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Autophagy-Mitophagy Related Serum Markers for the Early Detection of Hepatocellular Carcinoma

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Abstract

Background: HCC is the most common primary liver malignancy. Understanding the crosstalk of Mitophagy-related genes and apoptosis in the development of cancer can lead to finding out new biomarkers which will help in early detection of HCC.

Aim of Study: This study aimed to retrieve novel RNA based network related to mitophagy and apoptosis characteristic for HCC development from public microarray databases.

Material and Methods: Quantitative assessment of serum NFYA and TOMM40 gene expression was performed by qRT-PCR. Fifty-six patients diagnosed HCC, nineteen chronic hepatitis C cases and seventeen healthy volunteers.

Results: ROC curve analysis was performed to evaluate the diagnostic value for NFYA with AUCs=0.97, cutoff point of >1.3, sensitivity 96.5 %, specificity 94.4%, PPV 93.3%, NPV 97.1% and accuracy 95.7%. TOMM40 had AUC=0.83 and a cutoff point of >0.82, sensitivity 82.7%, specificity 77.7%, PPV 75%, NPV 84.8% and accuracy 80.2%. NFYA expression were significantly higher in HCC patients compared to CHC group and control group with p-value <0.001*. Also, TOMM40 level showed highly significant difference between HCC group and CHC group (p<0.01) and there was also a significant difference between CHC group and control group as regards fold change of serum TOMM40 (p<0.05).

Conclusion: Our data suggest that NFYA and TOMM40 expression levels could be highly accurate, early and non-invasive biomarkers in HCC diagnosis. It is promising as a general strategy for future panel biomarker development in the serum of HCC patients and CHC patients.This can overcome the lower reliability of single-gene biomarker experiments while maintaining high accuracy by combining signals from multiple genetic levels.

Key Words: *Hepatocellular carcinoma – Autophagy-mitophagy.*

Introduction

HEPATOCELLULAR carcinoma has a specific pattern of geographical distribution mirroring the areas of increased rate of infection by hepatitis B and C [1]. HCC is one of the leading causes of cancer death worldwide, with very poor five-year survival rates [2], mainly due to late diagnosis with overall detection rate reaching 41% [3], this is accompanied with a high rate of recurrence after treatment. In Egypt, liver cancer is the second cause of cancer mortality in both sexes. Hepatocellular carcinoma (HCC) represents 75% of malignant liver tumors [4], as the incidence of HCC has been nearly doubled over the last decade [5]. Hence, it drives the attention of scientists to determine its molecular pathogenesis and the possible genetic and epigenetic biomarkers that can be effective in early diagnosis, prognosis, and treatment of the disease [6].

Many studies depended on the examination of tissue biopsy in order to determine the pathogenesis and staging of the disease. Nowadays, according to the recent guidelines the diagnostic and the prognostic modality for HCC is hardly ever requiring a histological assessment, as the radiological and blood analysis taking the upper hand [7]. AFP is a weak screening method when used alone with a sensitivity of (41-65%) and specificity of (80-94%) [8]. It lacks sensitivity for early tumors and also could be elevated in liver cirrhosis with the absence of malignancy, which may explain the logic behind its removing in 2009 from the screening protocols of AASLD [7]. Thus, making it a hard journey to acknowledge which mutations could be targeted, except only in a restricted number of patients e.g., who underwent liver resection or transplantation where specimens could be obtained. As this leaves us with the closed door of tissue biopsy, another one opens; which is less invasive and equally effective; bioinformatics and mitophagy associated biomarkers [9].

Damage to the mitochondria or more catastrophic stresses can trigger the main signaling pathways

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of programmed cell death such as intrinsic/extrinsic apoptosis and regulated necrosis [10]. In addition, the cell redox state plays an important role in apoptosis since that reactive species produced by mitochondria can be involved in cell damage and death. On the other hand, autophagy seems to be one of the crucial cellular responses against stress situations and may contribute for cell adaptation and survival in adverse conditions [11]. Particularly, mitochondrial autophagy (also referred as mitophagy) appears to be a useful mechanism by removing the impaired mitochondria, thus avoiding the reactive species overproduction and this organelle-mediated cell death [12].

