MICRORNA UTILITY FOR DIAGNOSING ISCHEMIC STROKE IN EGYPTIAN PATIENTS.

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Worldwide, stroke is the second most common cause of mortality (Kumar *et al*., 2021). Ischemic stroke represents about 87% of strokes incidents (Guo *et al*., 2021). In Egypt, the incidence rate of stroke per year ranges between 137,000 and 250,000 as well as being one of the main reasons of mortality in our country (Abd-Allah *et al*., 2018). It has a high prevalence of stroke, accounting for 6.4% of all deaths. Most strokes are preventable and using predictive biomarkers for risk assessment and stratification of the population can help reduce the number of future strokes. Currently used neuroimaging techniques like CT and MRI are expensive and ineffective for early detection and not widely available in low-income countries. There is a need for a reliable, low-cost, and widely accessible diagnostic tool, prognostic marker and treatment strategy to improve stroke care and prevention (Aref *et al*., 2021).

Exploring the molecular mechanisms of stroke can develop better prevention strategies. Since they are protected from RNases, circulating miRNAs have been proven to have a high level of stability even under extreme conditions like boiling, long storage, and many freeze–thaw cycles, with extremely low or high pH (Elfert *et al*., 2022). Earlier studies showed a correlation between different miRNAs and stroke. The three brain-specific miRNAs (miR-9, miR-15a and miR-16) are found to be involved in the process of brain ischemic injury and can induce endoplasmic reticulum (ER) stress, which triggers the unfolded protein response (UPR) pathways to keep cellular homeostasis and protect cell survival (Mens *et al*., 2021).

As determined by microarray results, microRNA-9 (miR-9) is one of the most notable aberrantly expressed microRNAs (miRNAs) in IS; upregulated in serum; and even in CSF of IS cases (Sørensen *et al*., 2017). Moreover, miR-15a and -16 were studied before in serum of IS patients (Wu *et al*., 2015). The ele-

vated serum expressions of both are correlated with IS, and it is deducted that the overexpression of miR-15 and miR-16 may block vascular endothelial growth factor (VEGF)-mediated angiogenesis (Sun *et al*., 2018). Meanwhile, miR-16 is highly conserved and ubiquitously expressed across all tissues and cell types, with well-characterized roles in the negative regulation of cell proliferation and angiogenesis. It was reported to be upregulated in IS, as upregulated in small artery stroke (Vasudeva and Munshi, 2020).

The current study aimed to investigate- for the first time- the miRNA-IS panel (miR-9, miR-15a, and miR-16) in sera of Egyptian patients, and its use as potential non-invasive molecular biomarker for IS diagnosis.

SUBJECTS AND METHODS

Experimental design and selection of subjects

Subjects of this case-control study were 65 IS patients admitted to Kasr El-Ayni hospital- the emergency department- Cairo University, Egypt, with stroke suggestive symptoms and their diagnosis was confirmed by contrast MRI performed by 3.0 Tesla whole body imaging system with augmented clinical decision, while there were 30 apparently healthy subjects- recruited from blood bank of the same hospital- to represent the control group. Exclusion criteria for patients' group included: peripheral vascular disease, intracranial hemorrhage, neuropsychological disorders, myocardial infarction, tumours, blood disorders, immune diseases, infectious diseases, and psychiatric illness including schizophrenia and depression. The protocol of this study was in concordance with the ethical committee of Kasr El-Ayni hospital and all recruited subjects or the corresponding persons of charge for IS patients agreed with informed consents. The gold standard test for miRNA quantification is RT-qPCR, as has been demonstrated by previous studies (Bhattacharya *et al*., 2020). The present study was carried out at the Biochemistry and Molecular Biology Department, Faculty of Medicine- Cairo University, Egypt.

