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Research Article

Validation Method for Blood and Saliva Lithium Determination: Application for Therapeutic Drug Monitoring by Atomic Absorption Spectrophotometry

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Abstract

Background: Lithium is an effective medication used as first-line therapy for bipolar disorder. Due to its narrow therapeutic index, frequent monitoring is highly recommended.

Objective: This study was designed to validate the atomic absorption spectrophotometric method for serum and saliva lithium determination and examine its application for lithium therapeutic monitoring in patients with bipolar affective disorder.

Methods: The atomic absorption spectrophotometer (AAS) apparatus (Rayleigh, China) analyzes lithium in human blood and saliva samples. Precision, Recovery % (Accuracy), Detection Limit, and Specificity were evaluated to validate the used analysis method. The patient's study design was based on an observational study, where four patients with bipolar disorders type 1 and 2 (adults with bipolar disorder and on lithium therapy for one month) aged 32 to 64 years and weighing from 56 to 92 kg participated in this study. Four samples were collected from each patient, two samples of blood and another two of saliva.

Results: A linear relationship between the absorbance and lithium concentrations was obtained in 0.175 - 7 µg/mL with an R^2 of 0.99. Precision, Recovery % (Accuracy), Detection Limit, and Specificity are within the accepted limits. Ratio analysis of (Saliva/Blood) of C_{min}, C_{max}, and C_{pss} were 2.64, 3.77, and 3.54, respectively. The salivary lithium therapeutic range was 2.83- 4.25 mmol/L, which is much higher than blood. Only lithium Cmax in saliva was slightly affected by Body mass index (BMI), age, and creatinine clearance (CLcr), suggesting insignificant effects on lithium levels in general.

Conclusions: The AAS method used in this study is reliable and accurate for determining serum and saliva lithium concentrations. A higher correlation coefficient between lithium in saliva and blood suggests that saliva is an excellent candidate to replace blood for lithium therapeutic drug monitoring.

Keywords: Atomic Absorption Spectrophotometer; Lithium; Saliva; Therapeutic Drug Monitoring

Introduction

Bipolar disorders are chronic disorders that are estimated to affect more than 1% worldwide [1]. Lithium carbonate is the gold standard mood stabilizer for patients with bipolar disorder [2]. This medication can reduce the possibility of depression and mania in patients with type 1 bipolar disorder and reduce suicide attempts [3]. Thirst and excessive urination, nausea, diarrhea, and tremor are among the most popular side effects of being treated with this medication [4]. In addition, the presence of lithium in the salivary, mammary glands, blood, and others makes these glands the likely target for detection of its concentration in the body [5].

Like blood, saliva is rich in various biomarkers, such as DNA, RNA, protein, and easily detectable levels of microorganisms because these two biological fluids have many similarities in molecular makeup [6]. Saliva sampling offers several advantages over other routes for sampling.

Besides, it is non-invasive and has excellent potential for research to analyze the magnitude, time course, and response to medications [7]. Saliva enables the identification of many potentially valuable drugs, biomarkers, and molecular diagnostics [8,9]. The specific purpose of this study is to validate a method of detecting lithium in saliva using the Atomic Absorption Spectrophotometric Method and comparison to that concentration that can be detected in the blood.

Methodology and Patients

Assay methodology

This study used the atomic absorption spectrophotometer (AAS) apparatus (Rayleigh, China) to analyze lithium in human blood and saliva samples. To validate the lithium analysis method using the Atomic Absorption Spectrophotometer, the Calibration, precision,

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accuracy, sensitivity, specificity, and limit of quantitation were performed.

Calibration curves

Calibration Curves were prepared to check for the linearity of the method of analysis. AAS prepared and analyzed six different concentrations (0.175, 0.35, 0.7, 1.75, 3.5 and 7 µg/mL) of lithium standard in highly purified water. The calibration curve was also prepared by spiked blank blood with six different concentrations (0.175, 0.35, 0.7, 1.75, 3.5, and 7µg/mL) of lithium and analyzed by AAF. Blank saliva was also spiked six different concentrations (0.175, 0.35, 0.7, 1.75, 3.5, and 7µg/mL) of lithium and analyzed by AAS.

Precision

Precision was determined by choosing three different concentrations 0.35 (Low), 3.5 (Medium), and 7.0 µg/mL (High). The formerly mentioned solutions were prepared from the actual concentrations of standard lithium solution and doing five readings for each concentration. Evaluation of the measurements, whether close to each other, was performed by calculating the values of standard deviation, coefficient of variation percentage, and relative standard deviation and then evaluating if these values are located in the accepted range based on standard recommendations.

Accuracy

Three different concentrations, 0.35, 3.5, and 7.0 µg/mL were prepared and analyzed by AAS. Accuracy was determined as percent recovery and calculated by dividing the actual concentration of lithium by theoretical concentration (Calculated) multiplied by 100%.

