Chapter 4

The Molecular Background Associated with the Progression of Hepatitis C to Hepatocellular Carcinoma

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Additional information is available at the end of the chapter

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Abstract

Hepatocellular carcinoma (HCC) is a major health problem worldwide. The DNA PM of cancer-related genes plays an important role in the development and progression of HCC. The data reported in our studies provide evidence that PM of p73, p14, and O6-MGMT is associated with HCC, whereas PM of the APC gene is more common in chronic hepatitis (CH) cases. Thus, it could be used as a maker for early detection of HCV-induced chronic active hepatitis. A panel of four genes APC, p73, p14, and O6-MGMT independently affected the classification of cases into HCC and CH with accuracy (89.9%), sensitivity (83.9%), and specificity (94.7%). Also, the detection of PM of APC, FHIT, p15, p16, and E-cadherin in peripheral blood of HCV-infected patients is a highly sensitive and specific. Therefore, blood could be used as efficiently as tissue biopsies to assess PM of different genes. This could help in the follow-up of CH patients and early detection of HCC. We did not observe a significant difference in the methylation status according to the virus type HBV versus HCV. So, plasma DNA is a reliable resource for methylation studies in the future, irrespective of the type of hepatitis infection.

Keywords: hepatitis C virus-genotype 4, chronic hepatitis, hepatocellular carcinoma, promoter methylation

1. Introduction

Hepatocellular carcinoma (HCC) is a major health problem and it is the third most common cause of cancer-related death worldwide [1]. In Egypt HCC ranks the first in males and the second in females after breast cancer. It accounted for 33.6% in males and 13.5%

in females [2]. This high incidence of HCC is attributed to the high prevalence of HCV infection, especially genotype 4 in Egypt [3]. HCV infection has an estimated global prevalence of 2.5%, causing chronic liver disease in about 170 million people worldwide [4]. Although it has been estimated that 80% of HCC occurs in cirrhotic livers, the underlying molecular mechanisms of virus associated hepatocarcinogenesis are still unclear [5]. It has been suggested that HCV-encoded proteins may contribute to tumor progression through their direct and indirect interactions with host hepatic cells. Additionally, the generated status of chronic HCV inflammation is accompanied by immune-mediated destruction of infected hepatocytes, oxidative stress, virus-induced apoptosis and DNA damage leading to genomic instability and continuous regeneration that may be incorporated in liver cancer development [6].

Previous studies demonstrated that DNA methylation has a major role in the initiation and progression of various types of human cancers [7, 8]. Aberrant promoter methylation of tumor suppressor genes (TSGs), such as P14 or O6-methylguanine-DNA methyltransferase (O6MGMT) has been reported in relation to HCC development [9].

The term DNA methylation refers to the addition of a methyl group to the cytosine residue in the CpG islands. Normally, CpG islands are not methylated regardless of their transcriptional status, and methylation of the promoter regions of tumor suppressor genes (TSGs) or growth regulatory genes resulted in silencing of those genes, and cancer development. Since it was proven that different types of cancer showed distinct DNA methylation profiles, thus it could be possible to develop specific methylation signatures for those types of cancer [10].

The power of PM as a molecular marker is the ability of detecting its presence in a variety of sample types including fresh specimens, body fluids and archival paraffin-embedded tissues, as well as to the defined localization of the lesion in the CpG islands of the genes. Promoter methylation could be an important early event in the cascade of carcinogenesis and it can also be of important as prognostic and predictive marker [11]. The DNA methylation profiles in HCV-infected patients from Egypt have not been well studied yet, although it has the highest prevalence of HCV infection worldwide with approximately 14% of the population infected [12].

2. Concordance between tissue and plasma DNA methylation in HCC patients

Owing to the crucial effects of DNA promotor methylation in the development and progression of HCC, we investigated the role of DNA methylation events in the tissues of HCC patients for using five tumor suppressor genes: APC, FHIT, p15, p16, and E-cadherin. We also assessed the DNA methylation patterns of these genes in the plasma from the same patients and compared the tissue and plasma patterns [13]. This was done to investigate the concordance between tissue and plasma methylation patterns in Egyptian patients with HCV and/or HBV- associated HCC. Although liver biopsy is the current gold standard for detecting methylation events, imaging techniques are usually sufficient for liver cancer diagnosis and

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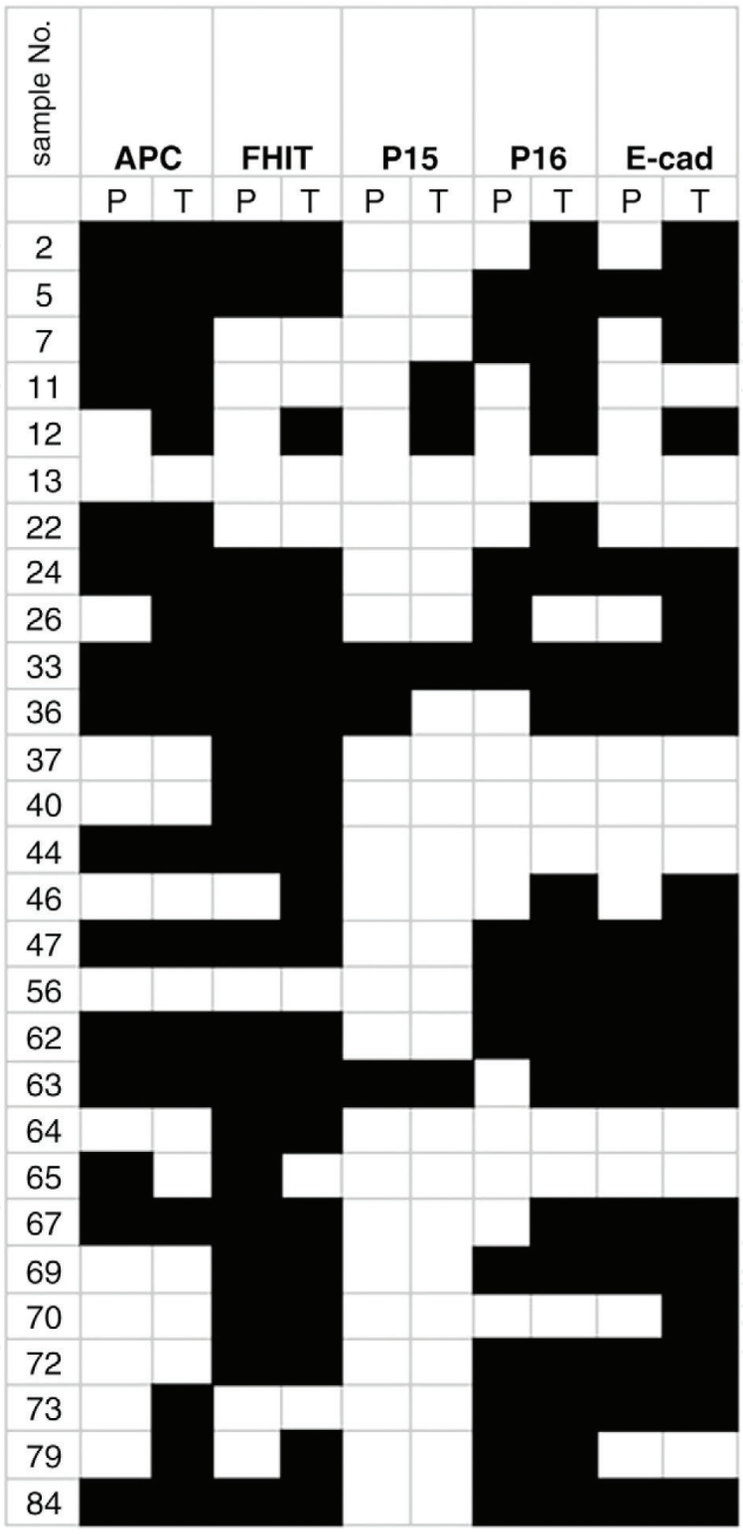

Figure 1. Summary of methylation analysis of APC, FHIT, p15, p16, and E-cadherin in 28 HCC samples and the corresponding plasma. Filled boxes indicate the presence of methylation and open boxes indicate the absence of methylation. T, tumor tissue; P, plasma.

therefore the need of tissue biopsy decreased markedly [14]. So it was essential to search for another tool for detection of promotor methylation in HCC by a simpler, easy and reliable technique.

Genes	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	*p*-Value
APC	77.8	90.0	93.3	69.2	0.001*
FHIT	85.7	85.7	90.5	66.7	0.001*
p15	50.0	95.8	66.7	92.0	0.045*
p16	60.0	87.5	92.3	46.7	0.037*
E-cadherin	68.4	100.0	100.0	60.0	0.0008*

PPV, positive predictive value; NPV, negative predictive value.*The concordance was significant for all five genes.

Table 1. The concordance between plasma DNA and tissue DNA.

We collected paired blood and tissue samples from 28 HCV and/or HBV- associated HCC patients from Egypt. DNA was extracted from those patients (tissue and blood) and the promotor methylation for ***APC, FHIT, p15, p16, and E-cadherin*** tumor suppressor genes were assessed using the EZ DNA Methylation-Direct TM Kit according to manufacturer's protocol.

