The Egyptian Journal of Biochemistry & Molecular Biology
VOL 37(N.1&2) 37-60 December. 2019

THE IMPACT OF CYCLOOXYGENASE-2 GENE POLYMORPHISM 899G/C AND CERTAIN INDICES ON HEPATITIS C RELATED LIVER FIBROSIS

Marwa A Gaber[1] , Naglaa K Idriss[1], Gehan S Seifeldein[2], Reem M Elkady[2], Rania Makboul[3], Hala M Imam[4], Lobna Abdel Wahid[4]

Departments of Medical Biochemistry[1], Diagnostic Radiology[2], Pathology[3] and Internal Medicine [4,] Assiut University, Assiut, Egypt

Received 19/12/2018– Accepted 5/1/2019

ABSTRACT

The link between cyclooxygenase-2 (COX-2) gene polymorphisms and liver diseases has been widely reported. Early and precise estimation and staging of hepatic fibrosis are crucial for prognosis and treatment decisions in those patients. We aimed in this study to clarify role of -899G/C polymorphism of COX-2 gene, alteration of CA 19-9 and CA 125 levels, and plasma protein pattern in staging of liver fibrosis comparing them to METAVIR stages of liver fibrosis. We recruited 103 patients with post-hepatitis C liver fibrosis and 42 healthy controls. COX-2 gene polymorphism was detected by PCR- TaqMan probes, while CA19-9, CA125 levels were estimated using quantitative ELISA. Plasma proteins were detected by the capillary electrophoresis method. The results revealed that the frequency of COX-2 -899G/C genotypes GG, GC, and CC were 68.0%, 28.2% and 3.9% in the fibrotic group; 97.06%, 2.4%, and 0.0% in healthy control group respectively. The percent of COX-2 expression for the fibrotic group and the healthy group were 32% and 2.3% respectively. COX-2 expression scores on mild- vs. sever-fibrosis stages (METAVIR stages 1, 2 vs. stages 3,4) were 18.2 %and 81.8% respectively (OR=48.00, 95%CI). The serum level of tested tumor markers were significantly higher in fibrotic patients than in control group (69.40 ±51.82, 13.41 ± 6.49 respectively for CA 19.9 and 59.16 ± 47.23, 10.90 ± 8.36 for CA 125) and in GC/CC genotypes than GG one (116.96 ± 55.00, 33.64 ± 28.39 respectively for CA 19.9 and 101.62 ± 51.29, 27.89 ± 25.51 respectively for CA 125). In conclusion, COX-2 -899 C allele carriers are more vulnerable to develop hepatitis C- related

* *Correspondence Author (Marwagaberbio@yahoo.com)*

hepatic fibrosis. The combined estimation of CA 19-9 and CA 125 levels are useful for identifying and staging patients with liver fibrosis.
Key words: COX-2 gene polymorphisms, CA 19-9, CA 125, protein electrophoresis, liver fibrosis.

INTRODUCTION

It is estimated that approximately three percent of the worldwide population are infected with the hepatitis C virus (HCV) and almost 80% of these cases are chronic **(Seeff, 2009).** Once a diagnosis of HCV infection is established, cirrhosis develops within 10– 20 years in approximately 20% of patients. In addition, disease progression, which may lead to hepatic decompensation, hepatocellular carcinoma, and death, is particularly common in these patients **(Afdhal 2004, Strader et al., 2004, Hofmann and Zeuzem, 2009, Seff, 2009).**

Though there is little operative treatment for those patients with end-stage cirrhosis except liver transplantation, early or intermediate liver fibrosis is emerging as a remediable complication **(Lotersztajn et al., 2005, Friedman and Bansal, 2006).** Consequently, precise estimation and staging of hepatic fibrosis, especially the early one, is crucial for estimation of prognosis, surveillance, and treatment decisions **(Castera, 2011, Germani et al., 2011).** Currently, liver biopsy is deliberated to be the gold standard for liver fibrosis assessment. However, it is an invasive procedure associated with sampling and interpretation errors, patient discomfort and the risk of complications. Thus, employment of alternative noninvasive tools in diagnosing and monitoring hepatic fibrosis became a real demand **[Bravo et al., 2001, Bedossa et al., 2015].**

The pro-inflammatory enzyme cyclooxygenase (COX), otherwise named prostaglandin endoperoxide synthases, renovates arachidonic acid into prostaglandins, which have a chief role in the inflammatory responds. Two isoforms, COX-1 and COX-2, are incorporated in the COXs family, where COX-1 is constitutively expressed in order to maintain a baseline prostaglandin for normal physiological functions **(Manning and Afdhal., 2008).** On the other hand, COX-2 is the isoform that is hardly ever expressed in cells and induced in response to diverse inflammatory and mitogenic stimuli **(Tan et al., 2007, Vidak et al., 2016).**

Chronic HCV infection may initiate liver inflammation and hence fibrosis by multifaceted and not yet well-understood molecular mechanisms **(Stattermayer et al., 2012, Valenti et al., 2012, Stattermayer et al., 2014).** Among the theory that explains HCV-induced inflammation is through upregulation of cyclooxygenase-2

(COX-2) **(Penin et al., 2004).** The HCV single-stranded RNA genome encoding for a single known proteins, 4 structural (C, E1, E2 and p7) and 6 non-structural (NS2, NS3, NS4A, NS4B, NS5A and NS5B) **(Zhang et al., 2005).** One of these proteins, namely NS5A encourages inappropriate upregulation of (COX-2) **(Penin et al., 2004).** Once induced, COX2 isozyme is contributing to chronic inflammation and fibrosis of the liver tissue through production of various prostaglandins **(Gretton et al., 2010).** This theory is supported by observing the biopsied liver in chronic HCV patients where the intrahepatic COX-2 was over expressed and was reported to be associated with progressive hepatic fibrosis **(Penin et al., 2004).**

Polymorphism of COX-2 genes result in modifying the enzyme's function and hence then individual's susceptibility to different diseases, making it a strong biomarker applicant for susceptibility screening, staging, therapy monitoring in these conditions **(Liu et al., 2010, Akkız et al., 2011, Fawzy et al., 2016).** Several studies have shown an association between COX-2 promoter polymorphisms and different diseases. Nevertheless, to the best of our knowledge, there is no research to study the relationship of COX-2, -899G/C polymorphism and hepatitis C-related liver fibrosis.

In addition, various plasma proteins are established to be elevated in cirrhotic patients such as γ–globulins, hyaluronic acid, α2-macroglobulin, amylase and lipase **(Naveau et al., 1994).** Also various tumor markers in serum as CA 19.9 and CA 125 have been suggested to be elevated nonspecifically in liver disease or non malignant conditions **(Zuckerman et al., 1999).**

Thus, we aimed in this study to clarify non-invasive rationale for staging of liver fibrosis using the combined role of -899G/C polymorphism of COX-2 gene, alternation of tumor markers CA 19.9 and CA 125 levels and plasma protein pattern comparing them to the fibrosis stages of liver biopsy using the METAVIR score in chronic HCV patients.

