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The Differential Diagnosis of Central Diabetes Insipidus by Arginine-Vasopressin Measurement Using High-sensitivity Radioimmunoassay

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Background: Plasma AVP evaluation is crucial for diagnosing central diabetes insipidus (CDI). From 2013, a new high-sensitivity radioimmunoassay (RIA) kit replaced the kit conventionally used for AVP measurement. However, it showed inferior sensitivity and cross-reactivity with AVP analogue, a remedy for DI. We investigated the clinical significance of AVP measurement using a new high-sensitivity RIA kit that shows no cross-reactivity (developed by the Yamasa Company) in the differential diagnosis of CDI.

Patients and Methods: Thirty-four patients visiting the Division of Endocrinology and Metabolism, Aichi Medical University Hospital, whose pituitary function was examined to evaluate their endocrine status were analyzed. Plasma AVP levels were measured using a Yamasa kit. This study received institutional ethics committee approval.

Results: The study population included patients previously diagnosed with CDI by high-sensitivity RIA (pre-2013; n＝12), patients without DI symptoms (n＝22), and hormone replacement therapy recipients (n＝10). All DI patients showed a low AVP response, despite AVP analogue treatment. Nine non-DI patients showed a normal baseline plasma AVP level, but the AVP response insufficiently increased during osmotic-stimulation. The remaining 13 patients showed a normal AVP response. Based on the response of AVP and clinical symptoms of DI, the subjects were classified into the DI, impaired response (IR), and normal response (NR) groups. The frequency of hypothalamus-pituitary disorder was high in the DI and IR groups.

Discussion: The plasma AVP level reflected the pathophysiology of DI patients, with impaired AVP secretion in response to osmotic-stimulation, and received no interference of AVP analogue. The IR patients showed subclinical AVP secretion impairment and background hypothalamus-pituitary dysfunction, since most IR patients had pituitary disorder and required hormone replacement.

Conclusion: Plasma AVP was low in CDI and insufficient secretion was detected in non-DI with hypothalamus-pituitary dysfunction. The Yamasa kit seems to be useful as a high sensitivity kit for AVP.

Key words: Diabetes insipidus, Arginine vasopressin, Hypertonic saline infusion, Radioimmunoassay, Subclinical

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INTRODUCTION

Arginine vasopressin (AVP), which regulates water reabsorption through the V2 receptors in the renal collecting duct, is produced in the nerve nucleus of the hypothalamus and is secreted from the posterior pituitary gland[1]. The secretion of AVP is regulated by osmotic and non-osmotic stimulation, and promotes water reabsorption[1]. The decreased secretion of AVP causes polyuria due to the insufficient reabsorption of water, and results in central diabetes insipidus (CDI).

CDI is diagnosed based on the assessment of the AVP secretion in response to osmotic stimulation. Thus, the accurate evaluation of the AVP response is a clinically significant issue. Conventionally, the plasma concentration of AVP was measured by a radioimmunoassay (RIA) using AVP-RIA Kit® (Mitsubishi old kit) (Mitsubishi Petrochemical Co., Ltd. Tokyo, Japan), the limit of detectability was 0.2 pg/ml. High-sensitivity AVP measurement enabled the detection of extremely low levels of plasma AVP, which contributed to differential diagnosis of partial or complete CDI, since endogenous AVP remains in patients with partial CDI. The AVP-RIA Neo Mitsubishi® kit (Mitsubishi new kit) (Mitsubishi Chemical Medience Co., Ltd. Tokyo, Japan) was subsequently adapted for the measurement of AVP, because of the depletion of anti-AVP serum in 2013. The minimum detectable level of the Mitsubishi new kit was 0.8 pg/mL and represented a lower sensitivity than that of the Mitsubishi old kit. In addition, because the Mitsubishi new kit shows cross-reactivity with 1-desamino-8-D-arginine vasopressin (DDAVP) which is used in the treatment of CDI, the plasma AVP values of patients under DDAVP treatment are high (Mitsubishi Chemical Medience Co. Attached document written in 2013), and it is a clinical challenge to accurately evaluate the endogenous AVP secretion of DI patients.

