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MiR-155 and MiR-665 role as potential non-invasive biomarkers for hepatocellular carcinoma in Egyptian patients with chronic hepatitis C virus infection

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ABSTRACT

Background and Objectives: Hepatocellular carcinoma (HCC) is the fourth leading cause of cancer associated death globally. Serum micro RNAs are full of potential as non-invasive biomarkers. Here, we aim to assess the performance of serum MicroRNA-155 and MicroRNA-665 as diagnostic biomarker for HCC comparing to AFP. **Methods**: Serum samples were collected from 200 subjects (40 healthy control, 80 chronic hepatitis C patients with cirrhosis and without HCC (LC) and 80 HCC patients currently infected by hepatitis C infection and didn't start the treatment). The HCC patients didn't include alcoholic liver disease, nonalcoholic fatty liver disease nor autoimmune liver disease. MicroRNA-155 and MicroRNA-665 expression were measured by real-time quantitative PCR (RT-qPCR), while AFP level was assessed by ELISA method. **Results**: Both miR-155 and miR-665 were significantly elevated in HCC group as compared to both control and LC groups. The comparison between LC and HCC patients revealed that the serum level of miR-155 was a significant increase in HCC patients compared to LC patients; however, the serum level of miR-665 didn't show any significant difference between the same two groups. MiR-665 expression level showed a direct correlation with tumor size in HCC patients. **Conclusions**: Using measurement against AFP level in serum, miR-665 is considered a promising serum biomarker for the diagnosis of HCC patients among the LC patients without HCC. MiR-155 didn't provide a better performance than serum AFP as a diagnostic biomarker among the same group. MiR-665 may serve as a good indicator for HCC prognosis.

Key words: hepatocellular carcinoma (HCC), chronic hepatitis with cirrhosis C and without HCC (LC), alpha-fetoprotein (AFP), micro RNAs (miRNA)

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INTRODUCTION

Hepatitis C virus (HCV), is one of the leading causes of chronic liver disease. Hepatocellular carcinoma (HCC) is a major complication associated with HCV virus infection, with significant mortality and morbidity rates.[1] Egypt holds the highest record of HCV infection globally with almost 15% of the population.[2]

Hepatocellular carcinoma is the most common type of liver cancer and a main cause of tumor-related mortality. According to global cancer statistics from 2012, HCC is the second leading cause of cancer-related deaths in men and the sixth in women, worldwide, and the HCC incidence is still rising globally.[3] The overall 5-year survival rate is very low because of the high aggressiveness of the cancer and the

limited therapeutic options. The most effective therapies as resection or liver transplantation only work in patients at an early stage of the disease. HCC in most cases occurs in the chronically inflamed and damaged liver.[4]

In screening and detection of HCC in high risk patients as chronic hepatitis C (CHC) patients, the most used method is combination of Ultrasonography and serum level of AFP. The disadvantages of ultrasonography are failure of detection of small tumors, operator dependence and diverse diagnostic accuracy.[5,6] As for the AFP serum level, it has many drawbacks as low sensitivity and specificity, the upregulation in some patients with cirrhosis or/and hepatic inflammation in absence of the tumor and no elevation in 80% of small tumor.[7,8]

Hepatocellular carcinoma development and progression are caused by the accumulation of genetic changes that results in tumor related genes expression: oncogenes, tumor suppressor genes, genes involved in many regulatory pathways, such as cell cycle control, apoptosis and angiogenesis. Expression of thousands of mRNAs can be measured at the same time due to advanced technology; this provides a thorough data for both diagnosis and therapy of HCC.[9]

MiRNAs are defined as small non–coding RNA molecules that mediate multiple physiological and pathological processes such as inflammation and cancer.[10] MiRNAs are present in body fluids as serum and plasma, mostly in exosomes or protein-RNA-complexes, which protect them against degradation caused by RNase and other harsh conditions.[11]

