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Relationship between serum cholinesterase level and urinary bladder activity in patients with or without overactive bladder and/or neurogenic bladder

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ABSTRACT

We compared the serum cholinesterase (ChE) level and various parameters between patients with or without overactive bladder (OAB) and/or neurogenic bladder (NB). A total of 258 patients who met the following criteria were enrolled: the presence/absence of OAB and/or NB was documented, laboratory data were available, and liver and renal functions were normal. Patients were divided into the 3 groups: 1) a NB^+/OAB^+ group who had both NB and OAB, 2) a NB^-/OAB^+ group who had OAB alone, and 3) an OAB^- group who did not have OAB. The relationship between the presence of OAB and various biochemical parameters were examined, as well as the therapeutic outcome in relation to the same biochemical parameters. Forty-three patients had both NB and OAB (NB^+/OAB^+), 66 patients had OAB without NB (NB^-/OAB^+), and 149 patients had no OAB (OAB^-). Serum ChE, total protein, and albumin levels were lower in the NB^-/OAB^+ group than the NB^+/OAB^+ group or the OAB^- group. In the NB^-/OAB^+ group, a higher serum albumin or ChE level was associated with a better therapeutic outcome. These results suggest that a decrease of serum ChE level is related to the occurrence of OAB and the poor response to treatment in OAB patients without NB.

The symptom of urinary urgency (usually combined with frequency and nocturia) with or without urge incontinence is characteristic of the overactive bladder (OAB) syndrome (1). OAB occurs in patients with neurogenic bladder (NB) caused by damage to the brain, spinal cord, or peripheral nerves, or in patients without neurogenic bladder, among whom it usually arises due to bladder outlet obstruction (BOO) secondary to conditions such as benign prostatic hyperplasia (BPH) and urethral stricture (2). Bladder smooth muscle cells undergo contraction when exposed to acetylcholine secreted from the parasympathetic nerve terminals, and the efficacy of anticholinergic agents is usually assessed on the basis of blocking the micturition reflex in animal studies. However, recent studies reported that acetylcholine is produced by the bladder epithelium as well as by nerve terminals (7, 23), and its production by the bladder epithelium increases after the occurrence of BOO (5, 22). It has also been suggested that the bladder epithelium express receptors for various neurotransmitters, including adrenergic, nicotinic and muscarinic receptors, and release not only acetylcholine but also nitric oxide, prostaglandin and adenosine triphosphate (ATP), and that muscarinic mechanisms may also have a role in urothelial sensory function (9). Clinically, anticholinergic agents are the main type of drug used for the treatment of OAB because these agents can usually

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improve the symptoms of OAB patients without inhibiting micturition (4). This suggests that anticholinergic agents may block the effect of acetylcholine released from the bladder epithelium on both the bladder smooth muscle cells and the afferent nerve terminals, or may block the effect of acetylcholine on the muscarinic receptors in the bladder epithelium during the urine storage phase of the micturition cycle.

Cholinesterase (ChE) is an enzyme that hydrolyzes choline esters, including acetylcholine, to form choline and acetic acid. There are two types of ChE, which are known as acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE). AChE mainly exists in the post synaptic area of cholinergic nerve terminals, while BuChE circulates in the blood and is usually measured as an index of hepatic protein synthesis. It has been reported that AChE inhibitor decreased the residual urine volume by restoring voiding function in rats with BOO (12), suggesting that AChE may have an important role in modulating acetylcholine-induced bladder contraction. In airway smooth muscles and pulmonary blood vessels, not only AChE but also BuChE play an important role in controlling acetylcholine-induced responses (2, 21). It has also been reported that co-application of both enzymes resulted in a 1330% prolongation in the decay of electric field stimulation-induced contractions, indicating that both enzymes are involved in the regulation of acetylcholine (3). However, there have been few investigations on the influence of ChE (including BuChE) on bladder function. Although AChE is mainly responsible for the local neuronal synaptic regulation of acetylcholine in the bladder, BuChE as well as AChE may also be responsible against an increased release of acetylcholine from the nerve terminals and bladder epithelium under the pathological conditions such as OAB. Therefore, we postulated that ChE circulating in the blood is involved in the hydrolysis of acetylcholine secreted by the bladder epithelium chronically, so that the serum ChE level is related to the occurrence of OAB.

