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# *MMP9* and *CEBPα* Genes as a New Prognostic Biomarker for Hepatocellular Carcinoma Caused by Infection with HCV-Genotype (4)

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ARTICLE INFO

*Article history:*
Received: 6 July, 2021
Accepted: 22 July, 2021
Published: 10 Aug, 2021

Keywords:
*HCV-genotype (4)*
*cirrhosis*
*HCC*
*MMP9*
*CEBPα*
*biomarkers*

ABSTRACT

Hepatocellular carcinoma (HCC) remains the main type of liver cancer. Understanding the molecular and immune mechanisms of HCC tumorigenesis are required to develop effective biomarkers. This study is designed to measure the circulating *MMP9* and *CEBPα* to provide a diagnostic and prognostic biomarker for HCV-genotype (4) induced liver cirrhosis and carcinogenesis. This study included one hundred Egyptian patients, divided into two groups 50 patients each. The first group: classified into Chronic Liver Disease (CLD) without cirrhosis (n=25) and CLD with cirrhosis (n=25). The second group: classified into CLD patients with HCC, (n=25), and healthy control (25 volunteers). The expression of *MMP9* and *CEBPα* genes were analysed using Real-Time PCR. Our results showed significant downregulation in *MMP9* and *CEBPα* genes in cirrhotic and HCC patients ($p< 0.001$ and $p<0.001$) respectively. There was a significant ($p< 0.001$) diagnostic capacity between HCC patients against CLD with or without cirrhosis patients. Bioinformatics analysis revealed a relationship between *MMP9* and *CEBPα* genes. In conclusion, the gradual decrease in the expression of *MMP9* and *CEBPα* gene during the progression of the disease recommended use of *MMP9* and *CEBPα* genes as a diagnostic and prognostic biomarker for both cirrhosis and HCC in HCV-genotype (4) patients.



## Introduction

Hepatocellular carcinoma (HCC) is considered as the primary type of liver cancer and the third main source of death because of cancer worldwide [1]. The development of HCC is mainly related to liver cirrhosis and chronic liver inflammation. The chronic infection with hepatitis C virus (HCV) is considered the principal cause of HCC [2]. Despite the successful treatment of HCV and the dramatic increase in number of patients cured from HCV, patients that suffer with cirrhosis remain at high risk for HCC [3-5]. Our knowledge and understanding of the pathogenesis of HCC has to be improved, and more data on the molecular and immune mechanisms of HCC tumorigenesis are required to develop effective biomarkers that provide new insights into clinical practice to increase survival in HCC patients [6-8].

Matrix metalloproteinases (MMPs) are a group of proteolytic enzymes. It is important in the proliferation, invasion and metastasis of cancer cells [9]. In addition, MMPs cause degradation of the extracellular matrix (ECM), hence permit the migration of cells to invade the adjacent tissue [10, 11]. Among MMPs family, MMP-9 activation is related to chronic hepatitis and promote the incidence and metastasis of HCC [12-15]. CCAAT/enhancer-binding protein (C/EBP) is a family of transcription factors that promote different mechanisms such as cell growth, metabolism, and immune response. Among C/EBP family, *CEBPα* is

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http://dx.doi.org/10.31487/j.COR.2021.08.02

most expressed in the liver and is important for the function of hepatocyte [16, 17]. The upregulation of *CEBPα* activity led to notable improvement in liver function and reduced HCC growth [18, 19]. Studying factors affecting the molecular and immune responses associated with HCC provides a great opportunity to develop both prognostic markers and new therapeutic approaches. Therefore, this work was planned to estimate and measure the circulating *MMP9* and *CEBPα* in order to provide a new latent biomarker for HCV-genotype (4) induced liver cirrhosis and HCC in Egyptian patients.

## Materials and Methods

### I Study Design

We planned a study of 25 subjects/group, and the patient's values were regressed against control. Depending on the prior data, which indicated 0.7, a standard deviation of control and 1.8 of the regression errors. Since the true slope of the line obtained by regressing patients against control is 1.7, with a 90% probability, the false discovery rate associated with this hypothesis was set to 0.05.

