Tumor Suppressor genes (P16 and RASSF1A) Hypermethylation in Hepatocellular Carcinoma and Chronic Hepatitis C Patients.

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Abstract:

Background: Hepatocellular carcinoma (HCC) is one of the most common cancer with high mortality rate requiring early diagnosis and treatment. Hepatocarcinogenesis is considered a multistep process in which DNA methylation is a type of epigenetic modification and the relationship between methylation and cancer have been the focus of molecular biology researches. **Aims:** This study aims to evaluate the frequency of tumor suppressor genes (P16 and RASSF1A) hypermethylation in liver specimens from HCC patients and liver specimens from non-HCC chronic liver disease patients (chronic HCV) and evaluate their clinical significance. **Materials and Methods:** This study was conducted on 50 participants (36 male, 14 female): 25 patients having HCV related chronic liver diseases without evidence of hepatocellular carcinoma and 25 patients with nodular hepatocellular carcinoma. All patients were selected from outpatient's clinic of Hepatology, Gastroenterology and Infectious diseases department, Benha University Hospital after approval by ethical committee of Benha faculty of medicine. **Results:** Methylated RASSF1A gene in liver specimens was detected in 76% of HCC cases in comparison to 12% of CLD (chronic HCV) group and the frequency of RASSF1A methylation was significantly higher in the HCC patients than CLD patients. Methylated P16 gene in liver specimens was detected in 68% of HCC cases in comparison to 28% of CLD group, and the frequency of P16 methylation was significantly higher in the HCC patients than CLD patients (chronic HCV). In the present study, the RASSF1A gene expression level was significantly lower in HCC patients who had methylated RASSF1A gene than those who had unmethylated RASSF1A gene. As regards P16 gene, its gene expression level was significantly lower in HCC patients who had methylated P16 gene than those who had unmethylated P16 gene. In the current study, RASSF1A methylation in tissue specimens of HCC group was analyzed by ROC curve showing area under the ROC curve (AUC) of 0.82, sensitivity and specificity of (76%) and (88%) respectively and its accuracy was 82%. As regards P16 methylation, P16 methylation in tissue specimens of HCC group was analyzed by ROC curve showing area under the ROC curve (AUC) of 0.70 , sensitivity and specificity of (68%) and (72%) respectively and its accuracy was 70%. **Conclusion:** The frequency of methylated genes (RASSF1A and P16) increases in a stepwise fashion from chronic hepatitis to peak in HCC that may suggest that epigenetic changes occur predominantly in the earlier stages of hepatocarcinogenesis. RASSF1A and P16 promoter hypermethylation play an important role in under expression of their related genes, but there was no significant relationship with the clinical staging of HCC. **KEYWORDS:** Hepatocellular carcinoma - tumor suppressor genes (p16INK4a and RASSF1A) - hypermethylation.

Introduction:

Hepatocellular carcinoma (HCC) is the fifth most common cancer and the third cause of cancer-related mortality worldwide. Its incidence is clearly arising comprised by the prevalence of major risk factors mainly hepatitis B and hepatitis C [1]. Hepatocellular carcinoma (HCC) is a major public health problem, accounting for about 600,000 deaths in the world in 2004 [2]. The burden of HCC has been increasing in Egypt, The annual proportion of HCC showed a significant rising trend from 4.0% in 1993 to 7.2% in 2002 [3]. This rising incidence of HCC in Egypt may be explained by the

increasing prevalence of risk factors such as the emergence of hepatitis C virus (HCV) over the same period of time [4] (Mostafa, 2004). Hepatocarcinogenesis is considered a multistep process involving subsequent mutations of genes subjected to continuous inflammatory and regenerative stimuli, starting from the initial phases of chronic hepatitis and then of liver cirrhosis [5]. HCC is known to be a result of the evolution process of a large number of genetic and epigenetic alterations, these alterations affect the proteins in certain major signaling pathways that control the cycle, proliferation, and cell survival [6]. DNA methylation is a type of epigenetic modification in the human genome, which means that gene expression is regulated without altering the DNA sequence, the relationship between methylation and cancer have been the focus of molecular biology researches [7]. RASSF1A is a tumor suppressor gene which belongs to Ras-Association Domain Family which comprises ten members from RASSF1 to RASSF10 [8]. RASSF1A regulates apoptosis via at least two pathways, overexpression of RASSF1A results in cell cycle arrest [9]. The evidence that RASSF1A is inactivated in a high percentage of human tumors is strong [8]. It has been proposed that since the RASSF1A methylation index showed a gradual increase from non-lesional liver to regenerative/hyperplastic conditions (chronic liver disease and focal nodular hyperplasia), to preneoplastic lesions to overt tumors, quantitative analysis of RASSF1A gene promoter methylation, rather than the detection of methylation bands per se, might be clinically relevant [10]. P16INK4A (also known as P16) a protein consisting exclusively of four ankyrin repeats, is recognized as a tumor suppressor mainly due to the prevalence of genetic inactivation of the p16INK4A (or CDKN2A) gene in virtually all types of human cancers. However, it has also been shown that elevated expression (up-regulation) of P16 is involved in cellular senescence, aging, and cancer progression, indicating that the regulation of P16 is critical for its function [11]. The relationship between p16 gene hypermethylation and the incidence of HCC has been verified by other studies that assessed p16 mRNA expression and its promoter CpG island methylation [12].