Gathering samples and extraction of total RNA

Five mL venous blood samples were collected by professional technicians from all subjects, blood samples were aliquoted. For miRNA assessment, serum separator tubes were used. Blood tubes had been standed for 15 minutes to clot, centrifuged for ten minutes (min) at 4x 10^3 round per min, then serum was kept at -80ºC until analyses. Hemolyzed serum samples were excluded. Total RNA was extracted from serum samples by using miRNeasy Mini Kit according to manufacturer instructions (Qiagen, Valencia, CA, USA). RNA samples were directed for RNA quantitation and purity assessment using the NanoDrop® (ND)-1000 spectrophotometer (NanoDrop Technologies, Inc. Wilmington, USA).

Quantitative Real Time- PCR (qRT-PCR) Assay

Two-step real time PCR (RT-PCR) was performed to convert miRNA to cDNA in a final volume of 20 uL RT reactions using miScript II RT Kit (Qiagen). Amplification and quantification of cDNAs were done by qRT-PCR system using miScript SYBR Green PCR kit according to manufacturer instructions (Qiagen). Due to the lack of an endogenous reference housekeeping gene of miRNAs in the serum, small nucleolar RNA C/D box 68 (SNORD 68) appeared to be a consistent normalization control that could be used in miRNA-PCR analysis, based on our preceding experience (Abd-Elkader *et al*., 2020).

The cycles for amplification were 40 cycles starting once with 15 minutes at 95ºC as initial activation, then 3 stages of reactions: DNA denaturation for 15 seconds at 94ºC, annealing for 30 seconds at 55ºC and extension for 30 seconds at 70ºC. After completion of qRT-PCR cycles, melting curves were analyzed to confirm and validate the specific expression of miRNAs in target.

Calculation

The fold change in miR-9, -15a, and -16 expression was calculated by the equation $2^{-\Delta\Delta Ct}$. The mathematical relationship between *Ct*, Δ*Ct*, ΔΔ*Ct* and Fold change (FC) or relative quantitation (Rq) is:

$$\Delta Ct_{(IS\ patients)} = Ct_{(miRNA)} - Ct_{(Endogenous\ ontrol)}$$

$$\Delta Ct_{(control)} = Ct_{(miRNA)} - Ct_{(Endogenous\ control)}$$

$$\Delta\Delta Ct = \Delta Ct_{(IS\ patients)} - \Delta Ct_{(Control)}$$

$$FC\ (Rq) = 2^{-\Delta\Delta Ct}$$

If the Fold Change is positive, it means that the miR-RNA is upregulated; if the Fold Change is negative, it means it is downregulated. Control value was assumed to equal 1, as $-\Delta\Delta Ct$ for control subjects equals zero and 2^0 equals one (ivak and Schmittgen, 2001).

Statistical analyses

Statistical analysis was carried out using statistical package of social science (SPSS) *version* 17. The data were represented as mean ± standard deviation (M±SD), numbers, and percentages. The student's t test, Man-Whitney test were used to compare the differences between two groups, and multiple comparisons between more than two groups were determined by the one-way analysis of variance (ANOVA). Diagnostic accuracy, cutoff values, sensitivity, and specificity for each miRNA were evaluated by the receiver operator characteristic (ROC) curve and the area under the curve (AUC). While Pearson's and Spearman's correlation tests were used for the correlation between the expression level of miRNAs and the biochemical investigations of IS patients, and between the expression level of miRNAs and the associated clinicopathological features of the diseased

group. *P*-value<0.05 was considered as a cutoff value for significance (Kothari, 2004).

RESULTS AND DISCUSSION

Demographic features of the studied groups

This prospective case-control study was carried out on ninety-five subjects, classified into two groups: Group I: 65 IS group, and Group II: 30 apparently healthy control group. There was no significant difference in age and gender between the two groups as shown in Table (1).

Clinicopathological features of the IS patients

Out of our 65 IS patients, 10 (15.38%) of our patients had ischemic heart disease (IHD) and 6 (9.24 %) atrial fibrillations (AF). 15 (23.1%) of them had atherosclerosis and 10 (15.4%) had stenosis. 36 (55.4%) had right side- injury while 29 (44.6%) had left side- injury in their brains. Only 12 (18.5%) of the IS patients were diabetic, 38 (58.5%) were hypertensive. Regarding severity, the National Institutes of Health Stroke Scale (NIHSS) is the most commonly used scoring system for stroke severity worldwide (Yao *et al*., 2016). According to NIHSS scoring, 18 (27.69%) of the IS group were considered severe and very severe, as illustrated in Table (2).