MATERIALS AND METHODS

Subjects:

This cross section case control study included 103 chronic hepatitis C virus (HCV) patients, positive HCV RNA detected by Polymerase chain reaction (PCR), with different degree of liver fibrosis and no prior exposure to antiviral therapy. They were attending the gastroenterology and hepatology outpatient clinic of internal medicine department of Assiut University Hospital, and El Ragahy Hepatology Hospital, Assiut

University. In addition, 42 healthy, age and sex matched volunteers, were included as a control group.

Patients with concomitant HBV/HIV infection, alcohol consumption (> 60g/day or biochemical feature of alcoholic hepatitis), acute/chronic liver disease were excluded from the study.

The study protocol has been revised and approved by the ethical committee of Faculty of Medicine, Assiut University and all participants signed a written informed consent.

Clinical and laboratory data:

All participants have undergone complete clinical assessment and routine lab working (liver function tests using a BM Hitachi 711 Chemistry Analyzer., prothrombin time was measured using a Bench Electronic coagulator, complete blood count (CBC) using a SYS-MEX K1000 device).

Plasma biochemical assessment of Serum CA19.9 and CA125 levels:

Venous blood samples were collected, centrifuged and serum was stored until the patch analysis. Serum CA19.9 and CA125 levels were measured using quantitative enzyme linked immunosorbent assay (ELISA), RayBio: Catalog #: ELH- Human CA19.9 ELISA Kit and RayBio: Catalog #: ELH-Human CA125 ELISA Kit respectively. We have considered 40 U/ml and 35 U/ml as the upper limits of ordinariness for CA 19.9 and CA 125 constantly.

Serum protein capillary electrophoresis (SPEP)

The blood serum was injected into a capillary with a negative surface charge under a high current. Albumin as it is negatively charged protein trying to move towards the anode while liquid buffer moves towards the cathode, dragging proteins with a weaker charge with it **(Keren, 2003).** Serum protein gears into five major fractions by size and electrical charge: serum albumin, alpha-1 globulins, alpha-2 globulins, beta1 and 2 globulins and gamma globulins (Beckman Coulter's PA 800 plus).

Genomic DNA extraction and genotyping

Genomic DNA was isolated from EDTA blood samples using the spin column method according to the protocol using QIAmp DNA Mini Kit (Cat.No.51306, Qiagen, Germany). DNA Polymorphism in COX-2 (-899G/C) was analyzed using polymerase chain reaction based TaqMan probe (PCR-TaqMan probe). The primers and TaqMan probe sequences (supplied by Analysis Biotechnology Com.) were as follow:

F: 5′-ACCCGTGGAGCTCACATTAACTAT-3′;

R: 5′-ATACTGTTCTCCGTACCTTCACCC-3′;

Probe (G) 5′-FA-M-CCTTTCCCGCCTCTCTTTCCA-TAMRA-3′;

Probe (C) 5′-TET-ACCTTTCCCCCCTCTCTTTCCA-AGA-TAMRA-3′ **(Jian-Hong et al., 2011).**

PCR-TaqMan reaction system was 25.5 μl, including: PCR mixture 12.5 μl (Qiagen) (1 unit Taq polymerase, 10 mM KCL, 10 mM (NH4)2 SO4, 20 mM Tris Hcl (pH 8.75), 0.1 % Triton X-100, 0.1 mg/ml BSA and 200 lm dTNPs), 1.0 μl of each PCR forward and reverse primers, 1.0 μl fluorescent probe solution, 0.5 μl ROX Reference Dye Ⅱ (50×), 7.0 μl DNA template, 1.5 μl dH_2O. PCR amplification conditions were an initial melting at 95°C for 10 minutes, followed by 40 cycles of denaturation at 95°C for 15s, annealing at 60°C for 60s, and extension at 72°C for 1 min. Deionized water was the negative control. **The reactions were performed by a Thermal Cycler (MJ Research, Inc., MA, USA).** Amplicons were subjected to 1% agarose gel electrophoresis (Biometra, P30-BI030090), stained with ethidium bromide and photographed under ultraviolet light to determine the presence or absence of PCR products for cyclooxygenase-2 (length of the DNA molecule-125 bp.) (Figure1).

Histopathogy:

Percutaneous liver biopsy was done to all HCV patients to assess degree of fibrosis using the METAVIR score. Fibrosis stage (F) was scored as F0 (absent), F1 (portal fibrosis), F2 (portal fibrosis with few septa), F3 (septal fibrosis), and F4 (cirrhosis) **(Germani et al., 2011)** (Figure 2).

Statistical analysis:

Data were verified, coded by the researcher and analyzed using SPSS version 21 (SPSS Chicago.IL. USA). The Shapiro–Wilkes test was used to determine whether or not data were normally distributed. Normally distributed data are expressed as mean and standard deviation (SD), non-normally distributed data as median and interquartile range. Groups were compared by one-way analysis of variance (ANOVA) or by use of the Kruskall–Wallis test, followed by Tukey's post-hoc test (with log transformation if necessary). Allele frequencies were estimated by the gene- counting methods. Logistic regression models were used for calculating odds ratios (95 % confidence interval (CI). Data were correlated by use of the Spearman rank method. A probability value of less than 0.05 was regarded as statistically significant.

RESULTS

Baseline characteristics of participants:

The clinical and biochemical characteristics of the studied groups are shown in Table1. Our results indicated that the age of cirrhotic group was 21–64 years (mean 41.06±11.54) and healthy control (HC) group 33–48

years (mean 41.52±3.91). There were no significant differences as regard age, gender, and smoking history among groups (P >0.05).

COX-2 -899G/C polymorphism*:*

The genotypes of COX-2 -899G/C were: GG, GC, and CC. The frequency of the three genotypes in studied groups demonstrated gradient alteration as GG, GC, and CC frequency were 68.0%, 28.2% and 3.9% in the fibrotic group; 97.06%, 2.4%, and 0.0% in HC one respectively (Figure 3,Table 2).

Relationship between COX-2 -899 G/C polymorphism and demographic characteristics*:*

To study any potential relationships between COX-2 -899G/C polymorphism and demographic characteristics including, we combined the GC and CC genotypes as polymorphic COX gene and compared them with the normal GG genotype. We found that males are less susceptible to carry an abnormal C allele (P <0.023).

Comparison of COX-2 expression in fibrotic group and healthy control group and with severity of hepatic fibrosis:

To examine the possible associations between COX-2 -899G/C polymorphism and occurrence of liver fibrosis, we combined both GC and CC genotypes and compared them with the GG genotype in both groups (Table 3). We found that the percent of COX-2 -899G/C genotype expression in fibrotic group, based on METAVIR staging using the full range of scores (from 0 to 4) and healthy group were 32% and 2.3% (p<0.001) respectively and that based on mild- vs. sever-fibrosis stages (METAVIR stages 1,2 vs. stages 3,4) were 18.2 % for the mild group and 81.8% for the sever stages of all fibrotic patient, (p = 0.001). In addition, CC genotype were only expressed in sever stages of fibrosis; while 79.3% of GC expression were in sever fibrosis (Table 3).