Mitophagy is an important form of macroautophagy, in which mitochondria are selectively targeted for degradation in autophagolysosomes [13]. However, mitophagy is not only limited to the turnover of dysfunctional mitochondria but also promotes reduction of overall mitochondrial mass in response to certain stresses, such as hypoxia and nutrient starvation. This prevents generation of reactive oxygen species and conserves valuable nutrients (such as oxygen) from being consumed inefficiently, thereby promoting cellular survival under conditions of energetic stress [14]. The failure to properly modulate mitochondrial turnover in response to oncogenic stresses has been implicated both positively and negatively in tumorigenesis [15].

In-depth analyses of autophagy-deficient tumors is revealed the presence of clearly dysfunctional mitochondria that exhibited altered morphology, ineffective fatty acid oxidation, reduced carbon flux through Krebs cycle, and lipid accumulation [16]. Fujiwara et al., reported that defective mitophagy is closely linked to HCC [17]. Targeting mitophagy may therefore offer opportunities to inhibit tumor progression more selectively to malignancy where one may take advantage of the acute sensitivity of tumor cells to mitochondrial dysfunction when combined with other drugs or stresses [18]. All in all, the crosstalk between apoptosis, autophagy/mitophagy and mitochondrial dynamics/biogenesis seems to be critical to the overall fate of cells towards death or survival [19].

The aim of the present work is retrieving novel RNA based network related to mitophagy and apoptosis characteristic for HCC development from public microarray databases and to evaluate their usefulness as molecular biomarkers for early HCC detection and to confirm that the sensitivity and specificity of the two genes are higher than the currently used biomarker AFP.

Subjects Material and Methods

Patients and samples:

This study included 65 participants, age from 37 to 82 years; samples were collected from Tropical Medicine Department, Ain Shams University Hospitals. Collection procedures were approved by the Research Ethical Committee HCC Department of Tropical Medicine at Faculty of Medicine, Ain Shams University from October 2017 - April 2018. no.: FWA 000017585. They were classified into three groups: Group I (Malignant cases of Hepatocellular carcinoma) consisted of 29 patients who all had chronic HCV infection. Proved diagnosis of HCC was according to the American Association for the Study of Liver Diseases (AASLD) practice guidelines [20]. Samples were collected from Tropical Medicine Department, Ain Shams University Hospitals from October 2017 to April 2019.

All diagnosed as having HCC by two elements: AFP higher than the cut-off limit [21], radiological characteristic features of HCC by ultrasound and confirmed by triphasic spiral CT scan. They were then subclassified into 26 patients with cirrhosis, 11 patients with Child-Pugh A, 14 patients with Child-Pugh B, and 4 patients with Child-Pugh C liver cirrhosis. And for BCLC stage, 25 patients with BCLC A (early stage) and 4 patients with BCLC C (late stage). Group II (Chronic hepatitis C cases) consisted of 19 patients who did not have HCC and 10 out of the 19 patients have liver cirrhosis. Group III consisted of 17 healthy normal volunteers with matching age and sex to the patients' groups after obtaining informed consent.

All studied participants were subjected to the following:

Evaluation of patients:

Complete medical history was taken from all members of the study groups. Full Clinical and radiological examination were performed.

Patients actively undergoing chemotherapy or radiation therapy, patients with other malignancies diagnosed or treated within the last 5 years, patients with any renal diseases and alcoholics were excluded from this study.

Laboratory investigations:

All groups were subjected to the estimation of serum alkaline phosphatase, total and direct bilirubin, albumin, ALT, AST and INR. Also, serum AFP (by ELISA technique), HCV antibodies and HBsAg were estimated by using ELISA technique.

Specimen collection:

Each individual provided serum and whole blood samples. Blood was collected by venipuncture into centrifuge tubes (red-topped tubes), allowed to clot at room temperature. Clotted blood samples were centrifuged at 1300xg at 4 °C for 20 min. Whole blood samples were collected in (Lavender topped tubes) containing EDTA.

Specimen storage and preparation:

Serum was removed carefully without disturbing the intermediate buffy coat layer, transferred to a polypropylene capped tube in 1mL aliquots and stored at –80 °C until assayed. None of the serum samples were allowed to thaw before analysis to minimize protein degradation and precipitation.

