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Clinical Utility of Micro RNA-148a in Egyptian Patients with Hepatocellular Carcinoma

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Abstract

Hepatocellular carcinoma (HCC) is the one of the most commonly diagnosed cancersworldwide and represents one of the leading causes of cancer-related death. HCC is associated with bad prognosis, as most patients present with advanced stages at the time of detection. Aberrant expression of miRNA-148a is found in HCC.This motivatesresearchersto evaluate miR-148a in HCC.The aim of our study was to assess the clinical utility of miR-148a as a non-invasive marker for early detection of HCC in addition to its prognostic value. This study was conducted on 64 subjects, undertitled into three groups: HCC, hepatitis C virus (HCV) and control groups. miR-148a was analyzed in serum by quantitative real time polymerase chain reaction (qRT-PCR). Results showed that miR-148a expression revealed a significant difference between groups with the HCC group revealing the lowest values. The HCC group revealed a negative correlation between miR-148a and primary tumor, tumor size, TNM staging andalpha-fetoprotein(AFP). Receiver operator characteristics (ROC) curve was constructed to estimate discrimination of HCC group versus the other groups through serum biomarker miR-148a and AFP. The best cut-off for miR-148a was ≤ 7.331 ($2^{-\Delta\Delta CT}$), with sensitivity 82.4 % and specificity 80 %. As for AFP, the best cut-off level was 25 ng/mL with sensitivity 70.6% and specificity 63.3%.As a conclusion, miR-148a was found to be downregulated in Egyptian patients with HCC. Our findings thus propose that miRNA-148a can be beneficial for early diagnosis of HCC, as well as having a prognostic value.

***Keywords*:**microRNA; microRNA-148a; hepatocellular carcinoma.

Introduction

Hepatocellular carcinoma (HCC) isone of the most common aggressive cancers worldwide [1]. Multifactorial risk factors contribute to HCC development. The processes of chronic inflammation and liver cirrhosis are considered as the major common links to hepatocarcinogenesis[2]. Hepatitis B virus (HBV), hepatitis C virus (HCV), aflatoxin exposure in food, alcohol, obesity, as well as other causes significantly promote the genesis of HCC[3].

In Egypt, HCC is the most common reason of cancer-related morbidity and death and it is also estimated to be the most common cancer in men[4]. The growing incidence in Egypt is proposed to be due to the improvedscreening programs conducted by the authorities with major concern on HBV and HCV as main risk factors [5,6].

Analysis of serum alpha-fetoprotein (AFP)and liver ultrasound are routinely advised to be performed in patients withcirrhosisfor early screening of HCC[7]. However, these currently used diagnosticmethods in practice lack satisfactory sensitivity [8].

Despite advances in molecular technology which have identified several genetic changes in HCC, more practical markers - with reasonable sensitivity and specificity -are required for early detection, determining prognosis and as therapeutic target for HCC[9].

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MicroRNAs (miRNAs) are tiny noncoding RNAs known to play a pivotalrole in the post-transcriptional control of messenger RNA (mRNA)[10]. miRNAs are related to post-transcriptional gene silencing through binding to the 3` untranslated region of mRNA and triggering translational repression or mRNA degradation [11].

About 2600 miRNAs are postulated to be encoded by the human genes[12]. Among them, certain miRNAs have been discovered to be dysregulated in HCC and contribute to the outgrowth and progress of HCC, in addition to influencing drug resistance in HCC [13].

Many researches have found that variousmiRNAsact as tumor suppressors thattarget oncogenes. These miRNAs have been found to be downregulated in HCC. However, other miRNAstargeting tumor suppressor genes,are found to be upregulated [14].

This sparkled scientists to extensively do researches on miRNAsas both diagnostic and prognostic biomarkers and therapeutic targets [15].

One of miRNAs of interest in HCC is miR-148a whichis a memberinthe miR-148/miR-152 family. This family includes miR-148a, miR-148b and miR-152. miR-148a is located in humans on chromosome 7[16].

