ORIGINAL RESEARCH

Analyzing the Role of Septin9 Gene Methylation in the Diagnosis and Treatment of Primary Liver Cancer in the Elderly

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ABSTRACT

Context • Because the early symptoms of primary hepatocellular carcinoma (PHC) aren't significant, it's difficult to diagnose it by routine inspection clinically, and if the lesion's diameter is small, less than 2.0 cm, false negatives can occur in pathological examinations. Researchers need to actively search for more diagnostic methods.

Objective • The study intended to detect and analyze the value of plasma Septin9 gene methylation for the diagnosis and therapeutic monitoring of PHC in older adults.

Design • The research team performed a prospective controlled study.

Setting • The study took place at the First Hospital of Qiqihar, an Affiliated Qiqihar Hospital at Southern Medical University in Qiqihar, China.

Participants • Participants were 32 patients with PHC and 28 with cholangiocarcinoma (CCA) who had been admitted to the hospital between January 2021 and July 2022 and 40 healthy individuals.

Groups • The research team divided participants into three groups: (1) patients with PHC, the PHC group; (2) patients with CCA, the CCA group; and (3) healthy individuals, the control group.

Outcome Measures • The research team: (1) determined the positive expression rate of Septin9 gene methylation; (2) measured liver function indicators—alanine aminotransferase (ALT), aspartate aminotransferase (AST), serum total bilirubin (TBIL), direct bilirubin (DBIL), alkaline phosphatase (ALP), γ-glutamyl transpeptidase (GGT), albumin (ALB); and (3) measured tumor markers—alpha-fetoprotein (AFP), carbohydrate antigen (CA) 199, CA125, and CA153. The team also: (1) established a binary logistic regression model based on levels of GGT and plasma Septin9 gene methylation to analyze risk factors and diagnosis accuracy, (2) created a receiver operating characteristic (ROC) curve to analyze diagnostic values; and (3) during followup, analyzed the negative conversion rate of Septin9 gene methylation in participants.

Results • The positive expression rate of Septin9 methylation in the PHC group was significantly lower than that that of the CCA group and significantly higher than that of the control group ($P < .05$). The PHC group's ALT, AST, TBIL, DBIL, ALP, and GGT were significantly higher than those of the control group but significantly lower than those of the CCA group (all $P < .05$). PHC group's ALB was significantly lower than that of the control group ($P < .05$). The PHC group's AFP, CA199, and CA125 were significantly higher than those of the control group, and the PHC group's CA199 and CA125 were significantly lower than those in the CCA group (all $P < .05$). The positive expression of Septin9 gene methylation and the high expression of GGT were risk factors for PHC (OR>1, $P < .05$). The AUC of the Septin9 gene methylation, the GGT level, and the combined detection of both variables (all AUC > 0.70), suggests that the variables have a diagnostic value in the detection of PHC, with the combined detection having the highest value. The negative conversion rate after surgery of Septin9 gene methylation was 87.10%, for 27 out of 31 participants in the PHC and CCA groups ($\chi^2 = 29.405$, $P < .001$).

Conclusion • Plasma Septin9 gene methylation is a sensitive molecular marker for the diagnosis and therapeutic monitoring of older adults with PHC, and combined with the serum GGT level, has a high diagnostic efficiency, which may reflect the treatment status of patients. (*Altern Ther Health Med.* 2023;29(4):194-199).

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Primary hepatocellular carcinoma (PHC) is a common malignant tumor occurring in hepatocytes or in intrahepatic bile duct cells. PHC is the second highest cause of cancer death in China, with a poor prognosis.[1] About 350 000 patients die of PHC every year in China, accounting for more than 50% of the fatal PHC worldwide.[2]

Because early symptoms aren't significant, it's difficult to diagnose PHC by routine inspection clinically. The pathological types of PHC include primary hepatocellular carcinoma, cholangiocarcinoma (CCA), and cancers with mixed types of liver-cancer cells, with different treatments and prognosis methods for each.

PHC Diagnosis

At present, clinicians mainly use imaging and pathological examinations for diagnosing PHC, such as a computed axial tomography (CAT) scans. The contrast medium flow in CAT scans can show the blood flow in liver tissue by enhanced scanning.