Extracellular miRNAs seem to be the by-products of dead or dying cells and stay in the extracellular space because of the high stability of the Argonaute2-miRNA complex[12] or the presence of lipoprotein complexes[13] or vesicles,[14] which may preserve stability. Setting aside of the mechanism, miRNAs can be detected and quantified by PCR using specific primers, making them potentially functional biomarkers.[12]

Dysregulation of miRNAs expression has been demonstrated in various types of cancer including HCC.[15] In addition, circulating miRNAs have been showed as a promising biomarkers for HCC diagnosis and/or good prognosis.[7]

There is a need for new biomarkers that are more disease reflective and more sensitive for identification of HCC patients. Recently, miR-665 was found to be elevated in the tissue of HCC.[16] In addition, miR-155 was linked to both CHC infection and HCC.[17,18]

Based on the previous information, the main aim of this study is to explore the usefulness of two serum miRNAs miR-155 and miR-665 as potential noninvasive biomarkers for the diagnosis of HCC.

PATIENTS AND METHODS

This study was approved by Department of Gastroenterology and Hepatology, Theodor Bilharz Research Institute. Serum samples were collected from a total number of 200 subjects. All patients were recruited from the Department of Gastroenterology and Hepatology, Theodor Bilharz Research Institute during the period from October 2017 to November 2018. Forty healthy volunteers were involved in the current study as a control group. Both patients and volunteers signed the consent documents allowing their clinical information to be gathered and analyzed for research purpose. Subjects were divided into 3 categories: control group (n = 40), liver cirrhosis group without HCC (LC) who had CHC more than 6 months of infection (n = 80) and HCC patients who had cirrhosis and were currently infected by HCV, but didn't start the treatments (n = 80). The staging of HCC patients was done using Okuda staging system respectively.

Venous blood samples (10 mL) were withdrawn from enrolled subjects by trained laboratory technicians. Each sample was divided into three portions: 4 ml were collected in tubes containing EDTA for processing total RNA extraction, 4 mL were left to clot at room temperature, centrifuged and sera were separated for determination of biochemical parameters) and 2 ml were collected in tubes containing EDTA for platelet count (PLT) by phoenix 3300.

The following biochemical tests were done for all involved subjects: Aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total bilirubin (T. Bilirubin) and albumin were assayed using OLYMPUS automatic analyzer AU 400 using original reagents produced by Olympus Diagnostics GmbH (Irish Branch, Lismeehan, Ireland). Serum AFP level was determined using sandwich Enzyme Linked Immunosorbent Assay (ELISA) using washer (State fax ®) reader (state fax chromate-3033®) and kit for AFP (Pointe Scientific, Inc. 4559 Research drive, Canton MI 48188 USA).

Determination of serum level of miR-155 and miR-665 by RT-qPCR

Total RNA extraction and purification was done using a miRNeasy Mini Kit; cat no: 217004 (Qiagen, Hilden, Germany) according to the manufacturer's protocol.

Reverse transcription: cDNA was synthesized by reverse transcription reaction using TaqMan MicroRNA Reverse Transcription Kit; cat no: 4366596 (Applied Biosystems, Foster city, USA) and the thermal cycler (Quanta Biotech).

Gene expression analysis: The quantification of miR-155 and miR-665 levels was amplified from cDNA using TaqMan universal Master Mix and TaqMan assay (has-miR-155; Catalog no: 4427975; Assay ID: 002623) and (hsa-miR-665; Catalog no: 4427975; Assay ID: 002681).The RNU6B snRNA was used as housekeeper gene (Catalog no: 4427975; Assay ID: 001093).