We reported a statistically significant concordance between plasma and tissue methylation profiles [13]. The frequency of promoter methylation in tissue and plasma samples for the five tumor suppressor genes was as follows: ***APC*** promoter methylation was accounted for approximately 64.2% for tissue (18/28), and 53.5% for plasma (15/28), ***FHIT*** promoter methylation was accounted for 75.0% for tissue (21/28), and 67.8% for plasma (19/28), ***p15*** promoter methylation was accounted for 14.2% for tissue (4/28), and 10.7% for plasma (3/28), ***p16*** promoter methylation was accounted for approximately 71.4% for tissue (20/28) and 46.4% for plasma (13/28), and ***E-cadherin*** promoter methylation was accounted for 67.8% for tissue (19/28) and 46.4% for plasma (13/28) (**Figure 1**).

Although detection of promoter methylation in the plasma DNA was highly specific, it was not as sensitive for the matching change in tissue DNA, suggesting that DNA promotor methylation in tissues might originate in tumor cells before appearing in the vascular spaces (blood or plasma). The positive predictive value (PPV) was higher than the negative predictive value (NPV) for ***APC, FHIT, p16,*** and ***E-cadherin*** whereas, the negative predictive value was higher for ***p15*** (**Table 1**). Therefore, a previous study by Huang et al., [15] concluded that it may be useful to combine the plasma DNA methylation status of ***ELF, RASSF1A, p16***, and ***GSTP1*** with serum AFP for HCC screening and several studies had confirmed these data [16, 17]. However controversial results were reported by Chang et al. [18] who found no agreement between plasma and tissue DNA samples. One possible explanation for the controversy in the results between the previously mentioned studies could be the small sample size in the study of Chang et al. (eight HCC patients only) and/or the use of RT-PCR which causes DNA degradation during amplification.

3. Methylation profile and viral status

Another interesting finding observed is that, there was no significant correlation between HBV or HCV infection and the incidence of promoter methylation, to suggest whether the viral status could be used to predict methylation and subsequent gene silencing for the five

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	HBV	HCV	HBV infection type*
APC	0.107	0.634	0.508
FHIT	0.545	1	0.508
p15	0.481	1	0.288
p16	0.295	0.639	1
E-cadherin	0.273	0.629	0.66

The methylation profile was not significantly associated with the HBV, HCV, or *HBV Infection type: past infection or immune.

Table 2. Statistical association of hepatitis viral status and promoter methylation.

aforementioned tumor suppressor genes [13]. Therefore, plasma DNA could be used as a reliable source for methylation detection in HCC patients irrespective of the type of hepatitis viral infection (**Table 2**).

4. Increasing DNA promoter methylation is associated with disease progression from chronic hepatitis C to cirrhosis and hepatocellular carcinoma

As a continuation of our previous studies, which showed a concordance between tissue and plasma DNA methylation, and hence the validity of using plasma DNA methylation profile as a marker for HCC [13], we had assessed the methylation frequency of three tumor suppressor genes (***P14, P15***, ***P73***) and a mismatch repair gene ***(O6MGMT)*** in the plasma of 516 Egyptian patients with HCV-related liver disease, during the period from 2010 to 2012, to identify candidate epigenetic biomarkers for prediction of HCC [19]. Subjects were divided into 4 clinically well-defined groups as follow: the HCC group (n = 208), liver cirrhosis group (LC; n = 108), chronic hepatitis C group (CH; n = 100), and normal control group (NC; n = 100). The methylation status of the target genes was analyzed in patients' plasma using EpiTect Methyl qPCR Array technology. According to the manufacturer's instructions, the four studied genes ***(P14, P15, P73 and O6MGMT)*** were considered methylated if >10% and intermediately methylated if >60%.

We found significant differences in the frequency of PM of all studied genes within the different stages of chronic liver disease and HCC (**Table 3** and **Figure 2**). The methylation frequency of ***P14*** gene was 48.1% (100/208) in HCC, 48.1% (52/108) in LC, 16% (16/100) in CH and 8% (8/100) in NC. Out of the studied patients 32/208 (15.4%), 16/108 (14.8%) and 8/100 (8%) were intermediately methylated in HCC, LC and chronic hepatitis C groups respectively, with a statistically significant difference between the studied groups (p = 0.008). Accordingly, p14 is preferentially methylated in HCV related HCC [20].

As for ***p15***, the methylation frequency was 44.2% (92/208) in HCC, 33.3% (36/108) in LC, 20% (20/100) in CH and 4% (4/100) in NC. While intermediate methylation was found in 32/208 (15.4%) of HCC, 20/108 (18.5%) in LC, in 8/100 (8%) CH and 4/100 (4%) in NC with a statistically significant difference between the studied groups (p = 0.006).

Gene	HCC	HCV with liver cirrhosis	Chronic Hepatitis C	Control	p value
	n = 208(%)	n = 108(%)	n = 100(%)	n = 100(%)	
P14	M 100 (48.1)	52 (48.1)	16 (16)	8 (8)	0.008
	U 108 (51.9)	56 (51.9)	84 (84)	92 (92)	
P15	M 92 (44.2)	36 (33.3)	20 (20)	4 (4)	0.006
	U 116 (55.8)	72 (66.7)	80 (80)	96 (96)	
O6MGMT	M 84 (40.4)	60 (55.6)	20 (20)	4 (4)	<0.001
	U 124 (59.6)	48 (44.4)	80 (80)	96 (96)	
P73	M 136 (65.4)	72 (66.7)	32 (32)	4 (4)	<0.001
	U 72 (34.6)	36 (33.3)	68 (68)	96 (96)	

Methylated (M); Unmethylated (U).

Table 3. Methylation frequency of P14, P15, O6MGMT and P73 genes in different studied groups.

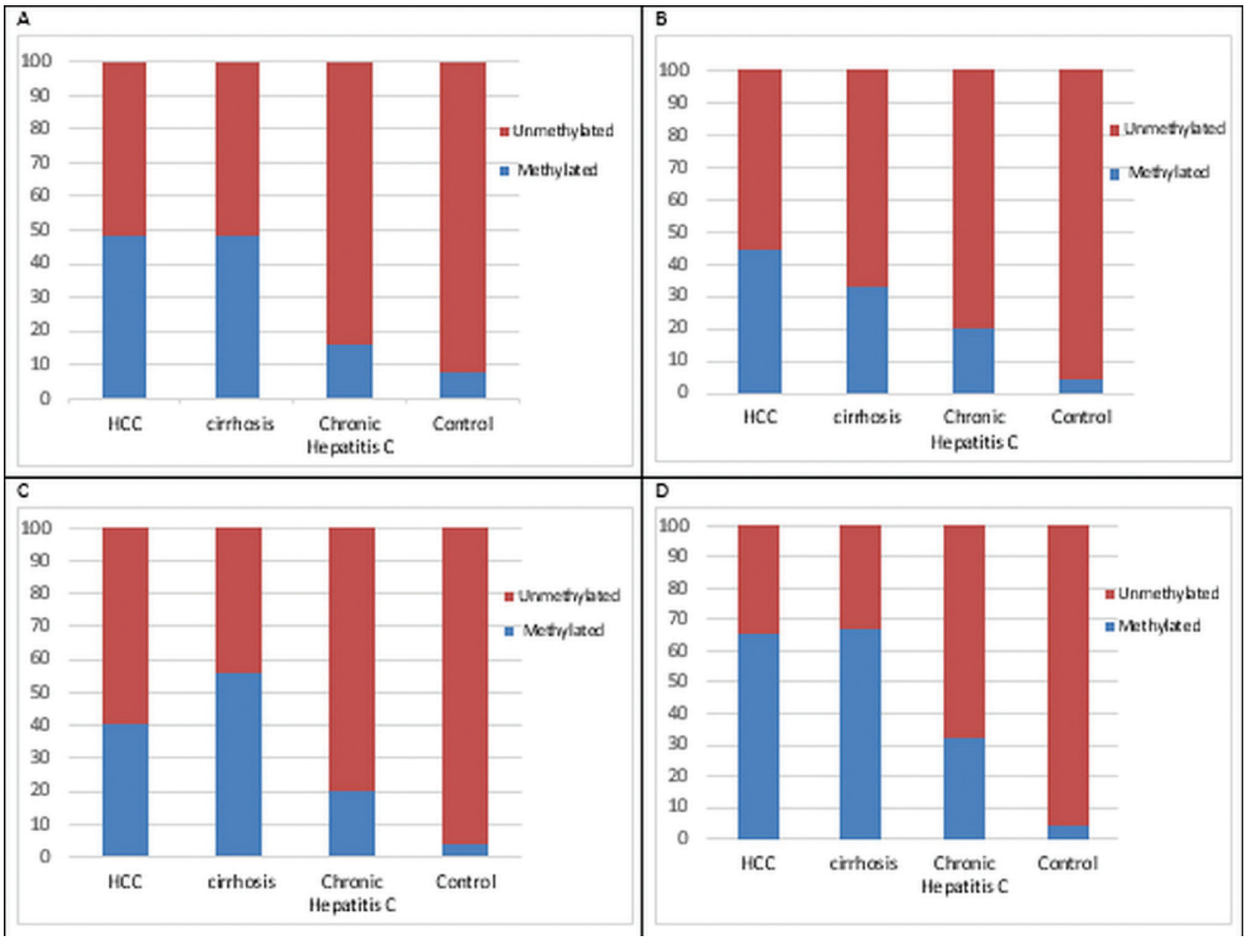

Figure 2. Methylation frequency of (A) P14 gene; (B) P15 gene; (C) O6MGMT gene; and (D) P73 gene in the studied groups.

