Estimation of Plasma Arginine Vasopressin Concentration Using Thirst Perception and Plasma Osmolality Values

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Summary: In human, thirst and antidiuretic hormone (ADH) are controlled by similar sensitive osmoregulatory mechanisms such that above a certain osmotic threshold (280-288 mOsm/kg H_2O) there is a linear relationship between the increase in plasma osmolality and increase in ADH and thirst. The purpose of this study was to estimate plasma arginine vasopressin (P_{AVP}) using thirst perception (TP) and plasma osmolality (P_{OSM}) values before and at 60 minutes in control or euhydrate (group A, 0.0 ml/kg body weight of distilled water), hydrated (group B, 7.1ml/kg body weight of distilled water) and dehydrated (group C, 0.0 ml/kg body weight of distilled water) subjects. A total of twenty five (25) subjects between the ages of 18 and 30 years were used for the study. Calculated P_{OSM} and TP values were used to estimate the P_{AVP} concentration. Data were presented as Mean ± SEM. Analyses of results were done using ANOVA and Student *t*-test. The estimated values of P_{AVP} using TP and P_{OSM} respectively at baseline levels were similar in euhydrate (2.22 ± 2.00 vs 2.40 ± 2.10 pg/ml), hydrate (2.22 ± 1.34 vs 2.40 ± 1.72 pg/ml) and in dehydrate (7.05 ± 1.70 vs 6.92 ± 1.94 pg/ml). Sixty minutes later, the values remained similar in euhydrate (3.29 ± 2.40 vs 4.16 ± 2.10 pg/ml), hydrate (1.92 ± 1.60 vs 1.79 ± 1.25 pg/ml) and in dehydrate (8.40 ± 1.40 vs 9.20 ± 1.50 pg/ml). The results show that there was a positive relationship between P_{AVP} calculated from TP and P_{OSM} values. We therefore concluded that plasma arginine vasopressin concentration may be estimated using thirst perception and/or plasma osmolality values. Estimation of P_{AVP} using plasma osmolar changes affected by glucose and urea may be inappropriate.

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INTRODUCTION

Arginine vasopressin (AVP) also referred to as antidiuretic hormone (ADH) is a nine amino acid peptide with a 6-member disulphide ring**.** It is structurally related to oxytocin but differ in two amino acids. It is synthesized majorly by the supraoptic nuclei and in a minute quantity by the paraventricular nuclei of the hypothalamus and eventually stored in the posterior pituitary. It has a very powerful antidiuretic action (Tijssen, 1985) and is the principal hormone involved in the conservation of water by the kidney. Its main effect is found in the collecting ducts where it causes insertion of aquaporin 2 (AQP2) water channels on the apical membrane (Nielson et al., 1993; Nielson et al., 1995; Fushimi et al., 1997) thus increasing permeability to water. It has been shown that AVP is released by both osmotic and non-osmotic stimuli (Clarke et. al., 1979; Malvin, 1971). It is also a potent pressor agent, through the activation of the vascular V1-receptors. Arginine Vasopressin secretion is regulated mainly by changes in the osmolality of blood and in extracellular fluid volume.

Disturbances of the secretion or function of vasopressin can cause profound clinical abnormalities in sodium and water homeostasis (Mckenna and Thompson, 1998). Serum AVP measurement is used clinically for studies involving diabetes insipidus, syndrome of inappropriate ADH secretion (SIADH), ectopic AVP production and psychogenic water intoxication (Haynes, 1958). These measurements are usually very expensive.

Robertson (1984) assessed the effects of osmotic stimuli on thirst mechanism and vasopressin secretion at various times during infusion of hypertonic sodium chloride solution in healthy adults. The result showed that the function, plasma arginine vasopressin concentration, $P_{AVP} = 1.48 (P_{OSM} - 284.7)$ and thirst perception, $TP = 9.06 (P_{OSM} - 293.5)$. According to his analysis, the osmotic threshold for the onset of thirst was 293.5mOsm/kg H_2O, which is approximately 10mOsm/kg H_2O above the osmotic threshold of vasopressin release. This analysis suggests that thirst rarely occurs in situations where plasma osmolality lies within the normal physiological range.

