"A CROSS SECTIONAL STUDY TO COMPARE SERUM LITHIUM, SALIVARY LITHIUM AND URINARY LITHIUM IN PATIENTS ON LITHIUM CARBONATE".

By

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LIST OF ABBREVIATIONS USED

AAS	-	Atomic absorption spectrometry
BMI	-	Body mass index
Da	-	Dalton
ECG	-	Electrocardiogram
EEG	-	Electroencephalogram
FDA	-	Food and drug administration
g or gm	-	Gram
hrs	-	Hours
HIV	-	Human immunodeficiency virus
Li^+	-	Lithium
L	-	Litre
L/h	-	Litre per hour
mmol/ L	-	Milli mol per litre
meq/ L	-	Milli equivalents per litre
ml/ h /Kg	-	Mililitre per hour per kilogram
ml	-	Mililitre
p	-	probability value
p.p.m	-	Parts per million
r	-	Correlation coefficient
S.R	-	Sustained release
SPSS	-	Statistical package for social sciences
S.E	-	Standard error
TSH	-	Thyroid-stimulating hormone
t ½	-	Half - Life

ABSTRACT

Background and Objectives:

Lithium carbonate is used in the treatment of psychiatric and non psychiatric disorders.Lithium has an established use in treatment of acute mania, prophylaxis of bipolar episodes and augmentation therapy for severe, refractory depression. The difference between toxic and therapeutic dose of lithium carbonate is narrow. Lithium therapy is monitored using series of serum determinations of lithium which requires repeated drawing of blood samples. An alternative method of determining lithium level is to assay salivary gland secretions or urine which has several practical advantages. This study is an attempt to explore the relationship between serum lithium, salivary lithium and urinary lithium, with the possibility of discovering alternate methods for monitoring patients who are on lithium therapy

Materials and methods:

Blood, saliva and urine samples were collected from 50 patients in the morning (12hrs after the last dose of lithium carbonate) before the patient consumed breakfast and the morning dose of lithium. Samples were collected under aseptic precautionary measures.

Estimation of serum, salivary and urine lithium was done using atomic absorption spectrophotometer. Statistical analysis was done using Pearson's correlation coefficient and linear regression analysis.

Results

Mean serum lithium was 0.75±0.25 mEq/L, mean salivary lithium was 1.91±0.80 mEq/L and mean urine lithium was 7.16±4.84 mEq/L. In males mean serum lithium was 0.75±0.26 mEq/L, mean salivary lithium was 1.91±0.80 mEq/L

and mean urine lithium was 7.53±5.26 mEq/L. Correlation between serum lithium and salivary lithium was statistically significant (r=0.695, p <0.001). This correlation was more in females(r=0.770, p <0.001) when compared to males (r=0.665, p <0.001). After doing linear regression analysis, the equation for calculating serum lithium from salivary lithium was: Y=0.332+0.221X (Y=serum lithium concentration, X=salivary lithium concentration).

Interpretation and conclusion:

This study showed that there is a significant correlation between serum and salivary lithium levels. By using more sophisticated technique, this correlation can be utilized to predict serum lithium levels from salivary lithium level.

The relationship found between serum and urine lithium was not statistically significant therefore urine lithium estimation may not be a suitable alternative for monitoring lithium therapy.

Key words: Lithium ,serum lithium, salivary lithium, urine lithium, therapeutic drug monitoring.

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Aim:

Aim of the present study was to explore the relationship between serum lithium, salivary lithium and urinary lithium, with the possibility of discovering alternate methods for monitoring patients who are on lithium therapy

Objectives:

1. Comparing serum lithium, salivary lithium and urinary lithium in patients on lithium carbonate.
2. Evaluate the possibility of replacing serum with salivary or urine lithium estimation for monitoring of patients on lithium carbonate

REVIEW OF LITERATURE

Lithium has an established use in three main indications: treatment of acute mania, prophylaxis of bipolar episodes and augmentation therapy for severe, refractory depression[10]. Although the psychopharmacological field of bipolar disorders has evolved rapidly during the last 10 years, lithium is still considered the 'gold standard' for these conditions and a first-choice mood stabilizer in recent guidelines[11-13]. Lithium and its augmentation by antidepressants, antipsychotics, and benzodiazepines had been the major approach for the management of bipolar disorder[14].

HISTORY

Lithium salts were used in the nineteenth century as a treatment of gout, sedative and as a putative anticonvulsant. Thereafter, lithium salts were unpopular until the late 1940s, when lithium chloride was employed as a salt substitute for cardiac and other chronically ill patients. This ill-advised use led to several reports of severe intoxication and death and to considerable notoriety concerning lithium salts within the medical profession[15].Cade, in Australia, while looking for toxic nitrogenous substances in the urine of mental patients for testing in guinea pigs, administered lithium salts to the animals in an attempt to increase the solubility of urates. Lithium carbonate made the animals lethargic, and in an inductive leap, Cade gave lithium carbonate to several agitated or manic psychiatric patients, reporting that this treatment seemed to have a specific effect in mania [3,16] .

Chemistry.

Lithium is the lightest of the alkali metals (group Ia); the salts of this monovalent cation share some characteristics with those of Na^+ and K^+. Li^+ is readily assayed in biological fluids and can be detected in brain tissue by magnetic resonance spectroscopy[17] . Traces of the ion occur normally in animal tissues, but it has no known physiological role. Lithium carbonate and lithium citrate currently are used therapeutically.

Pharmacological Properties

Therapeutic concentrations of lithium ion (Li^+) have almost no discernible psychotropic effects in normal individuals. It is not a sedative, depressant, or euphoriant, and this characteristic differentiates Li^+ from other psychotropic agents[15].The precise mechanism of action of Li^+ as a mood-stabilizing agent remains unknown, although many molecular and cellular actions of Li^+, as well as similarities of actions of other mood-stabilizing agents, including valproate, have been described.The main effect of lithium is probably to inhibit hydrolysis of inositol phosphate,therefore reducing the recycling of free inositol for synthesis of phophotidylinositides. These intracellular molecules are part of the transmembrane signaling system that is important in regulating intracellular calcium ion concentration which subsequently affects neurotransmitter release[18-20] .

Absorption

Water-soluble salts, such as chloride and sulphate,are rapidly and almost completely absorbed from the upper gastrointestinal tract, while the less soluble carbonate salt is absorbed more slowly[21].Absorption half-lives for standard- and sustained release forms of lithium carbonate is 0.78 ± 0.05 hours and 3.73 ± 0.37 hours, respectively[22].

Distribution

Li^+ initially is distributed in the extracellular fluid, then gradually accumulates in various tissues; it does not bind appreciably to plasma proteins. The concentration gradient across plasma membranes is much smaller than those for Na^+ and K^+. The final volume of distribution (0.7 to 0.9 liter per kilogram) approaches that of total body water and is much lower than that of most other psychotropic agents, which are lipophilic and protein bound. Passage through the blood-brain barrier is slow, and when a steady state is achieved, the concentration of Li^+ in the cerebrospinal fluid and in brain tissue is about 40% to 50% of the concentration in plasma[15].

