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Mini Review

Biomarkers in Overactive Bladder

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Nerve growth factor (NGF), brain– derived neurotrophic factor (BDNF), cytokines, stem cell factor *etc*. are some promising biomarkers for overactive bladder (OAB). To diagnose OAB urinary NGF/Creatinine (Cr) has 87.5% sensitivity and 100% specificity, and BDNF/Cr has 87.5% sensitivity and 83.3% specificity. In female OAB patient, urinary MCP-1 (monocyte chemotactic protein) was very high. In plasma 5 biomarkers IL-4 (Interleukin), TNF-α (Tumor necrosis factor), MIP-1β (macrophage inflammatory protein), SAA (Serum amyloid A), and Tie2 (Tyrosine kinase receptor) could significantly differentiate between OAB patients and control individuals. c-kit ligand, stem cell factor (SCF) found to be significantly more on overactive bladder than control. mRNA expression of connexin (Cx) and transient receptor potential (TRP) channel on urothelial cells from urine showed positive correlations of Cx26 *vs* urgency score, Cx40 *vs* nocturia, TRPM2 (Transient receptor potential melastatin 2) *vs* intermittency, TRPV1 (Transient receptor potential cation channel subfamily V member 1) *vs* urgency incontinence, and negative correlation of Cx40 *vs* intermittency. In plasma measurement of miR-98-5p (upregulation) + miR-139-5p (downregulation) seems to be a good biomarker (AUC = 0.839).

Keywords: Brain derived neurotrophic factor, Cyclooxygenase, MCP-1, Nerve growth factor, Nocturia, Stem cell factor, Urinary creatinine

Introduction

Overactive bladder (OAB) is a condition of urinary urgency, usually accompanied by increased daytime frequency and/or nocturia, with urinary incontinence (OAB-wet) or without (OAB-dry), in the absence of urinary tract infection or other detectable diseases. (International Continence Society, ICS)[1] Urinary frequency is defined as micturiting 8 or more times in a day (24 h). Nocturia is defined as waking to micturate 1 or more times per night[2].

Prevalence

EPIC study [European Prospective Investigation into Cancer and Nutrition (EPIC) study] one of the largest population-based surveys, revealed OAB prevalence as 11.8%, with similar rates in men and women[3]. NOBLE study (National Overactive Bladder Evaluation) in the USA revealed the prevalence of 16% (men) and 16.9% (women). With increasing age, women faced more urgency incontinence > 44 years whereas for men it is > 64 years[4]. In Asia, the overall prevalence of OAB is 53.1% and prevalence of urgency incontinence in the female is 11.4%[5]. In India the prevalence of urgency urinary incontinence in the female is 38%[6].

Patho-physiology

Detrusor muscle over activity is the root cause of OAB syndrome. Detrusor over activity (DO) may be due to - neurogenic, myogenic, or autonomous bladder activity[7].

Myogenic theory: Partial denervation of the detrusor myocytes may alter the properties (supersensitivity to neurotransmitters) of detrusor smooth muscle and leads to increased excitability and ability of activity to spread between cells. As a result, there are coordinated myogenic contractions of the whole detrusor[8,9].

Neurogenic theory: Cortical injury of the brain may cause the excitability of the bladder by reducing suprapontine inhibition. Spinal cord axonal pathways injury can lead to the emergence of primitive spinal bladder reflexes triggered by C-fiber bladder afferent neurons. Hence there could be bladder excitability[10,11].

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Abbreviation: AUC, Area under curve; Cr, Creatinine; DO, Detrusor Overactivity; ELISA, Enzyme-linked immunosorbent assay; MCP 1, Monocyte chemotactic protein 1; ROC, Receiver operating characteristic

Autonomous bladder theory: Detrusor muscle is arranged into modules which are circumscribed areas activated during the storage phase of the micturition cycle. The modules might be controlled by a peripheral myovesical plexus, comprised of intramural ganglia and interstitial cells. DO results from a shift in the balance of excitation and inhibition in smooth muscle modules[12].

The women suffering from OAB literary feel afraid of going out of home – if urgency comes and no toilet nearby! This has become a nightmare for them. Slowly they become depressed. The bothersome discomfort faced by the women suffering from overactive bladder has urged the researcher to seek for any biomarker (biological marker) which may diagnose or predict the possibility of overactive bladder and respective care may be addressed to that women.

