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ORIGINAL ARTICLE

Assessment of MiR-191-5p as a Predictive Marker of Early Diabetic Sensorimotor Polyneuropathy in Patients with Newly Diagnosed Type 2 Diabetes Mellitus

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ABSTRACT

Background: Diabetic sensorimotor polyneuropathy (DSPN) is the most common complication of type 2 diabetes mellitus (T2DM) and the major cause of nontraumatic lower limb amputation. We aimed in the current research to investigate miR-191-5p as a noninvasive predictive biomarker of DSPN among patients recently diagnosed with T2DM in associations with clinical and electrophysiological tests.

Methods: We conducted fifty cases recently diagnosed with T2DM and 50 healthy control subjects. contributors were evaluated by clinical and laboratory investigations in addition to nerve conduction studies. circulatory miR-191-5p assessed using the Real-Time PCR method.

Results: miR-191-5p levels were lower in patients with DSPN compared to patients without DSPN and controls. Remarkably, miR-191-5p levels were significantly negatively correlated with cardiometabolic risk factors and neuropathy scores; Neuropathy Disability Score (NDS), Neuropathy Symptom Score (NSS), and Toronto Clinical Scoring System (TCSS), p <0.001*. The linear regression test revealed that TCSS, HbA1c, and LDL were the main independent variables against Mir-191-5p levels in patients with DSPN, p <0.001*. To assess the predictive values of miR-191-5p we applied the ROC curve the cutoff values of miR-191-5p as a predictive marker for DSPN was (0.559), with a sensitivity of (95%) and a specificity of (98.7%), with the AUC was 0.958 (0.922-0.993), p <0.001*).

Conclusion: circulating miR-191-5p was significantly downregulated in patients recently diagnosed with T2DM, more specifically in patients with DSPN, and it could be used as a biomarker for predicting diabetes and DSPN.

Keywords: Diabetic sensorimotor polyneuropathy; microRNA; Neuropathy Disability Score; Neuropathy Symptom Score; Toronto Clinical Scoring System

INTRODUCTION

Diabetic sensorimotor polyneuropathy (DSPN) is the most common and costly diabetes-associated complication, occurring in around 50% of individuals with diabetes [1]. It is characterized by demyelination and axonal loss of peripheral sensory and motor nerves [2]. It has recently been shown that DSPN manifestations are variables that include sensory or motor loss [3]. Indeed, there are now several reports confirming that the symptoms of DSPN may be sporadic or constant. Additionally, these symptoms may lead to depression and sleep disorders [4].

As previously mentioned, the prevalence of DSSPN accounts for 60% of patients with T2DM [5]. It is well documented that chronic hyperglycemia ultimately leads to aberrant gene expression, inflammation, and oxidative stress which contributes to diabetic microvascular complications, particularly DSPN [6].

Increasing evidence suggests that miRNAs can be found in circulation, thus being available as predictive, diagnostic, and prognostic biomarkers for many diseases for example diabetic microvascular complications [5,7]

Recently, there has been a growing realization that there is insufficient evidence-based information about the epigenetic regulatory roles in particular miRNA in the pathogenesis of DSPN. However, the characteristics of miRNAs in the etiology and pathogenesis of patient with T2DM and DSPN has been reported in an interesting Egyptian study [8]. Additionally, interesting research was conducted to assess miR-191-5p's role in expecting the neurological outcome after cardiac arrest [9].

DSPN is correlated with huge costs for patients and society, and there is an increasing focus on prevention, and early detection with screening. Based on the above, we performed the current research to explore miR-191-5p as a noninvasive predictive biomarker of DSPN among patients with newly diagnosed T2DM and to assess its associations with clinical and electrophysiological tests.

METHODS

This case-control study was conducted on 50 patients with newly diagnosed T2DM [10] and 50 healthy subjects as controls. Patients with T2DM are classified into 2 groups patients without DPN (N=30) and 20 patients with DPN. The study groups were matched in gender and age.