Interestingly, althoughmiR-148a has been extensively studied in HCC, however contradictory results have been observed[17, 18]. Moreover, evaluation of miR-148a in HCC in Egyptian patients still need further studies for clinical implication.

Regarding miR-148a clinical implications in HCC, some studies stated that it is beneficial as screening marker for HCC [18]. Moreover, other researchesfound miR-148a to be associated with HCC progression and metastasis[19].The goal of our study was to evaluatethe clinical benefit of miR-148a as a diagnostic marker inEgyptian HCC patients, in addition to evaluation of its role in predicting the prognosis.

Subjects and methods

Subjects

The current case-control study was conducted over a 6-month period at Theodor Bilharz Research Institute (TBRI) upon 64 individuals. The TBRI Research Ethics Committee accepted the study (no. 192465). They were under titled in to three groups:

- **Group I** (HCC group; n=34).
- **Group II** (HCVgroup; n=20).
- **Group III** (control group; n=10)

Exclusion criteria:

- Patients with HCC who had undergone any kind of previous radiation or chemotherapy
- Other malignant tumors in addition to HCC.

Methods

All participants in the research were subjected to full history taking, clinical examination, abdominal ultrasound to assess hepatic focal lesions, liver cirrhosis, hepatosplenomegaly, ascites, portal vein invasion and thrombosis, and capsular tumor invasion, in addition to Computed tomography (CT) scan of abdomen for HCC patients.

Nine milliliters (9 mL) of venous blood were withdrawn from all participants. They were divided into different tubes to perform laboratory investigations whichinclude:

- Liver function tests:aspartate transaminase (AST) and alanine transaminase (ALT)were analyzed according to manufacturer's protocol on the automatedBeckman Coulter AU480 analyzer[1].
- Serum AFP: Sera from patients with HCV and HCC were analyzed for AFP on Immulite 2000 system analyzer using solid phase two sequential chemiluminescence immunometric assay according to manufacturer's protocol using kits provided by Siemens[2].
- Anti HCV antibodies was assayed by media enzyme immunoassay on Axsym auto analyzer using kits provided by ABBOTT[3].
- Analysis of the expression level of miRNA 148a in serum using qRT-PCR.

Four (4 mL) were collected on a plain vaccutainer and centrifuged at 4000 rpm for 15 minutes. The separated sera were transported to aliquots. Then, thesera were centrifuged again at 15,000 rpm for 15 minutes and supernatants were refrigerated at-70°C till time of PCR.

Genomic RNA extraction was done by miRNeasy Mini Kit[4] to isolate total RNA, including tiny RNA, from serum (cat. no. 217004). Reverse Transcription was done by miRCURY® LNA® RT Kit. Then, Real - time PCR quantification for miRNA-148a was carried out with miRCURY LNA SYBR® Green PCR Kit (catalogue numbers; 339345, 339346, and 339347) and miRCURY LNA miRNA PCR Assay (catalogue number 339306) on real-time cycler.

Results were released in relative quantification based upon the difference in thelevels of expression of a target gene and a reference gene which was SNORD68 in our research. The cycle threshold (CT) was used to calculate the level of miRNA-148a expression. Calculation is based upon comparing a distinct cycle determined by threshold values at a constant level of fluorescence.

The ΔCT value for every sample was calculated as the difference between the CT value of the target gene and the CT value of the reference gene. This was determined for the unknown sample and also for the calibrators. Then, ΔΔCT was calculated, where ΔΔCT = ΔCT (sample) – ΔCT (calibrator). SNORD68 was used as a reference gene since it is commonly used in human cancer studies [20], the relative expression (fold change) for each miRNA-148a was then calculated using the formula: $2^{-\Delta\Delta CT}$.

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[4] miRNeasy MiniKit; Trademarks: QIAGEN®,QIAzol®, RNeasy® (QIAGEN Group).

Statistical analysis

Sample size was estimated by Ain Shams University's Community Department using the G* power program version 3.1 (Universität Düsseldorf, Germany).The obtained data were reviewed, coded, and analyzed by Statistical Package for the Social Sciences soft-ware program version 23.0 (SPSS Inc., Chicago, Illinois, USA).

