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RESEARCH

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Early peripheral blood gene expression (Cyp4A11 and Cyp2E1) in cases of brain ischemia in addict cases admitted to Benha university hospital

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Abstract

Background Stroke is a major neurological disorder often exacerbated by substance abuse, including tramadol and cannabis. Understanding the molecular mechanisms underlying stroke pathogenesis in drug users can improve diagnosis and treatment. This study explores the roles of CYP4A11, CYP2E1, miR-27b, and miR-214-3p in the pathogenesis of stroke and their potential as diagnostic markers in individuals using tramadol and cannabis.

Results Our findings indicate that CYP4A11 and CYP2E1 are significantly upregulated in the brain tissues of stroke patients who use tramadol and cannabis. Additionally, miR-27b and miR-214-3p levels were markedly altered, suggesting their involvement in stroke pathogenesis. The combined analysis of these biomarkers provided a robust diagnostic model with high sensitivity and specificity for identifying stroke in the context of drug addiction.

Conclusions CYP4A11, CYP2E1, miR-27b, and miR-214-3p play critical roles in the pathogenesis of stroke in tramadol and cannabis users. These biomarkers hold promise as diagnostic tools, offering potential for early detection and personalized treatment strategies for stroke in drug-addicted populations. Further research is warranted to validate these findings and explore their therapeutic implications.

Keywords Stroke, Drud abuse, CYP4A11, CYP2E1, miR-214-3p, miR-27b

Background

Substance abuse has been implicated in the aetiology of both ischemic and haemorrhagic strokes. A multitude of underlying mechanisms have been delineated, including the induction of arrhythmias and cardioembolic events, tissue hypoxia, direct vascular toxicity, inducement of vascular spasm, and disruptions in the thrombotic pathways contributing to the onset of ischemic strokes. Conversely, haemorrhagic strokes have been associated with the emergent presentation of acute hypertension, the development and rupture of aneurysms, and pathological changes reminiscent of angiitis [1].

In the Egyptian context, irrespective of the sequence of consumption, cannabis and tramadol

The actual work was done at the Neurology and Psychiatry Department, Benha University Hospital, and the Medical Biochemistry and Molecular Biology Department, Faculty of Medicine, Benha University.

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are predominant among the substances utilized, as reported by the Fund for Drug Control and Treatment of Addiction (FDCTA). Ischemic strokes may arise from transient vasospasms attributed to synthetic cannabinoids, as noted by Abdel-Salam and colleagues (2017). Furthermore, there is an associated risk of precipitated aneurysmal rupture linked to tramadol abuse [2].

Correspondingly, drugs abusers have a 6.5 times increased risk of both hemorrhagic and ischemic stroke. In addition, while current treatment for ischemic stroke includes thrombolysis and mechanical thrombectomy, drug users may be at increased risk of complications such as iatrogenic vasospasm and hemorrhagic reperfusion injury following these therapies as a consequence of the damaging physiological changes on blood vessels provoked by substances of abuse [3, 4]. Yet, no difference in stroke management guidelines exists for ischemic stroke in the setting of drug abuse.

CYP4A11, an enzyme within the Cytochrome P450 superfamily, plays a pivotal role in synthesizing 20-hydroxyeicosatetraenoic acid (20-HETE), a compound with significant influence on vascular functionalities and the regulation of arterial tension [5]. The alteration of 20-HETE production causes decreased activation of peroxisome proliferator-activated receptor alpha, increased expression of proinflammatory cytokines such as TNF and IL1, and results in vascular inflammation and endothelial dysfunction [6].

Oxidative stress arises from a dysregulation between the generation of free radicals and the counteracting antioxidant mechanisms, serving as a pivotal pathological process in secondary brain damage subsequent to cerebral ischemia [7]. Elevated expression of CYP2E1 has been associated with hypoxic conditions and inflammatory responses. Research suggests that CYP2E1 plays a crucial role in mediating oxidative stress and inflammation subsequent to ischemic events [8].

MicroRNA-214-3p could be instrumental in neuroprotective mechanisms post-ischemia through its interaction with Phosphatase and Tensin Homolog (PTEN) deleted on chromosome 10. Enhanced expression of PTEN within the central ischemic zone may lead to augmented cellular demise and intensify the severity of ischemic damage [9].

Research indicates that AMP-activated protein kinase (AMPK) functions as a sensor for cellular energy levels and may mitigate cerebral ischemic damage by activating catabolic pathways while concurrently inhibiting ATP-consuming processes [10]. The impact of miR-27b on neurogenesis following ischemic stroke remains incompletely elucidated. While limited research has referenced the direct influence of miR-27b on AMPKα2, this is identified as the principal active AMPK isoform within the ischemic cerebral context [11].

This study explores the roles of CYP4A11, CYP2E1, miR-27b, and miR-214-3p in the pathogenesis of stroke and their potential as diagnostic markers in individuals using tramadol and cannabis.