Overactive bladder biomarker

Biomarkers are used to envisage the health status in individuals or across the populations so as to plan appropriate therapeutic intervention[13]. Recent years have seen the onset of novel technologies like proteomics, metabolomics, lipidomics, and urinomics, to develop many novel biomarkers[14]. Due to the complex pathophysiology of overactive bladder (OAB) researchers are searching biomarkers connected with bladder inflammation or nerve growth. Nerve growth factor (NGF), the brain–derived neurotrophic factor (BDNF), cytokines, stem cell factor *etc*. are showing promising results[15].

BDNF (Brain– derived neurotrophic factor), NGF (Nerve growth factor)

BDNF and NGF belongs to neurotrophin family of growth factors. They are dimeric molecules with 50% identical structures[16]. BDNF involves to axonal growth, pathfinding and in the modulation of dendritic growth and morphology[17] — thus may cause detrusor overactivity (Fig. 1).

The urinary levels of NGF/Cr were higher in OAB patients than in controls P <0.001)[19,20]. BDNF, NGF *etc*. levels are adjusted according to the urinary creatinine (Cr) level. This is done as there is varying hydration level which may give otherwise false data if not corrected[21]. Urinary creatinine assessment can be done by the commercially available kit *e.g*. Cayman (Ann Arbor, MI, USA) which has a dynamic sensitivity of 0-15 mg/dl Cr. Absorbance level per sample can be measured at 500 nm, with concentration calculation is done as per the standard curve. Before treatment urine NGF/Cr ratio and BDNF/Cr ratio were significantly higher in OAB[22,23]. NGF/Cr was found to have 87.5% sensitivity and 100% specificity, and BDNF/Cr was found to have 87.5% sensitivity and 83.3% specificity for OAB diagnosis. BDNF/Cr was more significant in treatment follow-up (decreased)[22]. In patients where there was no recurrence, urinary NGF/Cr decreased at the end of treatment in comparison to the baseline. This decreased level was maintained at 12 weeks after the end of treatment (P<0.05)[24]. Increased urinary NGF, BDNF, and ATP can help in identifying OAB phenotypes and choose patients for therapies directed to neurotrophic and purinergic pathways[25].

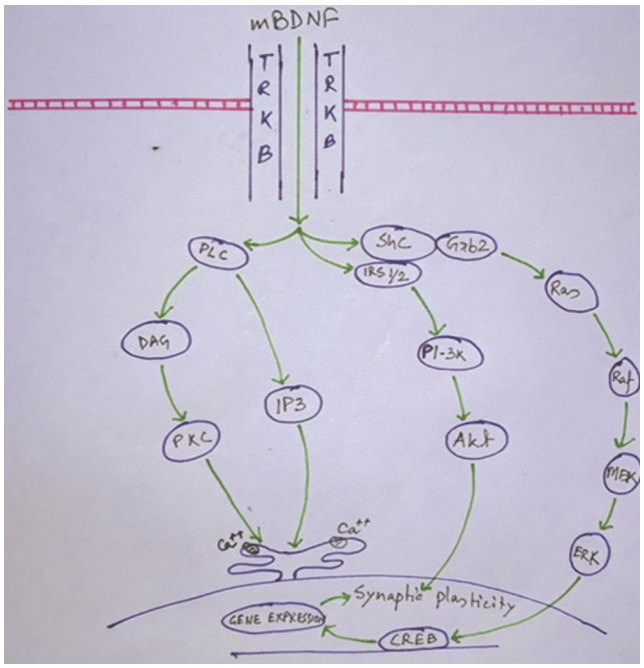

Fig. 1 — Signaling pathway of BDNF: Mature Brain– derived neurotrophic factor (mBDNF) binds to Tyrosine kinase b (TRKB) receptor → recruitment of proteins → activation of 3 signal transduction cascade → 1) sequential activation of IRS-1/2, PI-3K, Akt; 2) sequential activation of Shc/Grb2, Ras, Raf, MEKs, ERK; 3) activation of PLC, IP3, DAG, PKC. These signal transduction activates CREB and CREB-binding protein (CBP) → expression of genes encoding proteins involved in neural plasticity, stress resistance, and cell survival. [IRS-1/2 - Insulin receptor substrate-1, PI-3K - phosphatidylinositol-3-kinase, Akt - protein kinase B, Shc - Science. Src homology 2 domain-containing a structural domain in signal transduction proteins, GRB2 - Growth factor receptor-bound protein 2, RAF – Rapidly accelerated fibrosarcoma, MEK - mitogen-activated protein kinase, ERK - extracellular signal– regulated kinases, PLC - phospholipase C, IP3 - inositol (1,4,5)-trisphosphate, DAG – diacylglycerol, PKC - protein kinase C, CREB - cAMP-response-element-binding protein. (This figure is adapted and modified from Bathina S & Das UN[18].)