Patients and Methods:

This study was conducted on 50 participants (36 male, 14 female): 25 patients having HCV related chronic liver diseases without evidence of hepatocellular carcinoma and 25 patients with nodular hepatocellular carcinoma. All patients were selected from outpatient's clinic of Hepatology, Gastroenterology and Infectious diseases department, Benha University Hospital after approval by ethical committee of Benha faculty of medicine. The selected participants were categorized into two groups:

Group I: comprised 25 patients diagnosed as chronic liver disease (CLD) due to hepatitis C virus (HCV) affection based on clinical, laboratory, ultrasonographic examinations and / or liver biopsy.

Group II: comprised 25 patients with hepatocellular carcinoma (HCC) confirmed by spiral triphasic CT scan according to guidelines for the diagnosis and treatment of HCC [13].

Inclusion criteria-:

1. Patients with post hepatitic chronic liver disease due to HCV a ffection. 2- Patients with HCV related hepatocellular carcinoma.
2. Patients aging at least 18 years old.

Exclusion criteria-:

1. Patients aging less than 18 years old.
2. Any patient with any etiologic cause of chronic liver diseases other than chronic HCV infection as:
   - Co-infection with HBV. *Hemochromatosis. *Wilson`s disease. *Auto-immune liver disease. *Alcoholic liver disease.
   - Obesity induced liver disease. *Drug induced liver disease.

3. Patients with any cancer other than HCC.
4. Patient refusal.

All patients included in the present study (after getting an informed consent from each patient) were subjected to the following:

(1) Thorough history taking.
(2) Thorough clinical examination. 3-Routine laboratory investigations. 4-Radiological investigations.
5. Abdominal ultrasonography and Computed tomography)
6. Liver biopsies were done for patients in group (I) and group (II) admitted for tumor ablation" and taken for detection of methylated tumor suppressor genes by a semi-quantitative retro-

transcriptase polymerase chain reaction (PCR). The liver biopsies were stored at (-80°C) in sterile eppindorf tubes till further processing.

Molecular diagnosis which include:

A. Detection of promotor methylation status of RASSF1A and P16 in liver tissue using methylation specific PCR.

Detection of gene expression level of RASSF1A and P16 genes in liver tiaaue.

A. Detecting of promoter

methylation status of RASSF1A and P16 using methylation specific PCR:

1. DNA extraction from liver   biopsies.
2. Bisulfite conversion of extracted DNA.
3. Methylation specific PCR (MSP).

1. DNA extraction from liver biopsies: Using G-spin™ Total DNA Extraction kit (Korea INTRON Biotechnology) according to the manufacturer instructions [14].

2. Bisulfite conversion of extracted DNA: Using the EpiTect Bisulfite kit (QIAGEN®) according to the manufacturer instructions [15].

RASSF1A and P16 genes primers.

3. Methylation specific PCR (MSP): Using the EpiTect MSP kit (QIAGEN®) according to the manufacturer instructions [16].

B. Detection of gene expression level of RASSF1A and P16 genes in liver tissue

1. RNA extraction from liver biopsies.
2. cDNA synthesis using Reverse Transcription PCR (RT- PCR).
3. Quantitative PCR.

1.   RNA extraction from liver biopsies: by using the GF-Total RNA Extraction Kit (Vivantis, Eco-Life Science, Kowloon, Hong Kong) according to the manufacturer instructions [17].

2.   cDNA synthesis using RT PCR: Using Maxime RT Premix kit (Korea INTRON Biotechnology) according to the manufacturer instructions [18].

3.   Quantitative PCR: Using The Real MOD TM Real-time PCR master mix kit (Korea INTRON Biotechnology) [19], and the following primers for RASSF1A and P16 genes.

