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Original Article

Mathematical model for determination of colloid osmotic pressure: The role of albumin-globulin ratio

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Abstract

BACKGROUND: Colloid Osmotic Pressure (COP) is an important factor in the fluid balance of body compartments. COP is related to Total Protein (TP) concentration and Albumin: Globulin Ratio (A/G). The A/G was not included in pervious empirical models, and therefore the main objective of this study was to develop a mathematical model to determine the COP in terms of TP concentration and A/G.

METHODS: Sera with different A/G were prepared in-vitro, and COP was measured directly using colloid osmometer. The relationship between COP, TP concentration and A/G were determined mathematically. The validity of developed empirical models was confirmed by statistical comparison between measured and calculated COP in 122 serum samples obtained from hospitalized patients and healthy individuals.

RESULTS: By non-linear regression, the following relationships were found between COP, TP concentration and A/G. All coefficients were statistically significant ($p<0.05$): $COP = (4.0814\ A/G\ TP)/(A/G + 0.0153\ TP)$; $r^2 = 0.91272$. $COP = [5.3192\ A/G - 2.2252\ (A/G)^2 + 0.2939\ (A/G)^3]\ TP$; $r^2 = 0.94737$ No significant differences were indicated between measured COP and calculated one in clinical data.

CONCLUSIONS: The variation of A/G may be the most effective factor for the differences between calculated and measured COP. This parameter must be considered when the direct measurement of COP is unavailable.

KEY WORDS: Mathematical model, colloid osmotic pressure, oncotic pressure, albumin-globulin ratio.

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Colloid Osmotic Pressure (COP) or oncotic pressure plays an important role in the fluid balance between intravascular and interstitial fluid compartments. Almost one hundred years ago, Starling proposed that the net fluid volume movement across the membrane of capillary is based on the interaction between two opposing forces: the difference in hydrostatic pressure and the difference in the COP on either side of the membrane between the capillary and interstitial fluid spaces [1]. The COP monitoring has been suggested in many diseases to control the bodies' compartmental fluids. Observation on 99 consecutive and critical patients demonstrated a close relationship between COP and the patients survival time [2,3]. The lower incidence of pulmonary edema was observed in 128 critical patients with higher level of plasma COP [4]. Drummond et al showed that COP reduction per se can aggravate brain edema after a mechanical head injury [5]. Decreasing the COP level

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also indicates the occurrence of cerebral vasospasm following subarachnoid hemorrhage [6]. The lower COP difference across the tumor-microvascular wall indicates high COP level in the tumor which is consistent with the elevated interstitial fluid pressure [7]. Acute peritoneal dialysis may result in decreasing COP in peritoneal membrane. This phenomenon shifts the Starling equilibrium toward an absorptive state which is needed to be controlled [8]. The significant correlation also was reported between the improvement in visual field and the decrease in COP [9]. Accordingly, it is obvious that the monitoring of COP is extremely necessary, but the especial and expensive equipments are required to measure COP directly that is not available. Therefore, extensive attention was made to develop empirical and theoretical models to determine COP in term of Total Protein (TP) concentration. In an ideal condition, COP is given by Van't Hoff's formula:

$$COP = RTC \quad [1]$$

where C is the molar colloid concentration, R is the universal gas constant and T is the absolute temperature [10]. Practically, Landis and Pappenhrimer developed an empirical model to predict COP in human plasma in term of TP concentration [11]. This model is given as:

$$COP = 2.1\ TP + 0.16\ TP^2 + 0.009\ TP^3 \quad [2]$$

In this equation the first term represents the ideal limiting law of Van't Hoff while the second and third terms represent deviation from Van't Hoff law caused by Gibbs-Donnan's effects. The alternate equation described by Pappenheimer as following:

$$COP = aTP/(1\text{-}b\ TP) \quad [3]$$

where the empirical constant a = 2.4 and b = 0.046 are suggested [12]. Another study done by Navar and Navar proposed the following equation [13]:

$$COP = 2.265TP + 0.008TP^2 + 0.026TP^3 \quad [4]$$

More mathematical models also were developed by others [14-16], and among them the following simple formula also is available [16]:

$$COP = 4\ TP - 0.8 \quad [5]$$

All these equations could be used with confidence when Albumin: Globulin Ratio (A/G) is known to lie between normal limits. Geranton et al assessed the accuracy of four developed models [11,13-15] in renal patients to determine the potential role of liver-derived non-albumin proteins in the maintenance of COP, and they found strong correlation between measured and calculated COP, but in an absolute term, there was significant difference between measured and four calculated COP [17]. Miki et al designed an experiment to examine the effect of A/G on calculated COP [18]. They found that the difference between calculated and measured COP is considerably depending on A/G and they concluded that the main error caused by the prediction of COP comes from the difference between A/G of the sample. The greater the difference of the A/G, the more the discrepancy in the COP. Hoefs developed an equation to calculate COP from multivariate discriminate analyses of Albumin (A) and Globulin (G) concentrations [19], and he suggested the flowing equation based on COP and A ratio (COP/A).

$$COP/A = 1.058G + 0.163A + 3.11 \quad [6]$$

In general, A/G is not constant. Treated patients with proteins (A or G) may have abnormal A/G. The abnormal A/G, of course affects COP prediction. Including A/G to an empirical model for determination of COP will be helpful and beneficial to have the accurately calculated COP. Therefore, the main objective of this study was to develop a mathematical model to determine COP (based on TP concentration and A/G) with confidence in clinic for patients with normal and abnormal A/G.

