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The significance of miRNA-191-5P and miRNA-24-3P as novel biomarkers for multiple sclerosis: a case–control study



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Abstract

Background Multiple sclerosis (MS) is a long-term disease that can lead to disability. microRNAs (miRNA) can provide noninvasive markers allowing more frequent and easy testing in MS. Treatment methods based on manipulating miRNA activity can be innovative. The purpose of this work is to measure the serum expression of miRNA-191-5P and miRNA-24-3P in MS patients. The investigation was carried out on 80 patients with MS (68 patients with Relapsing-remitting multiple sclerosis (RRMS), 12 patients with Progressive MS) and 40 healthy controls. The serum expression of miRNA-191-5P and miRNA-24-3P was measured using real-time quantitative PCR. The expression of the studied miRNAs was relatively calculated using the Eq. $2^{-\Delta\Delta Ct}$.

Results Serum levels of miRNA-191 and miRNA-24 showed no difference between MS patients and healthy controls, and neither between RRMS and progressive MS groups. A negative correlation was detected between miRNA-191 and disease duration. Also, a positive correlation was detected between miRNA-191 and miRNA-24 expression. RRMS patients were significantly different from progressive MS patients regarding disease duration (*p* value 0.001) as well as expanded disability status scale score (*p* value < 0.001).

Conclusion The study uniquely analyzed the correlation between the miRNA-191 and miRNA-24, being expressed in all MS patients, and being positively correlated means they are influenced by the same factors and they can be therapeutically targeted in the same way, so further studies are required. The impact of disease duration on miRNA-191 expression encourages regular monitoring of miRNA-191.

Keywords Multiple sclerosis, miRNA-191, miRNA-24, Real-time PCR

Background

The neurodegenerative condition known as multiple sclerosis (MS) results in the demyelination of the central nervous system [1]. It is considered a main cause of impairment in young adults, primarily affecting young women between the ages of 20 and 40 [2]. Till now there

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are no markers specific for the diagnosis of MS that can discriminate MS from other diseases, such as some viral, neoplastic, genetic, metabolic, and other idiopathic inflammatory demyelinating syndromes (IIDD), which may resemble MS clinically and radiologically leading to diagnostic difficulty. The primary diagnostic tools for MS include neurological testing, magnetic resonance imaging (MRI) scans, and lumbar puncture-based cerebrospinal fluid (CSF) analyses. Therefore, it is crucial to develop a specific noninvasive definitive biomarker for



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early diagnosis, correct treatment choice, and long-term disability prevention [3].

Measurement of circulating miRNAs in MS patients' peripheral blood is one of the promising approaches as it can be a noninvasive alternative diagnostic protocol allowing early diagnosis by more regular and accessible testing [1]. miRNAs are remarkably stable in bodily fluids and are relatively simple to collect and quantify [4]. Moreover, a novel approach to therapy may be based on methods that regulate the activity of miRNAs. miRNAs are naturally occurring, non-coding RNA molecules (21–25 nucleotides). miRNAs play a part in the post-transcriptional regulation of gene expression and silencing of RNA [5]. miRNAs have been discovered to control some physiological processes, as apoptosis, proliferation, differentiation, and development [6].

Recent research has discovered that miRNAs contribute to the pathophysiology of MS, primarily influencing glial cells and the immune cells in the periphery. In recent years, miRNAs have become more prevalent as inflammation and demyelination process regulators in MS [7]. Many studies assessed the utility of miRNA signatures to diagnose and classify MS, while others assessed how disease-modifying treatments (DMTs) affected miRNA expression that was dysregulated in MS [8]. An analysis of active and inactive MS lesions using miRNA profiling revealed that miRNAs can control macrophage activity in lesions, increasing phagocytosis to remove myelin and debris [9]. Blood-brain barrier integrity is influenced by miRNAs, and endothelial cells from MS patients have an altered miRNA signature that may make the blood-brain barrier less effective [10].