Metabolism and Excretion

Lithium is not subject to metabolic transformation and is almost exclusively excreted via the kidney as a free ion. Similarly to sodium, it is able to freely cross the glomerular membrane. Eighty percent of lithium is reabsorbed by passive diffusion in the proximal tubules. Its clearance varies from 0.6 to 2.4 L/h with high interindividual variability[21]. Both creatinine clearance and bodyweight are important factors in predicting lithium clearance[23].

The plasma half life of lithium is dependent on the Volume of distribution, clearance and duration of lithium therapy [21]. Mean plasma half life of lithium ranged from 16 to 30 hours in subjects with normal renal function[22, 24-28].

Approximately 95% of a single dose of Li^+ is eliminated in the urine. From one- to two-thirds of an acute dose is excreted during a 6- to 12-hour initial phase of excretion, followed by slow excretion over the next 10 to 14 days. With repeated administration, Li^+ excretion increases during the first 5 to 6 days until a steady state is reached between ingestion and excretion. When therapy with Li^+ is stopped, there is a rapid phase of renal excretion followed by a slow 10- to 14-day phase. Since 80% of the filtered Li^+ is reabsorbed by the proximal renal tubules, clearance of Li^+ by the kidney is about 20% of that for creatinine, ranging between 15 and 30 ml per minute. This rate is somewhat lower in elderly patients (10 to 15 ml per minute). Less than 1% of ingested Li^+ leaves the human body in the feces, and 4% to 5% is secreted in sweat. Li^+ is secreted in saliva in concentrations about twice those in plasma, while its concentration in tears is about equal to that in plasma. Since the ion also is secreted in human milk, women receiving Li^+ should not breast-feed infants[15].

Influence of Intrinsic Factors on Lithium Pharmacokinetics

1. Age

Elderly patients (usually aged>65 years) require lower doses than younger adults to achieve a desired steady state plasma concentration[23] .

2. Renal disease

Renal clearance of lithium is decreased in patients with abnormal renal function therefore the risk of lithium intoxication in such patients are increased[21]. In severe renal insufficiency, the contraindication is definite and absolute[29].

3. Obesity

Lithium clearance was significantly greater for obese subjects than for the control group. The steady-state volume of distribution for the obese group was also significantly less than that for the control group.

4. Pregnancy

Lithium ion passes through the placental barrier[30]. It is recommended to balance the expected benefit of lithium versus the fetal risk[31].Lithium clearance increases by 30–50% in last month of pregnancy and at the time of delivery, clearance of the drug falls to pre-pregnancy levels. Therefore doses must be adapted and therapeutic drug monitoring must be carried out more frequently[30] .

Management of Lithium Therapy

Lithium has narrow therapeutic range therefore its use must confirm some strict rules. Therapeutic monitoring is the basis for optimal use and dosing of lithium.

Lithium doses should be adjusted on the basis of the concentration in serum drawn preferably 12 hours(security interval 10–14 hours) after the last dose[29]. Serum concentrations at this time are in the'flat part' of the pharmacokinetic curve [32].In patients receiving once-daily administration, the serum concentration at 24 hours should serve as the control value.

Lithium efficacy is dose dependent and reliably correlates with serum concentrations. The optimal serum lithium concentration for preventing mania and depression in maintenance treatment is not well established[32]. Typically, it has been considered that lithium concentrations should be maintained between 0.6 and 1.0 mmol/L but some authors still favour 0.8–1.2 mmol/L [21].

Before starting treatment with lithium personal and familial medical history, especially with respect to thyroid function, previous heart disease and comedications has to be noted. Following investigations should be done before starting lithium-creatinine clearance,blood TSH, free thyroxine, calcium, phosphorus,sugar and electrolytes; pregnancy test if appropriate;ECG in patients aged >40 years; and EEG in patients with a previous history of seizures. Suitable Contraceptive method should be started in female patients[21] .

Lithium therapy is initiated in divided doses. In the presence of normal kidney function, a total daily dose of 1,200 to 1,800 mg of lithium carbonate generally produces an antimanic serum concentration of 0.8 to 1.2 mEq/L. Maintenance levels of 0.6 to 1 mEq/L can usually be attained with 900 to 1,200 mg daily[4] . Sustained-release preparations can be given in a single dose and, to simplify therapeutic monitoring and are best taken at night[29] .

In case of sustained –release preparations slower increase in plasma concentrations and lower maximum plasma concentration result in benefits with respect to adverse effects such as tremor, upper gastrointestinal cramping and nausea, rashes, cognitive dulling, urinary frequency and neuromuscular slowing[33].

Measuring serum lithium concentration is usually recommended after 1 week of commencing

Therapy[34].. As a general rule, blood samples should be taken 12 hours (10–14 hours) after the last drug intake. In case of sustained-release preparations, bearing in mind the later peak of serum lithium concentration,it has been advised that serum concentrations should be maintained within the upper range (0.8–1 mmol/L), rather than within the 0.6–0.8 mmol/L range recommended for conventional formulations[25].

The frequency of blood sampling is usually every 1–2 weeks until a desirable serum concentration is achieved, then every 2–3 months for the first 6 months of treatment, and at least every 6–12 months thereafter[35].

After any change in dosage, and when there has been intercurrent disease or any change in co-medication, serum lithium concentration should be checked and adjusted to the desired range, after steady state has been reached [34,35].

During maintenance therapy, patients must be evaluated clinically, lithium levels determined periodically, and appropriate laboratory tests performed at regular intervals. Monthly visits are common early in treatment if the clinical course is uncomplicated. Patients who have been stable for extended periods may be seen at intervals of 3, 4, or even 6 months[4].

Serum creatinine must be measured every 6–12 months in order to check renal function. Measurement of serum calcium every 6–12 months and of thyroid status (TSH initially, every 6 months for the first 3 years, then once a year) must be performed[21].

Laboratory Monitoring

Lithium has been measured in virtually every body fluid[4]. Serum analysis is the most useful and is used in clinical practice[4,37]. Concentrations considered to be effective and acceptably

safe are between 0.6 and 1.25 mEq per liter. The range of 0.9 to 1.1 mEq per liter is favored for treatment of acutely manic or hypomanic patients. Somewhat lower values (0.6 to 0.75 mEq per liter) are considered adequate and are safer for long-term use for prevention of recurrent manic-depressive illness. Some patients may not relapse at concentrations as low as 0.5 to 0.6 mEq per liter, and lower levels usually are better tolerated[15]. The concentration of lithium can be determined by flame emission photometry,atomic absorption spectrometry or electrochemically using an ion selective electrode[37].

