Research Article

Comparison of measured ethanol and calculated ethanol using osmolal gap and osmolarity formulas

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Abstract

Objectives: Osmolality can be measured with an osmometer, and it can also be calculated using formulas that include the level of some osmotically active serum components. The difference between measured and calculated osmolality is referred to as the osmolal gap. The osmolal gap indirectly indicates the presence of osmotically active substances other than sodium, urea, and glucose. The aim of this study was to calculate the osmolal gap using 6 osmolarity formulas published in the literature and compare the measured ethanol concentrations with various ethanol calculation formulas that include the osmolal gap.

Methods: Serum ethanol, glucose, potassium, sodium, and urea levels were measured. Serum osmolarity was calculated with 6 formulas and converted to osmolality (mOsmol/L-mOsmol/kg H_2O) using a converting factor. The osmolal gap was determined using measured and calculated osmolality with 6 different formulas for each sample. The osmolal gap values were multiplied by the ethanol coefficient used to ascertain the effect of ethanol on serum osmolality in order to obtain the amount of ethanol in the samples.

Results: A positive correlation was observed between the 24 calculated ethanol levels and measured ethanol levels. No statistically significant difference was seen between the measured ethanol levels and 1 of the formulas, but there were systematic differences between them.

Conclusion: Estimating the ethanol concentration with this type of approach is particularly inappropriate in forensic cases. The osmolal gap should only be used for screening toxic alcohols as an adjunct to clinical decision-making in emergency departments when ethanol cannot be measured, as in a case of alcohol intoxication.

Keywords: Ethanol, Osmolal gap, Osmolarity formula

Osmolality is the concentration of the osmoles of solute dissolved per kilogram of pure water (mOsmol/kg H_2O), whereas osmolarity is the concentration of the osmoles of solute dissolved per liter of solution (mOsmol/L). These 2 terms can be used interchangeably, but osmolality is a more accurate term thermodynamically. Solution concentrations are expressed on the basis of weight, and therefore, it is independent of temperature and pressure. In contrast, solution concentrations are expressed on the basis of volume in osmolarity, and changes in volume can occur depending on the thermal expansion of the solution. As a result, osmolarity can lead to incorrect results in cases of hyperlipidemia or hyperproteinemia, or in the presence of osmotically active substances, such as alcohol or mannitol [1-3].

The osmolal gap is the difference between measured osmolality and calculated osmolality. Normally, the osmolal gap is less than 10 mOsmol/kg H_2O [4].

A high concentration of alcohol, such as ethanol, methanol, isopropanol, ethylene glycol, or propylene glycol causes hyperosmolality and a high osmolal gap in proportion with the

Table 1. Osmolarity formulas used in the study

Formula No	Osmolarity Formulas[a]	References
(OSM$_{C-1}$)	Osm=2 Na+(Glu/18)+(BUN/2.8)	(15)
(OSM$_{C-2}$)	Osm=1.86 (Na+K)" + " 1.15(Glu/18)+(BUN/2.8)+14	(16)
(OSM$_{C-3}$)	Osm=1.86 Na+1,38 K+1.08(Glu/18)+1.03(BUN/2.8)+7.45	(18)
(OSM$_{C-4}$)	Osm=1.897 Na+(Glu/18)+(BUN/2.8)+13.5	(19)
(OSM$_{C-5}$)	Osm=1.09[1.86Na+(Glu/18)+(BUN/2.8)]	(20)
(OSM$_{C-6}$)	Osm=1.86 Na+(Glu/18)+(BUN/2.8)+9	(17)

To convert osmolarity (mOsmol/L) to osmolality (mOsmol/kg), a coefficient of 0.995 (0.926/0.93) was used (0.926 is the osmotic coefficient for sodium, which is the most effective osmolite in serum, and 0.93 is the water content of serum).
[a]: Na and K: mmol/L; Glu and BUN: mg/dL.
BUN: Blood urea nitrogen; GLU: Glucose; K: Potassium; Na: Sodium; Osm: Osmolarity.

severity of exposure. Several studies have been performed calculating serum ethanol concentrations using various formulas and the osmolal gap as a practical approach when it would take time for the measurement of toxic alcohol or when these substances cannot be measured [5, 6].

Nowadays, ethanol is an easily obtainable substance, and therefore, in this study, the aim was to calculate the osmolal gap using 6 osmolarity formulas published in the literature and compare the measured ethanol concentrations with estimated ethanol concentrations obtained from various ethanol calculation formulas that include the osmolal gap.