Biomarkers' identification:

Bioinformatics' Analysis was done to retrieve promising biomarkers relevant to Hepatocellular Carcinoma based on previous microarray studies. This step included biomarkers' retrieval (TOMM40 and NFYA expression). The panel RNA-based biomarker was retrieved in two steps:

TOMM40 was selected according to public microarray databases, because this gene plays a major role in autophagy. To confirm the expression of (TOMM40 mRNA) in HCC cases depending on novelty and high tissue specificity; Gene Atlas database, Protein Atlas database and total cancer gene network were searched.

Secondly, autophagy regulatory network database was used to retrieve (NFYA mRNA) as a transcriptional regulator of TOMM40 mRNA. Lastly, we have confirmed the expression of them in HCC via exocarta database.

Serum samples were then used for detection of TOMM40 gene expression and NFYA gene.

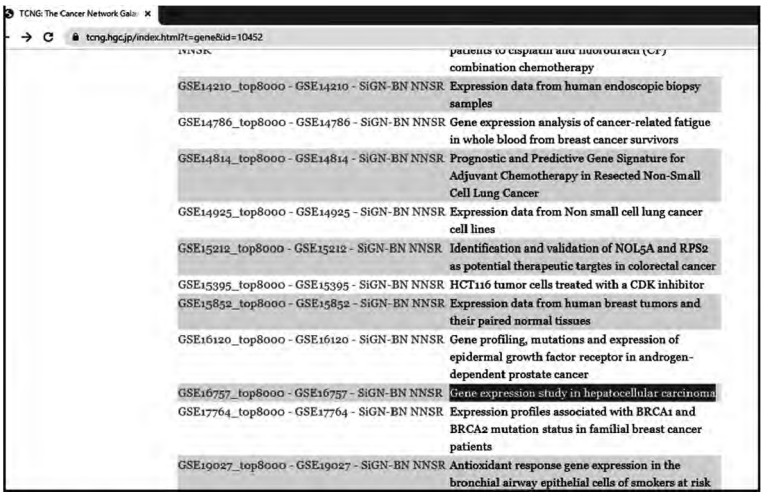


Fig. (1): Print screen from TCNG the Cancer Network Galaxy database showing the expression of Autophagy gene (TOMM40) in Hepatocellular Carcinoma.

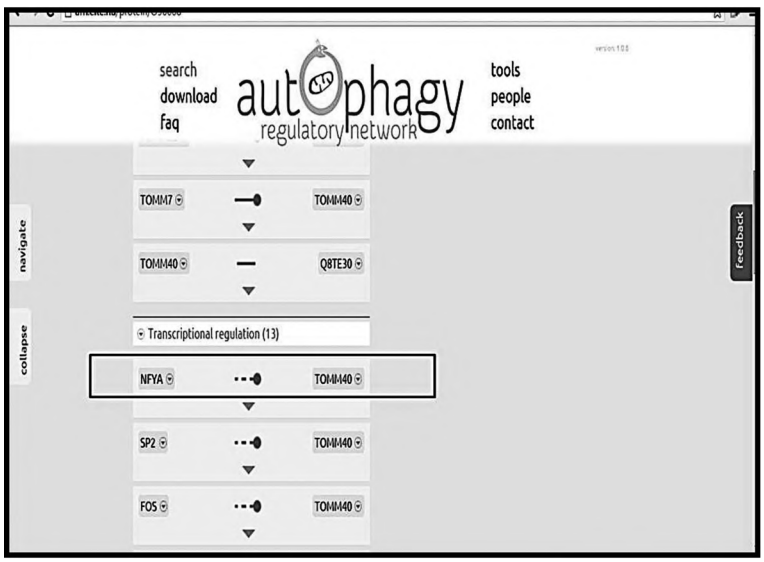
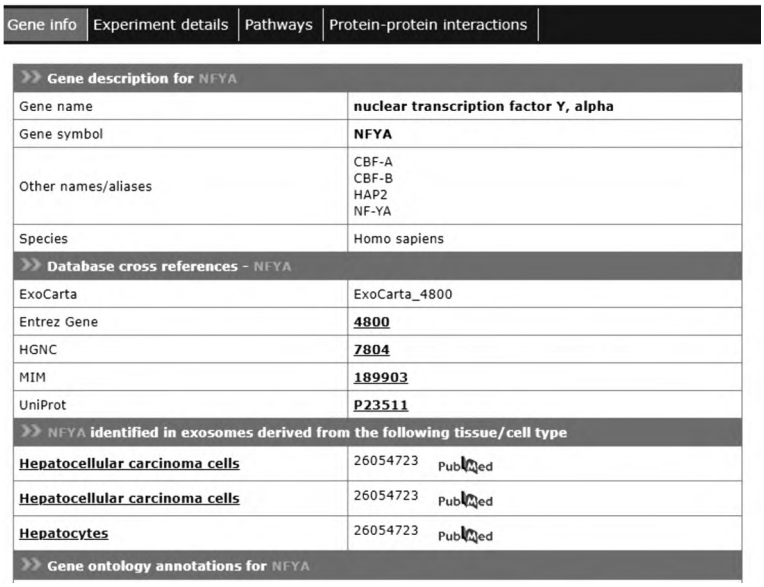