A study done on 20 patients on lithium carbonate investigated the correlations between serum and mixed saliva and parotid fluid lithium levels. High correlations from 0.90 to 0.95 were found between serum and saliva and serum and parotid fluid. A reproducible constant relationship of 2.26±10% was noted between mixed saliva and serum level. The study recommended that to use saliva most effectively in determining lithium levels the ratio between saliva and serum lithium levels should be calculated for each individual and the obtained ratio should be used to calculate subsequent determination for that individual[7].

A study was done on ten patients receiving lithium carbonate for affective disorder. 24 samples of serum and saliva were collected from them. Correlation coefficient between serum and salivary lithium was +0.88($P<0.01$).the salivary and serum lithium ratio was 2.22±0.5. The study found good degree of stability for saliva lithium levels. A therapeutic range of saliva lithium between 1.5-3 mEq/L was suggested to adjust lithium dosage[8].

The above two studies found high correlation between serum and saliva lithium levels but there was a high individual variation of paired results .To circumvent this problem a study was done using a naturally occurring marker in saliva and serum along with serially paired samples in individual patients. Thirty synchronous samples of serum and saliva was collected from thirty patients on lithium carbonate and lithium estimation was corrected for

potassium(natural marker) potassium . Three patients were monitored with a series of 7,6 and 8 paired results. Lithium was estimated using flame photometer. In 30 patients correlation coefficient was 0.71($P<0.001$). In series of paired samples correlation coefficient was 0.85($P<0.01$), 0.80($P<0.05$) and 0.63($P<0.1$).It was found that saliva levels variation for fixed serum levels were unacceptably large. The study showed satisfactory correlation from zero through the therapeutic range of lithium but was sceptical about such a correlation in excessive levels of serum lithium[38].

A study was done on 95 patients in india who received lithium carbonate. 309 samples of serum and saliva were collected. Flame photometer was used for lithium estimation. A positive and highly significant correlation was found[39].

A study was done to evaluate the relationship among plasma,RBC and saliva lithium levels using atomic absorption spectrophotometry. 30 synchronous samples of blood and saliva was taken from 9 subjects. High correlation($r=0.569, P<0,001$) was found between serum and salivary lithium levels. Correlation between RBC lithium and saliva was less compared to serum and saliva levels. The study found high interindividual and intraindividual correlation[40].

In 11 manic-depressive outpatients on chronic lithium therapy the relationship between serum and saliva lithium was studied. Trough serum and saliva lithium levels were measured using atomic absorption spectrometry every 3 or 4 weeks during clinic visits for a period of atleast 16 weeks. Clinical status of patient was rated according to level of mania. Intersubject analysis showed poor correlation($r=0.5$) intrasubject correlation was strong($r=0.72-0.94$). The study suggested that patient's saliva can be used to estimate serum lithium levels in clinically stabilised manic-depressive patients on prophylactic lithium therapy. In poorly stabilised patients serum concentration should be monitored[41].

Salivary and serum lithium concentrations were measured using atomic absorption spectrophotometry simultaneously in 118 manic-depressive patients. Lithium concentration in saliva was 2.24 ±0.35 times higher than in serum. An equation to calculate serum lithium concentration from salivary measurements was derived: Li serum = 0.36 Li saliva + 0.13. Psychotropic drugs had no effect on the salivary:serum ratio. Eighteen patients were followed for several weeks. A significant correlation coefficient($P<0.05$) between salivary and serum lithium concentrations was found in thirteen of the eighteen patients studied[42].

140 synchronous samples of serum and saliva was collected from 28 patients undergoing lithium therapy and was estimated using flame photometer. The mean saliva/serum ratio was calculated from 120 synchronous samples from 24 patients was found to be 2.68. Regression line equation calculated for same population came out to be $Y=0.325+0.22X$. Predictive value of saliva lithium was tested by applying this regression equation and the population mean ratio on 20 samples from the next 4 patients. Prediction was also tried in 24 patients who had given more than 3 synchronous samples using individual mean saliva/serum ratio. An individual's mean was calculated from the initial 3 synchronous samples and predictive value of saliva was tested on subsequent samples in the same patient by using his mean ratio. This method was found to be better than predicting on the basis of population figures[43].
The usefulness of salivary lithium values for monitoring long-term lithium prophylaxis was studied in 60 patients on lithium therapy. A total of 99 pairs of saliva and serum samples were obtained, and analysed using flame photometer. The correlation between serum and salivary lithium levels ($r = 0.73$) was found to be significant at the 1% level. The ratio of salivary serum levels to that of serum was found to range from 1.77 to 6.68. The ratio of 51% of the samples was between 3 and 3.99. The study suggested that it is important to identify

the subgroup of patients who show better correlation of salivary and serum lithium levels and use each individual's ratio to monitor only his or her lithium therapy[44].

The salivary composition and flow rate of 78 patients with primary affective disorders and of 49 healthy volunteers were examined. The former were divided into two groups: Group 1(n=57) patients receiving lithium carbonate and psychoactive drugs, and Group 2(n=21)patients receiving psychoactive drugs only. A significant correlation between salivary and serum lithium was found in patients on chronic lithium therapy. The use of saliva analysis for monitoring lithium dosage was recommended by this study[45].

Serum and saliva lithium levels were simultaneously determined using flame photometer in 14 prepubertal children being treated with lithium carbonate. Saliva and serum lithium levels were strongly correlated. The concentration of lithium in saliva was almost two times (1.82) that in serum. There was more variability in saliva levels than in serum levels. It was possible to predict serum levels from saliva levels using regression analysis. If dose was added to saliva as a second predictor variable, accuracy of the prediction was increased[46].

A study was been carried out to find out the reasons for the wide variations observed in the correlation between serum and saliva lithium levels. Serum/saliva lithium levels were monitored using flame photometer in 10 individuals on 6 occasions. The correlation coefficient (r) varied within individuals from 0.19 to 0.91 ($p < 0.025$) whereas when all the individuals were considered simultaneously, it was 0.59 ($p < 0.005$). It was concluded that prediction of serum lithium levels from salivary lithium levels would only be possible in

those individuals showing good correlation. The variability in this correlation could be mainly due to inter-individual variations in the particular sample of individuals studied[47].
In a study one tablet containing 755 mg of lithium tryptophanate (10.8 mEq of lithium) was administered to eight healthy volunteers. The main pharmacokinetic parameters for the group of subjects were estimated. Pharmacokinetic parameters (mean ± SD) from plasma and saliva were respectively: half life (t1/2) 17 ± 6 vs. 21.8 ± 14 h; mean residence time 23.7 ± 7.4 vs. 24.4 ± 15.3 h; total clearance 30.6 ± 9.3 vs. 28.6 ± 6.2 ml/h/kg; and apparent volume of distribution 0.71 ± 0.20 vs. 0.84 ± 0.37 L/kg. Although the mean pharmacokinetic parameters in plasma and saliva were similar, there was no significant correlation between the calculated parameters in the individual subject ($p > 0.05$). The study concluded that usefulness of monitoring salivary levels of lithium is questionable[48].

