

Review Report Form #2	Yes	Can be improved	Must be improved	Not applicable
Does the introduction provide sufficient background and include all relevant references?	(x)	()	()	()
Are all the cited references relevant to the research?	(x)	()	()	()
Is the research design appropriate?	(x)	()	()	()
Are the methods adequately described?	(x)	()	()	()
Are the results clearly presented?	()	(x)	()	()
Are the conclusions supported by the results?	(x)	()	()	()
Comments and Suggestions for Authors				

The paper presented for review concerns the potential use of miR-491-5p and miR-592 levels as a biomarker of the overactive bladder syndrome. The authors point to increased levels of microRNAs in patients compared to controls. The reviewer was interested in the issue of patients suffering from OAB.

We would like to extend our thanks for the insightful comments that helped to improve the manuscript.

I believe it requires a longer description of the symptoms in the introduction.

We thank the reviewer for this constructive comment. Symptoms and description of OAB were improved in the introduction, on page 1.

The authors indicated that samples from patients with and without treatment were selected for the study. The reviewer asks for an explanation of the differences in the levels of the examined indicators in both groups. Did the treatment significantly affect the obtained results?

We thank the reviewer for this comment. The main objective of the study was to assess the diagnostic value to these miRNAs. Future work will be done to validate these miRNA as potential biomarkers (for diagnosis and prognosis). Therefore, we collected only one sample of urine that matches the questionnaire response completed the day of urine collection. Comparisons were done between treated and untreated OAB patients (12 vs 8, respectively) and showed no difference in the questionnaires' scores nor the voiding diary (validated means to assess OAB symptoms), except for a significantly lower nocturnal urine volume in the treated OAB participants (Mossa et al, 2020). The miRNA comparisons between the two categories within the OAB patients (i.e. treated and untreated) showed no significant difference (as shown in the following table). We did not make measurements before and after treatments, neither did we measure the biological variation as we had only one sample (one date) per patient. So, we believe that OAB treatment did not affect the obtained results.

miRNA	Treatment within OAB group	Mean	Std. Deviation	P value (t-test)
miRNA592	Non-treated	0.42	0.17	0.99
	Treated	0.42	0.25	
miRNA132	Non-treated	0.66	0.55	0.48
	Treated	0.90	0.78	
miRNA491_5p	Non-treated	0.21	0.29	0.89
	Treated	0.23	0.29	
Let_7d	Non-treated	0.74	0.64	0.82
	Treated	0.66	0.73	
miR21_5p	Non-treated	0.76	0.56	0.56
	Treated	0.97	0.87	
Let_7b	Non-treated	1.30	1.54	0.73
	Treated	1.07	1.21	
miR98_5p	Non-treated	1.07	1.23	0.82
	Treated	1.24	1.65	
miR221_5p	Non-treated	1.38	1.32	0.81
	Treated	1.26	1.07	
miR212_5p	Non-treated	0.20	0.27	0.44
	Treated	0.33	0.35	
mir92a_3p	Non-treated	1.09	1.88	0.48
	Treated	1.78	2.14	
miR885_5p	Non-treated	0.10	0.11	0.09
	Treated	0.50	0.61	

Have the authors considered enlarging the group of patients and treating them separately - those with and without treatment? The treatment used can significantly affect the results.

Given the absence of an effect of the treatment on the tested miRNAs in the pilot sample (as shown above), we think that treatment is not a significant confounder in the observed difference. However, to expand this study, we are currently recruiting more patients to include a larger sample size as well as including both female and male participants aiming to explore and verify the effect of treatment on these potential biomarkers. We consider the effect of treatment in our expanded analysis. We are also going to compare the treated vs untreated patient in the same group (OAB) to ensure there are no influence on our data.

Apart from the indicated doubts, the work is very interesting.

Thank you for your thoughtful comments and efforts towards improving our manuscript.