Re-exploring Saliva as an Analyte in Estimation of Lithium in Stable Bipolar Patients – A Pilot Study in a Tertiary Care Hospital in West Bengal

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Abstract

Background: Lithium a frequently prescribed drug in the treatment of bipolar affective disorder, due to its inherent adverse potential and narrow therapeutic index, demands for regular therapeutic drug monitoring (TDM). Although the term TDM seems to be tedious, the most tedious job is to collect blood samples from patients with psychiatric illness. In this study, we aimed to find out an alternative method to serum lithium monitoring. Although saliva as an analyte is non-popular, the presence of lithium in the saliva in patients who consumes lithium makes procedure of collection easier. The rate of elimination of lithium is slower from saliva than from serum, which makes the concentration of lithium higher in saliva than in blood. Lithium elimination follows two compartment pharmacokinetic models, where important part of compartment is saliva and salivary glands. The trouble of repeated venipuncture can be done away with, if a non-invasive method for serum lithium concentration is taken into consideration. An alternative method of determining lithium level could be saliva. The aim of this study was to find out whether estimation of lithium in saliva can replace serum lithium estimation. **Materials and Methods:** In this cross-sectional study, 60 stable patients of bipolar disorder attending psychiatry outdoor on lithium therapy for 3 months or more were considered. After informed consent documentation serum and saliva samples were collected after 12 h of last dose of lithium carbonate intake. Assessment was done based on atomic absorption spectrometry. After statistical analysis, it was found that that there is a correlation between serum and saliva lithium level. The 60 patients were divided into two groups, consisting of 30 patients each. Linear regression was done in both the groups thereby the formula obtained from the first group, was utilized to calculate the saliva concentration form serum concentration. The mean values were compared at the last. **Results:** The mean serum lithium obtained was 0.99 ± 0.257 SD (mEq/L) and mean salivary lithium obtained was 1.63 ± 0.51 SD (mEq/L) in Group 1. Whereas, the mean serum lithium obtained was 0.79 ± 0.26 SD (mEq/L) and mean salivary lithium obtained was 1.67 ± 0.49 SD (mEq/L) in group 2. Lithium concentration from both the samples showed a positive correlation as obtained from the scatter plot. **Conclusion:** Salivary Li estimation seems to yield positive results, yet more research should be done in this regard. Since saliva method for estimation is non-invasive, the idea could be utilized in designing other devices.

Keywords: TDM, Bipolar disorder, Lithium, Pharmacokinetic model, Atomic absorption spectrometry

Introduction

Lithium remains a mainstay of treatment for bipolar disorder, especially for acute mania and maintenance treatment.[1] Lithium, an effective mood stabilizer, is used principally for the management of bipolar disorder (BD). Its administration is complex and often requires sophisticated management and time to time monitoring. In addition, lithium not only reduces the risk of suicide in patients with bipolar disorder[2,3] but also has the potential to reduce the risk of developing neurocognitive disorder.[4]

Lithium is rapidly absorbed through the gastrointestinal tract; food has no effect in lithium absorption.[5,6] Peak serum levels occur in 1–2 h with standard, immediate-release preparations of lithium, and within 4–5 h with slow-release preparations. Absorption of immediate-release lithium is complete within 6–8 h, and for slow-release preparations in approximately 8–12 h. Lithium being non-protein bound is distributed throughout total body water. Brain levels are highest within 2 h of peak serum levels. Steady state is achieved within 4–5 days after the last dose change. Lithium is not metabolized and is excreted almost exclusively through the kidneys.[5] Thus, lithium's elimination half-life is determined primarily by renal function. The half-life in healthy young patients is about 24 h and increases as renal function declines with age. Thus, before initiating lithium therapy, there are several basic points to discuss with patients including potential side effects, the need to

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take the medication as prescribed rather than on as-needed basis, and to expect that response and remission may not occur until a

few days to weeks have elapsed after a therapeutic dose/level has been achieved.