Haematological and biochemical parameters of IS and control groups

Clinical laboratory investigations for all subjects are illustrated in Table (3). INR and urea show a statistically significant difference between the two groups (*P*-values: <0.01 and <0.05), respectively.

Expression levels of miR-9, -15a and -16 in both groups

The expression level of the investigated miRNA panel is illustrated in Table (4). There is significant increase in serum level of each investigated miRNA in the IS group than the control group, which is consistent with other studies (Zhou *et al*., 2020 and Abdelaleem *et al*., 2022).

Different studies have provided conflicting results regarding the role of miR-9 in stroke and its use as a diagnostic and prognostic biomarker. While some studies have found increased levels of miR-9 in IS patients and a correlation with infarct volume and NIHSS score, others have found no differences in miR-9 expression between IS patients and healthy controls and a third party found it to be downregulated in IS group than control (Liu *et al*., 2015). Previous study by Liu *et al*. (2010) in which they explored stroke-specific miRNAs and found elevated levels of nervous system-specific miR-9 in serum after a stroke, which was also correlated with neural death and glial reaction. This suggests that miR-9 could be evaluated as a potential biomarker for human neural damage (Ogata *et al*., 2015).

Another explanation suggested that the regulation of ER stress through the modulation of endoplasmic reticulum metallopeptidase 1 (ERMP1) levels- which is a protein coding gene- and its downstream target miR-9. This was in line with the increasing evidence that ER stress is a significant signaling event triggered by ischemia (Xin *et al*., 2014). Later, a study by Grandi *et al*. (2016) demonstrated the involvement of ERMP1 protein in ER stress mediated by the unfolded protein response (UPR) in IS. These findings were confirmed by TargetScan analysis made by Chi *et al.* (2019) which predicted miR-9 as a target miRNA of ERMP1. The contradictive studies reported that miR-9 could rescue brain damage, cell viability reduction, and cell apoptosis in ischemic injury, which is associated with ERMP1-mediated ER stress (Wang *et al*., 2021). The upregulation of miR-9 has been shown to have a protective effect on the cellular response to ischemia and associated excitotoxicity by negatively regulating complex signal transduction networks. However, the relationship between miR-9, ER stress, and ischemic injury needs further investigation.

Wei *et al*. (2016) found that miR-9 regulates Bcl2l11 protein levels in the brain in a model of middle cerebral artery occlusion (MCAO); where Bcl-2-like 11 is a proapoptotic member of the B-cell CLL/lymphoma 2 family of proteins 15 and has emerged as a key modulator of apoptosis, meaning that suppressing miR-9 gene expression dramatically decreases neuronal apoptosis and improves post-stroke recovery in mice. The study found that the downregulation of miR-9 by the application of a miR-9 inhibitor led to an increase in Bcl2l11 protein levels, confirming the critical role of miR-9 in neuronal apoptosis during ischemic brain injury (Eyileten *et al*., 2018).

Liu *et al*. (2015) found conflicting results to ours, regarding the diagnostic value of miR-9 for ischemic stroke. The study showed no differences in miR-9 serum expression between ischemic stroke patients and healthy controls within the first 24 hours after the event. However, the study did find a negative correlation between miR-9 levels and infarct lesion volume, but not with the NIHSS score. These findings suggest that miR-9 may not have diagnostic value for ischemic stroke within the first 24 hours after the event but may have prognostic value based on its correlation with infarct lesion volume.