All participants underwent nerve conduction studies (NCSs) [11]. The neurological examination was evaluated according to the Neuropathy Disability Score (NDS) as shown in supplementary table 1[12] and the Neuropathy Symptom Score (NSS) as shown in supplementary table 2 [13], while the stages of DSPN were demarcated according to the Toronto Consensus criteria as shown in supplementary table 3 [14] as shown in the flowchart figure 1. Laboratory assessment was done for the examined contributors registered from the Departments of Internal Medicine and Neurology. Testing was done according to operating techniques in Zagazig University Hospital. The study protocol was approved by the Ethical Committee of the Faculty of Medicine, Zagazig University and the reference number was IRB (Ethics number. 10628). The study was performed in agreement with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving human.

Quantitative real-time RT-PCR for assessment of miR-191-5p:

The RNA was obtained from EDTA peripheral blood samples according to the company's directions. The relative expression of Mir-191-5p was calculated using $2^{-\Delta\Delta Ct}$ (with U6 sn RNA as the internal reference. The primer sequence is shown in supplementary table 4.

Statistical analysis*:* Data was analyzed by using SPSS Statistics for Windows, Version 26.0 (IBM SPSS Statistics for Windows, Version 26.0. Armonk, NY: IBMCorp), Pearson's coefficients to assess correlations of miR-191-5p with other studied parameters were done. Receiver operating characteristic (ROC) analysis was used to evaluate the potential diagnostic accuracy of miR-191-5p for the prediction of DSPN among diabetic patients. P value significant at <0.05.

RESULTS

Patients with T2DM, in particular patients with DSPN, had higher values of metabolic risk factors compared to the control group. It is interesting to note that metabolic disorders in the form of dyslipidemia, and HbA1C were significantly different between studied groups. Interestingly, there was a decline in nephrology tests in patients with DSPN compared to other groups. Table 1 (p<0.001*).

Comparison of miR-191-5p level in studied groups

To clarify the miR-191-5p levels in the studied group. we compare the results with the ANOVA test, and it is exciting to note that there were significantly lower values of miR-191-5p levels in patients with DSPN (0.43±0.09) compared to patients without DSPN (0.61±0.09) and controls (0.931±0.11), P value <0.001*, table 1 and supplementary figure 1

Electrophysiological tests of the studied groups

According to the nerve conduction velocities amplitude results, Median nerve [MNCV, SNCV, CMAP, and SNAP amplitude] values were meaningfully lowered in DSPN compared to others without DSPN, (p < 0.001). Additionally, CMAP and SNAP amplitudes of the Sural nerve were notably decreased in DSPN compared to others without DSPN P value <0.001*. However, all other

nerve velocities differences were not significant p>0.05. (Table 2)

Associations of miR-191-5p with neuropathy scoring system and other studied tests

In the DSPN group, the miR-191-5p level was significantly negatively correlated with body mass index, waist/hip ratio, TC, TG, LDL, HbA1c, and UACR, <0.001* (Table 3). On the other hand, it was significantly positively correlated with eGFR, P <0.001* (Table 3).

The most unique and interesting result of the current study was that there was a significant negative correlation between studied neuropathy scores; NDS, NSS, TCSS, and miR-191-5p**,** P value of <0.001* (Table 3).

Concerning miR-191-5p results obtained from the linear regression test*,* we found that among the studied parameters, only TCSS, HbA1c and LDL were the most unconventional parameters against Mir-191-5p in patients with DSPN, P <0.001* (Table 4).

Based on the ROC curve, the optimal cutoff values of miR-191-5p as a predictive marker for T2DM were projected to be (0.805), which yielded a sensitivity of (98%) and a specificity of (98%), with the AUC being 0.99 (0.972– 1.000). A p-value of <0.001*, figure 2.

The ROC curve results regard the optimal cutoff values of miR-191-5p as a predictive marker for DSPN were projected to be (be (0.559), which had a sensitivity of (95%) and a specificity of (98.7%), with the AUC 0.958 (0.922-0.993). P value of <0.001*, figure 3.

Table 1: Anthropometric and biochemical characteristics of the studied groups.

