STUDY OF THE EXPRESSION OF MICRORNA 181A AND ITS TARGET GENES: BCL2 AND NOVA1 IN MULTIPLE MYELOMA

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Introduction

Multiple myeloma (MM) is a plasma cell neoplasm accounting for 10% of hematological malignancies. It is characterized by clonal proliferation of plasma cells in the bone marrow resulting in monoclonal M protein production. This ultimately results in end organ damage presenting as: C (Hypercalcemia), R (Renal failure), A (Anemia), B (bone lesions).

MM is characterized by its complex genetic landscape, that tends to change over time owing to additional events, as somatic mutations, epigenetic and chromosomal copy-number changes.

The role of micro-RNAs, small non-coding RNAs, in myelomagenesis has been receiving increasing attention. They are found to manipulate the cross talk between myeloma cells and bone marrow micro-environment and thereby playing their role.

Studies have shown that miR-181a may play a role in lymphocyte proliferation and differentiation into B cells. At the same time, it has been suggested that the expression of miR-181a is increased in MM. The complex relationship between miR-181a and the various clinical and pathological factors of MM disease is yet to be clarified.

Aim of the work

The aim of this study was to investigate the expression patterns of miR-181a and its target genes: BCL2 and NOVA1 in multiple myeloma as well as their relations with different clinicopathological parameters.

Subjects and Methods

Subjects :

After approval of the Alexandria ethics committee, this study was conducted on 100 subjects including 50 newly diagnosed patients with plasma cell myeloma, and 50 matched age and sex control group.

Patients were diagnosed according to the international myeloma working group (IMWG) criteria.

Methods:

Total RNA, including miRNA, isolation from bone marrow samples was carried out using miRNeasy Mini kit (Qiagen, Maryland , USA).

miRNA 181a expression was studied using two-step RT-PCR. cDNA was reverse transcribed from RNA samples using specific miRNA primers from the TaqMan MicroRNA Assays and reagents from the TaqMan MicroRNA Reverse Transcription Kit (Applied Biosystems™, USA) using SimpliAmp thermal cycler. This was followed by relative quantification performed by TaqMan Fast Advanced MasterMix (Thermo Scientific, USA) using stratagene MX3000P PCR system.

For BCL2 and NOVA1 reverse transcription was done using High capacity cDNA Reverse Transcription Kit (Applied Biosystems™, USA) and relative quantification was performed using Maxima SYBR Green qPCR Master Mix (Thermo Scientific, USA), using stratagene MX3000P PCR system.

Results

There was a statistically significant difference between the MM cases and the controls regarding the expression level of miRNA 181a and it target genes:BCL2 and NOVA1.

miRNA181a expression in MM cases ranged from 1.52 to 22.78 with a median of 4.57, while in control group its expression ranged from 0.46 to 5.43 with a median of 0.84 and the difference was statistically significant (p<0.001).Table(1)

BCL2 gene expression level in PCM patients ranged from 0.60 to 293.25 with a median of 27.88, while in the control group it ranged from 0.78 to 5.88 with a median of 1.43 and the difference was statistically significant (p<0.001).Table(2)

NOVA1 gene expression level in PCM patients ranged from 0.21 to 33.59 with a median of 0.88, while in the control group it ranged from 0.21 –to 1.49 with a median of 0.50 and the difference was statistically significant (p<0.001).Table(3)

Table 1: Comparison between the two studied groups according to miRNA181a expression

miRNA181a	Cases (n=50)		Control (n=50)		Test of Sig.	p
	No.	%	No.	%		
Low (<4.57)	25	50.0	45	90.0	χ^2= 19.048*	<0.001*
High (≥4.57)	25	50.0	5	10.0		
Min. – Max.	1.52 – 22.78		0.46 – 5.43		U= 145.0*	<0.001*
Mean ± SD.	5.50 ± 3.74		1.33 ± 1.41			
Median (IQR)	4.57 (2.91–7.11)		0.84 (0.68–1.31)			

χ^2: **Chi square test** **U: Mann Whitney test**

p: p value for comparing between **the two studied groups**

*: Statistically significant at $p \leq 0.05$

Table 2: Comparison between the two studied groups according to BCL2 expression

BCL2	Cases (n=50)		Control (n=50)		Test of Sig.	p
	No.	%	No.	%		
Low (<27.88)	25	100.0	50	100.0	χ^2= 33.333*	<0.001*
High (≥27.88)	25	100.0	0	0.0		
Min. – Max.	0.60 – 293.25		0.78 – 5.88		U= 155.0*	<0.001*
Mean ± SD.	49.87 ± 60.81		1.94 ± 1.49			
Median (IQR)	27.88 (11.28–65.62)		1.43 (0.89–2.40)			

χ^2: **Chi square test** **U: Mann Whitney test**

p: p value for comparing between **the two studied groups**

*: Statistically significant at $p \leq 0.05$

Table 3: Comparison between the two studied groups according to NOVA1 expression

NOVA1	Cases (n=50)		Control (n=50)		Test of Sig.	p
	No.	%	No.	%		
Low (<0.88)	25	50.0	45	90.0	χ^2= 19.048*	<0.001*
High (≥0.88)	25	50.0	5	10.0		
Min. – Max.	0.21 – 33.59		0.21 – 1.49		U= 650.0*	<0.001*
Mean ± SD.	3.66 ± 6.29		0.55 ± 0.35			
Median (IQR)	0.88 (0.52–3.43)		0.50 (0.30–0.63)			

χ^2: **Chi square test** **U: Mann Whitney test**

p: p value for comparing between **the two studied groups**

*: Statistically significant at $p \leq 0.05$

Conclusion

The expression level of miRNA181a and its target genes was significantly different between the patients and control group. The diseased group showed upregulation of the expression of miRNA181a as well as its target genes BCL2 and NOVA1.