Serum protein levels among the studied population:

Studying the pattern of serum protein electrophoresis results revealed that total protein concentration was statistically decreased in the diseased group than healthy control (6.83±0.65 versus 7.16±0.5, respectively p=0.001). Compared to the healthy group, serum albumin was also lower in fibrotic patients (4.24±0.53 versus 3.73±0.7, respectively). Globulins comprise a much smaller fraction of the total serum protein content with no significant difference between levels in the studied groups (Figure 4, 5).

Serum tumor markers (CA19.9/CA125) Levels (ng/mL) in All Study Population According to COX-2 Genotypes and in relation to stage of fibrosis:

Serum CA19.9 and CA125 levels were significantly higher in fibrotic group than HC (p<0.001), and in sever fibrotic stages than in mild fibrosis group (p=0.000). In addition, our tumor markers levels were significantly increased in carriers of C allele (GC, CC) compared to those with G allele (p<0.001) (Table 4).

The Sensitivity, specificity and accuracy of our indices in detection of various stages of liver fibrosis and COX-2 -899G/C genotypes polymorphism at different cut-off levels were shown at (Table 5). For CA 19.9, a cutoff level *>14.5* ng/ml was able to diagnose the presence of liver fibrosis with a sensitivity of 87.38%, a specificity of 90.48% and the AUC was 0.898, a cutoff level *>75* ng/ml was able to distinguish between the mild and sever stages liver fibrosis with a sensitivity of 96.97%, a specificity of 100.00% and the AUC was 0.99, a cutoff level >69.3 ng/ml was able to diagnose the polymorphic COX-2 -899G/C with a sensitivity of 85.29%, a specificity of 90.99% and the AUC was 0.92. While that for CA 125, a cutoff level *>32.2* ng/ml was able to diagnose the presence of liver fibrosis with a sensitivity of 78.64%, a specificity of 100.00% and the AUC was 0.92, a cutoff level *>53* ng/ml was able to distinguish between the mild and sever stages liver fibrosis with a sensitivity of 93.94% , a specificity of 100.00% and the AUC was 0.98, a cutoff level >53 ng/ml was able to diagnose the polymorphic COX-2 -899G/C with a sensitivity of 73.53%, a specificity of 94.59% and the AUC was 0.89 (Table 5).

Table (1): Laboratory investigation of the studied groups:

	Patients (n= 103)	Control (n= 42)	P-value
Total bilirubin (mg/dl):			
Mean ± SD	2.04 ± 1.35	0.91 ± 0.37	<0.001*
Range	0.6-5.8	0.2-1.6	
Direct bilirubin (mg/dl):			
Mean ± SD	0.70 ± 0.63	0.30 ± 0.14	<0.001*
Range	0.1-2.8	0.1-0.6	
AST (IU/L):			
Mean ± SD	42.20 ± 18.51	14.95 ± 6.41	<0.001*
Range	7.0-90.0	3.0-30.0	
ALT (IU/L):			
Mean ± SD	45.56 ± 21.24	14.31 ± 5.77	<0.001*
Range	11.0-93.0	3.0-26.0	
ALP (IU/L):			
Mean ± SD	113.72 ± 46.82	78.86 ± 14.43	<0.001*
Range	54.0-250.0	46.0-101.0	
PC (%):			
Mean ± SD	90.65 ± 10.95	89.61 ± 11.07	0.458
Range	67.0-121.0	67.0-121.0	
PT (seconds):			
Mean ± SD	12.31 ± 0.97	12.35 ± 0.99	0.854
Range	10.0-14.3	10.0-14.3	
PCR (x1000 IU/ml):			
Mean ± SD	1253.59 ± 1643.25	--	--
Range	9.1-6540.0	--	
Platelets (x1000/mm^3):			
Mean ± SD	167.46 ± 99.25	267.75 ± 78.25	<0.001*
Range	66.4-960.0	147.0-458.0	

Continuous data was expressed in the form of mean ± SD and median (range) (compared with Student t test). *P value < 0.05 considered to be significant. AST: aspartate transaminase, ALT: alanine transaminase, ALP: alkaline phosphatase, PC: prothrombin concentration, PT: prothrombin time, PCR: polymerase chain reaction.

Table (2): Distribution of COX-2 -899G/C polymorphism genotypes and stages of liver fibrosis by METAVIR classification among studied groups:

	Patients (n= 103)		Control (n= 42)		X^2	P-value
	No.	%	No.	%		
Genotypes:						
CC	4	3.9	0	0.0	--	0.324
GC	29	28.2	1	2.4	12.08	0.001*
GG	70	68.0	41	97.6	14.62	0.000*
METAVIR:						
F0	0	0.0	42	100.0	145.00	0.000*
F1	21	20.4	0	0.0	10.01	0.002*
F2	49	47.6	0	0.0	30.18	0.000*
F3	3	2.9	0	0.0	--	0.557
F4	30	29.1	0	0.0	15.42	0.000*

Nominal data was expressed in form of frequency (percentage) (compared with Chi² test). F: fibrosis state. * *P* value < 0.05 considered to be significant.

Table (3): Distribution of COX-2 -899G/C polymorphisms genotype in studied groups in relation to the stages of liver fibrosis:

METAVER	Polymorphic genotype (GC+CC)		Normal genotype (GG)		OR (95% CI)	P-value
	No.	%	No.	%		
Fibrotic (F1.2.3.4)	33	97.1	70	63.1	19.33 (2.55-146.65)	<0.001*
Non fibrotic (F0)	1	2.9	41	36.9		
Sever (F3.4)	27	81.8	6	8.6		
Mild fibrosis (F1.2)	6	18.2	64	91.4		
	GC genotype		GG genotype		OR (95% CI)	P-value
	No.	%	No.	%		
Fibrotic (F1.2.3.4)	29	96.7	70	63.1	16.99 (2.23-129.38)	<0.001*
Non fibrotic (F0)	1	3.3	41	36.9		
Sever (F3.4)	23	79.3	6	8.6		
Mild fibrosis (F1.2)	6	20.7	64	91.4		
	CC genotype		GG genotype		P-value	
	No.	%	No.	%		
Fibrotic (F1.2.3.4)	4	100.0	70	63.1	0.295	
Non fibrotic (F0)	0	0.0	41	36.9		
Sever (F3.4)	4	100.0	6	8.6		
Mild fibrosis (F1.2)	0	0.0	64	91.4		

Nominal data was expressed in form of frequency (percentage) (compared with Chi^2 test). *P value < 0.05 considered to be significant.