### II Inclusion and Exclusion Criteria

This research on patients was approved by the Research Ethics Committee at Theodor Bilharz Research Institute (TBRI-REC), and a written informed consent was received from each patient. Patients included in the study were HCV RNA Positive for more than six months and have no treatment with specific drugs for HCV. Patients excluded if they had a history of any of the following diseases: schistosomiasis, chronic viral diseases other than HCV, dual HBV and HCV infection, nonalcoholic steatohepatitis (NASH), autoimmune hepatitis, biliary disorders, malignancies other than HCC, regular hepatotoxic drugs, alcohol abuse, diabetes and HCV-infected patients receiving DAAS or immunomodulatory interferon-α therapy.

According to the above criteria: hundred patients were involved in the current study classified as follows:

i. Twenty-five healthy adults (age- and sex- matched) served as control.
ii. Seventy-five HCV patients, classified into two major groups:
   A. **Group I:** chronic liver disease (CLD) without HCC (n=50); include two subgroups,
      a. Subgroub Ia represent CLD without cirrhosis (n=25).
      b. Subgroub Ib two subgroups: CLD without cirrhosis (n=25).
   B. **Group II:** CLD with HCC (n=25).

### III HCV Genotyping

i. Viral RNA Extraction; using high pure viral RNA kit (version 18, 2011), cat. No: (11858882001) (Link1).
ii. cDNA synthesis (Transcriptor First Strand); was done according to cDNA synthesis kit (Transcriptor first strand) (version 6.0, 2010), cat. No: (04379012001) (Link1).
iii. HCV Genotyping Detection; using hot start reaction mix detection for PCR using HybProbe probes with the light cycler carousel-based system (version 15, 2011), cat. No: (03003248001) (Link1).

### IV Target Gene Expression

i. Total RNA Extraction; was done according to high pure RNA isolation kit (version 12, 2011), Cat. No: (11828665001) (Link1).
ii. cDNA synthesis (Transcriptor First Strand); was done according to cDNA synthesis kit (FIREScript RT cDNA Synthesis Kit) cat. No: (6-15-00200) (Link2).
iii. Gene expression detections; was performed using lightcycler system with 5x HOTFIREPol EvaGreen qPCR SYBR green Master Mix, Cat. No: (08-36-00001) (Link2). Table 1 illustrates the sequences of the primer.

**Table 1:** Primers of genes included in the study.

| Gene | | Sequence | Tm | Reference |
|---|---|---|---|---|
| ***MMP9*** | | | | |
| | Forward | 5′- ACAGCATCTCACAGGTTGGG -3′ | 57.0ºC | (Link4) |
| | Reverse | 5′- TCTGGCACGTAGAAAGCACT -3′ | 57.0ºC | |
| ***CEBPα*** | | | | |
| | Forward | 5- GGAGGGTCTCTAGTTCCACG -3 | 57.0ºC | (Link4) |
| | Reverse | 5- CCCACAGCCAGATCTCTAGG -3 | 57.0ºC | |
| ***B-actin*** (an endogenous control) | | | | |
| | Forward | 5-AAATGAAGGGAGGCGATCAGG-3 | 57.0ºC | (Link5) |
| | Reverse | 5-AATTGGTGCCACATGGCTTG-3 | 57.0ºC | |

### V Bioinformatics Tools

Multiple Association Network Integration Algorithm (GeneMANIA) were used to analyse the gene association networks between *MMP9* and *CEBPα* genes in HCC cases in order to predict the possible functional interactions between the two genes (Link3).

### VI Statistical Analysis

Differences between the groups and themselves, as well as with the control in demographic characteristics were evaluated by the $\chi^2$ test (for categorical variables) or Student's *t* -test (for continuous normally distributed variables), while Mann-Whitney tests (for non-normally distributed variables). To compare the means of normally distributed variables between groups, the ANOVA was performed, and kruskal

wallis H test will be used in nonnormal variables. To determine the diagnostic performance of *MMP9* and *CEBPα* genes, the Receiver operating characteristic (ROC) curves was performed. HCC risk was estimated by computing odds ratios (ORs) and 95% confidence intervals (CIs) from logistic regression analyses. The data were analysed using Microsoft Excel 2016 and statistical package for social science IBM SPSS Statistics for Windows, version 26 (IBM Corp., Armonk, N.Y., USA).