Sensitivity

Measuring the lower limit of detection to check the degree of sensitivity of AAS for different concentrations from the standard lithium solution were prepared (0.10, 0.20, 0.05, and 0.01 µg/mL).

Specificity

The degree of specificity was done by ensuring no significant interferences are using blank purified water, blank blood, and blank saliva.

Patients study design

The patient's study design was based on an observational study conducted at Jordan University Hospital (JUH) after IRB (#33/2020) approval to determine the correlation coefficient between lithium concentrations in both blood and saliva to the dose for TDM using the method mentioned above. Patients with bipolar disorders (type 1 and 2) participated in this study. The study involves four patients aged 32 to 64 years (mean 49.5 years, ± SD 14.364), with the actual weight of these patients ranged from 56 to 92 kg (mean 73.25, ± SD 14.863). This investigation was conducted under the supervision of Dr. Radwan Banimustafa, associate professor of psychiatry at JUH. The inclusion criteria for patients involved in this study included: adults with bipolar disorder and being on lithium therapy for one month (to ensure a steady-state level of lithium). The consent form was also obtained and signed by each patient. The collection of data was done for each patient separately from specific medical files and direct measurements. Saliva and blood samples were taken, and private interviews were conducted.

Four patients consented to take part in this study. Four samples were collected from each patient, two samples of blood and another two of saliva. The first two samples of both saliva and blood were taken just before the first dose of the day to check the minimum concentration of lithium (C_{min}), and the second two samples were collected after taking the first dose by one hour to check the maximum concentration of lithium (C_{max}).

Data analysis

Pharmacokinetic analysis: Creatinine clearance (CLcr) was calculated using the Cockcroft-Gault equation [10].

$$Cl_{cr}[mL/\min] = \frac{(140 - age) \times wt[in\ \text{kg}]}{72 \times SCr\ [in\ \text{mg/dL}]} \rightarrow for\ male$$

$$Cl_{cr}[mL/\min] = \frac{(140 - age) \times wt[in\ \text{kg}] \times 0.85}{72 \times SCr\ [in\ \text{mg/dL}]} \rightarrow for\ female$$

Where *CLcr* is creatinine clearance in mL/min, age in year, *wt* is the actual weight in *kg*, and S_{cr}. is serum creatinine in mg/dL.

The total daily dose of lithium was calculated using the following equation:

Daily dose (mg/day) = 382.54 + (348.29 × desired lithium level) + (67.19×CLcr. × 0.06) [11].

C_{min} and C_{max} of lithium: the maximum lithium concentration at a steady-state was checked 1 hour after taking the morning lithium dosage (post-dose). The minimum lithium concentration is at a steady-state by withdrawing samples in the morning before taking the lithium pre-dose. Moreover, the average steady-state concentrations C_{ss} of lithium which is reached after 3 to 7 days (i.e., four half-lives), was calculated using the equation:

$$Css = \frac{C_{min} + C_{max}}{2}$$

Statistical analysis

Microsoft Excel program was used for descriptive statistics and correlation analysis to find a correlation between dose-normalized plasma and saliva levels of lithium. Analysis of variance was done using Systat V5 to investigate the effects of different factors on lithium levels. A P-value of 0.05 was adopted for a significant difference.

Results and Discussion

Calibration curves

A linear relationship between the absorbance and lithium concentrations was obtained over the concentration range of 0.175 – 7 µg/mL. Several calibration curves of inter and intra-day were done for the validation method on three different days; the results showed that each calibration curve had a correlation coefficient value of at least 0.99, which represented the acceptable criterion value for linearity of the calibration curve. A sample of calibration curve for blood spiked with lithium and saliva is presented in Figure 1 and 2, respectively.

Precision

Based on the precision results achieved average RSD% = 0.7 and 2.636, average SD = 0.0021 and 0.0047 and CV% = 0.046 and 0.147 in blood and saliva (Table 1). Standard deviation and CV% values were less than 15%, and RSD % were less than 5, indicating a high precision index based on the standard recommendations [12].

