

**Human telomerase as a novel serum tumor marker for detection of hepatocellular carcinoma**

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**Abstract:** Hepatocellular carcinoma (HCC) is one of the most common malignancies worldwide. Alpha-fetoprotein (AFP) and des-gamma carboxy prothrombin (DCP) are used for patients with HCC, but their results are controversial. Telomerase is a ribo-nucleoprotein enzyme that synthesizes telomeric DNA and adds this sequence to chromosomal ends. Thereby, it prevents telomere shortening and consequently protects the chromosomes from DNA degradation, end-to-end fusion, rearrangements, and chromosomal loss. Recently human telomerase reverse transcriptase mRNA (hTERT mRNA) has been demonstrated as a novel serum marker for HCC diagnosis. This study aimed to evaluate the diagnostic and prognostic performance of telomerase activity in the peripheral blood of patients with HCC and in those with non HCC cirrhosis and healthy controls as comparing with AFP and DCP. The study included 21 patients of proved HCC, 22 patients with cirrhosis without HCC (LC) in addition to 20 apparently healthy subjects were enrolled in the study as a control group. Patients and controls were subjected to full history taking, thorough clinical examination, routine laboratory and radiological assessment in addition to detection of hTERT mRNA expression in peripheral blood by real time PCR technique, and measurement of serum AFP and DCP levels. Results showed that, hTERT mRNA was detected in peripheral blood of 81% in HCC group, 40.9% in LC and 16.7% in healthy controls, its level was significantly higher in HCC group compared to cirrhosis and controls. Also, a significant elevation in AFP and DCP levels in HCC patients was detected compared to LC and control groups. The levels of each AFP, DCP and hTERT mRNA were positively correlated to tumor size only. at a cut-off level of 112.5 copies/ml, hTERT mRNA showed a sensitivity of 76.3%, specificity of 97.1%, PPV of 99%, NPV of 79% and diagnostic accuracy of 84% for HCC prediction. While. at a cut off level of 31.5 ng/ml DCP give a sensitivity of 95.2 %, and specificity of 100%, PPV of 100%, NPV 94% and diagnostic accuracy of 99.7%. At a cut-off level of 176 ng/ml, AFP give a sensitivity of 61.9%, specificity 100%, PPV 100%, NPV 79.2% and diagnostic accuracy of 84.6% for HCC prediction. Combined use of hTERT mRNA and/or AFP in prediction of HCC increased accuracy to 94.5% while combined use of DCP and/or AFP increase it to 100%. In conclusion: it was concluded that, the present study revealed that Real-time measurement of hTERT mRNA in the peripheral blood of patients with LC & cirrhosis could be used as a molecular marker for diagnosis of HCC.

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**1.Introduction**

Hepatocellular carcinoma (HCC) is a prevalent cancer that often develops in patients with chronic hepatitis and cirrhosis in association with hepatitis B or C viral infection (Yao *et al.*, 2007).

Most of HCC patients died quickly because of the rapid tumor progression, and hepatic resection or transplantation is the only potential curative treatment for HCC patients. Although the mortality of HCC has significantly decreased with the development of surgical techniques, about 60%-100% of the patients suffered from HCC recurrence ultimately even after curative resection, and it has become the most important factor that limits the long-term survival of HCC patients (Anzola, 2004).

The most urgent needs are to find sensitive markers for early diagnosis and monitoring of

postoperative recurrence of HCC, and to give adequate treatment for HCC patients. HCC has many characteristics, such as fast infiltrating growth, metastasis in early stage, high-grade malignancy, and poorly therapeutic efficacy. HCC prognosis is poor and early detection is of the utmost importance (Shi *et al.*, 2004).

Although serum alpha- fetoprotein (AFP) level is a useful tumor marker for the detection and monitoring of HCC, the false negative rate with AFP level alone may be as high as 40% for patients with early stage HCC. Even in patients with advanced HCC, the AFP levels may remain normal in 15%-30% of the patients. Furthermore, elevated AFP levels may be seen in patients with cirrhosis or exacerbations of chronic hepatitis i.e. low specificity (Sherman, 2007).

Other HCC marker, such as des-gamma-carboxy prothrombin (DCP); it is an abnormal prothrombin protein (Yao *et al.*, 2000), have been developed to improve the sensitivity, specificity, early detection, and prediction of prognosis; but the overall results have been unsatisfactory (Soresi *et al.*, 2003; Arrieta *et al.*, 2005).