Methods

Study design and population This case–control investigation was executed within the Neurology and Psychiatry Department at Benha University Hospital, alongside the Medical Biochemistry and Molecular Biology Departments at the Faculty of Medicine, Benha University. One hundred forty-seven participants were recruited from the Neurology and Psychiatry Department between June 2023 and June 2024, comprising 87 addict patients (with age 47.54 ± 9.36 years) presenting with stroke and 87 controls (with age 44.84 ± 11.27 years), the control group was sub divided into 37 healthy controls to analyze the relative changes in gene expression from real-time quantitative PCR experiments according to [12] and 50 patients presenting with stroke with no history of drug addiction in order to clarify the additive effect of addiction in stroke pathogenesis. The addict patients were diagnosed to have a dependence syndrome of cannabinoids and tramadol according to DSM 5 criteria for dependence syndrome as they use larger amounts over a longer period than was intended. There is a persistent desire and unsuccessful efforts to cut down or control cannabis and tramadol use. Other substances infrequently used by patients include cocaine, amphetamine and clonazepam.

Ethical Considerations The study was conducted in accordance with the guidelines of the Helsinki Declaration. Approval for this study was obtained from the Research Ethics Committee. All participants provided informed consent before blood sample collection, adhering to ethical standards for human research. For patients unable to give consent, written consents were taken from their relatives. The consent included information about the aim of the work, study design, site and time of the study, subjects involved, tools used, and confidentiality.

Participants The study group included 87 patients presenting with stroke, all above 18 years old, with a history of drug addiction (tramadol, cannabis, or other substances). We excluded patients presenting with stroke related to head trauma as cases of brain infarction resulting from cerebral vasospasm complicating traumatic subarachnoid hemorrhage or associated with hypertension, diabetes mellitus (DM), or heart diseases (confirmed by history & investigations as ECG & ECHO).

All patients were subjected to the following Medical history Taken from the patient if conscious, or from the patient's relatives if unconscious. This included the

duration of addiction and a history of other stroke risk factors (hypertension, diabetes, heart diseases). *General and neurological examination* Upon initial evaluation, comprehensive diagnostic imaging was performed for all patients, encompassing both brain computed tomography (by Toshiba Alixon 2013 CT) and magnetic resonance imaging (MRI and MRA by Simens, 1.5 T, Germany MRI). Laboratory investigations included liver function tests (AST, ALT, albumin, bilirubin), kidney function tests (urea, creatinine, uric acid), plasma glucose, drug screening, and genetic analysis. *Drug screening test* Performed using the Multi-Drug Rapid Test Panel (Urine) according to the manufacturer's instructions (Med Net, GmbH, Germany, Number: 145034113). Patients were classified as tramadol, cannabis, and multi-drug abuse addicts (tramadol, cannabis, cocaine, morphine) based on history and drug screening tests, with the duration of addiction being recorded. *Genetic study* Included assessment of the expression of CYP4A11, CYP2E1 genes, miR-214-3p, and miR-27b. Two ml venous blood samples were collected in vacutainer tubes and stored at − 80 °C until handling. Total RNA was isolated using the RNeasy Mini Kit (QIAGEN, USA) according to the manufacturer's instructions. Gene expression levels of CYP4A11, CYP2E1, miR-214-3p, and miR-27b were determined using Real-Time Quantitative Reverse Transcription–Polymerase Chain Reaction (RT–qPCR). The Total RNA purification kit (Cat No. PP-210S, Jena Bioscience, Germany) was used to extract total RNA from blood samples. Reverse transcription (RT) of RNA into complementary DNA (cDNA) was performed on a Veriti™ Thermal Cycler (Applied Biosystems). The qRT-PCR for detecting CYP4A11, CYP2E1, miR-214-3p, and miR-27b was conducted using Maxima SYBR Green qPCR Master Mix (2X) (Thermo Scientific, USA) as per the manufacturer's instructions [13], with the GAPDH gene used as the endogenous reference gene (Table 1).

The PCR reaction mix, prepared in a total volume of 20 µL per well, included 10 µL Maxima SYBR Green qPCR Master Mix (2X), 1 µL Forward Primer, 1 µL Reverse Primer, 4 µL cDNA, and nuclease-free water up to 20 µL. The amplification process was done using a StepOne Real-Time Cycler developed by Applied Biosystems in Singapore. After an initial holding phase of 3 min at 95 °C, the samples underwent a series of 40 cycles of denaturation for 15 s at 95 °C, annealing at 58 °C, and extension for one minute at 60 °C. The $2-\Delta\Delta Ct$ method was used to calculate the relative expression of CD70 and CXCL10 mRNA [12].