Materials and Methods

This research was designed as a prospective study. The study included 257 individuals (44 females, 213 males) who were admitted to the Ankara Numune Training and Research Hospital emergency department with a serum ethanol concentration between 2.60 and 80.31 mmol/L (12 mg/dL and 370 mg/dL). Lipemic, hemolyzed samples and diabetic, hypernatremic, hyperproteinemic, or mannitol-treated patients were excluded.

Between-day imprecisions (coefficient of variation [CV] %), mean, and standard deviation (SD) of ethanol, sodium, potassium, glucose, and urea parameters were determined using internal quality control materials. These parameters were measured using a Beckman Coulter AU680 autoanalyser (Beckman Coulter, Inc., Brea, CA, USA) and 6 different osmolarity formulas were used to calculate osmolarity values (Table 1). In the vast majority of publications, these formulas are used

to calculate serum osmolality, but in fact, these are formulas of serum osmolarity, because the unit used is mOsmol/L. For that reason, in this study, a correction coefficient of 0.995 was used to convert osmolarity to osmolality [7, 8].

There was no available quality control material for the osmometer, so we used 2 levels of serum pool created from patients' and normal individuals' sera. During the study period, between-day variations were evaluated using the samples obtained from the serum pool and mean, SD, and CV% values were calculated. For calibration, deionized water (0 mOsmol/kg H$_2$O) and a calibration solution (400 mOsmol/kg H$_2$O) supplied by the manufacturer were used.

Serum osmolality was measured with the osmometer (Knauer Wissenschaftliche Geräte GmbH, Berlin, Germany) using the freezing-point depression method. For each sample, 6 different osmolal gap values were determined using calculated and measured osmolality values. These osmolal gap values were used in the 4 formulas provided in Table 2, and the results were

Table 2. Formulas used to estimate ethanol concentration

Formulas	References
Ethanol (mg/dL)=OG×3.7	(10)
Ethanol (mg/dL)=OG×4.0	(9)
Ethanol (mg/dL)=OG×4.2	(11)
Ethanol (mg/dL)=OG×4.6	(5)

OG: Osmolal gap.

Table 3. Descriptive statistics for the concentration of selected substances in serum

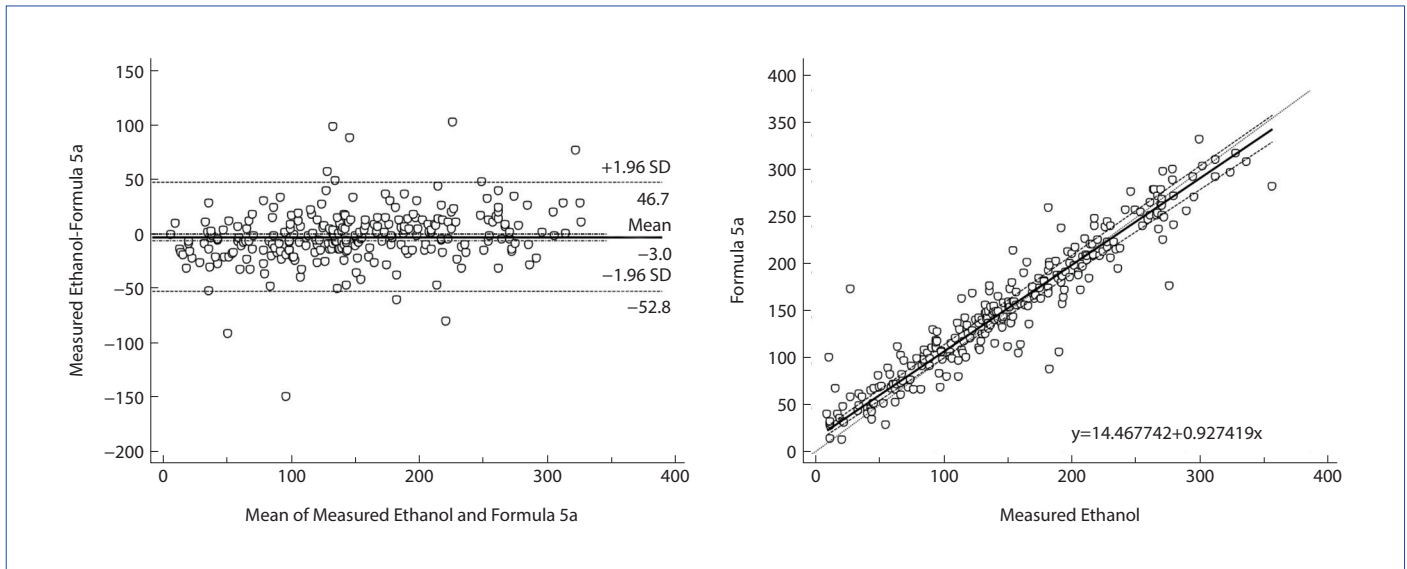|	Mean mmol/L (mg/dL)	±SD mmol/L (mg/dL)	Range mmol/L (mg/dL)
Sodium	140	±2.74	123-147
Potassium	3.95	±0.44	1.51-6.68
Glucose	5.77 (104)	±1.46 (±26.40)	2.50-16.04 (45-289)
BUN	0.45 (11.92)	±0.14 (±3.83)	0.11-1.34 (2.80-35.19)
Ethanol	34.38 (158.37)	±17.52 (±80.72)	2.60-80.31 (12-370)

BUN: Blood urea nitrogen.