Variables	Control group (*n* =50)	Patients without DSPN (n =30)	Patients out DSPN (n =20)	*P* value
Age (years)	46.12± 8.20	42.53± 9.302	46.7±7.6	0.127
Sex Male (n, %) Female (n,%)	 21(42%) 29(58%)	 11(63.3%) 19(35.7%)	 11(55%) 9(45%)	0.430
Body mass index	22.38±1.189	34.73 ± 2.32[&]	38.03 ± 4.96[$, £]	<0.001*
Waist/hip ratio	0.77±0.02	0.94 ± 0.23[&]	1.35 ± 0.22[$]	<0.001*
Systolic blood pressure	112.8±9.4	153.08 ± 27.45[&]	145.01 ± 16.2[$]	<0.001*
Diastolic blood pressure	76.6±5.7	84.52 ± 10.3[&]	104.5 ± 12.5[$, £]	<0.001*
Retinopathy	-	15(50%)	14 (70%)	0.160
Microalbuminuria	-	16(61.5%)	10(50%)	0.817
Stroke	-	6(20%)	3(15%)	0.652
CHD	-	14(46.7%)	6(30%)	0.239
Total cholesterol (mg/dl)	188.3±19.1	214.73 ±31.3[&]	232.61±28.2[$, £$]	<0.001*
Triglycerides (mg/dl)	142.26±13.2	232.86 ± 24.9[&]	341.4 ± 33.1[$, £]	<0.001*
LDL (mg/dl)	101.08±23.1	112.78 ± 29.3[&]	130.9 ± 24.7[$, £]	<0.001*
HDL (mg/dl)	56.48±4.8	37.78 ± 5.2 [&]	32.79 ± 5.6 [$, £]	<0.001*
FPG	77.48±14.8	111.78 ± 25.2 [&]	160.7 ± 33.6 [$]	<0.001*
HbA1c (%)	4.73±0.61	8.79±1.5[&]	9.99±3.8[$, £]	<0.001*
eGFR (mL/min)	93.37 ±12.5	70.19±3.2[&]	33.898[$, £]	<0.001*
Serum creatinine (mg/dl)	0.96±0.3	0.99±0.44	1.4±0.63[$, £]	0.757
UACR (mg/g)	8.72±2.1	145.97±93.4[&]	244.068[$]	<0.001*
NDS	0	4.79±1.32	8.63±0.62[$, £]	<0.001*
NSS	0	0.7± 0.66	2.1± 0.96[$, £]	<0.001*
TCSS	0.865±0.211	2.75 ± 1.96[&]	10.28 ± 2.33[$, £]	<0.001*
Mir-191-5p	0.931±0.11	0.61±0.09[&]	0.43±0.09[$, £]	<0.001*

FPG, fasting plasma glucose; eGFR, estimated glomerular filtration rate; UACR, urine albumin: creatinine ratio; NDS, Neuropathy Disability Score; NSS, Neuropathy Symptom Score; T2DM, type 2 diabetes mellitus; TCSS, Toronto Clinical Scoring System.

[&]Significant P values (P < 0.05) when comparing the control group with patients without DSPN group. Significant P values (P < 0.05) when comparing the control group with patients in the DSPN group.[$]

£Statistically significant P values (P < 0.05) when comparing patients without DSPN group with patients in the DSPN group.

Table 2: Electrophysiological tests among studied patients with T2DM.

Electrophysiological tests	Patients without DSPN, (n=30)	Patients with DSPN, (*n* =20)	*P* value
MNCV (m/s)			
Median	47.7±10.8 Normal	42.32± 3.6 Abnormal	<0.001*
CPN	46.96± 10.54 Normal	45.13± 6.47 Normal	0.496
PTN	47.96± 10.76 Normal	46.8±8.93 Normal	0.705
Ulnar	47.12± 8.6 Normal	46.8±10.5 Normal	0.912
SNCV (m/s)			
Sural	44.6±7.794 Normal	34.8±4.72 Abnormal	<0.001*
Median	48.6±4.4 Normal	41.78± 8.4 Abnormal	<0.001*
Ulnar	45.42± 3.4 Normal	44.91±4.6 Normal	0.832
CMAP amplitude (mV)			
Median	6.33±1.94 Normal	4.83±1.52 Abnormal	<0.001*
Ulnar	6.18±0.32 Normal	5.98±0.58 Normal	0.610
CPN	6.36±1.22 Normal	6.44±0.411 Normal	0.721
PTN	6.11±1.63 Normal	6.06±0.84 Normal	0.891
SNAP amplitude (*μ*V)			
Sural	5.67±1.33 Normal	3.79±1.22 Abnormal	<0.001*
Median	8.27±1.51	5.45±1.18 Abnormal	<0.001*
Ulnar	6.77±2.0 Normal	6.18±1.59 Normal	0.995