The major stimulus for thirst is an increase in plasma osmolality, which is regulated primarily by changes in the concentration of electrolytes in blood, measured as the osmolality of blood plasma. Oral fluid loads and dehydration show a consistent thirst perception in man (Obika et. al., 2009). In 2008, Igbokwe and Obika showed that thirst perception as

recorded with the VAS can be related to the concentration of plasma AVP in normal subjects. More recently, Amabebe et al. (2012) measured plasma AVP using AVP ELIZA and showed clearly that there is a relationship between plasma AVP concentration and thirst perception in man. Thus, thirst and ADH mechanism operate in such a way that the regulation of body fluid volume is dependent on the activity of the hormone ADH as well as an intact thirst mechanism.

A number of groups have employed the simple technique, the visual analogue scale, devised by Thompson et al. (1986) to measure thirst and showed that thirst perception does not change within the physiological range of plasma osmolality and that the thirst rating so obtained correlate closely with plasma osmolality. Thirst responses defined by this method are highly reproducible within an individual (Thompson et. al., 1991) and correlates well with the subsequent volume of water drunk (Thompson et. al., 1986; Obika et. al., 2009). Tiplady et al. (1998) established the validity and sensitivity of the visual analogue scale in healthy young and old subjects. The pattern of result obtained did not indicate any marked differences between the age groups in the use of VAS. The VAS, though, might be susceptible to a variety of personal and cultural influences; the method provides the best available description of the function of the thirst mechanism so far (Robertson, 1984).

The aim of this study was therefore to estimate plasma arginine vasopressin using TP and P_{OSM} values in an effort to ascertain the validity of the relationship between thirst perception and estimated plasma osmolality in normal subjects in three different states of hydration.

MATERIALS AND METHODS

SUBJECTS.
A total of twenty five (25) apparently normal subjects between the ages of 18 and 30 years were used for this study. Subjects were adequately informed of the experimental procedure and only those who gave their consent were enrolled. Exclusion criteria for this study were any history of diabetes and cardiovascular diseases. The subjects were divided into three groups: A, B and C.

Group A: This consists of the control or euhydrate subjects who did not receive any form of treatment. Anthropometric data were recorded, and they remained in the laboratory for the 60 minutes duration of the experiment.

Group B: This consists of hydrate subjects. On the day of the experiment, enrolled subjects entered the laboratory and their anthropometric data were obtained and were thereafter given 7.1ml/kg water

orally. They remained in the laboratory for the next 60 minutes.

Group C: This consists of dehydrate subjects who voluntarily went on 18-hour water deprivation. Prior to the day of the experiment, subjects were instructed not to drink water or any other form of fluid after 3.00pm till after the experiment the next day. They however continued to void and discard urine until 9.00pm. Thereafter and up until 9.00am on the next day (the day of the experiment), all urine was voided into a container provided. Only subjects who had a 12-hour urine volume of about 400ml were assumed to have complied with the dehydration procedure and therefore were included in this group. Anthropometric data were recorded and they remained in the laboratory for 60 minutes.

Collection of 12-hour urine sample. Prior to the day of the experiment, all the subjects in the three groups were asked to collect their 12-hour (from 9:00pm until 9:00am on the day of the experiment) urine sample into a container. Thus when they entered the laboratory by 9:00am, they were asked to empty the content of their bladder to complete the 12-hour urine sample collection.

Thirst perception rating. Thirst perception rating (cm) was obtained using the Visual Analogue Scale, VAS (Thompson et al., 1991). The VAS is an uncalibrated 10cm vertical line, with the top and base representing **"very thirsty"** and **"not thirst"** respectively.