Methods

1. Preparation of pooled serum:

Pooled human serum was obtained from Isfahan Blood Bank. TP and A concentrations were measured with the standard Biuret and Bromcresol Green methods (ready-to-use reagents from Pars Azmun Co, Iran) respectively. A/G was calculated using the following equation:

$A/G = A/(TP - A)$ [8]

2. Preparation of sera with different A/G:
Different amounts of human albumin 20% (albumin Berna, Swiss Serum and Vaccine Institute) and/or γ-globulin-KGCC 16.5% (Immune Serum Globulin, Human, Korean Green Cross Corp.) were added to the pooled serum. Fourteen serum samples with different A/G were prepared. Again TP and A concentrations of each sample were measured and the exact A/G of each sample was determined. The TP and A concentrations of each serum sample were measured twice and the mean value was calculated.

3. Preparation of sera with constant A/G:
Each serum sample from step 2 with specific A/G and known TP concentration was diluted stepwise with isotonic saline to the concentration ratio range of 1/10 to 10/10. Therefore, ten new samples were prepared with equal and constant A/G, but different TP concentrations. By the end of this step, 140 samples were prepared in 14 different A/G groups.

4. COP measurement:
A colloid osmometer (Knauer Co. Germany) was used to measure the COP of samples obtained from step 3. This membrane osmometer is impermeable to molecules larger than 30,000 Daltons. The instrument was calibrated with 5% standard solution of human albumin. The COP of each serum sample was measured twice and the mean value was calculated. The relationship between COP and TP concentration in samples with constant A/G was determined by the nonlinear curve estimation regression using SPSS software.

5. The relationship between COP, TP concentration and A/G:
Based on Van't Hoff's and Pappenheimer formulas (10,12) the following functions were suggested:

$COP = \varphi(A/G)TP$ [8]

$COP = aA/GTP/(A/G - bTP)$ [9]

where φ (A/G) is a function of A/G ; $\varphi = \alpha A/G + \beta(A/G)^2 + \delta(A/G)^3$, and a , b, α, β, and δ are constants to be determined numerically by non-linear curve fitting using data from steps 3 and 4.

6. Validity of the models:
In order to determine the validity of the models suggested in last step, 122 serum samples were collected from patients hospitalized in Burn Accidents Center of Isfahan and hemodialysis unit of the Al-Zahra Medical Center, and also healthy individuals. TP and A concentrations of each sample were measured and globulin was determined as described before. Each sample was directly subjected to COP measurement. The COP was also calculated from models suggested in step 5. The calculated and the measured COPs were statistically compared.

7. Data analysis:
The data are reported as mean ± SD. The data from calculated and measured COPs were tested by Kolmogorov-Smirnov test to find the normality of distribution. The COPs were compared using nonparametric test for two related samples (Wilcoxon test). P values below 0.05 were considered significant.

Results

1. The relationship between COP, TP concentration and constant A/G Ratio:
The relationship between COP and TP concentrations in 14 groups of serum samples with constant A/G are shown in table 1. Each of these relationships was subjected to numerical analytical procedures and analyses to determine best-fit curve lines and confident limits for each set of experimental conditions. The best of these relationships have been selected based on the best-fit correlation coefficients and statistical significance of the coefficients. The results indicated two suitable models for each A/G group as following:

$COP = aTP + bTP^2$ [10]

$COP = cTP/(1\text{-}d\ TP)$ [11]

where a, b, c, and d are constants. These coefficients were statistically significant in all equations ($P<0.05$).

Table 1. The relationship between COP and TP concentrations in 14 groups of serum samples with constant A/G. The coefficient of determination is demonstrated for each fitting curve. All coefficients (a, b, c, d) were statistically significant (P<0.05).

