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Micro-RNA-96 and interleukin-10 are independent biomarkers for multiple sclerosis activity

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ABSTRACT

Background: Micro-RNAs (miRNAs) are evolving as biological markers for multiple sclerosis (MS) both in activity and remission. miR-96 is associated with remission, however, the exact mechanism through which it contributes to the anti-inflammatory pathway is not clear.

Objective: To study the expression of miR-96 and IL-10 (anti-inflammatory mediator) in relapsing remitting (RR) MS.

Subjects and methods: A case control study including 32 RRMS patients from Kasr Al-Ainy MS clinic, Cairo University, Egypt, and 26 healthy controls (HC). Assessment of serum IL-10 by ELISA, and miR-96 via real time PCR was done during relapse and remission in patients, and in HC.

Results: IL-10 was higher in RRMS patients during remission and in HC compared with relapse ($P < 0.001$). miR-96 expression was higher in RRMS patients during remission compared with relapse and HC, and was higher in HC than in relapse ($P < 0.001$). IL-10 level in remission correlated positively with disease duration ($r = 0.41$; $P = 0.02$). Otherwise, no correlation was found between IL-10 and relapse number or EDSS ($P > 0.05$). miR-96 in relapse negatively correlated with EDSS in relapse ($r = -0.47$; $P = 0.007$), but no correlation was found with disease duration or relapse number, whereas, miR-96 in remission did not correlate with any clinical parameters ($P > 0.05$). No correlation was found between IL-10 and miR-96 either in relapse or remission ($P > 0.05$).

Conclusion: IL-10 and miR-96 are associated with MS quiescence, however, the lack of a significant correlation between them implicates that the influence of miR-96 may be exhibited through some pathway other than IL-10.

1. Introduction

Multiple sclerosis (MS) is an autoimmune-mediated disorder that affects the central nervous system (CNS) and often leads to physical or cognitive incapacitation in young adults [1]. The etiology of MS is considered multifactorial including a genetic predisposition combined with environmental factors [2]. Inflammation is the hallmark of relapsing remitting (RR) MS whose patients exhibit a complex system of inflammatory cytokines comprising pro-inflammatory, anti-inflammatory, and regulatory cytokines. Interleukin (IL)-10 is a pleiotropic, immunoregulatory cytokine which was initially characterized as a T helper (TH)2 specific cytokine [3,4]; however, further investigations revealed that IL-10 production was also associated with T-regulatory (Treg) cell responses and functions to promote immune homeostasis [3,5]. In MS patients, low levels of IL-10 mRNA in peripheral blood monocytes (PBMC) are associated with relapseand with secondary progressive disease [6,7].

Although dysregulation of microRNAs (miRNAs) expression has been characterized mostly in cancer, it has recently been studied in many other diseases. Specifically, playing a role as a key player in the pathogenesis of autoimmune and neurodegenerative diseases [8–10],as some of the functions attributed to the miRNA include stress response, immunomodulation and neuroprotection [10].

Recent literature has addressed the role of miRNAsin MS pathogenesis in relation to relapses, remissions, response to disease modifying therapies, and lesion pathology.[11]miRNAs are noncoding single-

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stranded RNA molecules that are pivotal in cellular and developmental processes by regulating gene expression at the post-transcriptional level. They suppress the expression of protein-coding genes by directing translational repression through base-pairing with complementary messenger RNA (mRNA) and/or by promoting degradation of target mRNA degradation [12,13].

Studies on the network wide role of miRNA have revealed that miRNAs are involved in T and B cell differentiation in the thymus and bone marrow, respectively [14].

Up to one half of innate immune genes are predicted to be under the direct regulation of miRNAs. With the capacity of miRNAs to regulate the survival and death of T and B cells, control over miRNA expression is essential to prevent adaptive immune cells from unregulated proliferation leading to cancer or autoimmunity [9,15].