Parametric numerical variables were presented as mean (standard deviation [SD]) and comparison between groups was done using student t test. Non-parametric numerical variables were presented as median (interquartile range [IQR]), and comparison between 2 groups were performed using Mann-Whitney test, while more than 2 sets of groups with skewed distribution were compared using Kruskal Wallis test.

Categorical variables were presented as number (%) and data were compared using the Chi-squared test. Correlations between non-parametric numerical variables were performed using the Spearman rank correlation coefficienttest.p value <0.05 was interpreted to be significant and p value <0.01 to be highly significant, while p value >0.05 was interpreted as non-significant.

ROC curve analysis was used to determine the diagnostic performance of the studied markers, wherespecificity % was plotted onthe x-axis and sensitivity % on the Y axis. The best cut-off value was determinedas the nearest point to the upper left corner of the curve.

Results

Descriptive and comparative statistics of all studied groups regarding different studied clinical and laboratory parameters are shown in **Table (1)**.

Descriptive Statistics of HCV and different studied pathological parameters in HCC patientsis shown in **Table (2).**

Descriptive and comparative statistics of the serum biomarker miRNA- 148a in all studied groups are shown in **Table (3) and Figure (1).** There was a highly statistically significant difference between groups according to the serum biomarker miR-148a.

In HCC group; a negative correlation was found between miR-148a with primary tumor,tumor size, TNM staging and AFP**(Table 4).**

In HCV group; a negative correlation was found between miR-148a with age and AFP**(Table 5).**

The ROC curve of serum biomarker miR-148a and AFP for discrimination of HCC group versus HCV group was constructed. The best cut-off level for AFP was 33 ng/mL.Regarding miR-148a, the best cut-offvalue was 3.7 ($2^{-\Delta\Delta CT}$). The sensitivity of miR-148a was 64.7% versus 58.8% for AFP. The specificity of miR-148a was 65% versus 60% for AFP(Figure 2and table 6).

Table (1): Comparison between the different studied groups regarding different studied parameters

Studied Parameter	Group I(HCC)(n=34)	Group II (HCV) (n=20)	Group III(Control) (n=10)	Test	p-value
Age (years) Mean± SD	57.8±9.5	57.3±14.1	55.2±8.8	F=0.212#	0.810
Sex Female Male	7 (20.6%) 27 (79.4%)	7 (35.0%) 13 (65.0%)	6 (60.0%) (%40.0) 4	x2=5.78*	0.056
AST (IU/L) Median (IQR)	69 (55-96)	52 (38-82)	30 (20-30)	H=27.697¥	<0.001
ALT (IU/L) Median (IQR)	87 (72-129)	76 (63-92)	28 (21-33)	H=26.64¥	<0.001
AFP (ng/mL) Median (IQR)	191 (52-543)	13 (9-32)	4 (3-8)	H=32.75¥	<0.001
WBCs (Thousands/ cmm) Median (IQR)	6.1 (4.4-7)	6.2 (5.-7)	8.2 (5.6- 9.2)	H=3.36¥	0.186
Hemoglobin (g/dL) Mean±SD	11.2±2.22	12.1±1.9	15.17±1.42	F=20.19#	<0.001
Platelets count (Thousands/ cmm) Median (IQR)	110 (73- 144)	167 (121 -227)	271.5 (222-376)	H=31.698¥	<0.001

Student-t test, * chi square test, ¥ Mann-Whitney test.

Table (2): Descriptive Statistics of HCV, Vascular invasion, Primary tumor (T), TNM staging and Tumor size (cm^2) Distribution Among HCC Group.