A/G	COP = aTP + bTP^2			COP = cTP/(1-d TP)		
	a	b	r^2	c	d	r^2
0.3877	1.6332	0.074	0.99929	1.6979	0.0294	0.99803
0.8577	1.8809	0.1513	0.99966	2.1880	0.0351	0.99966
1.1026	1.6966	0.1879	0.99938	2.2013	0.0372	0.99979
1.4216	2.0395	0.1792	0.99983	2.3780	0.0383	0.99979
1.4942	1.9666	0.2197	0.99972	2.5528	0.0376	0.99979
1.8812	2.0824	0.2018	0.99952	2.5123	0.0384	0.99977
1.9880	2.2472	0.2482	0.99965	2.8398	0.0394	0.99986
2.1438	2.3743	0.1777	0.99986	2.6267	0.0382	0.99962
2.4224	2.6085	0.2157	0.99993	2.8307	0.0452	0.99982
2.5258	2.5828	0.2425	0.99995	2.8352	0.0492	0.99993
2.6961	2.3926	0.2472	0.99989	2.7491	0.0469	0.99996
2.9512	2.4297	0.2663	0.99957	2.7783	0.0491	0.99979
3.2701	2.6746	0.2609	0.99969	2.9549	0.0499	0.99926
3.9306	2.7409	0.2681	0.99999	2.9587	0.0546	0.99991

2. The relationship between COP, TP concentration and A/G:

In order to determine the relationship between COP, TP concentration and A/G, the data were fitted to equations [8] and [9] by non-linear fitting curve. The following equations were obtained. All coefficients were statically meaningful (P<0.05) and the coefficients of determination were 0.91272 and 0.94737, respectively.

$$COP = (4.0814\ A/G\ TP)/(A/G + 0.0153TP);\ r^2 = 0.91272 \quad [12]$$

$$COP = [5.3192\ A/G - 2.2252\ (A/G)^2 + 0.2939\ (A/G)^3]TP;\ r^2 = 0.94737 \quad [13]$$

3. Comparison between measured and calculated COP in 122 human serum samples

The validity of the suggested empirical models [12] and [13] was obtained by comparison between measured and calculated COP in 122 human serum samples

(TP concentration =3.62 – 10.92 g/dl; A concentration = 2.07 – 6.953 g/dl; G concentration = 1.24 – 4.51 g/dl; A/G = 0.543 – 3.42). The measured COP was also compared by theoretical models given by Landis and Pappenhrimer (equation [2]), Navar and Navar (equation [4]), Lundsgaard-Hansen P (equation [5]) and Hoefs (equation [6]). The results indicated significant differences between measured COP and calculated COP when equations [2] and [4] were applied (P<0.05). However, no significant differences were seen between measured COP and calculated COP with equations [12] and [13] (table 2).

Discussion

The main objective of this study was to develop a mathematical model to determine the COP in patients plasma based on TP concentration and A/G. In human plasma, and under normal condition, the COP is a function of TP concentration. This relationship appears to fail in patients who are critically ill [20,21]. The variation of A/G may be the most effective factor for the differences between calculated and measured COP [18]. Although A/G was not considered in most empirical models [11-13,16], but its important role was mentioned [18]. In this regard, Hoefs suggested a

Table 2. The comparison between measured and calculated COP in 122 human serum samples.

Measured or Empirical Model	Model	Mean ± SD (mmHg)	Statistical P value *
Measured COP	Colloid Osmometer	23.97 ± 7.85	--
Calculated COP by equation [2]	$COP = 2.1\ TP + 0.16\ TP^2 + 0.009\ TP^3$	22.65 ± 7.57	0.00
Calculated COP by equation [4]	$COP = 2.265\ TP + 0.008\ TP^2 + 0.026\ TP^3$	22.26 ± 8.31	0.00
Calculated COP by equation [5]	$COP = 4\ TP - 0.8$	24.54 ± 5.53	0.01
Calculated COP by equation [6]	$COP/A = 1.058\ G + 0.163\ A + 3.11$	24.47 ± 8.32	0.065
Calculated COP by equation [12]	$COP = (4.0814\ A/G\ TP)/ (A/G + 0.0153\ TP)$	24.87 ± 4.93	0.120
Calculated COP by equation [13]	$COP = [5.3192\ A/G - 2.2252\ (A/G)^2 + 0.2939\ (A/G)^3]\ TP$	23.78 ± 5.71	0.541

*Compared with measured COP (23.97 ± 7.85 mmHg).

model including A and G concentrations [19]. Our experimental data indicated that the relationship between COP and TP concentration for each individual A/G is different (table 1). The difference was associated with the number of particles which create the COP and the varying effects of physical-chemical factors, such as Donnan effects for different types of proteins. The Donnan effect is responsible for COP generated as a result of combination of some positive ions with negative charges of proteins. It is much more pronounced at higher protein concentrations than the lower ones. In human serum, constant A/G will keep constantly the ratio of number of colloid particles to colloid concentration. On the other hand, because the COP is directly related to the number of osmotically active colloid particles, for constant A/G, the ratio of the COP to the colloid concentration (COP/TP) is constant. Accordingly, in condition of constant A/G, the non-linearity of the relationship is resulted from Donnan effect. When A/G is not constant in two different serums with equal TP concentration, the solution with higher A/G will create a higher COP. Therefore, COP is actually dependant on two major factors; TP concentration and A/G, and both of them must be considered for determination of COP (equations [12] and [13]). The importance of COP determination in clinic and clinical researches have been noticed in the literatures [6,8,22-30]. Therefore, an empirical model will be extremely helpful whenever the direct measurement is unavailable.

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