All the subjects were educated on how to use the VAS to estimate their level of thirst perception (TP). They were then asked to mark on the line rating scale in response to the question "**How thirsty are you NOW**?''. The reading obtained from the point of not thirsty to the point marked by the subject is a subjective record of the thirst rating measured in centimeters at that point of time. There was a separate sheet of paper for each subject to rate their thirst perception. Anthropometric data such as height, ht (m) and weight, wt (kg) were recorded using measuring rule and weighing scale respectively. Body mass index (BMI) was thereafter calculated from the formula,

$$BMI = \frac{Wt(kg)}{Ht^2(m^2)}$$

Baseline (resting) blood pressures (BP, mmHg) were measured with subjects in the sitting position and after 15minutes of rest in the laboratory at room temperature. Three basal readings were obtained by indirect auscultatory method using sphygmomanometer and stethoscope on each subject at 3-minutes interval. The mean of these readings was recorded as normal BP.

Sample collection and Analysis: Urine and blood samples as well as other data were collected when the subjects entered the laboratory. Then, 60min later samples were again collected. All urine samples were collected into a plain container to determine the volume and/or specific gravity, while all blood samples were collected and gently transferred (to avoid lysis) into the Lithium Heparin sample bottles for analysis of sodium and potassium concentrations after centrifugation; and Fluoride Oxalate sample bottles for analysis of glucose and urea concentrations respectively. All plasma, serum and urine samples were analyzed for sodium (Na^+), potassium (K^+), Glucose (Glu) and Urea (Ur) concentrations at the Chemical Pathology Laboratory of the University Benin Teaching Hospital, using standard procedures. Packed cell volume, PCV, urine volume (V), and specific gravity (SG) of urine were determined in the Physiology Laboratory of the University of Benin. PCV was determined using heparinised capillary tube, a centrifuge and haematocrit reader, while V and SG were determined using measuring cylinder and Urinalysis reagent strips respectively.

Calculations: Plasma arginine vasopressin (P_{AVP}) concentration was calculated using TP and P_{OSM} with the following equations:

Plasma Arginine Vasopressin, P_{AVP}, calculated with the formulae:

$$P_{AVP} = \frac{T(CM) - 1.2}{0.75} \quad \text{(Igbokwe and Obika, 2008) ... (1)}$$

and,

$$P_{AVP} = 0.43 \times (P_{OSM} - 284.3) \quad \text{(Thompson et al., 1986) (2)}$$

Plasma osmolality was obtained indirectly from thirst perception values using the equations:

$$P_{OSM} = 10TP/3 + 281 \quad \text{(Thompson et al., 1986)... (3)}$$

and from,

$$P_{OSM} = 2Na + \frac{Glucose}{18} + \frac{BUN}{2.8} \quad \text{(Purssell et al., 2001) (4)}$$

Statistical Analyses
Data were presented as Mean ± SEM. Analyses of results were done using ANOVA and Student *t*-test. Correlation between calculated P_{AVP} using TP and P_{OSM} were determined. P values less than 0.05 were considered statistically significant.

RESULTS

This study compared calculated P_{AVP} using TP and P_{OSM} in euhydrate (group A), hydrate (group B) and dehydrate (group C) subjects at zero minute (fifteen minutes after the subjects entered the laboratory) a well as between zero minute and after 60minutes in each group A, B and C. Results in table 1 show that the parameters in all the groups were comparable to each other.

Results in table 2 show that at baseline, the PCV was higher in dehydrate group than in both euhydrate and dehydrate groups, but not statistically significant. However, after 60 minutes PCV was significantly higher in group C compared to groups A and B. When compared to the baseline values, hydration (group B) caused a significant fall in PCV after 60 minutes, while in euhydrate and dehydrate subjects, the PCV remained unchanged. The table also shows that at baseline, specific gravity of urine (SG) was significantly higher in group C subjects than in both groups A and B. In addition, after 60 minutes, it was significantly higher when compared to the 60 minutes

Table 1: Anthropometric data in Euhydrate, Dehydrate and Hydrate subjects at zero min (Baseline). Values are Mean ± SEM.

