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DIAGNOSTIC TESTING FOR DIABETES INSIPIDUS

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Updated November 21, 2025

ABSTRACT

Diabetes insipidus (DI) is a disorder characterized by excretion of large volumes of hypotonic urine. The underlying cause is either a deficiency of the hormone arginine vasopressin (AVP) in the pituitary gland/hypothalamus [central DI **or Arginine Vasopressin Deficiency (AVP-D**)], or resistance to the actions of AVP in the kidneys [nephrogenic DI **or Arginine Vasopressin Resistance (AVP-R**)]. In most circumstances, DI is also characterized by excessive consumption of water (polydipsia). A third condition called primary polydipsia can clinically show overlapping features with DI. Both DI and primary polydipsia are collectively referred to as 'polyuria-polydipsia syndromes. Like other endocrine disorders, an accurate diagnosis of DI can be challenging. This is mainly because the results obtained from diagnostic testing can show significant overlap among the different forms of DI and primary polydipsia. When a case of DI is suspected, the initial step involves the confirmation of the presence of hypotonic polyuria, which is the hallmark of DI. Once hypotonic polyuria is established, the next step is to identify the type of polyuria-polydipsia disorder (central DI vs. nephrogenic DI vs. primary polydipsia). This can be determined either through the water deprivation test or through the copeptin stimulation tests using osmotic and non-osmotic AVP stimulants. Lastly, a detailed history and physical examination must be performed**,** and appropriate laboratory and imaging studies must be undertaken to identify the underlying etiology of DI. This chapter describes the diagnostic steps to be pursued to identify the presence of DI, distinguish the various forms of polyuria-polydipsia disorders, identify the underlying disorders responsible for the DI, the challenges faced with diagnostic testing for DI in clinical practice, and future prospects in the field of DI diagnosis.

INTRODUCTION

Diabetes insipidus (DI) is a disorder of water homeostasis that is characterized by excretion of large volumes of hypotonic urine either due to the deficiency of the hormone arginine vasopressin [AVP, also known as antidiuretic hormone (ADH)], or due to resistance to the action of AVP on its receptors in the kidneys (1, 2). Large volumes of urine excretion, also known as polyuria (typically over 4 L per day), is the hallmark of DI. The urine of DI has been classically described as insipid (tasteless), hypotonic, and dilute

(3). Polyuria is defined as excretion of a urinary volume >150 ml/Kg/24 hours at birth, >100-110 ml/Kg/24 hours up to the age of 2 years, and >50 ml/Kg/24 hours in older children and adults (1). DI is typically classified into 3 forms: 1. Central DI or **Arginine Vasopressin Deficiency (AVP-D),** 2. Nephrogenic DI **or Arginine Vasopressin Resistance (AVP-R**), and 3. Gestational DI. Primary polydipsia is a disorder that is characterized by excessive intake of water which results in hypotonic polyuria. Clinically, this condition can manifest with symptoms of DI and adds to the diagnostic challenge of identifying DI. Primary polydipsia and DI together are referred to as 'polyuria-polydipsia syndromes', characterized by hypotonic polyuria (4, 5).

Central DI is caused by a variety of disorders that arise from either the pituitary or the hypothalamus. These conditions are characterized by defective production, transport, or secretion of AVP (3, 6). This results in inappropriately low AVP levels in the setting of increased plasma osmolality. Nephrogenic DI is a form of DI that results from resistance by the kidney towards the action of AVP, due to AVP receptor defects or as an adverse effect of certain medications (3, 6). A third, rare form of DI occurs during pregnancy. Also known as gestational DI, this type of DI results from the enzymatic breakdown of the endogenous AVP by a placental cysteine aminopeptidase (3, 7). Therefore, this enzymatic degradation of plasma AVP during pregnancy can unmask sub-clinical DI in those women with borderline-low plasma AVP levels (8). Although primary polydipsia is not a true DI state, long-standing primary polydipsia can give rise to a DI-like picture on laboratory evaluation (described later) (4). There are several etiologies that give rise to each of these forms of polyuria-polydipsia syndromes (6). They have been listed in Table 1.

Table 1. Etiologies of the Various Polyuria-Polydipsia Syndromes
Central Diabetes Insipidus or Arginine Vasopressin Deficiency (involvement of pituitary and/or hypothalamus)
· **Neoplastic:** Craniopharyngioma Germinoma Meningioma Pituitary macroadenoma (invasive) Metastasis to the pituitary and/or the hypothalamus
· **Vascular:** Hypothalamic infarction/hemorrhage Cerebral infarction/hemorrhage Anterior communicating artery ligation Anterior communicating artery aneurysm Sheehan's syndrome Sickle cell disease
· **Trauma:** Deceleration injury Intracranial surgery Transsphenoidal/transfrontal surgery
· **Autoimmune/inflammatory:** Lymphocytic hypophysitis IgG4 disease Xanthogranulomatous hypophysitis

Anti-vasopressin neuron antibodies Guillain-Barré syndrome Systemic lupus erythematosus Scleroderma Granulomatosis with polyangiitis Anti-vasopressin neuron antibodies
· **Infectious:** Meningitis Encephalitis Tuberculosis Pituitary or hypothalamic abscess Toxoplasmosis
· **Granulomatous:** Sarcoidosis Granulomatous hypophysitis Langerhans' cell histiocytosis Erdheim-Chester disease
· **Drug/toxin-induced:** Phenytoin Ethyl alcohol Snake venom Tetrodotoxin
· **Congenital/genetic:** Autosomal dominant AVP-neurophysin II gene alterations Autosomal recessive: type a and b: AVP gene mutation type c: WFS1 gene mutation - Wolfram (DIDMOAD) syndrome type d: PCSK1 gene mutation (AVP-D + extreme obesity) Septo-optic dysplasia Schinzel-Giedion syndrome Culler-Jones syndrome Alstrom syndrome Hartsfield syndrome Webb-Dattani (WEDAS) syndrome X-linked recessive defects with subnormal AVP levels
Nephrogenic Diabetes Insipidus or Arginine Vasopressin Resistance
· **Metabolic:** Hypokalemia Hypercalcemia Starvation and low protein intake
· **Drug-induced:** Lithium Demeclocycline Methoxyflurane

Cisplatin Pemetrexed Aminoglycosides Amphotericin B Cidofovir Foscarnet Sevoflurance
· **Renal disease:** Chronic kidney disease Polycystic kidney disease Obstructive uropathy
· **Systemic disease:** Amyloidosis Sarcoidosis Sjogren's syndrome Multiple myeloma Hemochromatosis
· **Vascular:** Renal infarction Sickle cell disease Acute Tubular Necrosis
· **Congenital/genetic:** Autosomal recessive aquaporin-2 channel gene alterations X-linked recessive V-2 receptor gene alterations Polyhydramnios, megalencephaly, and symptomatic epilepsy (PMSE) syndrome Type 4b Bartter syndrome Inherited renal syndromes (nephropathic cystinosis, nephronophthisis)
Pregnancy-Induced/Gestational Diabetes Insipidus
· Increased vasopressin metabolism induced by placental cysteine aminopeptidase
Primary Polydipsia
Psychogenic polydipsia: Compulsive water drinking Psychosis-intermittent hyponatremia polydipsia (PIP) syndrome
· **Drug-induced:** Anticholinergics Phenothiazines
· **Intracranial etiology:** Hypothalamic tumors Tuberculous meningitis Intracranial surgery/trauma Sarcoidosis
Lowering of hypothalamic threshold for thirst ('Dipsogenic DI') Health Enthusiasts

The typical clinical presentation of DI is in the form of polyuria and polydipsia. On several occasions, co-existing conditions or other aspects of the patient's history can provide clues towards the possible etiology causing DI. Patients with hypophysitis can present with concomitant symptoms of anterior pituitary hormonal dysfunction including fatigue, erectile dysfunction, weight changes, loss of libido, galactorrhea, and amenorrhea (9). Headaches, visual field defects, or cranial nerve palsies can be observed along with DI in case of central nervous system (CNS) tumors, hypophysitis, or a pituitary adenoma (1, 9). In most circumstances, despite losing large volumes of water in the urine, individuals with DI do not manifest dehydration. This is due to the activation of the thirst center in the hypothalamus that induces a strong sense of thirst with rising plasma osmolality which results in the intake of large volumes of water (6). Therefore, although an intact thirst mechanism helps to compensate for the urinary water loss, the polyuria and polydipsia persist, and this can cause considerable distress for the patient unless the underlying DI is treated. A plasma osmolality of about 285 mOsm/Kg usually acts as a trigger for thirst, with further increments in plasma osmolality resulting in a linear increase in thirst (6, 10). DI becomes more challenging to manage if this thirst response is diminished or if the patient is unable to access water for drinking. Patients with an attenuated thirst response have reduced urge to drink water despite rising plasma osmolality and loss of large volumes of water in the urine, resulting in 'adipsic DI' (11). Although being classically associated with craniopharyngioma, adipsic DI can also manifest with CNS trauma, CNS tumors, or after CNS neurosurgical or neurovascular procedures (12). Moreover, the thirst mechanism may be lost in hypothalamic lesions, whereby DI may present acutely with signs and symptoms of dehydration. Patients who are unable to voluntarily access water include infants and young children, and individuals with altered consciousness. In young children, lack of adequate water intake can lead to dehydration, sleeplessness, irritability, enuresis, failure to thrive, and impaired growth (1). Similarly, among patients with altered/reduced consciousness, rapid output of large volumes of urine can result in dehydration and acute, sometimes severe, hypernatremia can ensue (2). The shrinkage of the brain induced by excessive water loss and severe hypernatremia could potentially lead to intracranial bleeding, obtundation, convulsions, or coma (13).

Diagnosing the type of DI/polyuria-polydipsia syndrome is essential for making the optimal treatment decision. A potential misdiagnosis and the resultant treatment can lead to catastrophic consequences (7). For instance, if primary polydipsia is misdiagnosed as central DI and desmopressin treatment is initiated, severe hyponatremia can occur (14, 15). There have been several tests that have been developed over the past century to diagnose the presence of DI and to differentiate the various types of polyuria-polydipsia syndromes. This chapter specifically comprises the description of different diagnostic tests utilized in the diagnosis of these conditions.