The methylation frequency of ***O6MGMT*** gene was 40.4% (84/208) in HCC, 55.6% (60/108) in LC, 20% (20/100) in CH and 4% (4/100) in NC. While intermediately methylated in 48/208 (23.1%) in HCC and 36/108 (33.3%) in LC, with a statistically significant difference between the studied groups (p value<0.001).

The methylation frequency of ***P73*** gene was detected in 65.4% (136/208) in HCC, 66.7% (72/108) in LC, 32% (32/100) in CH and 4% (4/100) in NC. While intermediate methylation was found in 88/208 (42.3%) in HCC, 56/108 (51.9%) in LC, 24/100 (24%) in CH and 4/100 (4%) in NC, with a statistically significant difference between the studied groups (p value<0.001). Statistically significant differences were reported among the four studied groups regarding the PM of all studied genes (**Table 4**).

Thus, it could be concluded that, the methylation frequency increases with the progression of liver disease and thus it that could be used to monitor whether a patient with chronic hepatitis C is likely to progress to liver cirrhosis or even HCC or not. Moreover, the process of PM does not represent an early event in hepatocarcinogenesis cascade but it increases and continues with disease progression to cancer.

Based on our data regarding the high methylation frequency of ***APC, FHIT, CDH1 and p16*** in the plasma and tissues of HBV and HCV-associated HCC patients from Egypt [13] we sought to confirm this data in a larger cohort of HCV-genotype-4 infected patients using a larger panel of 11 genes (***p14, p15, p16, p73, APC, FHIT, DAPK1, CDH1, RARb, RASSF1A, and O6MGMT***). The newly tested group included (1) asymptomatic carriers, (2) CH patients with cirrhosis and (3) HCC. PM of the 11 genes were assessed in the Peripheral Blood Lymphocytes (PBLs) and the tissues of 31 HCC with their adjacent normal tissue (ANT), 38 CH and 13 normal hepatic tissue (NHT); which represents the progression from NHT to HCC in the HCV genotype 4-infected persons [21]. Promotor methylation of these genes was assessed by methylation-specific PCR (MSP). APC and O6-MGMT protein expression was assessed by immunohistochemistry (IHC) in the studied HCC and CH tissue samples.

	HCC	**Cirrhosis**	**Chronic hepatitis C**	**Control**
P14	HCC	0.954[a]	0.035[b]	0.004[c]
	Cirrhosis		0.050[d]	0.004[e]
	Chronic C			0.546[f]
	Control			
P15	HCC	0.409[a]	0.090[b]	<0.001[c]
	Cirrhosis		0.554[d]	0.024[e]
	Chronic C			0.223[f]
	Control			
MGMT	HCC	0.328[a]	0.016[b]	0.003[c]
	Cirrhosis		0.002[d]	<0.001[e]
	Chronic C			0.189[f]
	Control			
TP73	HCC	0.858[a]	0.037[b]	<0.001[c]
	Cirrhosis		0.058[d]	<0.001[e]
	Chronic C			0.026[f]
	Control			

Table 4. Pairwise comparison among the studied groups.

5. Analysis of DNA methylation events of the 11 tested genes among the studied groups

A high methylation frequency was reported for all studied genes (except for p15) in the PBL and tissues with increasing methylation index as the disease progresses (**Figure 3**). The PM of the 11 tested genes assessed in 13 NHT samples showed no methylation events in ***p15, p73, RARb, RASS, F1A*** or ***O6MGMT***. However ***p14 PM*** was estimated in 46.2% of the cases followed by ***APC*** which was methylated in 30.8% of the cases. There was a significant difference in MF between NHT and CH groups regarding ***APC, FHIT, DAPK*** and ***RASSF*** genes. Also MF of ***p14, p73, RASSF1A, CDH1*** and ***O6MGMT*** was significantly higher in HCC and their ANT. However MF of ***APC*** was higher in CH (**Figure 4** and **Table 5**). Among the four groups enrolled (HCC, CH, ASC, NHT) binary logistic regression in PROC LOGISTIC for each gene was used. Our results indicate that there is a significant interaction between disease state (different groups) and DNA methylation of the tested genes (**Figure 5a–k**). As shown in **Figure 6**, there is a significant group effect for ***APC*** (ASC group is different from HCC Group, p = 0.0006). This interaction is explained by the fact that there is a bigger difference between methylation and un-methylation for the CH group compared to any other group, especially the NHT. For ***DAPK1*** (**Figure 5g**), there is a marginal group effect, not significant by our corrected level of

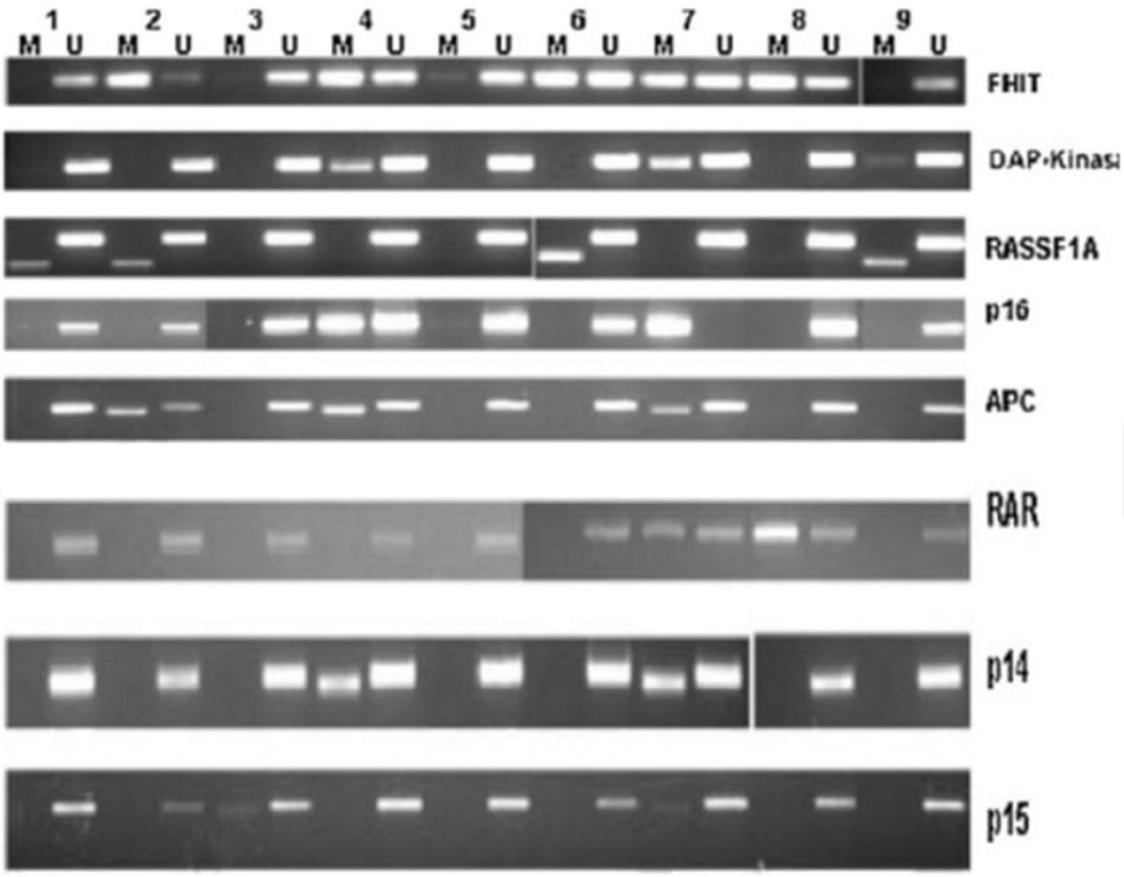

Figure 3. Methylation-specific PCR analyses of nine representative HCC samples (labeled 1–9 on the top). Each gene is indicated on the right. Both methylated (M) and unmethylated (U) reactions were amplified for each bisulfite-treated DNA and run in a 4% agarose gel.