**Table 2:** Descriptive data.

| | | | Control N=25 | Group I (CLD without HCC) N=50 | | Group II (CLD with HCC) N=25 | P. value |
|---|---|---|---|---|---|---|---|
| | | | | Subgroup Ia (CHC without Cirrhosis) N=25 | Subgroup Ib (CHC with Cirrhosis) N=25 | | |
| **Demographic data** | **Age** | | 45.3±9.1 | 47.7±7.1 | 61.8±10.0 | 58.2±8.9 | 0.001** |
| | **Sex** | **Female** | 2(8.0%) | 14(56.0%) | 13(52.0%) | 6(24.0%) | 0.001** |
| | | **Male** | 23(92.0%) | 11(44.0%) | 12(48.0%) | 19(76.0%) | |
| **Laboratory Investigation** | **HB** | | 13.2±0.8 | 11.2±4.1 | 10.2±2.1 | 11.3±0.9 | 0.001** |
| | **WBCs** | | 7.1±1.5 | 6.7±1.7 | 7.8±2.3 | 9.4±3.2 | 0.001** |
| | **Platelets X103** | | 268.2±84.7 | 243.5±69.9 | 110.2±48.0 | 155.2±74.1 | 0.001** |
| | **PT** | | 12.4(11.35 - 13.3) | 11.2(11.10 - 12.4) | 17.1(15.45 - 20.4) | 15.5(13.0 - 18.4) | 0.001** |
| | **PC** | | 98.4(77.9 - 100.0) | 99.0 (45.0 - 100.0) | 60.0(44.0 - 71.55) | 74.4(61.6 - 90.0) | 0.001** |
| | **INR** | | 1.01(1.0 - 1.16) | 1.01 (1.0 - 1.04) | 1.43(1.3 - 1.82) | 1.24(1.1 - 1.5) | 0.001** |
| | **UREA** | | 29.0(25.0 - 39.5) | 38.0(34.0 - 42.0) | 44.0(37.0 - 53.5) | 84.0(45.0 - 140.5) | 0.001** |
| | **CREAT** | | 0.9(0.65 - 1.00) | 1.0(0.75 - 1.1) | 1.1(1.0 - 1.32) | 1.87(1.01 - 3.2) | 0.001** |
| | **Albumin** | | 4.2±0.6 | 4.1±0.6 | 3.1±1.0 | 2.8±0.7 | 0.001** |
| | **T. Protein** | | 7.3±0.6 | 6.4±0.9 | 5.9±1.1 | 5.3±1.1 | 0.001** |
| | **T. Bil** | | 0.7(0.4 - 0.9) | 0.9(0.79 - 1.35) | 1.2(0.75 - 1.45) | 1.9(1.0 - 3.4) | 0.001** |
| | **D. Bil** | | 0.2(0.1 - 0.2) | 0.4(0.3 - 0.65) | 0.6(0.3 - 0.8) | 1.1(0.2 - 1.5) | 0.001** |
| | **ALT** | | 27.0(18.0 - 35.0) | 42.0(41.0 - 56.0) | 49.0(22.5 - 83.0) | 60.0(43.5 - 89.0) | 0.001** |
| | **AST** | | 30.0(21.0 - 40.5) | 38.0(26.0 - 45.0) | 53.0(30.0 - 65.5) | 82.0(36.5 - 106.5) | 0.001** |
| | **AFP** | | 5.4 (3.7 - 7.2) | 7.8(6.65 - 9.0) | 69.0(25.0 - 116.0) | 310.0(84.8 - 535.0) | 0.001** |
| **Clinical presentation** | **APRI** | | 0.29 (0.21 - 0.39) | 0.36(0.3 - 0.46) | 1.09(0.53 - 2.22) | 1.12(0.48 - 1.93) | 0.001** |
| | **U.S Finding** | **Cirrhosis** | 0 (0.0%) | 0 (0.0%) | 25 (100.0%) | 7 (28.0%) | 0.01* |
| | | **Splenomegaly** | 0 (0.0%) | 4 (16.0%) | 16 (64.0%) | 20 (80.0%) | |
| | | **Ascites** | 0 (0.0%) | 0 (0.0%) | 19 (76.0%) | 25 (100.0%) | |

Age, Haemoglobin (HB), White blood corpuscles (WBCs), Platelets, Albumin, and T. Protein are represented as mean ± SD; the data were analysed by ANOVA Test. While PT, PC and International normalized ratio (INR), Urea, Crat. T. Bilirubin., D. Bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alpha fetoprotein (AFP) and APRI core are represented as Median and interquartile range (25-75%); Kruskal Wallis tests were used for data analysis. Sex and U/S finding are represented as frequency and percent; the data were analysed by Chi square ($X^2$) Test.