Accordingly, we examined the relationship between serum ChE level and urinary bladder activity in patients with or without OAB and/or NB.

SUBJECTS AND METHODS

The subjects were selected from among outpatients who consulted the Department of Urology at Okinawa Kyodo Hospital between January and December 2004. Patients who met the following criteria were enrolled: 1) the presence/absence of OAB (urinary urgency > 1 time per week) within one year before the study was documented in their medical records, 2) laboratory tests (including serum ChE) had been performed within the past one year at a routine health check (after the onset of lower urinary tract symptoms), and 3) the serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and creatinine were within the normal range. Patients taking ChE inhibitors or cholinergic agents were excluded, as well as patients with bacterial cystitis, bacterial prostatitis, bladder cancer, or proteinuria. By examining the data in their medical records, these patients were divided into the following 3 groups: 1) a NB^+/OAB^+ group who had both NB and OAB, 2) a NB^-/OAB^+ group who had OAB alone without NB, and 3) a OAB^- group who did not have OAB (with or without NB). We examined the underlying disease, concomitant illnesses, medications, and progress of OAB, as well as the serum total protein (TP), serum albumin (ALB), liver function tests {AST, ALT, total bilirubin (T-Bil), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), zinc sulfate turbidity test (ZTT), γ-glutamine transpeptitase (γ-GTP), and leucine aminopeptidase (LAP)}, and serum ChE activity (dimethoxy benzoylthiocholine method, reference range: 100–240 IU/L) to assess the relationship between the presence of OAB (with or without NB) and these parameters, as well as the relationship between serum ChE and the other parameters. Each patient received suitable medical treatment for their disease.

At 3 months after starting treatment for lower urinary tract symptoms (LUTS), a qualified urologist comprehensively evaluated each patient's satisfaction with their therapy and assigned them to one of four categories (excellent: 3 points, good: 2 points, fair: 1 point, no change or worse: 0 points). Then the relationship between the therapeutic outcome and the above-mentioned parameters (including the serum ChE level) was examined. Results are reported as the mean ± standard deviation (SD).

Student's *t*-test for paired or unpaired data was used for statistical analysis, and $p < 0.05$ was considered to indicate statistical significance. Differences of categorical variables were assessed with the chi-square test.

RESULTS

The total number of outpatients examined during the study period was 1,362, and 258 patients who fitted the above criteria were enrolled. The NB^+/OAB^+

group comprised 43 patients (27 men and 16 women aged 75 ± 8 years), the NB^-/OAB^+ group contained 66 patients (49 men and 17 women aged 75 ± 7 years), and the OAB^- group included 149 patients (90 men and 59 women aged 67 ± 13 years). There was a significant difference of age between the OAB^- group and the NB^+/OAB^+ group ($p = 0.001$) or the NB^-/OAB^+ group ($p = 0.001$). In the NB^+/OAB^+ group, 15 patients (35%) had cerebrovascular diseases, 11 patients (26%) had spinal canal stenosis, and 5 patients (12%) had cervical spondylotic myelopathy. In the NB^-/OAB^+ group, all 49 men had BPH with or without non-bacterial chronic prostatitis, while 12 (71%) of the 17 women had non-bacterial chronic cystitis (including urethral syndrome). In the OAB^- group, 59 (66%) of the 90 men had BPH, 10 men (11%) had non-bacterial chronic prostatitis without BPH, and 10 men (11%) had NB without OAB, while 23 (39%) of the 59 women had non-bacterial chronic cystitis and 16 women (27%) had NB without OAB. Patient characteristics were shown on Table 1.