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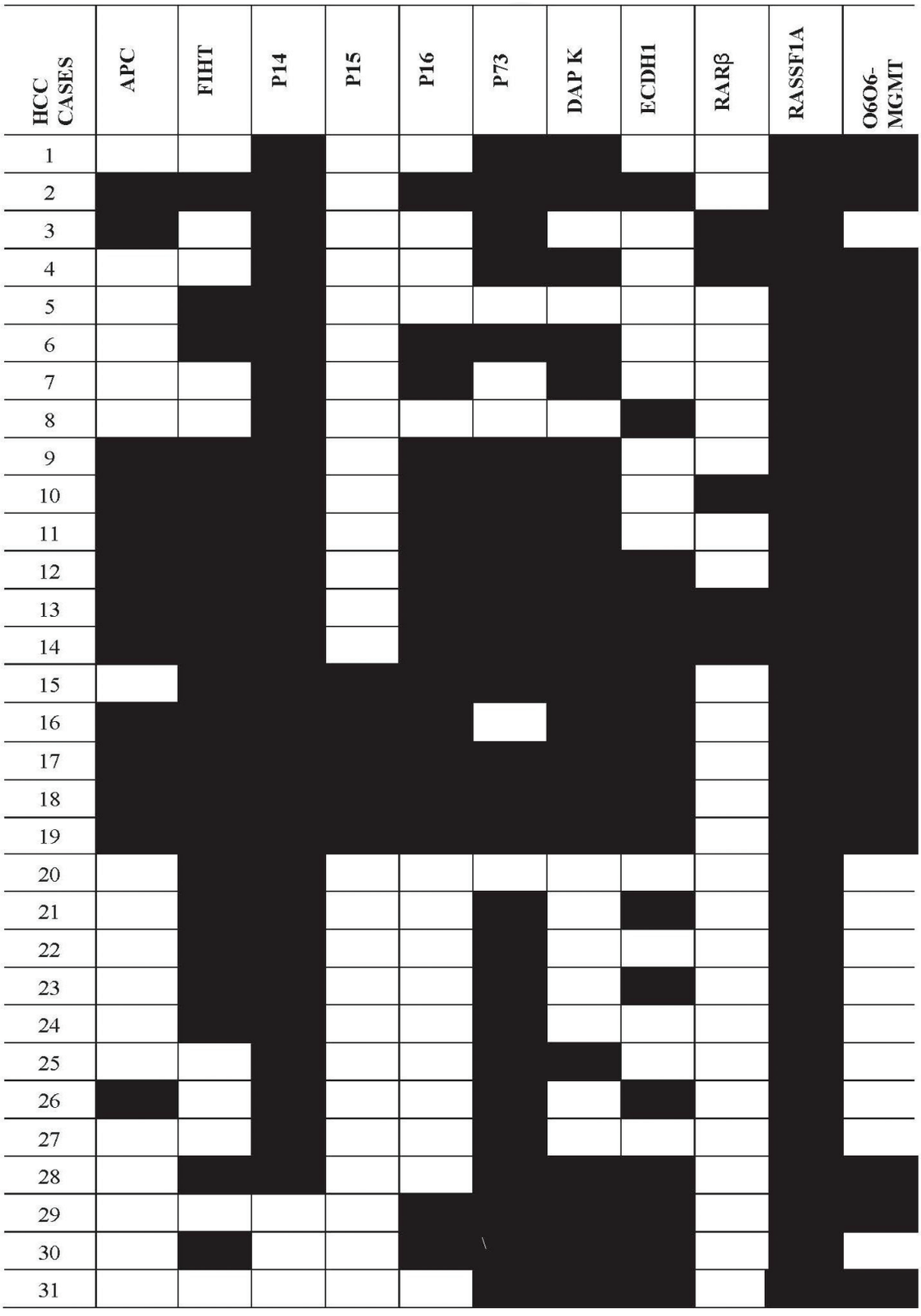
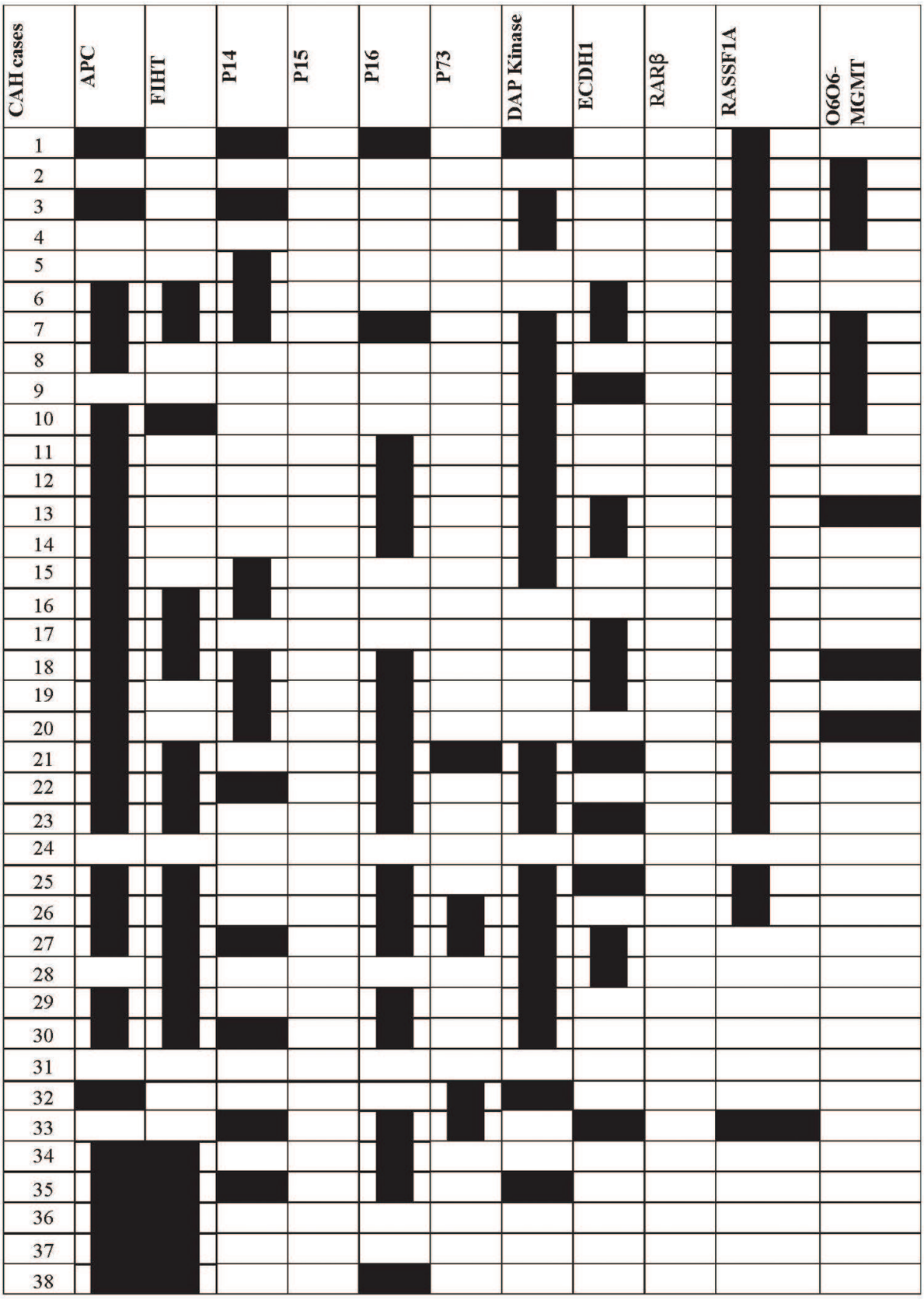
P = 0.004 (NHT is significantly different from HCC P = 0.007) and ***RARb*** (**Figure 5h**) (NHT is different from-HCC Group P = 0.007). In contrast, there were significant methylation effects for ***APC*** p < 0.0001), ***FHIT*** (P < 0.0001), ***p15***, (P = 0.003), ***p14*** (P < 0.0001), ***DAPK1*** (P < 0.0001), ***RARb*** (P < 0.0001) and ***E-cadherin*** (P < 0.0001).

(A)



(B)


CAH cases
APC
FIHT
P14
P15
P16
P73
DAP Kinase
ECDH1
RARβ
RASSF1A
O6O6-MGMT
1
2
3
4
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(C)

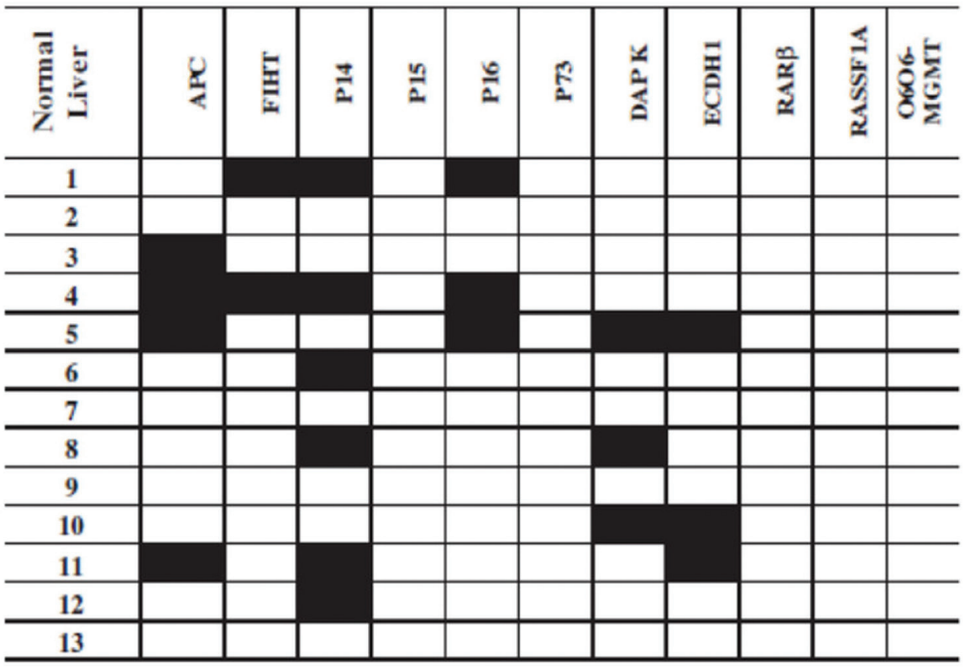

Figure 4. (A) Methylation of 11 genes in hepatocellular carcinoma patients; (B) Methylation of 11 genes in patients with chronic liver diseases; (C) Methylation of 11 genes in normal liver individuals. Dark squares depict methylation and blank squares depict unmethylation.

