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Original article

Correlation of serum biochemical parameters and saliva pH in healthy individuals

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ABSTRACT

Saliva has the potential to work alongside needles in standard medical diagnosis. Yet the number of studies aimed at deciphering the biochemical communication between saliva and the rest of the body's systems is still very limited. The aim of this study is to investigate the interfluid interaction between saliva and serum by determining the correlation between saliva pH and serum biochemical parameters under mild conditions. Ultimately, using saliva may provide a stress-free diagnostic tool, but more ambitiously, the pH of saliva could present a genuine cost-effective screening tool that may immensely benefit areas with limited access to health care and diagnostic labs. Saliva and blood samples were collected from 43 randomly selected children (7–12 years), living in Jeddah, free from obesity and chronic or systemic body and mouth diseases. A complete serum biochemical analysis was performed, and the salivary pH of all samples was measured immediately at the time of collection. The correlations between saliva pH and serum biochemical parameters were investigated using Univariate and multiple linear regression models. Our results showed that pH has a weak significant positive correlation with total protein and a negative weak significant correlation with urea. Weak correlations suggest the existence of more serum factors to be investigated for their effect on the pH using a stepwise multiple linear regression. The multiple linear models' calculated saliva pH values were close to the measured values, demonstrating its possible capacity to predict saliva pH using serum parameters. The regression model's successful prediction of saliva pH using serum biochemicals reflects the significant correlations between the body fluids' parameters and invites more research to elucidate these relationships.

1. Background

In adherence to Leonardo da Vinci's theory that everything is inextricably connected, this study aims to investigate the interfluid interaction between saliva and serum by determining the correlation between saliva pH and serum biochemical parameters under mild conditions. Understanding the biochemical relationship between saliva and the rest of the body's fluids and systems is the gateway to determining the potential for using saliva to diagnose various diseases. These body fluids and systems' biochemical relationships are complex and specifically regulated to maintain homeostasis. Elucidating these relationships could allow the use of one fluid parameter to predict others and to predict the status of the body's systems.

The body is an open system that is affected by and contributes to the external environment. Homeostasis refers to the dynamic mechanisms by which the body systems maintain vital ranges of

Abbreviations: Alb, Albumin; AlbBCG2, ATP-binding cassette super-family G member 2; ALKP, Alkaline phosphate; ALT, Alanine aminotransferase; AST, Aspartate aminotransferase; BiliT, Bilirubin serum total; BR, Bilirubin; BUN, blood urea nitrogen; Ca, Calcium; CaC, Coronary artery calcium; Chol2, Cholesterol; Cl, Chlorine; CL-C, Chloride; CR, Creatine; Crea 2, Creatinine; CK, Creatine kinase; CRP, C-reactive protein; GGT, Gamma-glutamyl transferase; Gluc, Glucose; HDL, High-density lipoprotein; K, Potassium; LDH, Lactate dehydrogenase; LDL, Low-density lipoprotein; Na, Sodiums; Na-C, Sodium; P, Phosphate; Phos, Phosphorus; TC, Total cholesterol; TP, Total protein; TG, Triglycerides; Urea 2, Urea; UA, Uric acid; UHDL, High density lipoproteins.

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temperature, pH, acidity, and ion strength and remain stable in the changing surroundings. Failure to maintain homeostasis may affect the functions of body systems and thus cause diseases (Billman, 2020). To ensure healthy living, we need to understand the mechanisms that maintain homeostasis, including how body fluids communicate and affect each other. Extracellular fluids, including saliva, are critical in maintaining homeostasis by providing stable conditions for cells to live in (Baptista, 2006). When under acute stress, the body's systems perform allostasis by implementing change mechanisms to maintain stability (Libretti & Puckett, 2022).

The content of saliva in the mouth is contributed to by the major and minor salivary glands, nonexocrine components, the mucosal fluid, and the gingival fluid. The gingival and mucosal fluids contribute substances from the circulatory system to the saliva (Boroumand, et al., 2021). The oral microorganisms produce most of the salivary metabolites which may enter the bloodstream (Hyvärinen, Kashyap, & Kullaa, 2023). Thus, saliva content may reflect the content of other body fluids and contribute to it, which can be useful in diagnosing various conditions such as Cushing's syndrome by measuring saliva cortisol (Bäcklund, et al., 2020), which showed variation in accuracy when compared to serum cortisol levels for Cushing's syndrome diagnosis (Vieira-Correa, et al., 2019). Other examples include detecting SARS-CoV-2 in the saliva for COVID-19 diagnosis (Azzi, et al., 2020; Teo, et al., 2021). Saliva is used to detect hormonal levels, like testosterone (Gao, Stalder, & Kirschbaum, 2015; Alvi & Hammami, 2020), estradiol, cortisol, cortisone, progesterone, corticosterone, dehydroepiandrosterone (Gao, Stalder, & Kirschbaum, 2015), and melatonin (Sundberg, et al., 2020). Saliva is used to detect vitamins (Clarke, et al., 2019), drugs (Desrosiers & Huestis, 2019), microbes (Gao, et al., 2018; Costalonga & Herzberg, 2014), and viruses (Eventov-Friedman, et al., 2019; Jamieson, et al., 2020; Khurshid, Zafar, Khan, Mali, & Latif, 2019; Zhou, Hua, & Liu, 2017).