Both T and B cells maturation, proliferation, differentiation, and activation are complex processes tightly controlled by different mechanisms including miRNA mediated posttranscriptional gene regulation [14]. The expression levels of all components involved in adaptive immune responses (cell surface receptors, transcription factors, cytokines, and their receptors) may be controlled by miRNAs. Moreover, miRNAs have been implicated in the development and function of natural killer (NK) cells which play an important role in the immune surveillance against viral infection and caner [16].

Dysregulation of a single or a few miRNAs or miRNA clusters can result from hormonalchanges, genetic variation, or environmental triggers including infections. Disruption of the entire miRNA network or specific miRNAs may lead to dysregulated immune system components including B cells, Th17, T-reg cells and inflammatory cytokines [8,16]. Additionally, Endogenous tissue repair mechanisms in MS such as gliogenesis, neurogenesis and myelin repair may also be modulated by specific miRNAs. Besides, complete loss of miRNA expression in the brain leads to neurodegeneration in several animal models as well as in human patients [17,18].

A network-based approach identified 4 miRNA (hsa-mir-96, hsa-mir-148a, hsa-mir-184 and hsa-mir-193) that could be interesting candidates related with the remission stage [11]. The proposed involvement of miRNA dysregulation in MS may give insights into the pathophysiology of MS and opens up a new therapeutic approach to explore and highlight some candidate biomarker targets in MS. [19,20]

This study aims at evaluating the level of expression miR-96 in relation to IL-10 in RRMS patients at time of relapse and during remission.

1.1. Study design and population

This is a case-control study carried out on 32 patients with recently diagnosed RRMS and 26 healthy controls (HC) matched for age and sex. They were recruited from Kasr Al-Ainy multiple sclerosis research clinic, Cairo University hospitals, Egypt, in the period from February 2016 to July 2017. Written informed consent was obtained from all patients and controls. The study was approved by ethical committee board of neurology department, Cairo University.

Included patients were diagnosed as RRMS according to the revised McDonald's criteria, 2010 [21]. They were evaluated at time of relapse and during remission. Relapse was defined as new symptoms appearance or worsening of previous symptoms over a minimum of 24 h and separated from a previous attack by at least 30 days. None of our patients was on disease modifying therapy (DMT) at time of study initiation.

Subjects were excluded if having other autoimmune disease, malignancy, diabetes mellitus, hypertension, cerebrovascular disease, any inflammatory or infectious diseases during the previous month, or pregnant females.

1.2. Method

1.2.1. Neurological assessment

The neurological disability status of the patients was evaluated using Expanded Disability Status Scale (EDSS) [22] at time of relapse (within 1 week from relapse onset) and during remission 3 months later.

1.2.2. Laboratory assessment

Five ml of venous blood were collected from all subjects. Patients'samples were collected during relapse, before receiving intravenous methylprednisolone (IVMP), and during remission, 3 months after receiving IVMP. Each blood sample was left for 30 min at 37 °C, and then centrifuged at 3000 rpm for 10 min. Sera were divided into 2 aliquots and were stored frozen at-80 °C (for miR-96) −20 °C (for IL-10) until use. Serum interleukin-10 was measured by ELISA using komabiotech, Inc., Korea, according to the manufacturer's instructions. Serum miR-96 was extracted using Real Time PCR kits manufactured by Stratagene, Qiagen, USA [23].

1.3. Statistical methods

The data were coded and entered using: the statistical package for social science version 15 (SPSS v 15). Student *t*-test was used for comparison between means of two groups of quantitative variables. Chi square test was used for comparison between two groups of categorical data or frequency of events. Paired sample t-test was used for comparison between means of two paired groups of quantitative variables. ANOVA test was used for comparison between means of three groups of quantitative variables. The Pearson correlation coefficient (r) was used to describe the degree of relationship between two variables. The sign of correlation coefficient (+,-) defines the direction of the relationship, either positive or negative. The probability/significance value (*P* value) ≥ 0.05 is not statistically significant and < 0.05 is statistically significant.