The starting dose of lithium for bipolar affective disease is usually 300 mg 2–3 times daily.[7-10] The total daily dose is then increased by 300–600 mg every 1–5 days based on response, tolerability, and body mass index. The goal is to reach a therapeutic serum level, which generally occurs with a dose of 900 mg–1800 mg/day. Dose increases generally occur more frequently at the beginning of treatment and less often as clinicians approach the target dose. The half-life of Li is approximately 24 h. Thus, it takes at least 4–5 days for serum lithium concentrations to reach steady-state after the dose is changed.

Lithium can be dosed either once daily or in a divided dose regimen. Clinicians usually initiate with a twice daily or 3 times daily dosing schedule to minimize side effects (especially nausea) early in treatment and consolidate the dose schedule to once daily after several weeks or months of treatment. Some patients may have to continue taking lithium in two or four divided doses to minimize peak level side effects. However, adherence generally decreases as the frequency of dosing increases.[11] The target serum level for acute phase management and maintenance treatment is between 0.8 and 1.2 mEq/L (0.8 and 1.2 mmol/L), and levels should usually not exceed 1.2 mEq/L (1.2 mmol/L).[10] Patients who cannot tolerate a level of 0.8 mEq/L (0.8 mmol/L) may respond to a level of 0.6 mEq/L (0.6 mmol/L).[11] After reaching the estimated therapeutic dose range (generally 900 mg–1800 mg/day), the serum lithium concentration should be checked. Subsequently, a level should be measured 5–7 days after each dose increase. In addition, if the dose is not changed and a level not checked for 2 or more weeks, a level should be checked before increasing the dose. An office-based instrument for finger prick test of lithium levels is available.[12]

Lithium levels should be drawn approximately 12 h after the last dose (12-h serum trough level) and generally collected in the morning, before the first dose of the day. Changes in the serum level per unit time are not dramatic 12 h after the last dose. Thus, a level drawn 11–13 h after the last dose, or even 10–14 h, provides meaningful information. In contrast, a serum level drawn a few hours after lithium ingestion is subject to marked fluctuation if the level is drawn one hour sooner or later, leading to unreliable information.

Since lithium being a drug with a narrow therapeutic index, the number of side effects related to lithium therapy potentiates the requirement of TDM. The number of side effects has resulted in poor patient adherence to treatment. Toxicity occurs at 1.5–2.0 mmol/L and is manifested by coarse tremor, apathy, hyperreflexia, hypertonia, nausea, myoclonus, seizures, acute renal failure, cardiac dysrhythmia, and coma. Hemodialysis is advised when serum concentration reaches to 3.5 mmol/L which are detrimental to life. TDM mainly aims at individualizing dosing schedules by measuring the plasma concentration of the drug. It is applied to drugs which have high inter-individual pharmacokinetic variability, a narrow therapeutic range and known relationship between plasma concentration and effect. The goal of TDM is to use drug concentrations to manage patient's medication regimen and optimize outcome.

Salivary drug concentration analysis is one of the non-invasive as well as patient complaint procedures in clinical pharmacology discipline. Lithium is not protein bound and thus has gained popularity in analytical research. Saliva lithium estimation may be an alternative to blood lithium estimation and even may replace it. In TDM, researches involving the use of saliva sampling as non-invasive qualitative and quantitative techniques have become increasingly important. Being readily accessible and collectible, saliva may have many advantages over "classical" biological fluids such as blood and urine. The growing interest in non-invasive procedures, this study evaluates the use of saliva in lithium estimation. New techniques for the collection and analysis of saliva as well as for identifying the components affecting drug concentrations in saliva are being explored. Our lack of knowledge of saliva as a biological specimen, saliva drug levels should be used concomitantly with recorded drug concentrations in other fluids, for example, plasma, to contribute to a more ideal interpretation of drug concentrations in clinical studies. In this pilot study, we did a comparison between serum lithium and saliva lithium concentration. Both the samples were analyzed using atomic absorption spectrophotometer Perkin Elmer A 400.