Telomerase is a ribo-nucleoprotein enzyme that synthesizes telomeric DNA and adds this sequence to chromosomal ends. Thereby, it prevents telomere shortening and consequently protects the chromosomes from DNA degradation, end-to-end fusion, rearrangements, and chromosomal loss. Telomerase activity has been found not only in germ line cells and stem cells, but also in various cancer specimens and almost in all human cancers including breast, bladder, ovary, prostate, colon, liver, stomach, brain and others (Shay *et al.*, 2008).

Telomerase consists of two essential components; human telomerase reverse transcriptase (hTERT) the catalytic subunit with reverse transcriptase activity and (hTR) human telomerase RNA template for telomeric DNA synthesis (Zu *et al.*, 2008). While hTR is expressed in almost all tissues irrespective to telomerase activity, hTERT is generally repressed in normal cells and up regulated in immortal cells (Masaki *et al.*, 2004).

Fabio *et al.* (2007) and Satra *et al.* (2007) who that telomerase activity is closely correlated with hTERT expression both in tissues & peripheral blood. That's why assessment of hTERT mRNA expression is considered a better evaluation of telomerase than the assay of the enzymatic activity itself, because, it avoids the variances in telomerase due to cell lyses, instability of the enzyme activity during storage and allowing the assay of expression levels compared to a house-keeping gene which prevents co-amplification of genomic DNA.

#### **Aim of the study:**

This study aimed to evaluate the diagnostic and prognostic performance of telomerase activity in the peripheral blood of patients with HCC and in those with non HCC cirrhosis and healthy controls as comparing with AFP and DCP.

#### **2. Patients and methods:**

This study was conducted on 43 patients of proved HCC and LC selected from the HCC Clinics and Hepatology department of National Liver Institute Hospital in the period from February 2010 to October 2011. Patients with history of other malignancies were excluded from the study. In addition, 20 apparently healthy individuals of comparable age and gender were taken as a control group.

They were divided into 3 groups as follows: Group: 21 HCC group, 15 males and 6 females, their

ages ranged from 49-67 years with a mean value of  $57.59 \pm 5.3$  years. Group II: 22 patients with LC without HCC, 13 males and 7 females, their ages ranged from 49 to 68 years with a mean value of  $56.27 \pm 5.73$  years. Group III: Includes 20 healthy volunteers, 14 males and 6 females with normal liver function tests & no history of liver disease, their ages ranged from 49 to 66 years old with a mean value of  $56.25 \pm 5.62$  years.

All patients in the study were subjected to full history taking, clinical examination, abdominal ultrasonography, abdominal CT scan and radiological examination (for HCC cases). Laboratory investigations were done including: Serum levels of AST, ALT, albumin, total and direct bilirubin, alkaline phosphatase and gamma glutamyl transpeptidase (GGT) were done using Integra-400 (Roche-Germany). Bilharzial antibody was done using hemagglutination kit. Prothrombin concentration was done by Fibrintimer (Roche-Germany). Complete blood counts were measured by Sysmix K-21 automatic cell counter (Japan). AFP serum level was measured by an automated Eleceyes (Roche-Germany). HBV markers and HCV antibodies were assayed by EIA (COBAS-Amplifire, Germany). HCV-RNA levels were analyzed by real time polymerase chain reaction using a commercial kit (Roche Diagnostic, Branchburg, NJ) according to the manufacturer's instructions.

Serum DCP (Miura *et al.*, 2007): done by a special automated analyser, UBASys™ DCP, Wako. Briefly, the reagent consists of anti DCP monoclonal antibody and anti-prothrombin monoclonal antibodies which are used as fab/molecules and a substrate for fluorophotometric measurement. When DCP in a sample reacts with anion conjugated anti-prothrombin monoclonal antibody and peroxidase labeled anti DCP monoclonal antibody which binds to all the present DCP molecules it forms an immune complex. The reaction mixture contain immune complex is introduced into anion exchange column and the immuno-complex fractions are eluted into the reaction cups. Then the fluorescence intensity of the complex is measured and compared to the fluorescence intensity of standard DCP.