Mature miR-155 Sequence:
UUAAUGCUAAUCGUGAUAGGGGU

Mature miR-665 Sequence:
ACCAGGAGGCUGAGGCCCCU

All samples were analyzed using the 5 plex Rotor-Gene PCR Analyzer (Qiagen, Germany). The $2^{\Delta\Delta Ct}$ method was conducted for the analysis of gene expression levels using TaqMan microRNA Control Assays RNU6B as an endogenous reference control for normalization purposes.[19]

Statistical analysis

GraphPad Prism5® software (version 5.0a; GraphPad Software, Inc., San Diego, Calif) was used for the analysis of the data. Qualitative data were presented as frequencies (n) and percentages (%). Chi-square ($\chi 2$) test was used for comparisons between the three groups regarding the gender. Quantitative data were presented as mean ± standard mean of error (SME). Comparison between the three groups used the one-way analysis of variance (ANOVA) test followed by Tukey test to compare the two groups. Comparison between three or more groups not normally distributed having quantitative variables used Kruskal-Wallis test (nonparametric test) followed by Mann-Whitney test to compare the two groups. Pearson's correlation coefficient was used to determine significant correlations between each miRNA-155 expression and miRNA-665 expression and other variables; the level of significant was < 0.05. The diagnostic value was described by plotting sensitivity against specificity to obtain the receiver-operating characteristic (ROC) curve and both sensitivity and specificity were calculated.

RESULTS

The demographic features of the studied groups are shown in Table 1. Both gender and age are matched among the three groups. Regarding the sex, there is a male predominance among HCC patients 64 males (80 %), which is matched in LC patients with 68 males (85 %) and controls group with 32 males (80%).

There was a significant increase in the serum levels of AST, ALT, ALP, total bilirubin and AFP in HCC group in comparison with LC group. The serum levels of albumin and platelets count were significantly decreased in both LC group and HCC group as compared to the control group. In addition, the serum level of albumin and platelets count levels in the HCC patients were significantly lower than in the LC patients.

Tumor-related characteristics of HCC patients are shown at Table 2. Regarding Okuda staging, 31.3% of HCC patients were at stage I, 32.5% of them at stage II and 36.2% at stage III. Moreover, 43.7% of patients had single focal lesion and 56.3% of them had multiple (2–3) focal lesions. The focal lesion site was 42.5% in right lobe, 32.5% in left lobe and 25% in both left and right lobes. As for the tumor size, 53.7% of HCC patients were equal or less than 3.5 cm and 46.3% of them were more than 3.5 cm.

As shown in Table 3, the mean serum level of miR-155 expression of both LC and HCC groups was significantly elevated to reach nearly (4-fold and 10-fold respectively) that of the control group. In addition, the mean serum level of miR-155 expression was significantly increased in the HCC group to reach nearly 3-fold of the LC group.

On the other hand, regarding the mean serum level of miR-665 expression, a marked escalation in the HCC group that reached about 14-fold of both LC and control group.

As illustrated in Table 4, in the patients of LC, there was a significantly positive correlation between the mean serum level of miR-155 expression and the mean serum level of each of AST, ALT, ALP and AFP. On the other hand, there was a significantly negative correlation between the mean serum level of miR-155 expression and the mean serum level of PLT.

As shown in Table 4, in the patients of HCC, there was a significantly positive correlation between the mean serum level of miR-155 expression and the mean serum level of each one of the following: ALT, ALP and AFP.

Regarding the mean serum level of miR-665 expression, there was a significantly positive correlation between the mean serum level of miR-665 expression and each of the mean serum level of AST, ALT, ALP, AFP and tumor size**.**

On the other hand, there was a significantly negative correlation between the mean serum level of miR-665 expression and the mean serum level of Albumin.