Data Analysis Statistical Package for the Social Sciences (SPSS) version 28 was employed to conduct data analysis. The absolute frequencies of categorical variables were recorded and contrasted using the chi-square test and Fisher's exact test, as appropriate. To verify parametric test assumptions, the Kolmogorov–Smirnov test was implemented. With respect to the nature of the data, quantitative variables were described using their median and interquartile range or their means and standard deviations. Independent sample *t*-tests (for normally distributed data) and Mann–Whitney tests (for not normally distributed data) were implemented to evaluate quantitative data between two groups. The Spearman rank correlation coefficient was employed to evaluate the magnitude and orientation of the association between two variables. ROC curves facilitated the identification of optimal thresholds for specific quantitative measures in the diagnosis of certain health conditions. Binary logistic regression analysis was utilized to pinpoint independent predictors related to specific health issues. Statistical significance was established at a threshold of $P < 0.05$, with distinctions deemed highly significant at $P \leq 0.001$.

Results

We recruited 87 cases presented with cerebral infarction as a complication of drug addiction. The mean age was 47.54 ± 9.36 years, ranging from 25 to 65 years. There were 83 males and 4 females. Of the cases, 40 had a history of tramadol addiction (46%), 28 had a history of cannabis addiction (32.1%), and 19 cases (21.8%) had multiple drug addictions. The mean duration of addiction was 9.51 ± 3.36 years. Regarding the size of the infarction,

Table 1 The CYP4A11, CYP2E1 genes, miR-214-3p and miR-27b GAPDH primers

Gene	Forward primer	Reverse primer
CYP4A11	5′-CTCAACACAGCCACGCT TTC-3	5′-ACAAGT CGTGCAATGGGGAT-3′
CYp2E1	5′-GCCATCAAG GATAGGCAAGA-3′	5′-TCCAGAGTTGGCACT ACGACT-3′
miR-214-3p	F: 5′-GCACAGCAGGCACAGACA-3′	5′-CAGAGCAGGGTCAGCGGTA-3′
miR-27b	5′GGCAAGCGCACCGAAGA3′	5′AGTGCAGGGTCCGAGGTATT3′
GAPDH	5′-GCA CCA CAC CTT CTA CAA TG3′	5′- TGC TTG CTG ATC CAC ATC TG3

CYP: Cytochromes P450, miR: microRNA, GAPDH: Glyceraldehyde-3-Phosphate Dehydrogenase

24.1% of patients had small infarctions, 46% had moderate infarctions, and 29.9% had large infarctions.

Brain imaging showed the following distribution of infarction locations: 23% of cases had isolated temporal lobe infarction, 18.4% had basal ganglia infarction, 11.5% had dual temporal and frontal lobe infarction, 10.3% had dual temporal and parietal lobe infarction, 9.2% had brainstem infarction, 8% had occipital lobe infarction, 8% had thalamic lobe infarction, 6.9% had frontal lobe infarction, 2.3% had occipital lobe infarction, 1.1% had infarction in the frontal, temporal, and parietal lobes, and 1.1% had infarction in the thalamic and basal ganglia regions (Table 2).

There was a statistically significant difference between both groups regarding CYP4A11 and miR-214-3p, where both showed downgrading among the case group, and CYP2E1 and miR-27b, which showed upgrading among the case group (Table 3 and Figs. 1, 2, 3, and 4).

There was a statistically non-significant correlation between the size of the infarction and the expression

Table 2 Comparison between the studied groups regarding baseline data

	Case group *n* = 87 (%)	**Control group *n* = 87 (%)**	**χ^2**	***p***
Sex				
Female	4 (4.6%)	6 (6.9%)		
Male	83 (95.4%)	81 (93.1%)	Fisher	0.747
Age (year) [mean ± SD]	47.54 ± 9.36	44.84 ± 11.27	t (1.179)	0.087
Substance abuse				
Cannabis	28 (32.1%)			
Tramadol	40 (46%)	–		
Multiple substances	19 (21.8%)			
Duration of addiction [mean ± SD]	9.51 ± 3.36	–		
Size of infarct				
Small	21 (24.1%)			
Moderate	40 (46%)	–		
Large	26 (29.9%)			
MRI findings				
Temporal lobe	20 (23%)			
Basal ganglia	16 (18.4%)			
Frontal lobe	6 (6.9%)			
Occipital	2 (2.3%)			
Parietal	7 (8%)			
Temporal, parietal	9 (10.3%)	–		
Temporal, frontal	10 (11.5%)			
Thalamic	7 (8%)			
Brainstem	8 (9.2%)			
Frontal, temporal, parietal	1 (1.1%)			
Thalamic, basal ganglia	1 (1.1%)			

χ^2: Chi square test, t independent sample t test

Table 3 Comparison between the studied groups regarding CUP4A11, CYP2E1, miR 214 and miR 27b

	Case group *n* = 87 Median (IQR)	**Control group *n* = 87 Median (IQR)**	***Z***	***p***
CYP4A11	0.03 (0.02–0.06)	1.01 (0.51–2.61)	− 11.414	< 0.001**
CYP2E1	1.34 (0.78–2.69)	0.38 (0.12–1.52)	− 5.483	< 0.001**
miR-214-3p	0.03 (0.02–0.06)	0.76 (0.39–2.07)	− 11.427	< 0.001**
miR 27b	91.29 (42–114.75)	0.96 (0.61–1.13)	− 11.399	< 0.001**