Within- and between-subject variability in serum and salivary lithium concentrations in nine psychiatric inpatients on stable drug regimens undergoing therapy was assessed using criteria for determining biologic variation. Estimation of lithium was done using flame photometer This allows separation of analytic from other measures of variance. There were marked differences in inter- and intra-subject variance for serum and salivary lithium concentrations for serum/salivary ratios. these variances were greater for salivary lithium than for serum concentrations. The results were used to assess analytical performance, the usefulness of the therapeutic range, and the reference change interval.the study found that despite greater variance for salivary concentrations, predicted serum concentrations from predetermined serum/saliva ratios were in good agreement with actual concentrations in most subjects[49].

A study was done to check if dialysis of saliva improves accuracy of saliva lithium determinations. Estimation of lithium was done using lithium sensitive electrode. Saliva has

two major components: the aqueous and the mucopolysaccharide portions. Since Li is likely to distribute only in the aqueous fraction, saliva was dialysed through a 3000 Da filter to isolate the aqueous component and determine the Li level in it. Lithium levels in the dialyzed saliva agreed more closely with plasma levels ($r = 0.901$, $p < 0.001$) than did whole saliva ($r = 0.775$, $p = 0.012$). the study concluded that dialysis of saliva may contribute to more accurate saliva Li levels[50].

In a study lithium ions concentration in human serum and saliva was determined using dry-slide technology Vitros 250 Analyser (Ortho Clinical Diagnostic) and atomic absorption spectrometry Perkin Elmer 403 (AAS). lithium ions were analysed in 100 serum and saliva specimens of patients after oral administration of lithium carbonate (3 x 300 mg) Jadran, Galen Laboratory Rijeka. Saliva and blood were taken 2 and 12 hours after the last dose. At the same time lithium ions at samples of blood and saliva were determined with both methods which showed high level of correlation. The mean difference of lithium ions between saliva and serum was statistically significant for $p<0.05$ using t student test. saliva constant of elimination Kel = 0.02(-1)h and elimination half life (t(1/2)) was t(1/2)=34.6 h. For serum t(1/2)= 24 h which means that lithium ions elimination is slower from saliva than from serum. That is the reason why probably concentration at saliva is higher then at serum. Lithium elimination is two compartment pharmacokinetic model where important part of compartment are saliva and salivary glands. The study concluded at a certain point in medical treatment it could be expected to use controlled determination of lithium ions in saliva with serum as control[51].

The relationship between the plasma concentration, saliva concentration and urinary excretion rate of lithium was investigated in a study and the possibility of using the saliva concentration or the urinary excretion rate for monitoring dosage was considered. The results in the study support the idea that saliva concentration of lithium could be useful in

monitoring dosage but, there are many difficulties to be overcome in using the urinary excretion rate of lithium due to inter-subject variation, intra-subject variation during the night and circadian variation in the renal excretion of lithium[52].

MATERIALS AND METHODS

Source of the data

The present study is a cross sectional study to compare serum lithium, salivary lithium and urinary lithium in patients on lithium carbonate.

Study period

The study was undertaken between December 2009 to March 2011.

SAMPLE SIZE

Sample size was determined to be 50.With α=0. 01 and β=0.2 sample size required to demonstrate correlation 'r'=0.6 minimum sample size of 33 is required.

STUDY POPULATION.

Consisted of in patients and out patients attending the Department of Psychiatry of KLE'S Dr. Prabhakar Kore Charitable Hospital and Medical Research Centre, Belgaum between the ages of 18-50 years who are on lithium carbonate for more than a week.

CRITERIA FOR SELECTION OF THE STUDY GROUP

Inclusion Criteria**:**

1) Patients who are on lithium carbonate therapy for at least a week.
2) Age: 18-50 years.
3) Renal function test within normal limits.
4) Urine analysis within normal limits.

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Exclusion Criteria:

1) Factors affecting normal salivary secretion.
2) Pregnancy
3) Age <18years and >50 years
4) Dehydration
5) Drugs affecting lithium pharmacokinetics :Nonsteroidal anti-inflammatory drugs, Diuretics, angiotensin-converting enzyme inhibitors, angiotensin IIreceptor type -1 antagoinists, metranidazole, sodium bicarbonate, propranolol.

APPROVAL FROM THE AUTHORITIES:

Permission to conduct the study was obtained from all the concerned authorities viz.

1. Institutional ethics committee on human subjects research of Jawaharlal Nehru medical college, Belgaum.
2. Director –clinical services for medical director and chief executive of KLE'S Dr.Prabhakar Kore Hospital and Medical Research Centre, Belgaum.(Annexure II)
3. University science instrument centre (USIC), Shivaji university, Kolhapur.

OBTAINING INFORMED CONSENT

Informed consent was taken from all the participants in the study.(annexure III)

SCHEDULING:

This study was carried out for a period of 14 months. It was undertaken during December 2009 to March 2011.

PILOT STUDY

Pilot study was conducted after taking serum, saliva and urine from 3 patients to assess the feasibility of the study and to standardise methods of sample collection.

Patient information

A structured proforma was used to collect sociodemographic and clinical information about the study participants.(annexure IV)

COLLECTION OF SAMPLE

Patient preparation:

Blood, saliva and urine samples were collected in the morning (12hrs after the last dose of lithium carbonate) before the patient consumed breakfast and the morning dose of lithium. Samples were collected under aseptic precautionary measures.

Serum

5 ml of blood was collected in a plain non vacuum tube and was allowed to stand in room temperature till clot was formed. Serum was separated within one hour of venipuncture by centrifuging the tubes at 3,000 r.p.m for 10 minutes. Serum was pipetted out into a sterile eppendorf tubes.

Saliva

Saliva was collected in a sterile container after asking the patient to rinse mouth with water. Subjects were asked to collect saliva in the mouth and then were asked to spit into the container till adequate amount was collected. Saliva was centrifuged to remove mucus.

Urine

Mid stream urine was collected in sterile container.

Sample collection was supervised by nursing staff. All the 3 samples were collected within 20 minutes.

All samples were stored in non vacuum sterile tubes at -20 ^{0}C till further analysis.

STATISTICAL TESTS USED

The following methods of statistical analysis have been used in this study. Data was entered in Microsoft excel and analyzed using SPSS (version 17).

Mean and standard deviation of serum lithium, salivary lithium and urinary lithium was computed and compared by using paired‘t’ test. Pearson’s correlation coefficient and Linear regression analysis was done to between three parameters was computed to know the strength of association between them was done.

MATERIAL USED IN THE STUDY

1. Non vacuum plain tube.
2. 5 ml Disposable syringe.
3. Tourniquet.
4. Sterile container.
5. Automated pipettes and tips.
6. Deionised water.
7. Microcentrifuge tubes.
8. Gloves.