	HCC group (n=34)
HCV	
Negative	6 (17.6%)
Positive	28 (82.4%)
Vascular invasion	
Negative	17 (50.0%)
Positive	17 (50.0%)
Primary tumor (T)	
T1	3 (8.8%)
T2	15 (44.1%)
T3	2 (5.9%)
T4	14 (41.2%)
TNM staging	
I	3 (8.8%)
II	13 (38.2%)
III	17 (50.0%)
IV	1 (2.9%)
Tumor size (cm^2)	
≤5 cm.	26 (76.5%)
>5 cm.	8 (23.5%)
Mean ±SD (Range)	4.68 ±3.55 (1.39-18.30)

HCV: Hepatitis C virus, TNM: tumors, nodes, metastasis

Table (3): Descriptive and Comparative Statistics of Serum Biomarker miR-148a in HCC, HCV and Control Groups Using Kruskal Wallis Test

Serum Biomarker [miR-148a]	Group I (HCC) (n=34)	Group II (HCV) (n=20)	Group III(Control) (n=10)	Test	p-value
Median	1.706	2.775	18.746	7.782	0.01
IQR	0.42-4.64	0.085-12.88	0.99-158.028		

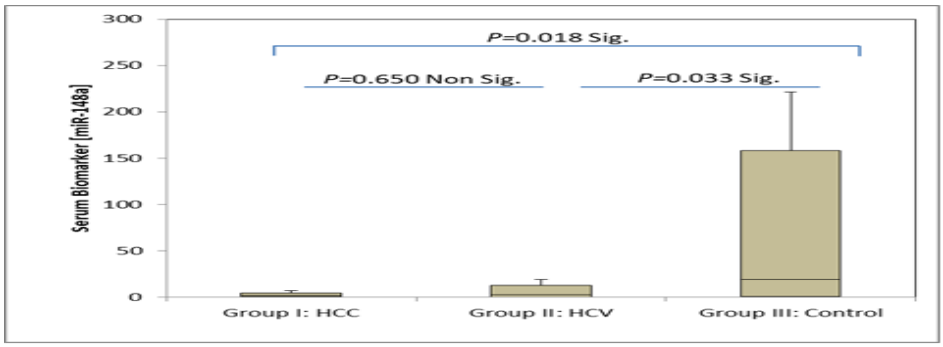

Figure (1): Box plot showing median levels of serum biomarker miR-148a in HCC, HCV and Control groups.

Table (4): Correlation study between patient's serum biomarker miR-148a and the other studied parameters in HCC group, using Spearman's rank correlation coefficient test

HCC group	Serum Biomarker (miR-148a)	
	R	p-value
Age (years)	0.052	0.77
AST (IU/L)	-0.088	0.62
ALT (IU/L)	-0.074	0.68
AFP (ng/mL)	-0.479	0.031*
WBCs (Thousands/ cmm)	0.200	0.26
Hemoglobin (g/dL)	0.125	0.48
Platelets count (Thousands/ cmm)	0.358	0.04*
Tumor size (cm^2)	-0.545	0.018*
Primary tumor (T)	-0.508	0.02*
TNM staging	-0.706	0.009*

Table (5): Correlation study between patient's serum biomarker miR-148a and the other studied parameters in HCV group, using Spearman's rank correlation coefficient (rs)test

HCV group	Serum Biomarker [miR-148a]	
	R	p-value
Age (years)	-0.600	0.005*
AST (IU/L)	0.151	0.53
ALT (IU/L)	0.308	0.19

AFP (ng/mL)	-0.374	0.038*
WBCs (Thousands/ cmm)	0.189	0.424
Hemoglobin (g/dL)	0.496	0.026*
Platelets count (Thousands/ cmm)	0.230	0.330