Z Mann Whitney test, *IQR* Interquartile range, ** $p \leq 0.001$ is statistically highly significant

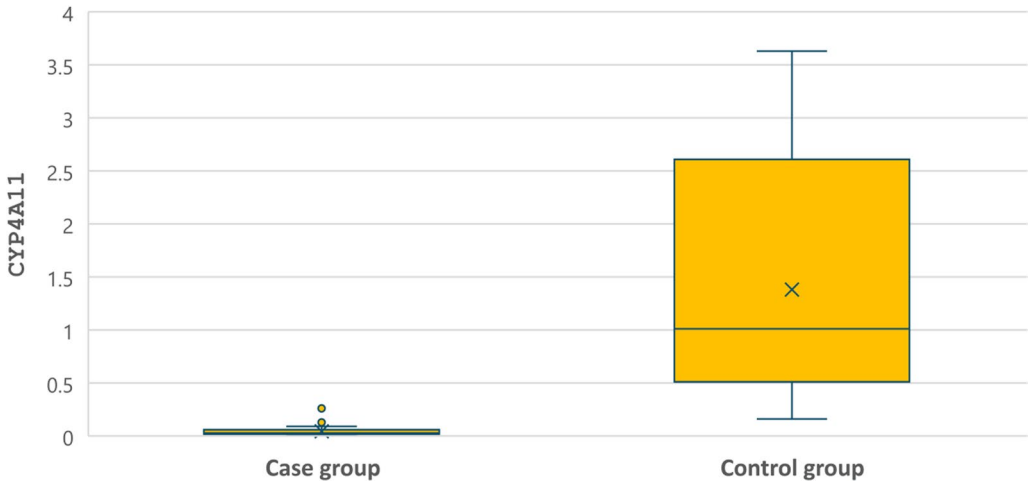

Fig. 1 Boxplot showing comparison between groups regarding CYP4A11

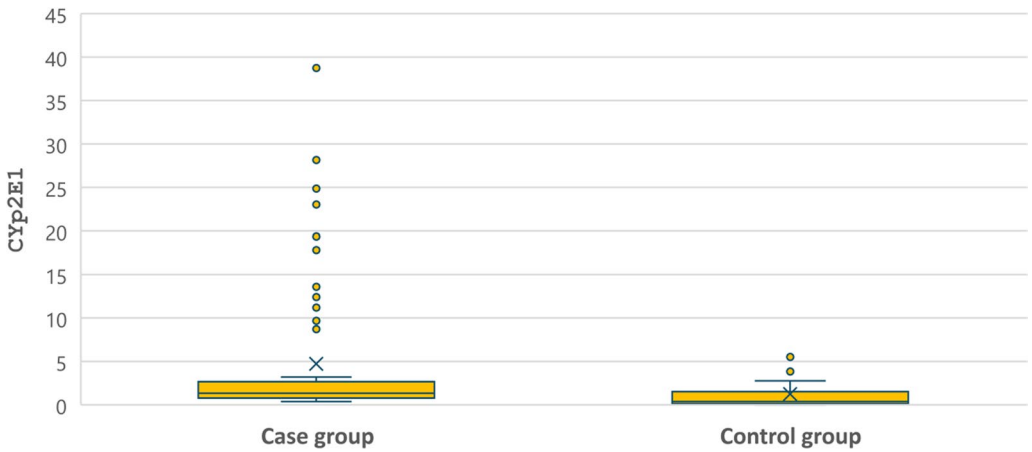

Fig. 2 Boxplot showing comparison between groups regarding CYp2E1

of CYP4A11, CYP2E1, miR-214-3p, or miR-27b. Similarly, there was a statistically non-significant correlation between the duration of addiction and the expression of CYP4A11, CYP2E1, miR-214-3p, or miR-27b (Table 4).

At a cutoff of ≤ 0.21, CYP4A11 can diagnose stroke with an area under the curve (AUC) of 1, 98.9% sensitivity, and 90.9% specificity. At a cutoff of ≥ 0.455, CYP2E1 can diagnose stroke with an AUC of 0.741, 90.8% sensitivity, and 55.2% specificity. At a cutoff of ≤ 0.215, miR-214-3p can diagnose stroke with an AUC of 1, 97.7% sensitivity, and 100% specificity. At a cutoff of ≥ 7.245, miR-27b can diagnose stroke with an AUC of 1, 100% sensitivity, and 97.7% specificity (Table 5, Figs. 5, 6, 7 and 8).

Discussion

Substance use disorder (SUD), identified as a risk factor for stroke, and poses an escalating global risk particularly among young adults. Prevention strategies are considered primary interventions (Schulte & Hser, 2014). In Egypt, the proliferation of substance abuse constitutes a significant public health challenge, with tramadol noted as one of the substances most frequently misused [14] (Fig. 9).