INSTRUMENTS USED IN THE STUDY

1. Centrifuge machine.

2. Flame atomic absorption spectrophotometer (Perkin Elmer Analyst 300).

ESTIMATION OF LITHIUM

Lithium estimation in serum, saliva and urine was done using Atomic Absorption Spectrophotometer (Perkin Elmer Analyst 300). Permission to use the instrument for lithium analysis was obtained from head of University science instrument centre (USIC), Shivaji University, Kolhapur.

SAMPLE PREPARATION

Serum

serum sample was diluted to 1:10 or 1:5 with deionized water(1, 2) . The dilution ratio was adjusted to ensure that concentrations fall within a suitable absorbance range.

Saliva

saliva sample was diluted to 1:5 with deionized water . The dilution ratio was adjusted to ensure that concentrations fall within a suitable absorbance range.

Urine

Urine sample was diluted to 1:50 with deionized water(1, 2). The dilution ratio was adjusted to ensure that concentrations fall within a suitable absorbance range.

Samples were taken in batches of twenty for analysis in flame atomic absorption spectrophotometer.

PRINCIPLE OF FLAME ATOMIC ABSORPTION SPECTROPHOTOMETER

Every element has a specific number of electrons associated with its nucleus. The normal and most stable orbital configuration of an atom is known as the "ground state." If energy is applied to an atom, the energy will be absorbed and an outer electron will be promoted to a less stable configuration known as the "excited state." Since this state is unstable, the atom will immediately return to the "ground state," releasing light energy.

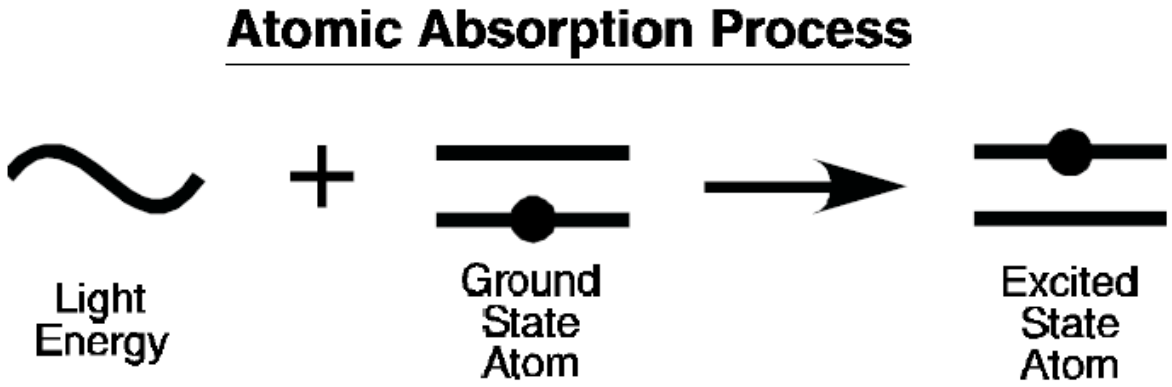


Figure 1: Atomic absorption process.

The "ground state" atom absorbs light energy of a specific wavelength as it enters the "excited state." As the number of atoms in the light path increases, the amount of light absorbed also increases. By measuring the amount of light absorbed, a quantitative determination of the amount of analyte can be made. The use of special light sources and careful selection of wavelengths allow the specific determination of individual elements.

ATOMIC ABSORPTION INSTRUMENTATION

There are five basic components of an atomic absorption instrument:

1. The light source that emits the spectrum of the element of interest
2. An "absorption cell" in which atoms of the sample are produced
3. A monochromator for light dispersion

4. A detector, which measures the light intensity and amplifies the signal.

5. A display that shows the reading after it has been processed by the instrument electronics[37].

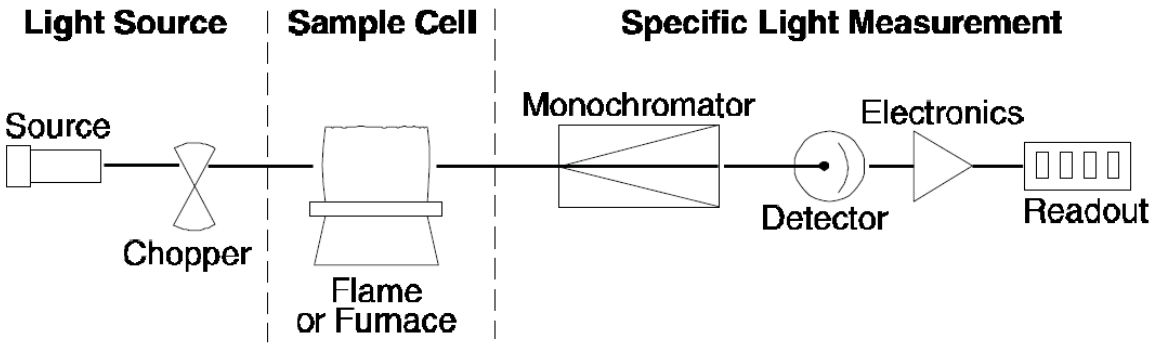


Figure 2: Schematic representation of flame Atomic absorption process.

PROCEDURE

Standard

Appropriate standards are prepared by diluting the lithium stock solution provided by the manufacturer.

Blank

Deionized water is used for blank solution.

ANALYSIS

Instrument was set in standard condition for lithium analysis.

Blank was aspirated first followed by a suitable standard and then the sample. Lithium standard was read after every five sample.

The readings were recorded in p.p.m. and then converted to meq/L.

PHOTOGRAPHS

Photograph 1: Flame atomic absorption spectrophotometer (Perkin Elmer Analyst 300)

Photograph 2: Investigator performing analysis of lithium on Flame atomic absorption spectrophotometer (Perkin Elmer Analyst 300).

RESULTS

A cross sectional study was done to compare serum lithium, salivary lithium and urinary lithium in patients on lithium carbonate to evaluate the possibility of replacing serum with salivary or urine lithium estimation for monitoring of patients on lithium carbonate.

The data obtained from the study was compiled, tabulated and subjected to statistical analysis. The results are presented here under the headings of the various parameters considered for the study.

Table 1:Distribution of age, serum lithium, salivary lithium, urine lithium and dose of lithium carbonate in 50 study subjects.

The mean age of study participants was 35.10±10.63. Their Mean serum lithium was 0.75±0.25 mEq/L, mean salivary lithium was 1.91±0.80 mEq/L and mean urine lithium was 7.16±4.84 mEq/L. Mean dosage of lithium carbonate prescribed to the subjects was 820±101.01 mg.

Table 2: :Distribution of Age, serum lithium, salivary lithium, urine lithium and dose of lithium carbonate in male and female.

The number of female participants were 18 and male participants were 32.The mean age of male participants was 36.50±10.50 years and that of female participants was 32.61±10.69 years. There was no statistically significant difference between the mean ages of males and females (p=0.22).

In males mean serum lithium was 0.75±0.2 mEq/L, mean salivary lithium was 1.91±0.80 mEq/L and mean urine lithium was 7.53±5.26 mEq/L. Mean dosage of lithium carbonate prescribed to the subjects was 806.25±94.82 mg.