Many different methods have been introduced for serum lithium determination. Initially, flame emission spectrometry (FES) and flame atomic absorption spectrometry (FAAS) were used to determine blood serum lithium concentration. A study says that both methods be it either emission spectroscopy or absorption spectroscopy, considered satisfactory, precise, and accurate and can be adopted for lithium quantification. In the comparison of quantitative results in lithium-treated patients through statistical tests, no significant differences were observed. Therefore, the methods for lithium quantification by flame atomic absorption spectrometry (FAAS) and flame atomic emission spectrometry (FAES) may be considered similar. In the late 1980s, ion selective electrodes (ISEs) were developed for lithium and recently a colorimetric method was developed. Flame photometer, atomic absorption spectroscopy, ion electrode method, and colorimetric methods are available for the estimation of serum lithium levels. Each method has its advantage over others; also disadvantages prevail in all of them. Although atomic absorption spectroscopy is used in determination of lithium with precision,[13] the cost of the procedure has restricted its use. Apart from AAS the other methods require sample processing which seems to be tedious to perform which includes the necessity of de-proteinization.[14] The other advantage is it can detect submicromolar endogenous concentration of lithium in human plasma.[15,16]

MATERIALS AND METHODS

Blood samples and unstimulated saliva samples were collected from the patients of bipolar disorder after 12 h of the last dose. The blood was centrifuged at the rate of 2500 revolutions per minute to separate serum. Serum and saliva samples were diluted 10 times, and diluted serum sample was then analyzed using AAS. The characteristic graph was obtained using 6 ppm, 8 ppm, and 8 ppm solution of lithium solution, while the standard lithium solution was 1000 ppm. Saliva samples were collected in sterile container. The collected saliva was ten diluted 10 times and diluted saliva sample was centrifuged. The supernatant fluid was then utilized for lithium estimation. Different methods are being utilized for estimation of lithium in blood samples. The atomic absorption spectrometry Perkin Elmer (Perkin Elmer) was used for the determination of lithium ions in serum and saliva. Lithium ions determination was made at wave light 670.8 nm. The light source used was hollow cathode lamp, with the cathode made of lithium. The values for lithium concentration obtained from AAS were

in ppm scale which was then converted to mEq/dl. The method was of estimation which was counter checked, using electrolyte analyzer. Serum lithium concentration obtained by AAS was compared to electrolyte analyzer method.

Study Site

The study was conducted at the Department of Clinical and Experimental Pharmacology, Calcutta School of Tropical Medicine, Kolkata, and Psychiatry Department Medical College Kolkata.

Study Population

All stable ambulatory patients of bipolar disorder on lithium monotherapy or polytherapy attending the Psychiatry outdoor, of Medical College and Hospital, Kolkata, satisfying inclusion criteria and willing to participate in this study was considered. Those patients belonging to the age group of 15–60 years were enrolled. Those patients who were suffering from xerostomia, having other diseases of oral cavity including dental caries, deranged renal function tests, and tobacco consumers were excluded from the study. This investigation was done respecting ethical standards stipulated in the Helsinki Declaration and a detailed protocol was approved by the Clinical Research Ethical Committee, STM Kolkata, and from Medical College and Hospital, Kolkata as well. Lithium estimation was done to the patients as and when advised by their treating psychiatrist, in the Department of Clinical and Experimental Pharmacology, Calcutta School of Tropical Medicine, Kolkata. All patients gave written and informed consent. All

patients were treated with lithium carbonate (supplied by Hospital Pharmacy store, MCH Kolkata) within a period from 3 months to 5 years.