Table (4): Levels of serum CA19.9/CA125 among studied groups and in fibrotic patients according to COX-2 -899G/C genotypes and METAVIR score of liver fibrosis:

	Patients	Control	P-value
CA19.9 (ng/ml):			<0.001*
Median (Range)	57.9 (0.6-221.5)	15.5 (2.2-33.6)	
CA125 (ng/ml):			<0.001*
Median (Range)	40.9 (5.9-165.0)	6.7 (4.1-32.2)	
	(GC+CC) genotypes	GG genotype	
CA19.9 (ng/ml):			<0.001*
Median (Range)	121.0 (12.9 - 221.5)	25.0 (0.6 - 135.6)	
CA125 (ng/ml):			<0.001*
Median (Range)	105.0 (7.6 - 165.0)	20.4 (4.1 - 155.3)	
	GC genotype	GG genotype	
CA19.9 (ng/ml):			<0.001*
Median (Range)	115.0 (12.9 - 221.5)	25.0 (0.6 - 135.6)	
CA125 (ng/ml):			<0.001*
Median (Range)	105.0 (7.6 - 160.0)	20.4 (4.1 - 155.3)	
	CC genotype	GG genotype	
CA19.9 (ng/ml):			0.001*
Median (Range)	150.5 (111.5 - 207.2)	25.0 (0.6 - 135.6)	
CA125 (ng/ml):			0.001*
Median (Range)	120.7 (98.3 - 165.0)	20.4 (4.1 - 155.3)	
	METAVIR (F 1 + 2)	METAVIR (F 3 + 4)	P-value
CA19.9 (ng/ml):			0.000*
Median (Range)	46.0 (0.6-75.0)	128.0 (57.9 - 221.5)	
CA125 (ng/ml):			0.000*
Median (Range)	37.7 (5.9-53.0)	125.3 (36.2 - 165.0)	

Continuous data was expressed in the form of median (range) (compared with Mann-Whitney test). * *P* value < 0.05 considered to be significant.CA19.9: cancer antigen 19.9, CA125: cancer antigen 125.

Table (5): The performance characteristics of serum CA19.9/CA125 for detection of various stages of liver fibrosis and COX-2 -899G/C genotypes polymorphism at different cut-off levels:

	Cut-off	Sensitivity	Specificity	PPV	NPV	Accuracy	AUC
CA19.9 (F1, 2, 3,4) vs. F0	**>14.5**	**87.38**	**90.48**	**95.7**	**74.5**	**88.3**	**0.898**
CA19.9 (F3, 4) vs. (F1, 2)	**> 75**	**96.97**	**100.00**	**100.0**	**98.6**	**99.0**	**0.992**
CA19.9 (CC ± GC) vs. GG	**> 69.3**	**85.29**	**90.99**	**74.4**	**95.3**	**89.7**	**0.918**
CA 125 (F1, 2, 3, 4) vs. F0	**> 32.2**	**78.64**	**100.00**	**100.0**	**65.6**	**84.8**	**0.922**
CA125 (F3, 4) vs. (F1, 2)	**> 53**	**93.94**	**100.00**	**100.0**	**97.2**	**98.1**	**0.977**
CA125 (CC ± GC) vs. GG	**> 53**	**73.53**	**94.59**	**80.6**	**92.1**	**86.4**	**0.893**

PPV: the positive predictive value, NPV: the negative predictive value, AUC: area under the curve. CA19.9: cancer antigen 19.9, CA125: cancer antigen125.

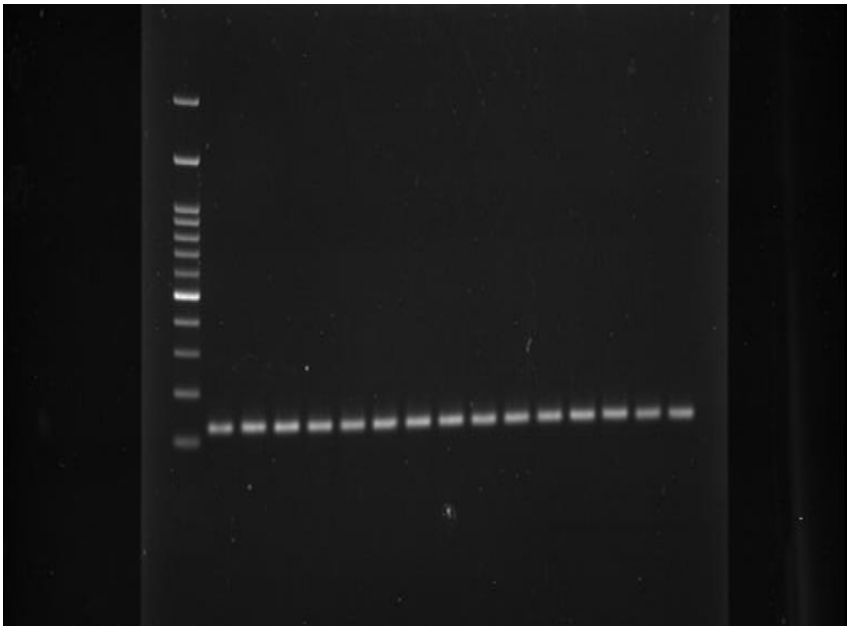


Figure 1: Agrose gel electrophoresis demonstrating that COX-2 -899G/C gene PCR products on the different blood samples was 125 bp (100 bp DNA marker was used).

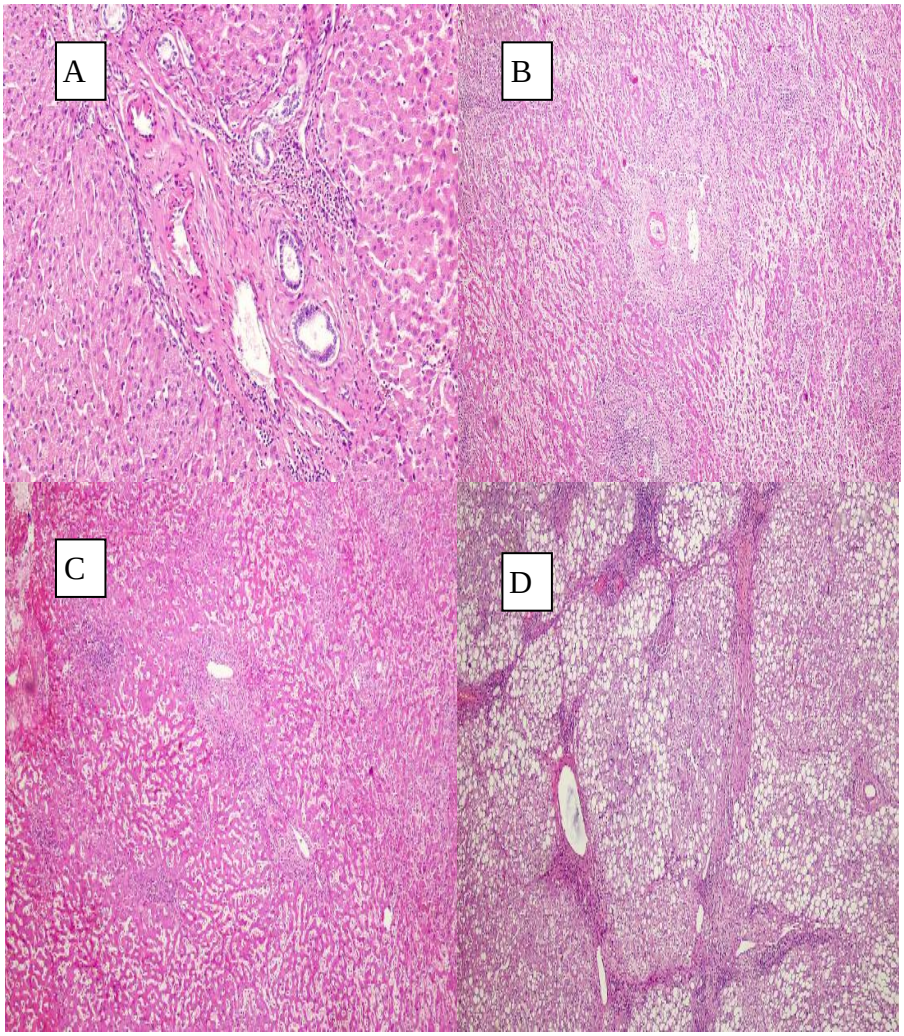


Figure 2: METAVIR scoring system of fibrosis (H&E stains):
A: F1 (portal fibrosis), B: F2 (portal fibrosis with few septa), C: F3 (septal fibrosis), and D: F4 (cirrhosis). The first image is x40, The other 3 images are x100

