnosis was 292.22 pg/mL, the sensitivity and specificity were 74.4% and 76.3%, respectively. The AUC of MMP-9 was 0.755 (95% CI 0.704–0.794, $p<0.05$), and when the optimal critical value of MMP-9 for diagnosis was 372.43 ng/L, the sensitivity and specificity were 63.1% and 67.2%, respectively (Figure 1).

Analysis of Influencing Factors

Among 158 patients, hepatic sclerosis combined with PVT was used as the dependent variable, and AFP, P-sel, and MMP-9 served as independent variables. Multi-classification logistic regression analysis showed that AFP (OR=2.244), P-sel (OR=3.179), and MMP-9 (OR=2.508) were the main risk factors ($p<0.05$) (Table III).

Immunohistochemical Expression of AFP, P-sel, and MMP-9 in the Observation and Control Groups

The positive expression rate of AFP, P-sel, and MMP-9 in patients with cirrhosis and PVT (observation group) was significantly higher than that in patients without PVT (control group) (Figure 2) (Table IV).

Correlation of AFP Level with P-sel or MMP-9 Level in Hepatic Sclerosis Patients with PVT

In the hepatic sclerosis patients with PVT, the expression level of AFP correlated positively with the expression of P-sel ($r=0.298$, $p<0.05$) and MMP-9 ($r=0.654$, $p<0.05$), respectively.

Pearson Correlation on the Level of AFP, P-sel, and MMP-9 in Serum and Tissue

There was a significantly positive correlation between serum levels of immunohistochemistry expression of AFP between the observation and

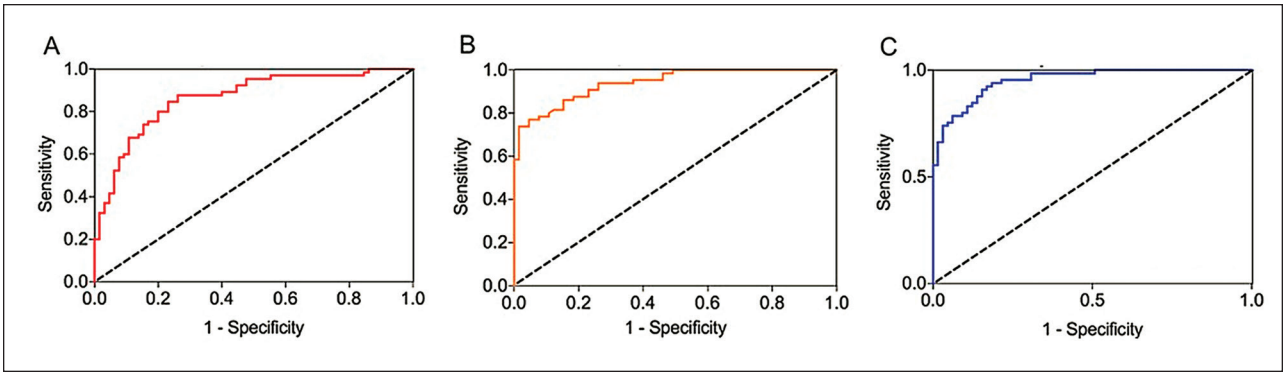

Figure 1 Value of the AFP diagnosis of hepatic sclerosis combined with portal vein thrombosis. (A) AFP, (B) P-sel, and (C) MMP-9.

Table III *Analysis of Factors Influencing Hepatic Sclerosis Combined with Portal Vein Thrombosis (n=158)*

Indicator	OR	95% CI	p Value
AFP	2.244	1.055–4.872	0.039
P-sel	3.179	1.043–9.702	0.042
MMP-9	2.508	1.059–5.949	0.037

Table IV *Comparison of the Optical Density Values of AFP, P-sel, and MMP-9 in Tissue Samples Between the Groups*

Target	Observation group	Control group
AFP	192.2±23.2*	15.22±7.1
P-sel	114.3±13.2*	11.22±5.3
MMP-9	127.71±20.5*	11.22±5.3

*p<0.05 as compared with those in the control group.

control groups (r=0.524, p=0.005), while a weak correlation was found between the serum levels of P-sel and MMP-9 and that of tissue expression in the 2 groups (r=0.281, p=0.049).

