

## **M1 and M2 Muscarinic receptor antibodies among patients with Postural Orthostatic Tachycardia Syndrome: potential disease biomarker**

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**Background:** Postural tachycardia syndrome (POTS) is characterized by exaggerated orthostatic tachycardia. Etiopathogenesis of POTS still remains unclear. We tested the hypothesis that patients with POTS have serum muscarinic receptor antibodies which may be contributing to clinical syndrome.

**Methods:** Sixteen POTS patients and twenty controls were examined for M1, M2 and M3 receptor antibodies by cell-based assay (immunofluorescence staining of CHO-K1 cells transfected with cDNA encoding M1, M2 and M3 receptors). The patients were selected based on clinical presentation and results of Autonomic testing (significant elevation of heart rate on tilt table testing). Untransfected CHO-K1 cells were also used as negative controls. Visual determination of positive or negative immunofluorescence was done by two independent observers. Quantification of fluorescence was performed using Image J software. Patients' demographic, clinical and laboratory data was also collected.

**Results:** Of the 16 POTS patients, 15 were females, while 55.5% (10) of controls were females. Among the controls; 4 were normal individuals; the remaining 16 had an underlying neurological conditions (cholinergic autonomic neuropathy, autoimmune epilepsy, Lambert-Eaton syndrome, non-specific dysautonomia, Multi-System Atrophy). Significantly higher proportions of POTS patients had M1 (87.5% vs 10%,  $p < 0.001$ ) and M2 (68.75% vs 15%,  $p < 0.005$ ) muscarinic receptor antibodies compared to the controls. M3 muscarinic receptor antibodies were detected in two POTS patients and two controls. Image J analysis also showed that serum from POTS patients had significantly higher fluorescence on M1 ( $p < 0.01$ , CI 8.38-8.45) and M2 ( $p < 0.001$ , CI 4.74-4.80) transfected cell lines compared to the controls. There was also a significantly higher fluorescence of POTS patients' serum on M1 ( $p < 0.001$ , CI 7.01-7.44) and M2 ( $p < 0.001$ , 3.01-3.37) transfected cell lines compared to untransfected CHO-K1 cells. Among the patients who had autonomic testing (20), presence of M3 receptor antibody was associated with abnormal QSART results ( $p < 0.01$ ). A high proportion (66.67%) of patients with cognitive changes had detectable M1 muscarinic receptor antibody ( $p = 0.245$ ). Among these 16 POTS patients, only 3 reported preceding infection or viral prodrome before symptom onset. Most of the patients (68.8%) had gradual ( $> 3$  months) onset and progression of symptoms.

**Conclusion:** M1 and M2 receptor antibodies can be detected in significant number of POTS patients. These antibodies may play an important role in the etiopathogenesis of this clinical syndrome, but further studies are needed to determine the significance of these antibodies.