Hepatitis Monthly 2009; 9(2): 114-121

ORIGINAL ARTICLE

Molecular Detection of Human Telomerase mRNA (hTERT-mRNA) in Egyptian Patients with Hepatocellular Carcinoma

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Background and Aims: Diagnostic modalities for hepatocellular carcinoma (HCC) as markers, sonography, and CT have contributed to the early detection of HCC but are still not sensitive enough. Human telomerase RNA subunit (hTERT-mRNA) has been identified in many cancers and claimed to be reactivated in HCC. To investigate hTERT-mRNA in the peripheral blood of HCC and chronic liver disease (CLD) patients and correlate its level with alpha feto protein (AFP), the serological marker for HCC.

Methods: The study was conducted on 44 patients selected from the National Liver Institute. Patients included Group I (22 patients diagnosed to have HCC), Group II (22 patients with CLD), and 12 apparently healthy volunteers as controls (Group III). All selected individuals were subjected to history taking, a clinical examination, abdominal sonography and laboratory investigations as liver function tests (LFTs), cell blood count (CBC), hepatitis viral markers, AFP, and real-time polymerase chain reaction (PCR) Quantitative detection of -mRNA expression, encoding for telomerase catalytic subunit.

Results: There was a significant elevation of AFP levels in the HCC group compared to both the CLD and control groups ($P < 0.00$, $P < 0.001$). The mean hTERT-mRNA expression in HCC patients was significantly higher than both CLD patients and controls ($P < 0.001$, $P < 0.001$). hTERT-mRNA was correlated with AFP and tumor size ($P < 0.05$, $P < 0.001$). The AFP cutoff level (185 ng/ml) resulted in a 63.6% sensitivity, a 85.3% specificity; a 89.3% positive predictive value (PPV) level, a 76.2 % negative predictive value (NPV) level and a 83.4% accuracy for HCC prediction. The hTERT-mRNA cutoff level (112.5 copies/ml) showed a 77.3% sensitivity, a 97.1% specificity, a 98% PPV level, a 79.2 % NPV level, and an accuracy of 84% for HCC prediction. Combining hTERT-mRNA and AFP increased diagnostic accuracy to 90.5%. Both markers had a 84.1% sensitivity, a 86.4% specificity, a 86.4% PPV level, and a 88.3 % NPV level.

Conclusions: hTERT-mRNA is thought to be superior to AFP in CLD patients and could be a marker for the early diagnosis of HCC. Combined hTERT-mRNA and AFP would augment early detection and successful follow-up of HCC patients.

Keywords: Hepatocellular Carcinoma, Telomerase, Chronic Liver Disease

Introduction

Hepatocellular carcinoma (HCC) is one of the most common malignancies worldwide. It is a prevalent cancer that often develops in patients with chronic hepatitis and cirrhosis in association with hepatitis B or C virus infection [(1)]. Although HCC is the fifth-most common cancer worldwide, the molecular pathogenesis of the disease has not been elucidated. Several studies have shown that telomerase activity and hTERT expression are

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Received: 22 Feb 2009 **Revised:** 24 Apr 2009
Accepted: 30 Apr 2009

Hepat Mon 2009; 9 (2): 114-121

increased in HCCs [2]. Recurrence of HCC after resection remains common and is considered the main cause of death after surgical resection. Diagnostic modalities such as tumor markers, ultrasonography, and CT have contributed to early diagnosis of HCC but are still not sensitive enough [3].

Numerous other genes and proteins that are expressed in patients with HCC might prove useful as clinical markers but are not well studied to date. These include alpha-1-fucosidase, alpha feto protein (AFP) mRNA, gamma glutamyl transferase (GGT) mRNA, human telomerase reverse transcriptase mRNA, vascular endothelial growth factor, tumor-specific growth factor, and others [4]. Telomeres are specialized structures at the ends of eukaryotic chromosomes and function in chromosome stabilization, positioning, and replication. Telomeric DNA (telomeric repeats GGTTAG) is shortened every time somatic cells divide, and when a few telomeric repeats remain, the cells stop dividing, leading to cell death due to chromosomal instability [5].