Hepatic-artery blood supplies PHC, so CAT scans can effectively increase the efficiency of the PHC diagnosis. However, CAT scans have a certain radiation effect, causing a small amount of blood-cell death and DNA rupture, which can damage the patient's body in subsequent treatments.[11]

The CAT scan is also weak in identifying the boundaries between liver lesions and adjacent tissues, leading to misdiagnoses.[3]

Imaging findings are similar for the three types, which make it easy for physicians to misdiagnose or miss the diagnosis of PHC and affect patients' prognoses.[4,5] Therefore, in addition to an examination using conventional imaging, it's necessary to coordinate those findings with those of other blood tests to further improve the diagnostic efficacy in PHC.

A pathological examination through puncture biopsy is the gold standard for PHC diagnosis. Puncture biopsy is an invasive treatment, and physicians can directly observe cancer cells after pathological examination. However, if the lesion's diameter is small, less than 2.0 cm, the physician may not be able to puncture the lesion tissue, resulting in false negatives, delaying patients' treatment and allowing development of the disease.[6,12] Therefore, improving the effectiveness of PHC diagnosis by laboratory examination is of great significance, and researchers need to actively search for more diagnostic methods.

Septin9 and γ-glutamyl transpeptidase (GGT)

Septin9 gene. Wang et al found that DNA methylation is closely related to the occurrence of tumors.[7] Chandrapalan et al found that the Septin family is related to the occurrence of diseases such as nervous-system diseases, tumors, and infections.[13] Septins are a conserved gene family, with guanosine triphosphatase (GTPase) activity. Researchers have found 14 Septin genes.

Wen et al found that the Septin9 gene belongs to the Septin family and can participate in biological processes, such as cell division and cytoskeleton formation.[5] Hitchins et al found that use of Septin9 gene methylation could achieve good results in the diagnosis of colorectal cancer.[14]

Lin et al found that necrotic or apoptotic cancer cells can release the methylated Septin9 gene into peripheral-blood circulation.[8] Yuan et al found that microRNA may interfere with the Septin9 gene, leading to incomplete division and the formation of binucleated cells and increasing the risk of carcinogenesis.[15]

Moreover, gene methylation can inhibit the normal expression of the Septin9 gene, resulting in a loss of the antitumor effect and the overexpression of hepatocytes, which can induce PHC.[16] Meanwhile, methylation in the "central bird nucleotide sequence domain" of the Septin9 gene can change the normal physical and chemical properties of cells, leading to abnormal regulation of cell division, and thereby, promoting carcinogenesis of hepatocytes and the risk of PHC.[17]

GGT. GGT is a plasma-membrane-bound glycoprotein in various tissues, mainly in hepatocytes and in the epithelial cells of intrahepatic bile ducts, and it's a common indicator of liver disease.[18] GGT is also the key to maintaining the intracellularly reduced glutathione level and to preventing cells from damage by oxidation and free radicals. An increased GGT level indicates that the oxidative imprinting reaction of the body is very serious and can cause damage to hepatocytes and increase the risk of carcinogenesis.[19,20] At the same time, cancer cells can also secrete GGT, suggesting that it may have an important role in the diagnosis of tumors.[21]

Current Study

The research team speculates that clinicians could diagnose PHC by detecting expression levels of Septin9 gene methylation. The current study intended to detect and analyze the value of plasma Septin9 gene methylation for the diagnosis and therapeutic monitoring of PHC in older adults.

METHODS

Participants

The research team performed a prospective controlled study. The study took place at the First Hospital of Qiqihar, an Affiliated Qiqihar Hospital at Southern Medical University in Qiqihar, China. Potential participants were patients with PHC or CCA who had been admitted to the hospital between January 2021 and July 2022 and healthy older adults.

The study included potential participants if they: (1) had undergone examination for Septin9 gene methylation, (2) hadn't received any relevant treatment before the examination; (3) had been diagnosed by liver puncture and pathological examination if they had PHC or CCA; and (4) had complete clinical data available.