Finally, there was a significantly positive correlation

Table 1: Demographic, biochemical data of the studied groups

	Control group (*n* =40)	LC group (*n* =80)	HCC group (*n*=80)	(*P*-value)
Gender				
Female *n* (%)	8 (20%)	12 (15%)	16 (20%)	0.670
Male *n* (%)	32 (80%)	68 (85%)	64 (80%)	
Age (years) Mean ± SEM	50.75 ± 1.275	49.28 ± 0.8622	52.03 ± 1.091	0.1247
AST (IU/L)	21.50 ± 1.151	75.39 ± 2.297[a]	113.9 ± 6.457[a,b]	< 0.0001
ALT (IU/L)	13.85 ± 0.5269	56.38 ± 3.154[a]	108.9 ± 5.519[a,b]	< 0.0001
ALP (IU/L)	60.60 ± 1.405	99.21 ± 3.106[a]	250.7 ± 10.73[a,b]	< 0.0001
Albumin (g/dL)	4.435 ± 0.08432	2.846 ± 0.09290[a]	2.305 ± 0.05676[a,b]	< 0.0001
T. Bilirubin (mg/dL)	0.6550 ± 0.03703	1.246 ± 0.04390[a]	2.546 ± 0.1368[a,b]	< 0.0001
PLT (×103/µL)	304.0 ± 10.18	192.9 ± 4.307[a]	137.7 ± 3.388[a,b]	< 0.0001
AFP (ng/mL)	6.523 ± 0.4850	19.94 ± 2.115[a]	228.3 ± 29.89[a,b]	< 0.0001
MiR-155 (RQ)	1.277 ± 0.1059	4.561 ± 0.4999[a]	13.20 ± 1.587[a,b]	< 0.0001
MiR-665 (RQ)	1.232 ± 0.1083	1.286 ± 0.1136	17.37 ± 1.138[a,b]	< 0.0001

Values are expressed as Mean ± standard error of mean (SEM).
AST: aspartate aminotransferase; ALT: alanine aminotransferase; ALP: alkaline phosphatase; T. Bilirubin: total bilirubin; PLT: platelets count; AFP: alpha fetoprotein.
[a] Significant difference from control group. [b] Significant difference from LC group.

Table 2: Tumor-related characteristics of HCC patients

Number of focal lesions	
• Single *n* (%)	35 (43.7%)
• Multiple (2–3) *n* (%)	45 (56.3%)
Site of focal lesions:	
• Rt. Lobe *n* (%)	34 (42.5%)
• Lt. Lobe *n* (%)	26 (32.5%)
• Both *n* (%)	20 (25%)
Tumor Size by CT:	
• ≤ 3.5 cm, *n* (%)	43 (53.7%)
• > 3.5 cm, *n* (%)	37 (46.3%)
Okuda staging:	
• Stage I	25 (31.3%)
• Stage II	26 (32.5%)
• Stage III	29 (36.2%)

CT: Computed tomography.

between the mean serum level of both miR-155 and miR-665 expression.

As shown in Figures 1-4, ROC analysis was conducted to identify the optimal levels of miR-155 and miR-665 expression for potential diagnostic value discriminating HCC among the high LC patients compared to the AFP level.

Alpha-fetoprotein best cut-off value was >10.45 with sensitivity of 57.5%, specificity of 58.8%, positive predictive value (PPV) of 58%, negative predictive value (NPV) of 59% and accuracy of 58%. On the other hand, miR-155 best cut-off value was > 4.30 with sensitivity of 80% and specificity of 62.5, PPV of 79%, NPV of 63% and accuracy of 71%. Moreover, miR-665 best cut-off value was > 2.23 with sensitivity of 92.5% and specificity of 86.3%, PPV of 93%, NPV% of 86% and accuracy of 89%.