Telomerase is a ribonucleoprotein enzyme responsible in most eukaryotes for the complete replication of the telomeres at the chromosome ends [6]. Its RNA subunit provides the template for the addition of the hexamer repeat (GGTTAG) to chromosome end. Human telomerase mRNA (hTERT-mRNA) has been identified in many cancers and is suspected to reactivate in HCC [7]. Telomerase is activated in about 80% of HCC cases. It is likely that increasing the activity of the telomerase promoter with a suicide gene will effectively eradicate the HCC because an increase in telomerase promoter activity may target hepatocellular carcinoma [8]. The aim was to investigate human telomerase mRNA (hTERT-mRNA) in the peripheral blood of HCC and chronic liver disease (CLD) patients. Also, the study attempted to detect hTERT-mRNA's possible role as a marker in HCC cases. Specifically, the study aimed to correlate the level of hTERT-mRNA with AFP, the traditional serological marker for HCC.

Patients and Methods

The study was conducted on 44 patients selected from Internal Medicine Department inpatients at National Liver Institute clinics and hospitals. Patients included 22 patients diagnosed as having HCC (Group I), and 22 patients with CLD (Group II). In addition, the study included 12 apparently healthy volunteers (Group III). All selected individuals were subjected to the following: a personal medical history, a clinical examination, an abdominal ultrasonography, routine laboratory investigations for CLD and HCC as liver-function tests, and a CBC using synchron CX5 and Pentra 80 instruments. Also, hepatitis viral markers were analyzed using enzyme immunoassay (EIA) Kits, and a serum AFP screening was conducted with an Axsym instrument. A specific laboratory investigation, a quantitative detection of mRNA expression encoding for the telomerase catalytic subunit hTERT was evaluated with a real-time polymerase chain reaction (PCR) measurement using the LightCycler® 2.0 Real-Time PCR System (Roche Diagnostics Germany) (Fig. 1).

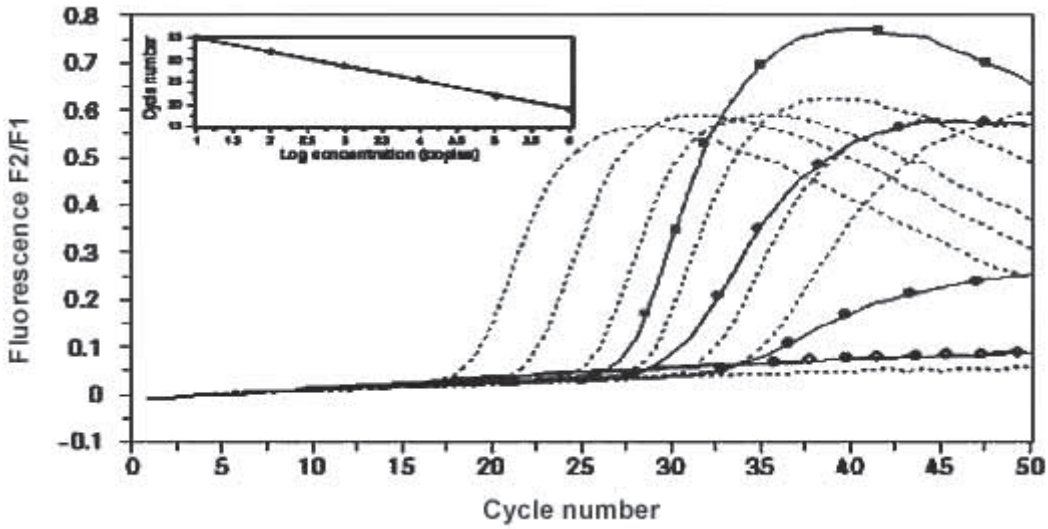

Figure 1. Sample curve and standard curve for a typical test.

Results

There was a significant elevation in AFP level in the HCC group compared to the CLD and control groups (P < 0.001 and P < 0.001, respectively). The mean level of hTERT-mRNA expression in the HCC group was significantly higher than the levels in both the CLD and control groups (P < 0.001 and P < 0.001, respectively). hTERT-mRNA expression was significantly correlated with AFP and tumor size (P < 0.05 and P < 0.001, respectively).

An AFP cutoff level of 185 ng/ml revealed that there was sensitivity of 63.6%, a specificity of 85.3%, a positive predictive value (PPV) level of 89.3%, an positive predictive value (NPV) level of 76.2 %, and a diagnostic accuracy of 83.4% for HCC prediction. An hTERT-mRNA expression cutoff level of 112.5 copies/ml predicted an HCC sensitivity of 77.3%, a specificity of 97.1%, a PPV level of 98%, an NPV level of 79.2 %, and a diagnosis accuracy of 84% for HCC prediction. Combining the hTERT-mRNA expression and AFP predictions of HCC cases increased diagnostic accuracy to 90.5%. In addition, for both markers sensitivity was 84.1%, specificity was 86.4%, PPV was 86.4%, and NPV was 88.3% (Figs 2 & 3).