miRNA-24 and miRNA-191 are abnormally expressed in tissues and cell types of different kinds of diseases and are found to be closely related to the development, diagnosis, and prognosis [11–15]. miR-24-3p was found to be overexpressed in Hodgkin Lymphoma and Protects Hodgkin and Reed-Sternberg Cells from Apoptosis [11] and it was also found to promote cell proliferation and regulate chemosensitivity in head and neck squamous cell carcinoma by targeting a potential tumor suppressor gene CHD5 [12]. miR-191 was found to directly repress Brain-derived neurotrophic factor (BDNF) through binding to their predicted sites in BDNF 3'UTR. BDNF is a secreted protein of the neurotrophin family that regulates brain development, synaptogenesis, memory, and learning [14]. miRNA-191 and miRNA-24 have been previously associated with MS but with different results and conclusions. Accordingly, in the current work, we studied the expression of miRNA-191-5P and miRNA-24-3P in MS patients and healthy controls. We compared their levels between the different MS subgroups. We also correlated their expression level to the clinical characteristics, radiological findings, disease progression, and disability of MS patients.

Materials and method

Study population

This case–control study purposed to uncover the role of miRNAs in MS patients. 120 individuals participated in the study and were split into 2 groups, Group I: included 80 patients with MS diagnosed based on the revised McDonald criteria recruited from the neurology department of Cairo University Hospital. They were 66 females and 14 males with a mean age of 32 ± 7.9 years. MS patients were further subdivided into two subgroups: 68 patients with RRMS and 12 patients with Progressive MS group. Group II: included 40 healthy individuals who were age- and sex-matched as a control group, with no history of any neurological or autoimmune disease. They were 33 females and 7 males with a mean age of 30.4 ± 8.2 years (Table 1). Patients with other neurodegenerative and autoimmune disorders were excluded.

All MS patients in this study were subjected to thorough history taking with particular focus on age, sex, disease duration, symptoms of the 1st presentation, and annual relapse rate (ARR). Personal interviews and medical records were used to gather information. They were also subjected to general examination and local neurological examination. The expanded disability status scale (EDSS) score was used to identify neurological

| Table 1 Demographic data of the M.S patients and healthy contro | ols: |
|---|------|
|---|------|

| Clinical data | | All MS (n = 80) | RRMS (n=68) | Progressive MS (n = 12) | Healthy control (n=40) |
|----------------|-----------|-----------------|-------------|----------------------------|------------------------------|
| Age (years) | Mean ± SD | 32±7.9 | 31.6±8 | 34.5±7 | 30.4±8.2 |
| Gender No, (%) | Female | 66 (82.5%) | 59 (86.7%) | 7 (58.3%) | 33 (82.5%) |
| | Male | 14 (17.5%) | 9 (13.2%) | 5 (41.6%) | 7 (17.5%) |

Age (years) is represented as Mean±SD

Gender is represented as number and percent

impairment. None of the patients experienced any relapses for at least 8 weeks before taking the sample.

MRI examination

MRI brain was done on all MS patients to detect the number, the site, and the enhancement of lesions. According to the number of lesions, there were 3 groups, those with 1–3 lesions, those with 4–5 lesions, and those with more than 10 lesions. Lesions were detected in the Periventricular, Juxtacortical, Cerebellum, Subcortical, Brainstem, and Basal ganglia. According to the enhancement of lesions, there was a group with T1 enhancement and one without T1 enhancement (Fig. 1).

Analysis of miRNA-191 and miRNA-24 gene expression

From each participant, 2mL of blood was drawn into a plain vacutainer and then centrifuged at 3000 rpm for 15 min. The supernatant was then collected in Eppendorf tubes. To precipitate cell debris from the samples, 15 min of centrifugation at 15,000 rpm was done. Until RNA extraction, the supernatants were stored at -80 °C. RNA was extracted using the miRNeasy Mini Kit from Qiagen (catalog no. 217004). The absorbance at 260 and 280nm using NanoDrop 1000A Spectrophotometer (NanoDrop Technologies, Waltham, MA) was measured to determine the concentration and purity of the extracted

RNA. For normalization, RNU-48 served as the reference gene.

Reverse transcription (RT) was performed using TaqMan MicroRNA Reverse Transcription Kit (Applied Biosystems, USA, cat no. 4366596). RT was done in 15 μ l reaction volume: 5 μ l of RNA (10 ng per reaction), 3 μ l stem-loop RT primer, and 7 μ l RT Reaction Master Mix. Sequences of the miRNAs and RNU-48 were determined from the miRbase (http://www.mirbase.org) and shown in Table 2. The tube was incubated for 5 min on ice, inserted into the thermal cycler, and subjected to the following temperatures: 16 °C for 30 min, 42 °C for 30 min, 85 °C for 5 min, and 4 °C on hold.