Discussion

PVT refers to thrombus at the main portal vein, extrahepatic portal vein system, and intrahepatic portal vein branch. Hepatic sclerosis can lead to PVT, which can further aggravate hepatic sclerosis, thus resulting in a vicious circle.[12,13] Epidemiological investigation has shown that, among all adult patients with PVT, approximately 25% of them were secondary to hepatic sclerosis and that the morbidity increases with the severity of the patient's liver function.[14] Clinical manifestations of the disease lack specificity, but clinical manifestations differ markedly among different patients. The patients with mild disease may not have obvious clinical symptoms, while those with severe

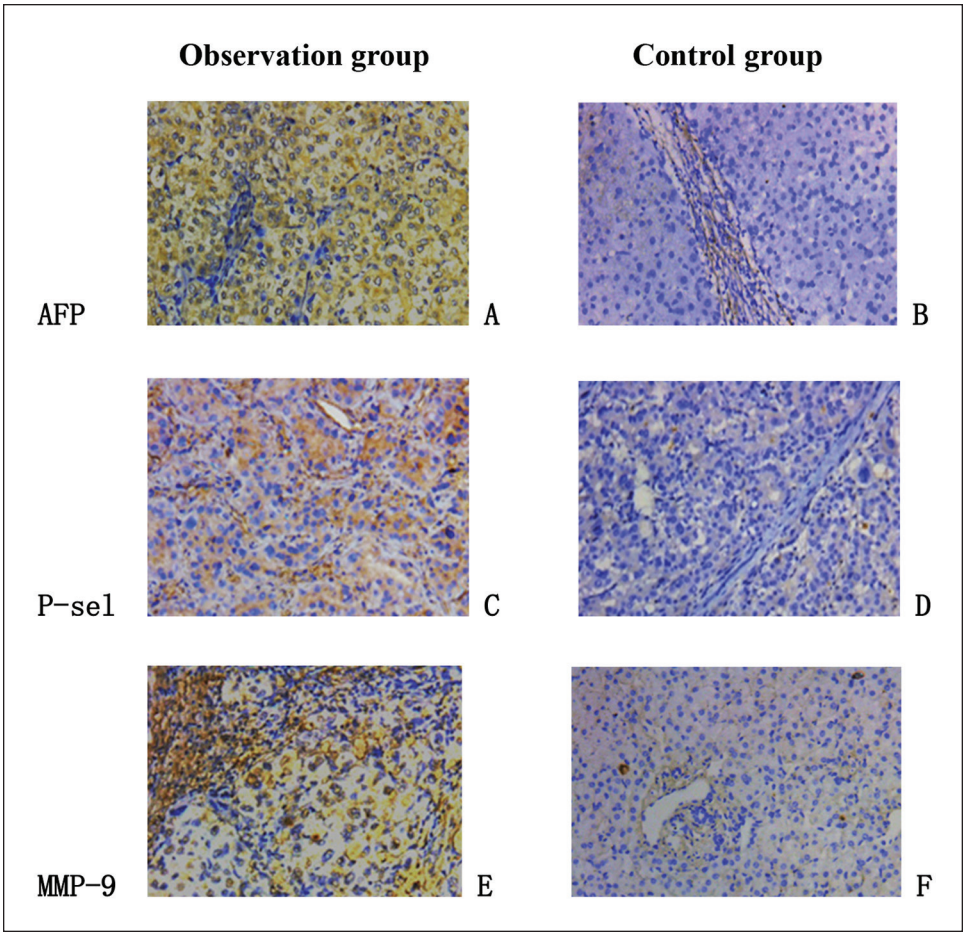

Figure 2
Immunohistochemistry of AFP, P-sel, and MMP-9 in tissues of hepatic sclerosis patients combined with PVT (A, C, and E) and without PVT (B, D, and F) (×200).

disease may face death.[15] Imagological examination is the main auxiliary examination method for PVT, but it largely depends on the operator's experience and technical level as well as the equipment. Additionally, the rate of misdiagnosis and missed diagnosis is high. Histopathological examination can help confirm the diagnosis, but it is an invasive examination and will cause certain trauma for patients.