DIAGNOSING DIABETES INSIPIDUS

The major challenge with diagnosing and classifying the type of DI arises from the fact that the various forms of polyuria-polydipsia syndromes can show overlapping features on diagnostic testing. The water deprivation test, also known as the indirect water deprivation test should potentially be able to distinguish the various forms of DI. Deprivation of water intake should allow patients with primary polydipsia to concentrate their urine while those with central or nephrogenic DI continue to excrete dilute urine. Administration of desmopressin (Deamino-8-D-arginine vasopressin), the synthetic analogue of AVP, after water deprivation should help with differentiating patients with central DI from those with nephrogenic DI as the former should be able to concentrate the urine once the deficient action of AVP is replaced with desmopressin, while the latter should not show a significant response due to end-organ resistance to

the action of AVP or its analogues. While water deprivation followed by desmopressin administration should be theoretically sufficient to identify the type of DI, in reality, the interpretation of these tests is more complicated, especially if a patient has partial central DI, partial nephrogenic DI or chronic primary polydipsia (4). Patients with partial central or nephrogenic DI retain some amount of response to water deprivation and desmopressin administration (4, 16). In the case of chronic primary polydipsia, long-standing water diuresis blunts the renal medullary concentration gradient and causes down-regulation of the aquaporin-2 channels in the proximal tubule and the collecting duct due to suppressed endogenous AVP, thus creating a state mimicking nephrogenic DI (4, 17).

The basic algorithmic approach for diagnosing any suspected case of DI usually involves the following steps: 1. Confirmation of hypotonic polyuria 2. Diagnosis of the type of polyuria-polydipsia syndrome and 3. Identification of the underlying etiology (6). Performing diagnostic testing in this order can potentially aide with establishing the appropriate diagnosis and with choosing the most relevant biochemical and imaging tests.

CONFIRMATION OF HYPOTONIC POLYURIA

Polyuria is defined as excretion of a urinary volume >150 ml/Kg/24 hours at birth, >100-110 ml/Kg/24 hours up to the age of 2 years, and >50 ml/Kg/24 hours in older children or adults. A hypotonic urine is typically defined as a urine with an osmolality of <300 mOsm/Kg. The primary objective in this step involves differentiating between conditions that give rise to polyuria resulting from osmotic diuresis (such as in hyperglycemia) and DI/primary polydipsia, in which polyuria predominantly involves water diuresis.

Conformation Of Polyuria

The first step is to confirm if a patient indeed has polyuria. Complaints of ‘polyuria’ can often be a misrepresentation of the actual symptoms of urinary urgency, nocturia, urinary incontinence, urinary tract infection, or prostatic hypertrophy (2). Once these symptoms have been ruled out, a 24-hour urine collection should be obtained. A 24-hour urine volume of <2.5 L could be reassuring and there is no concern for osmoregulatory disruption. Those patients without polyuria but with other above-mentioned urinary symptoms must be referred for urological evaluation. Alternatively, individuals can keep a diary for 24 hours making a note of the amount of their urine output (3). A 24-hour urine creatinine will help ensure an appropriate sample collection. Patients need not hold any medications that can cause polyuria, such as diuretics or sodium-glucose co-transporter-2 (SGLT-2) inhibitors for this step as the goal of this step is to establish the presence of polyuria.

Excluding Other Causes Of Polyuria

Once polyuria is confirmed, the next step would be to assess for urinary osmolality. The urine in cases of DI/primary polydipsia is hypotonic. A urine osmolality of >800 mOsm/Kg indicates optimal plasma AVP levels and appropriate renal response to AVP, therefore ruling out any form of DI (7, 18). In most cases, polyuria with isotonic/hypertonic urine is driven by glucose, sodium urea, radiocontrast dye**,** or medications such as diuretics or mannitol (19, 20). Glycosuria can result either from uncontrolled diabetes mellitus (DM), general hyperglycemia (from steroid administration, tube-feeding/parenteral nutrition), or by the use of SGLT-2 inhibitors (21). Administration of normal saline in large volumes for intravascular volume expansion can lead to sodium-induced polyuria (19). Sodium-induced polyuria is also seen with release of bilateral bladder obstruction and salt wasting nephropathies (20, 22). Urea-induced solute diuresis can be seen with high amounts of protein intake from TPN or tube feeds formula, tissue catabolism from high dose glucocorticoids, all of which result in production of urea from breakdown of proteins (19, 23). Administration of urea for treatment of hyponatremia, and recovery from azotemia can also result in urea-solute diuresis (19). Mannitol-induced

diuresis can result from treatment of increased intracranial pressure with mannitol (19).

INITIAL SERUM/PLASMA AND URINE INVESTIGATIONS

In individuals with established hypotonic polyuria or in individuals with urine osmolality of ≥300 mOsm/Kg and <800 mOsm/Kg, further evaluation must be undertaken through laboratory investigations. Serum sodium and plasma osmolality measurements could assist with indicating the type of the underlying polyuric state. A high serum sodium (>146 mmol/L) could point towards central or nephrogenic DI while a low normal or low sodium (<135 mmol/L) could indicate primary polydipsia as the underlying disorder (5, 24, 25). Similarly, a high plasma osmolality (≥300 mOsm/Kg) is typically seen in DI while a normal or low plasma osmolality (≤280 mOsm/Kg) is usually seen in primary polydipsia (4, 26).

As an alternative to urine osmolality, urine specific gravity is also useful in identifying a hypotonic polyuric disorder. For normal plasma osmolality, the urine specific gravity is between 1.003 to 1.030 (27). The specific gravity value depends on the number and the size of particles in the urine, unlike urine osmolality which solely depends on the number of particles in the urine. Because of this, although urine specific gravity and urine osmolality generally correlate well, presence of large molecules might elevate urine specific gravity despite the chance of the urine osmolality actually being low (28). Unlike falsely elevated urine specific gravity, false low values of urine specific gravity are uncommon, and a low urine specific gravity suggests DI or primary polydipsia. Both urine osmolality and specific gravity are easily measured on a urine specimen, but urine osmolality is more widely utilized in management of DI as osmolality is not affected by the size of the particles in the urine. Situations where urine specific gravity is suggested to be useful is when facilities for urine osmolality measurement are not available or if rapid results are required, especially in managing neurosurgical patients, and on rare occasions where DI co-exists with DM with hyperglycemia (as in craniopharyngioma, Wolfram syndrome, described later) (29). With uncontrolled DM, the urine osmolality and specific gravity should be high. But with co-existent DI and DM, the urine osmolality and the urine specific gravity can be inappropriately low (30). Some studies have also noted low urine specific gravity but normal urine osmolality with concomitant DI and DM (29). In clinical practice, some physicians rely solely on urine osmolality while others prefer to assess both urine osmolality and urine specific gravity for managing DI.

Any underlying renal dysfunction must be ruled out by measuring urine sodium, blood urea nitrogen and serum creatinine (2). Electrolyte abnormalities including hypokalemia and hypercalcemia can give rise to polyuria due to down-regulation of aquaporin-2 channels giving rise to a clinical picture of nephrogenic DI (31). Therefore, any serum potassium or calcium abnormalities must be appropriately corrected.

DIAGNOSIS OF THE TYPE OF DIABETES INSIPIDUS

Probably the most challenging step in the work-up of a suspected case of DI is to assign an accurate diagnosis: central DI vs. nephrogenic DI vs. primary polydipsia. These polyuric-polydipsic states can demonstrate substantial overlap, both in their clinical presentation and in their response to diagnostic testing. It is also possible for one or more forms of polyuria-polydipsia syndrome to co-exist in a single individual. An algorithm for the diagnostic approach for DI is described in Figure 1.

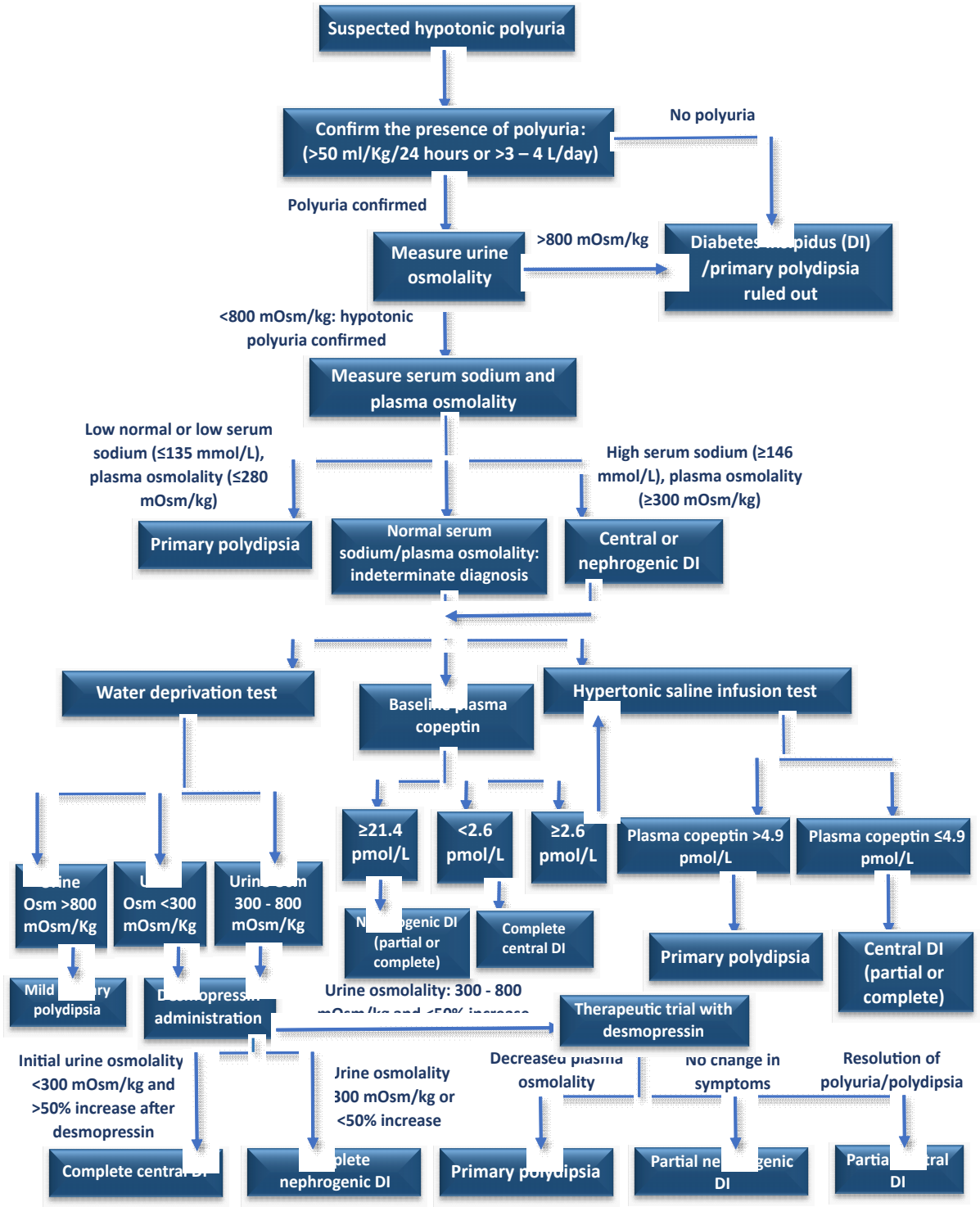

Figure 1. Algorithm for Diagnosis of the Various Types of Polyuria Polydipsia Syndromes