**p* value <0.05 is considered significant, ***p* value <0.01 is considered highly significant.

**Table 3:** Genes expression of *MMP9* and *CEBPα* genes in the studied groups.

| Biomarkers | Control N=25 | Group I (CLD without HCC) N=50 | | Group II (CLD with HCC) N=25 |
|---|---|---|---|---|
| | | Subgroup Ia (CHC without Cirrhosis) N=25 | Subgroup Ib (CHC with Cirrhosis) N=25 | |
| ***MMP9*** | 0 | 9.86(3.28- 26.48) | 2.78(0.61- 8.45) [aa] | 0.21(0.11- 1.65) [aa, bb, **] |
| ***CEBPα*** | 0 | 6.12(3.70- 7.75) | 2.80(1.07- 4.07) [aa] | 0.23(0.13- 0.32) [aa, bb, **] |

*MMP9* and *CEBPα* are represented as Median and Interquartile range (25-75%) of the fold change; the data were analysed by Mann-Whitney U test.

[a]*p* value is significantly different comparing with Subgroup Ia.

[b]*p* value is significantly different comparing with Subgroup Ib.

[*]*p* value is significantly different comparing with CLD group (I).

[1]Initial p value <0.05 is significant, [2]Initial p value <0.01 is highly significant.

## Results

The demographic characteristics and routine laboratory tests of the groups involved in the current study are described in (Table 2). Table 3 represent the expression of *MMP9* and *CEBPα* genes observed in serum of subgroup Ib and group II patients in comparison to the healthy control group, as well as when compared to subgroup Ia; in addition to the actual fold-change of the up-regulated and down-regulated genes amongst the subgroups Ia, Ib and group II. The present results showed a significant decrease (p< 0.001 and < 0.001) respectively of the *MMP9* expression in subgroup Ib and group II in comparison to subgroup Ia. Also, group II showed significant downregulation (p< 0.001) of the *MMP9* expression, in comparison to subgroup Ib as well as when compared to group I (Table 3, Figure 1A). As for the expression of *CEBPα* gene, our results showed a significant decrease (p< 0.001 and p < 0.001) respectively in subgroup Ib and group II, when compared to the subgroup Ia as well as when compared to subgroup Ib. In addition, in group II the expression of *CEBPα* downregulated significantly (p< 0.001) in comparison to subgroup Ib as well as when compared to group I (Table 3, Figure 1B).

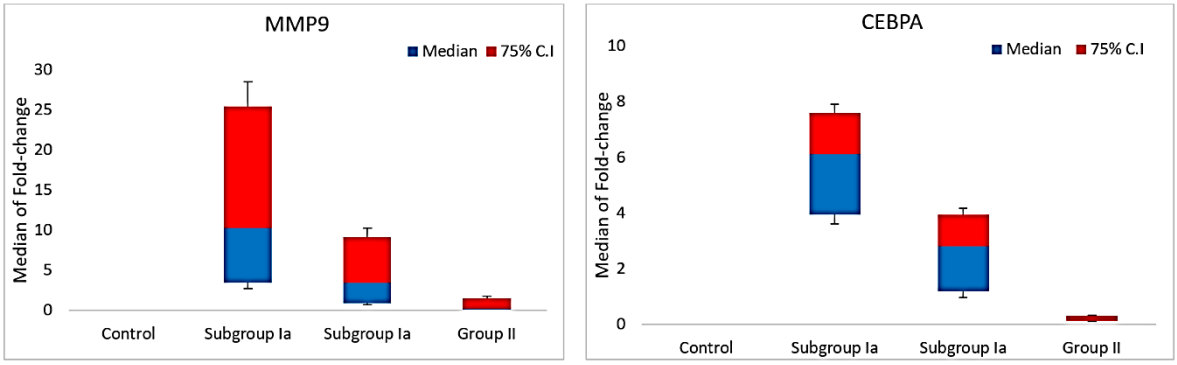


**Figure 1:** Box plot of the studied genes in the studied groups. **A)** Box plot of *MMP9* gene expression in the studied groups. **B)** Box plot of *CEBPα* gene expression in the studied groups.