Several studies have shown a correlation between the concentrations of biochemical parameters in saliva and blood, which makes saliva a suitable noninvasive diagnostic candidate. For example, the correlation between salivary and serum levels were shown for glucose (Andersson, Birkhed, Berntorp, Lindgärde, & Matsson, 1998; Abikshyeet, Ramesh, & Oza, 2012; Hartman, et al., 2014; Fares, Said, Ibrahim, Amin, & Saad, 2019), insulin (Fabre, et al., 2012; Gupta, Sandhu, Bansal, & Sharma, 2015; Vallejo, Mead, Gaynor, Devlin, & Robbins, 1984; Alqaderi, et al., 2022), C-reactive protein (CRP) (Punyadeera, Dimeski, Kostner, Beyerlein, & Cooper-White, 2011; Ouellet-Morin, Danese, Williams, & Arseneault, 2011; Browne, et al., 2013; Pay & Shaw, 2019; Alqaderi, et al., 2022), cytokines (Diesch, Filippi, Fritschi, Filippi, & Ritz, 2021; Novak, Hamedi, Bergmeier, Fortune, & Hagi-Pavli, 2021), adiponectin (Thanakun, Watanabe, Thaweboon, & Izumi, 2014; Riis, et al., 2017; Kalyani & Raghunath, 2020), cortisol (Vining, McGinley, Maksvytis, & Ho, 1983; Perogamvros, Keevil, Ray, & Trainer, 2010; Estrada-Y-Martin & Orlander, 2011), and antioxidants (Ahmadi-Motamayel, Goodarzi, Jamshidi, & Kebriaei, 2017; Punj, Shenoy, Kumari, & Pampani, 2017; Zovari, Parsian, Bijani, Moslemnezhad, & Shirzad, 2020).

In addition, saliva reflects the effect of dialysis on metabolic wastes. Seethalakshmi, et al. compared pre-dialysis and post-dialysis unstimulated whole saliva to serum in end-stage renal disease patients. Both samples showed a significant post-dialysis decrease in the concentrations of urea, creatinine, sodium, potassium, and phosphate (Seethalakshmi, Koteeswaran, & Chiranjeevi, 2014). The correlation between biochemical parameters in saliva and serum shows a biochemical association between fluids and makes saliva a potentially effective tool for diagnosis and biomarker analysis.

Saliva maintains stable pH and ionic conditions for enzyme and other immunological parameters. The maintenance of optimal pH is vital for protein structure and biochemical reactions. Prolonged inflammation alters the pH of tissues and body fluids and can cause damage. Koppolu, et al. showed that salivary pH is positively correlated with serum pH (Koppolu, et al., 2022). Researchers showed that saliva pH is significantly lower in type II diabetic patients (Puttaswamy, Puttabudhi, & Raju, 2017; Uthayasankar & Jayaraj, 2020), and in generalized chronic periodontitis patients (Baliga, Muglikar, & Kale, 2013). Moreover, one study (Tremblay, Brisson, & Gaudet, 2012) showed that salivary pH was significantly correlated with triglycerides (TG), apolipoprotein B (apo B), and plasma glucose. Since saliva can be collected noninvasively and stress-free, correlating the saliva pH to serum biochemical markers presents a cost-effective disease screening method. Despite these potential benefits, there is a shortage in studies that investigate this relationship.

The correlation between serum and saliva biochemicals and pH shows the association between human blood and saliva. To comprehend human biochemical homeostasis, the interactions and associations between systems and body fluids should be thoroughly examined and understood. This study contributes to this much-needed branch of research by studying the correlation between saliva pH and serum biochemical concentrations in healthy children. The understanding of the correlation between saliva pH and serum biochemicals may help in creating a cost-effective method to guide health care practitioners' diagnoses. It also may lead to adjusting body systems by prolonged adjustment of salivary pH.

2. Methods

2.1. Participants selection

For simplicity and cost-effectiveness, the study focused on a single gender. A-priori Sample Size Calculator for Multiple Regression (Soper, 2023) was used to calculate the minimum required sample size. The observed R^2 value of (0.8) was used to calculate the anticipated effect size (f^2). According to the equation ($f^2 = \frac{R^2}{1-R^2} = 4$), the f^2 is 4 which is considered a large effect size (0.59) according to Cohen's suggestion for regression effect size (0.14: Small, 0.39: medium, 0.59: large). The power analysis indicated that the minimum required sample size for the multiple regressions is 43, given the desired probability level of 0.05, the number of 13 predictors in the model, the anticipated effect size of 0.59, and the desired statistical power level of 0.8.

After determining the minimum required sample size for regression. The sample size was calculated using the Raosoft sample size calculator (Raosoft, 2020) with a confidence level of 95% and a margin of error of about 15%, using Jeddah's Population Size of 375,032 boys between 0 and 14 years old (Zhuji World.com. Jeddah, Saudi Arabia — statistics, n.d.).

Forty-three randomly selected boys living in Jeddah, Saudi Arabia between seven to twelve years old (mean ± SD, 10.31 ± 1.37810), and weighing 30.634 ± 5.7875 kg satisfied the inclusion and exclusion criteria.

This age group was selected to eliminate common factors in adulthood that may affect saliva and other biochemical conditions, such as smoking and drinking. To exclude the effect of extreme conditions such as metabolic syndromes, we selected children with average body weight for this age group who are free from any chronic or systemic diseases. Children with oral diseases that may affect saliva properties were also excluded from the selection process. The participants' parents signed a consent form.