Under some circumstances, a diagnosis of DI or primary polydipsia is established based on clear evidence of hypotonic polyuria and based on serum sodium and plasma osmolality values, with high serum sodium/plasma osmolality being consistent with DI and a low serum sodium/plasma osmolality being consistent with primary polydipsia (see Figure 1). But in most situations, the diagnosis is still unclear. Such

situations, where the initial diagnostic testing is indeterminate, include a urine osmolality value of 300 – 800 mOsm/Kg or a normal serum sodium/plasma osmolality. In these circumstances, there is a need to establish the diagnosis with more accuracy, and further testing must be considered. In this section, the various diagnostic tests specifically utilized to diagnose and identify the type of polyuria-polydipsia syndrome are discussed, along with their advantages and limitations.

WATER DEPRIVATION TEST

The water deprivation test is also known by the terms 'indirect water deprivation test' and 'dehydration test'. The term 'indirect' is utilized as this test generally does not involve 'direct' measurements of plasma AVP to diagnose and differentiate the various forms of DI. The water deprivation test is almost always followed with desmopressin administration to further characterize the type of polyuric polydipsic state. The basic principle behind the water deprivation test is that in individuals with normal posterior pituitary and renal function (or those with primary polydipsia), an increase in plasma osmolality from dehydration stimulates AVP release from the posterior pituitary which then leads to water reabsorption in the nephrons, thus resulting in concentration of urine and an increase in urine osmolality. In central or nephrogenic DI, the urine fails to optimally concentrate with water deprivation and there is persistent excretion of hypotonic urine. Once the diagnosis of DI is established, desmopressin administration can distinguish between central and nephrogenic DI. In central DI, once the deficient action of AVP is substituted with desmopressin administration, the urine osmolality should increase while in nephrogenic DI, as the desmopressin is ineffective due to lack of renal response to its actions, the low urine osmolality persists.

Testing Protocol

The test is performed either as an out-patient or preferably after admitting the patient to an in-patient ward in a controlled setting. For those individuals with milder polyuria (50 – 70 ml/Kg/24 hours), the test can be initiated in the evening, and the dehydration can be performed overnight as an out-patient. However, for those who experience significant polyuria or nocturia, the test is better performed during the day so that the patient can be supervised (3). The individual undergoing the test must not have undergone thirsting prior to the test. Any electrolyte abnormalities (potassium, calcium) must be corrected prior to the test. The patient has to discontinue any medications that can affect urine output (diuretics, SGLT-2 inhibitors, desmopressin, carbamazepine, chlorpropamide, glucocorticoids, non-steroidal anti-inflammatory drugs) 24 hours prior to initiation of dehydration, and refrain from activities such as smoking and caffeine intake that might affect AVP release or urine output (6, 32). Baseline plasma osmolality, serum sodium, urine osmolality (and plasma AVP or plasma copeptin where available) are obtained. In case of an out-patient overnight water deprivation, these baseline measures are obtained on the morning preceding the overnight water deprivation test (33).

The dehydration phase is initiated overnight among those patients in whom this phase is performed as an out-patient. The timing of initiation of overnight water deprivation for out-patient testing is determined based on the 24-hour urine output of the patient (which would have been previously determined in order to establish the diagnosis of a polyuria-polydipsia syndrome), with a goal of achieving approximately 3% loss of body weight.

The duration of overnight water deprivation (in hours) can be determined by using the following formula: patient's weight (Kg) x 0.03 x 1000 (ml) / urine output (ml/hour) (33). Clear, written instructions must be provided to the patient about terminating the dehydration phase in case of unseen adverse events such as nausea, diarrhea, dizziness, or syncope. Once the overnight dehydration phase is completed, the patient arrives at the hospital the following day around 07:00 – 08:00 am to obtain serum/plasma and urine studies. Patients are advised to bring along an

accompanying person for their hospital visit following the overnight water deprivation. In case the overnight dehydration is prematurely terminated for any reason (dizziness, or intractable thirst leading to consumption of water), then in these patients, in-patient water deprivation must be undertaken.

The dehydration phase for in-patient testing usually begins at 08:00 am. The patient voids prior to beginning the test and the baseline weight, blood pressure, and heart rate are measured prior to initiation of dehydration. Following the initiation of the test, the patient should have nothing by mouth. Weight, blood pressure, and heart rate must be recorded hourly as a cautionary measure due to the risk of severe water loss and dehydration in the setting of lack of access to drinking water. Adequate supervision is advised to watch for any undisclosed drinking of water. Every voided urine is recorded, and the osmolality of the urine is measured. Alternatively, for the convenience of measurement, urine output and urine osmolality can be measured once every 2 hours. Serum sodium and plasma osmolality are also obtained every 2 hours along with urine measurements. The dehydration phase should be discontinued if one of the following occur: loss of more than 3% of body weight, elevation of serum sodium to above normal limits (≥146 – 150 mmol/L as per most literature), orthostatic hypotension or orthostatic symptoms (dizziness), or intractable thirst (4).

The next phase of the test is the desmopressin phase, which involves administration of desmopressin following dehydration. This phase can be initiated if either one of the following end-points are achieved: 1. Dehydration phase is completed for 8 hours (except in those who need longer periods of dehydration), 2. Two consecutive urine osmolality measures do not differ by >10% and loss of 2% body weight, 3. Premature termination of dehydration phase due to loss of more than 3% of body weight, elevation of serum sodium to above normal limits, orthostatic hypotension, or intractable thirst (3). An injection of 2-4 µg desmopressin is administered either through intravenous, intramuscular or subcutaneous route and the above-mentioned urine and serum/plasma measures are obtained hourly for 1 – 2 hours after injection. Administration of desmopressin through oral or intranasal route does not result in predictable absorption, and especially where assigning an accurate diagnosis is crucial, optimal desmopressin concentrations in the blood must be ensured. So, oral or intranasal desmopressin administration is not recommended to be used as a part of water deprivation test. During this phase, the patient can eat and drink, even up to 1.5 – 2 times the volume of urine passed during the dehydration phase. The total duration of the test can vary based on the clinical presentation. In those patients with complete forms of DI, the test can be performed in less than 8 hours while in those with partial DI or a non-DI condition, the test could last longer, sometimes even over 18 hours (3).

Interpretation of Results

In normal individuals, with dehydration, the urine osmolality usually increases up to 800 – 1200 mOsm/Kg (3). If the dehydration phase is begun at 12:00 am, a morning (8:00 - 9:00 am) urine osmolality of 800 – 1200 mOsm/Kg excludes DI (3). A urine osmolality of <300 mOsm/Kg with a concomitant plasma osmolality of >300 mOsm/Kg or a sodium level above upper limit of normal following dehydration (>146 mmol/L) is suggestive of either central or nephrogenic DI (3, 4, 6). Desmopressin could be administered at this time (8.00 - 9:00 am). An increase of at least **>50%** in urine osmolality after desmopressin administration suggests complete central DI (the increase can be up to 200% to 400%) while a <50% increase points towards complete nephrogenic DI (3). An increase in urine osmolality over 300 mOsm/Kg prior to an increase in serum sodium/plasma osmolality suggests preservation of some endogenous AVP activity/renal response to AVP, suggesting either partial central or partial nephrogenic DI (5). Thus, in patients with partial DI (central or nephrogenic) the urine osmolality after water deprivation is usually between 300 – 800 mOsm/Kg and there can be <50% increase in urine osmolality following desmopressin administration.

In primary polydipsia, water deprivation results in an increase in urine osmolality, anywhere between 300 – 800 mOsm/Kg (usually up to 600 – 700 mOsm/Kg), without a substantial increase in plasma osmolality, but the increase in urine osmolality is not as substantial as in a normal response (3, 4, 6). Following desmopressin administration, an increase of <9% in urine osmolality is usually associated with primary polydipsia with a concomitant urine osmolality of 300 – 800 mOsm/Kg as per some literature (4). However, this amount of increase in the urine osmolality when the urine osmolality is between 300 – 800 mOsm/Kg can also be seen in partial nephrogenic DI and adds to the diagnostic conundrum, and these criteria alone should not be utilized to diagnose primary polydipsia. A graphical representation of the response of each of the polyuria-polydipsia disorders to the water deprivation test is provided in Figure 2.