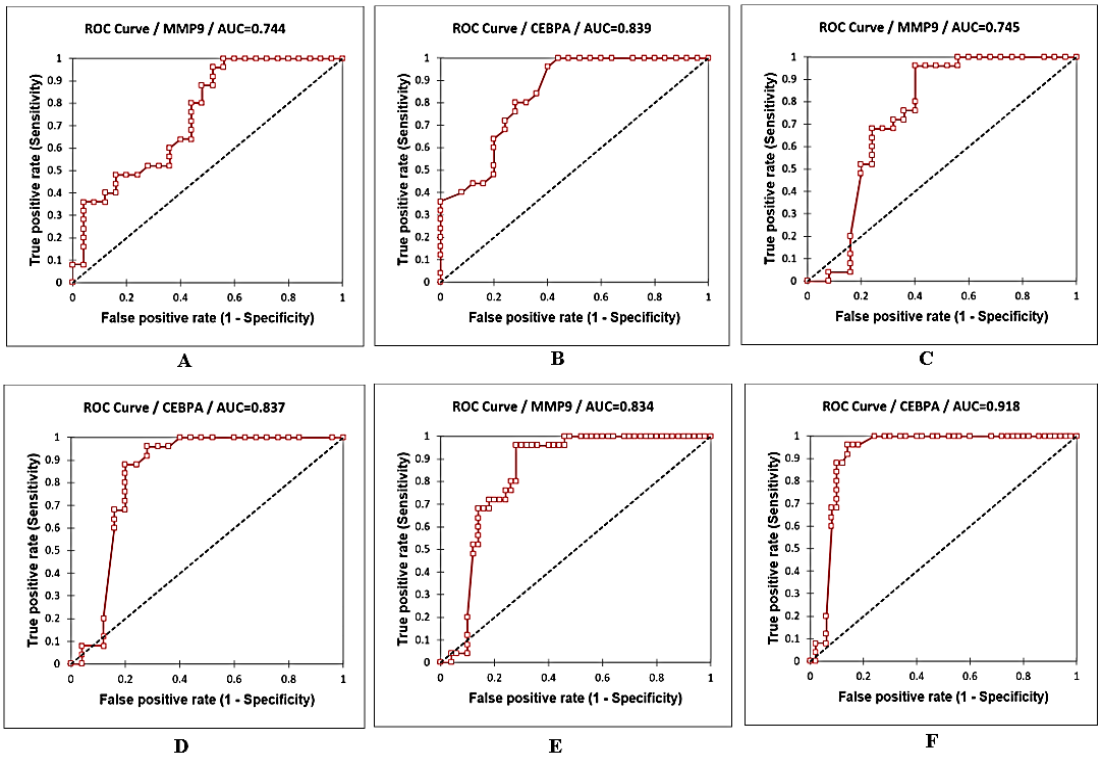


**Figure 2:** ROC Curve for the *MMP9* and *CEBPα* in the studied groups. **A)** Subgroup Ib Vs Subgroup Ia regarding *MMP9*. **B)** Subgroup Ib Vs Subgroup Ia regarding *CEBPα*. **C)** HCC Vs Cirrhotic regarding *MMP9*. **D)** HCC Vs Cirrhotic regarding *CEBPα*. **E)** HCC Vs CLD regarding *MMP9*. **F)** HCC Vs CLD regarding *CEBPα.*

The ROC curve was used to determine the diagnostic performance of the expression of *CEBPα* and *MMP9* genes as markers in cirrhotic and HCC patients at different cutoff points. The calculated sensitivity, specificity, and diagnostic accuracies for studied parameters are shown in (Table 4). To discriminate subgroup Ib from subgroup Ia, The *MMP9*, showed AUC value of 0.744 with (95% CI 0.608-0.880, p< 0.001); while the