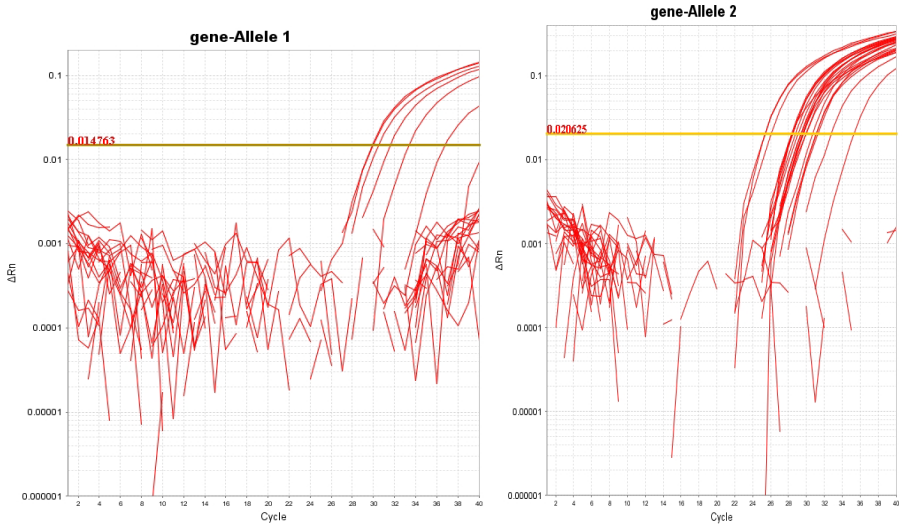
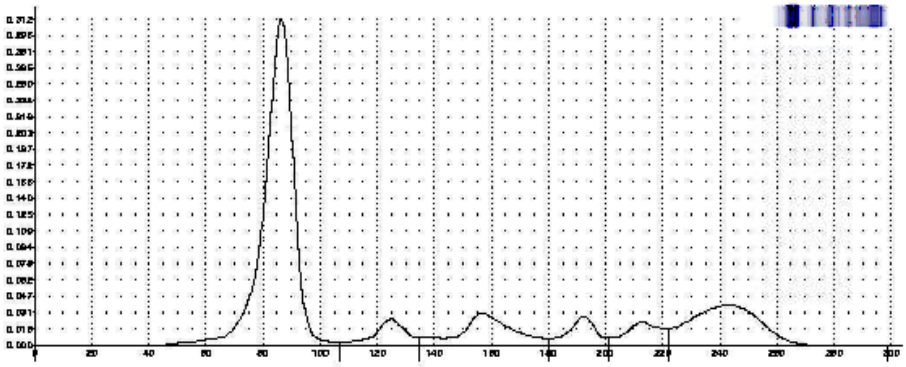


Figure 3: COX-2 -899G/C polymorphism amplification plot by PCR-TaqMan probes: Gene-Allele 1: amplification plots of the C allele, Gene-Allele 2: amplification plots of the G allele



Fractions	%	g/dl	Ref. g/dl
Albumin	58.3	4.3	3.2 - 5.0
Alpha 1	5.0	0.4	0.1 - 0.4
Alpha 2	9.6	0.7	0.5 - 1.0
Beta 1	4.8	0.4	0.4 - 0.7
Beta 2	5.0	0.4	0.2 - 0.6
Gamma	17.1	1.3	0.7 - 1.5

Figure 4: Serum protein electrophoresis of a healthy control with protein fractions were labeled according to the electrophoretic strip and the corresponding densitometry

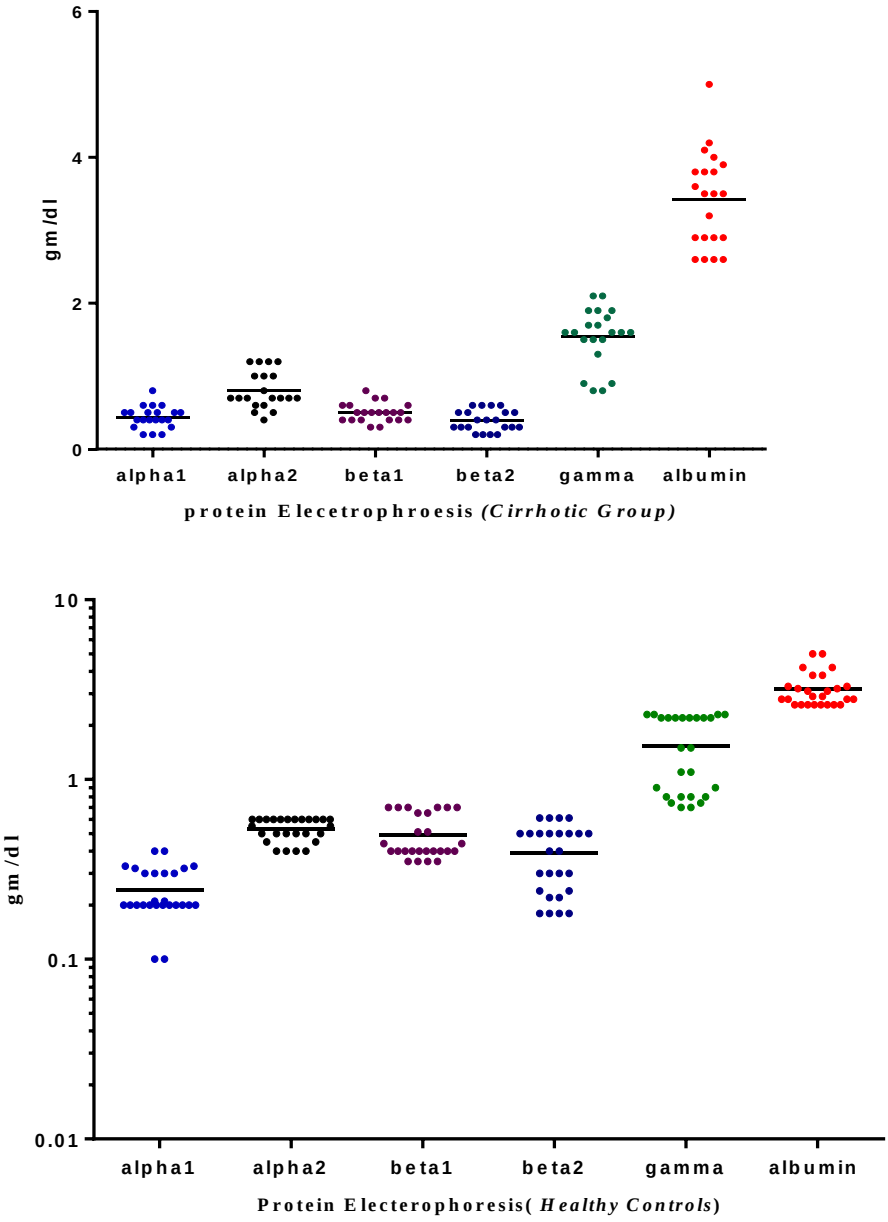


Figure 5: Serum proteins levels in the fibrotic group and healthy group.