Figure 1. (a) Bland-Altman plots for Formula 5a. The averages of the measured ethanol and the calculated ethanol are shown on the x-axis, and the differences between the measured ethanol and the calculated ethanol on the y-axis; (b) Passing-Bablok regression analysis between measured ethanol concentration and ethanol calculated with Formula 5a with a 95% confidence interval (results obtained from the formula on the y-axis, and results obtained from the measurement on the x-axis).

evaluated in estimations of ethanol concentration [5, 9-11].

The ethanol concentrations estimated from formulas were compared with ethanol concentrations measured using the enzymatic method. The usefulness of the osmolal gap coefficient and osmolality formulas was evaluated for estimating the ethanol concentration.

The Kolmogorov-Smirnov test was performed for normality. For normally distributed parameters, a paired sample t-test was used to evaluate the difference between the measured ethanol concentration and the calculated ethanol concentration. Pearson's correlation was used to measure the association between variables. To compare the coherence between ethanol measurements and formulas, Passing-Bablok regression analysis was performed. The Bland-Altman method was used to evaluate the agreement of differences between measured ethanol concentration and calculated ethanol concentration. Statistical significance was defined as $p < 0.05$.

Table 4. Mean value of OSM$_M$ and OSM$_C$ and the significance of the difference between OSM$_M$ and OSM$_C$

	Median (min-max)	P value
OSM$_M$ (mOsmol/kg)	333 (292-431)	
OSM$_{C-1}$ (mOsmol/L)	286 (254-299)	<0.001
OSM$_{C-2}$ (mOsmol/L)	293 (262-306)	<0.001
OSM$_{C-3}$ (mOsmol/L)	284 (253-297)	<0.001
OSM$_{C-4}$ (mOsmol/L)	289 (259-302)	<0.001
OSM$_{C-5}$ (mOsmol/L)	290 (258-304)	<0.001
OSM$_{C-6}$ (mOsmol/L)	279 (249-292)	<0.001

OSM$_C$: Calculated osmolality; OSMM: Measured osmolality.

Results

The mean age of the individuals was 32.6 years (range: 18-74 years). The mean age of the male patients was 33.2 years (range: 18-74 years) and the mean female patient age was 29.8 years (range: 19-53 years). Concentrations of glucose, sodium, potassium, BUN and ethanol in individuals' serum shown in Table 3.

For glucose, sodium, potassium, urea, and ethanol, the between-day imprecisions were 1.58%-1.20%, 1.01%-1.08%, 0.98%-1.11%, 1.6%-1.6%, and 2.71%-2.33% respectively (from internal quality control program level 1 and 2), and for osmolality, 0.88%-0.80% (from serum pool, normal and high level).

When osmolality was calculated, a statistically significant difference was observed between the calculated osmolality and the measured osmolality ($p < 0.001$) (Table 4).

The mean of the measured ethanol concentration was 34.38±17.52 mmol/L (158.37±80.72 mg/dL). A strong positive correlation was observed between the measured and the calculated ethanol concentrations (Table 5).

Formula 5a demonstrated a smaller mean difference of -0.65 mmol/L (-3.0 mg/dL), and a 95% limit of agreement, ranging from -52.8 to 46.7, and there was no significant difference between the mean of Formula 5a and measured ethanol ($p=0.057$) (Fig. 1a). But when Passing-Bablok regression analyses were performed, constant and proportional differences between the measurement and the Formula 5a result were observed (Fig. 1b).