Fig. (2): Print screen shows retrieval of NFYA mRNA from autophagy regulatory network database as a transcriptional regulator of TOMM40 mRNA.



Gene info | Experiment details | Pathways | Protein-protein interactions

Gene description for NFYA	
Gene name	**nuclear transcription factor Y, alpha**
Gene symbol	**NFYA**
Other names/aliases	CBF-A CBF-B HAP2 NF-YA
Species	Homo sapiens
Database cross references - NFYA	
ExoCarta	ExoCarta_4800
Entrez Gene	**4800**
HGNC	**7804**
MIM	**189903**
UniProt	**P23511**
NFYA identified in exosomes derived from the following tissue/cell type	
Hepatocellular carcinoma cells	26054723 PubMed
Hepatocellular carcinoma cells	26054723 PubMed
Hepatocytes	26054723 PubMed
Gene ontology annotations for NFYA	

Fig. (3): Print screen from ExoCarta database showing the expression of NFYA in liver HCC.

Experimental design:

Validation of the chosen biomarkers as diagnostic markers for early detection of Hepatocellular carcinoma.

Extraction of total RNA from serum samples:

Total RNA was extracted from serum using RNeasy® Serum/Plasma Kit (QIAGEN®, USA). QIAzol Lysis Reagent; included in the kit, is designed to facilitate lysis, denature protein complexes and RNases, and also to remove most of the residual DNA and proteins from the lysate. Final RNA used immediately for reverse transcription (RT); otherwise, they were stored at –80 °C.

Reverse transcription PCR:

The extracted RNA was reverse transcribed into cDNA in a total volume of 20 µl using QuantiTect Reverse Transcription Kit (Qiagen, USA). The RT reaction tubes were transferred to the thermocycler (7500 Fast Real-Time PCR system, Applied Biosystems, USA). The entire reaction took place at 42 °C and is then inactivated at 95 °C. The obtained cDNA was used immediately for amplification through quantitative Real Time-polymerase chain reaction (qRT-PCR).

Quantitative real time-PCR (qRT-PCR):

A two-stepReal-Time PCR System (Applied Biosystem, USA) was used to determine cDNA copy number. PCR reactions were set up in 25 µl reaction mixtures according to the manufacturer's instructions of QuantiTect SYBR Green PCR Kit (Qiagen, USA). The reaction program was allocated to 3 steps. First step was at 95.0 °C for 5min. Second step consisted of 40 cycles in which each cycle was divided to 3 steps: (a) at 94.0 °C for 15sec; (b) at 55.0 °C for 30 sec; and (c) at 72.0 °C for 30 sec.The third step (was carried out to obtain the melting curve) consisted of 71 cycles, which started at 60.0 °C and then increased about 0.5 °C every 10 sec up to 95.0 °C. The quantitative values of qRT-PCR of the two genes were normalized on the bases of ACTB expression. The relative expression of total NFYA gene and TOMM40 was analysed using the ΔΔCT method **[22]**.

Statistical analysis:

The statistical package for the social sciences (SPSS, version 20; SPSS Inc., Chicago, Illinois, USA) software computer program was used for analysis of the data. The obtained results were correlated with the different clinicopathological factors of the patients. Numerical data were expressed as mean ± SD. The results were analyzed using one way analysis of variance (ANOVA) followed by Mann-Whitney and the Kruskal-Wallis tests for statistical comparison of the variables between the different groups, Chi-square test which used to compare between qualitative data and find out the relation between various qualitative data, The χ^2-test was used to compare qualitative variables. The Receiver Operating Characteristics curve (ROC) was used to discriminate positive from negative results also determined the threshold value for optimal sensitivity and specificity. Evaluation of diagnostic value of the two genes was done by calculating sensitivity, specificity, positive predictive values (PPV), negative predictive value (NPV) and accuracy. All tests were two-tailed. A p-value <0.05 was considered significant.