In females mean serum lithium was 0.76±0.23 mEq/L, mean salivary lithium was 1.85±0.74 mEq/L and mean urine lithium was 6.50±4.06 mEq/L. Mean dosage of lithium carbonate prescribed to the subjects was 844.44±109.66 mg.

There was no statistically significant difference between the mean serum lithium (p=0.91) , salivary lithium(p=0.67), urine lithium(p=0.47) and dosages of lithium carbonate (p=0.2) between males and females.

Table 3:Correlation analysis of serum and saliva lithium levels

Correlation between serum lithium and salivary lithium was statistically significant (r=0.695, p <0.001). The serum lithium and salivary lithium was more strongly correlated in females (r=0.770, p <0.001) when compared to males (r=0.665, p <0.001).

Table 4(Graph 1):Linear regression analysis for serum and salivary lithium correlation.

Linear regression analysis for serum and salivary lithium was done with serum lithium as dependent variable, statistically significant correlation was found (r=0.695). The equation for calculating serum lithium from saliva lithium measurements was derived from the graph 1: Y=0.332+0.221X (Y=serum lithium concentration, X=salivary lithium concentration).

Table 4 (Graph 2): Linear regression analysis for serum and salivary lithium correlation for females

In females linear regression analysis for serum and salivary lithium was done with serum lithium as dependent variable, statistically significant correlation was found (r=0.770). The equation for calculating serum lithium from saliva lithium measurements was derived from the graph 2:Y=0.355+0.271X(Y=serum lithium concentration, X=salivary lithium concentration).

Table 4 (Graph 3): Linear regression analysis for serum and salivary lithium correlation for males

In males linear regression analysis for serum and salivary lithium was done with serum lithium as dependent variable, statistically significant correlation was found (r=0.665).

The equation for calculating serum lithium from saliva lithium measurements was derived from the graph 3: Y=0.259+0.204X(Y=serum lithium concentration, X=salivary lithium concentration).

Table 5:Correlation analysis of serum and urine lithium levels.

Correlation between serum lithium and urine lithium was not statistically significant (r=0.234, p =0.102). In males correlation between serum lithium and urine lithium was (r=0.319, p =0.08) in females (r=0.022, p =0.932).

Table 6(Graph 4):Linear regression analysis for serum and urine lithium correlation.

Linear regression analysis for serum and urine lithium was done with serum lithium as dependent variable. Correlation was found to be statistically not significant (r=0.234). Graph 4 showing serum versus urine lithium measurements shows a large deviation.

Table 1: Distribution of age, serum lithium, salivary lithium, urine lithium and dose of lithium carbonate in 50 study subjects.

Sl.No	Variable	Mean±S.D
1	Age (years)	35.10±10.63
2	Serum lithium (mEq/L)	0.75±0.25
3	Salivary lithium (mEq/L)	1.91±0.80
4	Urine lithium (mEq/L)	7.16±4.84
5	Dose of lithium carbonate (mg)	820±101.01

Table 2: Distribution of age,serum lithium,salivary lithium,urine lithium and dose of lithium carbonate in male and female.

Variable	Male (n=32)	Female (n=18)	t value	p value
Age	36.50±10.50	32.61±10.69	1.25	0.22
Serum lithium(mEq/L)	0.75±0.26	0.76±0.23	-0.12	0.91
Salivary lithium(mEq/L)	1.91±0.80	1.85±0.74	0.43	0.67
Urine lithium(mEq/L)	7.53±5.26	6.50±4.06	0.72	0.47
Dose of lithium carbonate (mg)	806.25±94.82	844.44±109.66	-1.29	0.20

Table 3:Correlation analysis of serum and saliva lithium levels.

	r	r^2	S.E	p value
Total study Subjects(n=50)	0.695	0.47	0.18	**<0.001***
Male (n=32)	0.665	0.42	0.20	**<0.001***
Female (n=18)	0.770	0.57	0.16	**<0.001***

*-statistically significant

Table 4:Linear regression analysis for serum and salivary lithium correlation.

	Unstandardized Coefficients		Standardized Coefficients	t	p value.	95.0% Confidence Interval for B	
	B	Std. Error	Beta			Lower Bound	Upper Bound
(Constant)	0.332	0.069		4.835	**<0.001***	0.194	0.470
Salivary Lithium	0.221	0.033	0.695	6.688	**<0.001***	0.155	0.288

Dependent Variable: Serum Lithium

*-statistically significant

Table 5: Correlation analysis of serum and urine lithium levels.

	r	r^2	S.E	p value
Total study subjects(n=50)	0.234	0.04	0.25	0.102
Male (n=32)	0.319	0.07	0.26	0.08
Female (n=18)	0.022	-0.06	0.25	0.932

Table 6:Linear regression analysis for serum and urine lithium .

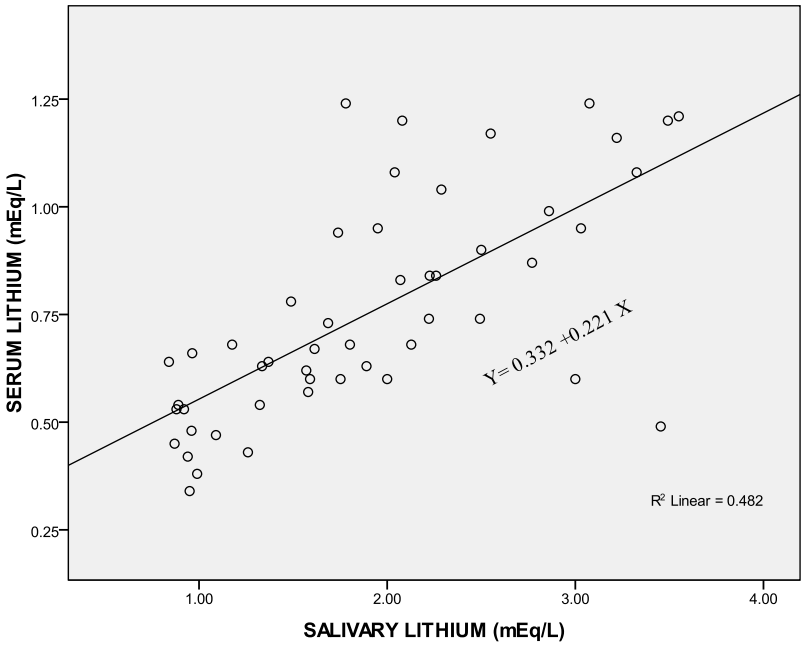
Model	Unstandardized Coefficients		Standardized Coefficients	t	p value
	B	Std. Error	Beta		
(Constant)	0.668	0.064		10.459	**<0.001***
Urine Lithium	0.012	0.007	0.234	1.667	0.102