Table 1 *Patient characteristics*

Group	Sub-group	Disease	No. Pts (%)
NB^+/OAB^+	Male	Cerebrovascular disease	9 (33)
		Spinal canal stenosis	5 (19)
		Cervical spondylotic myelopathy	3 (11)
		Parkinson's disease	3 (11)
		Brain atrophy	3 (11)
		Others	4 (15)
		Sub-total No. Male Pts	27 (100)
	Female	Cerebrovascular disease	6 (38)
		Spinal canal stenosis	6 (38)
		Cervical spondylotic myelopathy	2 (13)
		Others	2 (13)
		Sub-total No. Female Pts	16 (100)
		Sub-total No. Pts	43
NB^-/OAB^+	Male	BPH with/without non-bacterial chronic prostatitis	49 (100)
		Sub-total No. Male Pts	49 (100)
	Female	Non-bacterial chronic cystitis including urethral syndrome	12 (71)
		Others (unknown origin)	5 (29)
		Sub-total No. Female Pts	17 (100)
		Sub-total No. Pts	66
OAB^-	NB^-/OAB^- Male	BPH with/without non-bacterial chronic prostatitis	59 (66)
		Non-bacterial chronic prostatitis without BPH	10 (11)
		Urinary tract stones	3 (3)
		Hydrocele testis	2 (2)
		Idiopathic renal bleeding	2 (2)
		Others	4 (4)
	NB^+/OAB^- Male	Cerebrovascular disease	6 (7)
		Spinal canal stenosis	2 (2)
		Others	2 (2)
		Sub-total No. Male Pts	90 (100)
	NB^-/OAB^- Female	Non-bacterial chronic cystitis including urethral syndrome	23 (39)
		Stress urinary incontinence	3 (5)
		Urinary tract stones	3 (5)
		Idiopathic renal bleeding	3 (5)
		Nocturia	3 (5)
		Others	8 (14)
	NB^+/OAB^- Female	Cerebrovascular disease	10 (17)
		Spinal canal stenosis	2 (3)
		Cervical spondylotic myelopathy	2 (3)
		Others	2 (3)
		Sub-total No. Female Pts	59 (100)
		Sub-total No. Pts	149

Table 2 *Biochemical parameters of the NB⁺/OAB⁺ group, NB⁻/OAB⁺ group, and OAB⁻ group*

group	TP (g/dL)	ALB (g/dL)	T-Bil (mg/dL)	AST (IU/L)	ALT (IU/L)	ALP (IU/L)
NB^+/OAB^+	7.0 ± 0.5 *, **	4.0 ± 0.5 *, **	0.7 ± 0.3	24.8 ± 9.1	24.3 ± 10.1	194.2 ± 49.8
NB^-/OAB^+	6.7 ± 0.6	3.8 ± 0.5	0.8 ± 0.5	22.2 ± 6.4	20.7 ± 8.8	189.2 ± 31.3
OAB^-	7.0 ± 0.6	4.0 ± 0.5	0.7 ± 0.3	21.6 ± 6.3	21.3 ± 11.7	182.0 ± 34.3

group (n)	LDH (IU/L)	ZTT (U)	γ-GTP (IU/L)	LAP (IU/L)	ChE (IU/L)
NB^+/OAB^+	194.2 ± 49.8	9.3 ± 4.8	33.8 ± 31.7	48.1 ± 15.0	161.9 ± 37.3 **, **
NB^-/OAB^+	189.2 ± 31.3	7.9 ± 3.9	33.8 ± 26.0	46.7 ± 9.3	142.0 ± 32.3 **
OAB^-	182.0 ± 34.3	8.5 ± 3.9	32.9 ± 23.6	48.7 ± 11.5	156.6 ± 36.7 **

Mean ± SD, *: $p < 0.05$, **: $p < 0.01$.

NB: neurogenic bladder, OAB: overactive bladder, TP: total protein, ALB: albumin, T-Bil: total bilirubin, AST: aspartate aminotransferase, ALT: alanine aminotransferase, ALP: alkaline phosphatase, LDH: lactate dehydrogenase, ZTT: zinc sulfate turbidity test, γ-GTP: gamma glutamine transpeptitase, LAP: leucine aminopepitidase, ChE: cholinesterase.