DISCUSSION

The progression of chronic HCV into liver cirrhosis is correlated with an extensive fibrotic tissue that is primarily in periportal areas and in later stages completely surrounds the nodules of regenerating hepatocytes **(Bataller and Brenner 2005).** This progressive increase of the fibrotic matrix contributes both to the vascular disturbances in the form of irreversible portal hypertension and to the microenvironmental changes that ends by the occurrence of hepatocellular carcinoma. In addition, liver fibrosis is also a well-recognised negative prognostic factor of the viral response to interferon-based therapies **(Cheng et al., 2010).**

Although liver biopsy is the gold standard procedure for diagnosis of liver biopsy and is relatively safe, it is still associated with a significant morbidity, mortality and sample error with up to 24% false-negative results for cirrhosis **(Schöniger-Hekele and Müller, 2006).** There has been an explosion of research into noninvasive evaluation of liver fibrosis over the past decade, where they assessed combinations of serum biomarkers to increase diagnostic accuracy **(Boursier et al., 2009).**

In the present study we describe the association of COX-2 polymorphisms and the altered level of tumor markers (CA19.9 and CA125) with liver fibrosis. The results of our study demonstrated the presence of an association of the polymorphic C allele with liver fibrosis, as the COX-2 GC and CC genotypes were associated with a significantly elevated risk of fibrosis (OR = 19.33) which is more in sever stages**,** METAVIR stages 3,4, as the OR was 48.00. This Association of COX-2 polymorphism with liver fibrosis indicated that inflammation is crucial for the development of liver fibrosis. In addition, the COX-2 gene SNP may alter the individual susceptibility to liver fibrosis and probably be used as non invasive biomarkers of the disease.

It is well known that the promoter region of the COX-2 gene contains a variety of transcriptional regulatory elements, which may have profound effects on expression of the enzyme. The -899C SNP is inside a Sp-1 binding site and have no role in changing the consensus binding site, though, a NF-kB site is eliminated upstream of the SP-1 binding site by this SNP **(Panguluri et al., 2004).** This may partially explain the

implication of COX- 2 -899G/C gene polymorphism in developing liver fibrosis. We identify our self to be the first report that demonstrates the higher expression of COX-2 -899G/C gene polymorphism in fibrotic patients equated to healthy controls. On the other hand, few SNPs in other COX-2 promoter genes have been demonstrated to influence the progression of both hepatic inflammation and fibrosis in patients with chronic hepatic diseases. It have been reported that the -1195GG genotype in COX-2 is a genetic marker for liver disease progression **(Gu and Chen, 2012, Huang et al., 2013, Saad et al., 2013).** Besides, **Jeong et al. (2010)** clarified that the COX-2 expression was significantly higher in patients with liver cirrhosis compared to those with chronic hepatitis. In addition, the COX-2 expression scores according to Ishak's staging was significantly sophisticated in the advanced fibrosis stages. They concluded that COX-2 might play a role in the development of hepatic fibrosis. Moreover, expression of COX-2 has been demonstrated in liver generation after partial hepatoectomy, in different cirrhosis animal models and chronic hepatitis C infection **(Jeong et al., 2010).** Beside its role in fibrosis, other researchers validated that COX-2 polymorphism detected in different cancers indicating that inflammation is an important factor in the development of these cancers **(Miyashita et al., 2012, Xu et al., 2018).**

Based on, we can summarize that chronic liver patients with the polymorphic C allele are potentially susceptible to the development of liver fibrosis. Moreover, polymorphism research can be a significant future tool, as it will enable the accurate detection of the individuals most susceptible to the risk of liver disease.

In addition, our data revealed that serum CA19.9 and CA125 levels were significantly higher in fibrotic than non fibrotic HCV patients, and in the polymorphic (GC and CC) genotypes compared to the GG individuals. Also, serum CA19.9 and CA125levels were significantly higher in sever fibrotic patients than in mild fibrosis group. This is in accordance to several reports which illustrated an elevation of the tumor marker CA19.9 **(Bertino et al., 2007, Bertino et al., 2013, Haque et al., 2015)** and CA125 **(Deschenes et al., 2001, Edula et al., 2018)** in patients with liver cirrhosis. In addition, **Singhal et al. (2012)** clarified that CA 19.9 and CA

125 levels were elevated in 23% and 51.5% of patients with end stage liver disease, respectively, even though the exact etiology still uncertain.

Additionally **Schöniger-Hekele and Müller (2006)** studied the role of combined tumor markers in prediction of severe liver fibrosis. They noticed that CA 19.9, CA 125, and CA 15-3 levels were increased with stage of fibrosis and the best predictive ability of the combined elevation of CA 19.9 and CA 125 was for severe liver fibrosis (F3+F4). They conclude that the combined elevation of CA 19.9 and CA 125 may be useful for detecting patients with severe fibrosis or cirrhosis with high specificity. Actually, elevations of CA19.9 serum levels in fibrotic patients can be attributed to the necroinflammatory processes, small bile ducts alterations, the development of regeneration nodules, and to the increased production of raw collagen, which all are sequences in the progression of chronic hepatitis to liver cirrhosis.

Conclusion:

For the first time we considered the diagnostic value of a combination of non invasive markers (COX-2 -899G/C gene polymorphism, CA 19.9, CA 125 tumor markers and altered plasma proteins levels) available in clinical practice for precise detection and staging of liver fibrosis .Our results obviously identified that COX-2 −899G/C polymorphisms could be a risk marker for hepatic fibrosis from analyses of its genotype distributions among HCV patients and healthy controls in Assuit University Hospital. However, further confirmed by larger sample size investigation among different races and regions is mandatory. Likewise, abnormally elevated CA 19.9 and CA 125 levels should be considered in patients with liver cirrhosis. Finally, efforts to identify non invasive biomarkers providing suggestions for the accurate staging of liver fibrosis are important to reduce the number of the liver biopsies performed.

Author Contributions

All authors made substantial contributions to conception and design, or acquisition of data, or analysis and interpretation of data; the drafting of the article or revising it critically for important intellectual content; and final approval of the version to be published.

Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

REFERANCE

Afdhal N.H., (2004). The natural history of hepatitis C. Semin Liver Dis 24, 3–8.

Akkız H., Bayram S., Bekar A., Akgöllü E., Ülger Y. (2011). Functional polymorphisms of cyclooxygenase-2 gene and risk for hepatocellular carcinoma. Mol Cell Biochem 347, 201-208.

Bataller R. and Brenner D.A., (2005). Liver fibrosis. J Clin Invest115, 209–218.

Bedossa P., Patel K., Castera L., (2015). Histologic and non invasive estimates of liver fibrosis. Clinical liver disease 6, 5-8.

Bertino G., Ardiri A.M., Boemi P., Bruno C.M., Valenti M., Mazzarino M.C., et al., (2007). Meaning of elevated CA 19-9 serum levels in chronic hepatitis and HCV-related cirrhosis. Minerva Gastroenterol Dietol. 53(4), 305-9.

Bertino G., Ardiri A.M., Calvagno G.S., Malaguarnera G., Interlandi D., Vacante M., et al., (2013). Carbohydrate 19.9 Antigen Serum Levels in Liver Disease. BioMed Research International. Article ID 531640, 6 pages, 2013.

Boursier J., Vergniol J., Sawadogo A., Dakka T., Michalak S., Gallois Y., et al., (2009). The combination of a blood test and Fibroscan improves the non-invasive diagnosis of liver fibrosis. Liver Int. 29(10), 1507-1515.

Bravo A.A., Sheth S.G., Chopra S., (2001). Liver biopsy. N Engl J Med 344, 495-500.

Castera L., (2011). Invasive and non-invasive methods for the assessment of fibrosis and disease progression in chronic liver disease. Best Pract Res ClinGastroenterol 25, 291-303.

Cheng W.S., Roberts S.K., McCaughan G., Sievert W., Weltman M., Crawford D., et al., (2010). Low virological response and high relapse rates in hepatitis C genotype 1 patients with advanced fibrosis despite adequate therapeutic dosing. J Hepatol. 53, 616–623.

Deschenes M., Michel R.P., Alpert E., Barkun J.S., Metrakos P., Tchervenkov J., (2001). Elevation of CA-125 level is due to abdominal distension in liver transplantation candidates. Transplantation 72(9), 1519–1522.

Edula RG, Muthukuru S, Moroianu S, Wang Y., Lingiah V., Fung P., et al., (2018). CA-125 Significance in Cirrhosis and Correlation with Disease Severity and Portal Hypertension: A Retrospective Study. J Clin Transl Hepatol. 6 (3), 241-246.

Fawzy M.S., Elfayoumi A.R., Mohamed R.H., Fatah I.R., Saadawy S.F., (2016). Cyclooxygenase 2 (rs2745557) Polymorphism and the Susceptibility to Benign Prostate Hyperplasia and Prostate Cancer in Egyptians. Biochem Genet. 54(3), 326-36.

Friedman S.L. and Bansal M.B., (2006). Reversal of hepatic fibrosis-fact or fantasy? HEPATOLOGY 43, S82-S88.

Germani G., Hytiroglou P., Fotiadu A., Burroughs A.K., Dhillon A.P., (2011). Assessment of fibrosis and cirrhosis in liver biopsies: an update. Semin Liver Dis 31, 82-90.

Gretton S., Hughes M., Harris M., (2010). Hepatitis C virus RNA replication is regulated by Ras-Erksignalling. J Gen Virol 91, 671-680.

Gu K.S. and Chen Y., (2012). Mechanism of P-glycoprotein expression in the SGC7901 human gastric adenocarcinoma cell line induced by cyclooxygenase-2. Asian Pacific Journal of Cancer Prevention 13(5), 2379–2383.

Haque M.S., Sultana S., Mahtab M.A., Al-Mamun A., Rahman S., (2015). The Value of Serum CA19-9 Levels in predicting Extent of Hepatic Fibrosis in Patients with Chronic Hepatitis B. Euroasian J Hepatogastroenterol 5(1), 16-18.

Hofmann W.P. and Zeuzem S., (2009). Hepatitis C: new therapeutic strategies needed for advanced disease. Nat Rev GastroenterolHepatol 6 (6), 325–327.

Huang F., Lin C., Shi Y.H., Kuerban G., (2013). MicroRNA-101 inhibits cell proliferation, invasion, and promotes apoptosis by regulating cyclooxygenase- 2 in hela cervical carcinoma cells. Asian Pacific Journal of Cancer Prevention 14(10), 5915–5920.

Jeong S.W., Jang J.Y., Lee S.H., Kim S.G., Cheon Y.K., Kim Y.S., et al., (2010). Increased Expression of Cyclooxygenase-2 is Associated with the Progression to Cirrhosis. Korean J Intern Med 25, 364-371.

Jian-Hong H.E., Yu-Min L.I., Quan-Bao Z., Zhi-Jian R., Xun L., Wen-Ce Z., (2011). Cyclooxygenase-2 Promoter Polymorphism -899g/C Is Associated With Hepatitis B-Related Liver Cancer in a Chinese Population of Gansu Province. Chin Med J 124 (24), 4193-4197.

Keren D., (2003). Protein Electrophoresis in Clinical Diagnosis. Hodder Arnold. pp. 1–14. ISBN 0340 81213 3

Liu C.J., Hsia T.C., Wang R.F., Tsai C.W., Chu C.C., Hang L.W., et al., (2010). Interaction of cyclooxygenase 2 genotype and smoking habit in Taiwanese lung cancer patients. Anticancer Res 30 (4), 1195-1199.

Lotersztajn S., Julien B., Teixeira-Clerc F., Grenard P., Mallat A., (2005). Hepatic fibrosis: molecular mechanisms and drug targets. Annu Rev Pharmacol Toxico l45, 605-628.

Manning D.S. and Afdhal, N.H., (2008). Diagnosis and quantitation of fibrosis. Gastroenterology 134 (6), 1670 – 1681.

Miyashita M., Ito T., Sakaki M., Kajiwara A., Nozawa H., Hiroishi K., et al., (2012). Genetic polymorphism in cyclooxygenase-2 promoter affects hepatic inflammation and fibrosis in patients with chronic hepatitis C. J Viral Hepat. 19(9), 608-614.

Naveau S., Poynard T., Benattar C., Bedossa P., Chaput J.C., (1994). Alpha-2-macroglobulin and hepatic fibrosis. Dig Dis Sci 39(11), 2426–2432.

Panguluri R.C., Long L.O., Chen W., Wang S., Coulibaly A., Ukoli F., et al., (2004). COX-2 gene promoter haplotypes and prostate cancer risk. Carcinogenesis 25, 961-966.

Penin F., Dubuisson J., Rey F.A., Moradpour D., Pawlotsky J.M., (2004). Structural biology of hepatitis C virus. Hepatology 39, 5-19.

Saad N., Esa N.M., Ithnin H., (2013). Suppression of β-catenin and cyclooxygenase-2 expression and cell proliferation in azoxymethane-induced colonic cancer in rats by rice bran phytic acid (PA) Asian Pacific Journal of Cancer Prevention 14(5), 3093–3099.