Numerous genes have been implicated in the pathogenesis of stroke, yet only a select few have been definitively proven to affect susceptibility. Furthermore, considerable research has been directed towards understanding the role of microRNAs (miRNAs) in

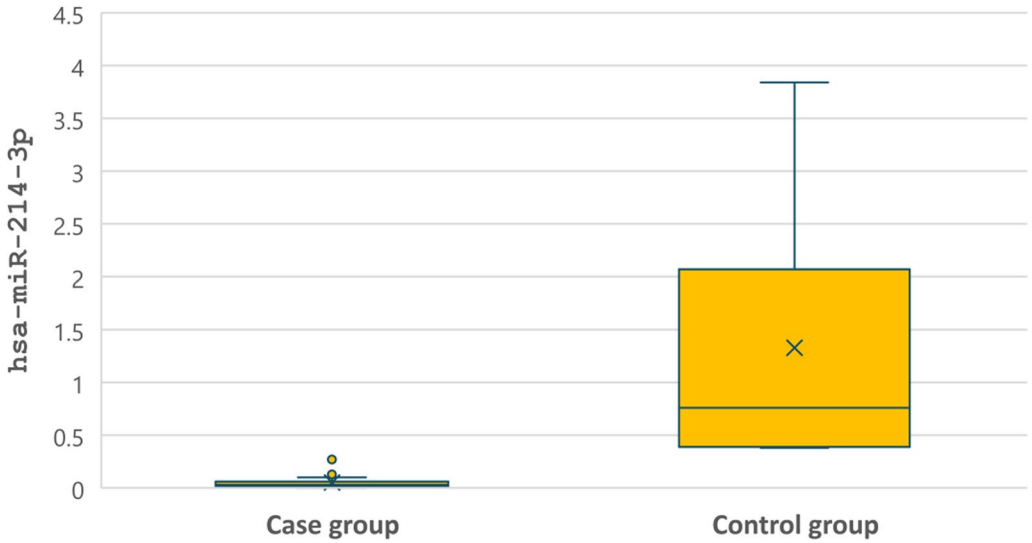

Fig. 3 Boxplot showing comparison between groups regarding hsa-miR-214-3p

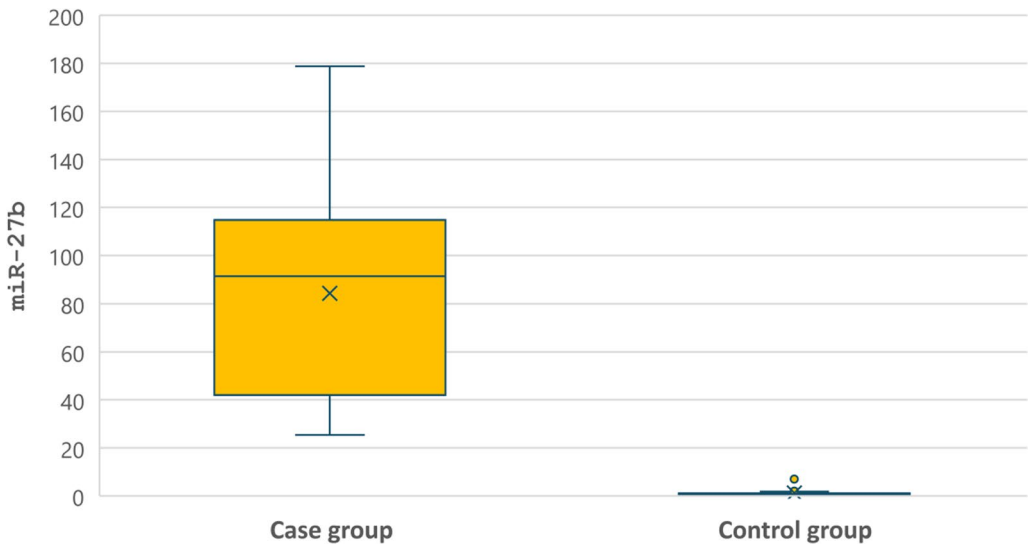

Fig. 4 Boxplot showing comparison between groups regarding hsa-miR-27b

Table 4 Correlation between size of infarction, duration of addiction and gene expression levels

	Size of infarction		Duration of addiction	
	r	*p*	*r*	*p*
CYP4A11	−0.015	0.89	0.075	0.491
CYp2E1	0.103	0.341	0.069	0.528
hsa-miR-214-3p	0.03	0.786	0.164	0.132
miR-27b	−0.048	0.661	0.011	0.919

r Spearman rank correlation coefficient

stroke, given their expression levels are correlated with the prognosis of this ailment [15].

The objective of this study was to clarify the potential significance of genetic factors (CYP4A11 and CYP2E1) in the diagnosis of stroke and to assess the association between miRNA (miR-214-3p and miR-27b) expression and stroke occurrence.