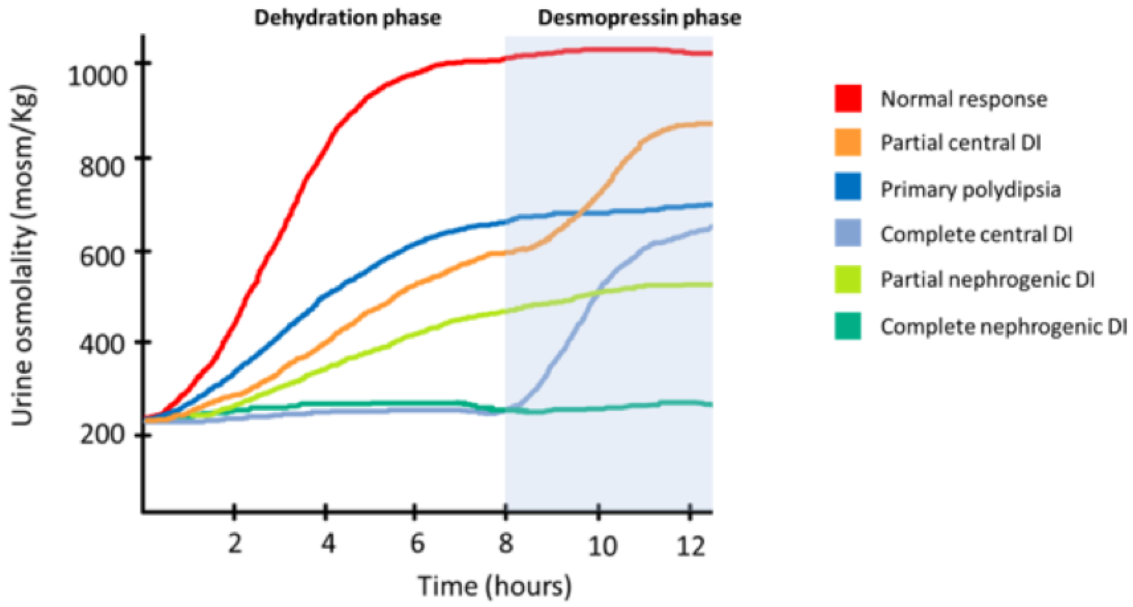

Figure 2. Graphical representation of the water deprivation test. Image courtesy: Sriram Gubbi, NIDDK, NIH

Limitations

Although **traditionally** widely regarded as the gold standard in literature for diagnosing DI, the water deprivation test does have its limitations (3, 24). The most common scenario being partial central or partial nephrogenic DI. Patients with partial central DI retain some degree of AVP secretory capacity that can be stimulated with dehydration leading to concentration of urine. In partial nephrogenic DI, maximal AVP secretion from water deprivation can overcome renal resistance to AVP's action and enable water reabsorption. In addition, long-standing central DI and chronic primary polydipsia can also give rise to false results on water deprivation test. With long standing central DI, due to chronic deficiency of AVP, the aquaporin-2 channels are down-regulated as AVP is required for the synthesis and membrane translocation of these channels (34). Therefore, administration of desmopressin after water deprivation in long-standing central DI might not result in an adequate increase in urine osmolality due to lack of adequate water reabsorption from insufficient aquaporin-2 channel availability, which could misleadingly suggest a picture of nephrogenic DI. On the other hand, chronic primary polydipsia can suppress the release of endogenous AVP due to increased intravascular volume and decreased

plasma osmolality from high volumes of water intake. In this situation, water deprivation might not lead to an adequate rise in plasma osmolality high enough to stimulate the release of endogenous AVP. This can result in a sub-optimal increase in urine osmolality, thus simulating a clinical picture of central/nephrogenic DI. Moreover, high volumes of water intake from chronic primary polydipsia can create a 'wash out' of the renal medullary osmotic gradient and also a down-regulation of the aquaporin-2 channels, and administering desmopressin in this situation may not result in adequate increase in urine osmolality (4, 17). Another confounding scenario occurs in patients with central DI and impaired glomerular filtration due to compensatory increase in expression of AVPR2 gene resulting in up-regulation of aquaporin-2 channels leading to a higher urine concentration than expected (19). Also, the water deprivation test must be performed in children only under the supervision of a pediatrician and the test should not be performed in infants (35). Adjunctive measurements of plasma AVP or copeptin levels can improve the diagnostic yield of the water deprivation test and are described in detail in the upcoming sections below. The protocol for the water deprivation test, interpretation of the results, and limitations are represented in Table 2.

Table 2. Summary of the Indirect Water Deprivation Test
Protocol
Preparation phase: · Test can begin either overnight (for out-patient testing) or at 08:00 am (for in-patient testing). · No thirsting prior to the test. Smoking and caffeine intake are avoided. · Any electrolyte abnormalities (potassium, calcium) are corrected. · Drugs that can affect urine output (diuretics, sodium-glucose co-transporter-2 inhibitors, glucocorticoids, non-steroidal anti-inflammatory drugs) must be held 24 hours prior to dehydration. · Baseline weight, blood pressure and heart rate are measured prior to dehydration. · Baseline plasma osmolality, serum sodium, urine osmolality (and plasma AVP or plasma copeptin where available) are obtained (these measures can be obtained on the morning prior to overnight dehydration in cases of out-patient water deprivation test).
Dehydration phase: · This phase usually lasts for 8 hours (can last longer in certain cases). · For out-patient testing, the duration (hours) of overnight dehydration can be determined using the formula: patient's weight (Kg) x 0.03 x 1000 (ml) / urine output (ml/hour). · Patient is allowed to have nothing by mouth. · Adequate supervision is necessary to watch for any undisclosed drinking (in case of in-patient testing). · Weight, blood pressure and heart rate are measured every 1 hour (in case of in-patient testing). · Urine output, urine osmolality, serum sodium and plasma osmolality are measured every 2 hours (for in-patient testing). Plasma copeptin is measured towards the end of the dehydration phase. (in case of out-patient overnight dehydration, serum/plasma and urine measures are obtained around 07:00 – 08:00 am the following morning) · Dehydration phase is discontinued if one of the following occurs:

o Dehydration is completed for 8 hours (not applicable for those who might need longer periods of dehydration). o Two consecutive urine osmolality measures do not differ by >10% and loss of 2% body weight. o The total body weight reduces by more than 3%. o Serum sodium increases to above upper limit of normal (preferably >150 mmol/L). o Orthostatic hypotension or orthostatic symptoms. o Intractable thirst (or if patient admits to drinking water overnight in case of out-patient testing).
Desmopressin phase: · An injection of 2 µg desmopressin is administered either through intravenous, intramuscular, or subcutaneous route (use of oral or intranasal desmopressin is not preferred due to unpredictable absorption). · The patient is allowed to eat and drink, even up to 1.5 – 2 times the volume of urine passed during the dehydration phase. · Urine output, urine osmolality, serum sodium and plasma osmolality are measured hourly for 1 – 2 hours after desmopressin administration.
Interpretation
Central DI: o Baseline urine osmolality of <300 mOsm/Kg and an increase in urine osmolality by >50% from baseline following desmopressin administration (complete central DI). o Baseline plasma copeptin of <2.6 pmol/L without prior dehydration (complete central DI). o The ratio of Δ copeptin from start till the completion of dehydration phase to serum sodium at the end of the dehydration phase of <0.02 pmol/L (indicates partial central DI).
Nephrogenic DI: o Urine osmolality fails to rise above 300 mOsm/Kg or by <50% after desmopressin administration (complete nephrogenic DI). o Baseline plasma copeptin of ≥21.4 pmol/L without prior dehydration (complete and partial nephrogenic DI). o Baseline plasma AVP of ≥3 pg/ml without prior dehydration (complete and partial nephrogenic DI).
Primary Polydipsia: o Urine osmolality usually increases (300 – 800 mOsm/Kg, usually up to 600 – 700 mOsm/Kg) without significant changes in plasma osmolality following dehydration. o The ratio of Δ copeptin from start till the completion of dehydration phase to serum sodium at the end of the dehydration phase of ≥0.02 pmol/L
Advantages
· Most extensively utilized and validated test. · No risks of hypertonic saline administration (thrombophlebitis, need for central line). · Plasma copeptin or AVP measurements are not necessary if the center does not have the facilities/assays to measure these peptides.
Disadvantages
· More time consuming than hypertonic saline infusion test.

· Can give overlapping results in cases of partial central DI, partial nephrogenic DI, or chronic primary polydipsia.
· Majority of the interpretation relies on urinary measurements, which can be affected by any of the above conditions due to their modulation of aquaporin-2 channel synthesis and expression.
· More burdensome for the patients and less convenient when compared with hypertonic saline infusion test.

MEASUREMENT OF PLASMA AVP

AVP is encoded by the arginine vasopressin gene (*AVP*, 20p13), which also encodes prepro-AVP (a single peptide), neurophysin II (NPII), and a glycoprotein, copeptin. The pro-hormone of AVP is synthesized in the magnocellular neurons of the hypothalamus (Figure 3). Due to the above mentioned limitations of the indirect water deprivation test, measurement of plasma AVP levels was suggested for its potential to be used as a 'direct' test in conjunction with water deprivation test to distinguish the various polyuria-polydipsia syndromes (36). In nephrogenic DI, as there is no deficiency of AVP, the plasma AVP levels is high, in order to overcome the resistance posed by the kidneys to the action of AVP. On the other hand, plasma AVP should be low or relatively low in central DI for the elevated plasma osmolality (4). In primary polydipsia, plasma AVP levels may be normal or can be suppressed in long-standing cases. Subnormal AVP levels in cases of central DI could be due to osmoreceptor dysfunction in the hypothalamus or due to defective AVP release from the neurohypophysis (37). Despite the initial promising results of its potential utility, plasma AVP is seldom utilized for diagnosing DI in clinical practice.

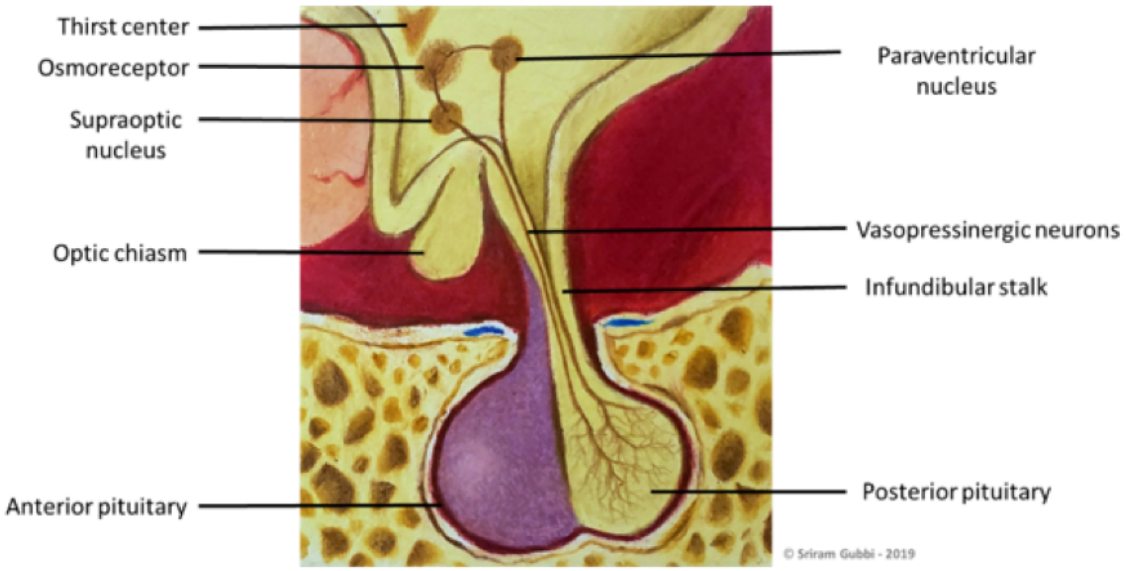

Figure 3. Anatomy of the pituitary gland and the hypothalamus. The pituitary gland comprises of two developmentally and functionally distinct parts: the anterior pituitary (adenohypophysis), derived from the Rathke's cleft and the posterior pituitary (neurohypophysis). The gland is attached to the hypothalamus through the posterior pituitary via a stalk. The posterior pituitary along with the hypothalamus and the stalk forms the functional unit of infundibuloneurohypophysis. This diagram emphasizes the neuronal network that supplies the posterior pituitary. The vasopressinergic neurons (magnocellular neurons) are present in the supraoptic and paraventricular nuclei of the hypothalamus.