Sun *et al*. (2018) confirmed our results in this context, as circulating miR-15a and miR-16 were found to be enriched in critical limb ischaemia (CLI) patients. It was hypothesized by Leung *et al*. (2014) that miR-16 would be elevated in response to the ischemic environment after stroke. They explained that the increase in circulating miR-16 may imply an induction of apoptosis in response to IS, and cell-free miR-16 may be liberated from cells undergoing apoptosis. Eyileten *et al*. (2018) were in line with our results too, where they mentioned miR-15a and miR-16 among 4 common miRNAs observed in

blood coagulation and platelet activation. In contrary to our results, miR-16 has been extensively studied for its potential role in ischemic reperfusion injury and has been shown to have a negative impact on the regulation of anti-apoptotic Bcl-2 (which is apoptosis regulator gene, encodes an integral outer mitochondrial membrane protein that blocks the apoptotic death of some cells such as lymphocytes) and Bcl-w (which is an anti-apoptotic protein too), promoting brain injury in ischemic conditions. Yang *et al.* (2017) noticed that the concentration of plasma miR-16 had been linked to patient's survival, with higher levels being indicative of better outcomes and lower levels being associated with poor clinical outcomes.

Expressed miRNAs levels in association with clinicopathological features among IS patients

Change in expression level of each of the investigated miRNAs in accompanied clinicopathological feature of the IS patients' group is illustrated in Table (5). All investigated miRNAs were non-significant with gender. Certain clinicopathological features, such as DM, HTN and severity of the disease had an impact on the serum expression level of miR-9. However, miR-9 showed response to the hypertension in the IS patients ($P< 0.05$) and DM showed a significant difference in diabetic IS patients ($P< 0.01$) than the non-diabetic IS ones. mir-9 was the only tested miRNA which showed a significant difference in its expression level due to the severity of the disease ($P< 0.01$). These findings are in line with previous studies (Wang *et al*., 2021 and Abdelaleem *et al*., 2022), as diabetes is considered as a major risk factor for stroke, especially for ischemic stroke, and managing glucose levels and other related risk factors can help prevent strokes.

Effective management of blood pressure and dyslipidemia, along with lifestyle changes or medication, can greatly reduce the risk of stroke (Olesen *et al*., 2019). This indicates that there may be a correlation between the level of miR-9 and the severity of stroke as measured by NIHSS scores. These results suggest that miR-9 levels may be useful for diagnosing IS patients. However, further studies with larger sample sizes and more robust methods are needed to confirm these findings and determine the potential use of miR-9 as a biomarker for stroke severity. Additionally, considering other confounding factors and co-existing diseases would help provide a clearer understanding of the relationship between miR-9 levels and stroke severity.

On the other hand, miR-15a showed significant difference in expression level with smoking ($P< 0.01$), and ($P< 0.05$) with DM presence in IS patients. These results are consistent with previous study by Hu *et al*. (2019). Our present study found that the level of miR-15a was higher in smoker patients and diabetic IS patients compared to non-smokers and non-diabetic IS patients, respectively.

Meanwhile, gender, smoking, DM, HTN and even NIHSS have no impact on miR-16 expression level, this may be referred to the small sample size of the diseased group in our work. ANOVA test was applied to examine the response of the 3 tested miRNAs to the presence of AF, or IHD; only miR-15a had been responded significantly to the IHD condition than the normal ECG patients. Atherosclerosis and stenosis had no effect on the expression level of any member of our panel. By applying independent t-test, we found that brain injury in right or left side had no impact on the expression level of any tested miRNA in the present work.

Correlation between miRNA panel and other parameters

Pearson's correlations showed a direct significant correlation was revealed between miR-9 and urea (P<0.05). On the other hand, miR-15a showed a direct significant correlation with creatinine (P<0.05). Meanwhile miR-16 has a direct significant correlation with total cholesterol (P<0.05), LDL (P<0.01), and an inverse correlation with creatinine (P<0.05), which agree with previous studies by Eyileten *et al*. (2018) and Deng *et al*. (2019). In our study, miR-16 had no correlation with HDL in contrast to another study made by Wu *et al*. (2015). Additionally, there were inter-correlation between miR-15a and miR-9 (r= 0.390, P<0.001), and between miR-15a and miR-16 (r= 0.319, P<0.01) as shown in Table (6).