According to our results, tramadol was the most commonly abused drug. This finding was also reported by Rizk and colleagues [16], who mentioned that tramadol addiction was observed in 53.7% of their patients.

Table 5 Performance of CYP4A11, CYP2E1, miR 214 and miR-27b in diagnosis of stroke

	Cutoff	AUC	95% CI	Sensitivity (%)	Specificity (%)	*p*
CYP4A11	≤ 0.21	1	1–1	98.9	90.9	< 0.001**
CYp2E1	≥ 0.455	0.741	0.665–0.816	90.8	55.2	< 0.001**
hsa-miR-214-3p	≤ 0.215	1	1–1	97.7	100	< 0.001**
miR-27b	≥ 7.245	1	1–1	100	97.7	< 0.001**

AUC area under curve, $^{**}p \leq 0.001$ is statistically highly significant

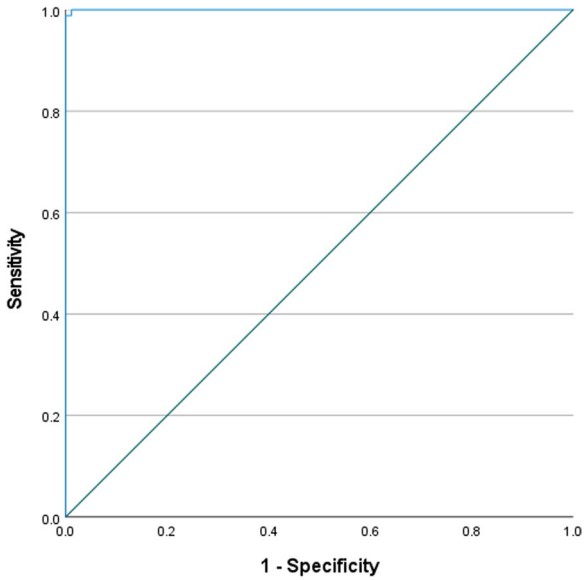

Fig. 5 ROC curve showing performance of CYP411A in diagnosis of stroke

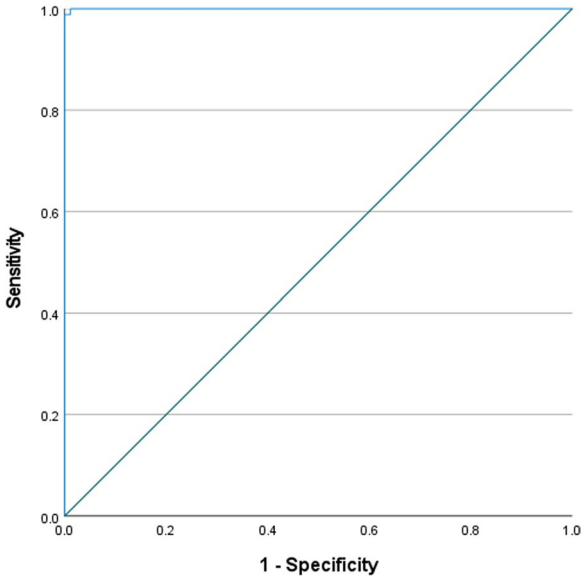

Fig. 7 ROC curve showing performance of hsa-miR-214-3p in diagnosis of stroke

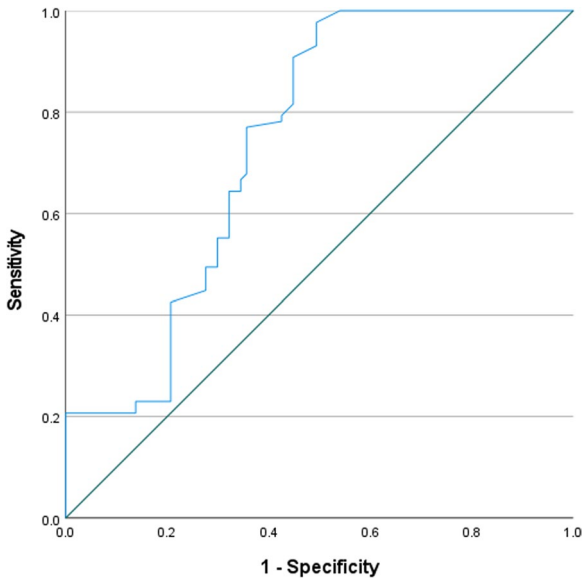

Fig. 6 ROC curve showing performance of CYP2E1 in diagnosis of stroke

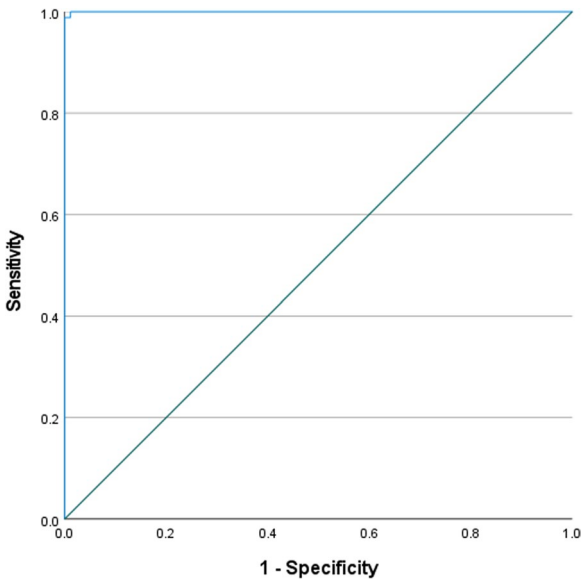

Fig. 8 ROC curve showing performance of miR-27b in diagnosis of stroke

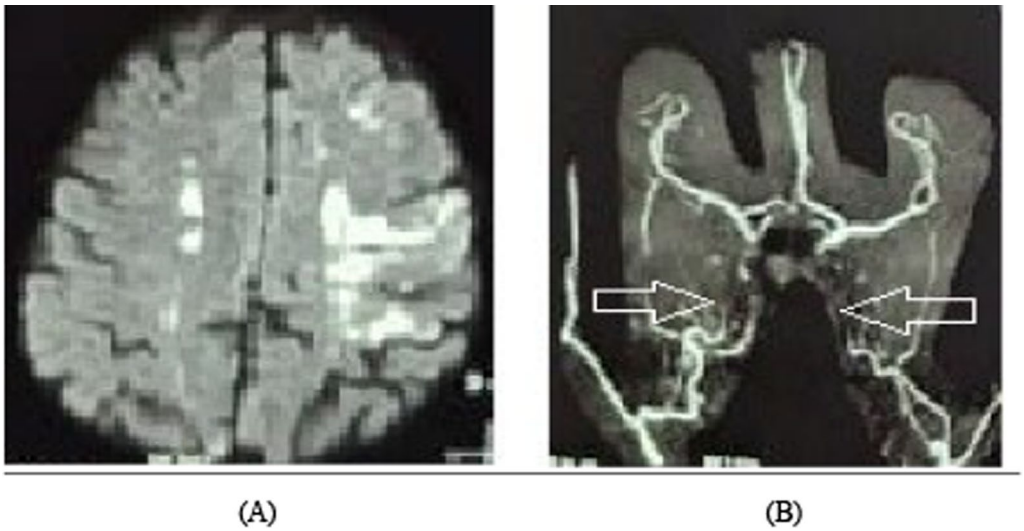

Fig. 9 MRI and MRA show bilateral parietal lobe infarctions in the distribution of the MCA. Diffusion_weighted axial image: **A** of the brain MRI showed acute infarcts in both parietal lobes, **B** MRA showed vasospasm of the right internal carotid artery and complete occlusion of the left internal carotid artery

The current investigation reveals that lesions may manifest across all cerebral regions, corroborating the observations made by Tsatsakis and colleagues [1], who noted that strokes attributable to drug abuse may arise in any area of the brain. Conversely, Middlekauff and colleagues [17] identified that cannabis-induced multifocal intracranial stenosis predominantly affects the posterior circulation.