*CEBPα*, showed AUC value of 0.839 with (95% CI 0.732-0.947, p< 0.001) (Figures 2A & 2B). To discriminate group II versus subgroup Ib, the *MMP9* showed AUC value of 0.745 (95% CI 0.595-0.895, p value < 0.001); while the *CEBPα* showed AUC was of 0.837 (95% CI 0.708-0.966%, p value < 0.001) (Figures 2C & 2D). We demonstrated that AUC for *MMP9* was 0.834 (95% CI 0.742-0.926, p value < 0.001), while for *CEBPα* was 0.918 (95% CI 0.850-0.986, p< 0.001) in case discrimination the group II (HCC) versus group I (CLD) in general (Figures 2E & 2F). For the characterization of *MMP9* and *CEBPα* as a predictive marker for cirrhosis progression, we performed univariate logistic regression analysis. Our data showed that, the increase in 1 degree of *MMP9* can lead to an increase in the odds of being cirrhosis by 1.126 with (95% C.I: 1.026-1.234, p=0.01). However, the increase in 1 degree of *CEBPα* expression level led to an increase in the odds of being cirrhosis by 2.172 with (95% C.I: 1.395-3.383, p=0.001).

**Table 4:** *MMP9* and *CEBPα* diagnostic performance.

| | | Cutoff | Sn. | Sp. | PPV | NPV | Accuracy | AUC | 95% C.I | | p-value |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | Lower | Upper | |
| **Subgroup (Ib) Vs Subgroup (Ia)** | ***MMP9*** | 15.51 | 96.0% | 48.0% | 64.9% | 92.3% | 72.0% | 0.744 | 0.608 | 0.880 | 0.001** |
| | ***CEBPα*** | 4.800 | 96.0% | 60.0% | 70.6% | 93.8% | 78.0% | 0.839 | 0.732 | 0.947 | 0.001** |
| **Group II Vs Subgroup (Ib)** | ***MMP9*** | 2.45 | 96.0% | 60.0% | 70.6% | 93.8% | 78.0% | 0.745 | 0.595 | 0.895 | 0.001** |
| | ***CEBPα*** | 0.930 | 88.0% | 80.0% | 81.5% | 87.0% | 84.0% | 0.837 | 0.708 | 0.966 | 0.001** |
| **Group II Vs Group I** | ***MMP9*** | 2.45 | 96.0% | 72.0% | 63.2% | 97.3% | 80.0% | 0.834 | 0.742 | 0.926 | 0.001** |
| | ***CEBPα*** | 1.390 | 96.0% | 86.0% | 77.4% | 97.7% | 89.3% | 0.918 | 0.850 | 0.986 | 0.001** |

Sn: Sensitivity; Sp: Specificity; PPV: Positive Predictive Value; NPV: Negative Predictive Value; AUC: Area Under Curve; C.I: 95% Confidence Interval. **p* value <0.05 is significant, ***p* value <0.01 is highly significant.

**Table 5:** Univariate study in the studied groups.

| | Biomarker | OR | 95% C.I | *p* value |
|---|---|---|---|---|
| **Subgroup (Ib) Vs Subgroup (Ia)** | ***MMP9*** | 1.126 | 1.026 - 1.234 | 0.01* |
| | ***CEBPα*** | 2.172 | 1.395 - 3.383 | 0.001** |
| **Group II Vs Subgroup (Ib)** | ***MMP9*** | 1.686 | 1.147 - 2.481 | 0.01* |
| | ***CEBPα*** | 4.462 | 1.871 - 10.641 | 0.001** |
| **Group II Vs Group I** | ***MMP9*** | 1.902 | 1.253 - 2.888 | 0.01* |
| | ***CEBPα*** | 5.429 | 2.303 - 12.798 | 0.001** |

OR: Odd Ratio, CI: Confidence Interval. *p* value calculated depend on logistic regression analysis. **p* value <0.05 is significant, ***p* value <0.01 is highly significant.