Detection of hTERT mRNA in peripheral blood: RNA extraction was done by RNA Isolation Kit (Gentra) catalogue number; 13355. Bayer Material Science AG, USA. RNA was isolated from whole blood according to manufacturer instructions. Real time PCR was done by TeloTAGGG hTERT Quantification Kit using LightCycler Instrument. Telomerase (hTERT)-encoding mRNA is reverse transcribed and a 198 bp fragment of the generated cDNA is amplified with specific primers in a one-step RT-PCR reaction. The amplicon is detected by

fluorescence using a specific pair of Hybridization Probes. One probe is labeled at the 5'-end with LightCycler Red 640, and to avoid extension, modified at the 3'-end by phosphorylation. The other probe is labeled at the 3'-end with fluorescein. Only after hybridization to the template DNA do the two probes come in close proximity, resulting in fluorescence resonance energy transfer (FRET) between the two fluorophores. The donor fluorophore, is excited by the light source of the LightCycler Instrument, and part of the excitation energy is transferred to LightCycler Red 640, the acceptor fluorophore. The emitted fluorescence of LightCycler Red 640 is then measured by the LightCycler Instrument. In a separate one-step RT-PCR, mRNA, encoding for porphobilinogen deaminase (PBGD) is processed for use as house-keeping gene. The reaction product serves as both a control for RT-PCR performance and as a reference for relative quantification.

### Statistical analysis:

Data were coded and summarized using SPSS (statistical package for Social Sciences) version 13.0 for Windows. Quantitative variables were described using mean  $\pm$  standard deviation and categorical data by using frequency and percentage. Comparison between groups was done using Chi square ( $X^2$ ) test for qualitative variables and Mann Whitney (U) test for non normally distributed variables. Kruskal-Wallis test was done to compare three or more of non normally distributed variables.  $P$  value  $<0.05$  was considered statistically significant.

### 3.Results:

All clinical and laboratory data are listed in tables (1-6) and figures (1-4). The prevalence of hepatitis markers and bilharzial antibody among the two

diseased groups (LC & HCC) shows no significant difference (Table 1).

A highly significant difference in the AFP level between the CLD and control group is shown, while the difference is more significant when comparing the HCC group with other groups. A significant difference in the DCP level between the LC and control group is shown, while a highly significant difference with both comparison between control and HCC groups and when comparing HCC and LC groups (Table 2).

Telomerase enzyme mRNA was detectable in 81.0% of HCC patients, 40.9% of LC patients and 16.7% of control subjects (Table 3). There is a highly significant increase in Telomerase enzyme mRNA level in HCC patients as compared to LC patients and controls, while no significant difference was found when comparing LC patients to the control subjects (Table 4).

Table (5) showed that, tumor size is the only parameter that is significantly positively correlated to both AFP and telomerase enzyme mRNA in HCC patients group[( $p < 0.05$ ) & ( $p < 0.0001$ ) respectively].

A level of AFP of 176.0 ng/ml is a cut-off point at which the prediction of HCC has a sensitivity of 61.9%, specificity of 100.0 %, PPV is 100.0%, NPV is 65.1 %, and diagnostic accuracy is 84.6 %. A level of DCP of 31.5 ng/ml is a cut-off point at which the prediction of HCC has a a sensitivity of 95.2%, specificity of 100 %, PPV is 100%, NPV is 94.8 %, and diagnostic accuracy is 99.7 %. A level of hTERT mRNA of 112.5 copies/ml is a cut-off point at which the prediction of hepatocellular carcinoma has a a sensitivity of 76.2 %, specificity of 97.1 %, PPV is 100%, NPV is 79.0 %, and diagnostic accuracy is 86.7 %. Area under the Roc curve (AUR) of AFP= 83.6; AUC of HTER mRNA= 85.7; AUC of DCP= 99.7) (Figure 3).

**Table (1): Comparison between the prevalence of hepatitis markers and bilharzial antibody among the diseased groups (LC & HCC)**

Variables	Studied groups				Fisher exact test	p- value
	HCC		LC			
	No.	%	No.	%		
<b>HCV Ab:</b>						
Positive	17	81.0	20	90.9	0.77	> 0.05
Negative	4	19.0	2	9.1		
<b>HbsAg:</b>						
Positive	4	19.0	1	4.5	2.00	> 0.05
Negative	17	81.0	21	95.5		
<b>Bilharzial Ab:</b>						
Positive	8	38.1	10	45.5	0.24*	> 0.05
Negative	13	61.9	12	54.5		
<b>Total</b>	21	100.0	22	100.0		

\* Chi square test.