MNCV, motor nerve conduction velocity; SNCV, sensory nerve conduction velocity; CPN, common peroneal nerve; PTN, posterior tibial nerve; CMAP, compound muscle action potential; SNAP, sensory nerve action potential. * *P* < 0.05

Table3: Correlations between miR-191-5p level with neuropathy scoring system and other studied parameters in patients with DSPN.

Variables	Mir-191-5p	
	R	p
Body mass index	-0.524	<0.001*
Waist/hip ratio	-0.350	<0.001*
Systolic blood pressure	-0.194	0.053
Diastolic blood pressure	-0.024	0.812
Total cholesterol (mg/dl)	-0.371	<0.001*
Triglycerides (mg/dl)	-0.432	<0.05*
LDL (mg/dl)	-0.159	< 0.001*
HDL (mg/dl)	0.178	0.077
FPG	-0.155	0.125
HbA1c (%)	-0.519	<0.001*
eGFR (mL/min)	0.614	<0.001*
Serum creatinine (mg/dl)	-0.024	0.812
UACR (mg/g)	-0.687	<0.001*
NDS	-0.343	<0.001*
NSS	-0.468	<0.001*
TCSS	-0.564	<0.001*

MNCV, motor nerve conduction velocity; SNCV, sensory nerve conduction velocity; CPN, common peroneal nerve; PTN, posterior tibial nerve; CMAP, compound muscle action potential; SNAP, sensory nerve action potential, * $P < 0.05$.

Table 4: linear regression analyses to test the influence of the main independent variables against Mir-191-5p levels (dependent variable) in patients with DSPN.

Model		Unstandardized Coefficients		Standardized Coefficients	t	p	95% C.I.	
		B	SE	Beta			Lower Bound	Upper Bound
Mir-191-5p	(Constant)	0.013	0.013		0.965	0.339	-0.014	0.039
	TCSS	-0.025	0.011	-0.984	-2.314	0.023	-0.047	-0.004
	BMI	-0.025	0.023	-0.246	-1.069	0.288	-0.071	0.021
	HbA1c	-0.104	0.042	-0.314	-2.489	0.015	-0.186	-0.104
	LDL	-0.025	0.011	-0.984	-2.314	0.023	-0.047	-0.025

* $P < 0.05$.