Results

Patients' characteristics:

In this work, sixty five participants were included. As shown in (Table 1), There was a highly significant difference among the three studied groups as regards the different laboratory parameters as AST, ALT, serum Albumin level, Total and Direct Bilirubin level, INR and AFP (p<0.01) using Kruskal-Wallis test.

Table (1): Comparison of the different laboratory parameters and the clinicopathological factors in different groups of the study.

	HCC (n=29)	CHC (HCV) (n=17)	Control (n=19)	p-value	$\chi^{2(a)}$
Age (years)					
Mean ± SD	58.4±8.1	55.8±5.0	53.7±4.7	0.206 NS	30.164
AST (U/L)	77.8±29	64.4±18.7	27.1±8.5	0.00**	95.475
ALT (U/L)	72.5±18	36.5±17.7	26.9±12	0.00**	71.807
Albumin (g/dL)	2.88±0.25	3.11±0.23	3.94±0.31	0.00**	62.136
Direct Bilirubin (mg/dL)	1.27±1.7	1.15±0.65	0.2±0.08	0.006**	66.42
Total Bilirubin (mg/dL)	2.4±1.3	2.5±0.9	0.9±0.19	0.011*	74.1
INR	2.1±0.76	1.9±0.69	1.08±0.12	.000**	75.139
AFP (ng/mL)	76.5±129	22.5±34.1	5.17±2.2	.003**	77.087
	(HCC) N (%)	CHC N (%)	Healthy Control N (%)	p-value	$\chi^{2(a)}$
Sex:					
Male (40)	20 (68.9%)	13 (68.4%)	7 (41.2%)	0.129	4.102
Female (25)	9 (31%)	6 (31.6%)	10 (58.8%)	NS	
Smoking:					.024
Smoker (28)	13 (44%)	8 (42.1%)	7 (41.2%)	0.98	
Non-Smoker (37)	16 (55%)	11 (57.9%)	10 (58.8%)	NS	
HCV-antibodies:					10.33
Positive (48)	29 (100%)	19 (100%)	0 (0%)		
Negative (17)	0 (0%)	0 (0%)	17 (100%)	0.006**	31.78
Cirrhosis:					
Cirrhotic (36)	26 (89.6%)	10 (52.6%)	0 (0%)		
Non-cirrhotic (29)	3 (10.3%)	9 (47.4%)	17 (100%)	.000**	

I : HCC.
II : CHC.
III: Controls.
HCC: Hepatocellular carcinoma.
p<0.001: Highly significant <0.05, significant.
p>0.05: Not significant.
CHC: Chronic hepatitis C (HCV positive).
AST : Aspartate Transaminase.
ALT: Alanine Transaminase.
INR: International Normalized Ratio.
AFP: Serum Alpha Fetoprotein.

Using Chi square test, the clinicopathlogical factors in different groups of the study were shown in (Table 1). There was no statistical significant difference among the study groups as regards age, sex and smoking, but there was a highly significant difference as regards positivity for HCV-antibodies and cirrhosis (p<0.01).

Differential expression of serum NFYA gene expression among the studied groups:

The mean value of the control group is 0.38 ± 0.59 and median value is 0.05, while in CHC group; the mean is 0.91±0.41 and median value is 0.95 and for HCC group the mean value is 65.7 ± 121.8 and the median value is 15.24, NFYA expression levels were significantly higher in HCC patients compared to CHC group and the control group with p-value <0.001, all described in Table (3). ROC (receiver operating characteristic) curve analysis performed to evaluate the diagnostic value for the NFYA with AUCs (area under the ROC curves) = 0.97. For discriminating malignant and non-malignant groups, ROC curve provides a cutoff point for diagnosis of the disease, above or equal (1.3) were considered as “Positive”, while those with expression level lower than 1.3 were considered as “Negative”. We also, found that sensitivity was 96.5 %, specificity was 94.4, PPV was 93.3%, NPV was 97.1% and accuracy was 95.7%.