( $P$  value significant  $< 0.05$ )

**Table (2): Comparison between AFP & DCP levels in the studied groups.**

	HCC n=21	LC n=22	Controls n=20	Kruskal Wallis Test	Tamhane post Hoc test
AFP (ng/ml)					$P1 < 0.001$

Mean rank (range)	394 3.7-31000	27.02 3.4-112	9.79 2.1-18	26.29	P2< 0.001 P3 <0.01
DCP(ng/ml) Mean rank (range)	94.00 36 – 200	19.45 1 – 23	12.71 1-19	40.28	P1 < 0.001 P2 < 0.001 P3 < 0.05

*P* value significant < 0.05, *p* value highly significant < 0.01 (*P*1 between HCC & CLD, *P*2 between HCC & Controls, *P*3 between CLD & Controls).

**Table (3): Results of Telomerase enzyme mRNA among studied groups.**

Telomerase hTERT mRNA	HCC N=21		LC n=22		Controls n=20		X <sup>2</sup>	P
	No	%	No	%	No	%		
Positive	17	81.0	9	40.9	3	15.0	14.09	<0.01
Negative	4	19.0	13	59.1	17	85.0		
Total	21	100	22	100	20	100		

(*p* value highly significant < 0.01)

**Table (4): Comparison of Telomerase enzyme mRNA level in the peripheral blood of studied groups**

hTERT mRNA (Copies/ml)	HCC n=21	LC n=22	Controls n=20	Kruskal Wallis Test	Tamhane post Hoc test
Mean rank (range)	240.14 0-621	22.27 0-156	17.25 0-112	22.99	P1 < 0.001 P2 < 0.001 P3 > 0.05

*P* value highly significant < 0.01 (*P*1 between HCC & LC; *P*2 between HCC & Controls; *P*3 between LC & Controls).

**Table (5): Correlation (Pearson's) between age, tumor size, CBC results & liver functions with each of AFP and telomerase expression in HCC group(n=21).**

Parameters of correlation	AFP		Telomerase	
	r	<i>p</i> - value	r	<i>p</i> - value
Age ( years)	0.04	> 0.05	0.43	> 0.05
Tumor size (cm)	0.47	< 0.05	0.92	< 0.01
HB in (gm/dl)	- 0.15	> 0.05	0.23	> 0.05
RBCs count ( millions/ C.C)	- 0.1	> 0.05	0.27	> 0.05
WBCs count (1000/ C.C)	- 0.04	> 0.05	- 0.14	> 0.05
Platelet count (1000/ C.C)	- 0.23	> 0.05	- 0.07	> 0.05
T. Bilirubin (mg/ dl)	- 0.05	> 0.05	0.19	> 0.05
D. Bilirubin (mg/ dl)	- 0.12	> 0.05	0.15	> 0.05
AST (U/ L)	- 0.1	> 0.05	0.05	> 0.05
ALT (U/ L)	- 0.13	> 0.05	0.01	> 0.05
ALP (U/ L)	- 0.22	> 0.05	0.38	> 0.05
GGT (U/ L)	- 0.18	> 0.05	0.41	> 0.05
Albumin (gm/dl)	0.09	> 0.05	0.15	> 0.05
T. Protein (gm/dl)	- 0.14	> 0.05	- 0.07	> 0.05
α- FP in ng/ ml	----	----	0.33	> 0.05

(*p* value significant < 0.05 and highly significant < 0.001)

**Table (6) Sensitivity, specificity, Positive predictive value (PPV), negative predictive value (NPV) and diagnostic accuracy of the three markers for HCC prediction.**

	Telomerase mRNA	AFP	DCP	Telomerase mRNA+ AFP	DCP + AFP	Telomerase mRNA and/ or AFP	DCP and/or AFP
Sensitivity	76.2 %	61.9 %	95.3%	47.6 %	57.1%	85.7 %	100.0 %
Specificity	97.1 %	100.0 %	100.0 %	100.0 %	100.0 %	100.0 %.	100.0 %
PPV	100.0 %	100.0 %	100.0%	100.0 %	100.0 %	100.0 %	100.0 %
NPV	79.0 %	65.1 %	94.8 %	75.5 %	79.1	91.9 %	100.0 %
Accuracy	86.7	84.6 %	99.7%	80.0 %	83.6 %	94.5 %	100.0 %
Cut off point	112.5 copies/ml	176.0 ng/ml	31.5 ng/ml	Both of 112.5 telomerase mRNA and 176.0 of AFP	Both of 31.5 DCP and 176.0 of AFP	Any of 112.5 of telomerase mRNA or 176.0 of AFP	Any of 31.5 of DCP or 176.0 of AFP