C-reactive protein

C-Reactive Protein (CRP) is not a typical marker of the patho-physiological processes of OAB. It is a non-specific marker of inflammation. Hence it has too poor sensitivity and specificity to become an isolated biomarker of OAB[15].

Cytokines

Certain cytokines and chemokines are found relevant as a biomarker for overactive bladder. Chemokines are chemotactic cytokines. Most important chemokines causing chemotactic migration of macrophages, eosinophils, and mast cells are monocyte chemotactic protein 1 (MCP-1; CCL2), macrophage inflammatory protein (MIP-1; CCL4), and eotaxin (CCL1)[26]. There is a linear relationship between the severity of the patient's OAB symptoms and the level of these urinary proteomics[27]. Urinary monocyte chemotactic protein 1 (MCP-1) is been studied in relation to OAB. It has been found that in female OAB patient urinary MCP-1 was very high in comparsion to the healthy individual. OAB-wet patients had a higher level in comparison to OAB-dry. Urinary MCP-1 levels measured by ELISA and normalised by urinary creatinine (Cr) levels[28]. More than 10-fold increase in the levels of MCP-1 and the soluble fraction of the CD40 ligand was seen in OAB patients in comparsion to control individuals. The five-fold increase was seen in the levels of MIP-1β, interleukin (IL)-12, p70/p40, IL-5, EGF, and growth–related oncogene-A and a 3-fold increase was seen in soluble IL-2Ra, and IL-10 in compare to control individuals[29]. In female with OAB, there were increased levels of IL-8 and /or MCP-1 in the urine[30]. Contrary to the above mentioned findings, another study found a significantly lower level of urinary IL-10, IL-12p70, and IL-13 levels in OAB patients in comparison to control[31]. IL 1 corresponded with worsening symptom distress on UDI-6 (Urinary Distress Inventory Questionnaire 6). This observation has warranted for further in-depth study of these cytokines in OAB.

Analysis of plasma by mesoscale discovery analysis revealed that 5 biomarkers (IL-4, TNF-α, MIP-1β, SAA, and Tie2) could significantly differentiate between OAB patients and control individuals. In OAB patients significantly lower value for IL-4, an anti-inflammatory cytokine, and Tie2, tyrosine kinase receptor involved with angiogenesis were observed whereas, significantly higher value for TNF-α, a proinflammatory cytokine/chemokine, SAA,(Serum amyloid A) an acute phase apolipoprotein important for recruitment of immune cells to inflammatory sites and MIP-1β in compare to control were observed[32]. An animal study reported the protective effects of IL-4 on the bladder wall during inflammation and detrusor overactivity. Vector-mediated expression of IL-4 with irritated bladder wall resulted in decreased bladder overactivity, as well as decreased inflammatory response and nociceptive behaviour in comparison to the untreated bladder[33]. That means IL-4 have an anti-inflammatory, protective effect on the bladder wall. The role of Tie2 in OAB is not properly known though its role in bladder carcinoma is well known - significantly lower than the control[34] (Fig. 2).

Prostaglandins

Prostaglandins (PGs) increase the afferent signals of the micturition reflex, leading to the occurrence of OAB. Preclinical trials have revealed that PGs instilled into the urethra induces its relaxation; while intravesical instillation induces detrusor contraction[20]. Levels of PGE_2 and $PGF_{2\alpha}$ in the urine of OAB patients are considerably higher than in healthy individual[36]. Cyclooxygenase (COX) inhibitors improve DO due to cerebral infarction by suppressing peripheral C fibre's without affecting urine production. The non-selective COX inhibitor is more