Reference range of each parameters, TP: 6.7–8.3 g/dL, ALB: 4.0–5.0 g/dL, T-Bil: 0.2–1.0 mg/dl, AST: 10–40 IU/L, ALT: 5–40 IU/L, ALP: 80–260 IU/L, LDH: 230–460 IU/L, ZTT: 2–12 U, γ-GTP: men < 70 IU/L, women < 30 IU/L, LAP: men 80–170 IU/L, women 75–125 IU/L, ChE: 100–240 IU/L.

None of the biochemical parameters showed a significant gender difference except for the serum level of γ-GTP (men: 36.3 ± 27.1 IU/L vs. women: 28.0 ± 21.8 IU/L, $p = 0.009$) in all patients. In the OAB^- group, there were no significant correlations between the age of the subjects and any of the biochemical parameters. There were no subjects whose ChE level was under 100 IU/L. Comparison of the various biochemical parameters among the 3 groups revealed that the serum ChE level showed the largest differences. Serum ChE was significantly lower in the NB^-/OAB^+ group (142.0 ± 32.3 IU/L) than in the NB^+/OAB^+ group (161.9 ± 37.3 IU/L, $p = 0.005$) or the OAB^- group (156.6 ± 36.7 IU/L, $p = 0.004$), although there was no difference between the NB^+/OAB^+ and OAB^- groups (Table 2 and Fig. 1). Serum TP and ALB levels were also significantly lower in the NB^-/OAB^+ group (TP: 6.7 ± 0.6 g/dL; ALB: 3.8 ± 0.5 g/dL) than in the NB^+/OAB^+ group (TP: 7.0 ± 0.5 g/dL, $p = 0.043$; ALB: 4.0 ± 0.5 g/dL, $p = 0.040$) or the OAB^- group (TP: 7.0 ± 0.6 g/dL, $p = 0.004$; ALB: 4.0 ± 0.5 g/dL, $p = 0.001$), again with no difference between the NB^+/OAB^+ and OAB^- groups (Table 2). There were no differences of the other biochemical parameters among the NB^+/OAB^+, NB^-/OAB^+, and OAB^- groups. When the OAB^- group was divided into two subgroups with (NB^+/OAB^-, $n = 26$) or without NB (NB^-/OAB^-, $n = 123$), significant differences of the biochemical parameters were not found between these two subgroups. However, serum TP, ALB and ChE levels were also significantly lower in the NB^-/OAB^+ group than in the NB^-/OAB^- subgroups (TP: 7.0 ± 0.6 g/dL, $p = 0.004$; ALB: 4.0 ± 0.5 g/dL, $p = 0.001$, ChE: 157.6 ± 34.2 IU/L, $p = 0.006$). There were no significant differences of the biochemical parameters between the NB^+/OAB^+ group and the NB^+/OAB^- subgroup (TP: 7.0 ± 0.6 g/dL, ALB: 4.0 ± 0.4 g/dL, ChE: 156.4 ± 37.3 IU/L).

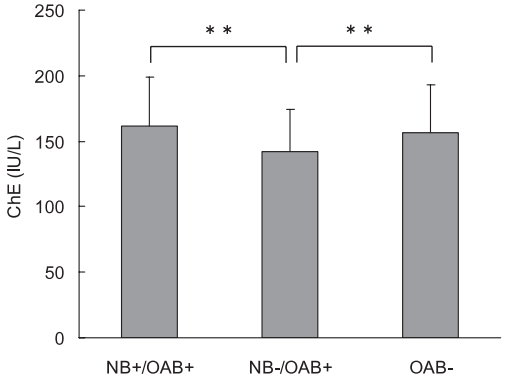

Fig. 1 Comparison of serum ChE levels among the NB^+/OAB^+ group, NB^-/OAB^+ group, and OAB^- group. Serum ChE was significantly lower in the NB^-/OAB^+ group than in either the NB^+/OAB^+ group or the OAB^- group, but there was no difference between the NB^+/OAB^+ and OAB^- groups. **: $p < 0.01$

We also examined relationship between serum ChE and the other biochemical parameters in all 258 patients. We found that the serum ChE level showed a positive correlation with the serum ALB level ($x = ChE$, $y = ALB$, $y = 0.006x + 3.06$, $r = 0.417$, $p < 0.001$), the TP level ($x = ChE$, $y = TP$, $y = 0.007x + 5.90$, $r = 0.391$, $p < 0.001$), and the LDH level ($x = ChE$, $y = LDH$, $y = 0.151x + 163$, $r = 0.208$, $p = 0.036$)(Fig. 2).