2. Results

Study groups demographics and disease characteristics of RRMS patients are shown in Tables 1–2 respectively. More than one third of the study cohort presented with motor weakness, and showed a relapse associated worsening (RAW) resulting in an EDSS of 4–6 at 3 months.

Regarding serumIL-10, its mean level was statistically significantly higher in RRMS patients during remission and HC compared with relapse (Table 3 & Fig. 1). As for miR-96, its expression was significantly higher in RRMS patients during remission compared with relapse and HC, and was significantly higher in controls than in RRMS patients in relapse (Table 3 & Fig. 2).)

2.1. Correlative results of IL-10

Only IL-10 level in remission correlated positively with disease duration ($r = 0.41$; $P = .02$). Otherwise, no correlation was found between IL-10 in either relapse or remission with total relapse number ($r = -0.321$; $P = .073$; $r = 0.319$; $P = 0.075$) or EDSS in relapse and remission ($r = 0.09$, $P = .62$; $r = 0.071$; $P = .64$ and r = 0.07, $P = .67$; $r = -0.07$, $P = .76$ respectively).

Table 1
Demographic characteristics of patients and controls.

Demographics		Patients ($n = 32$)	Controls ($n = 26$)	*P*-value
Age (y) range/mean (SD)		18–37/31.07(7.5)	18–35/35.7(13.7)	0.282
Sex	Male n (%)	20 (62.5%)	19 (73.1%)	0.47
	Female n (%)	12 (37.5%)	7 (26.9%)	

Table 2
Disease characteristics of multiple sclerosis patients.

	Range			Mean (SD)	Median
Disease duration (months)	1–13			5.00 (3.45)	5
Relapse number	1–3			1.66 (0.70)	2
EDSS during relapse	2.5–6.5			4.06 (1.44)	3.5
EDSS during remission	1–6	1–3.5	n = 21 (65.6%)	3.14 (1.93)	2
		4–6	n = 11 (34.4%)		
Presenting symptoms no. (%)					
Motor weakness	12 (37.5%)				
Sensory impairment	7 (21.9%)				
Optic neuritis	6 (18.8%)				
Cerebellar ataxia	5 (15.6%)				
Diplopia	2 (6.2%)				

2.2. Correlative results of miR-96

miR-96 in relapse showed a negative correlation with EDSS in relapse ($r = -0.47$; $P = .007$ respectively), but no correlation was found with total relapse number or disease duration ($r = -0.31$; $P = .088$; $r = -0.27$; 0.13). On the other hand, miR-96 in remission did not correlate with any clinical parameter ($r = 0.13$; $P = .48$ for disease duration; $r = 0.17$; $P = .353$ for relapse number; and $r = 0.30$; $P = .09$ for EDSS during remission).

2.3. Correlative results of study biomarkers

No correlation was found between IL-10 and miR-96 either in relapse or remission ($r = 0.09$, $P = .63$; $r = -0.17$, $P = .88$ respectively).

3. Discussion

Regarding the clinical characteristics, around one third of the study population showed an EDSS of 4–6 during the remission phase, though the disease duration was on the maximum 13 months. The reported high EDSS in such short duration reflects the aggressive nature of the disease in a number of cases. Motor and cerebellar deficits were the main presenting symptoms in almost half of our cohort, and both symptoms are predictors for poor outcome and aggressive disease course [24].

Hamdy et al., in their registry of Egyptian MS patients, have found that motor symptoms were the initial presentation in 43.9% of the studied population followed by sensory, visual manifestations and ataxia (33%, 28%, and 22%, respectively) [25]. Our findings were also in agreement with several studies previously conducted in the Middle East & North Africa (MENA) region [26–28].

In this study, IL-10 was higher in RRMS patients in remission than in relapse, which goes witha number of studies showing significant decrease in IL-10 during the relapse phase compared with remission [6,7]. A study evaluating how the therapeutic effect of interferon beta is mediated showed increase in IL-10 secreting cells after 1 year of treatment [29]. IL-10 promotes the development of an anti-inflammatory cytokine pattern by inhibiting the synthesis of many Th1-cell-related cytokines (IFN-gamma, TNF-alfa, interleukins (ILs)-1, −2, −6, and − 17) and T-cell proliferation, thus it does contribute to the remission phase of the disease [30].