These neurons synthesize arginine vasopressin (AVP), its precursors and co-peptides, and their axons project into the posterior pituitary where the AVP is stored along with oxytocin (produced by the oxytotic neurons present in the same nuclei) in the axonal terminals. These hormones are transported to the posterior pituitary through the supraoptic-hypophyseal tract. Another set of neurons of the paraventricular nucleus (also called parvocellular neurons) project into other areas of the brain and spinal cord, some of which secrete AVP. Image courtesy: Sriram Gubbi, NIDDK, NIH.

There are several limitations to measuring plasma AVP levels. AVP is rapidly cleared from the plasma, with a half-life of around 16 minutes (38). In addition, the pre-analytical instability of AVP in the plasma is high. A large amount of circulating AVP is bound to platelets through V1 receptors and failure to adequately segregate platelets from the plasma after blood sampling or prolonged storage of unprocessed blood samples can lead to wide fluctuations or even an increase in the measured plasma AVP levels (24, 39). For the AVP to be detectable by the currently available assays, an additional step of extraction/concentration of plasma is necessary, and this requires at least 1 ml of blood sample (24). Additionally, the current laboratory method employed in AVP measurement is radioimmunoassay (RIA), which has several limitations and analytical errors. Plasma concentration of AVP (measured in pg/ml) is one of the lowest among all hormones, and as AVP is a small peptide, sandwich assays cannot be effectively utilized to measure plasma AVP levels (5). A comparison study of RIA and liquid chromatography-tandem mass spectrometry (LC-MS/MS) done in Japan evaluating plasma AVP levels during hypertonic saline stimulation test demonstrated a lower detection limit (0.3 pg/mL) and a broader quantification range with LC-MS/MS than RIA (40). The AVP levels in the plasma can be stabilized up to 2 - 4 hours after blood sampling by storing the sample at 4ºC (22, 41, 42). However, the average turn-around time for the measurement of AVP is 3-7 days in most laboratories which would make maintenance of hormonal stability even more challenging (4, 43). For these reasons, plasma AVP measurements are not routinely utilized. A related peptide to AVP, copeptin is a more stable marker to diagnose the various hypotonic polyuric states.

MEASUREMENT OF PLASMA COPEPTIN

Copeptin (Carboxy-Terminal-Pro-vasopressin) is the C-terminal peptide of pro-vasopressin that is co-secreted with AVP in stoichiometric amounts from the posterior pituitary (4, 43). Both of these peptides are derived from a precursor molecule synthesized in the magnocellular neurons of the hypothalamus (Figure 3) (24). The post-transcription processing of AVP, copeptin, and related peptides are diagrammatically represented in Figure 4. Unlike plasma AVP measurement, which is technically challenging, copeptin measurement in the plasma is relatively less cumbersome and has several advantages: copeptin can remain stable for days after sampling of blood and can be measured relatively quickly (43). Plasma levels of copeptin strongly correlate with plasma AVP levels over a wide range of osmolalities, both in healthy individuals and those with DI or primary polydipsia (24, 44). Moreover, plasma copeptin demonstrates the same response to changes in plasma osmolality and plasma volume as does plasma AVP (5,24). Several studies have been conducted to validate the utility of plasma copeptin in the diagnosis of hypotonic polyuric states and to distinguish one form from the other (4, 5, 7, 24, 32).

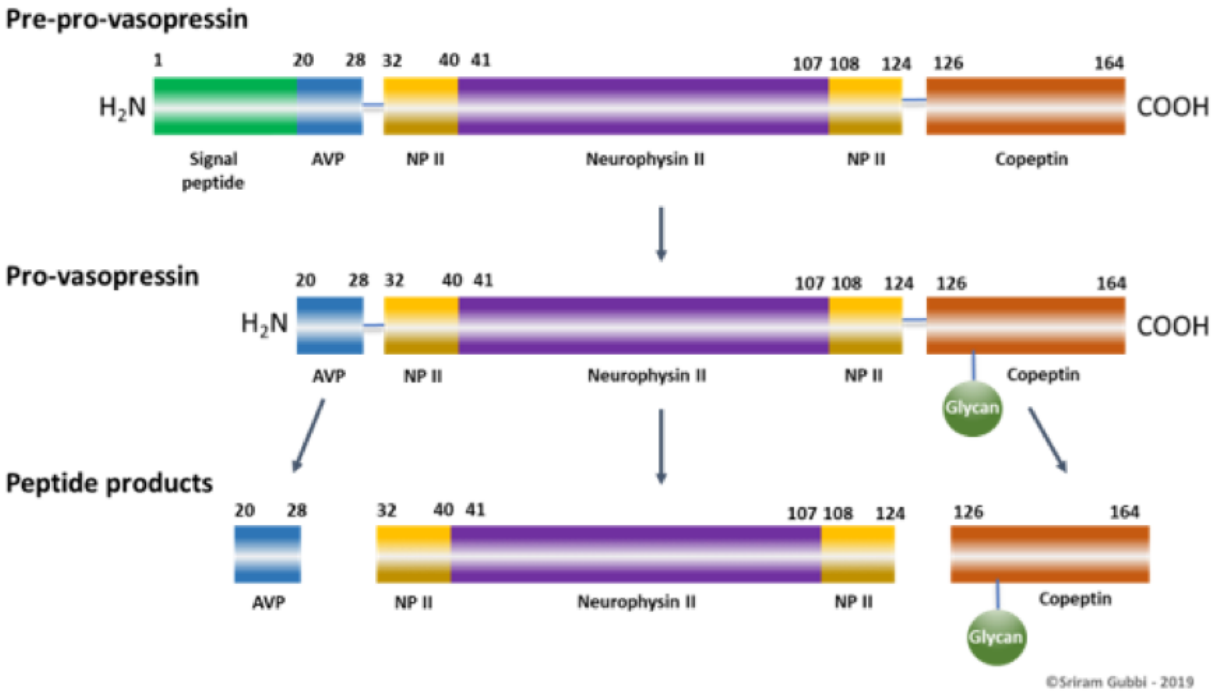

Figure 4. Post-transcription processing of vasopressin and related peptides. The pre-pro-vasopressin is a peptide consisting of 164 amino acids. This pre-pro-hormone is then converted to pro-vasopressin after the removal of the signal peptide and N-linked glycosylation of copeptin. Further processing of pro-vasopressin gives rise to the individual peptides: arginine vasopressin (AVP), neurophysin II (NP II), and copeptin. Image courtesy: Sriram Gubbi, NIDDK, NIH.

The next question logically is whether plasma copeptin is a better test when compared to plasma AVP with regards to diagnosing the various forms of polyuria-polydipsia syndromes. Without prior thirsting (without water deprivation/hypertonic saline infusion), baseline plasma copeptin of ≥21.4 pmol/L or a plasma AVP level of ≥3 pg/ml have been shown to distinguish nephrogenic DI (partial and complete) from other types of polyuria-polydipsia syndromes with 100% sensitivity and specificity (5, 45). Similarly, a single baseline plasma copeptin measurement of ≥2.9 pmol/L without prior water deprivation has demonstrated the ability to differentiate primary polydipsia from central DI with a sensitivity of 82% and specificity of 78%, while plasma AVP of ≥1.8 pg/ml can distinguish primary polydipsia from central DI with 54% sensitivity and 89% specificity (5).

Prior data has also shown that a plasma copeptin of <2.6 pmol/L can accurately distinguish between complete central DI from primary polydipsia with 95% sensitivity and 100% specificity (4, 24). Some studies have utilized a combined water deprivation/hypertonic saline infusion test to stimulate endogenous release of AVP and copeptin, and based on the results, plasma copeptin levels of ≥4.9 pmol/L can differentiate primary polydipsia from central DI (partial or complete) with 94% sensitivity and 96% specificity, while a plasma AVP of ≥1.8 pg/ml can do the same with 83% sensitivity and 96% specificity (5). In addition, the ratio of Δ copeptin from start till the completion of water deprivation (08:00 am to 04:00 pm) to the serum sodium at the end of water deprivation test has been shown to discern partial central DI (<0.02 pmol/L) from primary polydipsia (≥0.02 pmol/L) with a sensitivity of 83% and a 100% specificity, although newer data has demonstrated that the ratio of Δ copeptin to serum

sodium following water deprivation to be surprisingly of lower diagnostic accuracy than water deprivation alone (4, 32). Recent data suggests plasma copeptin measurement coupled with hypertonic saline infusion test provides more accurate results with regards to differential diagnosis of DI when compared with plasma copeptin measurement with water deprivation test (32)(see below, Hypertonic saline infusion test'). There is also emerging evidence for plasma copeptin as a valuable marker to predict post-operative risk for DI after pituitary surgeries, with plasma copeptin of <2.5 pmol/L predicting the risk of DI with a positive predictive value of 81% and plasma levels of >30 pmol/L on postoperative day 1 demonstrating a negative predictive value of 95% to rule out DI (46).

Based on these data, plasma copeptin measurement appears to be overall a better test when compared with plasma AVP measurement. However, it is the technical aspects of better pre-analytical stability, less cumbersome sample handling, and quicker reporting of results that make plasma copeptin a more attractive test. As of now, copeptin assays are not yet available worldwide and its utilization is limited to a few centers. Once the test becomes more widely available in the future, plasma copeptin level measurements are likely to be frequently pursued to diagnose and distinguish the various forms of polyuria-polydipsia syndromes.

COPEPTIN STIMULATION TESTS

The indirect water deprivation test is the most well tested and widely utilized as the standard diagnostic test for DI. Adjunctive measurements of plasma AVP or copeptin levels can enrich the diagnostic yield of the water deprivation test. But it is quite evident that the test has several limitations and, in many situations, just does not provide an accurate diagnosis. Therefore, several copeptin stimulation tests have been formulated in the recent years using osmotic stimulants of copeptin such as hypertonic saline and urea in addition to non-osmotic stimulants such as arginine and glucagon.