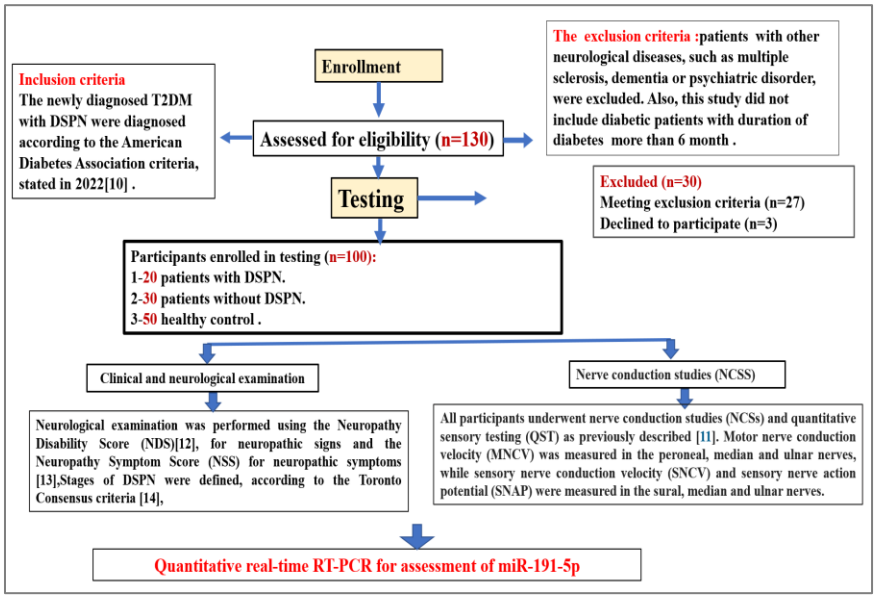
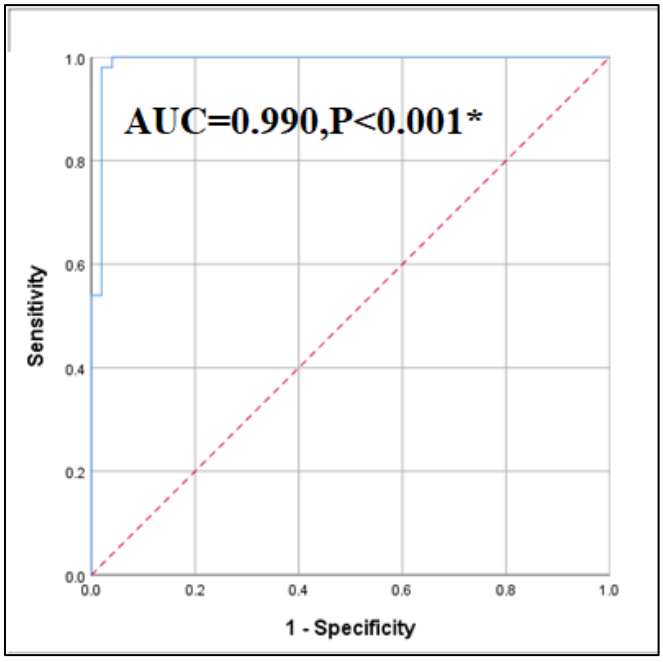


Figure (1): flowchart of the study



Figure(2): The accuracy of the relative expression levels of miR-191-5p level for discriminating patients with T2DM from control group by ROC analysis

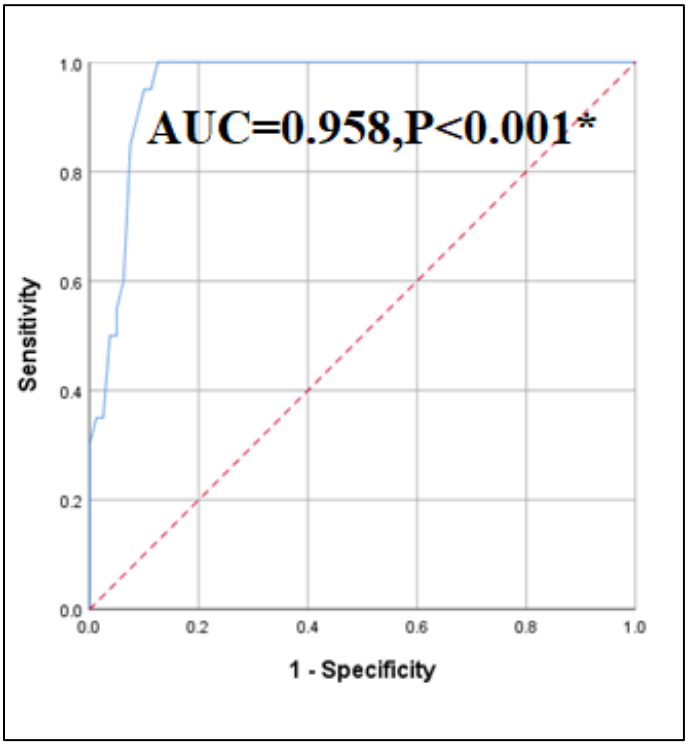


Figure (3): The accuracy of the relative expression levels of miR-191-5p level for discriminating patients with DSPN from patients without DSPN by ROC analysis.

DISCUSSION

As already mentioned in our previous research DSPN was detected to be the most common cause of lower limb amputation. Noteworthy, it leads to impairment of life [5]. Thus, we examined circulatory miR-191-5p values in newly diagnosed T2DM and explored their correlations with other studied parameters in the case group with DSPN.

In the current study, we examined 100 participants: 50 controls and 50 patients with newly diagnosed T2DM. As thought patients with DSPN had metabolic syndrome and DKD compared to other studied groups. According to the results of the NCS and neuropathy scores we divided our cases into 30 patients without DSPN and 20 patients with DSPN. The most affected nerves were the Median nerve [MNCV, SNCV, CMAP, and SNAP amplitude values] and the Sural nerve CMAP and SNAP amplitudes.