Recovery % (Accuracy)

Percent final recovery (Accuracy) achieved is approximately

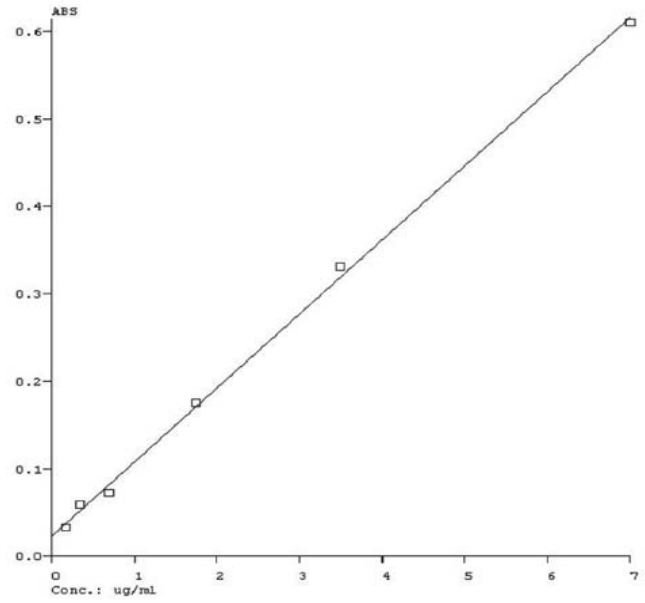

Figure 1: Calibration curve of standard blood spiked with lithium.

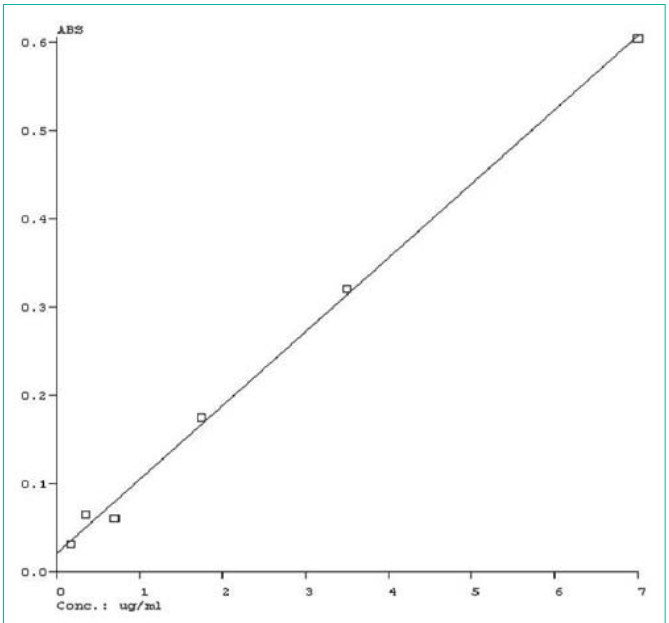

Figure 2: Calibration curve of standard saliva spiked with lithium.

99.723 and 99.91% in blood and saliva, which is within the accepted values (80% - 120%) (Table 2).

Detection limit (D.L)

Based on the results of this study, the value detection limit for standard blood and saliva lithium concentrations were 0.01 and 0.02 ug/mL respectively.

Specificity

No significant interferences are observed at the analyte or internal standard retention time, indicating high specificity of the (FAAS) apparatus.

Table 1: Precision based on standard blood and saliva lithium concentrations.

Sample	Actual concentration	Recalculated concentration	SD	CV%	RSD %
Blood					
1	0.35	0.3445	0.0001	0.029	0.21
2	3.5	3.5105	0.0018	0.05	0.83
3	7	6.995	0.0044	0.06	1.07
Saliva					
1	0.35	0.3498	0.0005	0.143	2.76
2	3.5	3.5716	0.0075	0.209	3.58
3	7	6.9661	0.0062	0.089	1.57

Table 2: Recovery % (Accuracy) based on standard blood and saliva lithium concentrations.

Sample	Actual concentration	Recalculated concentration	Recovery %
Blood			
1	0.35	0.3466	99.03%
2	3.5	3.5064	100.18%
3	7	6.997	99.96%
Saliva			
1	0.35	0.3488	99.66%
2	3.5	3.5031	100.08%
3	7	6.999	99.99%

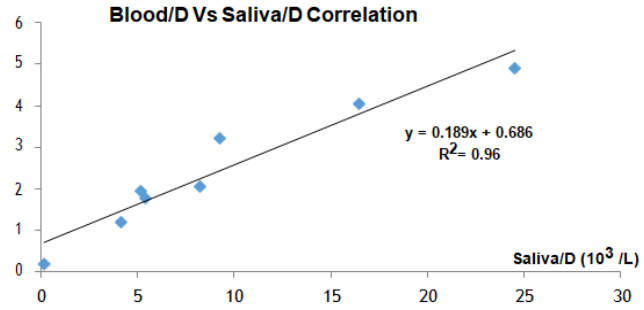

Figure 3: Correlation plot of Blood/Dose versus Saliva/Dose.

Blood and saliva lithium

The mean blood lithium concentrations steady-state ranged from 0.839 to 3.735 (µg/mL) and ratio between Cmax/Cmin of blood lithium = 1.49µg/mL. On the other hand, the mean steady- state saliva lithium levels ranged from 2.595 to 10.493 (µg/mL) ratio between Cmax/Cmin of saliva lithium = 1.84µg/mL as shown in Table 3.

