Gender Difference of Serum Micro RNA-155 in Multiple Sclerosis Patients

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Abstract

Background and Objectives

MS is becoming more commonplace globally, and its socioeconomic impact on society is becoming more significant. MS affects females nearly three times more frequently than males; male and female hormonal and genetic variations majorly influence health and disease. MicroRNAs, in particular, are of great interest because they can have a wide-ranging impact on downstream signaling pathways; variations in miRNA expression between men and women can make the biological and physiological disparities between the sexes easier to understand.

Methods

The level of miR-155 in the serum of 30 multiple sclerosis patients (21 females and 9 males) was assessed by real-time PCR.

Results

Serum expression of miR-155 was significantly higher in male MS patients than in females; no significant difference was detected between patients on disease-modifying therapy (DMT) and naïve ones.

Conclusion

MiR-155 sex-dependent differences suggest potential gender-specific roles in neuroinflammation.

Keywords

MiR-155, Multiple Sclerosis (MS), Gender/Sex Difference, DMT.

Abbreviations

MS=Multiple Sclerosis; **miR-155**= microRNA-155; **DMT**= Disease-Modifying Therapy; **PCR**=Polymerase Chain Reaction.

Introduction:

Multiple sclerosis (MS) is a chronic autoimmune disease in which immune cells invade the central nervous system, resulting in demyelination and neurodegeneration (1).

It is a cause of lifelong impairment among young adults (1). The global prevalence of MS is increasing, and the causes for this are unknown. Environmental factors like vitamin D, UVB exposure, EBV infection and genetic vulnerability play a role in MS development (2). The disease's radiological and histological changes, clinical presentation and progression, and therapeutic response vary significantly (3).

Currently, there is no reliable laboratory marker for MS diagnosis. Clinical characteristics, magnetic resonance imaging, and cerebrospinal fluid studies are being used (4). There is an urgent need for new

biomarkers that can aid in early diagnosis and help monitor the response to treatment, which can lead to improved control of the neurodegenerative nature of the disease (5).

MicroRNAs (miRNAs) are small RNA molecules that bind to mRNA to regulate gene expression, either by cleaving the mRNA or reducing the expression of proteins. They are important in post-transcriptional regulation and many biological processes (6). MiRNA synthesis can be dysregulated in both neurological and autoimmune disorders. MS is an autoimmune disorder that causes neurodegeneration. It is worth noting that a recent study has discovered that MS body fluids such as plasma, serum, and cerebrospinal fluid show dysregulation of circulating miRNAs (7).

The most frequently observed miRNA in MS, miR-155, was increased in eight studies involving immune compartments and CNS tissue. MiR-155 has previously been linked to inflammatory diseases and reactions, including immune cell activation, blood-brain barrier disruption, and neurodegeneration (8).

MS is a disease that predominantly affects women. The present sex ratio is 3:1, which has been growing (9). Male and female immunological capacities differ significantly. Innate and adaptive immune responses to poisons and diseases are frequently higher in females than males. Because of their better immune systems, females have a greater survival rate than males. However, there are drawbacks to the greater immune capacity in females, such as women being more likely to react strongly to self-antigens, which increases their vulnerability to most autoimmune inflammatory diseases (10).

Neurodegenerative diseases are related to hormonal control of microRNAs (11). Additionally, a theory suggests that sex chromosomes may control microRNAs in neurodegenerative diseases (12). We aimed to measure the difference in serum miR-155 expression level between males and females in multiple sclerotic patients and to assess the difference in expression level between naïve and patients on disease-modifying therapy (DMT).

Patients and Methods

Patients

Our study included 30 MS patients collected from patients in the neurology department at Assiut University Hospital. The laboratory analysis was done in the Medical Research Centre at Assiut University Hospital.

Each participant received an explanation about the study's aim and methodology, and informed consent was secured. Our study included patients diagnosed as MS patients according to the revised McDonald Criteria 2017 (13), aged 17 to 55 years. Patients with other autoimmune diseases, either neurological or non-neurological, and patients with renal or cardiac comorbidities or malignancy were all excluded.

Ethics Statement

The Ethical Committee of Assiut University's Faculty of Medicine accepted the study protocol (IRB: 17101160).