Differential expression of serum TOMM40 gene among the studied groups:

The mean value of the control group is 1.47 ± 0.64 and median value is 1.58, while in CHC group; the mean value is 1.07 ±0.41 and median value is 1.16 and for HCC group the mean value is 0.472 ± 0.49 and the median value is 0.24, TOMM40 expression levels were significantly higher in HCC patients compared to CHC group and the control group with p-value <0.001, all described in Table (2). ROC curve analysis performed to evaluate the diagnostic value for the TOMM40 with AUCs= 0.83. For discriminating malignant and non-malignant groups, ROC curve provides a cutoff point for diagnosis of the disease, below or equal (0.82) were considered as "Positive", while those with expression level above 0.82 were considered as "Negative". We also, found that sensitivity was 82.7%, specificity was 77.7%, PPV was 75%, NPV was 84.8% and accuracy was 80.2%.

Performance characteristics of Investigated Biomarkers for Detection of Hepatocellular carcinoma:

The sensitivity, specificity, positive predictive value, negative predictive value and accuracy of serum AFP, NFYA gene expression, TOMM40 gene expression, Combined NFYA and TOMM40 gene expressions, combined NFYA gene expression and AFP, combined TOMM40 gene expression and AFP and combined NFYA, TOMM40 gene expressions and AFP are showed in Table (3).

Table (2): Differential expression of serum NFYA and TOMM40 among different studied groups.

	HCC		CHC (HCV)		Healthy control		p-value	$\chi^{2(a)}$
	Mean ± SD	Median	Mean ± SD	Median	Mean ± SD	Median		
NFYA Expression	65.7±121	15.24	0.91±0.41	0.95	0.38±0.59	0.05	0.000**	48.2
TOMM40 Expression	0.47±0.49	0.24	1.07±0.41	1.16	1.47±0.64	1.58	0.000**	25.3

aKruskal-Wallis test. ** p<0.01: Is highly significant. * p<0.05: Is significant. CHC: Chronic HCV infection.

Table (3): Performance characteristics of investigated biomarkers for detection of HCC.

Investigated biomarker	Sensitivity	Specificity	PPV	NPV	Accuracy
AFP	72.4%	69.4%	69.4%	71.4%	74.6%
AFYA gene exp.	96.5%	94.4%	93.3%	97.1%	95.7%
TOMM40 gene exp.	82.7%	77.7%	75%	84.8%	80.2%
Combined NFYA and TOMM40 gene expression	100%	72.2%	77.7%	100%	85.9%
Combined NFYA and AFP	100%	63.8%	72.9%	100%	81.6%
Combined TOMM40 and AFP	94.2%	61%	70.2%	91.6%	77.4%
Combined NFYA, TOMM40 and AFP	100%	55.5%	68.6%	100%	77.4%

Discussion

Hepatocellular carcinoma (HCC) represents the most common type of primary liver malignancy; it ranks as the fifth most common cancer in men and the seventh in women **[23]**. Approximately 70%-90% of patients with HCC have an established background of chronic liver disease and cirrhosis, with major risk factors for developing cirrhosis including chronic infection with hepatitis B virus (HBV) and hepatitis C virus (HCV) **[24]**. Deaths due to HCC have increased from 2.4 per 100 000 in 1987 to 8 per 100 000 in 2010, accounting for 6000-7000 deaths per year (National Cancer Institute, Egypt) **[25]**. The low survival rate of HCC patients is attributed to late diagnosis, tumor recurrence, and metastasis, this highlighting the urgent need for identification of novel serum biomarkers for diagnosis of cancer, especially for detection and screening in early-stage cancer **[26]**.

Results of the present study showed that HCC was more prevalent in older age groups(≥566 years), this goes hand in hand with Asahina et al., who reported that the risk for HCC increases more than 15-folds after 65 years of age where the annual incidence of HCC is significantly higher in older patients than in younger patients (p<0.001) **[27]**.

The current study also highlighted higher prevalence of HCC among male patients who constituted 20 out of 29 patients with percentage 69% while female patients constituted 9 out of 29 patients with percentage31 %.. This agreed with Ferenci et al., who reported that older males are more susceptible getting HCC than females **[28]**.

The present study showed no association between cigarette smoking and HCC development. In other previous studies, it was found that smoking was not significantly associated with increased mortality in HCC [29].

This study revealed that HCC is dominant among patients with cirrhotic chronic liver disease that constituted 26 out of 29 HCC patients with percentage 89.6%. Similarly, previous studies have been reported that cirrhosis is the main risk factor for HCC and the annual incidence of HCC in patients with cirrhosis is 2-7% [30].