The interesting results reported by Lai et al found that patients with poorly controlled DM had decreased MNCV results regarding median, ulnar, peroneal, and tibial nerves compared to the normal population. Furthermore, they had decreased SNCV results of the sural nerve compared to normal subjects [15]. Also, Su and his colleagues detected that albuminuria, poor glycemic control, and glycemic variability were associated with diabetic neuropathy [16].

To date according to our information, this is the first research conducted to prove the theory that miR-191-5p levels downregulation in T2DM and DSPN. We investigated our genetic sample with the Real-Time PCR method. MicroRNA-191-5p is largely expressed by different cells [17]. Indeed, it has been reported that MicroRNA-191-5p regulates different cellular activities, by steering cell cycle-associated genes and transcription factors [18].

The most important findings of the current study were that there were significantly lower values of miR-191-5p levels DSPN compared to other studied groups of participants. Interestingly, miR-191-5p values were notably negatively associated with body mass index, waist/hip ratio, TC, TG, LDL, HbA1c, and UACR, $p<0.001$*. Conversely, it was significantly positively correlated with eGFR. The most exclusive and exciting results of the current study were that miR-191-5p values are inverse associated with NDS, NSS, TCSS, and miR-191-5p values.

Our findings are in concordance with a study conducted by Bellini et al. They found a decrease in the expression of miR-191-5p in patients with diabetic vascular complications [19]. Similar results

confirmed by Zampetaki et al discovered lower levels of serum miR-191-5p levels in T2DM compared with controls [20]. Cons, similarly, reports of Barutta et al detected lower levels of microRNA-191-5p in patients with chronic complications of diabetes [21].

Bothering miR-191-5p linear regression results, we discovered that TCSS, HbA1c, and LDL were the most unconventional tests associated with Mir-191-5p values in patients with DSPN. To assess the predictive values of miR-191-5p we applied the ROC curve, and we detected that the ideal cutoff values of miR-191-5p as a predictive indicator for T2DM were projected to be (0.805), with a sensitivity of (98%), a specificity of (98%), and the AUC being 0.99 (0.972– 1.000). The optimal cutoff values of miR-191-5p as a predictive marker for DSPN were projected to be (be (0.559), which had a sensitivity of (95%) and a specificity of (98.7%), with the AUC being 0.958 (0.922-0.993).

Interesting research conducted by Wang and his colleagues detected that the miR-30d-5p level was higher in the DPN patients than in other investigated participants [22]. Another study observed poor glycemic control and long duration of diabetes are linked with miRNAs [23]. Further progress in the understanding of the role of miRNA mutation in DPN was derived from a study conducted by Burada et al, this intriguing research detected that epigenetics in particular miRNA mutations were associated with T2DM and DPN [24].

In conclusion,

We detected decreased circulating miR-191-5p expression in patients with newly diagnosed T2DM in particular patients with DSPN. the miR-191-5p level was significantly correlated with neuropathy severity score and cardiometabolic risk factors. Thus, it could be used as a noninvasive predictor of DDSPN which could have pathophysiological and therapeutic implications.

Strengths and limitations of the study

There are strengths of this study that should be acknowledged. As far as we know, we examined and investigated our patients by both neuropathy clinical score and NCS. The small sample size of the study was the most important limitation of the current study. In addition, it will be important to follow up with the patients included in this study to be able to clarify whether those subjects with lower values of miR-191-5p eventually develop DSPN.