Patients in the NB^+/OAB^+ and NB^-/OAB^+ groups were treated by administration of anticholinergic agents, mainly propiverine hydrochloride, with or

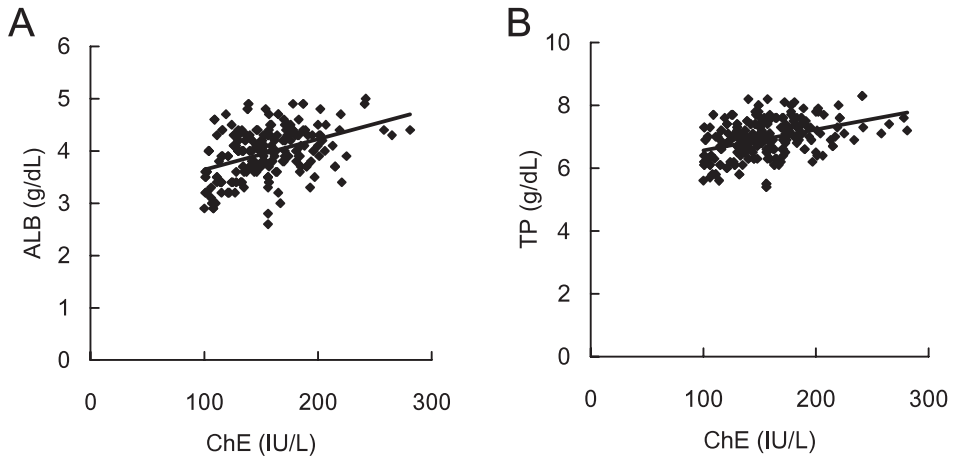

Fig. 2 Correlation between the serum ChE level and the serum ALB level (A) or TP level (B). Serum ChE showed a strong positive correlation with the serum ALB level (x = ChE, y = ALB, $y = 0.006\,x + 3.06$, $r = 0.417$, $p < 0.001$, A), as well as the serum TP level (x = ChE, y = TP, $y = 0.007\,x + 5.898$, $r = 0.391$, $p < 0.001$, B).