Gene	Forward	Reverse
RASSF1A	5'GCACTCTTTGAGCGAACTGA3'	5'AGCTCAGGGCTTTTTCACTG3'
P16	5' -CCCAACGCCCCGAACT-3'	5'GTGAACGTTGCCCATCATCA3'
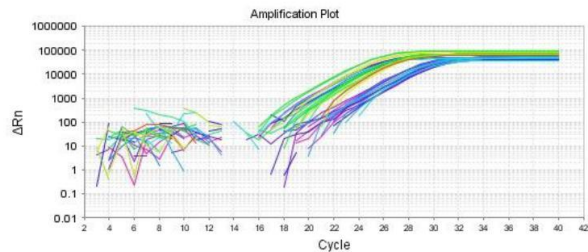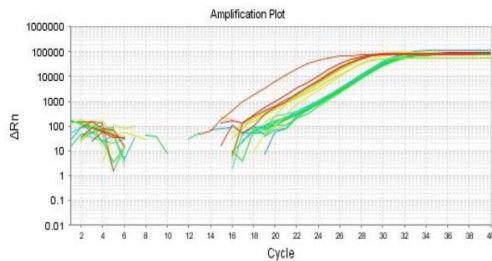
Results:

Table (1): Demographic data of the studied groups.

	Group I (CLD) N = (25)		Group II (HCC) N = (25)		P value
Age Range Mean ± SD	20-56 37.04±10.15		39-74 58.4±7.71		0.001 * S
	N	%	N	%	

Residence Urban Rural	6 10	24 76	5 20	20 80	0.733 NS
Occupation Farmer Non farmer	13 12	52 48	13 12	52 48	1.0 NS
Sex Male Female Male : female	. 18 7 2.5 : 1	. 72 28	. 18 7 2.5 : 1	 72 28	 1.0 NS

S= significant (p< 0.05), NS= non-significant (p > 0.05)

Table (2): RASSF1A methylation pattern in CLD and HCC groups.

	Group I (CLD) N = (25)		Group II (HCC) N = (25)		P value
	N	%	N	%	
Unmethylated	22	88	6	24	
Methylated	3	12	19	76	0.001 *S

Table (2) shows that the frequency of RASSF1A methylation was
significantly higher in the HCC patients than CLD patients. Methylated RASSF1A gene was detected in (76%) of patients with HCC in comparison to (12%) of CLD group.
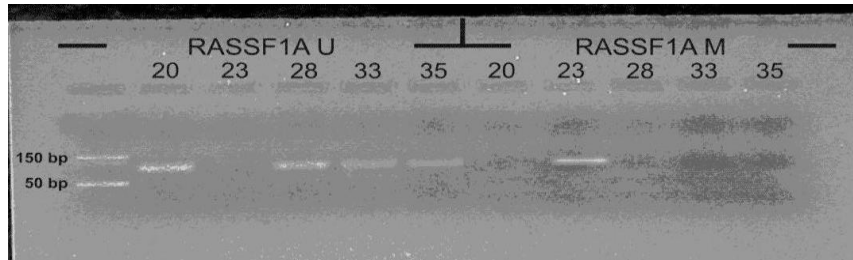
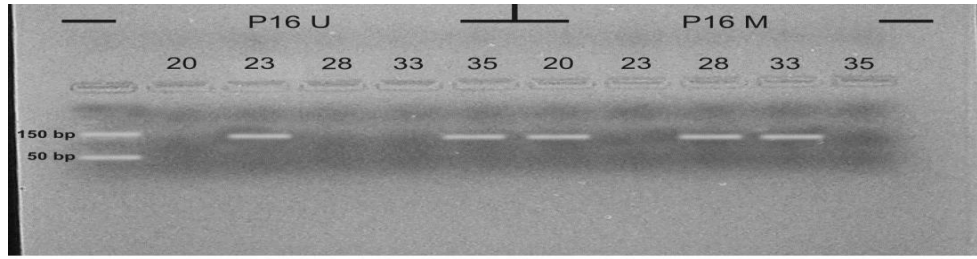*(U= Unmethylated, M= Methylated, bp= base pair)*

Fig. (3): Promotor methylation status of RASSF1A gene in selected samples.

Sample (23) shows methylated RASSF1A gene, while other samples showing the unmethylated status of RASSF1A gene.

Table (3): P16 methylation pattern in CLD and HCC groups.

P16	Group I (CLD) N = (25)		Group II (HCC) N = (25)		P value
	N	%	N	%	
Unmethylated	18	72	8	32	0.005
Methylated	7	28	17	68	

Table (3) shows that the frequency of P16 (INK4A) methylation was significantly higher in the HCC patients than CLD patients. Methylated P16 gene was detected in (68%) of patients with HCC in comparison to (28%) of CLD group.