Recently, a domestic company Yamasa (Yamasa Co., Chiba, Japan) developed a new RIA kit, AVP kit Yamasa® (Yamasa kit); its limit of detection is 0.25 pg/mL[2]. However, the measurement of AVP using the Yamasa kit has not been evaluated in the clinical setting. We investigated the clinical significance of AVP measurement using the Yamasa kit in the differential diagnosis of CDI.

PATIENTS AND METHODS

This clinical study was approved by the ethics committee of Aichi Medical University Hospital (YMS-2015-001); all of the patients gave their informed consent for the inclusion of their data in the present study.

PATIENTS

The study population included 34 patients who visited the Division of Endocrinology and Metabolism in Aichi Medical University Hospital between December 2015 and April 2016 and whose endocrine status had been evaluated based on the results of anterior pituitary function tests, a hypertonic saline (HS) infusion test and pituitary MRI, who received proper hormone replacement therapy, who were in a stable condition, and who gave their informed consent to participate in this study.

METHODS

The 5% HS infusion test, which has conventionally been used for the diagnosis of DI, was performed as a posterior pituitary function test[3]. Early in the morning, under fasting conditions, the HS infusion was initiated after a 30-minute rest period. HS (5%) was intravenously administered for 120 minutes at a rate of 0.05 mL/kg/min[3]. Before the start of the infusion,

6 ml of whole blood was collected in an EDTA-2 Na blood collection tube 5 times in total under resting bed every 30 minutes after blood collection.

A plasma sample was obtained by centrifuging at 4℃, at 3000 rpm, for 30 min and was preserved at a temperature of ≤−20℃ until measurement.

The AVP in the plasma samples was measured using a Yamasa kit. AVP was extracted from a plasma sample using the cold ethanol extraction method, reacted with an anti-AVP antibody (anti 8-arginine vasopressin rabbit polyclonal antibody serum), and then reacted with a fixed amount of 125 I-labeled AVP. Further, AVP—anti-AVP antibody complex was reacted with an antibody against anti-AVP antibody (anti-rabbit IgG goat polyclonal antibody serum) and PEG, and centrifuged. After the removal of the supernatant, the radioactivity in the precipitate was measured. The AVP concentration in the specimen was determined from a standard curve prepared using a known concentration of AVP solution[2].

The plasma AVP response was assessed by a conventional correlation diagram[4], suggesting that no or minimum response of AVP was for CDI. CDI was diagnosed according to the criteria of report of the Diencephalohypophysial Dysfunction of Research on Measures for Intractable Diseases Group of Japan's Ministry of Health, Labour and Welfare[5]. The anterior pituitary function was assessed according to the hypopituitarism criteria of the Diencephalohypophysial Dysfunction Research Group[6].

RESULTS

Twelve of the 34 subjects were previously diagnosed with CDI by the Mitsubishi old kit (before 2013). In the 12 patients, DDAVP treatment kept the water electrolyte balance within the normal range. Twenty-two had no DI symptoms and 10 were receiving hormone replacement therapy for pituitary-adrenal insufficiency (Table 1). Among the 34 patients, the adreno-cortical function and thyroid function remained within the normal range in 18 patients who received hormone replacement therapy with agents other than DDAVP (Table 1).

The subjects were classified into three groups according to the AVP response to HS (determined using the Yamasa kit) and the clinical symptoms of DI: the diabetes insipidus (DI) group; no or minimum response of AVP with CDI symptom (Patient No. 1–12), the impaired response (IR) group; insufficient response of AVP with no CDI symptom, (Patient No. 13–21), and the normal response (NR) group; enough response of AVP and non-CDI (Patient No. 22–34) (Table 1).