Genes	Normal liver N = 13 (%)	Chronic hepatitis (CH)		Hepatocellular carcinoma HCC		p-Value*	
		(Tissue) (38) (%)	(PBL) (20) (%)	(HCC) (31) (%)	(ANT) (31) (%)	(CH and HCC)	(CH and ANT)
APC	4 (30.8)	33 (86.8)	16 (80)	13 (41.9)	14 (45.2)	<0.001	<0.001
FHIT	2 (15.4)	20 (52.6)	6 (30)	21 (67.7)	20 (64.5)	0.204	0.005
P15	0 (0)	0 (0)	0 (0)	5 (16.1)	5 (16.1)	0.010	#
P73	0 (0)	8 (21.1)	1 (5.0)	26 (83.9)	23 (74.2)	<0.001	<0.001
P14	6 (46.2)	17 (44.7)	10 (50)	28 (90.3)	28 (90.3)	<0.001	<0.001
P16	3 (23.1)	15 (39.5)	9 (45)	14 (45.2)	19 (61.3)	0.634	0.390
DAPK	3 (23.1)	22 (57.9)	12 (60)	21 (67.7)	22 (71)	0.401	0.023
RARb	0 (0)	0 (00)	0 (0)	5 (16.1)	3 (9.7)	0.015	#
RASSF	0 (0)	26 (68.4)	20 (100)	31 (100)	31 (100)	0.001	<0.001
O6O6-MGMT	0 (0)	10 (26.3)	10 (50.0)	21 (67.7)	20 (64.5)	<0.001	<0.001
CDH1	3 (23.1)	7 (18.4)	8 (40.0)	17 (54.8)	14 (45.2)	0.002	0.004

*p-Values <0.05 are considered significant. #Numbers are too small for a valid statistical analysis.

Table 5. Methylation profile of the 11 genes in CH, HCC and normal liver tissues.

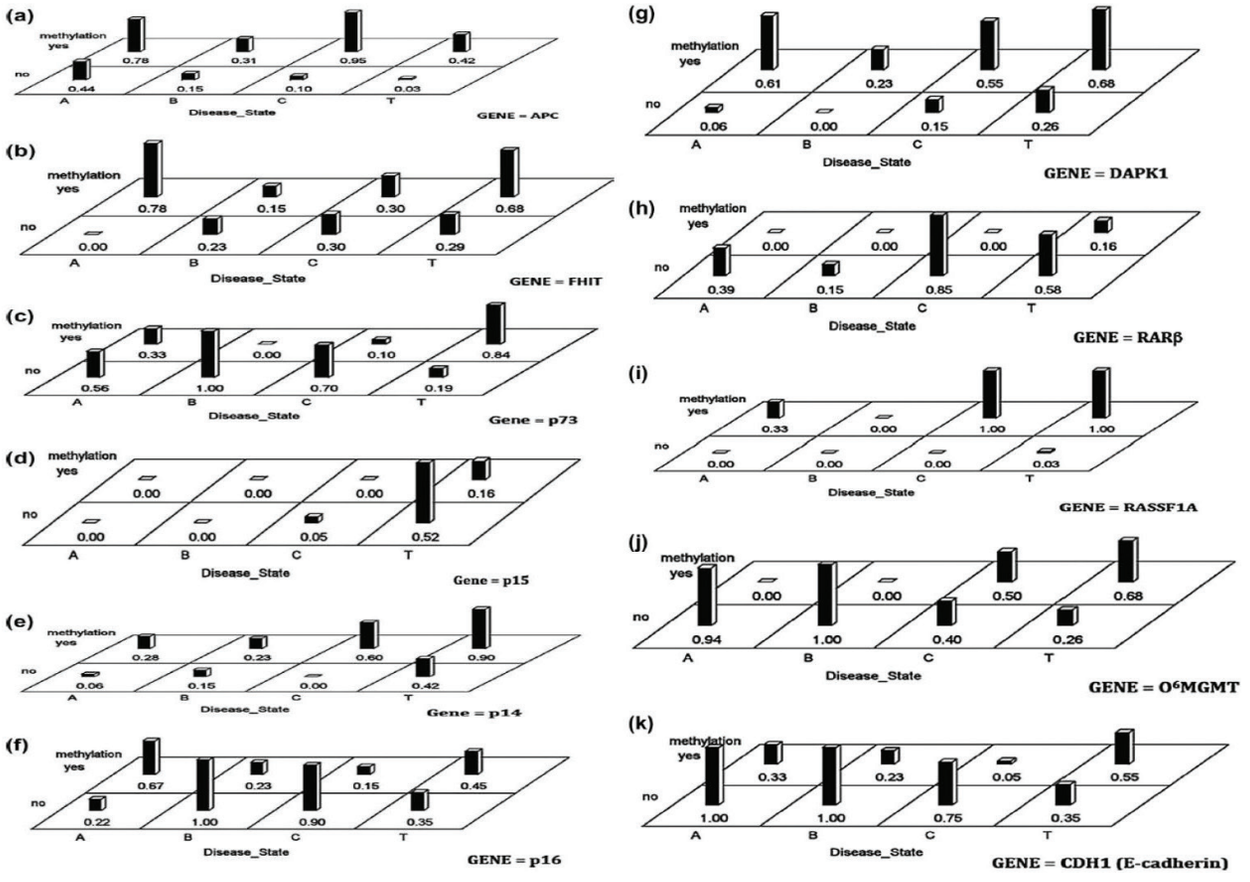

Figure 5. Differences in the methylation frequency among the four studied groups. (T = HCC, C = CAH with cirrhosis, A = asymptomatic carrier and B = normal hepatic tissue).

Our data regarding the ***p15*** gene confirms our previous study in which ***p15*** methylation was reported in 14.2% only of HCC cases [13, 19]. Within the studied groups, the methylation frequency of ***p14, p73, RASSF1A*** and ***O6MGMT*** was significantly higher in HCC and their ANT compared to CH and the NHT samples, whereas PM of ***APC*** was significantly higher in CH patients compared to all other groups. This was applied to PBL and tissues except for ***RASSF1A*** and ***O6MGMT*** in which the difference in the MF in PBL was statistically insignificant (**Figure 7).**

RASSF1A is a candidate TSG, which frequently shows PM and loss of heterozygosity (LOH) with consequent gene silencing in several human cancers [22]. The high MF reported here confirms the results of some recent studies including those of Qu and Lia [23, 24] who found PM of ***RASSF1A*** gene in 78 and 95% of HCC cases assessed. In our study, ***RASSF1A*** methylation was detected in all HCC cases and in 68.4% of CH cases (being second only to ***APC***). This finding is consistent with Araújo and Gioia et al. [25, 26] who reported an increase in ***RASSF1A*** PM with progression from regenerative conditions (e.g. cirrhosis) to hepatocellular nodules and HCC, as well as with Huang et al. [15] and Chan et al. [27] who reported ***RASSF1A*** methylation in the blood and tissues of HCC patients. Our results also showed an increasing frequency of ***p16*** PM from NHT to HCC which is in agreement with the earlier studies [28, 29].

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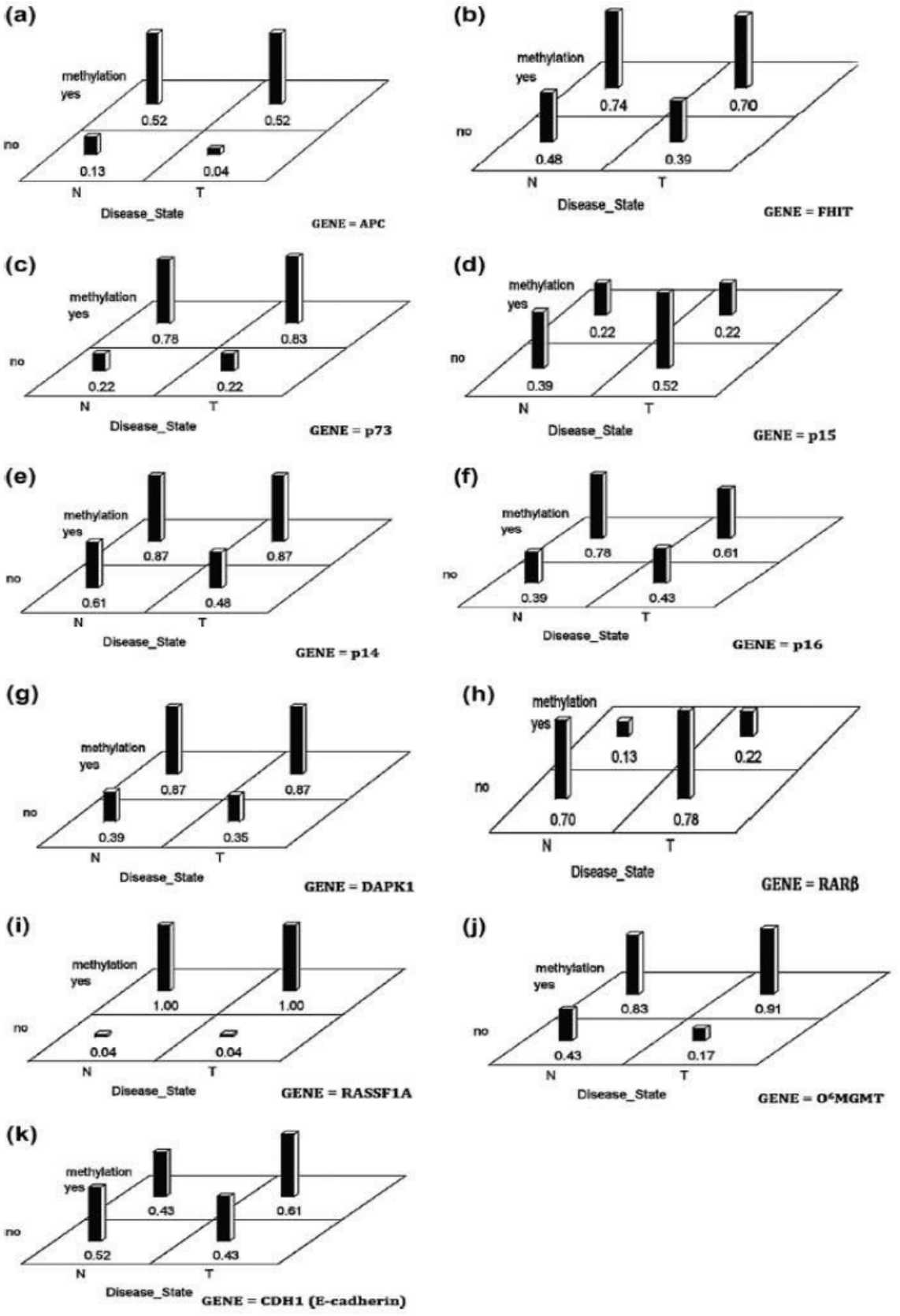