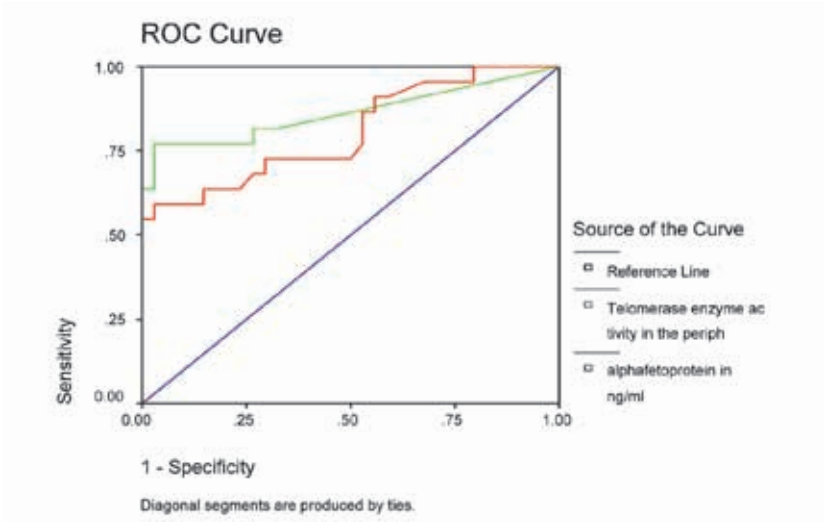

Figure 2. ROC curve for both AFP and hTERT-mRNA for HCC prediction.

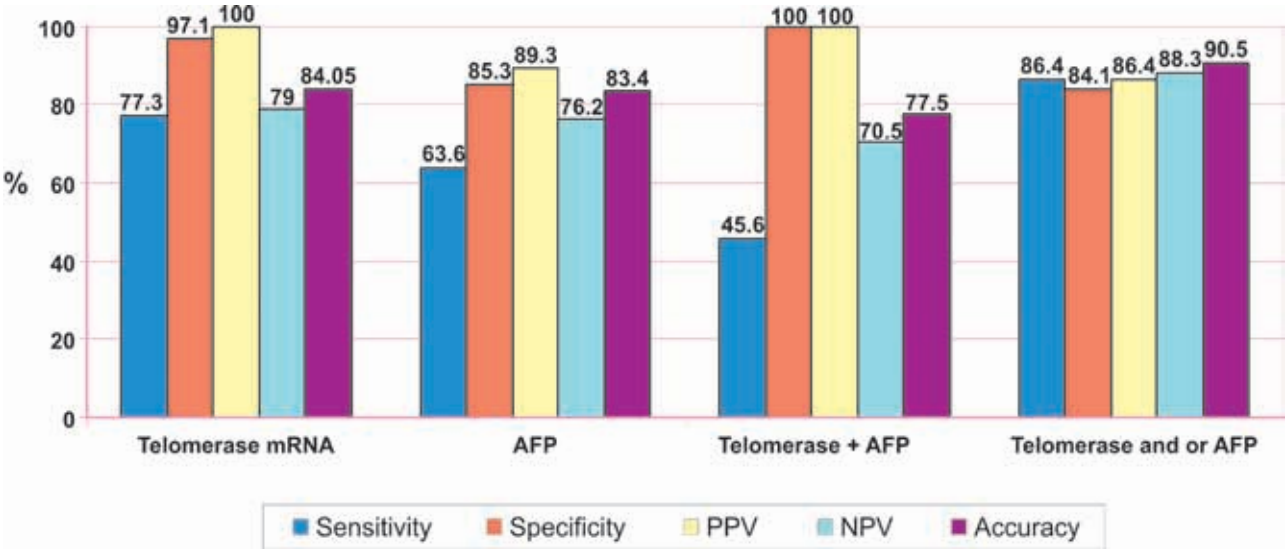

Figure 3. Sensitivity, specificity, PPV, NPV, and accuracy of hTERT-mRNA and/or AFP for HCC prediction.

Statistical analysis

Data were analyzed using SPSS (Statistical Package for the Social Sciences) version 11.0. Student's *t*-tests were conducted to test for normally distributed quantitative variables to measure means and standard deviations; $P < 0.05$ was considered significant. A correlation analysis was run through Spearman rank-correlation coefficients for two data series to compare between variables, and scatter plots were also made. A ROC curve was plotted to reveal AFP and hTERT-mRNA cutoff levels and also to calculate sensitivity, specificity, PPV, NPV, and accuracy.