Group	Age, yrs.	Ht, m	Wt, kg	BMI, kg/m²	SBP, mmHg	DBP, mmHg
A (N = 5)	21.6±0.81	1.65± 0.04	65.9± 3.6	24.40± 1.96	112.3±3.5	69.6± 3.2
B (N = 10)	20.8±0.47	1.82± 0.09	61.9± 3.0	20.98± 0.72	112.8± 2.0	73.2± 2.1
C (N = 10)	22.6±0.87	1.69± 0.03	69.2± 3.2	21.76±0.99	111.2± 2.4	76.8± 2.4

Table 2: PCV, Specific Gravity and 12-h urine volume in Euhydrate, Dehydrate and Hydrate subjects at zero minute (Baseline) and at 60 minutes Values are Mean ± SEM.

Group		PCV, %	SG (urine)	UV, ml
A (N = 5)	At Baseline	43.8 ± 3.8	1.014 ± 0.001	692 ± 10.2
	At 60 minutes	41.0 ± 3.7	1.016 ± 0.002[+]	-
B (N = 10)	At Baseline	42.7 ± 1.8	1.009 ± 0.001	652 ± 28.6
	At 60 minutes	40.8 ± 1.8 [+]	1.005 ± 0.001[+]	-
C (N = 10)	At Baseline	44.4 ± 2.2	1.021 ± 0.001*	327 ± 20.7*
	At 60 minutes	45.9 ± 2.2 [#]	1.022 ± 0.001[+]	-

*= p<0.05 between Dehydrate, Hydrate and Euhydrate subjects at baseline.
#= p<0.05 between Dehydrate, Hydrate and Euhydrate subjects at 60 minutes.
+= p<0.05 between baseline and after 60 minutes values in each group (A, B and C).

Table 3: Calculated P_{AVP} using Thirst perception and Plasma Osomolality values in Euhydrate, Dehydrate and Hydrate subjects at zero minute (baeline) and at 60 minutes. Values are Mean ± SEM

Group		Thirst perception, (TP) cm.	P_{AVP} (pg/ml) calculated from TP (Igbokwe & Obika, 2008).	Plasma osmolality, (P_{osm}) mOsm/kg (Thompson et. al., 1986).	P_{AVP} (pg/ml) calculated from P_{osm} (Thompson et. al., 1986).	Plasma osmolality (P_{osm}) mOsm/kg (Purssell et al., 2001).	P_{AVP} (pg/ml) calculated from Posm (Purssell et al., 2001).
A(N = 5)	0 min.	3.06 ± 1.4	2.27±2.00	291.06±4.8	2.91±2.10	274.22±1.93	-4.34±1.9
	60 min.	3.68±1.80	3.29±2.40	293.98±6.1[+]	4.16±2.60[+]	277.84±0.98[+]	-2.87±0.43
B (N = 10)	0 min.	2.80 ±0.9	2.22±1.34	290.33±2.9	2.40±1.72	286.16±3.66	0.80±1.6
	60 min.	2.64±1.20	1.92±1.60	289.74±4.0[+]	1.79±1.25	278.34±2.21[+]	-2.60±0.95
C (N = 10)	0 min.	6.43 ±1.3*	7.05±1.7*	300.46±4.5	6.92±1.94*	279.79±5.0	-1.96±2.06
	60 min.	7.50±1.05*[+]	8.4±1.40[+]	305.98±3.5[+]	9.20±1.50[+]	283.53±4.15[+]	-0.33±1.79

[*] = p<0.05 between Dehydrate, Hydrate and Euhydrate subjects at baseline
[+] = p<0.05 between baseline and after 60 minutes values in each group, (A, and C).
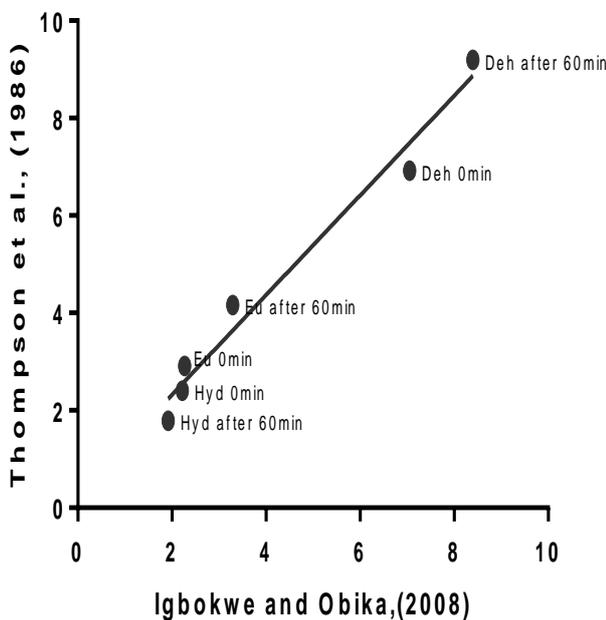