Inclusion Criteria:

- Boys in the age group of 7 to 12 years
- Free from any systemic or local diseases (such as submandibular duct canaliculi, asthma, and diabetes mellitus)
- Children with DMFT/deft score <3

Exclusion Criteria:

- History of current or recent (at least for the past 1 month) antibiotic usage
- Children who were severely ill
- Children who have difficulty in opening the mouth
- Abscess, draining sinus, cellulitis, or other conditions requiring emergency dental treatment
- Children with any orthodontic appliances or any removable/-fixed prostheses
- Children with any negative oral habit (e.g., mouth breathing)
- Children with early childhood caries

2.2. Biological material collection and analysis

Saliva and blood serum were used in these analyses. Blood from veins was taken and after the coagulation samples were centrifuged (7 min, 6000 rpm), in order to separate the serum in which biochemical parameters were determined.

Saliva collection protocol was adopted from Henson and Wong's published protocol (Henson & Wong, 2010). As follows: Subjects were on an 8-hour fast (refrained from eating, drinking, or oral hygiene procedures for at least 8 h prior to the collection). Subjects were given distilled drinking water and asked to rinse their mouth out well for 1 min. Subjects then expectorate the water. After this oral rinse, subjects were asked to spit into a 50 mL sterile tube.

All biochemical laboratory work and results analyses were performed in the National Guard centers between KSAU-HS and King Khalid Hospital.

The complete biochemical analysis was performed for the concentrations of the following parameters: phosphate (P), calcium (Ca), sodium (Na), potassium (K), chlorine (Cl), total protein (TP), bilirubin (Bili T), C-reactive protein (CRP), albumin (Alb), creatinine (CR), urea (Urea2), uric acid (UA), aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), creatine kinase (CK), alkaline phosphatase (AlkP), lactate dehydrogenase (LDH), glucose (Gluc), total cholesterol (TC), triglyceride (TG), and high-density lipoprotein (HDL).

The concentration of biochemical parameters was determined using an automated clinical chemistry analyzer Architect c8000 (Abbott, Abbott Park, IL, USA) at the clinical biochemistry laboratory at King Abdulaziz Medical City, King Khalid Hospital for National Guard, Jeddah, Saudi Arabia, that is associated with KSAU-HS. Studies were conducted based on guidance on the ARCHITECTc System, using the ARCHITECT Reagents. Quality control samples were run daily, and they were within acceptable limits. Research samples were monitored by using internal and external quality control samples. Tests were evaluated rigorously for precision, accuracy, and linearity before implementation.

The salivary pH of all samples was measured immediately at the time of collection using a digital pH meter (pH 211 Microprocessor, Hanna Instruments). To obtain accurate measurements, the pH meter was dipped in 0.1 M HCl solution for one day and then calibrated using buffers of pH 7 for neutral solution, pH 10 for basic solutions, and pH 4 for acidic solutions. After adjusting the pH, the probe was dipped in the Falcon tube containing the sample, where it remained until the reading was stable and the screen showed ready, thus yielding a pH reading. The pH meter was washed with deionized water after measuring each sample.

2.3. Statistical analysis

The statistical software programs RStudio v 4.2.1 and SPSS v 25 were used to perform the statistical analysis. A-priori Sample Size Calculator for Multiple Regression (Soper, 2023) was used to calculate the minimum required sample size. The normality of the variables was tested using the Shapiro-Wilk test. Multiple linear regressions were used to describe how the response variable changes by the independent variables and stepwise regression was used to find the best model that represents the data. The Durbin-Watson test was used to check the independence assumption. The non-constant variance score was used to test the constant variance assumption. Finally, Spearman's rho was used to find the correlation for non-normal variables, and Pearson's Correlation was used to find the correlation for normal variables.

3. Results

3.1. Descriptive and correlation analysis

As depicted in Fig. 1, the sample population had a minimum saliva pH of 6.75, a maximum of 7.44, and a median equal to 7.12. Of the sample population, 15% had a pH of <6.96, and 75% had a pH of <7.24.

After testing the normality of parameters, the correlations between the pH and normal parameters were tested using Spearman's rho for non-normal variables (Table 1) and Pearson for normal variables (Table 2). As seen in Tables 1 and 2, except for the Urea and TP parameters, correlations with the pH were weak and not significant. The relationship between pH and TP is weakly negative, and significant since the P-value of 0.050 equals 0.05. The relationship between pH and Urea2 is weakly positive, and significant since the P-value of 0.040 is <0.05.

Pearson's product-moment correlation was used for testing the significance of the strong relationships: A significant association was found between AST and ALT (p-value = $2.666x10^{-7}$ < 0.05), LDH and CK (p-value = $4.096x10^{-5}$ < 0.05), and LDH and AST (p-value = $7.137x10^{-5}$ < 0.05).

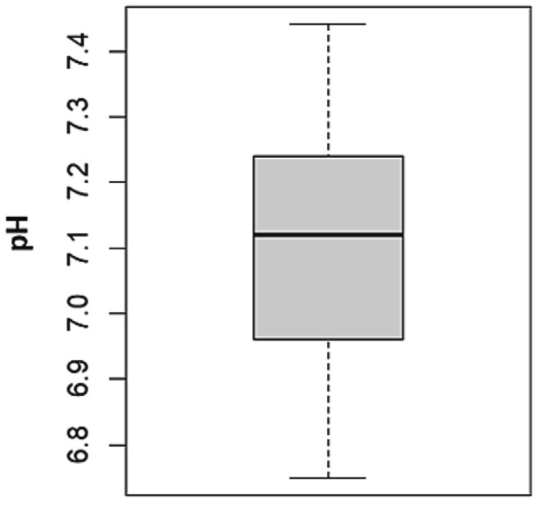

Fig. 1. Box plot showing saliva pH distribution, maximum and minimum values.