Spearman's rho program was performed to examine correlations between each member of the examined miRNA-panel and clinicopathological features within the diseased group. miR-9 was correlated with DM (r=-0.538, P<0.01), and with HTN (r=-0.431, P<0.05). miR-15a revealed highly significant inverse correlation with smoking (r=-0.458, P<0.01), while miR-16 was correlated only with HTN (r=0.362, P<0.05). There was no correlation between miR-9 and NIHSS which agrees with Liu *et al*. (2015) and disagrees with Abdelaleem *et al*. (2022) who found that miR-9 was positively correlated with NIHSS. miR-15a showed no correlation with NIHSS too, which is in accordance with Xiang *et al*. (2017). miR-16 showed also no correlation with NIHSS in contrary to Leung *et al*. (2014) who reported a correlation of miR-16 with mild stroke, defined as NIHSS <5. These correlations are illustrated in Table (6) too.

Diagnostic performance of miRNA panel for IS

ROC curves were applied to establish the diagnostic potential for the investigated microRNAs to IS; miR-9 had 0.99 AUC at cutoff value equals 12.58 FC with 71.56% sensitivity and 99.0% specificity as in Fig. (1a) and Table (7). Regarding miR-15a, its AUC was 0.96 at cutoff value equals 5.123 FC with 82.11% sensitivity and 95.5% specificity as in Fig. (1b) and Table (7). Finally, the AUC value of miR-16 was 0.99, with 76.61% sensitivity and 98.8% specificity at cutoff value= 5.628 FC as in Fig. (1c) and Table (7). miR-9 was superior in specificity and miR-15a

was superior in sensitivity, concerning IS diagnosis in normal cohort.

Bejleri *et al.* (2021) reported that miR-16-5p has been shown to have both diagnostic and prognostic utility in IS patients. This highlights the potential of miR-16 as a biomarker for stroke diagnosis and prognosis. The extravagant ROC curve results in the present work may be referred to comparing patient's group with totally normal control group; real value of this panel may be achieved when people at risk for IS act as the control group.

CONCLUSION

This is a groundbreaking study that aims to evaluate the expression level of miRNA-panel specific to ischemic stroke. Our study revealed that this panel (miR-9, miR-15a, and miR-16) seems to be a potential molecular biomarker for the diagnosing ischemic stroke and hence its treatment. However, further validation and large-scale studies are needed to establish their clinical utility in IS.

SUMMARY

Stroke is a major public health concern in Egypt with a high overall prevalence rate and a significant impact on mortality. The identification of genetic and molecular factors involved in the development of ischemic stroke (IS) can lead to the identification of new diagnostic and therapeutic targets and the development of personalized medicine approaches for the prevention and treatment of this debilitating disease. MicroRNAs (miRNAs) could regulate cell proliferation and apoptosis and accumulating published data have showed a correlation of miRNA with stroke pathogenesis. This work aimed to evaluate the expression level of three brain-specific miRNAs in serum: miR-9, miR-15a and miR-16 as a panel, among newly diagnosed IS-Egyptian patients compared to apparently healthy control subjects and to examine its use as diagnostic molecular biomarker for this silent disease. Serum expression level of the tested miRNA panel was detected in all subjects using quantitative real-time polymerase chain reaction (qRT-PCR). The expression levels of the investigated miR-9, miR-15a, and miR-16 had shown to be significantly (P<0.001) increased by 12.85, 5.15, and 5.89-fold change (FC), respectively in IS sera than the control group. Diabetes mellites (DM), hypertension, and NIHSS scoring of the stroke was found to have significant implication on the expression level of miR-9. DM and smoking had significant implications on the expression level of miR-15a. miR-16a had significant correlation to total cholesterol and LDL. Diagnostic potentiality of this panel for IS was tested by performing receiver operating characteristic (ROC) curve analysis, which revealed specificity for the newly tested miRNAs ranging from 95.5% to 99%, with a sensitivity between 71.56% and 82.11%. Finally, we concluded that miR-9, miR-15a, and miR-16 may play a crucial role in ischemic injury, suggesting their promising role as molecular biomarkers for early diagnosis of IS. Additional studies are recommended- on larger IS cohort compared to risky

subjects- to confirm the role of this panel in this context.