Figure 1. Relationship between the calculated P_{AVP} using the formula of Igbokwe and Obika (2008) and that of Thompson et. al. (1986).

values in groups A and B subjects. Values at baseline and at 60 minutes in groups A and B remained similar. Furthermore, the 12-h urine volume (UV) was significantly lower in group C subjects when compared the values in both group A and B subjects. Results in table 3 show that TP was significantly higher in group C subjects than in both group A and B subjects at baseline. After 60 minutes, there was an increase in TP in group A and C subjects but this was significantly higher only in group C while in group B, it was lower but not significant.

P_{OSM} (calculated from the formula of Thompson et al., 1986) was not significantly different between the three groups at baseline. It was however significantly higher after 60 minutes in both A and C groups and significantly lower in group B compared to baseline.

P_{AVP} calculated from TP (Igbokwe and Obika, 2008) and that calculated from P_{OSM} (Thompson et al., 1986) were significantly higher in C group at baseline. After 60 minutes, P_{AVP} was significantly higher in group A (from only the formula of Thompson et al., 1986) and in group C from both formulae, but there was no significant difference in group A from the formula of Igbokwe and Obika, (2008) and in group B from both formulae.

When the formula of Pursell et al., (2001) was applied in the calculations of both P_{OSM} and P_{AVP}, there was no significant difference in the three groups at baseline and the results were not consistent. After 60 minutes, P_{OSM} of subjects in groups A and C were significantly higher while that of the subjects in group B was significantly lower. There was no significant difference in P_{AVP} of the three groups after 60 minutes. There was a positive and significant (p<0.01) linear relationship between between the calculated P_{AVP} using the formula of Igbokwe and Obika (2008) and that of Thompson et. al. (1986) as shown in figure 1.

DISCUSSION

These experiments were designed to estimate P_{AVP} using TP and P_{OSM} values. Therefore we set out to develop an alternative method to estimate plasma arginine vasopressin concentration without measuring it or measuring plasma osmolaity.

Edwards (1971) reported that the normal P_{AVP} concentration measured by immunoassay is about 1-5 pg/ml. The Cayman Chemical Company (2011), using the arginine vasopressin Enzyme immunoassay (EIA) kit showed that normal levels of AVP in serum are between 0.4 and 5.2pg/ml. Kamath (2010) had shown that in healthy adults who had no fluid restrictions and had a normal activity level, the normal plasma concentration of ADH is between 0.35-1.94 ng/l (0.32-1.80pmol/l). Our result agrees with these findings, where P_{AVP} in euhydrate and hydrate subjects at baseline calculated from the

formula of Thompson et al. (1986) and that of Igbokwe and Obika (2008) were similar. The result as obtained with the formula of Purssell et al. (2001) were however negative and not within the same range, except for the hydrate subjects at zero minute. The probable explanation for this is that the equation involves substances that affect osmolality without affecting other substances that influence plasma osmolality like sodium and urea concentrations. And since it has been established that sodium is a major determinant of serum osmolality, it implies that P_{AVP} concentration will be indirectly affected.

Nevertheless the calculated P_{AVP} from TP (Igbokwe and Obika, 2008) and P_{OSM} (Thompson et al., 1986) were similar in the three groups (euhydrate, hydrate and dehydrate subjects), even when the calculated P_{AVP} in euhydrate and dehydrate subjects increased after 60 minutes and decreased in hydrate subjects after 60 minute. This implies that application of the stimulus (dehydration) increased both P_{AVP} concentration and TP, while removal of the stimulus (hydration) suppresses them. When P_{AVP} concentration was calculated from plasma osmolality using Purssell et. al. (2001) equation, the values also increased in euhydrate and dehydrate subjects while in hydrate subjects it reduced but as mentioned earlier these values were negative except for the hydrate subjects at baseline.