Table 1
Spearman's correlation between pH and various normally distributed serum parameters.

Speaman's rho pH		**BiliT (umol/L)**	**TG (mmol/L)**	**AlbBCG2 (g/L)**	**Cl-C (mmol/L)**	**Na-C (mmol/L)**
	Correlation Coefficient	−0.071	0.330	−0.312	0.116	−0.023
	Sig. (2-tailed)	0.685	0.052	0.068	0.509	0.896
		ALT (U/L)	**UHDL (mmol/L)**	**AST (U/L)**	**CRP32 (mg/L)**	**CK (U/L)**
Speaman's rho pH	Correlation Coefficient	−0.015	−0.327	−0.153	0.044	0.014
	Sig. (2-tailed)	0.931	0.056	0.381	0.802	0.935

Table 2
Pearson correlation between pH and various serum parameters that do not follow the normal distribution.

	TP (g/L)	**GluC (mmol/L)**	**CaC (mmol/L)**	**K-C (mmol/L)**	**Chol2 (mmol/L)**	**UA (umol/L)**
Pearson Correlation	−0.334*	−0.004	0.008	0.106	−0.197	0.136
Sig. (2-tailed)	0.050	0.981	0.964	0.544	0.257	0.435
	GGT (U/L)	**LDH (U/L)**	**Crea2 (umol/L)**	**Urea2 (mmol/L)**	**Phos (mmol/L)**	**AlkP (U/L)**
Pearson Correlation	−0.100	−0.125	−0.183	0.348*	−0.047	0.048
Sig. (2-tailed)	0.569	0.474	0.293	0.040	0.787	0.786

3.2. Regression analysis

3.2.1. Multiple linear regression model

A multiple linear regression model was used to describe how the response variable (pH) changes by the independent variables. The fitted regression model is:

$$\hat{y} = b_0 + b_1x_1 + b_2x_2 + \cdots + b_nx_n$$

- $\hat{y}$: the predicted value of the response variable (pH).
- b_0: the intercept (value of pH when all other variables are equal to zero).
- b_1: the first regression coefficient.
- x_1: the first independent variable.
- b_2: the second regression coefficient.
- x_2: the second independent variable.
- b_n: the n regression coefficient.
- x_n: the n independent variable.

Model 1 (Table 3) describes the relationship between the independent variable (biochemical parameter) and the (pH).

The coefficients in the equation describe the relationship between each independent variable and the dependent variable (pH). It shows the amount by which a change in one unit of the independent variable affects the response variable (pH). The p values for the coefficients (See Supplementary Table S2) indicate whether these relationships are statistically significant. As the p-value in Table S2 shows, the biochemical parameters that were statistically significant are Trig, Cac, Cl-C, Crea2, Urea2, K-C, Chol2, AlkP, CK and Phos. Among these biochemical parameters Trig, CaC, Cl-C, K-C, CK, urea2, and AlKP were positively correlated whereas Crea2, Chol2, and Phos were negatively correlated.

As an example, the model suggests that a 1 mmol/L increase in the CaC may increase the mean of the pH by 1.7678 while keeping all other parameters constant, whereas a 1 mmol/L increase in the Phos decreases the mean of the pH by 0.3775, while keeping all other variables constant.

The following five assumptions of multiple linear regressions were tested and confirmed: linear relationship between dependent and independent variables, independence or no correlation between the independent variables, constant variance assumption, normality of error terms, and no multicollinearity.

3.2.2. Univariate and multiple linear regression models of Pearson's significantly correlated variables

Pearson's product-moment correlation was used for testing the significance of the relationships. The relationships of pH with Urea2 and TP are significant; pH and Urea2 are weakly positively correlated (P-value = 0.04035 <0.05), whereas TP and pH are weakly negatively correlated (P-value = 0.04993 <0.05).

Two univariate linear regression models (Table 4; equations 2 & 3) were used to describe how the response variable (pH) changes by the independent variables (TP) and (Urea2). The fitted regression model is:

$$\hat{y} = b_0 + b_1x_1$$

Table 3
Multiple linear regression analysis of serum parameters and saliva pH.

Model equation	R^2	Dependent	b_0	Independents	β	P-value
1	0.808	pH	−5.6321	Trig (mmol/L)	0.584	0.000338
				GluC (mmol/L)	−0.167	NS
				CaC (mmol/L)	1.768	0.000444
				Cl-C (mmol/L)	0.088	0.00018
				K-C (mmol/L)	0.229	0.008272
				Chol2 (mmol/L)	−0.104	0.009850
				CK (U/L)	0.0005	0.037634
				Crea2 (umol/L)	−0.023	0.000475
				Urea2 (mmol/L)	0.071	0.001200
				Phos (mmol/L)	−0.378	0.038895
				CRP32 (mg/L)	0.008	NS
				AlkP (U/L)	0.001	0.018954
				UHDL (mmol/L)	−0.109	NS

Table 4
Regression analysis of serum parameters that correlate with saliva pH.

Model equation	R^2	Dependent	b_0	Independents	β	P-value
2	0.121	pH	6.8122	Urea2 (mmol/L)	0.059	0.0499
3	0.112	pH	8.4042	TP (g/L)	−0.017	0.0404
4	0.194	pH	7.928	Urea2 (mmol/L)	−0.014	
				TP (g/L)	0.050	
5	0.121	Urea2 (mmol/L)	−9.5327	pH	2.029	0.0404
6	0.112	TP (g/L)	123.514	pH	−6.584	0.0499

- $\hat{y}$: the predicted value of the response variable (pH).
- b_0: the intercept (value of pH when TP is equal to zero).
- b_1: the first regression coefficient.
- x_1: the first independent variable.