All patients with DI showed a low plasma level of AVP in spite of the administration of an AVP analogue and no response to osmotic stimulation during HS testing (Fig. 1). In the IR

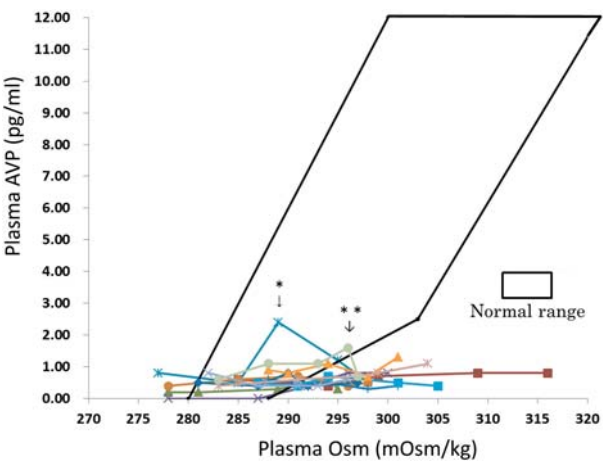

Fig. 1. AVP Secretion during Hypertonic Saline Infusion Testing in Patients with Diabetes Insipidus
Ten of twelve patients in the DI group showed no AVP response during HS testing, two (arrow* and arrow**) of twelve had a low AVP response, and all twelve deviated from the reference range under high osmolality.

Table 1. The Clinical Characteristics and Classifications of the Patients

No	Group	Age	Sex	Cause of symptom	Complication	Hypothalamic/Pituitary function	DDAVP	Degree of hormone replacment
1	DI	52	F	Idiopathic	DI	Normal	Administration	Single
2	DI	36	M	Craniopharyngioma	DI, Panhypopituitarism	Dysfunction	Administration	Multiple
3	DI	18	M	Craniopharyngioma	DI, Panhypopituitarism	Dysfunction	Administration	Multiple
4	DI	19	F	Craniopharyngioma	DI, Panhypopituitarism	Dysfunction	Administration	Multiple
5	DI	67	M	Idiopathic	DI, Eosinophilic granuloma	Normal	Administration	Single
6	DI	74	M	Lymphocytic hypophysitis	DI	Normal	Administration	Single
7	DI	54	M	Idiopathic	DI, Panhypopituitarism	Dysfunction	Administration	Multiple
8	DI	39	M	Pineal tumor	DI, Panhypopituitarism	Dysfunction	Administration	Multiple
9	DI	75	M	Non-functioning pituitary adenoma	DI, Panhypopituitarism	Dysfunction	Administration	Multiple
10	DI	46	M	Craniopharyngioma	DI, Hypopituitarism	Dysfunction	Administration	Multiple
11	DI	43	F	Craniopharyngioma	DI, Panhypopituitarism	Dysfunction	Administration	Multiple
12	DI	83	M	Idiopathic	DI, Benign prostatic hyperplasia	Normal	Administration	Single
13	IR	73	F	Non-functioning pituitary adenoma	Vitamine D insufficient	Normal	No administration	None
14	IR	43	M	Non-functioning pituitary adenoma	Adult GH deficiency	Dysfunction	No administration	Single
15	IR	79	F	Non-functioning pituitary adenoma	Panhypopituitarism	Dysfunction	No administration	Multiple
16	IR	72	M	Lymphocytic hypophysitis	Iatrogenic adrenal insufficiency	Dysfunction	No administration	Multiple
17	IR	48	M	Chiari malformation	Hypothalamic dysfunction	Dysfunction	No administration	Single
18	IR	40	M	Lymphocytic hypophysitis	Hashimoto's disease	Dysfunction	No administration	Single
19	IR	56	F	None	Hypothalamic dysfunction	Dysfunction	No administration	Single
20	IR	62	F	Rathke's cleft cyst	Hypothalamic dysfunction	Dysfunction	No administration	Single
21	IR	66	F	None	Hypothalamic dysfunction	Dysfunction	No administration	Multiple
22	NR	48	M	Non-functioning pituitary adenoma	Hyperlipidemia	Normal	No administration	None
23	NR	53	M	Non-functioning pituitary adenoma	Essential hypertention	Normal	No administration	None
24	NR	64	F	None	Osteoporosis	Normal	No administration	None
25	NR	48	F	Idiopathic	Hyperprolactinemia	Normal	No administration	None
26	NR	59	M	None	Primary aldosteronism	Normal	No administration	None
27	NR	52	F	None	Iatrogenic adrenal insufficiency	Normal	No administration	Multiple
28	NR	49	F	None	Hypothalamic dysfunction	Dysfunction	No administration	None
29	NR	60	F	Growth hormone producing adenoma	Hypothalamic dysfunction	Dysfunction	No administration	None
30	NR	70	M	None	Neurofibromatosis	Normal	No administration	None
31	NR	51	F	None	Idiopathic hypothalamic dysfunction	Dysfunction	No administration	None
32	NR	25	F	None	Idiopathic hypothalamic dysfunction	Dysfunction	No administration	None
33	NR	59	F	None	Idiopathic hypothalamic dysfunction	Dysfunction	No administration	Single
34	NR	24	F	Idiopathic	Hyperprolactinemia	Normal	No administration	None