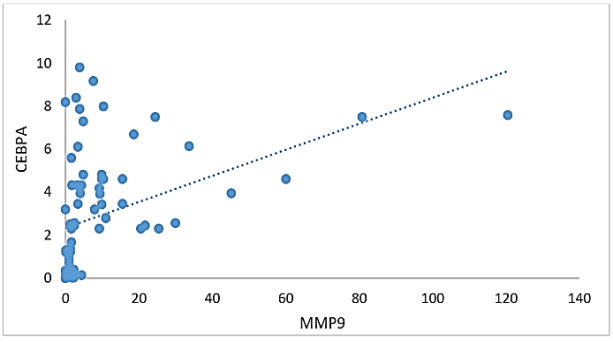


**Figure 3:** Correlation between *MMP9* and *CEBPα* in the studied group.

On evaluating the *MMP9* and *CEBPα* predictor value for the progression of HCC, our results showed that the increase in 1 degree of *MMP9* initiated an increase in the odds of being HCC by 1.686 with (95% C.I: 1.147-2.481, p=0.01). Nevertheless, an increase in 1 degree of *CEBPα* expression level initiated an increased the odds of being HCC by 4.462 with (95% C.I: 1.871-10.641, p=0.001). Moreover, in group I the increase in 1 degree of *MMP9* expression induced an increase in the odds of becoming HCC by 1.902 with (95% C.I: 1.253-2.888, p= 0.01), likewise the increase in 1 degree of expression of *CEBPα* induced the odds of becoming HCC by 5.429 with (95% C.I: 2.303-12.798, p=0.001) (Table 5). Correlation analysis revealed a direct significant correlation of *MMP9* with *CEBPα* (r=0.465 and p< 0.001) as general through the studied groups (Figure 3).

Regarding the Gene networks bioinformatic analysis via GeneMANIA server, our study indicated several functional interaction genes between *MMP9* and *CEBPα* genes. Which showed the direct interaction between *MMP9, CEBPα* and *FAM126A* genes and various functional genes are related to *MMP9* and *CEBPα* genes. This result showed an interaction between *MMP9* and *CEBPα* genes and a predictive interrelationship to HCC disease (Figure 4) (Link3).

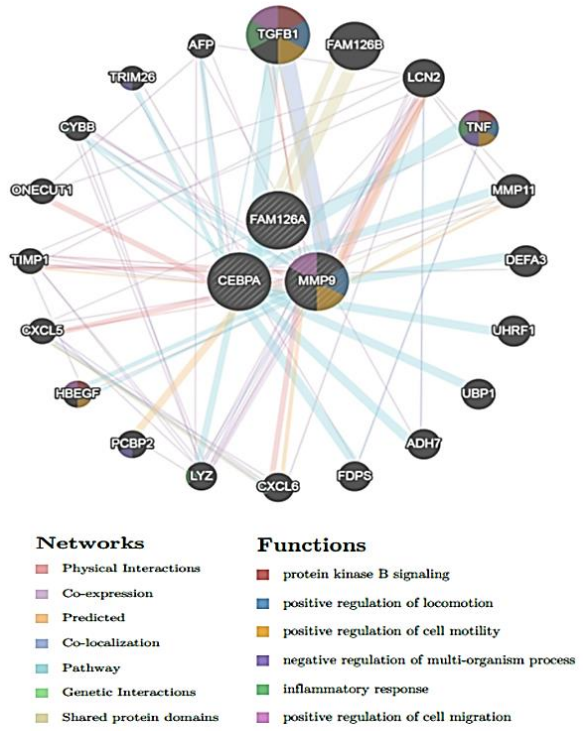


**Figure 4:** GeneMANIA: showing possible functional related genes to *MMP9* and *CEBPα* in HCC disease.

## Discussion

HCC relating to cirrhosis is considered to be one of the major global health problems. Cirrhosis pathogenesis is a consequence of CLD, that can be distinguished by the unnecessary growth in the protein of ECM. A substantial role of different types of MMPs including MMP-1, 2, 8 and 9 have been reported as in the progression in CLD and HCC [20]. *CEBPα* is recognized as a tumor suppressor gene. Mutations in some domains which minimize or void the function of *CEBPα*, along with overall inhibition of its expression, can enhance tumorigenesis and tumor progression [21]. Hence, this trend may lead us in thinking that it is potential to use both genes as indicative and/or predictive biomarkers for both CLD and HCC. Therefore, we planned to perform this study with cases of HCV-genotype (4) induced liver cirrhosis and carcinogenesis of Egyptian patients compared to healthy controls in order to investigate whether the circulating *MMP9* and *CEBPα*, expressions were associated with HCC or not, hoping to discover new non-invasive prognostic biomarkers for HCC.