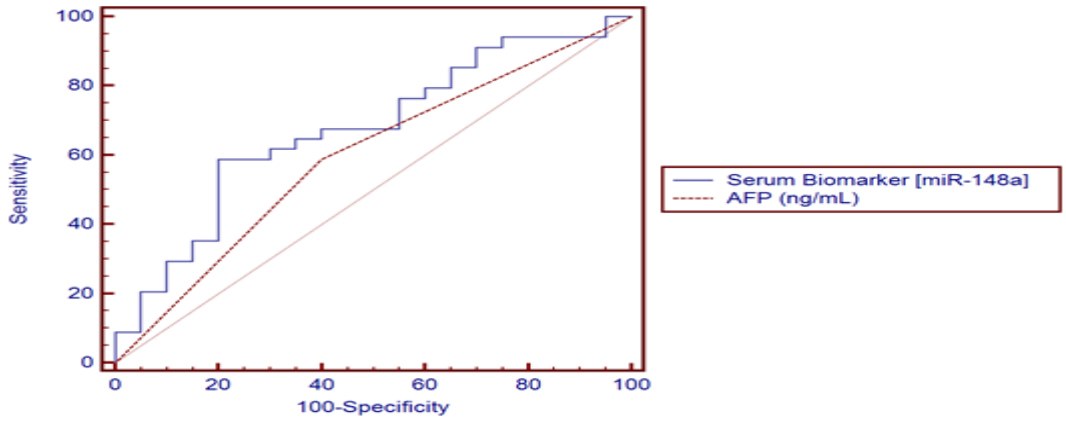

Figure (2): Receiver-operating characteristic (ROC) curve for diagnostic performance of serum biomarkers miR-148a and AFP in discrimination of HCC versus HCV groups.

Table (6): The diagnostic performance of AFP and miRNA-148a in discriminating HCC group from HCV group:

Parameters	Cut-off	AUC	Sensitivity	Specificity	PPV	NPV
miRNA-148a($2^{-}_{\Delta\Delta CT}$)	≤ 3.7	0.675	64.7 %	65 %	75.9 %	52 %
AFP (ng/mL)	≥ 33	0.594	58.8 %	60 %	71.4 %	46.2 %

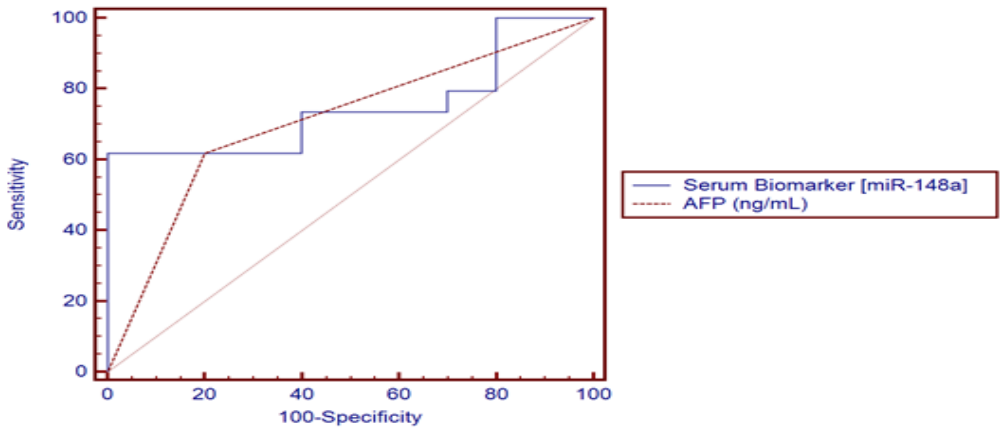

Figure (3): Receiver-operating characteristic (ROC) curve for diagnostic performance of serum biomarkers miR-148a and AFP in discrimination of HCC group versus control group.

Table (7): The diagnostic performance of AFP and miRNA-148a in discriminating HCC group from control group:

Parameters	Cut-off	AUC	Sensitivity	Specificity	PPV	NPV
miRNA-148a ($2^{-\Delta\Delta CT}$)	≤ 1.9	0.771	73.5 %	90 %	96.2 %	60 %
AFP (ng/mL)	≥ 15	0.684	61.7%	80%	91.3 %	38.1%