DI, diabetes insipidus; IR, impaired response; NR, normal response; M, male; F, female

The classifications, according to the AVP response, and the clinical characteristics of the patients are presented. Thirty-four patients were classified into the DI, IR, and NR groups. The patient number, age, sex, cause of symptoms, complications, hypothalamic or pituitary function, presence or absence of DDAVP, and degree of hormone replacement are presented. The rate of hypothalamus-pituitary dysfunction in the DI and IR groups was higher than that in the NR group. Furthermore, the proportion of patients who received hormone replacement therapy with agents other than DDAVP was high in the DI and IR groups (average number of hormone replacement 3.4 and 1.5, respectively). The proportion in the DI group was higher than that in the IR group.

group, although the AVP tended to increase slightly under high osmolality, the increase was not as great as that observed in the NR group. In addition, the IR group had no clinical symptoms of DI, and a decreased AVP response to osmotic stimulation during HS testing (Fig. 2). In contrast, the NR group showed a normal AVP response to osmotic stimulation (Fig. 3).

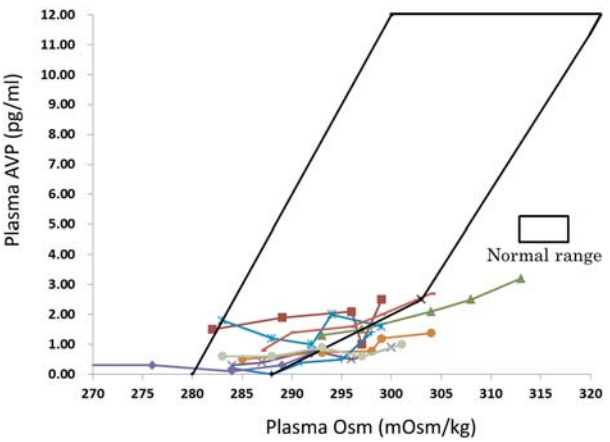

Fig. 2. AVP Secretion during Hypertonic Saline Infusion Testing in Patients with an Impaired AVP Response
Although the plasma AVP tended to show a slightly increased response under high plasma osmolality, the increase was not as great as that in the normal response group. In addition, under high plasma osmolality the plasma AVP response decreased and deviated from the reference range with low AVP values.