As shown in equation 2 (Table 4), a 1 g/L change in the TP decreases the mean of the pH by 0.0167 (P-value = 0.0499). Equation 3 shows that a 1 mmol/L change in the Urea2 increases the mean of the pH by 0.0597 (P-value = 0.0404).

Assumptions of univariate linear regression were verified, and the three models (equations 2, 3, and 4) were accepted with 95% confidence. Then, new models were created to solve for ×, the serum parameter.

- Equation 5 shows that the amount that a one-unit change in the pH affects the mean of the Urea2 by 2.0299 mmol/L (P-value = 0.0404)
- Equation 6 shows that the amount that a one-unit change in the pH affects the mean of the TP by −6.584 g/L (P-value = 0.0499)

3.3. Validation and verification

To check the validity of the models, we compared the measured pH values with the calculated values (Table S3). We superimposed both into a histogram and scatter plot. Fig. 2 shows overlaying histograms of measured pH values and calculated pH values. Only the histograms of data obtained from equation 1 (Fig. 2a) show a similar mean and a very close distribution of measured data.

The scatter plot in Fig. 3 shows the distribution patterns of the pH values. As seen in the red and green scatter plots (Fig. 3a) both plots center around the same positive slope, and many of the values are superimposed on each other. The other scatter plots (Fig. 3b, 3c, and 3d) show that calculated pH values were not aligned with the measured ones. The results show that the model in Equation 1 was better than the other models at reproducing the pH values for children between the ages of 7–12 years.

4. Discussion

This study did not put much weight on predetermining the sample size (Sim, Saunders, Waterfield, & Kingstone, 2018), since it exceeded the minimum required sample size for regression (N > 25) (Jenkins & Quintana-Ascencio, 2020). We believe available data with high significant correlations can be used to construct knowledge without overestimating the individual value as a relevant unit. This data can be used for abstract theoretical constructs such as homeostasis, inter fluid correlations, osmolarity, and balance. This study's findings and the current knowledge of these concepts can be built upon to logically make justifiable connections to form models and theories. These models and theories can then be tested and validated on larger samples. We invite colleagues to use constructivism and logic to further advance our knowledge of homeostasis, inter fluid correlation, and mechanisms of the body systems interactions.

The above steps may be used as a scaffold to frame the thinking process. Further logical analysis of the weak and non-significant correlations in our model is as essential as the analysis of the statistically significant correlations. The analysis may advance our understanding of mechanisms by which each component is reflected in the saliva pH.

Sample size influences the statistical significance (Royall, 1986; Collins & Morris, 2008; Khalilzadeh & Tasci, 2017). A small sample size could fail to ascertain statistical significance that may appear in a larger size (saturation effect). That is why the results of this study are valuable, as the results show correlations in small sample sizes and mild conditions. There is solid ground here to have confidence in these correlations and to logically seek interpretations. This is supported by Emmel's statement, "It is not the number of cases that matters, it is what you do with them that counts" (Emmel, 2013; Sim, Saunders, Waterfield, & Kingstone, 2018). Furthermore we can ask questions about thresholds that allow a particular parameter (like glucose) to be reflected by our response variable (saliva pH), and the roles that the selected parameters play on each other to affect the pH.

As apparent from the correlation table in normal, heathy children (See Supplementary Table S1), the correlations between serum biochemicals and saliva pH are weak and non-significant, except for urea and TP. Our results show that the correlation between the pH and TP is significant (p-value = 0.050 = 0.05) and weakly negative. On the other hand, the relationship between pH and urea2 is significant (p-value = 0.040 < 0.05) and weakly positive. This suggests that urea has a direct impact on pH or pH has a direct impact on urea, whereas TP and pH have an inverse impact on each other. However, since the correlation is weak but significant, it suggests the existence of other important determinants in this correlation. Considering the correlation between urea2 and pH, we cannot rule out the existence of a lurking variables that affects both of them directly.

The circulatory system spans the whole body, where plasma encounters nearly all body cells. Plasma proteins may reflect individual health status (Enroth, 2021). Moreover, plasma proteins act as weak acids to maintain blood pH homeostasis. The specific role of blood proteins in acid-base balance is still a topic of inquiry. Researchers found that lowering the plasma protein concentration increased the pH (alkalosis), whereas increasing the plasma protein concentration decreased the pH (acidosis) (Rossing, Maffeo, & Fencl, 1986). In other studies, researchers calculated the charges attributed to proteins and observed the acid-base effect of protein in plasma at physiological pH = 7.4 (Figge, Rossing, & Fencl, 1991). They developed a mathematical model that produced results that aligned well with data collected from human serum and concluded that proteins other than albumins have little effect on total anionic charge (Figge, Rossing, & Fencl, 1991). The negative correlation between saliva pH and serum protein agrees with the correlation between blood pH and protein concentration (Rossing, Maffeo, & Fencl, 1986; Figge, Rossing, & Fencl, 1991). The fact that this correlation appeared regardless of the small study population and mild physiological conditions increased our confidence in the potential ability of saliva pH to be used as a screening tool to reflect on