Fig (4): P16 methylation pattern in CLD and HCC groups.
(U=Unmethylated, M= Methylated, bp= base pair).

Table (4): Relationship between RASSF1A methylation pattern and its gene expression level in HCC group.

HCC group	Unmethylated RASSF1A (6)		Methylated RASSF1A (19)		P value	Significance
Variant	Mean	±SD	Mean	±SD		
RASSF1Agene expression level (RQ)	0.131	0.051	0.045	0.032	0.001	S

Table (4) shows that the gene expression level patients was significantly lower in HCC who had methylated RASSF1A gene (0.045±0.032 RQ) than HCC patients who had unmethylated RASSF1A gene (0.131± 0.051RQ).

**Table (5): Relationship between P16 methylation pattern and its gene expression level in HCC group.**

HCC group	Unmethylated P16 (8)		Methylated P16 (17)		P value	Significance
Variant	Mean	±SD	Mean	±SD		
P16gene expression level (RQ)	0.929	0.039	0.498	0.117	0.005	S

Table (5) shows that the gene expression level was significantly lower in HCC patients who had methylated P16 gene (0.498±0.117) than HCC patients who had unmethylated P16 gene (0.929±0.039).

Table (6): Sensitivity and specificity of RASSF1A methylation (ROC curve) in HCC group.

AUC	0.82
Sensitivity	76%
Specificity	88%
PPV	86.4%
NPV	78.6%
Accuracy	82%

Table (6) shows that the sensitivity of RASSF1A methylation in HCC group was (76%) and the specificity was (88%).
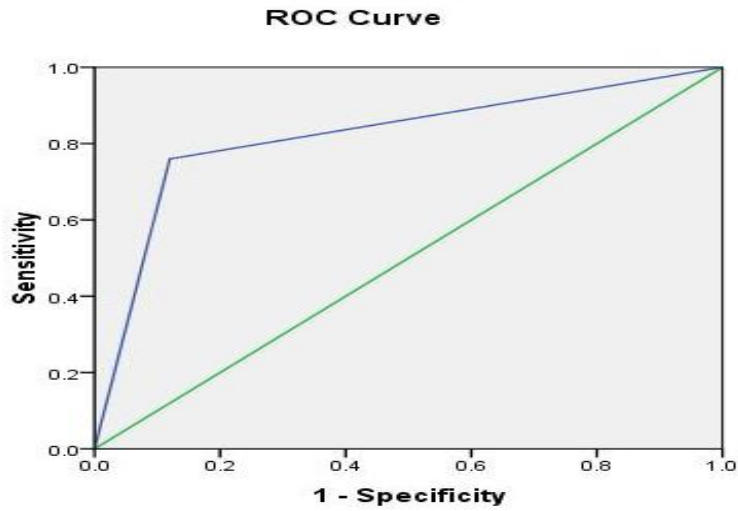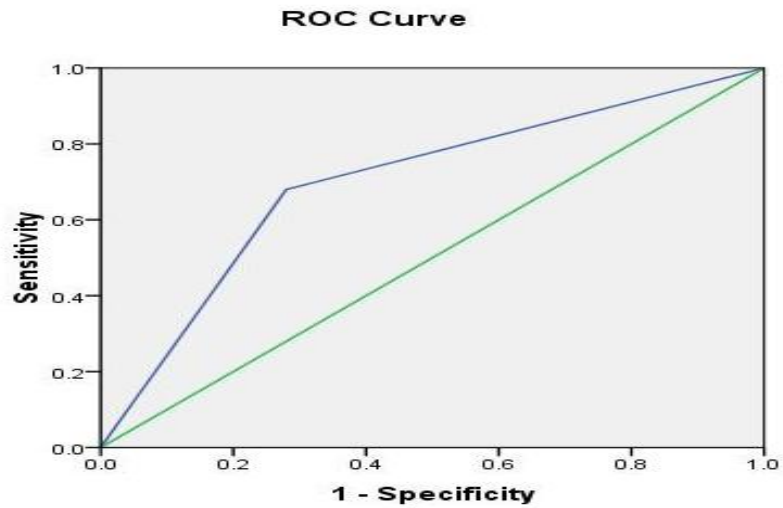
ROC Curve

Diagonal segments are produced by ties.

Table (7): Sensitivity and Specificity of P16 methylation (ROC curve) in HCC group.