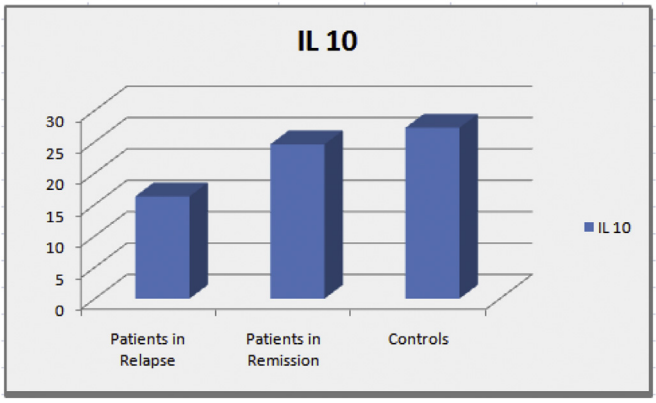

Fig. 1. IL-10 in RRMS patients and healthy controls.

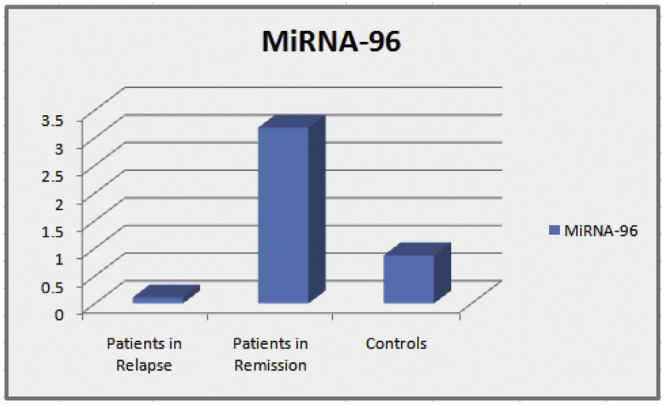

Fig. 2. miR-96 in RRMS patients and healthy controls.

As for miR-96, our results revealed thatits expression was higher in RRMS patients in remission than in relapse as well as in controls, and was significantly higher in controls than in RRMS patients in relapse. Many miRNAs have been studied in MS, however, the studies addressing miRNA-96 are scarce. In accordance with our results, Otaegui et al. showed that miRNA-96 is more expressed in remitting samples than in controls, and less in relapse samples than in remitting. So, they concluded that miR-96 might be characteristic of the remitting phase of the disease. The genes targeted by miR-96 are thought to be involved in immunological pathways such as interleukin signaling [11].

Profiles of blood cells and CNS lesions of MS patients have revealed that miRNA expression is dysregulated in MS. [31] In whole blood of MS patients, 165 miRNAs were reported to be significantly up- or down-regulated and miR-145 was identified as a highly sensitive, specific, and accurate miRNA [32], also, significantly decreased levels of miR-17 and miR-20a have been detected in all MS subtypes compared with normal

Table 3
IL-10 and miRNA-96 in MS patients and healthy controls.

Biomarker	IL-10			miR-96		
Group	RRMS patients in Relapse	RRMS patients in Remission	Controls	RRMS patients in Relapse	RRMS patients in Remission	Controls
Range	7.27–35.89	8.97–46.85	8.29–46.87	0.002–0.50	1.84–6.82	0.03–1.38
Mean(SD)	16.29 (9.62)	24.59 (11.52)	27.22(10.3)	0.108 (0.15)	3.18 (1.41)	0.87(0.47)
Median	11.49	24.56	27.74	0.035	2.47	1.05
95% CI	12.83–19.77	20.44–28.75	23.06–31.38	0.06–0.16	2.68–3.69	0.68–1.06
P- value	$<0.001_*$			$<0.001_*$		

* Statistically significant.

individuals [33]. miR-155 overexpression has also been shown to be associated with MS pathogenesis [34].