AFP is a single polypeptide chain molecule in the early stage of embryonic development and belongs to the albumin family. It is mainly synthesized in fetal liver cells and the yolk sac. Its main components include protein and carbohydrate. The main factor causing the rise in the serum AFP level is pregnancy in a normal physiological status. However, when there is irreversible liver damage and an increased risk of live cancer, high amounts of AFP may exist in the patient's serum. Additionally, AFP can be detected in serum a half year before clinical symptoms appear.[16] The current detection of the serum AFP level is easy and convenient, and no traumatic harm will be caused to patients. P-sel is a membrane protein on the surface of platelet alpha particles and is also an important marker of platelet activation. P-sel can mediate the adhesion between activated platelets and a variety of endothelial cells, promote blood into the hypercoagulable state, and regulate the adhesion of neutrophils to endothelial cells.[17] MMP-9 plays an important role in thrombosis, and it plays an important role in the synthesis and degradation of the extracellular matrix.[18] This study showed that the serum AFP content of the observation group was higher than that of the control group, and the difference between the two groups was statistically significant ($p<0.05$). Mechanistically, considering the positive mediating effect on N-ras, c-fos, p53, and other proto-oncogenes, AFP, P-sel, and MMP-9 can induce the reduction of CD4+T lymphocytes and dysfunction, induce T-lymphocyte apoptosis, and make the proportion of CD4+/CD8+ T-lymphocyte subpopulation imbalanced, thus leading to immunoreaction function disorder. Moreover, AFP induces immune cell apoptosis through upregulating apoptosis factor expression, thus influencing hepatocellular Fas/FasL system expression and escaping immunological surveillance by the body.[19]

PVT is a hepatic sclerosis complication that easily can be ignored. With the development of imaging diagnosis technology, the detection rate of hepatic sclerosis combined with PVT is increasing.[20] Patients with early AFP screening may not show obvious symptoms. AFP screening is relatively specific for the diagnosis of liver cirrhosis, but some patients still show negative serum AFP expression in the clinic.[21] This study showed that the optimal critical values for AFP, P-sel, and MMP-9 diagnoses of hepatic sclerosis combined with PVT were 25.22 IU/mL, 292.22 ng/mL, and 372.43 ng/mL, respectively, and the AUC values were 0.779, 0.722, and 0.755, respectively. Current studies also showed that AFP has become an important auxiliary indicator in the diagnosis of liver cancer. Except for early screening and the diagnosis of liver cancer, AFP can also reflect invasion capacity and the differentiation degree of tumors. Additionally, it can be used to predict relapse and prognosis.[22] Some studies have shown that, for patients with suspected PVT, their serum AFP, P-sel, and MMP-9 levels should be dynamically monitored to determine the change and development of disease conditions. At the same time, multiple serum markers can be combined for detection to improve the diagnostic sensitivity and specificity.[23]

The risk factors for PVT are complex and diversified. The combined actions of multiple factors can lead to this disease. In this study, multiclassification logistic regression analysis showed that AFP (OR=2.244), P-sel (OR=3.179), and MMP-9 (OR=2.508) were the main risk factors for PVT ($p<0.05$). The latest research indicates that liver regeneration and tumor cell proliferation can be inhibited by AFP, P-sel, and MMP-9, thus inducing disease deterioration. AFP, P-sel, and MMP-9 can be combined with an anti-tumor effect and exert a tumor suppression effect through the transfer to tumor cells via endocytosis. Vascular endothelial abnormality can easily occur in diabetic patients, and it can activate the coagulation system and promote platelet activation, thus facilitating thrombosis.[24] Splenectomy is an important means to treat portal vein hypertension, but it can lead to vessel endothelium damage of the portal vein, reduction of blood flow velocity, blood platelet rise, and coagulation system activation after the operation, thus inducing PVT.[25] This study also has some defects, and AFP, P-sel, and MMP-9 level changes in the same patient were not observed dynamically. The sample size was small, and other relevant serum markers were not stud-

ied. Thus, further analysis will be carried out in the future.