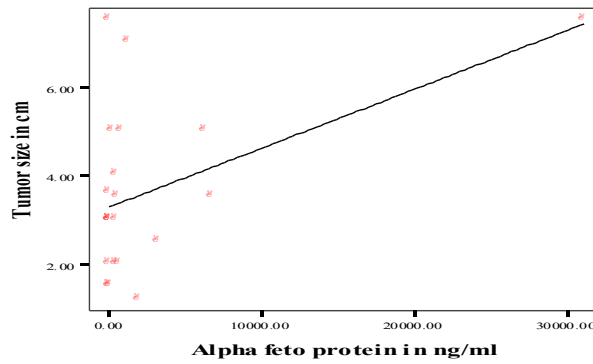


Figure (1) Positive correlation between tumor size & AFP.

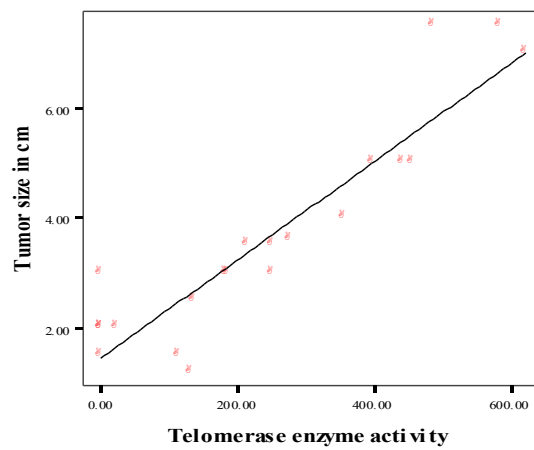


Figure (2) Positive correlation between tumor size & hTERT mRNA.

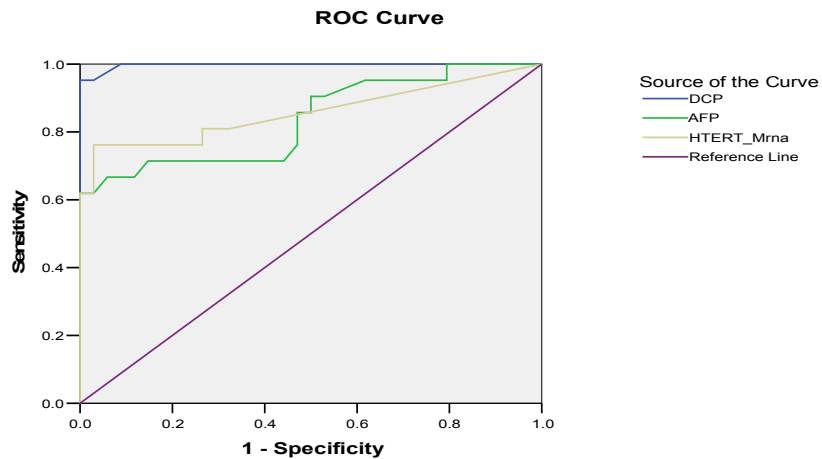


Figure (3): ROC curve for AFP , DCP and telomerase enzyme mRNA as a predicting factors for HCC. Area under the Roc curve (AUR) of AFP= 83.6; AUC of HTER mRNA= 85.7; AUC of DCP= 99.7

4. Discussion:

The incidence of HCC is rising, and the number of patients with HCC is expected to be more than double over the next 1 to 2 decades (**Santmaria et al., 2007**). AFP has been used as a serum marker for HCC for many years. Several biomarkers such as DCP, AFP-L3, human hepatocyte growth factor, and insulin like growth factor-1 are promising, but none of these markers has been validated enough for clinical use (**Marrero et al., 2009**). Thus, there is an urgent need for new biomarker for the detection of early HCC (**Li et al., 2007**).