The excluded potential participants if they: (1) had immune system diseases; (2) had liver cancer that metastasis of other malignant tumors to the liver had caused; (3) had colorectal diseases; (4) had poor control of underlying diseases; or (5) had PHC or CCA complicated with acute or chronic infection.

Patients and their families signed informed consent forms. The Medical Ethics Committee of the hospital approved the study's protocols.

Procedures

Detection for methylation. The research team: (1) collected 10 ml of participants' fasting, peripheral-elbow, venous blood upon admission and divided it into two test tubes; (2) centrifuged the whole blood for 12 min with a centrifugal force of 1350 ± 150 relative centrifugal force; (3) after obtaining the plasma, centrifuged it again for 12 min with the same centrifugal force as used previously; (4) transferred the plasma to the conical bottom centrifuge tube (Shanghai Dahua Medical Device, approval number: 20153100282, Shanghai, China); (5) extracted the free DNA from the plasma; (6) obtained sulfite transformed DNA (Bis-DNA) after successively lysing, DNA binding, nitrite transformation, and other steps; (7) using real-time polymerase chain reaction (RT-PCR) StepOne from Life Technologies Holdings, approval number: 20173220816), detected Septin9 gene methylation with a detection kit from Bolcheng Beijing Technology, approval number: 20153401481, Beijing, China); (8) tested the Bis-DNA from the effectiveness of PCR reaction with control samples. If the positive control was ≤32.1 and the Septin9 cycle number was ≤41.0, the research team considered the result of the Septin9 gene methylation detection to be positive.

Outcome measures. The research team: (1) determined the positive expression rate of Septin9 gene methylation, (2) measured liver function indicators, and (3) measured tumor markers. The team also: (1) established a binary logistic regression model based on levels of GGT and plasma Septin9 gene methylation to analyze risk factors and diagnosis accuracy, (2) created a receiver operating characteristic (ROC) curve to analyze diagnostic values; and (3) during followup, analyzed the negative conversion rate of Septin9 gene methylation in participants.

Outcome Measures

Liver function. The research team detected: (1) the levels of serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) using the enzymatic method and (2) the levels of serum total bilirubin (TBIL), direct bilirubin (DBIL), alkaline phosphatase (ALP), γ-glutamyl transpeptidase (GGT) and albumin (ALB) using the colorimetric method.[9]

Tumor markers. The research team detected participants' levels of serum alpha fetoprotein (AFP) and carbohydrate antigen (CA) 199, CA125, and CA153 using the enzymatic and colorimetric methods. Siemens provided the kit, and the research team followed the steps in strict accordance with the manufacturer's instructions.

GGT and Septin9 gene methylation. The research team: (1) set the occurrence of the disease as the dependent variable: 0 = control group, 1 = PHC group and (2) used the levels of serum GGT and plasma Septin9 gene methylation as the independent variables. The variables for the Septin9 gene methylation were categorical variables and for the GGT were continuous variables, with 1 = positive and 0 = negative for both.

Statistic Analysis

The research team employed the SPSS 25.0 software (company, city, state, country) for statistical analysis. The team analyzed: (1) measurement data, statistically expressing the data as means and standard deviations (SDs) (2) count data, recorded as numbers and percentages; (3) comparisons between groups used univariate analysis; (4) comparisons between baseline and postintervention within groups using the least significant difference (LSD) test; (5) the relationship between Septin9 gene methylation and older adults with PHC using binary logistic regression with the χ^2 test; and (6) drew the receiver operating characteristic (ROC) curve and calculated the area under the curve (AUC) value to test the diagnostic value of Septin9 gene methylation expression in older adults with PHC. $P < .05$ was considered to be statistically significant.

RESULTS

Participants

The research team included and analyzed the data of 100 participants, 32 in the PHC group, 28 in the CCA group, and 40 in the control group. Table 1 shows that no significant differences existed between the groups at baseline ($P > .05$).

Positive Expression Rate

As Table 2 shows, the positive expression rate of Septin9 gene methylation in the PHC group was significantly lower than that of the CCA group ($P < .001$) and significantly higher than that of the control group ($P < .001$).