Conclusion

Serum AFP, P-sel, and MMP-9 present high expression in hepatic sclerosis combined with PVT, with high diagnosis sensitivity and specificity, and they are also the main risk factors leading to PVT.

References

1. Shi SJ, Wang DL, Hu W, Peng F, Kang Q: Ex vivo liver resection and autotransplantation with cardiopulmonary bypass for hepatoblastoma in children: A case report. Pediatr Transplant 2018;7(12):e13268
2. Frakes JM, Abuodeh YA, Naghavi AO, Echevarria MI, Shridhar R, Friedman M, Kim R, El-Haddad G, Kis B, Biebel B, Sweeney J, Choi J, Anaya D, Giuliano AR, Hoffe SE: Viral hepatitis associated hepatocellular carcinoma outcomes with yttrium-90 radioembolization. J Gastrointest Oncol 2018;9(3): 546-552
3. Carr BI, Akkiz H, Guerra V, Üsküdar O, Kuran S, Karaoğullarından Ü, Tokmak S, Ballı T, Ülkü A, Akçam T, Delik A, Arslan B, Doran F, Yalçın K, Altıntaş E, Özakyol A, Yücesoy M, Bahçeci HI˙, Polat KY, Ekinci N, Şimşek H, Örmeci N, Sonsuz A, Demir M, Kılıç M, Uygun A, Demir A, Yilmaz S, Tokat Y: C-reactive protein and hepatocellular carcinoma: Analysis of its relationships to tumor factors. Clin Pract (Lond) 2018;15(Spec Issue):625-634
4. Ruiqi S, Donghua S, Meilin H, Guowei L: HNF1α rs7310409 polymorphism is associated with serum α-fetoprotein level in Chinese healthy adults. Chinese J Lab Diagn 2018;22(3): 397-401
5. Goto Y, Uchino Y, Sasaki S, Shirahama N, Nomura Y, Akiba J, Ishikawa H, Akagi Y, Tanaka H, Okuda K: Complete spontaneous necrosis of hepatocellular carcinoma accompanied by portal vein tumor thrombosis: A case report. Int J Surg Case Rep 2018;10(44):220-225
6. Chen ZW, Lin ZY, Chen YP, Chen J, Chen J: Clinical efficacy of endovascular radiofrequency ablation in the treatment of portal vein tumor thrombus of primary hepatocellular carcinoma. J Cancer Res Ther 2018;14(1):145-149
7. Dongwu S, Jinhua P, Xiaoli Q, Songlin W: Influence of TACE combined with radioactive particle interstitial radiotherapy on malignant biological indexes in patients with primary hepatocellular carcinoma. Pract J Cancer 2018;33(3): 416-418
8. Abouchaleh N, Gabr A, Ali R, Al Asadi A, Mora RA, Kallini JR, Mouli S, Riaz A, Lewandowski RJ, Salem R: ^{90}Y Radioembolization for locally advanced hepatocellular carcinoma with portal vein thrombosis: Long-term outcomes in a 185-patient cohort. J Nucl Med 2018;59(7):1042-1048
9. Guifang J, Baojiang L, Qingfeng X, Junping P: The value of combined detection of tumor markers in the diagnosis of primary liver cancer. Chinese J Primary Med Pharmacy 2018;25(12):1600-1602
10. Muñoz D, Serrano MK, Hernandez ME, Haller R, Swanson T, Slaton JW, Sinha AA, Wilson MJ: Matrix metalloproteinase and heparin-stimulated serine proteinase activities in post-prostate massage urine of men with prostate cancer. Exp Mol Pathol 2017;103(3):300-305
11. Douketis JD, Murphy SA, Antman EM, Grip LT, Mercuri MF, Ruff CT, Weitz JI, Braunwald E, Giugliano RP: Peri-operative adverse outcomes in patients with atrial fibrillation taking warfarin or edoxaban: Analysis of the ENGAGE AF-TIMI 48 trial. Thromb Haemost 2018;118(6):1001-1008