According to the manufacturer guidelines to perform real-time PCR, 1.33 µl of RT products, 10 µl of TaqMan[®] Universal PCR Master Mix II (Applied Biosystems, USA,

 Table 2
 Target miRNA and control Sequences to be studied and amplified by qRT-PCR

| Target MiRNA | MiRbase No | Mature sequence |
|--------------|------------|---|
| MiR-191 | MI0000465 | CAACGGAAUCCCAAAAGCAGCUG |
| MiR-24 | MI000080 | UGGCUCAGUUCAGCAGGAACAG |
| RNU-48 | | GATGACCCCAGGTAACTCTGAGTGTGT CGCTGATGCCATCACCGCAGCGCTCTG ACC |



Fig. 1 MRI brain showing (a) Axial FLAIR WIs showing subcortical (black arrow) & juxta-cortical (white arrow) white matter plaques. b Sagittal FLAIR WIs showing periventricular plaques (white arrow). c Post-contrast sagittal T1 WIs showing incomplete ring enhancement of one of the periventricular plaques suggesting activity

cat no. 4440042), 1 µl of TagMan MicroRNA Assay and 7.67 µl of nuclease-free water were combined in a final volume of 20µl. The following conditions were used to run each reaction using the StepOne real-time PCR system (Applied Biosystems, USA): 95 °C for 10 min, 45 cycles of 95 °C for 1 s, and 60 °C for 60 s. The comparative cycle threshold (Ct) approach was used to measure the relative expression of miRNAs. Then, using the Eq. 2 $^{-\Delta\Delta CT}$, the fold change of each potential miRNA was determined.

Statistical analysis

IBM SPSS[®] Statistics version 26 (IBM[®] Corp., Armonk, NY, USA) was used for the statistical analysis. The expression of the numerical data was done by using the mean, standard deviation, or median and range. Frequency and percentage were used to express qualitative data. The relationship between the various qualitative factors was investigated using Pearson's Chi-square test. In order to compare two groups of quantitative data, either the Student t-test or the Mann-Whitney test (a non-parametric t-test) was used, depending on whether the data were normally distributed or not. Kruskal-Wallis test (nonparametric ANOVA) was used for comparison between the three groups, and a post-hoc test (based on Kruskal-Wallis distribution) was employed for pair-wise comparison. To examine the relationship between the numerical variables, the Spearman-rho method was employed. All tests were two-tailed. A p value of 0.05 or lower was considered significant.

Results

All the MS patients in the study underwent clinical, neurological, and radiological examination and the descriptive findings are shown in Table 3. The Symptoms of initial presentation in all MS patients were in the form of sensory involvement in 25 patients (31.3%), motor involvement in 22 patients (27.5%), visual manifestations in 16 patients (20%), brainstem manifestations in 6 patients (7.5%), Ataxia and incoordination in 6 patients (7.5%) and bladder manifestations and others in 5 patients (6.3%).

RRMS patients were statistically significantly different from progressive MS patients in disease duration (p value (0.001) as well as EDSS (*p* value < (0.001)), but no significant difference was detected regarding ARR. Median levels of miRNAs' fold changes (FC) in the examined groups are shown in Table 4. The comparison of miRNA-24 and miRNA-191 expression levels between all M.S patients and the healthy control group revealed no difference between both groups with a p value of 0.147 and 0.517 respectively. Further analysis was done by comparing the miRNA expression levels between the different MS subgroups, as this comparison may help to distinguish the clinical phenotypes of MS, also we compare the levels of miRNAs in each MS subgroup with the controls. The study didn't detect any difference between the RRMS group and the progressive MS group regarding the

| | Table 3 | Clinical, neurological, and | d radiological findings in MS patients |
|--|---------|-----------------------------|--|
|--|---------|-----------------------------|--|

| Clinical data | All MS (n=80) | RRMS (n = 68) | Progressive MS (n = 12) |
|-----------------------------------|-----------------------|---------------|----------------------------|
| Disease Duration (years) | 2 (1–22) | 2 (1-22) | 6.5 (2–18) |
| EDSS | 1.5 (1–6.5) | 1.5 (1–6) | 5.5 (1–6.5) |
| ARR (relapses/year) | 1 (1-4) | 1 (1–4) | 1.5 (1–2) |
| Site of lesion | Frequency and percent | | |
| Periventricular | 13 (16.3%) | | |
| Periventricular and Juxtacortical | 50 (62.5%) | | |
| Others | 17 (21.3%) | | |
| Number of lesions | Frequency and percent | | |
| 1–3 lesions | 42 (52.5%) | | |
| 4–9 lesions | 14 (17.5%) | | |
| > = 10 lesions | 24 (30%) | | |
| T1 Enhancement | Frequency and percent | | |
| with T1 enhancement | 5 (6.3%) | | |
| without T1 enhancement | 75 (93.8%) | | |