Dependent Variable: Serum Lithium

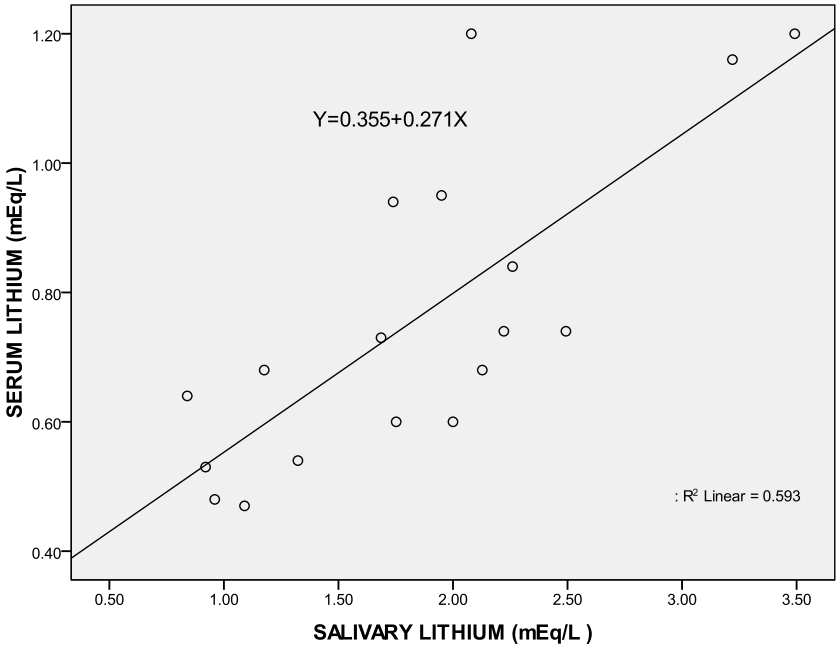
*-statistically significant

GRAPHS

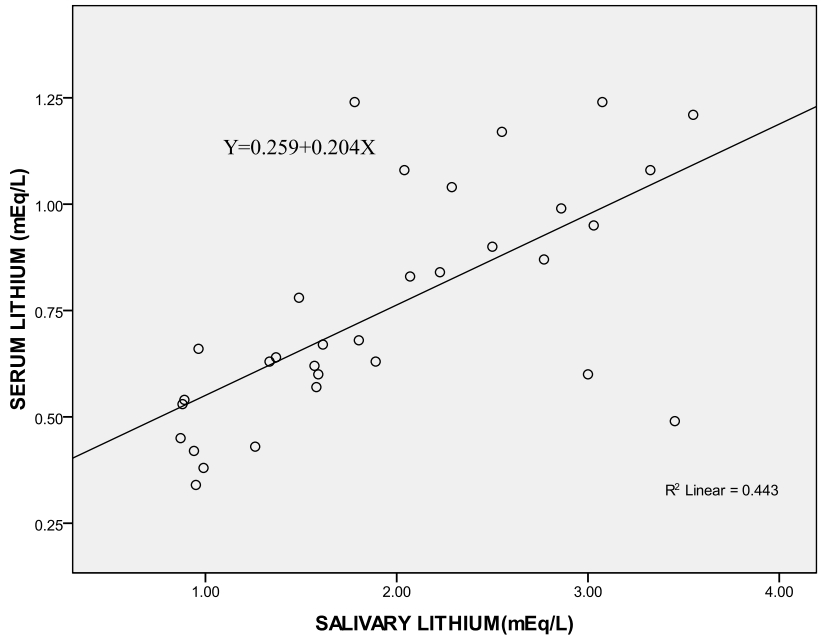
Graph 1: Linear regression analysis of serum versus salivary lithium of 50 study participants



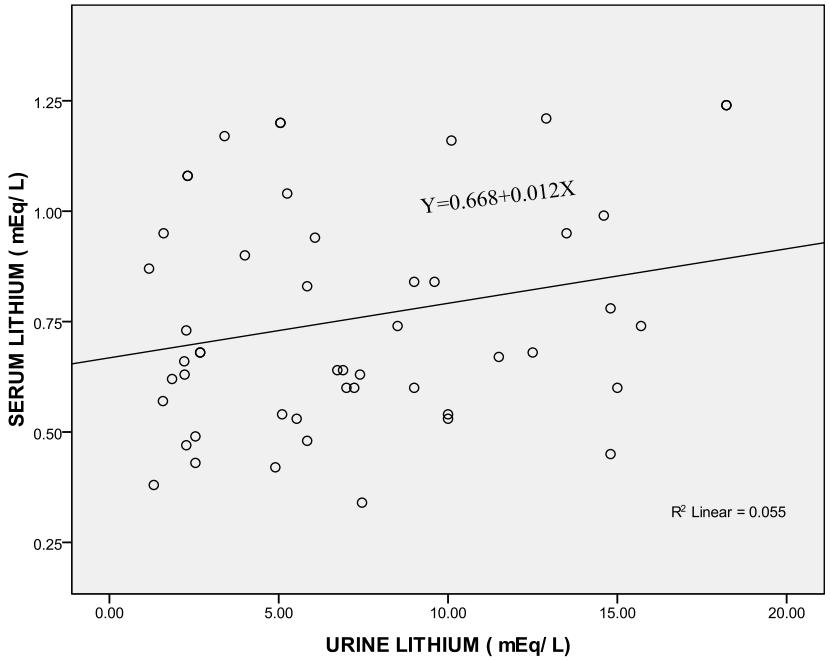
Graph 2: Linear regression analysis of serum versus salivary lithium in female participants.



Graph 3: Linear regression analysis of serum versus salivary lithium in male participants.



Graph 4: Linear regression analysis of serum versus urine lithium of 50 study participants



DISCUSSION

Lithium carbonate is used in the treatment of psychiatric and non psychiatric disorders[53]. Lithium has an established use in treatment of acute mania, prophylaxis of bipolar episodes and augmentation therapy for severe, refractory depression[10]. The difference between toxic and therapeutic dose of lithium carbonate is narrow. Lithium therapy must be guided by monitoring plasma concentration it has been considered that lithium concentrations should be maintained between 0.6 and 1.0 mmol/L[21]. At plasma concentrations above 1.5mmol/L is associated with signs of intoxication mainly affecting gastrointestinal and neurological system. Frank toxicity is associated with plasma concentration greater than 2mmol/L which is an acute medical emergency. Therefore when patients are on lithium strict monitoring of serum levels are done since the amount of lithium required to achieve a therapeutic response depends on concentration of ion in serum which reflects its distribution throughout the body rather than on actual amount required per day[7] .

The frequency of blood sampling for lithium monitoring is usually every 1–2 weeks until a desirable serum concentration is achieved, then every 2–3 months for the first 6 months of treatment, and at least every 6–12 months thereafter[35]. These series of lithium monitoring requires repeated drawing of blood samples.