AUC	0.70
Sensitivity	68%
Specificity	72%
PPV	70.8%
NPV	69.2%
Accuracy	70%

Table (7) show that the sensitivity of P16 methylation in HCC group was (68%) and the specificity was (72%).

ROC Curve

Diagonal segments are produced by ties.

Discussion:

Hepatocellular carcinoma (HCC) is the fifth most common cancer worldwide and the third cause of cancer-related mortality. Its incidence is clearly rising comprised by the prevalence of major risk factors mainly hepatitis B and hepatitis C [1].The incidence of HCC increases progressively with advancing age in all populations, reaching a peak at 70 years [20], [21]. In Egypt, the annual proportion of HCC showed a significant rising trend from 4.0% in 1993 to 7.2% in 2002 [5]. This rising incidence of HCC in Egypt may be explained by the increasing prevalence of risk factors such as the emergence of hepatitis C virus (HCV) over the same period of time [4]. Early HCC diagnosis is feasible in 30–60% of cases in developed countries and this enables the application of curative treatments [22]. Patients diagnosed at an early HCC stage are optimal candidates for resection, liver transplantation or percutaneous ablation [13]. Hepatocarcinogenesis is considered a multistep process involving subsequent mutations of genes subjected to continuous inflammatory and regenerative stimuli, starting from the initial phases of chronic hepatitis and then of liver cirrhosis [5]. Aberrant promoter hypermethylation of tumor suppressor genes is a common event during the pathogenesis of human cancers and one of the important epigenetic mechanisms in carcinogenesis. It has been shown that methylation of multiple tumor suppressor genes in HCC may contribute to the pathogenesis of this disease [23]. In the current study, the mean age of patients with HCC was (58.4±7.71) years, ranging from (39-74). This result agreed with [24], who reported in a study including 215 consecutive patients with hepatocellular carcinoma that, the mean age of HCC patients was 58 years . Also, Atta and his colleagues reported in another Egyptian study including 41 HCC patients that, the mean age of HCC patients was 57.95 ± 8.41 years [25]. Similarly, [26] reported in a large study including 1305 patients that the mean age of HCC patients was 58.4 years On the other hand, [21] reported that the age incidence of HCC is higher in Japan (70–79 years). This difference may be partially attributed to the distribution of the risk factors among patients with hepatocellular carcinoma which is highly variable, depending on geographic region, race or ethnic group [27]. In the current study, HCC is presented more frequently in males than females with a male to female ratio (2.5:1). This male predominance came in

agreement with Keng and his colleagues who reported that the universal estimated male/female ratio of HCC is (2.5:1) [28]. Male predominance for HCC development was reported also by [29], who concluded that, the male to female ratio in HCC incidence is about (2.4: 1). [25], coincided with this result and reported male to female ratio of (3.6 : 1) , also [30] pronounced male predominance of hepatocellular carcinoma worldwide. On the other hand, [31] reported a non significant difference in sex distribution between HCC patients.Higher HCC rates among males is related to their greater exposure to the different risk factors for chronic liver disease, such as HCV infection and to environmental carcinogenic factors[27]. In addition, an experimental study on rats demonstrated that male animals had a two to eight times higher chance to develop HCC, possibly supporting the hypothesis that androgens may influence the progression of HCC and that exposure to risk factors may not be the only feature related to predominance of the disease among males [32]. Among the tumor suppressor genes studied previously, the Ras association domain family 1A (RASSF1A) gene has been extensively investigated. RASSF1A is located at 3p21.3 and is implicated in the Ras signaling pathway, which plays a pivotal role in cell cycle control, microtubule stabilization, cellular adhesion, cell motility, and apoptosis. Loss of RASSF1A expression is one of the most common events in human cancer, with aberrant promoter methylation reported in a variety of tumor types, including HCC [33]. During the past decade, there have been an increasing number of investigations focusing on the role of RASSF1A promoter methylation in HCC [34]. P16 gene is another important tumor suppressor gene which located on chromosome 9p21 and it is one of the most frequently altered genes observed in various human neoplasms. The inactivation of the p16 gene resulting from methylation of the p16INK4A gene, has been reported in HCC which leads to disruption of p16-mediated cell cycle control and play a role in hepatocarcinogenesis [12]. In the current study, methylated RASSF1A gene in liver specimens was detected in 76% of HCC cases in comparison to 12% of CLD (chronic HCV) group and so the frequency of RASSF1A methylation was significantly higher in the HCC patients than CLD patients . This result goes in agreement with [35] , who stated that the methylation frequency for RASSF1A was