Correlation analysis was done after dose normalization. A plot of lithium dose-normalized blood versus saliva levels yielded a straight line with an equation: Y = 0.186X + 0.868 and a good correlation coefficient of 0.96, as shown in Figure 3.

Also, from the straight-line equation, blood lithium level may be predicted from saliva lithium level. These results are consistent with previous studies regarding the correlation between lithium in saliva and blood. The Correlation Coefficient of some of these studies achieved ranged from 0.53 - 0.78 [13-15]. This study indicates a higher correlation coefficient between lithium in saliva and blood,

Table 3: C_{min}, C_{max}, and steady-state lithium levels summary (µg/mL).

Parameters	C_{min} (µg/mL) blood, saliva	C_{max} (µg/mL) blood, saliva	C_{pss} (µg/mL) blood, saliva
Mean	1.64, 4.82	2.44, 8.86	2.04, 6.84
SD	1.46, 4.24	1.06, 2.6	1.26, 3.28
CV	0.90, 0.88	0.44, 0.29	0.62, 0.48

suggesting that saliva is an excellent candidate to replace blood for lithium therapeutic drug monitoring.

Ratio analysis of this study showed that the ratio of dose-normalized (Saliva /Blood) of C_{min}, C_{max}, and C_{pss} were 2.64, 3.77, and 3.54, respectively. Based on the calculated Saliva/Blood ratio, the normal salivary lithium therapeutic range can be calculated by multiplying the lithium therapeutic range in the blood (0.8-1.2 mmol/L) by Saliva / Blood Cpss ratio of 3.54. Salivary lithium therapeutic range = (0.8-1.2 mmol/L) µg/mL X 3.54 = (2.83- 4.25) mmol/L. Since lithium levels in saliva are much higher than in the blood and lithium exists mostly un-bound (high fu) in the body and high permeability coefficient, lithium is considered SECS class I based on Salivary Excretion Classification System [16].

Body mass index (BMI), age and creatinine clearance (CLcr) are vital variables that can affect lithium pharmacokinetic variability [17,18]. Analysis of variance was done after log transformation, as shown in Table 2. Only lithium Cmax in saliva was slightly affected by such factors, suggesting insignificant effects on lithium levels in general.

In general, it is well documented that therapeutic drug monitoring (TDM) of drugs with a narrow therapeutic index was a helpful tool for dosage adjustment, avoiding adverse side effects, ensuring safety, efficacy, and patient adherence 18. Because of lithium's narrow therapeutic range, therefore therapeutic drug monitoring is recommended through frequent blood lithium level measurement [19,20]. Although blood samples (serum or plasma) are traditionally used to measure drug levels for TDM, saliva samples as an alternative for blood are a promising approach [21-23]. Saliva sampling has several advantages: inexpensive, non-invasive, easy sampling, and drug excreted in saliva as free (unbound) [21]. Several investigators measured lithium levels in saliva as an alternative for blood by several investigators [13,24,25]. This study aimed to use saliva samples instead of blood to monitor lithium levels in Jordanian psychiatric patients and develop a validated method of analysis for lithium in saliva and blood using an atomic absorption spectrophotometer. This study documented a validated method of measurement of lithium concentration in both saliva and blood using atomic absorption spectrophotometer with linearity, precision, and recovery within the recommended standard values [26].

This study also revealed that lithium salivary level is much higher than blood, which agrees with several published articles [13,24,25]. Literature data revealed a higher concentration of lithium in saliva than in serum, which was explained by the fact that lithium ions are eliminated slower from saliva than from serum and by the active transport to saliva [27,28]. The salivary excretion classification system is another explanation for the higher saliva lithium level 16. Since lithium has high effective intestinal permeability and high fraction unbound (protein binding less than 10%), lithium is considered class I based on SECS. The data of this study showed a perfect correlation between lithium saliva/dose and serum/dose (r^2 = 0.96). Most of the previous studies obtained a good result, but the variable correlation between serum lithium and salivary lithium was because they did not correct the dose of individual patients [25,29,30]. There were no differences between lithium concentrations in saliva and serum concerning gender, except in the one study, a higher correlation between lithium levels in saliva and serum was found in women [31]. It is worth mentioning that high variability in saliva lithium/serum lithium level ratio was reported [14,29,32]. This variability in saliva level was higher in intra-subject than inter-subject, which may be attributed to many factors, like saliva sampling (stimulated versus un-stimulated), saliva handling (centrifuged versus un-centrifuged sample), method of analysis, time of sampling, duration of lithium treatment 33 reported that centrifugation or dialysis of saliva improves the quality of lithium measurements. In this study, un-stimulated saliva was used, reported to a better saliva sample for analysis [34].

Limitation of the Study

Even though few patients (because of COVID-19) were used in this study, we consider these results primal, and more sample sizes will be used in future studies.

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