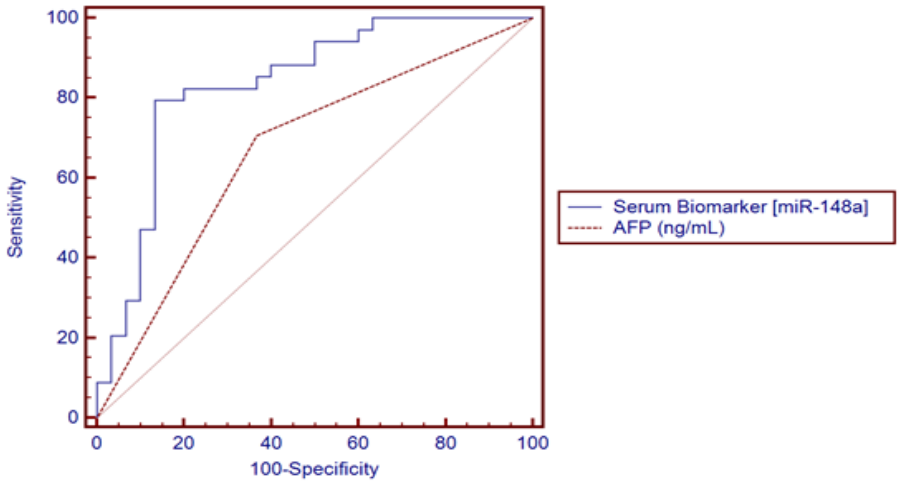

Figure (4): Receiver-operating characteristic (ROC) curve for determination ofdiagnostic performance of serum biomarker miR-148a and AFP in discrimination of HCC group versus HCV and control groups

Table (8): Diagnostic performance of miRNA-148a and AFP in discrimination between HCC group from HCV and controlgroups

Parameters	Cut-off	AUC	Sensitivity	Specificity	PPV	NPV
miRNA-148a ($2^{-\Delta\Delta CT}$)	≤ 7.33	0.835	82.4%	80 %	82.4 %	80 %
AFP (ng/mL)	≥ 25	0.67	70.6 %	63.3%	68.6 %	65.5 %

Discussion

The arising role of miRNAs in HCCis demonstrated by manyresearchesas they revealed their association with tumor progression, metastatic dissemination, as well as being potential therapeutic targets[21]. Among miRNAs studied,increasing evidence highlighted that miR-148a show aberrant expression in a variety of malignant tumorswith crucial roles in regulating hepatocarcinogenesis[22].

Based on the previous data, our research was designed to investigate the relation between miR-148a and HCC. Results of our study showed that the majority of patients were males, as 79.4 % and 65 % were males in the HCC and HCV groups, respectively. It is quite evident that HCC is one of the sex- biased cancers. Gender discrepancies may be explained by variations in biology of HCC cells, in addition to environmental and behavioral risk factors as men tend to consume greater quantities of alcohol and smoke cigarettes[23]. Gender-related effect of sex hormones on development and growth of HCC has been hypothesized, however, the mechanism by whichandrogens promote HCC remain unclear[24, 25].In this context, it should be mentioned that Estrogen is proposed to protect women from hepatocarcinogenesis[26].Moreover, several studies proposed sex- specific molecular etiologieswith differential gene expression, that require further studies to beproven [26, 27]. Our results agree with Wu et al., (2018) study; as 74.5% of patients with HCC were males [23]. Also, Mu et al., (2020) and El Ghandour et al. (2021) showed comparable results [28, 29].

Regarding AFP, AST and ALT,our study found a highsignificant difference between the studied groups. Similar results were found in El Ghandour et al. (2021) study, as AFP was much higher in the HCC group than in the HCV group [29]. In Abed El-Aziz et al.(2016) study, the serum AFP levels showed significant increase in HCC patients than in the liver cirrhosis,HCV, and control groups [30].

Spaniel et al. (2013) also stated that ALT in patients with HCC was substantially greater than in the control group, which is in concordance with our study results [31].Shoaib et al. (2014) also demonstrated that ALT and AST showedsignificantly higher values in cases with HCC compared to cases with HCV and the controls[32].This may be attributed to the fact that the majority of HCC patients also have cirrhosis of the liver, which is a process of longstanding hepatic inflammation [33].