higher in HCC than in (chronic hepatitis/cirrhosis) patients and [36], who reported in a study including 47 HCC patients that methylation rate of RASSF1A gene was 63.8%. Also [37] , reported that RASSF1A promoter methylation was detected in 31 cases (88.6%) in 35 HCC specimens. Similarly,[38] reported that RASSF1A gene was methylated in (88%) of HCC cases and Lia and his cooleages reported methylation prommotor of RASSF1A gene in 78–95% of the studied HCC cases [39]. The methylation of RASSF1 gene which present in 12% of HCV related chronic liver disease cases could be explained by the fact that, chronic HCV infection may act as a powerful epi-mutagen that induce methylation and accelerate the methylation process during hepatocarcinogenesis. So some degree of methylation was demonstrated in HCV cases but less frequent than HCV related HCC cases [40]. In the current study, methylated P16 gene in liver specimens was detected in 68% of HCC cases in comparison to 28% of CLD group, and the frequency of P16 methylation was significantly higher in the HCC patients than CLD patients (chronic HCV). This was in agreement with [41], who observed higher frequency of p16 methylation in HCV related HCC (65%). Also, [42] reported that methylation of p16 promoter was detected in 35 (69%) of 51 HCC cases. In concordance with this result, [43], in an Egyptian study compared tumor methylation profile for the tumor suppressor gene p16 in tumor tissues and plasma. To evaluate the concordance between the two types of specimen from the same HCC patients and found high methylation frequency of p16 in the plasma and tissues from HCV-associated HCC Egyptian patients (67.9%). Also, [44] in another Egyptian study showed that p16 methylation was detected in 45.2% of HCC cases (14 from 31 cases). Similar results were also reported by other studies which showed high frequency of P 16 methylation, [45] (82.7%), [46] (62.5%), [47] (72.7%), and [48] (82%). The results reported by [49] showed that p16 hypermethylation was present in 58% (29 of 50) of the examined HCCs and 16% (6 of 38) of the preneoplasic lesions (including chronic hepatitis and cirrhotic tissues). And stated that aberrant p16 methylation may contribute to hepatocarcinogenesis from early stages and persistent hepatitis virus infection may play a role in the induction of p16promoter methylation in hepatocarcinogenesis. Also [50], reported that promoter methylation of different kinds of tumor suppressor genes including *p16*

and *RASSF1A*, has been demonstrated in premalignant conditions as chronic hepatitis or liver cirrhosis. Moreover, the frequency of aberrant promoter methylation increases during the progression from precancerous lesion to HCC. In this study, there was no statistically significant relationship between RASSF1A methylation pattern and age in HCC group. These results agreed with [37] and [36] , who reported that there was no association between tissues DNA RASSF1A methylation and age . Also, [51] agreed these results concerning age and showed that no relationship was apparent between RASSF1A methylation and patient′ age. On the other hand, [52] reported that RASSF1A methylation was higher in younger HCC cases. Concerning correlation with P16 gene and the age, it was significantly higher in HCC patients who had methylated P16 gene (62.24±5.36) than HCC patients who had unmethylated P16 gene (50.25±5.15). This result was in agreement with [41] and [53] who reported that p16 methylation was more frequently observed in elderly HCC patients. Similar results were also reported by [49] who showed that hypermethylation of the p16 was more frequent in HCCs from older patients. On the other hand, [54] disagreed with these results and showed no relationship between age and P16 methylation. This difference may be attributed to the fact that the mean age of HCC cases in their study was younger (48 years) than the present study (58.4 years). In the present study, there was no statistically significant relationship between RASSF1A methylation pattern and sex in HCC group. This result goes in agreement with [55], who reported no association between RASSF1A methylation and patient′ s sex. In the present study, there was no statistically significant relationship between P16 methylation pattern and sex in HCC group. This result goes in agreement with [54], who showed that no significant correlation was found between abnormal P16 methylation in HCC and gender. In the current study, there was no statistically significant relationship between RASSF1A methylation pattern and characters of focal lesion (number and diameter) in HCC group. These results go in agreement with [37] and [36], who reported the same results.On the other hand, [52] found that methylation level of RASSF1A gene was correlated with the tumor size and showed that HCC tumor larger than 6 cm had higher methylation frequency. This difference may be related to the fact that none of the focal lesion of studied HCC cases exceed 6 cm in size in this study.