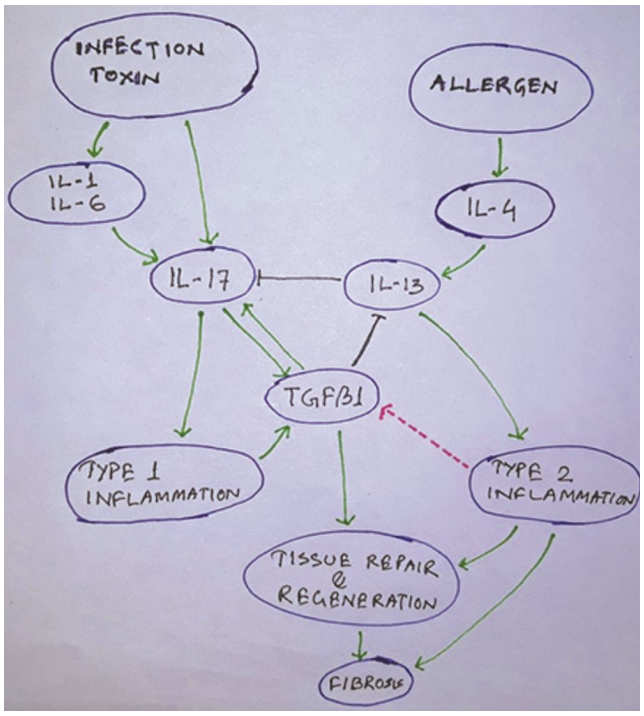

Fig. 2 — Role of Interleukin in inflammation and repair (Figure adapted and modified from Gieseck RL III *et al.*[35])

efficient than the selective COX-1 or COX-2 inhibitor alone[37]. Hence PGs in the diagnosis of OAB is still had to go many more miles.

Stem cell factor

On immunohistochemical study c-kit ligand, stem cell factor (SCF) was found to be significantly more on overactive bladder than control[38]. Interstitial cells of Cajal (ICC) which is present in the gastro-intestinal tract, act as primary pacemaker cells that transmit depolarizing currents into neighbouring smooth muscles to start spontaneous slow waves and phasic contractions[39]. ICC expresses proto-oncogene c-kit, which is used as an identification marker for ICC[40]. In whole urinary tract starting from renal pelvis upto urethra c-kit positive ICCs are found – they are also called as interstitial cells (IC), ICC-like cells, or myofibroblasts[41]. In the urinary bladder ICCs may be responsible for signal transmission between smooth muscle bundles - from efferent nerves to muscles, and from urothelium to afferent nerves[42]. Now the stem cell factor gets bonded to ICCs *via* c-kit and affect the controlling of bladder function. In OAB patient median and IQRs of urinary SCF and SCF/Cr level was 85.9 pg/mL and 1.3 in compare to 18.9 pg/mL and 0.26 in control individuals[38].

mRNA expression

mRNA expression of connexin (Cx) and transient receptor potential (TRP) channel on urothelial cells from urine was measured using quantitative real-time reverse transcription– polymerase chain reaction. It revealed positive correlations Cx26 *vs* urgency score, Cx40 *vs* nocturia, TRPM2 (Transient receptor potential melastatin 2) *vs* intermittency, TRPV1 (Transient receptor potential cation channel subfamily V member 1, also known as the capsaicin receptor and the vanilloid receptor 1) *vs* urgency incontinence, and negative correlation Cx40 *vs* intermittency[43]. In plasma of OAB patients up-regulation of let-7b-5p, miR-92a-3p, miR-98-5p, miR-142-3p, and miR-200c-3p and down-regulation of miR-139-5p was found. miR-98-5p showed the highest diagnostic accuracy alone (AUC = 0.79) in ROC curve analysis. Combined miR-98-5p + miR-139-5p is seem to be a good indicator (AUC = 0.839). This knowledge may help in inventing drugs for OAB also[44]. Urinary bladder wall transfection of the miR-132 plasmid in absence of acetic acid exposure is independently able to induce overactivity, hypertrophy, and upregulation of NGF and other cytokines[45].

Conclusion

Bladder inflammation and nerve growth factors are believed to be associated with OAB. BDNF, NGF, NGF/ Cr ratio, CRP, cytokines, prostaglandins, stem cell factors, mRNA expression of Cx and TRP channel on urothelial cells from urine are found to be associated with OAB. Five cytokines (IL-4, TNF-α, MIP-1β, SAA, and Tie2) in plasma significantly differ in OAB patients from healthy individuals. However, combined use of several biomarkers may yield a better level of sensitivity and specificity than a single one. New horizons are coming up regarding biomarkers of overactive bladder and some of them have the promise of helping in developing therapeutic agents.

Conflict of Interest

All authors declare no conflict of interest.

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