The currentstudy highlighted chronic infection with hepatitis C virus is a major risk factor for HCC which supported by Donato et al., [31] who reported that HCC risk increases to 17-fold in HCV-infected patients compared with HCV-negative subjects and was also supported by El-Nady et al. [32], who explained that HCV may play a direct role in hepatic carcinogenesis.

ALT levels in this study were markedly higher in HCC patients than those of chronic hepatitis C patients followed by those of control group which was highly statistically significant (p<0.00). This with an agreement with Okonkwo et al., [33] who stated that the serum ALT level showed a statistically significant difference between the HCC group and the non-HCC groups.

Regarding the other liver function tests, serum albumin, bilirubin, and INR they showed a highly significant difference between the HCC group, CHC group and control group, the bilirubin being higher, the albumin lower, and INR was prolonged in the first group; this is in agreement with Durazo et al. [34] who stated that HCC patients had higher levels of serum bilirubin (p=0.0059), international normalized ratio (p<0.0001), and lower albumin levels (p<0.0001) compared with non-HCC patients.

AFP is the most widely used biomarker for HCC with sensitivity of 60% at a cutoff value of 20ng/ml [35]. In this work, the AFP serum level showed a highly significant elevation in HCC patients, and this agreed with Baghdady et al. [36], who stated a highly significant difference among the three study groups (p<0.01), He found that the mean serum AFP was higher in HCC group (334.40 ±311.30ng/ml) than in chronic hepatitis C group (4.82±2.18ng/ml) and followed by the control group. This also agreed with Jasirwan et al. [37], who found that the serum level of AFP above 1 0ng/ml were seen in 82.6% of HCC patients and 29% of non-HCC patients. ROC curve analysis was performed to evaluate the diagnostic value for the AFP with AUCs=0.709 and a cutoff point of (≥8.1 IU/ml). We found that sensitivity was 72.4%, specificity was 69.4%, PPV was 69.4%, NPV was 71.4% and accuracy was 74.6%. Our results were closely similar to those of Abdel-Hamid et al. [38], who calculated the AFP cut-off value for HCC diagnosis (≥6.46IU/ml) with sensitivity 80% and specificity 85%. As a direct result to this poor sensitivity and specificity, AFP cannot be used alone as a tool for HCC screening and diagnosis.

The results of the current study revealed that TOMM40 gene expression was down regulated in HCC patients (median RQ 0.24) when compared to chronic hepatitis C patients (median RQ 1.16) and normal healthy control individuals where there was a highly significant difference among the three study groups as regards fold change (RQ) of serum TOMM40 gene expression (p<0.01). These results support the fact that autophagy is often downregulated in cancer cells. This agrees with Chen et al., who reported that defective autophagy plays a vital role in tumorigenesis [39]. Accordingly, TOMM40 gene is assigned as a tumor suppressor gene which goes hand in hand with Yang et al., who provided strong evidence that autophagy has a tumor suppressor function in liver based on findings that mice lacking the autophagy gene Atg5 develop liver tumors that increase in size and frequency as the animals age [40]. ROC curve analysis was performed to evaluate the diagnostic value for the TOMM40 with AUCs=0.83 and a cutoff point of (≤0.82). In the current studythe sensitivity was 82.7%, specificity was 77.7%, PPV was 75.0%, NPV was 84.8% and accuracy was 80.2%. Thus, TOMM40 gene expression mRNA could be used as a sensitive biomarker for early diagnosis of HCC. When combining both serum TOMM40 gene expression and AFP we obtained sensitivity, specificity, PPV, NPV and accuracy of (94.2%, 61%, 70.2%, 91.6%, 77.4%) respectively while they were found to be (93.5%, 61%, 67.4%, 91.6%, 76%) respectively in early stage of HCC (BCLC stage A).