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Table (1): Demographic characteristics and medical history of IS and control groups.

Parameters		**IS group (n=65)**		**Control group (n=30)**		***P*-value**
Age (years)		62.40±2.36		60.33±5.72		N.S.
		Count	%	Count	%	
Gender	Female	20	30.7%	11	36.6%	N.S.
	Male	45	69.3%	19	63.4%	
Smoking:	Yes	42	(64.6%)			<0.01
	No	23	(35.4)			
DM:	Yes	12	(18.5%)			<0.01
	No	53	(81.5%)			
HTN:	Yes	38	(58.5%)			<0.05
	No	27	(41.5%)			

Age is represented as M±SD; gender is represented as number and percentage (%); DM: diabetes mellitus; HTN: hypertension.

Table (2): Clinicopathological features associated with IS patients.

Clinicopathological feature	Frequency (n=65)
ECG:	
AF	6 (9.24%)
IHD	10 (15.38%)
Normal	49 (75.38%)
Carotid Doppler:	
Normal	40 (61.5%)
Abnormal (Atherosclerosis)	15 (23.1%)
Abnormal (Stenosis)	10 (15.4%)
CT Brain:	
Right site- brain injury	36 (55.4%)
Left site- brain injury	29 (44.6%)
NIHSS:	
Mild & Moderate	47 (72.31%)
Severe & Very severe	18 (27.69%)

Data are represented as number and percentage (%); ECG: electrocardiogram; AF: atrial fibrillations; IHD: ischemic heart disease; CT: computerized tomogrphy; NIHSS: The National Institutes of Health Stroke Scale.

Table (3): Clinical analyses for IS and control groups.

Laboratory Test		IS group (n=65)	Control group (n=30)	*P*-value
Hgb	(gm/dL)	13.00±0.23	13.17±0.22	N.S.
INR	(ratio)	1.01±0.06	0.99±0.08	<0.01
Total chol.	(mg/dL)	166.01±5.87	173.35±6.85	N.S.
TG	(mg/dL)	144.03±8.28	152.20±9.43	N.S.
LDL	(mg/dL)	107.86±4.91	108.73±4.88	N.S.
HDL	(mg/dL)	32.70±0.96	34.46±0.97	N.S.
Urea	(mg/dL)	52.83±65.72	29.02±5.31	<0.05
Creatinine	(mg/dL)	1.10±0.55	1.01±0.09	N.S.

All data are represented as M±SD; Hgb: haemoglobin; INR: international normalized ratio; chol.: cholesterol; TG: triglycerides; LDL: low density lipoprotein; HDL: high density lipoprotein; N.S.: non-significant; *P*-value <0.05: significant; *P*-value <0.01= highly significant.

Table (4): Expression level of the newly-tested miRNAs in IS and control groups.

Biomarkers	**IS group (n=65)**	**Control group (n=30)**	***P*-value**
miR-9 (FC)	12.85±0.287	1.010±0.09	<0.001
miR-15a (FC)	5.15±0.227	1.009±0.05	<0.001
miR-16 (FC)	5.89±0.234	1.030±0.02	<0.001

All data are represented as M±SD; FC: Fold Change; *P*-value <0.001= very highly significant.

Table (5): Impact of some clinicopathological on the expression level of the tested-miRNAs among IS patients.

Parameters		**miR-9**	***P*-value**	**miR-15a**	***P*-value**	**miR-16**	***P*-value**
Gender	Female	13.53±0.53	N.S.	5.42±0.44	N.S.	5.48±0.39	N.S.
	Male	12.54±0.33		5.02±0.26		6.00±0.26	
Smoking	Yes	12.94±0.40	N.S.	4.38±0.21	<0.01	5.81±0.34	N.S.
	No	12.74±0.40		5.93±0.35		5.87±0.27	
DM	Yes	11.10±0.24	<0.01	4.84±0.24	<0.05	5.34±0.27	N.S.
	No	13.24±0.32		5.21±0.27		5.96±0.26	
HTN	Yes	12.46±0.31	<0.05	5.45±0.32	N.S.	6.26±0.27	N.S.
	No	13.38±0.51		4.70±0.28		5.26±0.34	
NIHSS	Mild &Moderate	17.24±1.17	<0.01	7.98±1.29	N.S.	7.08±1.19	N.S.
	Severe & Very severe	15.61±1.43		7.35±1.29		6.79±1.40	

N.S.= non-significant; *P*-value<0.05= significant; *P*-value <0.01= highly significant.