Discussion

Human telomerase, a ribonucleoprotein reverse transcriptase, catalyzes the addition of a telomeric repeat (TTAGGG) at the telomere's end of each chromosome. Most somatic cells do not contain detectable levels of active telomerase. Cells enter cell arrest and die once a critically short length of telomeric DNA is reached. In contrast, telomerase activity is detectable in over 90% of known human tumor cells, enabling these tumor cells to escape senescence and to proliferate at a higher rate. Thus, understanding telomerase biology and its complex regulation may shed light on how tumor cells acquire their capability for unlimited replication (immortality), and, consequently, the potential of several therapeutic approaches to block telomerase activity [9].

In the present study, hTERT-mRNA was detected in 18 out of 22 patients (81.8%) in the HCC group, in 9 out of 22 patients (40.9%) in the CLD group, and in 2 out of 12 patients (16.7%) in the control group. There was a significant difference between the HCC group and the other two groups ($P < 0.001$, see Table 1). Similar detection rates for telomerase expression in HCC, CLD, and controls were reported by Wu *et al.* [10] (83%, 43.2%, and 10%, respectively). Also, Yao *et al.* [11] found rates of 85.5%, 45%, and 15.4%, respectively, for the same patient groups and healthy controls. On the other hand, the present study showed that 10–15% of cancer patients tested negative for hTERT-mRNA expression. Most likely, the patients' tumors maintained their telomere length via a poorly understood mechanism called (ALT) or *(Alternative Lengthening of Telomerase).*

The mean level of hTERT-mRNA expression was significantly higher in the HCC group compared to controls ($P < 0.001$) and the CLD patients ($P < 0.001$, see Table 1). The same findings were reported by Kumi *et al.* [11], Tatsuma *et al.* [12], Miura *et al.* [13], Wu *et al.* [10], Miura *et al.* [14], Yao *et al.* [15], Satra *et al.* [16], and Miura *et al.* [17]. Specifically, all of these studies observed the parallel result that telomerase expression was significantly increased in HCC patients compared to CLD patients and controls. Although telomerase per se is not carcinogenic, it plays a direct role in oncogenesis by allowing the precancerous cells to proliferate continuously and become immortal, which can be explained by the positive correlation between telomerase activity and tumors of different histological origins and types [18]. In addition, the present work demonstrates that there were no significant differences in hTERT-mRNA expression between the CLD and control groups ($P > 0.05$, see Table 1), which coincides with the results reported by Tatsuma *et al.* [12] and Miura *et al.* [13]. This finding suggests that normal hepatocytes may express a negligible amount of hTERT-mRNA and that inflamed hepatocytes may still express more weakly than HCC cells.

On the other hand, Xue-Gang *et al.* [19] reported a significantly lower telomerase level by tartrate-resistant acid phosphatase–polymerase chain reaction–enzyme-linked immunosorbent assay (TRAP-PCR-ELISA) in the peripheral lymphocytes of patients with CLD when compared to controls; the authors explained this finding as immune dysfunction due to premature aging of the immune system in hepatic patients, which decreases

Table 1. Telomerase enzyme hTERT-mRNA expression level in the peripheral blood of the studied groups.

hTERT-mRNA (copies/ml)	HCC (n = 22)	CLD (n = 22)	Controls (n = 12)	Kruskal-Waillis test	P value	
Median (range)	232.5 0-621	56.4 0-156	38.5 0-112	24.27	0.001	P1 < 0.001 P2 < 0.001 P3 > 0.05

P1 is between HCC & CLD, P2 is between HCC & Controls, and P3is between CLD & Controls

lymphocyte functions and consequently suppresses telomerase activity. This theory may affect the hTERT-mRNA in isolated lymphocytes but not the whole-blood hTERT-mRNA in HCC and CLD patients originating from lymphocytes as well as from injured replicating and immortal hepatocytes [20]. These cells may secrete hTERT-mRNA into circulation or circulate in a hematogenous pattern [21]. Also, the real-time PCR technique used in the present study was corroborated by findings from Francisco *et al.* [21], who used a more sensitive analysis than TRAP assay for telomerase activity assessment.