There was a statistically significant difference between both groups regarding CYP4A11 and hsa-miR-214-3p expression. CYP4A11 expression was downregulated with a highly significant difference from the control group, showing a diagnostic value with an area under the curve (AUC) of 1, 98.9% sensitivity, and 90.9% specificity. This finding agrees with Solodilova and colleagues [18], who mentioned that CYP4A11 could be a novel genetic marker of susceptibility to coronary artery disease (CAD). Sirotina and colleagues [6] also mentioned that the polymorphism rs9332978 of CYP4A11 could be a novel marker of genetic susceptibility to CAD, at least in Europeans.

MicroRNA-214-3p was downregulated with a highly significant difference from the control group, showing a diagnostic value with an AUC of 1, 97.7% sensitivity, and 100% specificity. This is consistent with Ping and colleagues [19], who mentioned that miR-214 inhibits neuronal apoptosis by negatively regulating Bax, consequently attenuating ischemic injury in MCAO mice. These findings may provide a potential therapeutic target for stroke treatment. Shen and colleagues [20] also showed that the injection of miR-214 agomir promoted the expression of SOD and relieved the apoptosis of brain cells. Wu and colleagues [21] reported analogous findings, indicating that the suppression of miR-214-3p negates the pronounced efficacy of Hypo-Exo's (exosomes sourced from mesenchymal stem cells cultured in hypoxic conditions) in facilitating the recovery process of the ischemic brain.