Also, [56] disagreed with these results and reported that the promoter methylation of RASSF1A in HCC cases was correlated to the tumor size. This difference may be attributed to the fact that this correlation was observed with serum methylated RASSF1A and not with the tissue RASSF1A as in this study. In the current study, there was no statistically significant relationship between RASSF1A methylation pattern and level of alfa fetoprotein. Xu and his colleagues (2013) agreed these results and stated that there is no correlation between RASSF1A methylation pattern and level of alfa fetoprotein [52]. Similar results were reported by [53], [37], [36]. Concerning survival of HCC cases, there was no statistically significant relationship between RASSF1A methylation pattern with the overall and progression-free survival of patients in HCC group. This was in agreement with [52] who concluded that hypermethylation of the RASSF1A promoter in HCC tissues did not affect the overall survival of patients. Also, [53] and [51] stated that there is no difference in the overall and progression- free survival between patients with and without RASSF1A hypermethylation. This study showen no statistically signficant relationship between characters of focal lesion (number and diameter) in HCC group which came in agreement with [53], whose results showed that neither tumor number nor diameter had correlation with P 16 methylation. Also, [41] and [57] reported no assosiation between diameter of focal lesion and P16 methylation. In the current study, there was no statistically significant relationship between P16 methylation pattern and level of alfa fetoprotein. This goes in agreement with [49] and [53]) who reported the same results. As regards survival of HCC cases, there was no statistically significant relationship between P16 methylation pattern with survival of patients in HCC group. This result was consistent with [58], who performed a systematic review and meta-analysis of studies assessing the impact of p16 methylation on overall survival and progression free survival to clarify this issue. This study found that p16 hypermethylation did not have significant correlation with survival of hepatocellular cancer but had significant association with poor survival of non-small cell lung cancer (NSCLC) and colorectal cancer. Also, [53] showed that the relationship between overall or progression free survival and the methylation status of P16 gene was not statistically significant. In the present study, the RASSF1A gene expression level was significantly lower

in HCC patients who had methylated RASSF1A gene than those who had unmethylated RASSF1A gene. This result goes in agreement with [37] who reported that the gene expression level of RASSF1A is reduced and lost in HCC by promoter methylation. Similar results were also reported by [59] and [60]. As regards P16 gene, its gene expression level was significantly lower in HCC patients who had methylated P16 gene than those who had unmethylated P16 gene. This result was in agreement with [12], who reported that p16 gene methylation might play an important role in p16 gene inactivation, similar results were also reported by [47] and [48]. In the current study, RASSF1A methylation in tissue specimens of HCC group was analyzed by ROC curve showing area under the ROC curve (AUC) of 0.82, sensitivity and specificity of (76%) and (88%) respectively and its accuracy was 82%. These results were nearly similar to [36], who showed that sensitivity the specificity of RASSF1A methylation tissue specimens of HCC cases was (61.7) and (85.1) respectively with AUC (0.802) .These results indicated RASSF1A methylation in liver tissue might be a useful diagnostic biomarker of HCC patients with HCV-related CLD. As regards P16 methylation, P16 methylation in tissue specimens of HCC group was analyzed by ROC curve showing area under the ROC curve (AUC) of 0.70 , sensitivity and specificity of (68%) and (72%) respectively and its accuracy was 70%.These results indicated P16 methylation in liver tissue might be a useful diagnostic biomarker of HCC patients with HCV-related CLD. There is no available data for sensitivity and the specificity of P16 methylation in liver tissue to compare with these results , but [61], reported that the sensitivity and the specificity of P16 methylation in serum was 84% and 94%, respectively with AUC (0.89). This study concluded that, the frequency of methylated genes (RASSF1A and P16) increases in a stepwise fashion from chronic hepatitis to peak in HCC, which may suggest that epigenetic changes occur predominantly in the earlier stages of hepatocarcinogenesis. These molecular changes may be a valuable biomarker for early detection of HCC, risk assessment in high-risk populations and provide clues to develop potential prevention strategies for the subset of HCC that develop through the epigenetic pathway. RASSF1A and P16 promoter hypermethylation play an important role in under expression of their related genes,

but there was no significant relationship with the clinical staging of HCC.

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الملخص العربى

يعد سرطان الكبد من أكثر الاورام الخبيثة انتشارا حُيث يحتل المرتبة الخامسة على مستوى العالم، والمرتبة الثالثَّة فيَّ مصر. يُختلف معدل انتشار المرض من منطقة إلى أخري تبعا للعدُيد من العوامل مثل انتشار الفُيروسات الكبدُية و التعرض لبعض المواد المسرطنة، وُيعد التشخُّص المبكر للإصابة بسرطان الكبد أحد أهم عوامل النجاح فى العلاج. وقد ثبت حدوث اختلال فى نشاط الجُينات المثبطة للأورام التى تسبب أمراض السرطان بما فُيها سرطان الكبد.