Our study found that the majority of patients with HCC (82.4%)were confirmed to be HCV positive. Carcinogenesisby HCV is proposed to be rather indirect, crucially through chronic inflammation and the establishment of a pro-tumorigenic environment[34]. HCV is also directly involved in hepatooncogenesis, as it is claimed that the core protein of HCV is found to have an oncogenic capability [35].In Chen et al. (2011) study, 54 % of HCC patients were positive for HCV [36]. Ali et al. (2024) claimed through their meta-analysis that50-80% of the globally chronic HCV cases leadto cirrhosis andHCC [37]. The variation in the incidence of HCV-related HCC is attributed to both ethnicity and geographical distribution [38, 39].

Regarding tumor size, luckily, 76.5% of patients revealed tumor size to be less than 5 cm. Lim et al. (2014) declared comparable findings, with 65% of HCC patients having tumor size less than 5 cm [40]. However, Wu et al. (2018b) found 53 % of patients showing tumor size to be less than 5 cm in size [41]. Moreover, Shehata et al.(2024) found 26.7% of patients only with tumor size less than 5 cm. Several explanations are proposed for such discrepancy in results [42]. About 20% of cases with HCC are recognized to develop in non-cirrhotic liver. Those patients often present at late stages as surveillance is not done to non-cirrhotic liver[43].In this context,Chartampilas et al. (2022) stated that ultrasound's sensitivity may only be 65% for tumors less than2 cmin size which delay diagnosis till tumor size gets bigger [44].

The present study observed a highly statistically significant difference in miR-148a expression among different groups ($p<0.05$).The HCC group had the lowest values, followed by the HCV group, while the highest values are revealed inthe control subjects. Comparable results were observed by Han et al. (2019) which reported that HCC patients had substantially lowered plasma miR-148a levels than those with liver cirrhosis or controls [17]. Many studies claimed that miR-148a markedly diminished the growth of HCC [45, 46].It is strongly proposed that miR-148a acts as a tumor suppressor miRNA, and its down regulation plays a pivotal role in the carcinogenesis and progress of HCC [47].It is suggested that miR-148a inhibits HCC growth through down regulation of certain pathways as epithelial-to-mesenchymal transition and the phosphatidylinositol 3-kinase /protein kinase B signaling pathways by targeting death receptor which plays a crucial role in apoptosis[48].

In our study, association between miR-148a and different clinico-pathological and laboratory parameters was detected. A strong negative relation was observed between serum miR-148a andprimary tumor (T), tumor size, TNM staging andAFP in cases with HCC. This in in agreement with Heo et al.(2014), where the downregulation of miR-148a has been correlated withhigh levels of AFP and TNM stage [49].In addition, Wang et al. (2016) found that serum levels of miR-148a and miR-148bwere low and were correlated with tumor sizes and TNM stages[50]. Moreover,Pan et al. (2014) studies revealed that miR-148a was inversely related with TNM stages and capsular invasion in HCC[51].Severalstudies related their results to the evidence that miR-148/152 family are important modulators of HCC growth and progression [52].

On contrary, according to Ji et al. (2018), increased miRNA expression in HCC was correlated with high AFP levels and pathological grade [53]. Moreover, Ajdarkosh et al. (2016) found in his results that miR-148a expression was not related to tumor size[54].

In our study, ROC analysis of miR-148a and AFP in HCC group versus HCV and control groups showed statistically significant difference between both markers with sensitivity 82.4%, specificity 80 % and AUC 0.835 for miR-148a. Our results were in accordance to Ajdarkosh et al. (2016) who performed his study on 96 HCC patients. The AUC of miR-148a was 0.837 with the sensitivity and specificity were 80 % and 62.2 %, respectively [53]. In another study, the AUC for miR-148a was 0.919, with sensitivity 89.6 % andspecificity 89.0% for HCC patients versus liver cirrhosis [17]. The ROC analysis of miR-148a in predicting HCC recurrence after liver transplantation in the study of NG et al.(2016) showed that AUC was 0.727, sensitivity 88.9% and specificity 56.6% [55]. Results of our study and the published results of Ajdarkosh et al. (2016)and Han et al. (2019) revealed that miR-148a may be used as a marker for detection and follow up of HCC [17, 54, 55].