Figure 6. Differences across methylation profiles between HCC\cases and their ANT# samples with 0.0045 as a cut-off for significance. *HCC = T. # ANT = N. a-k: names of the studied genes.

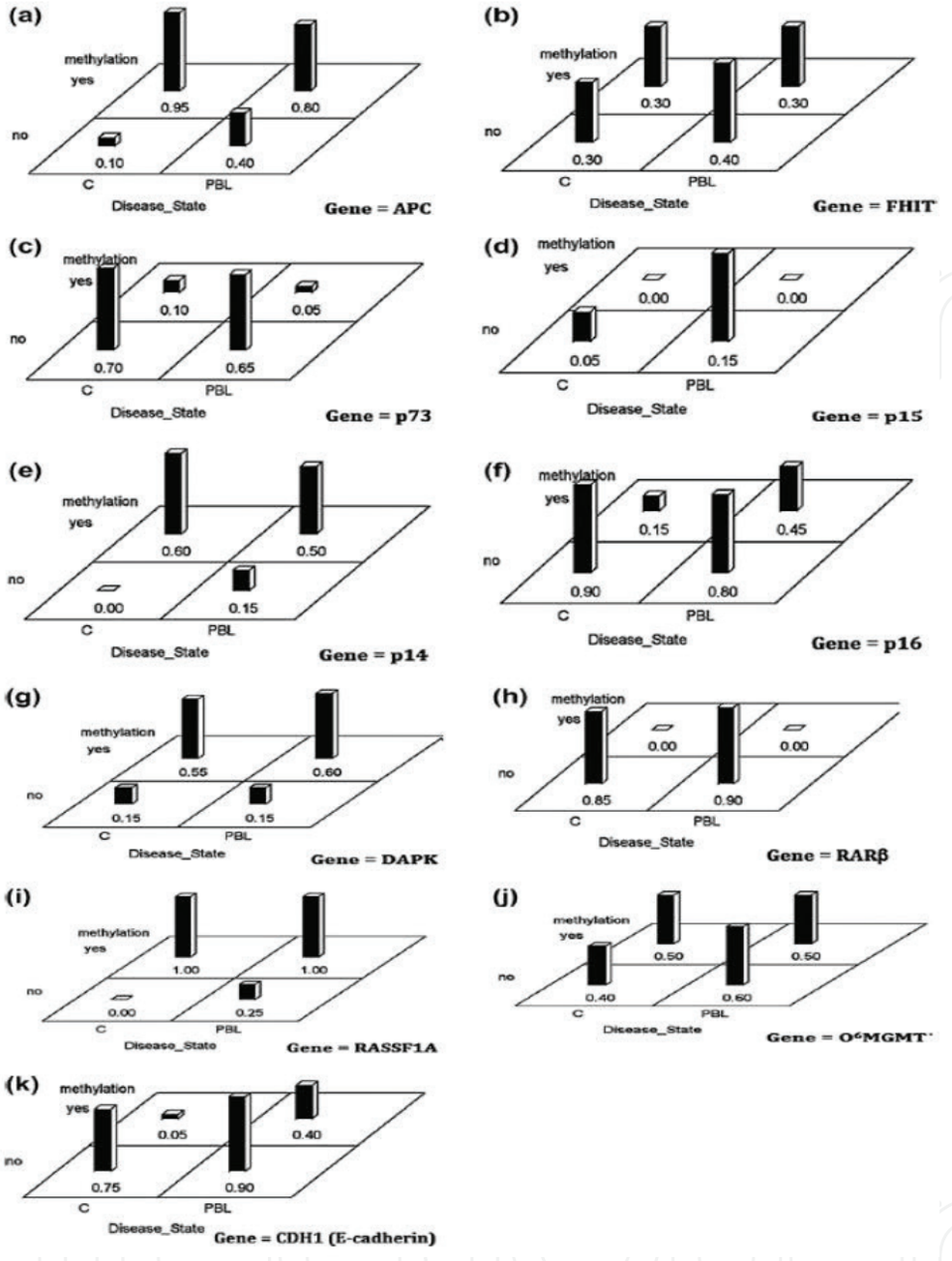

Figure 7. Differences across methylation profiles within CAH\cases between tissues and PBL. a-k: names of the studied genes.

6. Genes methylation could be used as a biomarker for diagnosis of HCC and CH

The Coordination of methylation at the 11 tested genes was analyzed in our study using the Mann–Whitney U test through comparing the status of each gene (M or U) with the MI calculated with the remaining genes (**Table 6**). The combined effect of the studied methylated genes as biomarkers for diagnosis of HCC and CH has been determined (assessed) using the stepwise logistic regression, and accordingly only ***APC, p73, p14 and O6MGMT*** independently affected the classification of cases into HCC and/or CH (**Table 7**). Together, these four genes (combined) give an accuracy of 89.9%, sensitivity 83.9% and specificity 94.7%.

Factor	Concordance, n=31 (n (%))	Kappa[#]	p-Value*
APC	28 (90.3)	0.803	<0.001
FHIT	24 (77.4)	0.497	0.006
P15	31 (100.0)	1.000	<0.001
P73	18 (58.1)	−0.248	0.150
P14	31 (100.0)	1.000	<0.001
P16	24 (77.4)	0.558	0.001
DAPK	22 (71.0)	0.318	0.076
RARb	27 (87.1)	0.431	0.012
RASSF	31 (100.0)	—	—
O6O6-MGMT	26 (83.9)	0.640	<0.001
CDH1	22 (71.0)	0.425	0.016

– Numbers are too small for a valid statistical analysis.[#]Kappa measure of agreement.
*p-Values<0.05 are considered significant.

Table 6. Summary of methylation specific PCR results and concordance tests of each locus in HCC samples.

Parameter	Regression estimate	P value	Odds ratio	95% CI	for OR
APC	−3.606	0.003	0.027	0.003	0.287
p73	3.671	0.001	39.302	4.752	325.017
P14	3.638	0.009	38.014	2.492	579.829
O6-MGMT	2.589	0.014	13.311	1.685	105.132

Table 7. Stepwise logistic regression for HCC.

Within the identified genes panel which independently affected the classification of cases into HCC and CH in this study, p14 only showed a high MF in HCC cases. Our data in this context confirmed those of Anzola et al. [30] and Yang et al. [20] who reported that ***p14*** PM is an important factor contributing for the development of HCV-induced HCC. The fact that we were able to detect ***p14***-PM in NHT and CH with almost the same frequency, suggests that it might be an early event in the cascade of HCV-induced HCC. On contrary, we could not find the same profile for ***p16***PM suggesting that ***p14*** and ***p16*** are may be regulated by different promoters [31].