In the current work, no significant correlation ($P > 0.05$) was discovered when comparing the CLD and control groups' relationships of age, CBC results, and liver-function tests with AFP and hTERT-mRNA expression (Tables 2 & 3). Trevisani *et al.* [22] concluded that AFP showed no significant correlation to any of the clinico-pathological variable in CLD patients and controls. Also, Kim *et al.* [23] reported that elevated AFP in CLD patients was independently associated with elevation of aminotransferase enzymes in cases of acute viral exacerbation without malignant transformation. For the HCC group in the current work, the findings revealed that AFP and hTERT-mRNA expression levels were positively correlated with tumor size ($P < 0.05$ and $P < 0.001$, respectively, Figs 4 & 5).

These results are parallel to those reported by Kishimoto *et al.* [24], Wu *et al.* [10], Miura *et al.* [14], and Farinati *et al.* [25] who reported that hTERT-mRNA in HCC patients were not related to any of clinico-pathological variables but tumor size and degree of differentiation of HCC. Moreover, both hTERT-mRNA and AFP levels were significantly decreased in HCC patients after reduction of tumor size via transcatheter arterial embolization (TAE), suggesting the value of following up these parameters in HCC therapy [10]. Opposing data have been shown by the possibility of variability in technique and low telomerase enzymatic activity in tissue samples taken from necrotic malignant tissue [26]. Hisatomi *et al.* [27] demonstrated that telomerase enzymes were detected in 89% of tumor tissue from HCC patients but that the enzyme level was not related to tumor size. Also Huang *et al.* [28] reported that neither telomere length nor telomerase activity in tissues from HCC patients related to any clinical parameters, tumor size or AFP level in serum at time of diagnosis.

For HCC patients, the present work used a cutoff level of 185 ng/ml AFP and revealed a sensitivity of 63.6%, a specificity of 85.3%, a PPV level of 89.3%, an NPV level of 76.2%, and a diagnostic accuracy of 83.4% for HCC prediction (ROC AUC 0.652 95%, CI 0.551 -0.861, Fig. 2). Parallel to these results, Miura *et al.* (14) reported an AFP sensitivity of 69.3%, specificity 60% PPV 81.2%, NPV 59% prediction of HCC. Another related study by Arrieta *et al.* [3] reported that, at a cutoff level of 400 ng/ml AFP, the universally accepted level for HCC diagnosis, the analysis showed a sensitivity level of 17.5% and a specificity of 100%, which would lead to late diagnosis of many cases. Other factors that limit diagnosis falsely suggest that AFP sensitivity is lower in certain races (e.g., African Americans) [10] and is significantly decreased in CLD patients receiving interferon therapy for HCV (18). May be that's why Trevisani *et al.* [22] reported that in CLD patients, AFP monitoring misses many HCC cases and inappropriately arouses suspicion of malignancy

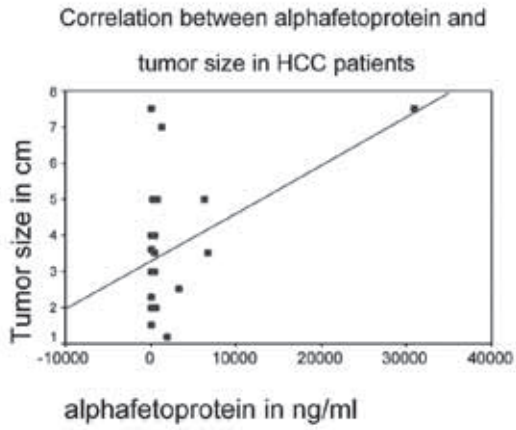

Figure 4. Correlation between tumor size and AFP.

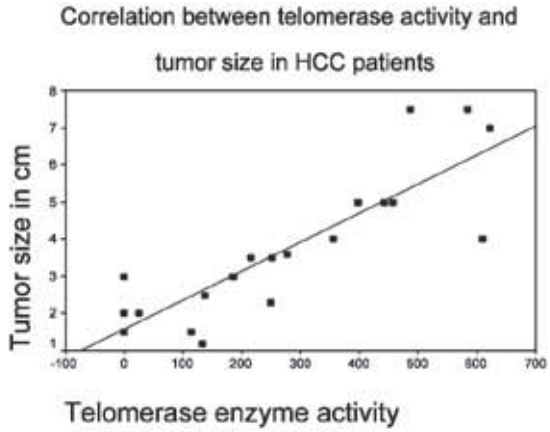

Figure 5. Correlation between tumor size and hTERT mRNA.