Our study showed a significant gradual decrease in *MMP9* gene expression in patients with cirrhosis and HCC when compared to CLD without cirrhosis patients ($p<0.001$ and $p<0.001$ respectively), as well as in CLD with HCC patients compared to CLD without cirrhosis patients ($P< 0.001$), and CLD as general ($P< 0.001$), These results are in accordance to Badra G *et al.* (2010), who reported a significant decrease in serum MMP-9 through the development of chronic HCV to cirrhosis with the minimum level in the group with cirrhosis [22]. Concerning *CEBPα*, it has exposed a significant gradually decreased in cirrhotic and HCC patients when compared to CLD without cirrhosis patients ($p< 0.001$ and $p< 0.001$ respectively), along with in CLD with HCC patients compared to CLD without cirrhosis patients ($P< 0.001$), and CLD as general ($P< 0.001$). These results are in agreement with Lu G *et al.* (2010), who reported that HCV patients with HCC expressed *CEBPα* in a decreased level [23]. The ROC curve accuracy of *MMP9* genes expression as a biomarker in patients with cirrhosis at different cutoff points showed a diagnostic performance of 72.0% ($p= 0.001$). While the accuracy of HCC patients was 78.0%, ($p=0.001$). Although the accuracy of HCC patients than CLD patients as one group was 80.0% ($p=0.001$). Concerning the *CEBPα* gene in cirrhotic patients, accuracy of 78.0% ($p=0.001$). For HCC patients, the accuracy was 84.0% ($p=0.001$). While there is accuracy 89.3% ($p=0.001$) regarding HCC patients than CLD patients as one group. These results indicated that *MMP9* and *CEBPα* could be considered as a diagnostic parameter with a significant result and could be used for discrimination between CLD without cirrhosis, CLD with cirrhosis and HCC.

Stratification of the predictive values, deduced from the ROC curve, as a biomarker for the progression of the disease indicated that *MMP9* gene expression gradually declines with the progression of disease in a disparate process (15.51, $p< 0.001$) and (2.45, $p< 0.001$) respectively. In addition, the expression of *CEBPα* gene decreased with disease progression (4.800, $p< 0.001$) and (0.930, $p< 0.001$) respectively. These results are in consistence with both Yang and colleagues, who reported that *MMP9* could be used as a chemotherapeutic drug and Setten and colleagues, who reported that the expression of *CEBPα* declined sharply in solid tumors and assumed that restoration of *CEBPα* in HCC showed a clinical capacity [21, 24]. Evidently, the regression analysis indicated that *MMP9* and *CEBPα* expression possibly represent as substantial prognosticators related to the changes of CLD without cirrhosis and CLD with HCC versus CLD with cirrhosis patients. In addition, the *MMP9* and *CEBPα* expression levels improved the chances of being cirrhosis when designated as significant predictors related to odds of diagnosing HCC against cirrhosis patients.

Regarding the correlation analysis, there were significant direct correlation between *MMP9* gene expression and *CEBPα* gene expressions ($r=0.465$ and $p=0.001$). This relationship indicates that there is a direct interaction between these genes that act as tumor suppressors genes, and which are likely to have an effect and/or interaction on many other tumor suppressors genes. Interestingly, the results of bioinformatics analysis by Gene networks analysis using GeneMANIA server showed evidence of the existence of the relationship not only between the studied genes, but also with many other genes like FAM126A and their relations with TGFB1, FAM126B, LCN2 and TNF, that deserve study and consideration (Figure 4).

## Conclusion

The gradual decline in the expression of *MMP9* and *CEBPα* genes with disease progression recommended the use of these parameters as a non-invasive predictive biomarker for HCV induced HCC in HCV-genotype (4) patients. However, this research has limitations, and a study of the relations between the interactions of other genes are recommended. Such as TGFB1 and TSGs (*MMP9* and *CEBPα*) and the methylation pattern in its promoter regions in HCV-related HCC and evaluate their relationships with its gene expressions, in addition to the remaining

genes that appeared in the gene network like (FAM126A, TGFB1, FAM126B, LCN2 and TNF).

## Compliance with Ethics Requirements

All procedures followed were in accordance with the ethical committee of the Theodor Bilharz Research Institute (TBRI), GIZA, Egypt. (Ethical Approval Number: PT: 523) An informed consent was obtained from patients and/or their guardian. Which is independent on the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008 (5).

## Informed Consent

Informed consent was obtained from all patients for being included in the study.

## Conflicts of Interest

None.

## Funding

None.

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