RESULTS

In this present cross-sectional study, a comparison was done between blood serum and saliva samplers using AAS. The study completed with 27 (45%) males and 33 (55%) females (age: 15–56 years). We took serum and saliva samples simultaneously after 12 h of the last dose of lithium intake. The mean serum lithium concentration for 60 patients was found to be 0.784 ± 0.268 SD (mEq/L), and that of mean saliva concentration was found to be 1.657 ± 0.55 SD (mEq/L). Linear regression analysis was performed which showed a positive correlation.
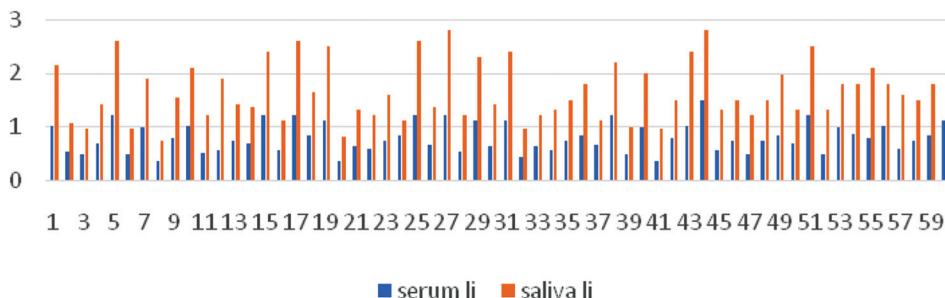
For our better understanding, the 60 patients were grouped in two groups, Group 1 and Group 2, each group containing 30 patients. The mean serum lithium obtained was 0.99 ± 0.257 SD (mEq/L) and mean salivary lithium obtained was 1.63 ± 0.51 SD (mEq/L) in Group 1. Whereas, the mean serum lithium obtained was 0.79 ± 0.26 SD (mEq/L) and mean salivary lithium obtained was 1.67 ± 0.49 SD (mEq/L) in Group 2. A scatter plot was made which clearly shows a positive correlation with the two data sets.

The linear regression formula as obtained from the Group 1 was utilized to find out the salivary lithium concentration, for Group 2. The mean value for salivary lithium concentration for Group 2 was found to be 1.67 ± 0.49 SD (mEq/L), and while applying the formula obtained from Group 1 the salivary Li concentration,
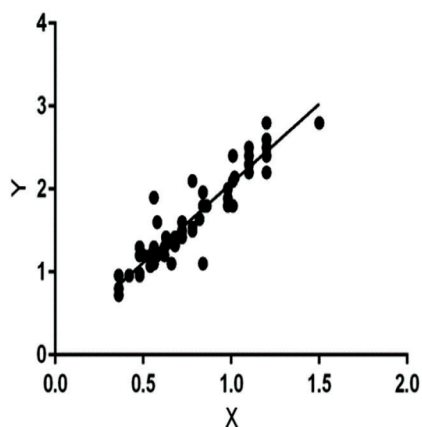
Best-fit values	
Slope	1.913 ± 0.09659
Y-intercept	0.1565 ± 0.08005
X-intercept	-0.08178
1/Slope	0.5227

95% Confidence Intervals	
Slope	1.720 to 2.107
Y-intercept	-0.003811 to 0.3167
X-intercept	-0.1833 to 0.001818

Goodness of Fit	
R square	0.8712
Sy.x	0.1992

Is slope significantly non-zero?	
F	392.3
DFn,DFd	1,58
P Value	< 0.0001
Deviation from horizontal?	Significant