Karkare (2010) showed that if serum osmolality is more than 290 mOsm/kg H_2O, the ADH levels should be around 2-12pg/ml, and when it is less than 290mOsm/kg H_2O, the ADH levels should be less than 2pg/ml. Our results also agree with these findings as shown in tables 3 and 5 where the plasma osmolality calculated from Thompson et al., (1986) was 290.33mOsm/kg H_2O, and 289.8mOsm/kg H_2O, with the corresponding ADH values of 2.36pg/ml and 1.79pg/ml respectively.

However, when the formula of Purssell et al., (2001) was used, the values obtained were not within the physiological range of plasma ADH concentration, except for hydrate subjects at zero minute. This suggests that this formula may not be ideal for the estimation of P_{AVP} concentration in certain situations.

The baseline TP reported in these studies are similar to earlier reports of Obika et al., (1996) in young healthy non dehydrated subjects. Obika et al. (2009) also showed that after a period of dehydration, normal subjects showed an increase in TP. The results reported here agree with these earlier reports.

The subjects who dehydrated for 18hours (group C) had a decrease in urine volume and an increase in specific gravity indicating that there was increase in urine osmolality and consequently increase in P_{AVP} secretion and TP. Furthermore, previous studies by Thompson et al. (1986), Takamata et al. (1994) and

Baylis and Robertson (1980) showed that the increase in TP during dehydration correlates positively with increase in packed cell volume. Our work agrees with these findings where PCV was higher in dehydrate subjects compared to both euhydrate and hydrate subjects

Amabebe et al. (2012) using the arginine vasopressin Enzyme Immunoassay (EIA) to determine P_{AVP} concentration showed that a linear relationship exists between measured P_{AVP} and TP (cm) and from the present study, linear relationship also exists between calculated P_{AVP} and TP (cm) in that after a period of dehydration, P_{AVP} increased concomitantly with TP and decreases as well with hydration.

The validity of measurements of subjective rating of thirst has been previously reported as follows: thirst correlates positively with P_{OSM} (Baylis and Robertson, 1980). Thirst ratings using VAS was also found to correlate positively with P_{OSM} (Thompson et al., 1986; Takamata et al., 1994). From our study, it was shown that TP and P_{OSM} were higher in dehydrate subjects than in both euhydrate and hydrate subjects, also suggesting that after dehydration, there is a concomitant increase in TP and P_{OSM}. and vice versa. These findings thus agree with the earlier reports of Baylis and Robertson (1980), that above certain osmotic threshold (280-288 mOsm/kg H_2O), there is a linear relationship between increase in P_{OSM} and increase in ADH and thirst.

In summary, this study shows that there was a positive relationship between P_{AVP} calculated from TP (cm) and that calculated from P_{OSM}; between TP (cm) and P_{OSM}; between calculated P_{AVP} and TP (cm) as well as between calculated P_{AVP} and P_{OSM}. This work therefore was found to validate the findings and equations put forward by Igbokwe and Obika, (2008) and Thompson et al. (1986), and further established that there is a linear relationship between plasma arginine vasopressin, P_{OSM} and thirst.

We can therefore conclude that plasma arginine vasopressin concentration may be estimated using thirst perception and/or plasma osmolality values. However, caution should be taken when other factors that may not directly affect osmolar changes are involved.

REFERENCES

Amabebe, E., Idu, F and Obika, L.F.O. (2012): Relationship between thirst perception and plasma arginine vasopressin concentration in Man. *Nig. J. Physiol., Sci.* 27: 3 – 10.

Baylis, P.H and Robertson, G.L. (1980): Plasma Vasopressin Response to Hypertonic Saline infusion To Assess Posterior Pituitary Function. *J. Roy Soc. Med.,* 73: 255 – 260.

Clarke, G., Wood, P., Merrick, L and Lincoln, D.W. (1979): Enkephalins co-exist with Oxytocin and Vasopressin in Nerve Terminals of Rat Neurohypophysis. *Nature*, 282: 746.