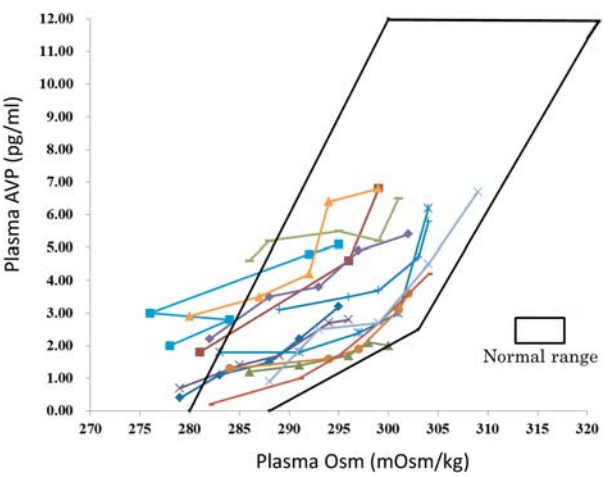

Fig. 3. AVP Secretion during Hypertonic Saline Infusion Testing in Patients with a Normal AVP Response
The change in plasma AVP was within the reference range, and the plasma AVP level increased in response to the increase in plasma osmolality.

Four of the 12 subjects in the DI group had idiopathic DI; 8 had secondary DI. The causes of secondary DI included pituitary tumor and lymphocytic hypophysitis. The proportion of patients in the IR group with organic diseases such as pituitary tumor (3 patients), Rathke's cleft cyst (1 patient), lymphocytic hypophysitis (2 patients), and Chiari malformation (1 patient) was as high as 7 of 9 patients (Table 1). In addition, 8 of 9 patients in this group required hormone replacement therapy. Eight of the 12 subjects in the DI group, 8 of the 9 subjects in the IR group, 5 of the 13 subjects in the NR group had hypothalamus-pituitary dysfunction, as assessed by examinations of the pituitary function. In other words, both the DI and IR groups showed an impaired AVP response, and clinically, the rate of hypothalamic pituitary dysfunction in these groups was higher than that in the NR group. Eight patients in the DI group received an average of 3.4 kinds of hormone replacement treatments, while 8 patients in the IR group received an average of 1.5 kinds of hormone replacement treatments; the DI group had a higher degree of hormone replacement than the IR group (Table 1).

DISCUSSION

According to Yamasa's report[2], the plasma AVP concentration of DI patients during DDAVP treatment is 0.38±0.09 pg/ml, which is significantly lower than that of healthy subjects drinking water ad libitum (1.43±0.66 pg/ml). The simultaneous reproducibility over several measurements has also been confirmed[2].

The AVP response in the NR group was within the normal range of the correlation diagram during the HS test[4], and no symptoms of DI were observed. The patients who showed a normal AVP response according to the Yamasa kit were considered to be healthy subjects with a

normal AVP response.

The alteration of AVP secretion in DI patients obviously reflects the pathology of DI; in these patients, no correlation was observed between plasma AVP and osmolality during HS testing[4]. It was also considered that it is possible to evaluate the AVP response during HS testing with the Yamasa kit, using the normal range referred in the correlation diagram between plasma AVP and osmolality[4]. It is also possible to accurately measure low AVP values in patients with DI because of the high sensitivity of the Yamasa kit[2]. Furthermore, the Yamasa kit had low cross-reactivity with AVP analogues. The cross-reactivity between endogenous AVP and analogue AVP in measurements with the Yamasa kit was 0.09% with 8-arginine vasotocin (AVT), 0.001% with 8-lysine vasopressin (LVT), $\leq$ 0.002% with oxytocin (OXT), and $\leq$0.001% with DDAVP[2]. Our result in DI also supported that the administration of AVP analogues did not interfere with the diagnosis of CDI, and cross-reactivity seemed to have a negligible effect on the measurement of endogenous AVP secretion. The cross-reactivity between endogenous and exogenous AVP was reported based on the study in vitro not in plasma of CDI patients under DDAVP replacement[2]. Our study confirmed that the cross-reactivity of AVP analogue was negligible and not interfered with endogenous AVP, since the AVP concentration was still low in CDI patients with DDAVP treatment.

Ten of the 12 patients in the DI group showed no AVP response during HS testing, while 2 had a low AVP response. This suggests that 10 patients could be diagnosed with complete DI and 2 could be diagnosed with partial DI[7][8].

The patients with no DI symptoms (*e.g.* polyuria or thirst), but with a low AVP response during HS testing were considered to have an impaired response (IR). The analysis of the relationship between the plasma AVP and osmolality revealed that the AVP response in the IR group did not have sufficient increase like the NR group, despite the lack of DI symptoms.