Table 2. Correlation (Pearson's) between age, CBC results, and liver functions with each AFP and hTERT-mRNA expression level in the CLD patients group (n = 22).

	Telomerase expression		AFP	
	P value	r	P value	r
Age	> 0.05	0.22	> 0.05	0.17
Hb (mg/dl)	> 0.05	-0.07	> 0.05	-0.09
RBCs count (millions/CC)	> 0.05	-0.02	> 0.05	-0.24
WBCs (1000/CC)	> 0.05	0.03	> 0.05	0.21
Platelet count (1000/CC)	> 0.05	-0.06	> 0.05	-0.31
Total bilirubin (mg/dl)	< 0.05	0.07	> 0.05	0.26
Direct bilirubin (mg/dl)	> 0.05	0.07	> 0.05	0.05
AST (U/L)	< 0.05	0.02	> 0.05	0.26
ALT (U/L)	> 0.05	0.19	> 0.05	0.14
ALP (U/L)	> 0.05	0.20	> 0.05	0.20
GGT (U/L)	> 0.05	0.14	> 0.05	0.02
Albumin (mg/dl)	> 0.05	-0.07	> 0.05	-0.07
Total protein (mg/dl)	> 0.05	-0.13	> 0.05	-0.04
AFP (ng/ml)	---	0.15	> 0.05	---

Table 3. Correlation (Pearson's) between age, tumor size, CBC results, and liver functions with each AFP and hTERT-mRNA expression level in the HCC patients group (n = 22).

	Telomerase expression		AFP	
	P value	r	P value	r
Age	> 0.05	r	> 0.05	0.04
Hb (mg/dl)	< 0.05	0.39	< 0.0001	0.47
RBCs count (millions/CC)	> 0.05	0.86	> 0.05	-0.14
WBCs (1000/CC)	> 0.05	-0.14	> 0.05	-0.09
Platelet count (1000/CC)	> 0.05	-0.20	> 0.05	0.06
Total bilirubin (mg/dl)	> 0.05	0.05	> 0.05	-0.21
Direct bilirubin (mg/dl)	> 0.05	-0.11	> 0.05	-0.05
AST (U/L)	> 0.05	0.18	> 0.05	0.11
ALT (U/L)	> 0.05	0.09	> 0.05	0.09
ALP (U/L)	> 0.05	0.01	> 0.05	0.12
GGT (U/L)	> 0.05	0.04	> 0.05	0.19
Albumin (mg/dl)	> 0.05	0.26	> 0.05	0.15
Total protein (mg/dl)	> 0.05	0.29	> 0.05	0.11
AFP (ng/ml)	> 0.05	-0.06	> 0.05	-0.11

in many patients. Also some studies reported that AFP has limited utility in differentiating HCC from benign hepatic disorders for its high false positive and false negative rates, and also patients with acute viral exacerbation may have remarkably elevated AFP levels but no HCC [25, 26].

The analysis in the present study revealed that hTERT-mRNA at a cutoff 112.5 copies/ml for HCC prediction showed a sensitivity of 77.3%, a specificity of 97.1%, a PPV level of 98%, an NPV level of 79%, and a diagnostic accuracy of 84% (ROC AUC 0.760 95%, CI 0.750- 0.851, Fig. 2). Similar results were reported by Miura *et al.* [14], which showed a sensitivity of 88.2%, a specificity of 72.4%, a PPV level of 86.2%, and an NPV level of 87%. Also Yao *et al.* [15] reported a 68.4% sensitivity, a 92% specificity, and a 75.7% PPV level. Higher sensitivity rates of hTERT-mRNA in the prediction of HCC were reported by Masaki *et al.* [29] who reported a sensitivity of 100%, but their study measured hTERT-mRNA in HCC tissue and not in the peripheral blood, which might indicate that the locally expressed hTERT-mRNA may be a more sensitive predictor of HCC in tissue than in peripheral blood, but still blood samples are much more easily obtained for monitoring HCC-vulnerable patients than tissue biopsies. Some HCC cases had hTERT-mRNA below the calculated cutoff level. However, cirrhosis is frequently the underlying cause for HCC, causing a high level of TGF-B, which promotes apoptosis of immortalized hepatocytes [30]. Also, nonsteroidal anti-inflammatory drugs may inhibit hTERT-mRNA [31], and Genistein presents that soya bean represses telomerase activity via both transcriptional and post-transcriptional mechanisms [32]. Lastly, immortal hepatocytes may acquire an "Alternative Lengthening of Telomerase" (ALT) mechanism for maintenance of their chromosomal stability [33]. Yao Fu *et al.* [15] also found that AFP combined with telomerase expression in peripheral blood could increase the accuracy of HCC diagnosis to 92.6%. Data in present work revealed that the combination increased accuracy of HCC to 90.5%, with a sensitivity for hTERT-mRNA and AFP of 86.4%, a specificity of 84.1%, a PPV level of 86.4% and an NPV level of 88.3% (Fig. 3).