DISCUSSION

MiR-155 is an important immune response regulator of pro-inflammatory activities and it is considered as a linking factor between inflammation and carcinogenesis.[20] Therefore, the analysis of miRNA-155 expression may be useful in the prognosis of the course of CHC and the development of fibrosis, cirrhosis, and HCC.[17]

Table 3: Correlation of miR-155 and miR-665 level with clinical variables in both LC and HCC groups

	LC group				HCC group			
	MiR-155		MiR-665		MiR-155		MiR-665	
	r	*P*-value	*r*	*P*-value	*r*	*P*-value	*r*	*P*-value
AST (IU/L)	0.438	<0.0001	0.014	0.903	0.216	0.054	0.392	0.0003
ALT (IU/L)	0.272	0.015	-0.168	0.137	0.282	0.011	0.475	<0.0001
ALP(IU/L)	0.281	0.011	0.033	0.773	0.293	0.008	0.371	0.0007
Albumin (g/dL)	-0.238	0.033	-0.172	0.127	-0.205	0.069	-0.441	< 0.0001
T. Bilirubin (mg/dL)	-0.090	0.424	-0.126	0.265	0.065	0.569	0.066	0.561
PLT (×103/µL)	-0.257	0.021	-0.099	0.381	-0.048	0.674	-0.155	0.170
AFP (ng/mL)	0.378	0.0006	0.179	0.112-	0.315	0.004	0.518	< 0.0001
Tumor size (cm)	-	-	-		0.063	0.577	0.533	< 0.0001
MiR-155 (RQ)	-	-	0.172	0.126	-	-	0.423	< 0.0001
MiR-665 (RQ)	0.172	0.126	-	-	0.423	< 0.0001	-	-

AST: aspartate aminotransferase; ALT: alanine aminotransferase; T. Bilirubin: total bilirubin; PLT: platelets Count; AFP: alpha fetoprotein

Table 4: Diagnostic performance of AFP, miR-155, miR-665 for discriminating HCC patients from LC patients

	Cut-off	Sensitivity %	Specificity %	AUC	*P*-value	95% Confidence Interval
AFP(ng/mL)	> 10.45	57.5	58.8	0.608	0.18	0.507 to 0.710
MiR-155 (RQ)	> 4.30	80	62.5	0.743	< 0.0001	0.666 to 0.820
MiR-665 (RQ)	> 2.23	92.5	86.3	0.930	< 0.0001	0.879 to 0.980

In our investigation, HCC patients didn't include alcoholic liver disease, nonalcoholic fatty liver disease nor autoimmune liver disease. Our results showed that the mean serum level of miR-155 expression was significantly higher in both LC and HCC patients compared to the controls. In addition, the mean serum level of miR-155 expression was significantly elevated in the HCC group in comparison with that of the LC group.

Our finding came in agreement with Ezzat *et al.*, 2016[20] whose work found that there was an overexpression of miR-155 in the serum of both LC and HCC patients in comparison with the controls.

Also, our results were in accordance with Guan *et al.*, 2016[21] who measured the level of miR-155 expression in HCC tissue against the neighboring free cancer cells and declared its elevation in the cancer cells.

Generally, these findings further support the idea that miR-155 forms an important bridge between inflammation represented by CHC patients with cirrhosis group and tumorigenesis represented by HCC group indicating that miR-155 has an important role in the diagnosis of HCC in LC patients previously infected by HCV.

There is a direct positive correlation between miR-155 and TNF-α where both factors are considered a positive regulator for the other.[22] TNF-α is an important cytokine in the inflammation process and regulated by the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-ϰB) transcription factor at the level of transcription. NF-ϰB pathway is induced by some ligands and receptors as Toll-like receptors (TLRs).[23] Regarding the HCV infection, hepatitis C virus core protein and Non-structural protein 3 causes a direct increase in the production of TNF-α in B cells. In addition, HCV induces the NF-ϰB pathway by interacting with TLR 8 and 4 where HCV as a single stranded RNA virus interacts with TLR 8 and non-structural protein 5 interacts with TLR 4 in Kupffer cells.[22]

Regarding the elevation of miR-155 in HCC patients, a possible explanation for these results may be presented by Li *et al.*, 2018,[24] who showed that stimulation of Tumor Growth Factor-β1 results in the raise of miR-155 expression in HCC cells and this escalation of miR-155

promotes epithelial-mesenchymal transition (EMT), invasion and migration of HCC cells.