12. Qifeng S, Zhiqiang Z, Tong Z, Qiwen Z: Serum tumor marker changes after laparoscopic partial hepatectomy in patients with primary liver cancer. J Pract Hepatol 2018;21(1):121-122
13. Shen J, Wang LF, Zou ZY, Kong WW, Yan J, Meng FY, Chen FJ, Du J, Shao J, Xu QP, Ren HZ, Li RT, Wei J, Qian XP, Liu BR: Phase I clinical study of personalized peptide vaccination combined with radiotherapy for advanced hepatocellular carcinoma. World J Gastroenterol 2017;23(29):5395-5404
14. Biederman DM, Posham R, Durrani RJ, Titano JJ, Patel RS, Tabori NE, Nowakowski FS, Fischman AM, Lookstein RA, Kim E: Outcomes of radioembolization for unresectable hepatocellular carcinoma in patients with marginal functional hepatic reserve. Clin Imaging 2018;1(47):34-40
15. Xueke F, Liangjun S, Lin G: Comparative analysis of short-term and long-term results of laparoscopic guided radiofrequency ablation and laparoscopic liver resection for hepatocellular carcinoma. J Clin Exp Med 2018;17(14):1540-1544
16. Zeng Y, Ye X, Liao D, Huang S, Mao H, Zhao D, Zeng H: Orphan nuclear receptor TR3/Nur77 is a specific therapeutic target for hepatic cancers. J Clin Exp Oncol 2017;6(3):184
17. Bolognesi M, Sacerdoti D, Bombonato G, Chiesura-Corona M, Merkel C, Gatta A: Arterioportal fistulas in patients with liver cirrhosis: Usefulness of color Doppler US for screening. Radiology 2000;216(3):738-743
18. Primignani M, Tosetti G, La Mura V: Therapeutic and clinical aspects of portal vein thrombosis in patients with cirrhosis. World J Hepatol 2015;7(29):2906-2912
19. Kim TS, Kim JH, Kim BH, Lee YS, Yoo YJ, Kang SH, Suh SJ, Jung YK, Seo YS, Yim HJ, Yeon JE, Byun KS: Complete response of advanced hepatocellular carcinoma to sorafenib: Another case and a comprehensive review. Clin Mol Hepatol 2017;23(4):340-346
20. Zhihong S, Xiao Z, Zhifen B: Association of Helicobacter pylori infection with clinical stage, cell differentiation and pathological types of liver cancer. J Pract Oncol 2018;33(2): 162-164
21. Choi HJ, Kim DG, Na GH, Hong TH, Bae SH, You YK, Choi JY, Yoon SK: The clinical outcomes of patients with portal vein tumor thrombi after living donor liver transplantation. Liver Transpl 2017;23(8):1023-1031
22. Xian H, Yuanfu Q, Jianhai Z, Jiayang C, Lizhen L, Xingrong Z: The correlation analysis of serological indexes alpha fetal protein, total bilirubin, alanine aminotransferase and prothrombin time activity with the process of liver fibrosis. J Pract Med Techn 2018;25(2):125-127
23. Kuo YH, Wu IP, Wang JH, Hung CH, Rau KM, Chen CH, Kee KM, Hu TH, Lu SN: The outcome of sorafenib monotherapy on hepatocellular carcinoma with portal vein

tumor thrombosis. Invest New Drugs 2018;36(2):307-314

24. Jianping L, Yong X, Yaogang F, Deqin L, Junzhou W, Yigang W, Su L, Changqin S, Jianping Z: Effects of microwave ablation combined with transcatheter arterial chemoembolization on immune function, AFP level and survival time in patients with hepatocellular carcinoma. Chinese J Gerontology 2018;38(3):554-556
25. Abdelmaksoud AH, Mandooh S, Nabeel MM, Elbaz TM, Shousha HI, Monier A, Elattar IA, Abdelaziz AO: Portal vein thrombosis in unresectable HCC cases: A single center study of prognostic factors and management in 140 patients. Asian Pac J Cancer Prev 2017;18(1):183-188