Data	
Number of XY pairs	60
Equation	$Y = 1.913*X + 0.1565$

Chart Title

■ serum li   ■ saliva li

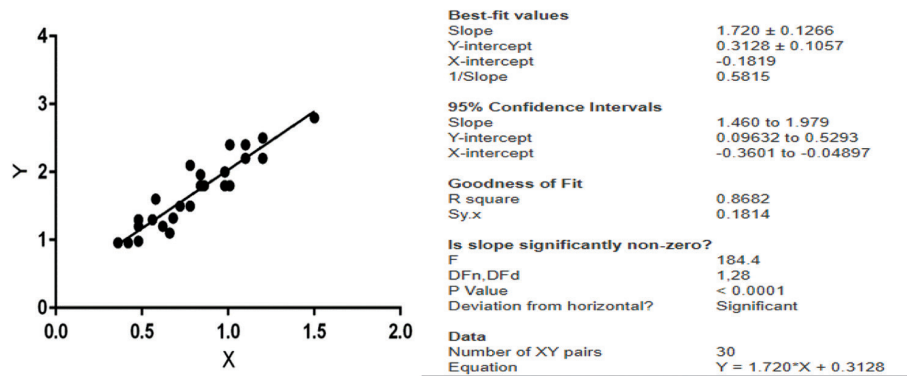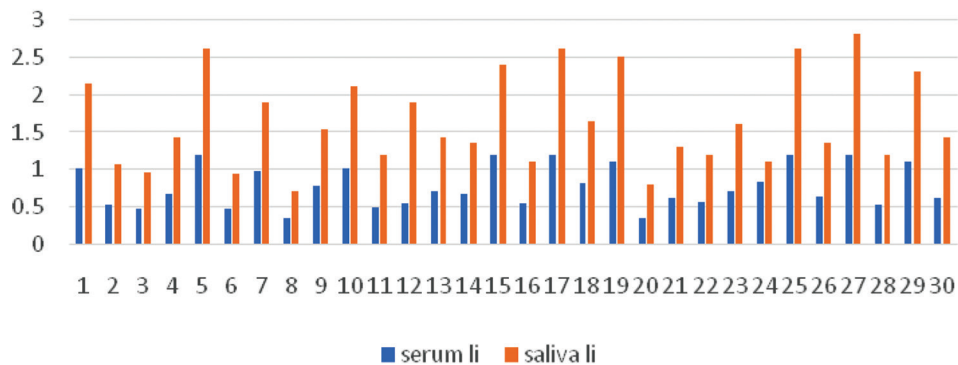| Best-fit values	
Slope	2.093 ± 0.1415
Y-intercept	0.01366 ± 0.1164
X-intercept	-0.006528
1/Slope	0.4778
95% Confidence Intervals	
Slope	1.803 to 2.383
Y-intercept	-0.2248 to 0.2521
X-intercept	-0.1387 to 0.09505
Goodness of Fit	
R square	0.8866
Sy.x	0.2098
Is slope significantly non-zero?	
F	218.8
DFn,DFd	1,28
P Value	< 0.0001
Deviation from horizontal?	Significant
Data	
Number of XY pairs	30
Equation	Y = 2.093*X + 0.01366

Scatter Plot for Group A, X-axis: serum Li conc., Y-axis: saliva Li conc

serum vs salivary li conc. in group 1

■ serum li   ■ saliva li

Best-fit values	
Slope	1.720 ± 0.1266
Y-intercept	0.3128 ± 0.1057
X-intercept	-0.1819
1/Slope	0.5815
95% Confidence Intervals	
Slope	1.460 to 1.979
Y-intercept	0.09632 to 0.5293
X-intercept	-0.3601 to -0.04897
Goodness of Fit	
R square	0.8682
Sy.x	0.1814
Is slope significantly non-zero?	
F	184.4
DFn,DFd	1,28
P Value	< 0.0001
Deviation from horizontal?	Significant
Data	
Number of XY pairs	30
Equation	Y = 1.720*X + 0.3128

Scatter Plot for Group B, X-axis: serum Li conc, Y-axis: saliva Li conc.

serum li vs salivary li in Group 2

■ serum li   ■ saliva li
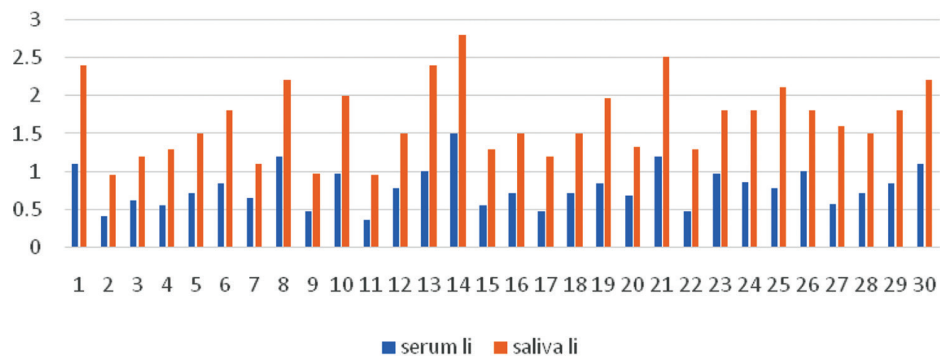
was found to be 1.69 ± 0.55 (mEq/L). This study revealed higher concentration of lithium in saliva than in serum. The explanation can be made, since lithium ions are eliminated slower from saliva than from serum as well as by the active transport to saliva (Langman 2007, Serdarevic 2006).[17,18] However, there are several factors which are to be kept in mind while considering saliva as an analyte. The collection process must be corrected if the patient has been suffering from xerostomia, and other diseases confined to oral cavity; moreover, brushing of teeth and fasting status should be well counseled a priori.