Liver Function

As Figure 1 shows, the PHC group's ALT, AST, TBIL, DBIL, ALP, and GGT were significantly higher than those in the control group and significantly lower than those in the CCA group (all $P < .05$). The PHC group's ALB was significantly lower than that of the control group and significantly higher than that of the CCA group (both $P < .05$).

Tumor Markers

As Figure 2 shows, the PHC group's AFP, CA199, and CA125 were significantly higher than those of the control group, and the PHC group's CA199 and CA125 were significantly lower than those of the CCA group ($P < .05$).

GGT and Septin9 Gene Methylation

Table 3 shows that a positive expression of Septin9 gene methylation and a high expression of GGT were risk factors for PHC (OR > 1, $P < .05$).

Diagnostic Value

Figure 3 shows the ROC curve. In the diagnosis of PHC, the AUC of the Septin9 gene methylation expression, the GGT

Table 1. Participants' Demographic and Clinical Characteristics by Group (N = 100)

Variables		PHC Group (n = 32) n (%) Mean ± SD	CCA Group (n = 28) n (%) Mean ± SD	Control Group (n = 40) n (%) Mean ± SD	χ^2/t	*P* value
Gender	Male	18 (56.25)	13 (46.43)	22 (55.00)	0.685	.710
	Female	14 (43.75)	15 (53.57)	18 (45.00)		
Age, y		68.27 ± 2.67	67.95 ± 3.52	68.49 ± 4.25	0.185	.831
BMI	≥24mg/m^2	13 (40.63)	11 (39.29)	20 (50.00)	0.985	.611
	<24mg/m^2	19 (59.38)	17 (60.71)	20 (50.00)		
Complicated With Hypertension	Yes	11 (34.38)	8 (28.57)	6 (15.00)	3.824	.148
	No	21 (65.63)	20 (71.43)	34 (85.00)		
Complicated With Diabetes	Yes	4 (12.50)	3 (10.71)	4 (10.00)	0.117	.943
	No	28 (87.50)	25 (89.29)	36 (90.00)		
Complicated With Coronary Heart Disease	Yes	3 (9.38)	2 (7.14)	2 (5.00)	0.524	.770
	No	29 (90.63)	26 (92.86)	38 (95.00)		
Smoking	Yes	14 (43.75)	15 (53.57)	17 (42.50)	0.909	.635
	No	18 (56.25)	13 (46.43)	23 (57.50)		
Drinking	Yes	21 (65.63)	16 (57.14)	19 (47.50)	2.391	.303
	No	11 (34.38)	12 (42.86)	21 (52.50)		

Abbreviations: BMI, body mass index; CCA, cholangiocarcinoma; PHC, primary hepatocellular carcinoma.

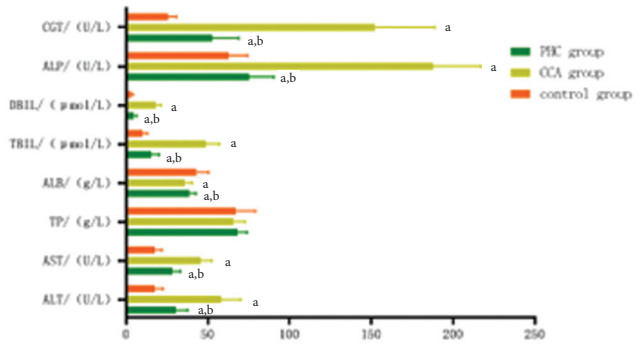
Table 2. Comparison of Positive Expression Rate Among the PHC, CCA, and Control Groups (N = 100)

Expression Rate	PHC Group (n = 32) n (%)	CCA Group (n = 28) n (%)	Control Group (n = 40) n (%)	PHC Group Vs CCA Group		PHC Group Vs Control Group		CCA Group Vs Control Group	
				χ^2	*P* value	χ^2	*P* value	χ^2	*P* value
Positive	21 (65.63)	11 (39.29)	1 (2.50)	4.163	.041[a]	33.385	<.001[b]	15.336	<.001
Negative	11 (34.38)	17 (60.71)	39 (97.50)						

[a]*P* < .05, indicating that the PHC group's positive expression rate was significantly lower than that of the CCA group
[b]*P* < 0.05, indicating that the CCA group's positive expression rate was significantly higher than that of the control group

Abbreviations: CCA, cholangiocarcinoma; PHC, primary hepatocellular carcinoma.