Analytical Methods

Three ml of venous blood samples were collected from participants in a plain tube. Blood samples were processed within 2 hours of sampling (centrifugation at 3000 rpm for 15 min), and serum was preserved at –80°C until future use.

Estimation of miRNA-155 in serum by real-time polymerase chain reaction

MiRNA Extraction:

MiRNA extraction was done using the MiRNeasy Mini kit (QIAGEN, Cat No: 217004, Germany) according to the manufacturer's instructions, keeping an RNase-free environment in whole steps.

Reverse transcription (cDNA synthesis):

Reverse transcription was done with Applied Biosystems™ High-Capacity cDNA Reverse Transcription Kit (USA).

Quantitative real-time PCR:

Thermo Scientific Maxima SYBR Green/ROX qPCR Master Mix (2X) (Catalogue no. #K0221) was used to prepare qPCR under sterilized conditions in accordance with the manufacturer's

specified protocols. The comparative CT method converted the resulting threshold cycle (CT) values into relative quantities. Next, the measured levels were normalized against housekeeping genes (U6-snRNA) and internal control genes.

Statistics

The data was analyzed using IBM-SPSS 24.0 (IBM-SPSS Inc., Chicago, IL, USA)*. Descriptive Statistics: The mean, standard deviation, median, and range were determined. If appropriate, the normality of continuous variables was determined using the Kolmogorov-Smirnov or the Shapiro-Wilk tests. Student t-test / Mann-Whitney U analysis was used to compare the means and medians of dichotomous data. P-values < 0.05 were considered significant.

Results

Patient Characteristics

Our study included 21 female MS patients and 9 MS males. The mean age of females was 32.10±8.5 years, while males were 31.29±8.2 years; no statistically significant difference was detected between the two groups. 11 MS patients were on DMT, and 19 were without treatment. The age and gender distribution of the patients are shown in Table 1, Figure 1.

Table (1): Patient Demographics

	Female (n=21)	Male (n=9)	P-value
Age/years			
Mean ± SD	31.29±8.2	34±9.4	=0.435* NS
Median (R)	30 (21-55)	33 (17-48)	

*Independent Samples T-test was used to compare the mean difference between groups, SD=standard deviation, R=range, NS=not significant

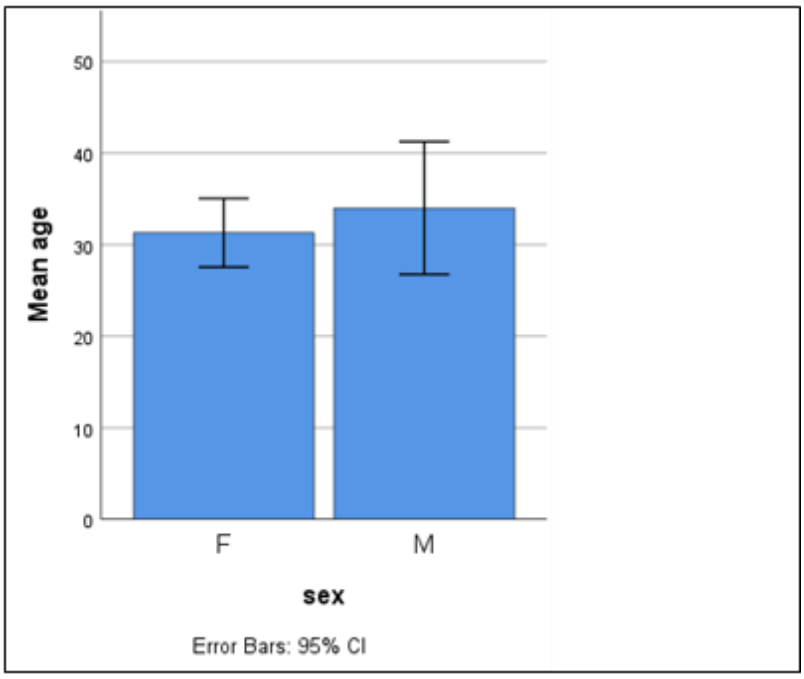

Fig.1 Age distribution of MS patients.
F=females, M=males

Gender difference of MiR-155 expression (Table 2, Figure 2)
The median fold change in females was 7 (2.4-76.6), while in males it was 28 (3.9-44.3). Higher levels of miR-155 were detected in males compared to females (p-value = 0.005).