Discussion

Sixty patients diagnosed with bipolar disorder on lithium carbonate were studied, to revisit the role of saliva in therapeutic drug monitoring. The patients were on lithium carbonate preparations as supplied by the hospital pharmacy store. Saliva were collected from these patients by standard methods under supervision. The concentration of lithium in saliva was higher than those in serum [Table 1]. Even though few studies yield reasonable evidence to support salivary lithium monitoring, while others dispute the usefulness of this method probably because of methodological differences in saliva collection and patient selection.[19,20] There are several factors which contribute to the variability of salivary lithium concentrations. These include lithium ion concentration in blood, stimulation of salivary glands, and aspects of lithium administration such as dosage and duration of treatment. A potentially important variable is the presence of

the mucopolysaccharide in saliva. The relationship between the lithium levels of ultrafiltrate and plasma is stronger than when saliva is centrifuged and unfiltered supernatant is measured.[21]

Special Findings

Although all patients were stable, and were in their maintenance therapy, it was found that nine out of 60 patients had lithium concentration below therapeutic range, no patients had supratherapeutic lithium concentration and well-tolerated therapy as prescribed to them.

Conclusion

Salivary Li estimation seems to yield positive results, yet more research should be done in this regard. Since saliva method for estimation is non-invasive, the idea could be utilized in designing other devices. A protype device in the name of NaLiK has already been made, which can be utilized in estimating sodium, lithium, and potassium simultaneously. The device was so designed so that it could be utilized in the outpatients' department, as a point of care device. The study on salivary lithium estimation seems to be very scanty in number. The other issues like methods of obtaining saliva and its biochemical analysis, collecting samples in a specific time frame from the last dosage of lithium as well as inter-subject or intra-subject measurements should be kept in mind.

Table 1: Comparison of lithium values in both saliva and serum in two cohorts

Cohort 1			Cohort 2		
Sl. No			Sl. No		
	Serum Li meq/l	Saliva Li meq/l		Serum Li meq/l	Saliva Li meq/l
1	1.02	2.142	1	1.1	2.4
2	0.54	1.07	2	0.42	0.96
3	0.48	0.96	3	0.62	1.2
4	0.68	1.42	4	0.56	1.3
5	1.2	2.6	5	0.72	1.5
6	0.48	0.955	6	0.84	1.8
7	0.98	1.89	7	0.66	1.1
8	0.36	0.72	8	1.2	2.2
9	0.78	1.54	9	0.48	0.98
10	1.01	2.1	10	0.98	2
11	0.5	1.2	11	0.36	0.96
12	0.56	1.9	12	0.78	1.5
13	0.72	1.42	13	1.01	2.4
14	0.68	1.36	14	1.5	2.8
15	1.2	2.4	15	0.56	1.3
16	0.56	1.1	16	0.72	1.5
17	1.2	2.6	17	0.48	1.2
18	0.82	1.64	18	0.72	1.5
19	1.1	2.5	19	0.84	1.96
20	0.36	0.8	20	0.68	1.32
21	0.62	1.3	21	1.2	2.5
22	0.58	1.2	22	0.48	1.3
23	0.72	1.6	23	0.98	1.8
24	0.84	1.1	24	0.86	1.8
25	1.2	2.6	25	0.78	2.1
26	0.65	1.36	26	1.01	1.8
27	1.2	2.8	27	0.58	1.6
28	0.54	1.2	28	0.72	1.5
29	1.1	2.3	29	0.84	1.8
30	0.63	1.42	30	1.1	2.2

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