فقد لوحظ اختلال وظُيفة العدُيد من الجُينات المثبطة للسرطان على سبيِّل المثال جُين RASSF1A وجين P16 أحد الجُينات التى غالبا ماّتم تثبيُطها نتُيجة عملُية مِيثِّلة الـ DNA ويشترك هذا الجُين فى تنيظُم العدُيد من العمليات الحيُوية داخل الخلُية مثل دورة الخلُية ،الانقسام المِيتُوزي،انتحار الخيلُية المنظم وغُيرها من العملُيات المهمة على الهُيكل الخلوي والمحتوى الجُيئِى للخلية وقد وجد أن عملُية زيادة مِيثِّلة جُين RASSF1Aتسبب اختلال وظُيفته مما يُنتج عنه حدوث طفرات جُيِنية قد تؤدى إلى حدوث العدُيد من الامراض السرطانية منها سرطان الكبد.

و كذلك يشارك جُين P16 أُيضا فى تنظُيم العديد من العمليات الحيُوُية داخل الخلُية مثل دورة الخلُية ولهذا فإن توقف هذا الجُين عن العمل نتُيجة زيادة عملية المِيثِّلة تؤدى إلى ظهور الخلُايا السرطايُة ونموها.

ونتُيجة لذلك فانَّ التغيُّر فى عملُية مِيثِّلة الجُينات قد يمثل أحد الوسائل التىّيمكن تطبيقُها من أجل إتاحة التشخُّيص المبكر أو متابعة أمراض السرطان وذلك عن طرُيق استخدام الخلايا السرطانيُّة ذاتها.

الهدف من البحث:

وقد كان الهدف من الدراسة الحالَّة هو تحدُيد مستوى مِيثِّلة DNA الخاص بجُين RASSF1A وكذلك جيُن P16 فى الخلايا السرطائُية ، ودراسة مدى ارتباط زيادة عملُية المِيثِّلة بمختلف المتغُيرات الاكليُنِّكية فى مرضى سرطان الكبد و كذلك تحدُيد المستوى الجُينى الناتج عن عِيملُة المِيثِّلة.

خطوات البحث:

تم إجراء هذه الدراسة على 25 مرُضى مصابُين بسرطان الكبد، تم اخيُارهم من مرضى قسم الكبد والجهاز الهضمىَّ والامراض المعدُية بمستشفى بنها الجامعىَّ، بالاضافة إلىَّ 25 مرُيضا آخرُين من مرضى الكبد المزمن الالتهاب الكبدى بفُيروس (س).

جميُع المرضَّى تم فحصهم إكليُنِّكيًا ومعملُيا بصورة دم كاملة، ووظائف كبد و كلَّ دلالات الفُيروسات الكبدُية، نسبة البروثرومبيُن، وفحص بالموجات فوق الصوتُية على البطن ، كما تم التأكد من جميُع حالات سرطان الكبد بالاشعة المقطعُة ثلاثية المراحل. أخُيرا تم فحص جميُع المشاركين عن طرُيق اجراء عُينة كبدية من البؤرة الورميَّة للكبد و ذلك لتحدُيد مستوى مِيثِّلة DNA الخاص بجُين RASSF1A وكذلك جيُن P16فى الخلايا السرطانيُّة و كذلك تحدُيد المستوى الجُينى الخاص بهما.

نتائج البحث:

أوضحت هذه الدراسة أن هناك زُيادة ذات دلالة إحصائُة فى مستوى مِيثِّلة كل من جُين RASSF1A و جيُنP16 فى المصابُين بسرطان الكبد وذلك عند مقارنتهم بالمجموعة الخاصة بمرضى الكبد المزمن الفُروسى (س) ،كما اظهرت الدراسة عدم ارتباط عمليُة المِيثِّلة بمختلف المتغُيرات الإكليُنِّكيُة فى مرضى سرطان الكبد كما اظهرت الدراسة أُيضا تأثُير مِيثِّلة DNA الخاص بجيُن RASSF1A وكذلُك جيُن P16 فى الخلايا السرطانيُّة على انخفاض المستوى الجُينى الخاص بهما.

الخلاصة:

ونستخلص من هذه الدراسة أن عميلُة مِيثِّلة DNA فى كل من جيني RASSF1A و P16 تؤدي إلى اختلال وظيفة الجُينات المثبطة للسرطان وكذلك تلعب دورا مؤثرا فى عميلُة بدّاية وتكاثر الخلايا السرطانيُة.