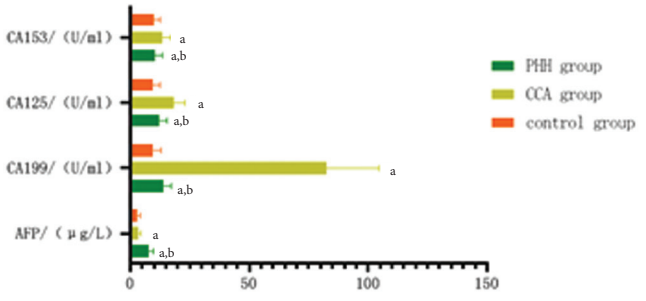
Figure 1. Comparison of liver function in three groups



[a]*P* < .05, indicating that the PHC, CCA group's positive expression rate was significantly higher than that of the control group
[b]*P* < .05, indicating that the PHC group's positive expression rate was significantly lower than that of the CCA group

Abbreviations: CCA, cholangiocarcinoma; PHC, primary hepatocellular carcinoma.

Figure 2. Comparison of three groups of tumor markers



[a]*P* < .05, indicating that the PHC, CCA group's positive expression rate was significantly higher than that of the control group
[b]*P* < .05, indicating that the PHC group's positive expression rate was significantly lower than that of the CCA group

Abbreviations: CCA, cholangiocarcinoma; PHC, primary hepatocellular carcinoma

Table 3. Binary Logistical Regression Analysis of GGT and Septin9 Gene Methylation (N = 100)

Variables	β	SEM	Wald χ^2	*P* value	OR	95% CI
Septin9 gene methylation	4.801	1.303	13.584	<.001[a]	121.603	9.467-1562.072
GGT	0.150	0.038	15.974	<.001[a]	1.080	1.251
Constant	-4.961	1.190	17.391	<.001[a]	-	-

[a]*P* < .001, indicating that positive expression of Septin9 gene methylation and a high expression of GGT were risk factors for PHC

Abbreviations: GGT, γ-glutamyl transpeptidase

Table 4. The Diagnostic Value of GGT and Septin9 Gene Methylation in PHC (N = 100)

Variables	AUC	Cut-off	95% CI	*P* value	Specificity	Sensibility	Jordan Index
Septin9 gene methylation	0.746	-	0.651-0.841	<.001[a]	0.975	0.517	0.492
GGT	0.800	28.065U/L	0.709-0.890	<.001[a]	0.725	0.783	0.508
Combined detection	0.910		0.846-0.973	<.001[a]	0.925	0.850	0.775

[a]*P* < .001, indicating that the Septin9 gene methylation expression, the GGT level, and the combined detection of both variables have a diagnostic value in the detection therapeutic monitoring of PHC

Abbreviations: GGT, γ-glutamyl transpeptidase; PHC, primary hepatocellular carcinoma

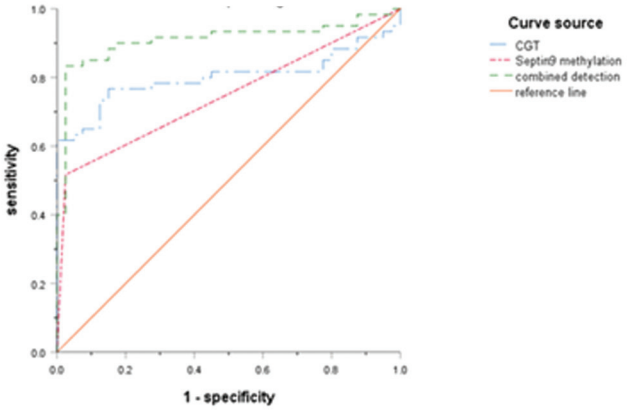
Table 5. Negative Conversion Rate of Septin9 Gene Methylation in PHC and CCA Participants After Surgery (N = 31)