Similar to ***p14, O6MGMT*** plays an important role in cytoprotection by preventing DNA damage and triggering DNA repair mechanisms [32]. Our results showed a significant increase in the frequency of ***O6MGMT*** PM from CH (26%) to HCC (67.7%) providing an evidence that this gene could be used to differentiate between CH and HCC. We have also reported that ***O6MGMT*** PM is significantly higher in non-responder to antiviral therapy, and consequently ***O6MGMT*** could be used as a predictor for antiviral response [33]. Literature reviews shows different frequencies of ***O6MGMT*** PM in HCC ranging from 0% to 22–39% [34, 35]. This variability in the results among different studies could be attributed to several factors including the sensitivity and type of PCR, the primer sequences used, the site of CpG islands, the geographical and the underlying etiological factors that promoting HCC development

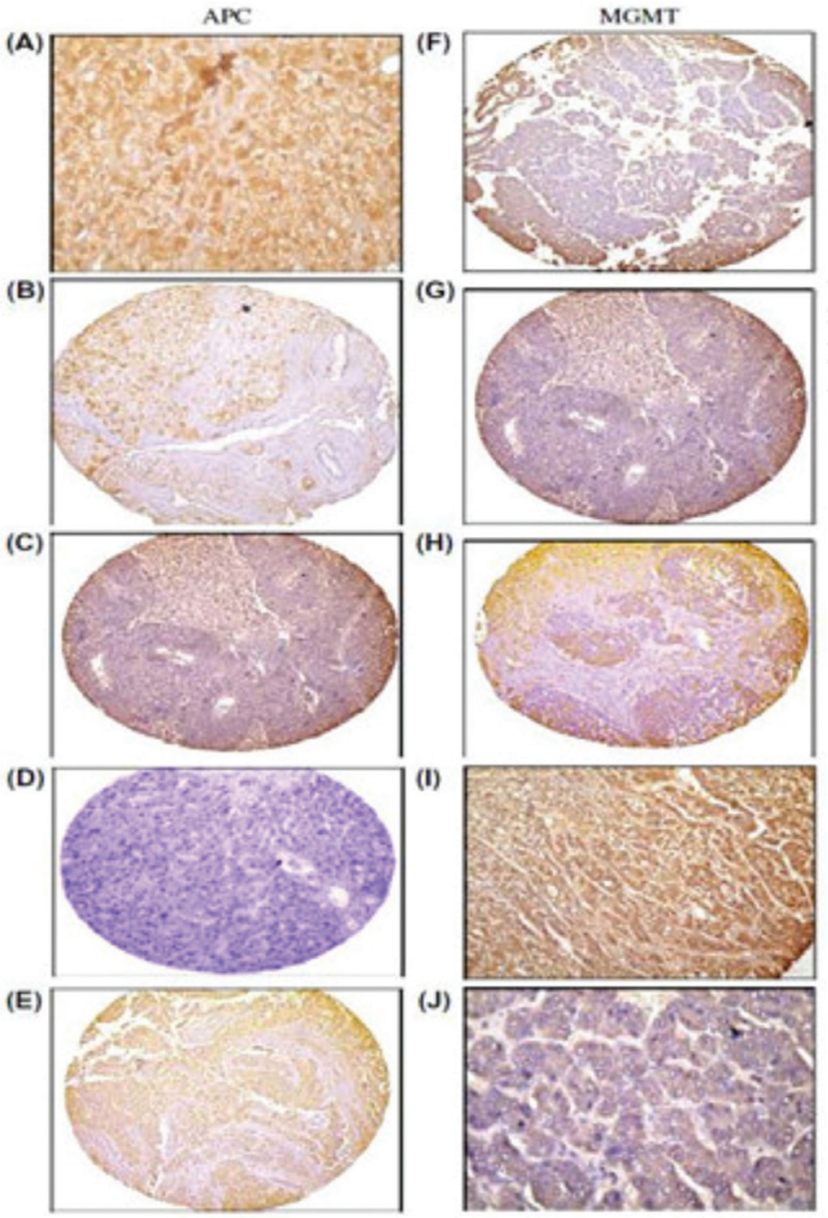

Figure 8. (A) Normal hepatic tissue sample showing positive cytoplasmic immunostaining for APC (200×). (B) A case of HCV induced chronic hepatitis showing mild focal cytoplasmic immunostaining for APC (100×). (C) A case of HCV induced chronic hepatitis with cirrhosis negative for APC (100×). D: A case of HCV-associated HCC negative for APC (100×). (E) A case of HCV-associated HCC with positive cytoplasmic immunostaining for APC (40×). (F) Normal hepatic tissue negative for MGMT (100×). (G) A case of HCV-induced chronic hepatitis with cirrhosis negative for MGMT (100×). (H) A case of HCV-induced chronic hepatitis with cirrhosis positive for MGMT immunostaining (100×). (I) A case of HCV-induced HCC with marked cytoplasmic immunostaining for MGMT (200×). (J) A case of HCV-induced HCC showing faint cytoplasmic immunostaining for MGMT (200×).

[24, 35]. And finally, ***p73*** PM was reported in 83.9% of the HCC cases assessed in our study compared to 21.1% in CH and none in the NHT samples. Thus ***p73*** PM could be used to differentiate between CH and HCC cases even in patient's blood [32].

A significant difference in the MFs of ***APC*** and ***CDH1*** were found between CH and HCC cases. APC was more frequent in the CH and ***CDH1*** in HCC. ***APC*** and ***CDH1*** PM was reported by Yang et al. [20] who demonstrated that PM of ***APC*** and ***CDH1*** are more frequent in HBV and

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HCV-positive HCC than in HBV and HCV negative ones. Nomoto et al. [34] founded ***APC*** PM in 88.2% of the NHT and 21.6% in CH with cirrhosis compared to 82.4% in HCC. They explained that ***APC*** loss in cirrhotic and inflammatory cases could be occurred due to the presence of inflammatory cells and fibroblasts. However in contrast, we reported a high ***APC*** MF in the blood and tissues of CH patients. This contradictory between our results and those of Nomoto et al. could be attributed to (a) their smaller sample size (19 cases only); (b) the samples of CH and cirrhosis were obtained from HCC cases in their study or (c) a possibly different underlying etiology as viral infection was not mentioned in their study.

7. Concordance between PM and protein expression of APC and O6MGMT

We assessed the protein expression of ***APC*** and ***O6MGMT*** in 20 NHT samples, 20 HCC and 20 CH tissues as well as in another group of samples including 40 NHT, 52 CH and 107 HCC tissue samples for confirmation of the methylation results [21]. In the original set, cytoplasmic immunostaining for the ***APC*** was detected in 11 cases of NHT (55%), with loss of staining in 10 CH cases (50%), and 15 cases of HCC group (75%). As for the confirmatory set, cytoplasmic immunostaining for the APC was present in 50% of NHT (20 cases), with loss of staining in 57.7% of CH (30 cases), and 72% of HCCs (77 patients). Nuclear immunostaining for ***O6MGMT*** protein was detected in 13 patients with NHT (65%), with loss of expression in 11 patients with CH (55%) and 16 patients with HCCs (80%), from the original set. In the confirmatory set ***O6MGMT*** protein was lost in 26 patients with CH accounting for 50%, and 70 patients with HCC accounting for 65.4% (**Figure 8**).

8. Conclusion

We conclude that DNA PM of multiple cancer-related genes plays an important role in the development and progression of HCC and therefore, it could be detected in different stages of disease progression from hepatitis to HCC. The data reported in our study provide evidence that PM of *p73, p14, O6- MGMT* is associated with HCC whereas PM of the *APC* gene is more common in CH cases compared to other groups. Therefore, *APC* PM could be used as a maker for early detection of HCV-induced chronic active hepatitis patients.

Moreover, a panel of four genes *(APC, p73, p14, O6-MGMT)* independently affected the classification of cases into HCC and CH with high accuracy (89.9%), sensitivity (83.9%) and specificity (94.7%). In addition, detection of PM of certain genes *(APC, FHIT, p15, p16, and E-cadherin)* in the PBL of HCV-infected patients is a highly sensitive and specific, noninvasive way (technique) and therefore, blood could be used, as efficiently as tissue biopsies, to assess PM of different genes. This could help in the follow-up of chronic hepatitis patients and possibly for early detection of HCC. We did not observe a significant difference in the methylation status according to the virus type (HBV versus HCV infection). Therefore, plasma DNA could be used as a reliable resource for methylation studies in the future, irrespective of the type of hepatitis infection.

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References

[1] Siegel RL, Miller KD, Fedewa SA, Ahnen DJ, Meester RG, Barzi A, Jemal A. Colorectal cancer statistics, 2017. CA: A Cancer Journal for Clinicians. 2017 May 6;**67**(3):177-193. DOI: 10.1186/s13012-017-0668-7

[2] Ibrahim AS, Khaled HM, Mikhail NN, Baraka H, Kamel H. Cancer incidence in Egypt: Results of the national population-based cancer registry program. Journal of Cancer Epidemiology. 2014;**2014**

[3] Zekri AR, Hafez MM, Bahnassy AA, Hassan ZK, Mansour T, Kamal MM, Khaled HM. Genetic profile of Egyptian hepatocellular-carcinoma associated with hepatitis C virus genotype 4 by 15 K cDNA microarray: Preliminary study. BMC Research Notes. 2008 Oct 29;**1**(1):106. DOI: 10.1186/1756-0500-1-106

[4] Petruzziello A, Marigliano S, Loquercio G, Cozzolino A, Cacciapuoti C. Global epidemiology of hepatitis C virus infection: An up-date of the distribution and circulation of hepatitis C virus genotypes. World Journal of Gastroenterology. 2016;**22**:7824-7840. DOI: 10.3748/wjg.v22.i34.7824

[5] Chen SL, Morgan TR. The natural history of hepatitis C virus (HCV) infection. International Journal of Medical Sciences. 2006;**3**:47-52. DOI: 10.7150/ijms.3.47