CYP2E1 was upregulated with a highly significant difference from the control group, showing a diagnostic value with an AUC of 0.741, 90.8% sensitivity, and 55.2% specificity. Yu and colleagues [22] documented that the inhibition of Cytochrome P450 CYP2E1 mitigates damage from cerebral ischemia–reperfusion.

miR-27b was upregulated with a highly significant difference from the control group, showing a diagnostic value with an AUC of 1, 100% sensitivity, and 97.7% specificity. This finding is consistent with the results of Xu and colleagues [23], who determined that the inhibition of miR-27b could diminish neurological deficits by curtailing neuroinflammation and decreasing cellular mortality. This concurs with the observations by Wang and colleagues [11], who reported that inhibiting miR-27b enhances recuperation following ischemic stroke through modulation of AMPK activity.

Our result reveals that there was statistically non-significant correlation between size of infarction and CYP4A11, CYP2E1, hsa-miR-214-3p or miR-27b. This finding is consistent with the results of Ceren E and colleagues [24], who determined that miRNA-target gene networks based on inflammatory response, blood coagulation, platelet activation resulting in large artery stroke, small artery stroke, stroke due to undetermined cause and cardioembolic stroke.

Finally, our study had some limitations, the study focused on a specific set of molecular markers (CYP4A11, CYP2E1, miR-214-3p, and miR-27b). However, other CYP enzymes and miRNAs might also be involved in stroke pathogenesis in addicted patients. Future research should consider a broader range of molecular markers to provide a more comprehensive understanding. Future studies with larger, more diverse populations are needed to validate these results.

Given their potential as biomarkers, research should aim to validate the clinical utility of CYP4A11, CYP2E1, miR-214-3p, and miR-27b in predicting stroke risk among addicted patients. Large-scale cohort studies and clinical trials are needed to assess their predictive value and reliability. Investigating the possibility of targeting these pathways therapeutically could offer new avenues for stroke prevention and treatment in addicted individuals. Pharmacological modulation of CYP enzymes and miRNAs may reduce stroke incidence and improve outcomes in this high-risk group.

Limitations

We acknowledge that there are a number of limitations in this review including.

1. ESUS work up including Holter ECG, TEE, collagen battery, autoimmune markers and thrombophilia protocol should be done and documented as the mean age of patients according to the study is 47 years.
2. TCD studies should be done for all patients to assess the mean MCA/intracranial ICA diameter to document the vasospasm.

Conclusions

Our findings suggest that alterations in the expression levels of CYP4A11, CYP2E1, miR-214-3p, and miR-27b make them candidate biomarkers for stroke risk in addicted patients, providing insights into personalized treatment strategies so prophylactic antiplatelet and anticoagulant therapy can provide protection for these patients. However, the exact mechanisms by which these factors contribute to stroke pathogenesis require further investigation. Understanding these molecular pathways could lead to the development of targeted therapies aimed at mitigating the increased stroke risk in this vulnerable population.

Abbreviations

SUD	Substance Use Disorder
CYP4A11	Cytochrome P450 4A11
CYP2E1	Cytochrome P450 2E1
miRNA	MicroRNA
miR-27b	MicroRNA-27b
miR-214-3p	MicroRNA-214-3p
FDCTA	Fund for Drug Control and Treatment of Addiction
20-HETE	20-Hydroxyeicosatetraenoic Acid
TNF	Tumor Necrosis Factor
IL1	Interleukin 1
PTEN	Phosphatase and Tensin Homolog deleted on chromosome 10
AMPK	AMP-activated Protein Kinase
CT	Computed Tomography
MRI	Magnetic Resonance Imaging
MRA	Magnetic Resonance Angiography
DWI	Diffusion Weighted Imaging
MCA	Middle cerebral artery
AST	Aspartate Aminotransferase
ALT	Alanine Aminotransferase
RT–qPCR	Real-Time Quantitative Reverse Transcription–Polymerase Chain Reaction
cDNA	Complementary DNA
SPSS	Statistical Package for the Social Sciences
ROC	Receiver Operating Characteristic
AUC	Area Under the Curve
MCAO	Middle Cerebral Artery Occlusion
SOD	Superoxide Dismutase
Hypo-Exo	Exosomes derived from mesenchymal stem cells cultivated under hypoxic conditions
CAD	Coronary Artery Disease
GAPDH	Glyceraldehyde-3-Phosphate Dehydrogenase
IQR	Interquartile Range
CI	Confidence Interval
ECG	Electrocardiography
ECHO	Echocardiogram
ESUS	Embolic stroke of undetermined source
TEE	Transesophageal ECHO
TCD	Transcranial Doppler
ICA	Internal Carotid Artery

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Author contributions

All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by NEA, AMN, and ORM. The first draft of the manuscript was written by NEA and GMM, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Availability for data and materials

The data is available upon reasonable request from the authors.

Declarations

Ethics approval and consent to participate

The study was approved from the institutional ethical committee, Benha University. After receiving written informed consent from each subject, the study was carried out with their permission after receiving approval from the Institutional Review Board (IRB) of the Benha University Faculty of Medicine (Approval number: RC24-5-2023).

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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