Telomeres are special functional complex of specific DNA sequence repeats at the end of eukaryotic chromosomes (**Cong et al., 2002**). These terminal structures have an essential role in protecting chromosomes from end-to-end fusion & DNA degradation (**Salhab et al., 2007**). Telomerase is a ribo-nucleoprotein enzyme that synthesizes telomeric DNA and adds this sequence to chromosomal ends. Thereby, it prevents telomere shortening and consequently protects the chromosomes from DNA degradation, end-to-end fusion, rearrangements and chromosomal loss (**Wu et al., 2005**).

Human telomerase reverse transcriptase mRNA (hTERT mRNA) has also been demonstrated to be a novel and available marker for HCC diagnosis, the expression of hTERT mRNA in the serum of HCC patients is significantly higher than that in the serum of healthy adults or patients with nonmalignant liver diseases (**Miura et al., 2007**). Telomerase re-expression was found in 85% of human malignant tumors (**Satyanarayana et al., 2004**).

The present study spot a beam of light on the role of telomerase in HCC and LC to find its possible role in prediction of HCC as compared to the commonly used markers such as AFP and DCP. This study included 21 patients with proved HCC, in addition to 22 patients with LC as well as 12 apparently healthy subjects were enrolled in the study as a control group, liver function tests, CBC, imaging tools of abdomen (Ultrasonography and abdominal CT scan for HCC patients), serum AFP, DCP & hTERT mRNA were performed for all patients and control subjects.

The present work showed no significant difference between HCC & LC regarding the presence of HBV, HCV infections or bilharzial antibodies in serum, These findings coincide with those reported by **Tatsuma et al. (2000)**, **Miura et al. (2005)**. These may be explained due to the fact that liver cirrhosis is present in most of cases of HCC, and the commonest cause of cirrhosis in Egypt is viral hepatitis especially in the presence of bilharzial infection (**El-Zayadi et al., 2005**).

Moreover, the present work showed highly significant elevation in AFP level in HCC group compared to LC and control groups. **Miura et al.**

**(2005)** and **Lin et al. (2007)** reported similar results regarding AFP level being significantly elevated in HCC group when compared to LC and control groups.

Data against these were reported in a study by **Sun-yong et al. (2009)**; the presence of AFP was not a useful prognostic marker for HCC. **Jourge et al. (2009)** stated that AFP had the best performance of all markers for all stages include stage zero HCC, the detection of which is the main goal of a surveillance program, while **Deng et al. (2007)** reported less performance of AFP in early stages and small size HCC (less than 3 cm).

Our study showed a significant elevation in the DCP level in the HCC group when compared to LC and control groups. Results are parallel to those reported by **Norimase et al. (2007)** and **Francisco et al. (2008)** who reported DCP has the highest sensitivity for HCC diagnosis and was not elevated in any patients without HCC. **Jourge et al. (2009)** added that DCP perform better as HCC marker in those with viral etiology.

In the present study, hTERT mRNA was detected in peripheral blood of 81% in HCC group, 40.9% in LC and 16.7% in controls, but the frequency of positive cases was significantly higher in HCC group when compared to LC group and controls. Similar detection rates for telomerase expression in HCC, CLD and controls was reported by **Wu et al. (2005)** 83%, 43.2% and 10% respectively, and by **Deng Fu et al. (2006)** 85.5%, 45% and 15.4% respectively for the same patient groups and healthy controls. **Marrero et al. (2009)** reported that, DCP is a well recognized tumor marker for its high sensitivity and specificity in the screening and diagnosis of HCC, 44 to 81% of HCC patients have elevated serum DCP levels.

In the present study, although hTERT mRNA expression level was detectable in HCC as well as LC & control groups, the mean level was significantly higher in HCC group than both in controls and CLD group. **Tatsuma et al. (2000)**, **Wu et al. (2005)**, **Miura et al. (2005)**, **Yao et al. (2006)**, **Satra et al. (2007)** and **Miura et al. (2007)** all reached the same final results parallel to results of present study that is; telomerase is significantly increased in HCC compared to LC and control subjects. This is to be explained by the striking positive correlation between high telomerase activity and tumors of different histological origins and types. That's why 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 (**Xochtil & Maurizio, 2007**).