Patients with DI required an increased number of hormone replacement treatments required for hypothalamus-pituitary disorder in comparison to the patients with IR. This suggests that the patients with DI had a greater degree of hypothalamus-pituitary dysfunction and impaired AVP secretion in comparison to the patients with IR. The AVP response in IR indicated the subclinical impairment of AVP secretion and a background of hypothalamus-pituitary dysfunction, since most patients in the IR group had organic pituitary disease and required hormone replacement therapy due to pituitary dysfunction. Our result indicated that HS testing, using Yamasa AVP kit, can detect asymptomatic impairment of AVP secretion in patients with disturbed hypothalamus-pituitary axis. It is clinically significant to comprehend the pathophysiologic state in patients with impaired ability of remained AVP secretion, since these patients tend to deplete AVP, resulting in symptomatic CDI. Therefore, the assessment of AVP secretion by HS testing on the patients with hypothalamus-pituitary dysfunction is supposedly effective in the meaning of clinical management as avoiding the trigger of dehydration in the elderly specially.

DI has been reported to be caused by direct invasion and/or the mass effect of pituitary tumors, craniopharyngioma, and inflammatory changes accompanying lymphocytic hypophysitis and Rathke's cleft cyst[9][10]. If the pathology of the complicated hypothalamus-pituitary lesion progresses in IR, the disordered AVP secretion progresses and is presumed to represent a

disease state in which there is a higher probability of transitioning to obvious DI in comparison to patients without a disordered AVP response.

Several factors are considered to be associated with the results of the IR group. Masked DI can significantly affect water electrolyte metabolism. Glucocorticoids inhibit the secretion of AVP from the posterior pituitary gland[11], and the secretion of the remaining vasopressin increases due to the presence of adrenocortical hypofunction. Thus, symptoms of DI (*e.g.* polyuria) are masked. However, when glucocorticoid replacement therapy is started, the secretion of AVP is suppressed and CDI manifests with polyuria. In addition, hyperresorption of sodium from the renal collecting tubules occurs with supplementation with glucocorticoids, and sodium elevation is promoted, leading to a sudden change in the metabolism of water electrolytes. Under these conditions, central pontine myelinolysis, which represents a severe complication, may occur[8]. There are other reports that hypothyroidism also affects the secretion of AVP[12][13].

Based on the above findings, when a patient presents a declining anterior pituitary function (especially in patients with adrenocortical dysfunction and hypothyroidism) it is necessary to reevaluate the AVP secretion ability again after adequate hormone supplementation. However, the patients in the present study received adequate glucocorticoid and thyroid hormone replacement and had no physical or biological abnormalities; thus, the AVP secretion of the patients in the present study was unlikely to have been affected by adrenal and thyroid dysfunction.

Generally, it is reported that the cause of DI is due to direct invasion and/or the mass effect of a tumor and inflammation[9][10]. Therefore, the impaired production of AVP and the obstruction of the AVP secretory pathway due to physical compression caused by hypothalamus-pituitary tumors and inflammation were considered to have been the main reasons for the disordered AVP secretion of the patients in the present study.

In conclusion, the measurement of the plasma AVP concentration by a newly developed RIA kit with high-sensitivity was useful for the diagnosis of CDI in patients who were treated with AVP analogues, and enabled the detection of an incomplete response of AVP to osmotic regulation in patients with hypothalamus-pituitary dysfunction who lacked the symptoms of DI. The results of the present study indicate that this assay may be useful for the clinical evaluation of such patients.

Acknowledgments

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The authors declare no conflicts of interest in association with the present study.

Abbreviation

AVP: arginine vasopressin
AVT: 8-arginine vasotocin
CDI: central diabetes insipidus
DDAVP: 1-desamino-8-D-arginine vasopressin
HS: hypertonic saline
LVT: 8-lysine vasopressin
OXT: oxytocin
RIA: radioimmunoassay

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