Table (2): Serum expression of miR-155 between males and females

	Female (n=21)	Male (n=9)	P-value
MiR-155 fold change			
Median (R)	7 (2.4-76.6)	28 (3.9-44.3)	=0.005*

*Mann Whitney U-test was used to compare the median differences between groups
miR=microRNA-155, R= range.

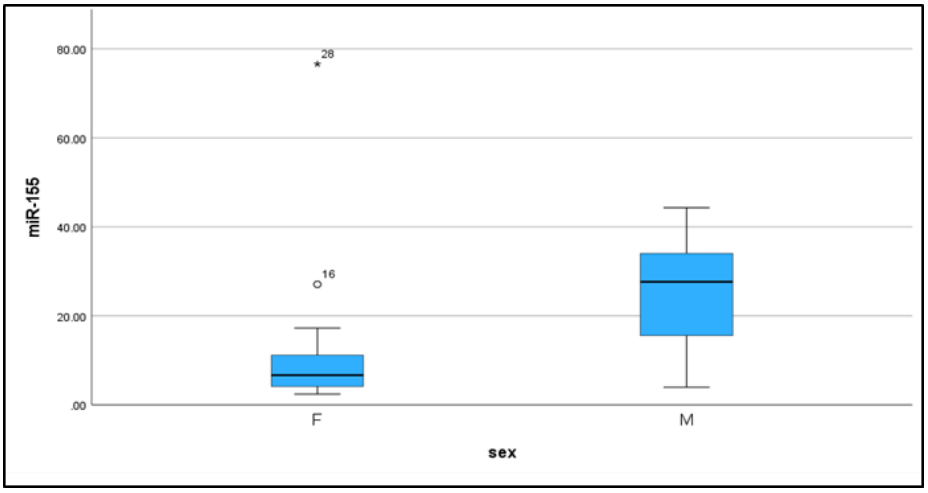

Fig.2 MiR-155 fold change between MS patients.
F=females, M=males

Difference of miR-155 expression levels with treatment (Table 3, Figure 3)
The median fold change of miR-155 was 7.5 (2.5-16) in patients on DMT and 11 (3-76.5) in those without treatment. The comparison yielded a P-value of 0.232, indicating no statistically significant difference.

Table 3: Relationship between treatment and miR-155 expression among MS cases

	Patients on DMT (n = 11)	No DMT (n = 19)	P-value
miR-155 Fold Change			= 0.232* NS
Median (R)	7.5 (2.5-16)	11 (3-76.5)	

*Mann Whitney U-test was used to compare the median differences between groups: miR-155= microRNA-155, DMT=disease modifying therapy, R=range, NS=not significant.