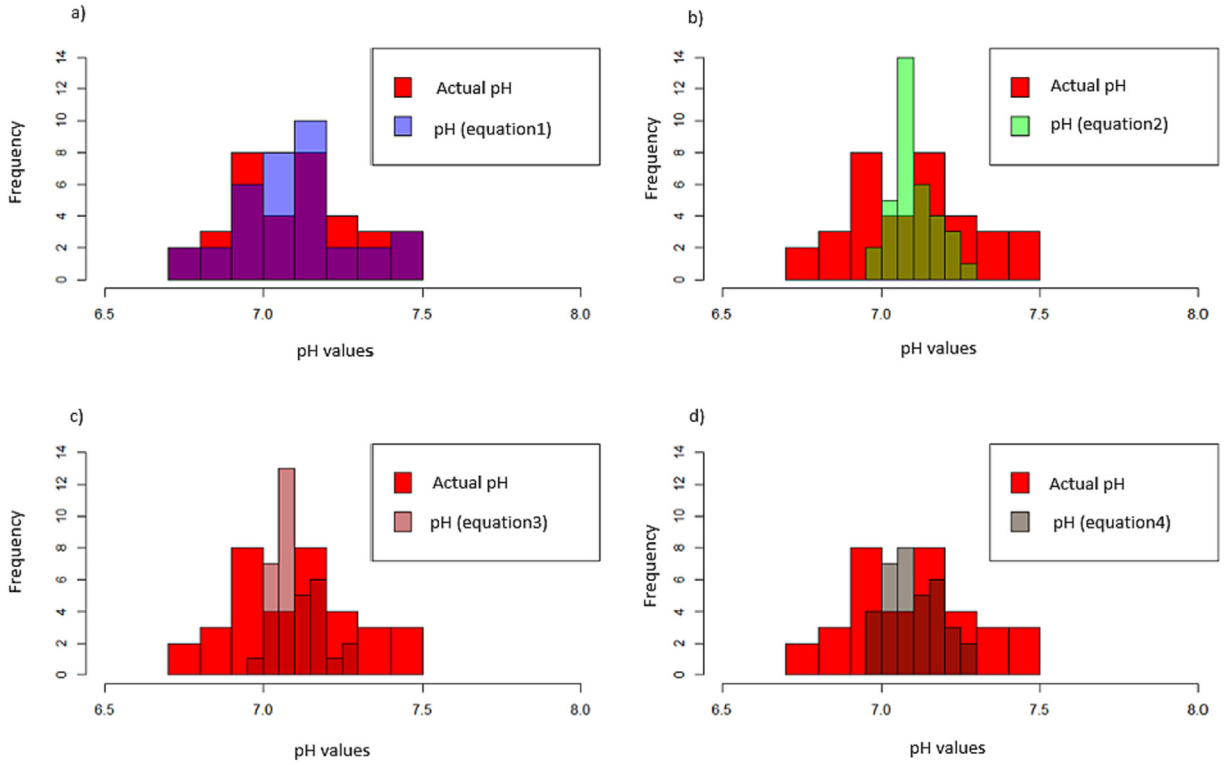

Fig. 2. Histogram showing the frequency of pH values superimposed on the frequencies of the calculated pH values using the four equations. The frequency of pH values (in red) superimposed on the frequencies of the calculated pH values using equation 1 (blue seen on a), equation 2 (green seen on b), equation 3 (pink seen on c), and equation 4 (gray seen on d). We can see that almost all the pH values have the same mean, but the kurtosis is different except for pH calculated using equation 1(Fig. 2.a). The purple shaded area in (Fig. 2.a) reflects data from both measured and calculated pH using equation 1. The majority of the histogram's bars are shaded purple.