Studies isolating peripheral blood mononuclear cells (PBMCs) from MS patients during both relapses and remissions, have identified differential expression of miR-18b, miR-493, and miR-599, which have been implicated in interleukin signaling. miR-21, miR-146a, and miR-146b have also been reported to be significantly increased in RRMS patients compared with controls [35].

A recent multicenter study showed that5 miRNAs (miR-484, miR-140-5p, miR-320a, miR-486- 5p, and miR-320c) had a significant difference between patients with MS and healthy individuals; among these, miR-484 remained significant after accounting for multiple comparisons. But, no comparison was done between patients in relapse and remission. Four miRNAs (miR-320a, miR-337-3p, miR-199a-5p, and miR-142-5p) correlated with disability measured by the EDSS, where miR-337-3p, miR-199a-5p, and miR-142-5pshowed a negative correlation and miR-320a positively correlated. miR-337-3p was the most statistically significant miRNA that correlated with the EDSS in three of the MS cohorts tested [36].

In our cohort, miR-96 in relapse also correlated negatively with EDSS during relapse. This finding may point to a potential role of this miRNA dysregulation, namely under-expression, not only in the inflammatory process contributing to relapse occurrence, but also to relapse severity.

Though both IL-10 and miR-96 expression were higher in RRMS patients in remission compared with relapse, yet no significant correlation between biomarkers during relapse or remission was found. The lack of such correlation may indicate that the influence of miR-96 might be exhibited via another anti-inflammatory pathway rather than IL-10 signaling.

3.1. Limitations

Though miRNAs constitute an enormous number with influences on many immune factors, yet, this study investigated only a single miRNA, as it was self-funded.

Another limitation was that MS patients were DMD naïve, so the effect of DMDs on miRNA expression was not tested. However, this was intended to see the miRNA and cytokine levels independent from the potential effect of DMDs.

3.2. Strengths

This work investigated two biological factors that are involved in aborting the dysregulated immune response in MS; the miR96 which may be involved in switching off the whole dys-immune cascade, and IL-10, the regulatory and anti-inflammatory cytokine, that plays a crucial role in balancing the immune system and paving the way to the remission phase.

4. Conclusion

This work highlights the significance of miRNA expression in the molecular mechanisms implicated in MS disease pathogenesis. miR-96 was found to be associated with disease remission and lower relapse number and relapse severity. Lack of a significant correlation between miR-96 and IL-10 implicates that the influence of miR-96 may be exhibited through another pathway other than IL-10.

4.1. Recommendations

1. Studying the pattern of MS in Egyptian patients to find out whether this ethnicity is characterized by more aggressive forms of the disease.
2. A broader analysis of miRNAs expression could highlight further candidate miRNAs involved in MS pathology viastudying the role of miRNAs in regulating the expression of proteins involved in immune responses, intracellular signaling, modulation of chemokines and cytokines, costimulatory and adhesion molecules.
3. Designing a longitudinal study to test the effect of DMDs on the levels of the studied biomarkers.
4. Analysis of the expression of miRNAs in both active and inactive MS lesions and control white matter in post-mortem brain tissue samples.
5. Comparing miRNAs expression changes in multiple sclerosis versus other demyelinating disorders including ADEM and neuromyelitis optica spectrum disorder (NMOSD).
6. Studying the expression of miRNAs in CSF of MS patients with radiologically active disease.
7. Correlating miRNAs expression changes with different inflammatory and neurodegenerative biomarkers in MS.

4.2. Clinical implications

Generally, understanding the role of miRNAs in MS will help the use of miRNAs as promising diagnostic biomarkers for assessment of disease risk, differentiating disease subtypes, monitoring disease progression, predicting treatment responses to DMDs and innovation of potential therapeutic agents that tackle the disease process from the origin. As for this study, we may suggest implementing the miRNA-96 and IL-10 as biomarkers to differentiate true from pseudo-relapses.

Disclosure

Authors report no conflict of interest.
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