Also in the present work, no significant differences in hTERT mRNA expression were detected between LC and controls, which coincides

with results reported by **Tatsuma et al. (2000)** and **Miura et al. (2007)** and explained by normal hepatocytes that may express negligible amount of hTERT mRNA and inflamed hepatocytes still express more weakly than hepatocellular carcinoma cells. (**Miura et al. ,2005**). Also the technique used in the present study (Real-time PCR) proved to be more sensitive than TRAP assay for assessment of telomerase activity (**Francisco et al., 2008**).

Similar results for AFP levels were concluded by **Cedrone et al. (2000)** and **Trevisani et al. (2001)**; that AFP showed no significant correlation to any of the clinicopathological variables in CLD and controls.

When studying HCC group, the current work revealed that AFP, DCP and hTERT mRNA expression level were positively correlated only to tumor size. These results are parallel to those reported by **Wu et al. (2005)**, **Miura et al. (2005)**, **Farinati et al. (2006)**, **Jourge et al. (2009)** and **Francisco et al. (2008)** who reported that hTERT mRNA in HCC patients were not correlated to any of the clinicopathological variables but tumor size and degree of differentiation of HCC. **Wu et al. (2005)**, reported that both hTERT mRNA and AFP levels were significantly decreased in HCC patients after reduction of tumor size via Tran catheter arterial embolisation suggesting the value of these parameters in follow up for HCC therapy.

The present work revealed that, at a cut-off level of 176 ng/ml AFP, a sensitivity of 61.9%, specificity 100%, PPV 100%, NPV 79.2% and diagnostic accuracy of 84.6% for HCC prediction. Parallel to our results of AFP in prediction of HCC, **Miura et al. (2005)** reported a sensitivity of 69.3%, specificity 60%, PPV 81.2% and NPV 59%. In another related study by **Arrieta et al. (2005)**, it was reported that, at a cut-off level of 400 ng/ml AFP –the universally accepted level for HCC diagnosis– showed a sensitivity of 17.5% and a specificity of 100%. This would lead to late diagnosis of many cases.

Other factors which limit the diagnostic sensitivity of AFP included ; its being less sensitive in certain races e.g. African Americans (**Nguyen et al., 2002**), and being significantly decreased in LC patients receiving Interferon therapy for HCV (**Murashima et al., 2006**).

Our work revealed that at a cut off level of 31.5 ng/ml DCP, a sensitivity of 95.2 %, and specificity of 100%, PPV of 100%, NPV 94% and diagnostic accuracy of 99.7%. This data are parallel to the results of the study of **Francisco et al. (2008)** reported a sensitivity of 87.2 %, and specificity of 85.0%, PPV of 86.0%, NPV 81.6%. Also, in the present study showed that hTERT mRNA at a cut-off level of 112.5 copies/ml, prediction of HCC showed a sensitivity of 76.3%, specificity of 97.1%, PPV of 99%, NPV of

79% and diagnostic accuracy of 84%. Similar results were reported by **Miura et al. (2005)** which showed sensitivity of 88.2%, specificity 72.4%, PPV 86.2% and NPV 87%. Also **Deng-Fu et al. (2006)** reported 68.4% for sensitivity, 92% for specificity and 75.7% for PPV. In an Egyptian study, **Atia et al. (2008)** reported a sensitivity of 77.3% , specificity 96.8% , PPV 98% , NPV 78% and diagnostic accuracy 83%.

Additionally, higher sensitivity rates of hTERT mRNA in prediction of HCC was reported by **Masaki et al. (2004)** who reported a sensitivity of 100%, but their study measured hTERT mRNA in HCC tissue & not in 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 sample is much more easily obtained for monitoring vulnerable subjects for HCC than the tissue biopsy which need an expert hand and may be contraindicated in suspected HCC patients.

Furthermore, the current study revealed that combined use of hTERT mRNA and/or AFP in prediction of HCC increased accuracy to 94.5%. **Attia et al. (2008)** reported that combined hTERT mRNA and/or AFP could increase accuracy for HCC diagnosis to 90.5%. **Deng Fu et al. (2006)** also reported that AFP in combination with telomerase expression in peripheral blood could increase accuracy for HCC diagnosis to 92.6%.

**In conclusion:** Finally, it was concluded that, the present study revealed that Real-time measurement of hTERT mRNA in the peripheral blood of patients with LC & cirrhosis could be used as a molecular marker for diagnosis of HCC.

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