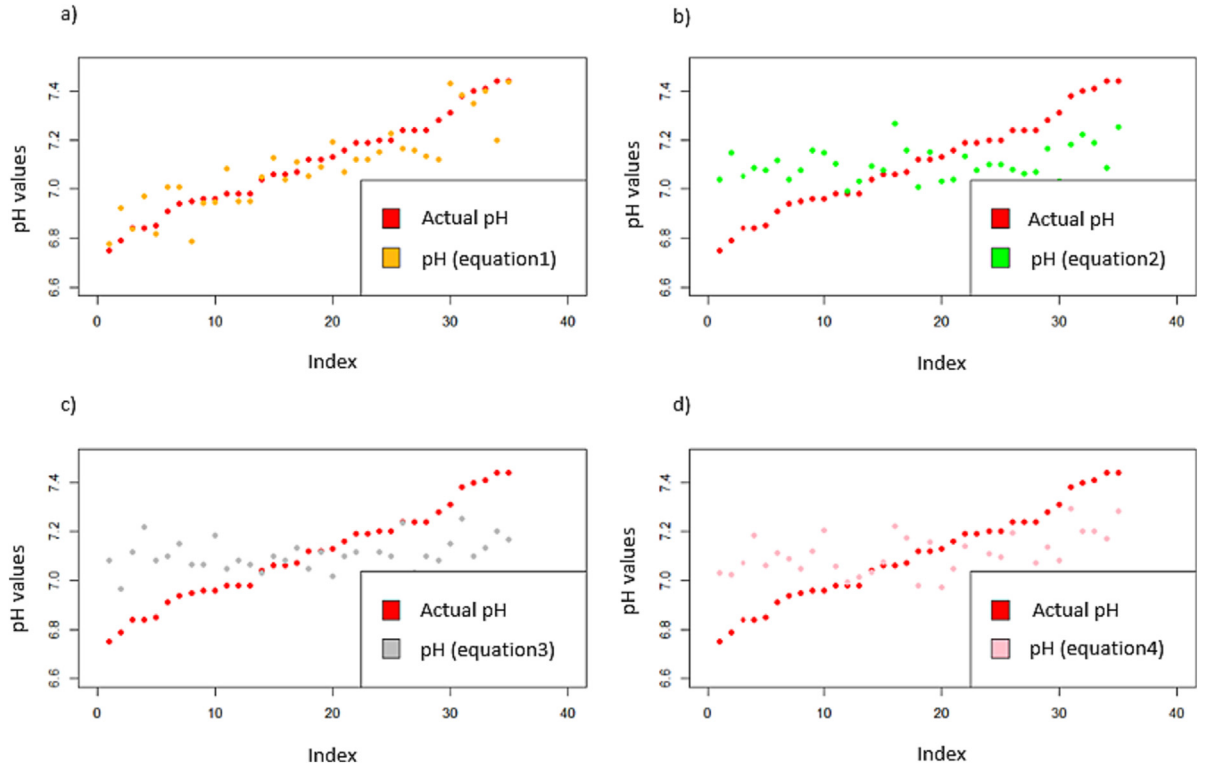

Fig. 3. Scatter plot showing the trend of the distribution of actual pH values in comparison with the calculated pH values. The distribution of actual pH values (red) in comparison with the calculated pH values obtained using a) equation 1 (yellow) b) equation 2 (green) c) equation 3 (gray), and d) equation 4 (pink).

the serum protein status. However, more studies are needed on a larger population, in different age groups, and various health conditions. These findings shed light on many questions raised, such as, what is the mechanism by which serum protein affects saliva pH?

The blood urea nitrogen test (urea2) measures the amount of urea in circulation. The body detoxifies blood from ammonia through the hepatic urea cycle that uses it to synthesize urea which contributes to the acid-base balance (Seifter & Chang, 2016). It is logical to expect a correlation between blood urea and pH, since urea is one of the major plasma solutes, and its concentration contributes to the calculation of serum osmolarity (Elgart, 2004). The correlation between saliva pH and serum urea2 concentrations presents another potential for saliva pH to be used as a screening tool in kidney disease.

The weak significant correlation between TP and urea2 and pH indicates the contribution of other factors to this correlation. To elucidate this effect, we applied a stepwise linear regression to find the best model that represents the relationship between parameters and pH. This model sought to determine how serum components affect the saliva pH, so saliva pH was set to be the response variable, and all other measured serum parameters were predictors. The model starts by screening the predictors for the strength of their relationship with saliva pH and then inputting them into the model from strongest to weakest with proper coefficients. The final model is achieved when no more parameters can be justifiably added or removed from the model.

The achieved stepwise regression model is:

$$\begin{aligned} Salivap H = & -5.6321 + 0.5838 Trig - 0.1674 Gluc + 1.7678 CaC \\ & + 0.0879 ClC + 0.2298 KC - 0.1035 Chol + 0.0005 CK \\ & - 0.0231 Crea2 + 0.0705 BUN - 0.3775 Phos \\ & + 0.0084 CRP32 + 0.0010 AlKP - 0.1097 UHDL \end{aligned}$$

According to the model, the following serum-measured parameters have an effect on the value of saliva pH: TG, Gluc, CaC, ClC, KC, Chol, CK, Crea2, BUN, Phos, CRP, ALKP, UHDL. Except for Gluc, CRP, and HDL, all other parameters were significant predictors of the saliva pH. The non-significant parameters were left in the original model to avoid the biased effect of significant variables. In observational studies, setting regression adjustments may be used to compensate for inherent differences between the covariate distributions of different groups (Chin, 2019). Non-significant variables may serve as a regression adjustment function. This adjustment works for causal identification of the population, not sample level (Pearl, 2010). So, a variable could be non-significant in the sample estimate but not in the population. Non-significant variables may have a weak influence on other independent variables in the model.

Further evidence that shows the importance of keeping the non-significant variable in the proposed stepwise multiple linear regression model appears when examining the calculated versus the measured pH values. To further investigate the significant correlation between saliva pH and serum urea2 and TP only, 3 models were developed. A stepwise multiple linear regression model that takes in consideration TP and urea only (equation 4), and two univariate linear regression models were created. One of the univariate regression equations presents the relationship between pH and Urea2 (equation 2), and one univariate model shows the relationship between pH and TP (equation 3). Since it is important to determine which factor is to be dependent, pH was the response variable in both models as it changes in response to changing fluid solutes. We validated the models by using them to calculate pH (Table S3). Using the models of a significant variable only (equations 2, 3, and 4) gave data that was less accurate than the data obtained from equation 1 (Table S3). As seen in the scatter plot, the pH values of equation 1 followed the same pattern and were the closest to the actual values in comparison to equations 2, 3, and 4. We can see in Fig. 2 that almost all the pH values have the same mean, but the kurtosis is different except for pH calculated using equation 1 (Fig. 2a). As evident from the pH assessment using univariate regression models, the equation could not accurately predict one variant using the other in isolation from other factors. Similarly, a model to predict the significantly correlated serum biochemicals urea (equation 5) and TP (equation 6) with saliva pH could not accurately predict the actual values of urea (Table 4) and TP (Table S5). Overlaying the scatter plot to visualize the variation between measured versus calculated urea (See Supplementary Figures S1 and S2) and TP values (Supplementary Figures S3 and S4) shows a clear variation between measured and calculated variables.