In this study, mRNA NFYA gene was picked up via computational bioinformatics tools. Currently NF-Y is emerging as a regulatory factor for many genes overexpressed in several different kinds of cancer [41]. The results of this study revealed that NFYA expression was up regulated in HCC patients (median RQ 15.24) when compared to chronic hepatitis C patients (median RQ 0.95) and normal healthy control individuals (median RQ .05) where there was a highly significant difference among the three study groups as regards

fold change (RQ) of serum NFYA expression ($p<$ 0.01). ROC curve analysis was performed to evaluate the diagnostic value for the NFYA with AUCs =0.97 and a cutoff point of (› 1.3). Sensitivity was 96.5%, specificity was 94.4%, PPV was 93.3%, NPV was 97.1% and accuracy was 95.7% which is better than the currently used biomarker AFP which is less sensitive and less specific in diagnosis of HCC. Consequently, NFYA expression could be used as a sensitive biomarker for early diagnosis of HCC. Remarkably, when combining both serum NFYA expression and AFP we obtained sensitivity, specificity, PPV, NPV and accuracy of (100%, 63.8%, 72.9%, 100%, 81.6%) respectively, while they were found to be (100%, 63.8%, 70.4%, 100%, 80.5%) respectively in early stage of HCC (BCLC stage A).

This high sensitivity of the combined biomarker NFYA and AFP reaching 100% even in early stage of HCC recommends it to be a sensitive reliable biomarker for early diagnosis of HCC. The combined biomarker of the current AFP together with the TOMM40 gene expression and NFYA showed sensitivity, specificity, PPV, NPV and accuracy of (100%, 55.5%, 68.6%, 100%, 77.4%) respectively while in early stage of HCC (100%, 55.5%,69.9%, 100%, 76%) respectively.

The current study presented a novel approach that enables a reliable integration of differential TOMM40 gene expression with the selected epigenetic regulator. This approach has been shown to generate interesting biomarkers (NFYA and TOMM40) for HCC diagnosis. It is promising as a general strategy for future panel biomarker development in the serum of HCC patients and HCV patients. This can overcome the lower reliability of single-gene biomarker experiments while maintaining high accuracy by combining signals from multiple genetic levels. These findings expand the existing knowledge of RNA-RNA crosstalk characteristics and provide new tools to explain disease processes and offer new targets for HCC therapy.

Conflicts of Interest:

The authors declare that they have no conflict of interests.

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الدلالات المتعلقة بالبلعمه والالتهام الميتوكوندیری للتشخيص المبكر لسرطان الكبد

ورم الكبد الأولى الخبيث (HCC) هو الأكثر شيوعاً. ويمكن أن يؤدى فهم العلاقة المتبادلة للجينات المرتبطة ب Mitophagy لفهم تطور السرطان إلى اكتشاف مؤشرات حيوية جديدة ستساعد فى الكشف المبكر عن HCC. والغرض من هذه الدراسة هو استرداد شبكة جديدة قائمة على الحمض النووى الريبى والتى تتعلق بخاصية Mitophagy و apoptosis لتطوير HCC بالاستعانة بقواعد بيانات microarray العامة. وقد تم إجراء التقييم الكمى لـ NFYA المصل والتعبير الجينى TOMM40 بواسطة qRT-PCR على عدد ستة وخمسين من المرضى الذين تم تشخيصهم بـ HCC وتسعة ، عشر حالة مزمنة من التهاب الكبد C وسبعة عشر متطو عاً أصحاء. وقد تم إجراء منحنى ROC لتقييم القيمة التشخيصية لـ NFYA مع AUCs=0.97، ونقطة القطع من ≤1.3، والحساسية 96.5%، والنوعية 94.4%، NPV 97.1%، PPV 93.3% والدقة 95.7%، كان لدى TOMM40 AUC=0.83 ونقطة فاصلة ≥0.82، وحساسية 82.7%، وخصوصية 77.7%، و PPV 75%، و NPV 84.8% ودقة 80.2%، كان تعبير NFYA أعلى بكثير فى مرضى HCC مقارنة بمجموعة CHC ومجمو عة التحكم مع قيمة $p<0.001$. كما أظهر مستوى TOMM40 فرقاً ذا دلالة إحصائية عالية بين مجموعة HCC ومجموعة CHC ($p<0.01$) وكان هناك أيضاً فرق كبير بين مجموعة CHC ومجموعة التحكم فيما يتعلق بتغيير أضعاف المصل TOMM40 ($p<0.05$). وتشير بياناتنا إلى أن مستويات التعبير و يمكن أن تكون مؤشرات حيوية دقيقة للغاية ومبكرة وغير غازية فى تشخيص HCC. إنه واعد كاستراتيجية عامة لتطوير المؤشرات الحيوية المستقبلية فى مصل مرضى HCC ومرضى CHC.