Conclusions

hTERT-mRNA expression in patients with CLD could be a satisfactory molecular marker for early diagnosis of HCC. hTERT-mRNA is thought to be superior to AFP for early detection and follow-up of HCC. The combined use of peripheral-blood hTERT-mRNA and AFP would increase the accuracy of early detection of HCC in susceptible patients.

References

1. Bae JS, Park SJ, Park KB, *et al*. Acute exacerbation of hepatitis in liver cirrhosis with very high levels of alpha-fetoprotein but no occurrence of hepatocellular carcinoma. *Korean J Intern Med*. 2005;**20**(1):80-5.
2. Iliopoulos D, Satra M, Drakaki A, Poultsides GA, Tsezou A. Epigenetic regulation of hTERT promoter in hepatocellular carcinomas. *Int J Oncol*. 2009;**34**(2):391-9.
3. Arrieta O, Cacho B, Morales-Espinosa D, Ruelas-Villavicencio A, Flores-Estrada D, Hernandez-Pedro N. The progressive elevation of alpha fetoprotein for the diagnosis of hepatocellular carcinoma in patients with liver cirrhosis. *BMC Cancer*. 2007;**7**:28.
4. Bertuch AA, Lundblad V. The maintenance and masking of chromosome termini. *Curr Opin Cell Biol*. 2006;**18**(3):247-53.
5. Bryce LA, Morrison N, Hoare SF, Muir S, Keith WN. Mapping of the gene for the human telomerase reverse transcriptase, hTERT, to chromosome 5p15.33 by fluorescence in situ hybridization. *Neoplasia*. 2000;**2**(3): 197-201.
6. Davis GL, Dempster J, Meler JD, *et al*. Hepatocellular carcinoma: management of an increasingly common problem. *Proc (Bayl Univ Med Cent)*. 2008;**21**(3):266-80.
7. Ancelin K, Brunori M, Bauwens S, *et al*. Targeting assay to study the cis functions of human telomeric proteins: evidence for inhibition of telomerase by TRF1 and for activation of telomere degradation by TRF2. *Mol Cell Biol*. 2002;**22**(10):3474-87.
8. Song J, Kim C, Ochoa ER. Sleeping Beauty-mediated suicide gene therapy of hepatocellular carcinoma. *Biosci Biotechnol Biochem*. 2009;**73**(1):165-8.
9. Perez JM, Grimm J, Josephson L, Weissleder R. Integrated nanosensors to determine levels and functional activity of human telomerase. *Neoplasia*. 2008;**10**(10):1066-72.
10. Wu W, Yao DF, Qiu LW, *et al*. Abnormal expression of hepatomas and circulating telomerase and its clinical values. *Hepatobiliary Pancreat Dis Int*. 2005;**4**(4):544-9.
11. Nagao K, Tomimatsu M, Endo H, Hisatomi H, Hikiji K. Telomerase reverse transcriptase mRNA expression and telomerase activity in hepatocellular carcinoma. *J Gastroenterol*. 1999;**34**(1):83-7.
12. Tatsuma T, Goto S, Kitano S, Lin YC, Lee CM, Chen CL. Telomerase activity in peripheral blood for diagnosis of hepatoma. *J Gastroenterol Hepatol*. 2000;**15**(9):1064-70.
13. Miura N, Shiota G, Nakagawa T, *et al*. Sensitive detection of human telomerase reverse transcriptase mRNA in the serum of patients with hepatocellular carcinoma. *Oncology*. 2003;**64**(4):430-4.
14. Miura N, Maeda Y, Kanbe T, *et al*. Serum human telomerase reverse transcriptase messenger RNA as a novel tumor marker for hepatocellular carcinoma. *Clin Cancer Res*. 2005;**11**(9):3205-9.
15. Yao DF, Wu W, Yao M, *et al*. Dynamic alteration of telomerase expression and its diagnostic significance in liver or peripheral blood for hepatocellular carcinoma. *World J Gastroenterol*. 2006;**12**(31):4966-72.
16. Satra M, Gatselis N, Iliopoulos D, Zacharoulis D, Dalekos GN, Tsezou A. Real-time quantification of human telomerase reverse transcriptase mRNA in liver tissues from patients with hepatocellular cancer and chronic viral