In addition, MiR-155 targets adenomatous polyposis coli (APC) gene, which is a negative regulator of Wnt signaling, which is an important signaling factor implicated in the control of cell apoptosis and proliferation and is reported to be one of the main cascades that regulates cancer development.[25–27]

Moreover, miR-155 has a dual action in case of cancer – it may act as onco-suppressor or oncogene, which is the case in hepatocellular carcinoma.[28]

In HCC, miR-155 acts as an oncogene resulting in tumor progression by the inhibition of multiple genes that act as tumor suppressors and this offers a third mechanism or for the escalation of serum miR-155 expression level in HCC patients.

Cytosine-cytosine-adenosine-adenosine-thymidine-enhancer-bindingproteins (CCAATor C/EBPs) was often increased in HCC; hence, it was assessed as the first tumor suppressor that high level of miR-155 resulted in its knock down.[29]

In addition, sex-determining region Y-gene related high-mobility-group box gene (SOX6) is another tumor suppressor that was suppressed due to the increase in miR-155 expression level. The inhibition of SOX6 leads to the inactivation of P21 growth regulator promoting HCC development.[30]

Moreover, suppressor cytokine signaling 1 (SOCS1) is a tumor suppressor that is also considered as a target for miR-155. Downregulation of SOCS1 by miR-155 elevation causes the activation of Signal transducers and activators of transcription 3 (STAT3) that inhibits apoptosis in cancer cells including HCC.[31,32]