Cayman Chemical Company, (2011): Arginine Vasopressin EIA Kit. Cayman Chemical Item Number 583951. Ann Arbor, USA, pp. 1-11.

Edwards, C.R. (1971): Measurement of Plasma and Urinary Vasopressin by Immunoassay. *Proc. R. Soc. Med.,* 64(8): 842-844.

Fushimi, K., Sasaki, S and Marumo, F. (1997): Phosphorylation of serine 256 is required for cAMP – dependent regulatory exocytosis of the aquaporine-2 water channel. *J. Biol. Chem*., 272: 14800 – 14804.

Haynes, R.C. (1958): Mechanism of action of cyclic 3', 5'- adenosine monophosphate on Steroid Hydroxylation in Adrenal Homogenates. *J. Biol. Chem.,* 233: 1220.

Igbokwe, V.U and Obika, L.F.O. (2008): Thirst Perception and Dryness of Mouth in Healthy Young Adult Nigerians. *Afri. J. Biomed. Res.,* 11: 39-46.

Kamath, A.M. (2010): Endocrinology answers (Internet). Available from *http://www.doctorslounge.com*

Karkare, A. (2010): Antidiuretic Hormone – The body's water Regulator (Internet). Available from *http://www.ezinearticles.com*

Malvin, R.L. (1971): The Effects of Vasopressin on Pituitary Oxytocin Content and Plasma Renin Activity in Rats with Hypothalamic Diabetes Insipidus (Brattleboro strain). *Fed. Proc.,* 30: 1383.

Mckenna, K and Thompson, C. (1998): Osmoregulation in Clinical Disorders of Thirst Appreciation. *Clin. Endocrinol.,* 49: 139-152.

Nielson, S., DiGiovanni, S.R., Christensen, E.I., Knepper, M.A and Harris, H.W. (1993): Cellular and subcellular immunolocalization of vasopressin regulated water channels in rat kidney. *PNAS*., 90: 11663 – 11667

Nielson, S., Chou, C.L., Marple, D., Christensen, E.I., Kishore, B.K and Knepper, M.A. (1995): Vasopressin increases water permeability of kidney collecting duct by inducing translocation of aquaporin- CD water channels to plasma membrane. *PNAS*., 92: 1013–1017.

Obika, L.F.O., Idu, F.K., George, G.O., Ajayi, O.I and Mowoe, R.S. (2009): Thirst Perception and Drinking in Euhydrate and Dehydrated Humans. *Nig. J. Physiol. Sci.,* 24(1): 25-32.

Purssel, R.A., Pudek, M., Brubacher, J and Abu-Laban, R.B. (2001). Derivation and validation of formula to calculate the contribution of ethanol to Osmolal Gap. *Ann. Emerg. Med.,* 38(6): 653-659.

Robertson, G.L. (1984): Abnormality of Thirst Regulation. *Kidney International.,* 25: 460-469.

Takamata, A., Mark, G.W., Gillen, C.M. and Nadel, E.R. (1994): Sodium Appetite, Thirst and Body Fluid Regulation in Humans during Rehydration without Sodium Replacement. *Am. J. Physiol.,* 266(35): R1493-R1502.

Thompson, C.J., Bland, J., Burd, J. and Baylis, P.H. (1986): The Osmotic Thresholds For Thirst and Vasopressin Release Are Similar In Healthy Man. *Clin. Sci.,* 71: 651-656.

Thompson, C.J., Selby, P. and Baylis, P.H. (1991): Reproducibility of Osmotic and Non-osmotic Test of Vasopressin Secretion in Man. *Am. J. Physiol.,* 260: R533-R539.

Tiplady, B., Jackson, S.H.D., Maskrey, V.M and Swift, C.G. (1998): Validity and Sensitivity of Visual Analogue Scales in young and older healthy subjects. *Age and Ageing,* 27: 63-66.

Tijssen, P. (1985): Practice and Theory of Enzyme Immunoassays. Publishers: Elsevier. Amsterdam. pp. 1 – 19.