Furthermore, Pan et al. (2014) reported in their ROC curve analysis that the AUC of miR-148a was 0.761, with sensitivity 76.3% and specificity 50.6%[51].

Several miRNAs have been studied to determine their role as diagnostic and/or prognostic markers for HCC, like miR-21, miR-101, miR-3677, miR-421, miR-326, miR-424 and miR-511-2 [56].Expression of miRNAsvaryas some show down regulation, and others are upregulated in HCC. Down regulated miRNAs includelet-7g, miR-22, miR-26, miR-29, miR-99a,

miR-122, miR-124, miR-139, miR-145, miR-23b-3p and miR-199b. On the other side, upregulated miRNAs in HCC include miR-10b, miR-331-3p, miR-17-5p, miR-135a, miR-155, miR-182, miRNA 486-5p, miR-221, and miR-222 [14].

A study upon miR-221 in HCC revealed in their ROC analysis that miR-221 yielded 87% sensitivity and 40% specificity in differentiating HCC from cirrhotic patients [57].Another study revealed that miR-23b-3p yielded 80% sensitivity and 74% specificity andmiR-331-3p yielded 66% sensitivity and 61% specificity and AFP yielded 64% sensitivity and 61% specificity of 61% at cutoff value 148 ng/mL in discrimination between HCC patients from control group [58]. Furthermore, a research found that miRNA 486-5p had a sensitivity and specificity of 60% and 76%, respectively to diagnose HCC, while AFP yielded a sensitivity of 36% and specificity of 76% at a cut off value of 100 ng/mL [59].

Interestingly, although several miRNAs have been evaluated in HCC, yet no single miRNA have been determined to be reliable enough to be used in clinical practice as a diagnostic or prognostic marker for HCC. In their study, Alemayehu et al. (2024) stated that miRNA panels showed superior performance in detecting HCC compared to single miRNAs (AUC 0.94 versus 0.88). This could be attributed to the involvement of several gene mutations and epigenetic genetic alterations in the progress of HCC [60].

A study performed on two miRNAs panel (miR-21-5p and miR-423) revealed significant difference between the HCC group and chronic liver disease group. Furthermore, this study found out that combining established protein biomarkers such as AFPto miRNAs did not improve the diagnostic performance [56].

Moreover, another research concluded that the panel of the three miRNAs (Let-7a, miR-221, and miR-222) showed much better diagnostic performance than a single miRNA and better than AFP alone (AUC for the panel = 0.961, AUC for each miRNA separately; AUC for let-7a = 0.801, AUC for miR-221 = 0.786), AUC for miR-222 = 0.758 and AUC for AFP alone= 0.766)[61].

Limitations of the current study include sample size being small and the need to follow up the patients to determine the role of our marker as prognostic marker. In addition, the technical reliability of miRNAs analysis in HCC requires further evaluation. Moreover, in our study, one particular miRNA was evaluated, however, due to heterogeneity of HCC as mentioned; several biomarkers have to be co-studied with well designated studies. Furthermore, the aberrant expression of miR-148a is not only found in HCC, but it is also detected in other cancers, thus, in clinical practice, it is of extreme importance to use a combination of biomarkers to ensure appropriate diagnosis.

Conclusion

In our current study, miR-148a was down regulated in HCC and it has a high sensitivity and specificity that makes it a potential biomarker over AFP in detection of HCC or to supplement the limitations of AFP. Furthermore, miR-148a was correlated to HCC aggressiveness and progress. Down regulation of miR-148a was significantly related to the poor adverse features of HCC such as tumor size, primary tumor, tumor stage and high AFP level that makes it a prospective marker capable of predicting the prognosis of HCC patients. In this regard, it is highly recommended to follow up the patients and compare miR-148a levels to patient outcomes e.g. survival and treatment response

Conflict of interest

There are no conflicts of interest to declare.

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