1. Osmotic Stimulation tests (Hypertonic Saline Infusion test, Urea stimulation test).
2. Non-osmotic stimulation tests (Arginine stimulation test, Glucagon stimulation test).

Hypertonic Saline Infusion Test

Although relatively newer than the water deprivation test, some of the earliest reports on the potential utility of hypertonic saline infusion in the differential diagnosis of DI dates to the 1940s (47). Hypertonic saline (3% saline, 513 mOsm/L) infusion coupled with plasma copeptin measurement is an alternative test that is now being recommended by many experts in the field of DI as the preferred test to be used in place of water deprivation test.

TESTING PROTOCOL

The test overall lasts for about 3 hours and can be initiated at 08:00 am. Medications that have diuretic or anti-diuretic effects (diuretics, SGLT-2 inhibitors, desmopressin, carbamazepine, chlorpropamide, glucocorticoids, non-steroidal anti-inflammatory drugs) need to be discontinued 24 hours prior to testing (32). The patient lies in a supine position. Two intravenous cannulas are inserted, one for infusion and the other for blood sampling. Before commencing the intravenous infusion, venous sampling is performed to obtain plasma copeptin, serum sodium, glucose, urea, and plasma osmolality (32). Hypertonic saline infusion is then commenced, initially with a bolus dose of 250 ml given over 10 – 15 minutes, followed by a slower infusion rate of 0.15 ml/Kg/min. Serum sodium and osmolality are measured every 30 minutes. The infusion is terminated once the serum sodium is ≥150 mOsm/L (32). Typically, protocols allow up to a maximum of 3 hours of infusion (32,48). At this point, a plasma copeptin is measured and the patient is asked to drink water at 30ml/Kg within 30 minutes (32, 48). This is followed by intravenous infusion of 5% glucose (dextrose) at 500 ml/hour for one hour (32, 48). Serum sodium is measured once more after completing the 5% glucose infusion to ensure its return to normal values. Vital parameters,

including blood pressure and heart rate must be constantly assessed, preferably on a monitor.

TEST INTERPRETATION

A plasma copeptin level of <4.9 pmol/L after hypertonic saline infusion indicates central DI (partial and complete) while a level of ≥4.9 pmol/L indicates primary polydipsia (5, 32). It is likely that this cut-off value might be changed to 6.5 pmol/L in the future due to its higher diagnostic accuracy, based on more recent data (described below) (32). Baseline copeptin value of >21.4 pmol/L is indicative of nephrogenic DI (partial and complete) and <2.6 pmol/L indicates complete central DI (4, 5, 32).

The major advantage with the hypertonic saline infusion test is that it basically excludes the renal component out of the equation and any potential down-regulation of aquaporin-2 channels will not affect the diagnosis. This test depends only on two aspects: 1. Increasing the plasma osmolality, which is a strong stimulus for endogenous AVP synthesis and release and 2. Measurement of endogenous AVP secretory capacity by measuring plasma copeptin, which is secreted in equimolar proportions from the posterior pituitary along with AVP (3, 4). Thus, unlike water deprivation test which is mainly a test based on urine output and osmolality, the hypertonic saline infusion test is free from any requirements to measure urinary indices. In fact, when compared with water deprivation test with desmopressin administration with/without plasma copeptin measurement, the hypertonic saline infusion test with plasma copeptin measurements has been demonstrated to be the superior test for diagnosing the various forms of polyuria-polydipsia syndromes (32). Hypertonic saline infusion test also takes less time to perform, causes less patient burden, and is more convenient and tolerable when compared with indirect water deprivation test (32).

A modified hypertonic saline infusion test followed by water deprivation has also been previously described when the diagnosis after water deprivation is inconclusive (49). In this scenario, administration of hypertonic saline (as described in the protocol) followed by measurements of plasma AVP, serum sodium, plasma and urine osmolality can potentially differentiate the types of DI when the plasma AVP levels are plotted on a nomogram relating plasma AVP values to either urine osmolality or serum sodium/plasma osmolality (normal plasma AVP for urine osmolality or serum sodium/plasma osmolality suggests primary polydipsia, low plasma AVP for serum sodium/plasma osmolality suggests central DI, and low urine osmolality for plasma AVP levels suggests nephrogenic DI). However, this test is more cumbersome than either indirect water deprivation test or hypertonic saline infusion test alone and requires measurements of plasma AVP levels which is also challenging (as described above. Measurement of plasma AVP'). Therefore, this combined method is not utilized in routine clinical practice. With the advent of plasma copeptin assays and with the ability to eliminate urine measurements out of the equation for the regular hypertonic saline infusion test, the plasma AVP measurement-based combined water deprivation and hypertonic saline infusion test is likely going to be obsolete in the future.

Recent data by Fenske et al. have demonstrated the diagnostic accuracy of hypertonic saline infusion test combined with plasma copeptin measurement to be higher (96.5%) than that of water deprivation test (76.6%) in correctly identifying the type of polyuria-polydipsia syndrome (32). Even when it came to differentiating those with partial central DI from patients with primary polydipsia, the hypertonic saline infusion test with plasma copeptin measurement had a higher accuracy (95.2%) as compared to water deprivation test without plasma copeptin measurement (73.3%) (32). Data from the same study has also shown that a plasma copeptin value of 6.5 pmol/L provides the best diagnostic accuracy (97.9%) for differentiating central DI (partial and complete) from primary polydipsia (vs. 96.5% for the plasma copeptin cut-off level of 4.9 pmol/L), with a value of >6.5 pmol/L being consistent with primary polydipsia. Surprisingly, in the same study, water deprivation test

followed by plasma copeptin measurement demonstrated a lower diagnostic accuracy when compared to water deprivation test alone without plasma copeptin measurement (44% vs. 76.6%). These results show that combining hypertonic saline infusion and plasma copeptin measurements currently offers the best diagnostic capability with distinguishing the various forms of hypotonic polyuric states. The protocol for the hypertonic saline infusion test, interpretation of the results, and limitations are presented in Table 3.

Table 3. Hypertonic Saline Infusion Test (with plasma copeptin measurement)
Protocol
Preparation phase: · Test can begin at 08:00AM. · Drugs that can affect urine output (diuretics, sodium-glucose co-transporter-2 inhibitors, glucocorticoids, non-steroidal anti-inflammatory drugs) must be held 24 hours prior to dehydration. · Patient lies in a supine position. Two intravenous cannulas are placed: one for blood sampling and the other for infusion. · Baseline serum sodium, glucose, urea, plasma osmolality and plasma copeptin are obtained prior to infusion.
Hypertonic saline infusion phase: · Hypertonic saline infusion is commenced: Bolus dose of 250ml given over 10 – 15 minutes, followed by 0.15 ml/Kg/min. · Serum sodium and osmolality are measured every 30 minutes. · The infusion is stopped if: o The serum sodium raises to >150 mmol/L. o The infusion is completed for 3 hours. · Plasma copeptin is measured after the infusion is stopped. · Heart rate and blood pressure are continuously monitored throughout the phase.
Hypotonic fluid administration phase: · Patient is asked to drink water at 30ml/Kg within 30 minutes. · This is followed by intravenous infusion of 5% glucose at 500ml/hour for 1 hour. · Serum sodium is measured after the completion of 5% glucose infusion to ensure its return to normal values. · Heart rate and blood pressure are continuously monitored throughout the phase.
Interpretation
Central DI: o Baseline plasma copeptin <2.6 pmol/L prior to hypertonic saline infusion (complete central DI). o Plasma copeptin level of ≤4.9 pmol/L after hypertonic saline infusion (partial or complete). *
Nephrogenic DI: o Baseline plasma copeptin ≥21.4 pmol/L prior to hypertonic saline infusion (partial or complete).
Primary polydipsia: o Plasma copeptin level of >4.9 pmol/L after hypertonic saline infusion. *
Advantages

· Takes less time to perform than water deprivation test. · Eliminates the need to obtain urine studies which can be affected by aquaporin-2 channel availability, thus reducing the chances of confounding results. · Hypertonic saline is a stronger osmotic stimulus when compared to dehydration and is therefore more potent with causing AVP release in cases of partial central DI or chronic primary polydipsia. So, this is likely to be a better test to utilize in cases of partial central DI and chronic primary polydipsia. · Distinguishes central DI (complete and partial) from primary polydipsia with a higher accuracy when compared with water deprivation (with/without plasma copeptin) test. · Less burdensome and more convenient for the patients than water deprivation test.
Disadvantages
· Several centers mandate the use of central lines for hypertonic saline administration. · Hypertonic saline infusion has the theoretical risk of causing superficial thrombophlebitis. · Higher risk for hypernatremia when compared to water deprivation test. Hypertonic saline infusion should be used in caution or avoided in individuals with heart failure, uncontrolled hypertension, and seizure disorder · Plasma copeptin assays are currently of limited availability.
*A higher cut-off value of 6.5 pmol/L distinguishes central DI from primary polydipsia with increased accuracy based on newer data, with a value of >6.5 pmol/L being consistent with primary polydipsia (Fenske et al., 2018).

LIMITATIONS

The challenges with utilizing hypertonic saline infusion test are as follows:

1. Hypertonic saline has been claimed to cause thrombophlebitis when administered through peripheral intravenous line. However, this risk is overestimated and is rarely observed in practice (50).
2. Several institutions require placement of a central venous catheter and admission to an intensive care unit for administration of hypertonic saline. Central line insertion is a more invasive and time-consuming procedure when compared to a peripheral intravenous line and can cause more bleeding or infections, and an admission to an intensive care unit exerts substantial expenditure on the patient and the institute.
3. Hypertonic saline should be avoided in individuals with heart failure, poorly controlled hypertension, and seizure disorder where changes in plasma osmolality can trigger seizures.
4. Copeptin assays are not yet commercially available in several countries.