Discussion

Gas chromatography is the reference method for ethanol measurement, but it is not available in typical laboratories. This

Table 5. Mean and correlation between measured and calculated ethanol concentration			
Formula	**Test**	**Mean±SD mmol/L (mg/dL)**	**Correlation coefficent**
	Measured Ethanol	34.38±17.52 (158.37±80.72)	
1a	OG1X3.7	38.79±16.20 (178.72±74.62)	0.950
1b	OG1X4.0	41.93±17.57 (193.19±80.70)	0.950
1c	OG1X4.25	44.55±18.61 (205.25±85.77)	0.950
1d	OG1X4.6	48.22±20.15 (222.15±92.83)	0.950
2a	OG2X3.7	33.18±16.18 (152.87±74.53)	0.951
2b	OG2X4.0	35.87±17.49 (165.27±80.58)	0.951
2c	OG2X4.25	38.11±18.58 (175.61±85.62)	0.951
2d	OG2X4.6	41.25±21.21 (190.07±92.70)	0.951
3a	OG3X3.7	40.29±16.20 (185.61±74.64)	0.951
3b	OG3X4,0	43.56±17.51 (200.68±80.67)	0.951
3c	OG3X4.25	46.28±18.61 (213.21±85.73)	0.951
3d	OG3X4.6	50.09±20.13 (230.77±92.76)	0.951
4a	OG4X3.7	36.02±16.22 (165.98±74.72)	0.951
4b	OG4X4.0	38.95±17.52 (179.45±80.74)	0.951
4c	OG4X4.25	41.38±18.63 (190.63±85.84)	0.951
4d	OG4X4.6	44.79±20.17 (206.35±92.93)	0.951
5a	OG5X3.7	35.03±16.20 (161.39±74.62)	0.950
5b	OG5X4.0	37.89±17.51 (174.54±80.67)	0.949
5c	OG5X4.25	40.25±18.60 (185.44±85.72)	0.950
5d	OG5X4.6	43.56±20.14 (200.68±92.79)	0.950
6a	OG6X3.7	43.80±16.24 (201.79±74.81)	0.951
6b	OG6X4.0	47.35±17.55 (218.15±80.88)	0.951
6c	OG6X4.25	49.72±18.43 (229.08±84.91)	0.951
6d	OG6X4.6	54.45±20.17 (250.86±92.94)	0.951

OG: Osmolal gap.

method requires expensive equipment and educated technical staff; therefore, ethanol is generally measured with the enzymatic method. In some laboratories, the enzymatic method is not possible, and so the calculated osmolal gap provides a practical approach, but it must be remembered that there are several conditions that can increase the osmolal gap other than ethanol, such as diabetes mellitus, dehydration, or hypernatremia [12-14].

In the literature, many formulas have been defined for osmolality calculation and ethanol estimation; however, there is still no consensus on which formula is most accurate [15-20].

The coefficient of 1/4.6 is used to calculate the contribution of ethanol to serum osmolarity, caused by ethanol's molecular mass. Ethanol is a nonideal liquid, like serum; therefore, the phrase "1 mmol/L of the compound would produce 1 mOsm/L of osmolarity" would be invalid for ethanol and makes the aforementioned coefficient of ethanol open to debate.

In this study, we evaluated osmolality formulas and ethanol estimation formulas, and a statistically significant difference between measured osmolality and calculated osmolality was observed (p<0.001). This result revealed the presence of osmotically active substances that were not included in osmo-

lality formulas. A strongly positive correlation was found between 24 ethanol estimation formulas and enzymatic ethanol measurements (Table 5). When examining the mean difference, there was no significant difference between Formula 5a and measured ethanol, but there were constant and proportional differences between the measurement and the Formula 5a result.

Our results indicating that the coefficient for ethanol should be greater than 1/4.6 agreed with the findings of several studies [9-11]. But it may vary with increasing ethanol concentrations, as ethanol may affect the solubility of solutes in serum as a solvent. Although Formula 5a would achieve the closest value to measured ethanol concentration, using ethanol formulas instead of ethanol measurement is inappropriate due to of systematic (constant and proportional) differences between measurement and calculation.

The osmolal gap may be close to zero in healthy individuals but the presence of ethanol or another osmotically active substance (methanol, ethylene glycol, etc.) or their metabolites may change the solubility of other ingredients in the serum. As a result, with the calculated ethanol value, a higher or lower ethanol concentration than expected may be observed. Fur-

thermore, the absence of consensus on molal-molar conversion can be another reason for differences between results.

Conclusion

Due to the presence of various osmolarity formulas in the literature and contradictions between osmolality and osmolarity usage, the use of the osmolal gap is limited. For this reason, estimating ethanol concentrations with this type of approach is particularly inappropriate in forensic cases. Considering these variable factors and the nonideal nature of ethanol, the osmolal gap should only be used for screening toxic alcohols as an adjunct to clinical decision-making in emergency departments, such as in a case of alcohol intoxication, since it is the metabolites of alcohol that cause alcohol toxicity rather than the alcohol itself, and it takes time for toxicity symptoms to appear. When ethanol is measured, it can be used with the osmolal gap to evaluate the residual osmolal gap to reveal other toxic substances.

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