Table (6): Correlation between expression level of the miRNA-panel members and associated parameters.

Parameter	miR-9		miR-15a		miR-16	
	r	***P***	***r***	***P***	***r***	***P***
Hgb	0.177	N.S.	0.070	N.S.	0.186	N.S.
INR	0.153	N.S.	0.202	N.S.	-0.148	N.S.
Total chol.	-0.057	N.S.	-0.066	N.S.	0.298	**<0.05**
TG	0.101	N.S.	-0.117	N.S.	-0.142	N.S.
LDL	-0.077	N.S.	0.026	N.S.	0.348	**<0.01**
HDL	0.151	N.S.	-0.142	N.S.	0.283	N.S.
Urea	0.273	**<0.05**	0.207	N.S.	0.080	N.S.
Creatinine	0.148	N.S.	0.256	**<0.05**	-0.258	**<0.05**
miR-9	1	------	0.390	<0.001	0.136	N.S.
miR-15a	0.390	<0.001	1	------	0.319	<0.01
miR-16	0.136	N.S.	0.319	<0.01	1	------
Smoking	------	------	-0.458	<0.01	------	------
DM	-0.538	<0.01	------	------	------	------
HTN	-0.431	<0.05	------	------	0.362	<0.05
NIHSS	------	------	------	------	------	------

The correlation is significant at the 0.05 level. N.S.= non-significant; *P* <0.05= significant; Hgb: haemoglobin; chol.: cholesterol.

Table (7): Diagnostic performance of the tested miRNA panel- members in the IS group.

Biomarkers	AUC	Cut-off value	Sensitivity	Specificity	95% CI
miR-9 (FC)	0.99	12.58	71.56%	99%	1.000-1.000
miR-15a (FC)	0.96	5.123	82.11%	95.5%	1.000-1.000
miR-16 (FC)	0.99	5.628	76.61%	98.8%	0.751-0.975

FC: fold change; AUC: area under curve; C.I.: confidence interval.

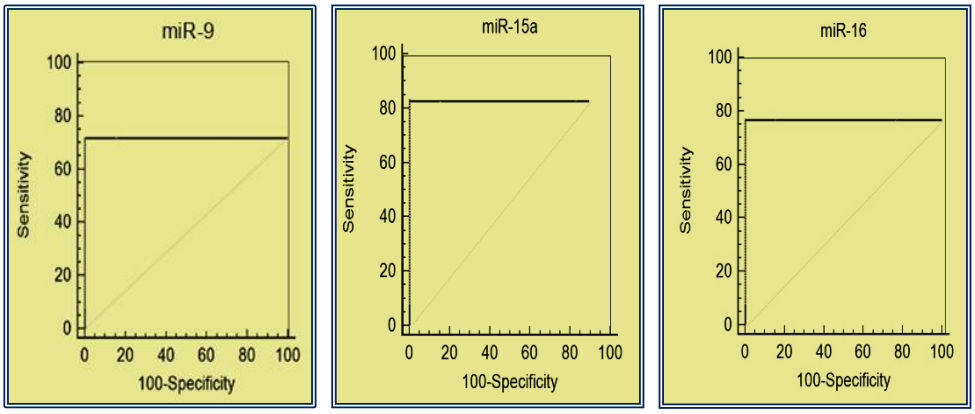

Fig. (1a): ROC curve for miR-9 biomarker in IS group.

Fig. (1b): ROC curve for miR-15a biomarker in IS group.

Fig. (1c): ROC curve for miR-16 biomarker in IS group.

Fig. (1): ROC curve analyses for miR-9, miR-15a and miR-16 biomarkers for diagnosing IS.