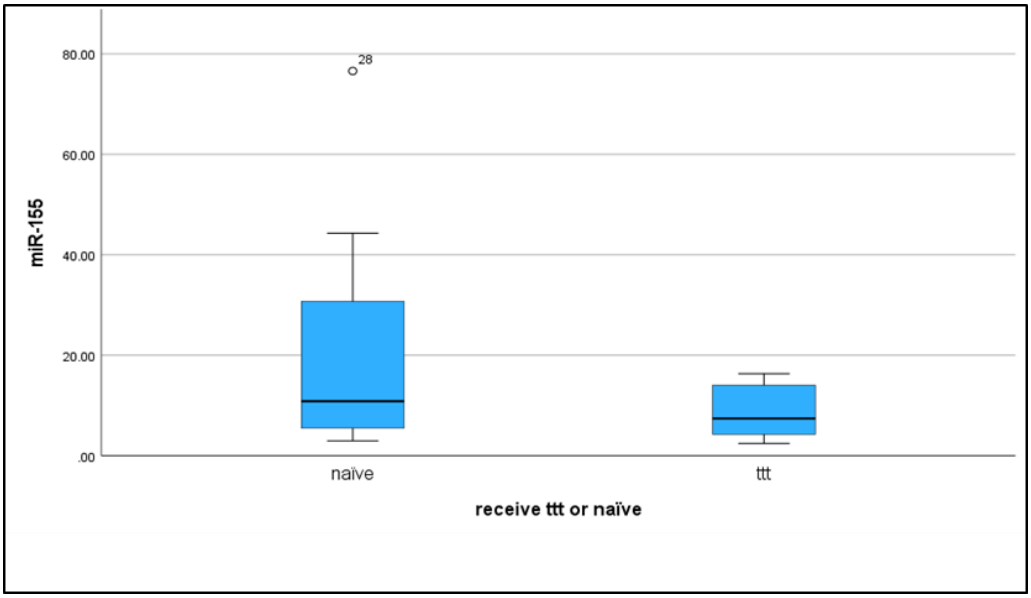

Fig.3 Difference in miR-155 expression with treatment

Discussion

Multiple sclerosis (MS) is a prevalent cause of neurologic disabilities that negatively impacts a patient's quality of life and productivity (14).

Our study's findings demonstrated that women are more likely than men to have MS, with 70% of female patients compared to male patients. This finding supports the widespread belief that women are more likely than men to develop MS (15). As a result, considerable research has been done on the immunological and neurological systems of males and females. These differences may be caused by gonadal hormones, genetic variants, and the different environmental exposures and modern lifestyles that men and women lead (16). The expression levels of several genes and miRNAs can be greatly influenced by a second X chromosome in females, which may be essential for the emergence of female-biased autoimmunity (17).

Our study revealed a significant difference in the serum expression miR-155 between males and females (P= 0.005). Our findings are supported by data on miR-155, a powerful activator of inflammation and an important microRNA in the pathogenesis of autoimmune diseases due to its influence on myeloid cell polarization to a phenotypic and functional pro-inflammatory form (18, 19), the higher level in males may be attributed to that men. However, having a reduced risk of developing MS, they are more likely to have the progressive type of the disease if they do (20) and are expected to fare worse than women. Additionally, they show worse rates of brain volume loss, worse cognitive impairment, slower recovery from MS relapses, and higher development of disabilities (21). Prior research also revealed the function of miR-155 in many pathophysiological MS processes and symptoms, which may also signal a severe condition course and a poor prognosis in MS patients (22-24).

The role that microRNAs play in influencing sex biases in disorders is not

well understood or acknowledged (25, 26). There is limited information specifically addressing the difference in miR-155 expression between males and females in MS. Numerous human research have identified sexually dimorphic variations in miRNAs in patients with amyotrophic lateral sclerosis (ALS), frontotemporal dementia (FTD), and Parkinson's disease (PD). MiR-155 was one of a group of miRNAs involved in ALS that was shown to be significantly upregulated in males than in females (27). Recently, it was shown that numerous inflammatory miRNAs in brain CD11b+ cells isolated from naïve and traumatic brain injury (TBI) mice showed sex-specific changes and greater levels of miR-155 were observed in male brain CD11b+ cells derived from naïve mice (28). In contrast, among the lupus-related miRNAs, female NZB/WF1 mice were shown to have considerably higher levels of miR-155 than male mice (29).

Lower levels of miR-155 were observed in patients on DMTS compared to naïve patients, but the difference was not statistically significant. Consistent with our findings, Keller et al. revealed that IFN-β and glatiramer acetate did not affect miR-155 expression levels (30). In contrast to our findings, Giuseppe Mameli et al. detected significant down-regulation of miR-155 and miR-26a in MS patients before and after six months of natalizumab therapy (31). One possibility is that the sample size of our study may have been relatively small, which may have limited the statistical power of the analysis in detecting significant differences. Other confounding factors were found, such as disease severity or duration differences between the two groups. This could have affected the biomarker levels and masked any differences that may have been present.

It's also crucial to remember that clinical importance and statistical significance are not always the same. Even if the differences observed between the DMT and naive groups were not statistically significant, they may still be clinically meaningful. Therefore, further studies with larger sample sizes and more comprehensive evaluations of biomarkers and clinical outcomes are required to understand better the relationship between treatment and diagnostic biomarkers in MS.

Conclusion

Considering sex as a potential factor in MS diagnosis and management is important, and further research is recommended to understand the mechanisms and clinical implications of sex-related differences in miRNA levels.

Conflicts of Interest

No conflicts of interest exist.

Acknowledgments

Not Applicable.

Declarations:

I state that the thesis is an original report of my research, that I wrote it, and that I haven't submitted it for consideration for another degree or professional certification.

Consent for Publication:

All authors agreed to submit the work for publication.

Availability of Data and Materials: Available.

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