Urea-Stimulated Copeptin Test

Urea is another osmotic stimulant for arginine vasopressin release from posterior pituitary. Therefore, another diagnostic approach to distinguish central DI from primary polydipsia can be urea-stimulated copeptin test. Lustenberger et al. (51) conducted a randomized, double-blind, placebo-controlled cross-over trial which showed that copeptin levels increased from 4.6 pmol/L at baseline to 10.1 pmol/L at 120 min after ingestion of a single dose of urea (0.5g/kg; minimum 30g, maximum 45g), while the levels remained stable at 3.8 pmol/L in the placebo group. In patients with primary polydipsia the median copeptin levels peaked after 150 min at 7.4 pmol/L (4.3, 10.3) from baseline of <2.7 pmol/L with maximum median change of +4.1 pmol/L (+1.3, +6.1) while in patients with central DI the median copeptin remained below detection limit throughout the test with baseline of <2.7 pmol/L and maximum median change of ±0 pmol/L (±0, +0.5). The best copeptin cutoff was observed at 3.5 pmol/L after 120 min of ingestion of urea to distinguish central DI from primary polydipsia with sensitivity and specificity of 92% (CI, 77 to 100%).

Maximum specificity and sensitivity of 100% was observed at a copeptin level of 2.7 pmol/L and 4.7 pmol/L respectively (51). If validated, this oral urea-based test could potentially serve as simple and cost-effective, initial oral diagnostic test in diagnosis of polyuria-polydipsia syndrome which can be safely carried out in primary care settings. Ingestion of urea is usually well-tolerated except for a very common side effect of dysgeusia which can now be reduced with availability of more palatable formulations.

Arginine-Stimulation Test

With regards to diagnostic testing, further refinements are implemented to the copeptin-based testing, such as utilization of non-osmotic neurohypophyseal secretagogues, to minimize adverse effects or discomfort related to iatrogenic hypernatremia induced by hypertonic saline.

Arginine, a known growth hormone secretagogue, is also a non-osmotic stimulus to the posterior pituitary, and it increases plasma copeptin levels in healthy individuals (52, 53). Arginine-stimulated copeptin measurement is another diagnostic tool to differentiate central DI from primary polydipsia. In a prospective study by Winzeler et al. (53), intravenous arginine-stimulation was able to distinguish patients with central DI, and primary polydipsia, and healthy controls using the 60-minute post-stimulation plasma copeptin levels, with high diagnostic accuracy of 93% using the cutoff of 3.8 pmol/L (53).

In a head-to-head trial by Refardt et al. (54), the diagnostic accuracy for arginine-stimulated copeptin (using cutoff of 3.8 pmol/L) was 74.4% (95% CI, 67.0 to 80.6) as compared to 95.6% (95% CI, 91.1 to 97.8) with hypertonic saline–stimulated copeptin (using cutoff of 4.9 pmol/L) with estimated difference of −21.2 percentage points (95% CI, −28.7 to −14.3). Arginine-stimulated copeptin levels ≤3.0 pmol/L and >5.2 pmol/L led to diagnosis of AVP deficiency with a specificity of 90.9% and primary polydipsia with a specificity of 91.4% respectively (54). A secondary post-hoc analysis of the initial study by Winzler et al validated these newly proposed cutoffs. A 60-min arginine-stimulated copeptin level of ≤3.0 and >5.2 provided specificity of 95% (95% CI, 0.86 to 1.00) and 100% (95% CI 0.99-1.00) for diagnosing central DI and PP respectively, and a 90-min arginine-stimulated copeptin ≤3.0 and >6.0 provided specificity of 92% (95% CI, 0.84 to 1.00) and 100% (95% CI, 0.99 to 1.00) for diagnosing central DI and PP respectively (55). Using these cutoffs 69% of patients with PP and 71% of the patients with central DI were correctly diagnosed.

The hypertonic saline-stimulated copeptin test is currently considered the most accurate method for diagnosing central DI. However, owing to the challenges that hypertonic saline-stimulated copeptin test presents (see Table 3), the arginine-stimulated copeptin test can be considered a well-tolerated and simple option for initial diagnosis. Atila et al further suggest that the 30% of the patients with copeptin values between these cutoffs or those who are intolerant to arginine due to nausea or vomiting should undergo further testing using the hypertonic saline-stimulated copeptin test or indirect water deprivation test (55).

A recent single-center cohort study utilized multivariable approach to enhance the diagnostic accuracy of arginine-stimulation test. Serum sodium level ≥141 mmol/L at the end of arginine-stimulation test was the best predictor of central DI with sensitivity, specificity, and accuracy of 87.5%, 100% and 94.7% respectively (AUC 0.989) (56). In cases falling between serum sodium 140-142 mmol/L, serum copeptin ≤4.1 pmol/L, UOsm ≤ 428 mOsm/kg, or absent posterior pituitary hyperintense signal achieved 100% diagnostic accuracy (56). This multivariable approach can offer better accuracy without measuring copeptin levels (which remain widely unavailable) and can make arginine-stimulation test more simple and feasible option. However, the study had several limitations including but not limited to small sample size, retrospective nature, lack of validation by using hypertonic saline-stimulation test.

The most common reported adverse effect with arginine-stimulated hypertonic saline is mild nausea (53,54). More than 70% of the patients preferred testing with arginine as compared with hypertonic saline (54). However, arginine is not widely available limiting its use.

PROTOCOL

Preparation Phase:
Overnight fast with fluids allowed until 6 am (2 hours before testing begins)
Start stress dose steroids for those on chronic steroid therapy.
Begin the test at 8am.

Arginine Phase:
An infusion of L-arginine hydrochloride (21%) at a dose of 0.5 g/kg of body weight (maximum, 40 g) diluted in 500 ml of 0.9% normal saline administered over a 30-minute period.
Blood samples for copeptin measurement obtained at baseline before the infusion and 60 and 90 minutes after the start of the infusion.

Interpretation:
Central DI
Baseline plasma copeptin <2.6 pmol/L prior to arginine infusion (complete central DI).
Plasma copeptin ≤3.8 pmol/L after arginine infusion (partial or complete).

Nephrogenic DI
Baseline plasma copeptin >21.4 pmol/L prior to arginine infusion (partial or complete).

Primary Polydipsia
Plasma copeptin >3.8 pmol/L after arginine infusion.

Advantages:
Takes less time to perform as compared to water deprivation test.
Less cumbersome and more convenient for the patients as compared to Hypertonic saline infusion test.
Can be preferred where use of hypertonic saline is contraindicated.
Eliminates the need to obtain urine studies.

Disadvantages:
Less accurate than hypertonic saline infusion test to distinguish central DI (partial or complete) from primary polydipsia.
Limited availability of Arginine.

Glucagon-Stimulated Copeptin Test

Glucagon, another known growth hormone secretagogue and neurohypophyseal stimulant, was also evaluated in a double-blind, placebo-controlled, randomized trial for differential diagnosis of central DI and PP (57). Subcutaneous injection of 1mg Glucagon led to significant increase in plasma copeptin levels measured at baseline and 30, 60, 90, 120, 150, and 180 minutes in healthy controls compared to placebo, and while there was minimal increase of 0.55 pmol/L in plasma copeptin in central DI patients, there was a substantial increase of 15.70 pmol/L in copeptin levels in patients with primary polydipsia. Sensitivity and specificity were measured at 100% (95% CI, 100 to 100) and 90% (95% CI, 70-100) respectively using cutoff copeptin level of 4.6 pmol/L (57).

Further studies performing head-to-head prospective comparisons with hypertonic saline infusion test are required to identify the best copeptin-based diagnostic test.

THERAPEUTIC TRIAL

If none of the modalities succeed in establishing an appropriate diagnosis of DI, a therapeutic trial with desmopressin can be undertaken (3, 49). The therapeutic trial can be provided in the form of desmopressin tablets of 100 µg by mouth every 8 hours for 48 hours. If this treatment abolishes polyuria, normalizes serum sodium/plasma osmolality or at least brings down serum sodium to near normal levels, resulting in elimination of thirst, the diagnosis is most certainly central DI. If there is cessation of polyuria and

there is slight normalization of serum sodium/plasma osmolality without reduction in polydipsia/thirst, the most likely diagnosis is primary polydipsia. In nephrogenic DI, therapeutic trial with desmopressin does not result in any significant changes in serum sodium or plasma and urine osmolality (49). This method can distinguish between various forms of DI with 90% accuracy (49). However, caution is advised when utilizing desmopressin for diagnostic testing due to the risk of hyponatremia, which can sometimes be severe in patients with primary polydipsia and the test is preferably done in a monitored in-patient setting (49).

OTHER POTENTIAL DIAGNOSTIC MARKERS

Macimorelin, another growth hormone secretagogue which also has stimulatory effect on prolactin, does not stimulate copeptin (58). Apelin is an endogenous hormone present in magnocellular neurons of hypothalamic supraoptic and paraventricular nuclei together with arginine vasopressin and oxytocin (59). Apelin plays an important role in water homeostasis by inhibiting the release of AVP from posterior pituitary and counteracting its anti-diuretic effect on the kidneys. It augments the diuresis by antagonizing the effects of angiotensin II on the afferent arterioles and is degraded by angiotensin converting enzyme type 2 (59). The patients with central DI have increased apelin-to-copeptin ratio while those with PP have a normal ratio at baseline (59). A post hoc secondary analysis of a multi-center cross-over trial showed that after stimulation with either hypertonic saline or arginine, the apelin levels and apelin-to-copeptin ratio decreased in all patients with greater reduction seen in patients with PP (59). Possible explanations could be concomitant deficiency of apelin in patient with central DI or appropriately suppressed apelin levels to limit water diuresis. The sub-group analysis of this study showed that the change in apelin-to-copeptin ratio can improve the accuracy of arginine-stimulated copeptin test (59).

ROLE OF DIAGNOSTIC SCORING SYSTEM

A diagnostic score using laboratory data and clinical parameters for distinguishing central DI from PP was recently developed by Atila et al (60). Data from 2 international multicenter studies was used and excluded the patients with nephrogenic DI with basal copeptin levels. The score includes the sum of

1. Basal laboratory score (basal serum sodium multiplied by serum osmolality, divided by 100),
2. Extended laboratory score (-50 points for plasma copeptin >4.9 pmol/L),
3. Clinical score (+50 points for anterior pituitary dysfunction, +50 points for history of pituitary surgery, +30 points for drinking >1L at night, +20 points for sudden onset of symptoms, +20 points for ≥3 episodes of nocturia or +10 points for 2 episodes of Nocturia), and
4. Clinical MRI score (+40 points for thickening of pituitary stalk and +10 points absence of posterior bright spot). A high-sensitivity threshold of <415 points excludes central DI, whereas high-specificity threshold of >461 points identifies patients with a high likelihood of Central DI (60).

The clinical score was only slightly less precise without incorporating copeptin levels, therefore can still be used in the clinical practices where copeptin measurements are not available (60).