hepatitis. *J Viral Hepat*. 2007;**14**(1):41-7.
17. Miura N, Maruyama S, Oyama K, *et al*. Development of a novel assay to quantify serum human telomerase reverse transcriptase messenger RNA and its significance as a tumor marker for hepatocellular carcinoma. *Oncology*. 2007;**72 Suppl 1**:45-51.
18. Cortez-Gonzalez X, Zanetti M. Telomerase immunity from bench to bedside: round one. *J Transl Med*. 2007;**5**:12.
19. Fan XG, Huang Y, Tang FQ, Yi H. Telomerase activity of peripheral blood lymphocytes in patients with chronic hepatitis B. *Immunol Lett*. 2000;**73**(1):7-11.
20. Yao DF, Dong ZZ, Yao M. Specific molecular markers in hepatocellular carcinoma. *Hepatobiliary Pancreat Dis Int*. 2007;**6**(3):241-7.
21. Dasi F, Lledo S, Garcia-Granero E, *et al*. Real-time quantification in plasma of human telomerase reverse transcriptase (hTERT) mRNA: a simple blood test to monitor disease in cancer patients. *Lab Invest*. 2001;**81**(5):767-9.
22. Trevisani F, D'Intino PE, Morselli-Labate AM, *et al*. Serum alpha-fetoprotein for diagnosis of hepatocellular carcinoma in patients with chronic liver disease: influence of HBsAg and anti-HCV status. *J Hepatol*. 2001;**34**(4):570-5.
23. Kim KA, Lee JS, Jung ES, *et al*. [Usefulness of serum alpha-fetoprotein (AFP) as a marker for hepatocellular carcinoma (HCC) in hepatitis C virus related cirrhosis: analysis of the factors influencing AFP elevation without HCC development]. *Korean J Gastroenterol*. 2006; **48**(5):321-6.
24. Fu J, Zhang W, Jin S. [Telomerase activity in liver cancer and the adjacent tissue]. *Zhonghua Zhong Liu Za Zhi*. 1998;**20**(6):434-6.
25. Farinati F, Marino D, De Giorgio M, *et al*. Diagnostic and prognostic role of alpha-fetoprotein in hepatocellular carcinoma: both or neither? *Am J Gastroenterol*. 2006;**101**(3):524-32.
26. Suda T, Isokawa O, Aoyagi Y, *et al*. Quantitation of telomerase activity in hepatocellular carcinoma: a possible aid for a prediction of recurrent diseases in the remnant liver. *Hepatology*. 2003;**27**(2):402-6.
27. Hisatomi H, Kawaoto Y, Ohmura Y, Kuwahara M, Yamauchi T, Hikiji K. Detection of telomerase activity in hepatocellular carcinoma by fluorescence-based TRAP method. *International Hepatology Communications*. 1996;**6**(1):29-35.
28. Huang GT, Lee HS, Chen CH, *et al*. Telomerase activity and telomere length in human hepatocellular carcinoma. *Eur J Cancer*. 1998;**34**(12):1946-9.
29. Shimojima M, Komine F, Hisatomi H, Shimizu T, Moriyama M, Arakawa Y. Detection of telomerase activity, telomerase RNA component, and telomerase reverse transcriptase in human hepatocellular carcinoma. *Hepatol Res*. 2004;**29**(1):31-8.
30. Cavin LG, Romieu-Mourez R, Panta GR, *et al*. Inhibition of CK2 activity by TGF-beta1 promotes IkappaB-alpha protein stabilization and apoptosis of immortalized hepatocytes. *Hepatology*. 2003;**38**(6):1540-51.
31. He H, Xia HH, Wang JD, *et al*. Inhibition of human telomerase reverse transcriptase by nonsteroidal antiinflammatory drugs in colon carcinoma. *Cancer*. 2006;**106**(6):1243-9.
32. Jagadeesh S, Kyo S, Banerjee PP. Genistein represses telomerase activity via both transcriptional and posttranslational mechanisms in human prostate cancer cells. *Cancer Res*. 2006;**66**(4):2107-15.
33. Nittis T, Guittat L, Stewart SA. Alternative lengthening of telomeres (ALT) and chromatin: is there a connection? *Biochimie*. 2008;**90**(1):5-12.