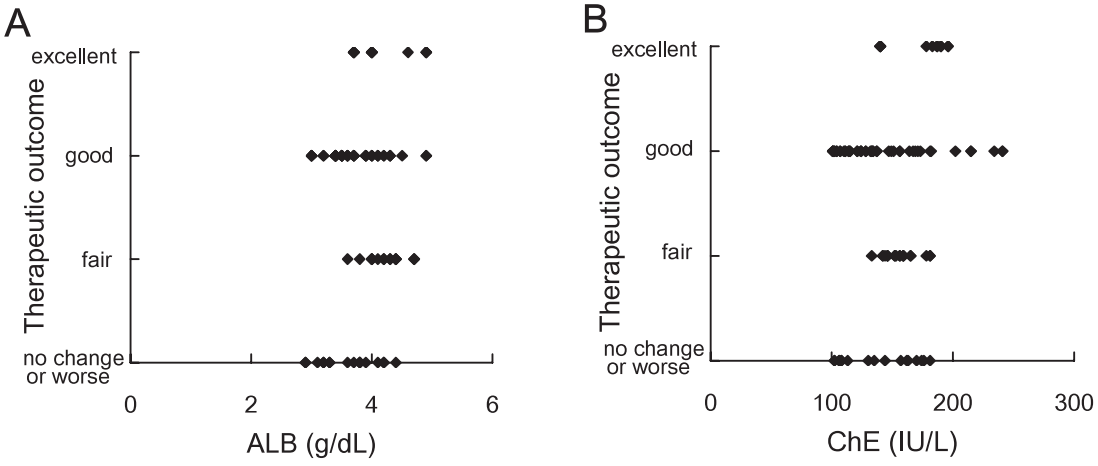

Fig. 3 Correlation between the serum ALB level (A) or the ChE level (B) and the outcome of treating LUTS (including OAB) in the NB^-/OAB^+ group. Patients with higher serum ALB or ChE levels had a better outcome (excellent: 3 points, good: 2 points, fair: 1 point, no change or worse: 0 points) (A: x = ALB, y = therapeutic outcome, $y = 0.402\,x + 0.487$, $r = 0.274$, $p = 0.038$. B: x = ChE, y = therapeutic outcome, $y = 0.006\,x + 1.117$, $r = 0.263$, $p = 0.039$).

without adrenergic alpha-1 receptor antagonists and/or Chinese herbal medicines (Chorei-to or Gosha-jinki-gan). The therapeutic outcome was evaluated in 36 patients from the NB^+/OAB^+ group and 62 patients from the NB^-/OAB^+ group. An excellent or good outcome was achieved in 7 patients (19%) from the NB^+/OAB^+ group and 54 (87%) patients from the NB^-/OAB^+ group. The therapeutic outcome was significantly ($p < 0.001$) superior in the NB^-/OAB^+ group compared with the NB^+/OAB^+ group. Within the NB^-/OAB^+ group, a better therapeutic outcome was obtained for patients with a higher serum ALB level (x = ALB, y = therapeutic outcome, $y = 0.402\,x + 0.487$, $r = 0.274$, $p = 0.038$) and for those with a higher serum ChE level (x = ChE, y = therapeutic outcome, $y = 0.006\,x + 1.117$, $r = 0.263$, $p = 0.039$) (Fig. 3).

DISCUSSION

In the present study, we found that the serum ChE level was significantly lower in the NB^-/OAB^+ group than in the NB^+/OAB^+ group or the OAB^- group, as were the serum TP and ALB levels although liver function of those patients was normal. Higher serum ALB and ChE levels were associated with a better therapeutic outcome in patients from the NB^-/OAB^+ group. These results suggest that a

low serum ChE level was related to the occurrence of OAB, as well as being related to a poor response to treatment in OAB patients without NB.

Bladder smooth muscle cells undergo contraction when exposed to acetylcholine secreted from the parasympathetic nerve terminals, and the efficacy of anticholinergic agents is assessed from the extent of blocking the micturition reflex in animal studies. Therefore, anticholinergic agents have been thought to improve OAB by inhibiting the parasympathetic innervation of bladder smooth muscle through blockade of the actions of acetylcholine. However, anticholinergic agents mainly act during the storage phase of the micturition cycle to decrease urgency and increase bladder capacity, but there is normally no parasympathetic activity during this phase (15). This implies that massive release of acetylcholine (such as occurs during micturition) would reduce the response to these drugs and that impairment of bladder contraction would eventually lead to urinary retention. Although high doses of anticholinergic agents can cause urinary retention, there is little evidence for a significant reduction of bladder contraction in the dose range with a beneficial effect on OAB (6). The reason for this discrepancy has been unclear for a long time.

It was recently reported that the bladder epithelium secretes acetylcholine (7, 23), and that its production is increased by BOO due to conditions such as BPH (5, 22). In addition, a role of muscarinic mechanisms in urothelial sensory function has been proposed because activation of the muscarinic receptors in the urothelium releases substances that modulate afferent nerves and smooth muscle activity (10). Some animal studies have indicated that activation of muscarinic receptors in the urothelium or in suburothelial afferent nerves may promote the spinal micturition reflex mediated by afferent C fibers (9, 13, 20), and that cholinergic nerves exist in close proximity to urothelial cells in the rat bladder (8). These findings suggest that secretion of acetylcholine from the bladder epithelium or activation of muscarinic mechanisms related to urothelial sensory function may stimulate bladder smooth muscle cells and activate the afferent nerve terminals to promote the micturition reflex, thus causing OAB. In addition, anticholinergic agents may decrease sensory symptoms in OAB at least partly by blocking muscarinic receptors located in the urothelium or on afferent nerves.