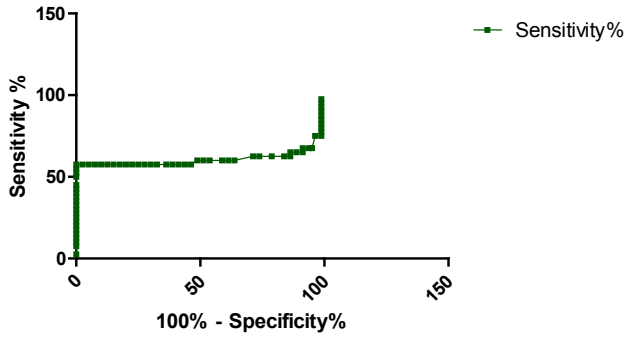

Figure 1: ROC curve of AFP in discriminating HCC group from LC group

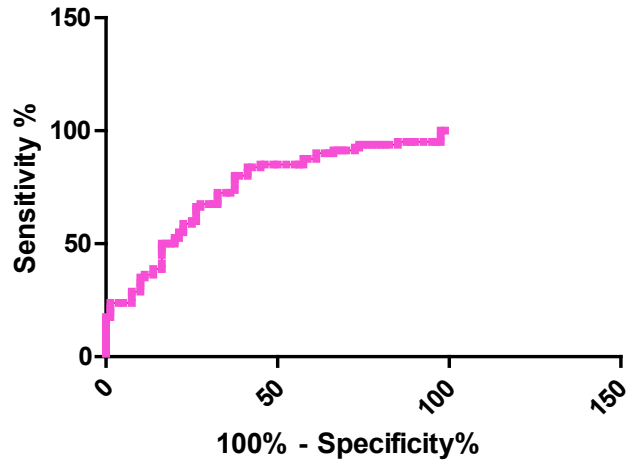

Figure 2: ROC curve of RQ of miR-155 in discriminating HCC group from LC group

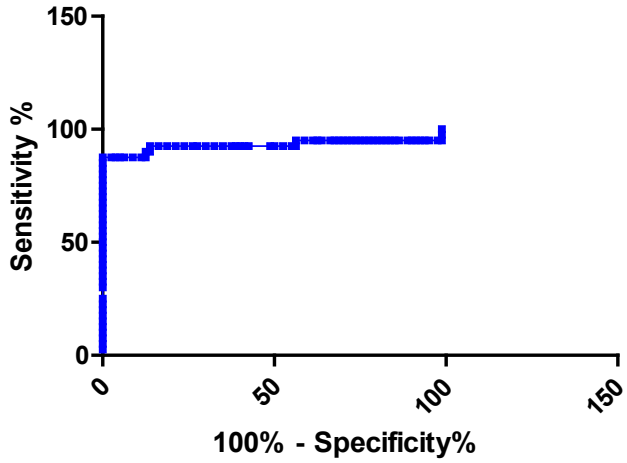

Figure 3: ROC curve of RQ of miR-665 in discriminating HCC group from LC group

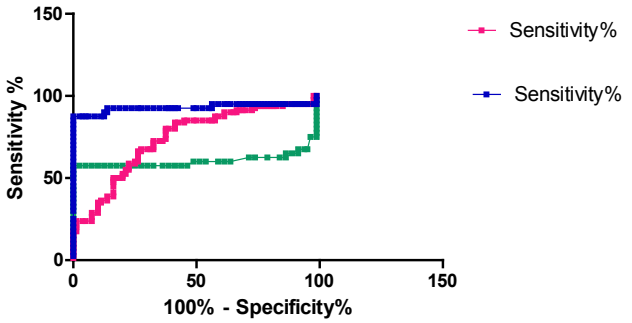

Figure 4: Combined ROC curves of RQ of miR-665, RQ of miR-155 and AFP in discriminating HCC group from LC group

Furthermore, the fourth tumor suppressor that is inhibited by miR-155 uprising is called F-box/WD repeat-containing protein 7 (FBXW7). FBXW7 is believed to be the center of multiple pathways including controlling cell growth, cell differentiation and tumor genesis.[33,34]

Finally, another tumor suppressor that was examined as destination for miR-155 was suppressing Phosphatase and Tensin homolog (PTEN) through inhibiting the action of class I phosphatidylinositol 3-kinases (PI3K)/Akt pathway.[18]

Regarding miR-665 expression level, it was increased in some tumors as intestinal gastric adenocarcinoma [35] but decreased in others as osteosarcoma.[36] This may suggest that miR-665 exerts a different biological function according to the type of tumors. As for the expression of miR-665 in HCC, previous studies reported that there was an increase in the serum exosomal expression of miR-665 in HCC patients in comparison to people in good health.[37,38]

The current study showed that the serum level of miR-665 expression in the HCC group was markedly higher than both LC and control groups. However, there was no significant difference between the LC patients and the control group.

Another study by Qu *et al.*, 2017[39] demonstrated that serum level of exosomal miR-665 in HCC group of patients was significantly upregulated when measured against a healthy group of people.

In addition, Hu *et al.*, 2018[16] worked on HCC tissue cells and illustrated that there was a significant elevation of miR-665 expression in the HCC cells when compared to the surrounding non-cancerous cells.

However, our results showed that there was a significant increase of miR-665 expression in the free serum of HCC patients in comparison with the control group, which gives the benefit of removing the step of differential centrifugation and density-gradient centrifugation required for serum exosomal extraction.

The increase in miR-665 expression in HCC could be explained by Qu *et al.*, 2017[38] who showed that exosomes extracted from hepatocellular tumor cells can use its content of miR-665 to activate the MAPK/ERK pathway. The activation of the MAPK/ERK pathway through miR-665 is considered an important intracellular proliferative and anti-apoptotic pathway and takes part in the malignant proliferation of tumor cells by affecting the activity of effector molecules, such as downstream cell cycle regulatory proteins and apoptosis-related proteins.[39]

In addition, another possible explanation for the elevation of miR-665 expression in HCC was introduced by Hu *et al.*, 2018[16] who illustrated that miR-665 is targeting a tumor suppressor called protein tyrosine phosphatase receptor type B (PTPRB). This gene can modulate Hippo signaling activity, which is a central mechanism that suppresses the overgrowth of the tissue leading to the inhibition of carcinogenesis. Based on these reasons, miR-665 promotes hepatic cancer proliferation, migration, and invasion by inhibition of Hippo pathway activity through PTPRB.