Table 4. Arginine Vasopressin Deficiency (Central Diabetes Insipidus) Diagnostic Score Developed by Atila et al 2025 (ref. 60)		
Basic Laboratory Score	**Serum sodium (mmol/L) x Plasma Osmolality (mOsm/kg) Divided by 100**	**<415 excludes central DI** **>461 suggests high likelihood of central DI**
Extended Laboratory Score	**-50 points for Copeptin >4.9 pmol/L**	
Clinical Score	**+50 points for anterior pituitary dysfunction** **+50 points for history of pituitary surgery** **+30 points for drinking >1L at night** **+20 points for sudden onset of symptoms** **+20 points for ≥3 episodes of nocturia or +10 points for 2 episodes of Nocturia)**	
Clinical MRI Score	**+40 points for thickening of pituitary stalk** **+10 points absence of posterior bright spot**	

IDENTIFICATION OF THE UNDERLYING CAUSE OF THE DI

Once the type of polyuria-polydipsia syndrome is identified, efforts must be undertaken to diagnose the underlying pathology responsible for this clinical presentation. A detailed list of the potential causes for each type of DI and primary polydipsia is provided in Table 1. In cases of central DI, a thorough clinical history should be obtained, and a detailed physical exam needs to be performed to evaluate for the signs and symptoms of hormonal deficiencies (or excess in cases of hyperprolactinemia) from other pituitary axes (9, 61). Biochemical evaluation must include a morning plasma measurement of pituitary hormones (growth hormone, prolactin, ACTH, TSH, FSH, and LH), and the hormones from their target organs (insulin-like growth factor 1, cortisol, free thyroxine, total and free testosterone, estradiol). An MRI of the sella and suprasellar regions with gadolinium needs to be obtained to evaluate for any anatomical disruptions of the pituitary or hypothalamic anatomy (macroadenomas, empty sella, infiltrative diseases, surgery) (19, 23). The normal posterior pituitary demonstrates hyperintensity on T1 images (also known as the 'bright spot'), suggested to be due to phospholipid-rich granules storing AVP and oxytocin (Figure 5) (62). The absence of this bright spot could indicate an absence of posterior pituitary function. However, this should not be used as a sole criterion to attribute a pituitary etiology as causing the DI, because absence of the bright spot on the pituitary MRI is also seen in up to 25% of normal individuals and may disappear with aging (63). Also, an enlarged/thickened pituitary infundibular stalk can be found on the MRI, which may be seen in cases of hypophysitis, granulomatous disorders, tuberculosis, craniopharyngioma, germinoma or a metastasis to the

sella or suprasellar region (3, 9, 19, 64). The hypothalamus is the site of AVP synthesis (Figure 3) and any pathological involvement of the region (inflammatory, infectious, vascular, or neoplastic process) can certainly result in the destruction of the hypothalamus leading to deficiency of AVP synthesis (3). This is specifically important among patients with adipsic DI as the thirst center might be disrupted due to the above-mentioned etiologies that can involve the hypothalamus. Any history of cranial trauma or intra-cranial surgery could also give rise to central DI.

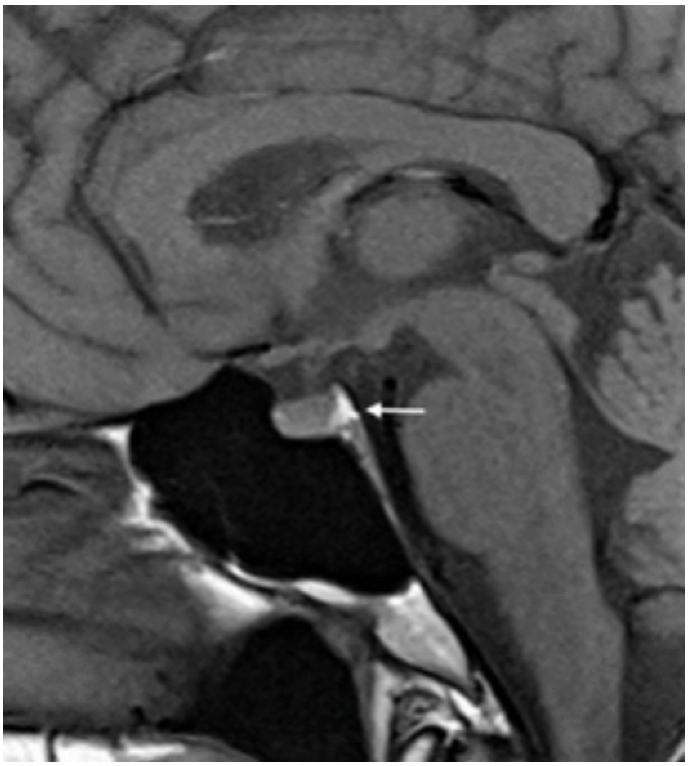

Figure 5. Magnetic resonance imaging (MRI) of the pituitary gland. The above image is a non-contrast T1 MRI image of a normal pituitary gland. The white arrow points towards the 'bright spot' seen in the posterior pituitary. This finding is a result of phospholipid-rich granules that store arginine vasopressin (AVP) and oxytocin. Image courtesy: NIDDK, NIH.

In cases of DI with onset during infancy or early childhood, genetic/congenital causes must be suspected. Some of the genetic conditions causing DI include AVP-neurophysin II gene alterations (*AVP-NP II,* autosomal dominant), Wolfram syndrome, an autosomal recessive disorder caused by alterations in *WFS1*(4p16.1) associated with diabetes insipidus, diabetes mellitus, optic atrophy, and deafness (OMIM 22300, DIDMOAD syndrome), and other autosomal recessive disorders due to production of mutant, weaker forms of AVP, and X-linked recessive disorders resulting in sub-normal plasma AVP levels (23, 65-68). Congenital malformations, such as septo-optic dysplasia (*HESX1, OMIM* 601802, 3p14), Schinzel-Giedion midface retraction syndrome (*SETBP1*, OMIM 611060, 18q12), Culler-Jones syndrome (*GLI2,*OMIM 165230, 2q14), Alstrom syndrome (*ALMS1, OMIM* 606844, 2p13), Hartsfield syndrome (*FGFR*, OMIM615465, 8p11), Webb-Dattani syndrome (*ARNT2*, OMIM 606036, 15q25), amongst others, can also give rise to childhood-onset DI (69-73).

Nephrogenic DI in most cases is acquired, usually in the setting of intake of certain drugs like lithium, demeclocycline, pemetrexed, cisplatin, and others (6,

7, 19, 23, 74). Therefore, a review of the patient's medication intake history can lead to the identification of the potential culprit medication, which can then be discontinued. Use of osmotic diuretics or electrolyte abnormalities such as hypercalcemia or hypokalemia and low caloric intake especially low protein intake must be investigated in cases of nephrogenic DI (6, 19, 31). Any underlying acute or chronic renal disease (vascular, inflammatory, or neoplastic processes, polycystic kidney disease, acute tubular necrosis), obstructive uropathy, and systemic diseases such as amyloidosis, hemochromatosis, or sickle cell disease can also give rise to nephrogenic DI and prompt evaluation for these disorders is necessary (6, 7, 19, 23). Congenital causes for nephrogenic DI include mutations in the gene for aquaporin-2 receptor (autosomal recessive) and the gene for V-2 receptor (X-linked recessive inheritance), and must be suspected in childhood-onset nephrogenic DI (7). Other genetic causes of nephrogenic DI include; polyhydramnios, megalencephaly, and symptomatic epilepsy (*STRADA*, OMIM 608626, 17q23) and some Barter syndrome subtypes, including Bartter syndrome, type 4b, digenic forms (*CLCNKA*, OMIM 602024, 1p36.13) and other inherited renal syndromes (nephropathic cystinosis, nephronopthisis) (23, 75, 76).

Primary polydipsia or dipsogenic DI is often seen in individuals on treatment for mood disorders or schizophrenia (77). The dry mouth caused by intake of medications with strong anticholinergic properties to treat these disorders is most likely the cause for excessive water intake (77). Hypothalamic disease (sarcoidosis, tuberculosis, trauma, neoplasms) can alter the thirst response by lowering the thirst threshold, either by disruption of the thirst center or through osmoceptor dysfunction, which leads to polydipsia (3, 7). Primary polydipsia which results from this subset of cases with low thirst threshold in hypothalamus, is also referred to as 'dipsogenic DI'. In other cases, individuals are health enthusiasts or just habitual compulsive drinkers of large volumes of water without any underlying organic or pharmacologic cause (6, 23). Another etiology is psychosis-intermittent hyponatremia polydipsia (PIP) syndrome (23).

A unique presentation of DI which is worthy of note, is the one that occurs during pregnancy. Also known as gestational diabetes insipidus, this disorder occurs during pregnancy due to the enzymatic breakdown of the endogenous AVP by a placental cysteine aminopeptidase (6, 7). In women with borderline-low plasma AVP levels, pregnancy can unmask sub-clinical DI from enzymatic degradation of AVP (8). However, work-up for other etiologies of DI must be considered when appropriate in this situation.

CONCLUSIONS

Making an accurate diagnosis of DI and ascertaining its type and the underlying etiology poses a significant challenge to this day. In clinical practice, under some circumstances, a diagnosis of DI is established based on serum/plasma and urine studies alone and based on the history and clinical presentation, investigations to identify the underlying etiology are pursued. However, an accurate diagnosis of the type of DI or any polyuria-polydipsia syndrome in general, is difficult to establish as there can be a significant overlap in the results among the various forms of polyuria-polydipsia syndromes on diagnostic testing. Specific testing protocols, such as the indirect water deprivation test or the hypertonic saline infusion test can assist with providing a diagnosis with increased accuracy. Measurement of plasma AVP levels is not routinely performed due to several pre-analytical considerations and lack of widespread availability of assays. The combination of hypertonic saline infusion coupled with plasma copeptin level measurement has achieved diagnostic accuracies that have not been previously attained by any other testing modalities with regards to differential diagnosis of polyuria-polydipsia syndromes (32). But the newer non-osmotic, neurohypophyseal secretagogue (arginine-) based plasma copeptin measurements holds the promise of being the safer, less cumbersome testing modality. However, more randomized trials comparing these modalities with hypertonic saline infusion are

necessary, with cost and regional availability of these agents also taken into consideration. Therefore, hypertonic saline infusion, plasma copeptin-based approach and arginine-stimulation test could potentially become the standard of practice in the future to accurately establish the diagnosis of DI and related polyuria-polydipsia syndromes.

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