MS Multiple sclerosis, RRMS relapsing-remitting multiple sclerosis, EDSS expanded disability status Scale, ARR annual relapsing rate

Disease duration, EDSS, and ARR are expressed in median (range)

MRI data is presented by frequency and percentage

| Table 4 m | 1RNA-191 | and miRNA-24 | expression | levels in [•] | the different | study gro | ups and he | ealthy contro | ls |
|-----------|----------|--------------|------------|------------------------|---------------|-----------|------------|---------------|----|
|-----------|----------|--------------|------------|------------------------|---------------|-----------|------------|---------------|----|

| | All MS (n = 80) | RRMS (n = 68) | Progressive MS (n = 12) | Healthy control (n=40) | |
|--|-----------------------|----------------------|-------------------------|------------------------|--|
| miRNA-191 | 1.01 (0.0058–1601.27) | (0.0058-1601.27 | 0.85 (0.0060–32) | 1 (0–2076.59) | |
| miRNA-24 | 2.38 (0.0002–680.29) | 2.38 (0.0083–680.29) | 1.99 (0.0002–49.18) | 1 (0.0439–179.77) | |
| All data are presented as median (min.—max.) | | | | | |

*Fold change formula: Fold-change=2 - $\Delta\Delta$ CT

Table 5 Relationships of the studied miRNAs with different parameters of the MS patients

| Clinical Parameters of MS (n=80) | s Median values of miRNAs | | | | | | <i>p</i> value |
|-------------------------------------|---------------------------|---------------------|--------------------|-----------|--------|----------------|----------------|
| Sex | Male | Female | | | | | |
| miR-191 | 0.92 (0.01–1601.27) | 1.05 (0.01–389.37) | | | | | 0.79 |
| miR-24 | 2.85 (0–137.66) | 2.34 (0–680.29) | | | | | 0.929 |
| Initial presentation | Motor | Sensory | Visual | Brainstem | Ataxia | Bladder/others | |
| miR-191 | 0.98 | 0.71 | 2.02 | 18.54 | 4.52 | 0.95 | 0.113 |
| miR-24 | 0.92 | 2.43 | 4.58 | 10.27 | 50.07 | 0.99 | 0.13 |
| Number of lesions | 1–3 | 4–9 | >=10 | | | | |
| miR-191 | 0.92 (0.01–1601.27) | 1.01 (0.01–47.50) | 1.01 (0.01–350.92) | | | | 0.702 |
| miR-24 | 2.38 (0–137.66) | 0.99 (0.02–114.56) | 1.75 (0–680.29) | | | | 0.991 |
| MRI Enhancement | Without T1 enhancement | With T1 enhancement | | | | | |
| miR-191 | 0.99 (0.01–1601.27) | 3.73 (0.18–47.50) | | | | | 0.225 |
| miR-24 | 2.38 (0–680.29) | 3.71 (0.08–114.56) | | | | | 0.817 |

p value significant: $p \le 0.05$.

p value highly significant: $p \le 0.001$.

Table 6 Correlation of miRNA-191 and miRNA-24 expressionlevels with different parameters of the MS patients

| N=80 | miRNA 19 | 1 | miRNA 24 | | |
|------------------|----------|-------|----------|-------|--|
| | R | Р | R | Р | |
| Age (years) | -0.046 | 0.686 | -0.149 | 0.189 | |
| Disease Duration | -0.251 | 0.025 | -0.160 | 0.157 | |
| EDSS | -0.078 | 0.492 | -0.127 | 0.263 | |
| ARR | 0.109 | 0.338 | 0.113 | 0.318 | |

p value significant: $p \le 0.05$.

p value highly significant: $p \le 0.001$.

measured miRNAs, also no significant differences were found when comparing RRMS and Progressive MS with the control.