[6] Zhang C, Li H, Zhou G, Zhang Q, Zhang T, Li J, et al. Transcriptional silencing of the TMS1/ASC tumour suppressor gene by an epigenetic mechanism in hepatocellular carcinoma cells. The Journal of Pathology. 2007;**212**(2):134-361. DOI: 10.1002/path.2173

[7] Ye M, Huang T, Ying Y, et al. Detection of 14-3-3 sigma (sigma) promoter methylation as a noninvasive biomarker using blood samples for breast cancer diagnosis. Oncotarget. 2017;**8**:9230-9242. DOI: 10.1002/path.2173

[8] Paska AV, Hudler P. Aberrant methylation patterns in cancer: A clinical view. Biochemical Medicine (Zagreb). 2015;**25**:161-176. DOI: 10.11613/BM.2015.017

[9] Li CC, Yu Z, Cui LH, et al. Role of P14 and MGMT gene methylation in hepatocellular carcinomas: A meta-analysis. Asian Pacific Journal of Cancer Prevention. 2014;**15**:6591-6596

[10] Teodoridis JM, Hardie C, Brown R. CpG island methylator phenotype (CIMP) in cancer: Causes and implications. Cancer Letters. 2008;**268**(2):177-355. DOI: 10.1016/j.canlet.2008.03.022

[11] Tsou JA, Galler JS, Wali A, Ye W, Siegmund KD, Groshen S, et al. DNA methylation profile of 28 potential marker loci in malignant mesothelioma. Lung Cancer. 2007;**58**(2): 220-230. DOI: 10.1016/j.lungcan.2007.06.015

[12] Lehman EM, Wilson ML. Epidemiology of hepatitis viruses among hepatocellular cases and healthy people in Egypt: A systematic review and meta-analysis. International Journal of Cancer. 2009;**24**:690-697. DOI: 10.1002/ijc.23937

[13] Iyer P, Zekri AR, Hung CW, Schiefelbein E, Ismail K, Hablas A, Seifeldin IA, Soliman AS. Concordance of DNA methylation pattern in plasma and tumor DNA of Egyptian hepatocellular carcinoma patients. Experimental and Molecular Pathology. 2010 Feb 28; **88**(1):107-111. DOI: 10.1016/j.yexmp.2009.09.012

[14] Bongiovanni M, Casana M. Non-invasive markers of liver fibrosis in HCV mono-infected and in HIV/HCV co-infected subjects. Medicinal Chemistry. 2008;**4**(6):513. [PubMed: 18991734]

[15] Huang W, Li T, Yang W, Chai X, Chen K, Wei L, Duan S, Li B, Qin Y. Analysis of DNA methylation in plasma for monitoring hepatocarcinogenesis. Genetic Testing and Molecular Biomarkers. 2015 Jun 1;**19**(6):295-302. DOI: 10.1089/gtmb.2014.0292

[16] Shen J, Wang S, Zhang YJ, Kappil M, Wu HC, Kibriya MG, Wang Q, Jasmine F, Ahsan H, Lee PH, Yu MW. Genome-wide DNA methylation profiles in hepatocellular carcinoma. Hepatology. 2012 Jun 1;**55**(6):1799-1808. DOI: 10.1002/hep.25569

[17] Liu JB, Zhang YX, Zhou SH, Shi MX, Cai J, Liu Y, Chen KP, Qiang FL. CpG island methylator phenotype in plasma is associated with hepatocellular carcinoma prognosis. World Journal of Gastroenterology: WJG. 2011 Nov 14;**17**(42):4718. DOI: 10.3748/wjg.v17.i42.4718

[18] Chang H, Yi B, Li L, Zhang H, Sun F, Dong S, et al. Methylation of tumor associated genes in tissue and plasma samples from liver disease patients. Experimental and Molecular Pathology. 2008;**85**(2):96. [PubMed: 18691570]

[19] Zekri AE, Nassar AA, El-Rouby MN, Shousha HI, Barakat AB, El-Desouky ED, Zayed NA, Ahmed OS, Youssef AS, Kaseb AO, El-Aziz AO. Disease progression from chronic hepatitis C to cirrhosis and hepatocellular carcinoma is associated with increasing DNA promoter methylation. Asian Pacific Journal of Cancer Prevention. 2013;**14**(11):6721-6726. PMID: 24377595

[20] Yang B, Guo M, Herman JG, Clark DP. Aberrant promoter methylation profiles of tumor suppressor genes in hepatocellular carcinoma. The American Journal of Pathology. 2003;**163**(3):1101-1107. DOI: 10.1016/S0002-9440(10)63469-4

[21] Zekri AR, Bahnasy AA, Mohamed WS, El-Dahshan DH, Ali FT, Sabry GM, Dasgupta N, Daoud SS. Methylation of multiple genes in hepatitis C virus associated hepatocellular carcinoma. Journal of Advanced Research. 2014 Jan 31;**5**(1):27-40. DOI: 10.1016/j.jare.2012.11.002

[22] Hesson LB, Cooper WN, Latif F. The role of RASSF1A methylation in cancer. Disease Markers. 2007;**23**:73-87. PMCID: PMC3850810

[23] Qu Z, Jiang Y, Li H, Yu DC, Ding YT. Detecting abnormal methylation of tumor suppressor genes GSTP1, P16, RIZ1, and RASSF1A in hepatocellular carcinoma and its clinical significance. Oncology Letters. 2015 Oct 1;**10**(4):2553-2558. DOI: 10.3892/ol.2015.3536

[24] Lia Z, Zhangb H, Yangb J, Haoc T, Li S. Promoter hypermethylation of DNA damage response genes in hepatocellular carcinoma. Cell Biology International. 2012;**36**:427-432. DOI: 10.1042/CBI20100851

[25] Araújo OC, Rosa AS, Fernandes A, Niel C, Villela-Nogueira CA, Pannain V, Araujo NM. RASSF1A and DOK1 promoter methylation levels in hepatocellular carcinoma, cirrhotic and non-cirrhotic liver, and correlation with liver cancer in Brazilian patients. PLoS One. 2016 Apr 14;**11**(4):e0153796. DOI: 10.1371/journal.pone.0153796

[26] Gioia S, Bianchi P, Destro A, Grizzi F, Malesci A, Laghi L, et al. Quantitative evaluation of RASSF1A methylation in the non-lesional, regenerative and neoplastic liver. BMC Cancer. 2006;**89**(6):1471-2407. DOI: 10.1186/1471-2407-6-89

[27] Chan KC, Lai PB, Mok TS, Chan HL, Ding C, Yeung SW, et al. Quantitative analysis of circulating methylated DNA as a biomarker for hepatocellular carcinoma. Clinical Chemistry. 2008;**54**:1528-1536. DOI: 10.1373/clinchem.2008.104653

[28] Lv X, Ye G, Zhang X, Huang T. p16 Methylation was associated with the development, age, hepatic viruses infection of hepatocellular carcinoma, and p16 expression had a poor survival: A systematic meta-analysis (PRISMA). Medicine. 2017 Sep;**96**(38):e8106. DOI: 10.1097/MD.0000000000008106

[29] Vivekanandan P, Torbenson M. Epigenetic instability is rare in fibrolamellar carcinomas but common in viral-associated hepatocellular carcinomas. Modern Pathology. 2008;**21**:670-675. DOI: 10.1038/modpathol.2008.32

[30] Anzola M, Cuevas N, López-Martinez M, Saiz A, Burgos JJ, Martinez de Pancorbo M. p14ARF gene alterations in human hepatocellular carcinoma. European Journal of Gastroenterology & Hepatology. 2004;**16**:19-26. PMID: 15095848

[31] Esteller M, Tortola S, Toyota M, Capella G, Peinado MA, Baylin SB, et al. Hypermethylation-associated inactivation of p14 (ARF) is independent of p16 (INK4a) methylation and p53 mutational status. Cancer Research. 2000;**60**:129-133. PMID: 10646864

[32] Lenz G, Hutter G, Hiddemann W, Dreyling M. Promoter methylation and expression of DNA repair genes hMLH1 and O6-MGMT in acute myeloid leukemia. Annals of Hematology. 2004;**83**:628-633. DOI: 10.1007/s00277-004-0925-0

[33] Zekri AR, Raafat AM, Elmasry S, Bahnassy AA, Saad Y, Dabaon HA, El-Kassas M, Shousha HI, Nassar AA, El-Dosouky MA, Hussein N. Promotor methylation: Does it affect response to therapy in chronic hepatitis C (G4) or fibrosis? Annals of Hepatology: Official Journal of the Mexican Association of Hepatology. 2014 Sep 1;**13**(5):518-524. PMID: 25152984

[34] Nomoto S, Kinoshita T, Kato K, Otani S, Kasuya H, Takeda S, et al. Hypermethylation of multiple genes as clonal markers in multicentric hepatocellular carcinoma. British Journal of Cancer. 2007;**97**:1260-1265. DOI: 10.1038/sj.bjc.6600743

[35] Matsukura S, Soejima H, Nakagawachi T, Yakushiji H, Ogawa A, Fukuhara M, Miyazaki K, Nakabeppu Y, Sekiguchi M, Mukai T. CpG methylation of MGMT and hMLH1 promoter in hepatocellular carcinoma associated with hepatitis viral infection. British Journal of Cancer. 2003 Feb 24;**88**(4):521-529. DOI: 10.1038/sj.bjc.6600743