In the current study and in the matter of the correlation between the two biomarkers and the other variables in patients of LC, there was a significantly positive correlation between the mean serum level of miR-155 expression and the mean serum level of each of AST, ALT, ALP and AFP. On the other hand, there was a significantly negative correlation between the mean serum level of miR-155 expression and the mean serum level of PLT.

Our data was consistent with RIAD *et al.*, 2015,[40] who reported a positive correlation between the serum level of miR-155 and both ALT and AST.

Also, a previous study by Kałużna, 2014 [17] showed that hepatic expression of miRNA-155 correlates with the serum level of biochemical markers of liver damage.

In the present study and in the matter of the correlation between the two biomarkers and the other variables in patients of HCC, there was a significantly positive correlation between the mean serum level of miR-155 expression and the mean serum level of each of ALT, ALP and AFP.

Regarding the mean serum level of miR-665 expression, there was a significantly positive correlation between the mean serum level of miR-665 expression and each of the mean serum levels of AST, ALT, ALP, AFP and tumor size**.**

On the other hand, there was a significantly negative correlation between the mean serum levels of miR-665 expression and the mean serum level of Albumin.

Finally, there was a significantly positive correlation between the mean serum level of both miR-155 and miR-665 expression.

Our results agreed with Guan *et al.*, 2016,[21] who revealed the absence of association between miR-155 expression and tumor size in HCC patients however, in contrast to our results, they showed no association between miR-155 expression and AFP level.

In addition, our results came in agreement with Ezzat *et al.*, 2016,[20] who found that was no significant impact of tumor characteristics regarding number; site; size and serum values of miRNA-155.

Moreover, our results were in consistence with Qu *et al.*, 2017,[38] who reported that a high-expression of miR-665 was found in the higher clinical stages patients of HCC; also a high-expression of miR-665 was associated with larger tumor size.

The most used marker in diagnosis of HCC is the AFP serum level in spite of its humble sensitivity (39%–65%).[8]

Regarding the discrimination of HCC patients from the high risk LC patients, both miR-155 and miR-665 had the ability to differentiate between the two groups with sensitivity 80%, specificity 62.5 %, PPV 79%, NPV 63% and accuracy 71%. and sensitivity 90%, specificity 95 %, PPV 93%, NPV% 86% and accuracy 89% respectively in comparison with AFP with sensitivity 60%, specificity 70%, PPV 58%, NPV 59% and accuracy 58%.

According to the previous results, miR-155 didn't show a better ability in the diagnosis with specificity of only 62.5% than the common marker AFP, while miR-665 approved to be a good diagnostic biomarker with good sensitivity, specificity, PPV, NPV and diagnostic accuracy.

Another study by Ezzat *et al.*, 2016 [20] reported that in the discrimination of HCC patients from CHC patients, miR-155 serum expression had 95% sensitivity, 76.5% specificity compared to the AFP serum level with 100% sensitivity, 69.2% specificity.

STUDY LIMITATION

Hepatocellular carcinoma group didn't include patients with alcoholic liver disease, nonalcoholic fatty liver disease and autoimmune liver disease, and we recommend future involvement of this condition.

CONCLUSION

The following conclusions can be drawn from the present study:

Serum miRNA-665 is a good noninvasive diagnostic biomarker for detection of HCC in patients with LC as compared to AFP serum level as a reference.

Serum miR-155 didn't show a better diagnostic ability than serum AFP due to relative low specificity that makes it hard to be used in clinical practice in differentiation between HCC patients and high-risk LC patients.

The positive correlation between serum miR-665 and the tumor size of HCC is suggesting a promising role of miR-665 in the prognosis of the disease.

Conflict of Interest

The authors declare no conflicts of interests.

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