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Ts 1: Revised neuropathy disability score (NDS)

NDS items	Description
Vibration sensation (128 Hz tuning fork)	0 = present, 1 = reduced/absent
Temperature sensation (cold tuning fork)	0 = present, 1 = reduced/absent
Pin- prick	0 = present, 1 = reduced/absent
Ankle reflex	0 = normal, 1 = present with reinforcement, 2 = absent per side

Ts 2: Neuropathy Symptom Score(NSS)

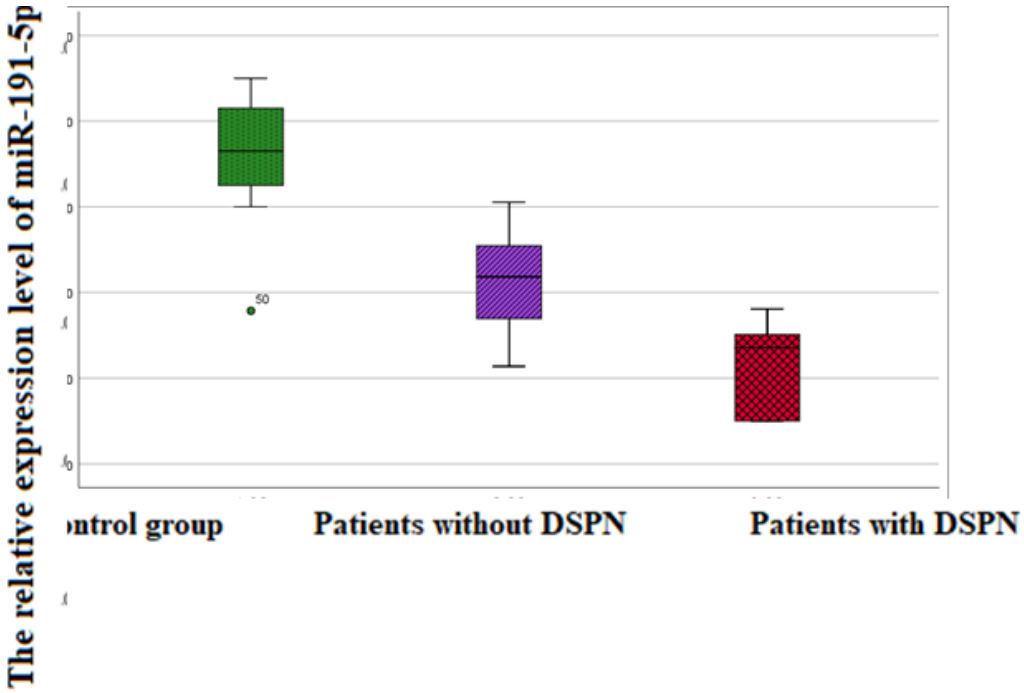
Task	Points
Presence of mono- or hemiparesis	**1**
Inability to walk on a 3-cm-wide beam	**1**
Inabil to walk on a 2-cm-wide beam	**1**
Inability to walk on a 1-cm-wide beam	**1**
Inability to balance on a I-cm-wide beam	**1**
Inability to balance on a round stick 10.5 cm diameter)	**1**
Failure to exit a 30-cm-diameter circle (fer 2 min)	**1**
Inability to walk straight line	**1**
Less of startle behavior	**1**
Less of seeking behavior	**1**
Maximum total	**10**

Ts 3: Toronto clinical scoring system (TCSS)

TCSS items		Description
Symptoms score	Pain	0 = absent, 1 = present
	Numbness	0 = absent, 1 = present
	Tingling	0 = absent, 1 = present
	Weakness	0 = absent, 1 = present
	Ataxia	0 = absent, 1 = present
	Upper- limb symptoms	0 = absent, 1 = present
Reflex score	Knee reflexes	Score for each side: 0 = normal, 1 = reduced, 2 = absent
	Ankle reflexes	Score for each side: 0 = normal, 1 = reduced, 2 = absent
Sensory test score	Pinprick	0 = normal, 1 = abnormal
	Temperature	0 = normal, 1 = abnormal
	Light touch	0 = normal, 1 = abnormal
	Vibration sense	0 = normal, 1 = abnormal
	Position sense	0 = normal, 1 = abnormal

Ts 4: The primer sequence of the studied miR-191-5p and U6

Gene	Forward primer (5'-3')	Reverse primer (5'-3')
miR-191-5p	5′-CGGAATCCCAAAAGCAGCTG-3′	5′-TGTCGTGGAGTCGGCAATTG-3′
U6	5′-ATGACGTCTGCCTTGGAGAAC-3′	5′-TCAGTGTGCTACGGAGTTCAG-3′



Fs 1: Comparison of miR-191-5p level in studied groups