In the present study, we measured serum ChE levels in patients with or without OAB and/or NB. ChE can be divided into acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE). AChE mainly exists in the synaptic cleft of cholinergic synapses, and binds selectively with acetylcholine to catalyze its hydrolysis into choline and acetic acid. On the other hand, BuChE is usually considered to be an index of hepatic protein synthesis, and it binds non-selectively with various choline esters (including acetylcholine) to catalyze their hydrolysis into choline and acetic acid. Therefore, serum ChE may also partly hydrolyze the acetylcholine secreted by the bladder epithelium. Indeed, both AChE and BuChE play an important role in controlling acetylcholine-induced responses in airway smooth muscles and pulmonary blood vessels (2, 21). In rat urinary bladder, tetraisopropylpyrophosphoramide (a BuChE inhibitor) can potentiate acetylcholine-induced contraction as does neostigmine (a non-selective ChE inhibitor) although the effect of neostigmine is stronger (16). It has also been reported that each AChE and BuChE had some effect to canine tracheal muscle, and that co-application of both enzymes resulted in a 1330% prolongation in the decay of electric field stimulation-induced contractions and the development of a sustained contracture (3). In human isolated bronchial preparation, neostigmine or tetraisopropylpyrophosphoramide retarded the degradation of acetylcholine, and a marked reduction of acetylcholine degradation was observed in the presence of both inhibitors (18). Therefore, even though the decrease of the BuChE level is small, whole ChE activity may decrease relatively greatly. Of course, the amount of circulating ChE is not sufficient for rapid removal of the large acetylcholine burst secreted by the parasympathetic nerve terminals in the bladder when the micturition reflex is activated, and the activity of AChE is higher than that of BuChE in intact urinary bladder preparations (16). However, it is possible that OAB develops when non-synaptic acetylcholine secreted by the bladder epithelium increases in the presence of BOO and exceeds the level of BuChE production by the liver. AChE is mainly responsible for the local neuronal synaptic regulation of acetylcholine in the bladder. But under the pathological condition such as OAB, BuChE as well as AChE are also responsible against an increased release of acetylcholine from the nerve terminals and bladder epithelium. In the present study, the serum ChE level was significantly lower in the NB^-/OAB^+ group than in either the NB^+/OAB^+ group or the OAB^- group. These results suggest that a decrease of the serum ChE level is related to the occurrence of OAB in patients without NB. In addition, the serum ChE level of the $NB^+/$

OAB^+ group did not differ from that of the OAB^- group, so BOO may not be closely related to the occurrence of OAB in patients with NB.

Propiverine hydrochloride is the main anticholinergic agent used for the treatment of OAB at our department, and the level of patient satisfaction achieved by treatment of OAB and BPH with this drug plus doxazosin (an adrenergic alpha-1 receptor antagonist) is reported to be 81% (14). In the present study, a good therapeutic outcome for LUTS (including OAB) was achieved in the NB^-/OAB^+ group, with patient satisfaction reaching 87%, perhaps because adrenergic alpha-1 receptor antagonists or Chinese herbal medicines (Chorei-to or Gosha-jinki-gan) were often added to the basal anticholinergic therapy. Sympathetic activity induces contraction of the internal urethral sphincter via an alpha-1 action, and also promotes the activity of the afferent pathway from the bladder at the level of the spinal cord (19). Since Gosha-jinki-gan decreases sympathetic activity (17), addition of adrenergic alpha-1 receptor antagonists and/or Chinese herbal medicines to anticholinergic agents may have improved the outcome in our NB^-/OAB^+ group. There were also significantly positive correlations between the serum levels of ChE and ALB or TP. Therefore, it may be possible that high-protein diet assist with the treatment of OAB in patients without NB.

In conclusion, a low serum ChE level was related to the occurrence of OAB and to a poor therapeutic response of LUTS (including OAB) in patients without NB. These results suggest that an increase of acetylcholine production by the bladder epithelium during the urine storage phase of the micturition cycle is related to the occurrence of OAB in patients without NB.

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