Groups	Positive Numbers at Baseline n	Positive to Negative Changed n (%)	Remained Positive Changed n (%)	χ^2/t	*P* value
HCC Group, n = 32	20	18 (90.00)	2 (10.00)		
CCA Group, n = 28	11	9 (81.82)	2 (18.18)		
Total, n = 60	31	27 (87.10)	4 (12.90)	χ^2 = 29.405	<.001[a]

[a]*P* < .001, indicating that the conversion from positive Septin9 gene methylation at baseline to negative Septin9 gene methylation was significant after surgery

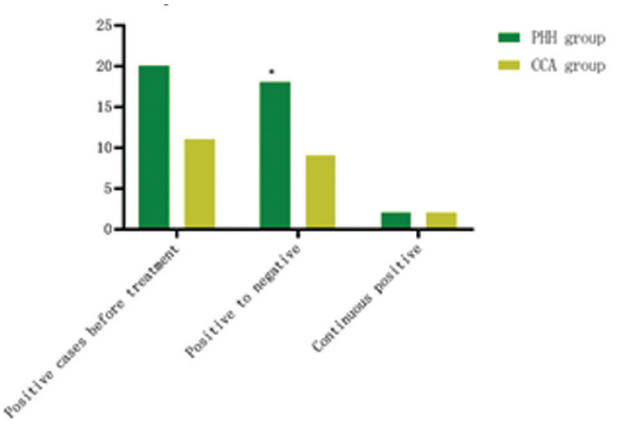
Abbreviations: CCA, cholangiocarcinoma; PHC, primary hepatocellular carcinoma.

Figure 3. Receiver operating characteristic curve



Abbreviations: AUC, area under the curve; GGT, γ-glutamyl transpeptidase; PHC, primary hepatocellular carcinoma.

Figure 4. The negative conversion rate of Septin9 gene methylation in HCC patients after treatment



level, and the combined detection of both variables were 0.746, 0.800, and 0.910, respectively (Table 4). This suggests that the variables have a diagnostic value in the detection of PHC, with the combined detection having the highest value for diagnosis and therapeutic monitoring of PHC in older adults.

Negative Conversion Rate

Table 5 and Figure 4 show that 18 out of 20 PHC participants (90.00%) and 9 out of 11 CCA participants (81.82%) who had positive Septin9 gene methylation at baseline—27 of 31 participants (87.10%)—had turned negative after surgery ($\chi^2 = 29.405$, $P < .001$).

DISCUSSION

In the current study, the positive expression rate of Septin9 gene methylation and GGT level in the PHC and CCA groups were significantly higher than those in the control group. Binary logistic regression analysis showed that the positive expression of Septin9 gene methylation and high expression of GGT were risk factors for patients with PHC. The results indicated that Septin9 gene methylation and GGT level were closely related to the development of PHC.

In the current study, the ROC curve further showed that the expression of Septin9 gene methylation, the GGT level and combined detection of the two variables (all AUCs > 0.70) had a detection value. Septin9 gene methylation and GGT can effectively diagnose PHC in older adults. After surgery, the number of patients with positive expression of Septin9 gene methylation was lower than that at baseline.

Septin9 gene methylation expression is also of certain value in evaluating the treatment of older adults with PHC. Therefore, physicians can further improve the clinical efficacy of PHC diagnosis by detecting Septin9 gene methylation expression in older adults with highly suspected PHC and can evaluated clinical efficacy using Septin9 gene methylation expression after treatment.

The current study some shortcomings. It was a single-center study, and the patients were all local patients, and they may be different from patients in other regions. The second study included fewer patients.

CONCLUSIONS

Plasma Septin9 gene methylation is a sensitive molecular marker for the diagnosis and therapeutic monitoring of older adults with PHC, and combined with the serum GGT level, has a high diagnostic efficiency, which may reflect the treatment status of patients.

AUTHORS' DISCLOSURE STATEMENT

The Septin9-gene methylation expression and treatment monitoring research project of the Heilongjiang Provincial Health Commission (research project No. 2020-423) supported the study. The authors have no potential conflicts of interest to report relevant to this study.

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