The relationships of miRNA-191 and miRNA-24 with different clinical, neurological, and radiological data of MS patients of the study were done and shown in Tables 5 and 6 They revealed no statistically significant relationship apart from a negative correlation between miRNA-191 and disease duration, the longer the disease duration is, the less expressed miRNA-191 is (r = -0.251 *p* value = 0.025) (Fig. 2). Also, a positive correlation,

which is statistically significant, was detected between miRNA-191 and miRNA-24 expression (r=0.703 and *p* value < 0.001).

Discussion

In the context of the studied miRNAs' expression, the median FC of miRNA-191 and miRNA-24 showed no significant difference between all MS cases and controls. However, the expression of miRNA-24 was higher in MS cases (median FC=2.38) than in controls (median FC=1), it didn't reach statistical significance. Contrary to our results, Vistbakka J. et al., (2018) revealed statistically significant overexpression of miRNA-191-5p and miRNA-24-3p in sera of MS cases in comparison to healthy control [16]. Also, Ehya F. et al., (2017) on blood samples of 33 MS patients and 30 healthy individuals found that the expression level of miRNA-24 was statistically upregulated in MS patients versus healthy control [1].

In another research by Guerau-de-Arellano M. et al., (2015), miRNA analysis was performed using nanostring technology on NAWM (normally appearing white matter) of MS cases and control brain tissues and they observed miRNA-191 as one of 14 miRNAs considerably reduced in the MS patients [17]. The difference in the



Fig. 2 Correlation of miRNA-191 and disease duration

findings of this research and the previous 2 studies may be related to measuring the miRNAs in brain tissues rather than in blood. Similar results with miRNA-184 and miRNA-127-3p in Alzheimer's disease have been described, where their levels were decreased in CSF but elevated in blood when compared to people with no neurological diseases [18].

Although its precise role in immune systems is still unclear, it has been demonstrated that miRNA-191 promotes the survival of naive, memory, and regulatory T-cells, which helps to sustain immunological balance [19]. miRNA-24 reduces the expression of interferon (IFN)- γ [20] and IL-4 [21] indicating its function in controlling the Th1/Th2 balance and the regulation of inflammatory responses. Additionally, miRNA-24 was shown to control the synthesis of aldosterone and cortisol by inhibiting the function of the messenger RNAs (mRNAs) CYP11B1 and CYP11B2 [22]. Aldosterone is involved in the inflammatory processes, it encourages autoimmune damage and controls the synthesis of vascular endothelial growth factor (VEGF)-A in neutrophils, which is a powerful disruptor of the bloodbrain barrier [23].

Further analysis was done by comparing miRNA-191 and miRNA-24 expression in the different subgroups of MS and the healthy group. There was no difference when comparing RRMS vs control, progressive MS vs controls, and RRMS vs Progressive MS. Our results were not matching with Vistbakka J. et al.'s (2018) results regarding the comparison between RRMS vs control and progressive MS vs controls, but they were matching regarding the comparison between RRMS vs Progressive MS. Vistbakka J. et al., (2018) detected overexpression of miRNA-191 and miRNA-24 in RRMS (P=0.01, P=0.01, respectively) and in primary progressive MS (P<0.001, p=0.01) in comparison with healthy controls [16].

In our investigation, an examination of the possible associations and correlations of miRNA-191 and miRNA-24 levels with different clinical, neurological, and radiological characteristics of all MS patients was done. We could not detect any association between the studied miRNAs and sex, initial presentation symptoms, MRI enhancement, and the number of lesions by MRI. Moreover, no significant correlation was detected between the circulating miRNA expression levels and age, EDSS, and ARR. However, a significant negative correlation was observed between miRNA-191 and disease duration. This encourages regular monitoring of miRNA-191 during the disease course and will help in following up with the patient as this suggests a potential impact on immune mechanisms throughout the whole duration.

Similarly, Vistbakka J. et al., (2018) didn't find a significant relationship between the investigated miRNAs in all MS cases and age, initial presentation symptoms, and EDSS. Contrary to our results, he didn't find a correlation with disease duration [16]. On the other hand, matching our observation on the effect

of disease duration on miRNAs expression, Samandari N. et al., (2018) detected a significant change in the expression of miRNAs with type 1 diabetes progression in children and the observation was associated with variations in cytokine and pancreatic antibodies at different time points throughout the disease course, suggesting a potential impact on immune mechanisms through the whole T1D duration [24]. Assessment of associated cytokines and autoantibodies in MS while testing miRNA expression can help in fully understanding the impact of illness duration on the expression of miRNA.