Schöniger-Hekele M. and Müller C., (2006). The combined elevation of tumor markers CA19.9 and CA125 in liver disease patients in highly specific for severe liver fibrosis. Dig Dis Sci. 51(2), 338-345.

Seeff L.B., (2009). The history of the "natural history" of hepatitis C (1968–2009). Liver Int. 29 (Suppl 1), 89–99.

Singhal A., Lander E., Karachristos A., Daly E., Dowling P., Patel V., et al., (2012). Elevation of CA125 and CA19.9 in patients with end-stage liver disease. Int J Biol Markers 19; 27(2), e147-e151.

Stattermayer A.F., Rutter K., Beinhardt S., Scherzer T.M., Stadlmayr A., Hofer H., et al., (2012). Association of the IL28B genotype with insulin resistance in patients with chronic hepatitis C. J Hepatol. 57, 492–498.

Stattermayer A.F., Rutter K., Beinhardt S., Wrba F., Scherzer T.M., Strasser M., et al., (2014). Role of FDFT1 polymorphism for fibrosis progression in patients with chronic hepatitis C. Liver Int. 34, 388–395.

Strader D.B., Wright T., Thomas D.L., Seeff LB., (2004). American Association for the Study of Liver Diseases, diagnosis, management, and treatment of hepatitis C. Hepatology 39 (4), 1147 – 1171.

Tan W., Wu J., Zhang X., Guo Y., Liu J., Sun T., et al., (2007). Associations of functional polymorphisms in cyclooxygenase-2 and platelet 12-lipoxygenase with risk of occurrence and advanced disease status of colorectal cancer. Carcinogenesis 28, 1197-1201.

Valenti L., Aghemo A., Stattermayer A.F., Maggioni P., De Nicola S., Motta B.M., et al., (2012). Implications of PNPLA3 polymorphism in chronic hepatitis C patients receiving peginterferon plus ribavirin. Aliment Pharmacol Ther. 35, 1434–1442.

Vidak H.K., Ivković T.C., Vidak Z, Kapitanovic S., (2016). COX-1 and COX-2 polymorphisms in susceptibility to cerebral palsy in very preterm infants. Mol Neurobiol. 54 (2), 930-938.

Xu W., Huang Y., Zhang T., Zhao L., Fan J., Li L., (2018). Cyclooxygenase-2 gene polymorphisms and susceptibility to hepatocellular carcinoma: A meta-analysis based on 10 case-control studies. J Cancer Res Ther. 14, S105-S113.

Zhang X., Miao X., Tan W., Ning B., Liu Z., Hong Y., et al., (2005). Identification of functional genetic variants in cyclooxygenase-2 and their association with risk of esophageal cancer. Gastroenterology; 129, 565–576.

Zuckerman E., Lanir A., Sabo E., Rosenvald-Zuckerman T., Matter I., Yeshurun D., et al., (1999). Cancer Antigen 125: A sensitive marker of ascites in patients with liver cirrhosis. Am J Gastroenterol 94, 1613–1618.

الملخص العربى

تاثير التعدد الشكلى لجين السيكلوكسجينيز-٢ ٨٩٩ جى/سى وبعض المؤشرات الاخرى على التليف الكبدى المصاحب للالتهابى الكبدى الفيروسى سى

د/ مروه عبد النعيم جابر[١]، د/ نجلاء كمال ادريس[١]، د/ جيهان سيف الدين[٢]، د/ ريم القاضى[٢]، د/ رانيا مقبول[٣]، د/ هاله مصطفى امام[٤]، د/ لبنى عبد الواحد[٤]
اقسام الكيمياء الحيوية الطبية[١] ، الاشعة التشخيصية[٢]- الباثولوجي[٣]، الباطنة العامة[٤]، كلية الطب ، جامعة أسيوط ، أسيوط

قامت العديد من الابحاث بدراسة العلاقة و الارتباط بين التعدد الشكلى لجين سيكلوكسيجينيز -٢ (COX-2) وأمراض الكبد. يعتبر التقدير المبكر والدقيق لمراحل التليف الكبدي من الأمور الحاسمة فى قرارات العلاج وكذلك التنبؤ بالنتائج في هؤلاء المرضى. لقد استهدفنا فى هذه الدراسة توضيح دور التعدد الشكلى لجين سيكلوكسيجينيز -٢ C٨٩٩ / G ، وتغيير مستويات الدم من دلالات الاورام CA 19-9 و CA 125 ، كذلك نمط بروتين البلازما في حدوث المراحل المختلفة لتليف الكبد باستخدام تقسيم METAVIR لتليف الكبد. استهدف البحث ١٠٣ من المرضى اللذين يعانون من تليف الكبد اللاحق للاصابة بالالتهاب الكبدى الفيروسى سى و ٤٢ من الاصحاء كمجموعة ضابطة. تم الكشف عن تعدد الأشكال الجيني COX-2 بواسطة PCR- TaqMan ، في حين تم تقدير مستويات الدم من دلالات الاورام CA19-9، CA125 باستخدام الاليزا الكمية. تم تعيين بروتينات البلازما بواسطة طريقة الفصل الكهربائي. أوضحت النتائج أن تواتر التراكيب الوراثية GG (- COX-2) و GC و CC كانت ٦٨,٠٪ و ٢٨,٢٪ و ٣,٩٪ في المجموعة المصابة بالتليف الكبدى، بينما كانت ٩٧,٠٦٪ ، ٢,٤٪ ، و ٠,٠٪ في المجموعة الضابطة على التوالي. كانت النسبة المئوية لتعبير COX-2 للمجموعة المرضى ومجموعة الاصحاء هي ٣٢٪ و ٢,٣٪ على التوالي. كانت درجات تعبير COX-2 على المراحل المتوسطة من التليف مقابل المراحل المتقدمة ١٨,٢٪ و ٨١,٨٪ على التوالي. كان مستوى المصل من دلالات الاورام التي تم اختبارها أعلى بشكل ملحوظ في المرضى الذين يعانون من تليف الكبد مقارنة بالمجموعة الضابطة (٦٩,٤٠ ± ٥١,٨٢ ، ١٣,٤١ ± ٦,٤٩ على التوالي لـ CA 19.9 و ٥٩,١٦ ± ٤٧,٢٣ ، ١٠,٩٠ ± ٨,٣٦ لـ CA 125) وفي الأنماط الجينية GC / CC مقابل GG (55.00 ± 116.96) و ٣٣,٦٤ ± ٢٨,٣٩ على التوالي لـ CA 19.9 و ١٠١,٦٢ ± ٥١,٢٩ و ٢٧,٨٩ ± ٢٥,٥١ على التوالي لـ CA 125. نخلص من هذة الدراسة بان الاشخاص الحاملين للاليل C 899- COX-2 أكثر عرضة للإصابة بالتليف الكبدي المصاحب للفيروس سى. كذلك إن التقدير المشترك لمستويات CA19-9 و CA125 مفيدان في تشخيص وتقسيم المرضى الذين يعانون من تليف الكبد.