Our results verify that the model in Equation 1 was the best in describing the relationship between pH and serum parameters. The variables that were not significant in the model are serum variables that have been reported to be correlated with saliva pH directly, like glucose (Puttaswamy, Puttabudhi, & Raju, 2017; Uthayasankar & Jayaraj, 2020; Tremblay, Brisson, & Gaudet, 2012) and HDL (Suzuki, et al., 2020) or indirectly, like CRP through being linked to obesity and metabolic syndromes (Choromańska, et al., 2015; Ouellet-Morin, Danese, Williams, & Arseneault, 2011). The fact that these blood factors were shown to be correlated with saliva pH in abnormal conditions gives justifiable supportive evidence for including them in the model. However, we cannot provide an explanation for the model in equation 1 excluding TP, when it showed a weak but significant correlation with pH.

The proposed regression model suggests multidimensional effects of variables on saliva pH in healthy children. An increase in TG, CaC, ClC, KC, CK, BUN, CRP, or ALKP concentration may increase the saliva pH to be more alkaline. On the other hand, an increase in Gluc, Chol, Crea, Phos, or UHDL concentration may shift the pH to be more acidic. In healthy children with average weight, pH can be predicted using the concentration of the included blood parameters.

Serum calcium concentration, CaC, has the highest coefficient (1.7678) in the equation, which suggests that its concentration plays the strongest role in saliva pH prediction. One mmol/L increase in the calcium concentration will increase the pH by 1.7678, keeping every other variable constant. The weak positive correlation between saliva pH and serum CaC is in contrast with the negative correlation between serum pH and serum calcium concentration (Agenes, Sartorelli, Bisso & Dominoni, 1993). We have no justifiable explanation for this weak positive effect of calcium concentration on saliva pH, considering the reported positive correlation between the serum and the saliva calcium concentration (Bravo, et al., 2022). The level of pH is the most important factor that affects calcium binding to proteins. The higher the pH, the more bound Ca which causes a decrease in the free calcium concentration (Goldstein, 1990; Peterson, Feigen, & Crismon, 1961). Some studies reported no relationship between calcium serum concentration and serum pH (Hodgman, Marraffa, Wojcik, & Grant, 2017) Researchers recommended using the regression equation to express the concentration of ionized calcium due to variations between calculation and raw data (Lam, Dhaliwal, & Mamo, 2013). However, a difference between serum and saliva calcium was observed in diabetic children (Moreira, Passos, Sampaio, Soares, & Oliveira, 2009) which suggests a mechanism by which pH is related to calcium levels in different body fluids. It is important to note that our findings propose an effect of calcium in the network of other serum parameters in mild conditions. We believe more studies are needed to elucidate the correlation between CaC and saliva pH.

The majority of the variable coefficients were small, indicating a minimal effect of a single serum variable on saliva pH estimation. However, the collective effects of serum variables concentrations were able to predict the pH value. To test the model in equation 1, it was applied on a population of healthy children with known serum variables concentrations and successfully produced very close pH values. Our study proposes taking this model into consideration to investigate serum markers that affect pH levels.

This research has multiple advantages. First, the participants were strictly chosen to be healthy and free from any chronic or systemic diseases. Secondly, the study selected random samples from single gender, a precisely defined age group, and with an average weight and BMI, which eliminates the presence of a common confounding effect. Third, it uses linear regression analysis which can isolate correlations from confounding effect (Pourhoseingholi, Baghestani, & Vahedi, 2012). This allows for a clear path to investigate the potential of using saliva pH as a non-invasive cost-effective screening tool to estimate serum parameters.

Nonetheless, this study has the limitation of sample size and tight intervals of some of the measured analytes in the normal distribution, as it was intended as the first step to pilot the correlations. Further, larger randomized studies are required to confirm the relevant findings. Furthermore, due to technical challenges, this study did not estimate the salivary flow rate, which should be investigated in further studies. Moreover, the salivary pH is influenced by very many variables, therefore more experimental data on saliva samples, such as proteomic, metabolomic, and biochemical profiling of saliva analysis are needed to guide further investigations. For more extensive characterization of saliva, future studies should include saliva physico-chemical analysis, the assessment of a group of chemokines, or molecules related to certain signaling pathways. Despite these limitations, this study brings attention to the necessity of dedicating more research efforts and resources to understanding the relationship between saliva pH and body fluids.

5. Conclusion

Our data shows a correlation between saliva pH predicted by serum variables and measured saliva pH. With the addition of more variables and adjustment to the model there may be a way to reliably predict saliva pH. It also provides means by which urea and TP concentrations can be correlated to the saliva pH value.

This study could not determine an unambiguous way to calculate serum TP or urea concentration using saliva pH. Thus, the study does not recommend using saliva pH to estimate serum variables. Nonetheless, the study's findings provide a great opportunity to dedicate more efforts to using saliva as a true stress-free, cost-effective diagnostic tool. Such a tool is of particular importance in areas where medical care is expensive and laboratory tools are inaccessible. Once the relationship between saliva pH and health is clearly understood, it may even be possible to adjust body systems by prolonged saliva pH adjustment.

6. Institutional review board statement

This study (NRJ22J/241/09) was approved by the Institutional Review Board (IRB) for King Abdullah International Medical Research Center (KAIMRC), Jeddah, Saudi Arabia. (IRB/2428/22), and all methods were performed in accordance with the relevant guidelines and regulations. Informed consent was obtained from all subjects and/or their legal guardian(s).

7. Informed consent statement

Informed consent was obtained from all parents of the children volunteered in the study.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.sjbs.2023.103793.

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