Regarding miRNA-24, the findings of Ehya F. et al., (2017) did not match with what we found, there was a negative correlation between EDSS and the expression level of miRNA-24 from the MS patients [1].

We also uniquely analyzed the correlation between miRNA-191 and miRNA-24 in MS patients. A positive correlation was detected between the two miRNAs (r = 0.703 and p value < 0.001). Because these two different variables move in the same direction, they theoretically may be influenced by the same factors.

The present study included 80 patients with an unknown history of treatment. A larger study group with a proper history of treatment and continuous follow-up of its effects on miRNA expression may be recommended. Levels of miRNAs were measured only in the serum so further studies should be done on the comparison of miR-191 and miR-24 expression levels in the blood and CSF of patients with MS. Measuring the different cytokines and messenger RNAs that may be affected by the change in miRNA levels would add a great value to the study. Added to that more studies on miRNA-191 and miRNA-24 during relapses and remissions are recommended and more studies on these miRNAs not only in MS patients but also in other autoimmune diseases are also recommended.

The significant ethnic and regional heterogeneity in MS incidence among the various communities may be the cause of the disparity between the findings of the present research and those of previous investigations. The potential circadian rhythmicity of the circulating miRNAs and the bio-oscillations in miRNA biomarker investigations are additional factors [25]. Up to date, only minimal studies on the measured miRNAs in multiple sclerosis are present, so more studies should be researched in the future, in a larger scope and in terms of age, ethnicity, and disease severity to elucidate their level of expression and their role in MS pathogenesis, subgrouping, and disability progression.

Conclusion

To sum up, our study revealed that miRNA-191 and miRNA-24 were expressed in all MS patients with different phenotypes and in the healthy controls, but they couldn't discriminate between these groups. Relationships between the estimated miRNAs and the clinical, neurological, and radiological parameters of MS patients, that denote disease progression and severity, were done. A negative correlation between miRNA-191 and disease duration was detected, so this encourages regular monitoring of miRNA-191 during the disease course. The positive correlation that was detected between the two miRNAs means they theoretically are influenced by the same factors so they can be targeted in the same way. Further investigations are required to detect these factors and their roles in disease pathogenesis. Recent findings suggest that it may eventually be possible to treat some neurological disorders by restoring or inhibiting miRNAs altered by disease pathology. Both viral delivery and administration of modified oligonucleotides mimicking or inhibiting specific miRNAs have been effective in model systems [26].

Abbreviations

| MS | Multiple sclerosis |
|------------|---|
| miRNA-/miR | MicroRNA |
| BDNF | Brain-derived neurotrophic factor |
| IIDD | Idiopathic inflammatory demyelinating disorders |
| MRI | Magnetic resonance imaging |
| CSF | CerebroSpinal fluid |
| DMT | Disease modifying therapies |
| ARR | Annual relapsing rate |
| EDSS | Expanded disability status scale |
| RT | Reverse transcription |
| RRMS | Relapsing remitting multiple sclerosis |
| FC | Fold change |
| NAWM | Normally appearing white matter |
| IFN | Interferon |
| mRNA | Messenger RNA |
| VEGF | Vascular endothelial growth factor |

Acknowledgements

This work was supported by Cairo University. The authors owe a particular depth of gratitude to their colleagues and staff members who taught them how to walk in the depth of knowledge with steady steps and took on their own to support these steps all through the way.

Author contributions

All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by SA, HT, LS, AS and MT. Interpretation of data and the first draft of the manuscript was written by NS and MS and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Funding

The authors declare that no funds, grants, or other support were received during the preparation of this manuscript.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate

The current study had been approved by the Research Ethics Committee of the Faculty of Medicine, Cairo University with ethical Committee approval number MD-220–2020. The study adhered to the tenets of the Declaration of Helsinki. Informed consent was obtained from all individual participants included in the study.

Consent to publication

The authors affirm that human research participants provided informed consent for publication.

Competing interests

The authors have no relevant financial or non-financial interests to disclose.

Received: 5 November 2023 Accepted: 18 December 2023 Published online: 03 January 2024

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