To monitor health status, disease onset and progression, and treatment outcome noninvasively is a most desirable goal in the health care delivery and health research. There are three prerequisites necessary to reach this goal

1. A non-invasive method for collecting biological samples.
2. Specific biomarkers associated with health or disease.
3. A technology platform to rapidly discriminate the biomarkers[54].

Diagnostic tests based on fluid generally use blood and urine and less frequently the esoteric fluids such as saliva, sweat, and tears. Saliva's popularity has suffered because it

lacks "the drama of blood, the sincerity of sweat and the emotional appeal of tears.'" Sweat and tears, however, are difficult to obtain in sufficient quantities for routine testing, and urine will always lack the charisma of the other fluids. Saliva, by default, therefore becomes the most favoured alternative to blood[55].

Most molecules present in blood or urine can also be detected in salivary secretions. Their concentrations in saliva are usually one tenth to one thousandth of those in blood. Although highly sensitive methods of detection are required, technical advances have made this feasible. Studies of the correlation between concentrations in blood and saliva have found examples of excellent concordance (ethanol, cortisol, theophylline, and antibodies to HIV)[55].

Lithium prophylaxis of bipolar disorder is prolonged with the duration of treatment extending over several years. The narrow therapeutic index of lithium combined with long duration of treatment necessitates that adequate blood levels be maintained[44]. The use of saliva or urine collection versus blood collection has many advantages like ease of collection, safety, acceptance by the patient and cost effectiveness.

Totally valid comparisons could not be done between our study and other studies in literature due to paucity of researches devoted to finding correlation between serum, salivary and urine lithium. Also there was a wide variation with respect to age group, study design methodologies employed and instruments used. However a sincere attempt is being made to compare and discuss to the extent possible and permissible.

In the present study all the study subjects were on lithium carbonate (Tablet. Lithosun SR, Sun pharmacy). The mean dose was 820±101.01mg/day (range 600-1200mg). Dose of lithium carbonate was in the range of 900-1800mg/day(eskalith,smith kline & French)in study done by Rosman.A.W et al(41);300-1800mg/day in study done by Ben-Aryeh. H et al[42], mean 1050mg/day with range 500-1600mg/day in study done by Mckeage.M.J[56].

Estimation of lithium can be done by flame emission photometry, atomic absorption spectrometry and ion selective electrode[37]. Atomic absorption spectrophotometer is superior to colorimetric and flame emission spectrophotometric methods for measuring lithium in serum and urine because of its relative lack of susceptibility to interfering substances[57]. Therefore in the present study lithium estimation was done by using atomic absorption spectrometry. Flame emission photometry was used for lithium determination in some studies [7, 8, 38, 39, 42-44, 46, 47, 49]. Atomic absorption spectrometry was used in lithium estimation in earlier studies [40, 41, 52, 56]. Ion selective electrode was used in lithium estimation in study done by El-Mallakh RS et al[50].

Saliva lithium concentration was more than serum lithium concentration. The mean ratio of salivary lithium to serum lithium was 2.57±0.91. Similar results were observed in previous studies[7, 8, 41, 43, 47].Higher ratio of 3.64±1.04 was reported by Khare CB et al[44]. while Weller EB et al[46] found lower ratio 1.82±0.29 when compared to the present study. The reason behind getting a higher saliva : serum lithium ratio might be that we employed unstimulated saliva for estimation. Similar technique was used by Khare C B et al[44] . Unstimulated saliva was preferred over stimulated saliva in this study as this unstimulated flow, is what is secreted by the salivary glands in normal physiological conditions. Lithium in unstimulated saliva was found to be directly proportional to serum concentration. lithium in stimulated saliva tends to be a negative function of flow rate[58].

Mean serum lithium was 0.75±0.25 mEq/L in the present study. Lower mean serum lithium was found to be by Sankaranarayanan A et al(0.59±0.19 mEq/L) and by Khare CB et al 0.65±0.24 mEq/L[44, 47] .Higher levels were found by Weller EB et al(1±0.2 mEq/L) and by Rosman AW et al(0.91±0.27 mEq/L)[41, 46] .These differences can be attributed to differences in pharmacological preparations, dosage and duration of lithium therapy.

Mean salivary lithium was 1.91±0.80 mEq/L in the present study. Salivary lithium concentration was found to be 1.56 mEq/L by Neu C et al[7] , 1.37±0.97 mEq/L[47] by Sankaranarayanan A et al[47] which was lesser than the present study. Higher saliva lithium was found by some other studies. The slight differences which were found can be ascribed to differences in saliva collection technique and dosage of lithium.

In the present study Correlation between serum lithium and salivary lithium was statistically significant (r=0.695, p <0.001). Similar correlations were found in studies done by Sankaranarayanan A et al[47] (r=0.59, p <0.005), Khare CB et al[44] (r=0.73) and Nataraj G et al [43] (r=0.71, p <0.001)[43, 44, 47] . Higher correlations were found in other studies [7, 8, 45, 50] . Lower correlations were found in studies done by Prakash,R.S et al (r=0.41, p<0.001) and Rosman AW et al(r=0.5) [39, 41].

The linear regression equation derived was: **Serum lithium = 0.332 + 0.221 X Salivary lithium**. This equation was utilized to calculate the serum lithium levels from salivary lithium levels measured. The correlation between the calculated and the measured serum lithium was highly significant(r=0.695, p<0.001).Our results support the assumption of several previous reports[7, 8, 42] that recommend salivary lithium measurements for monitoring serum lithium. Other finding in studies done by Sims A et al[38] and Mathew RJ et al[40] dispute the usefulness of salivary lithium for monitoring probably because of methodological differences in saliva collection and patient selection.

Several factors have been found to contribute to variability of salivary lithium concentrations. These include blood ion concentration, stimulation of salivary glands and

aspects of lithium administration such as dosage and duration of treatment. Another potential variable can be mucopolysaccharide content. El Mallakh RS et al[50] found that when all mucinous material is removed from the saliva by filtration the agreement between the lithium levels of ultrafilterate and plasma is stronger than when saliva is centrifuged and unfiltered supernatant is measured.

More correlation was found in females($r=0.770$, $p<0.001$) than in males($r=0.665$, $p<0.001$).Exact reason for this finding is not known.

Correlation of ($r=0.234$, $p= 0.102$) was found between serum lithium and urine lithium levels which had no clinical or statistical significance. There is scarcity of literature demonstrating any correlation between serum and urine lithium levels. In a study done by Kyroudis A et al [52] , a good intra -subject correlation between urinary excretion rate and plasma concentration was found. Intersubject variability might have accounted for a statistically insignificant correlation in our study which used inter-subject design. It can be deduced that there are many difficulties to be overcome in using the urinary excretion rate of lithium due to inter subject variation, intra-subject variation during the night and circadian variation in the renal excretion of lithium.

Rosman AW et al[41]suggest that one must use the linear regression equation derived from intrasubject not intersubject data. These intrasubject estimates of serum lithium concentrations can be used safely in the clinically stabilized manic patient on prophylactic lithium therapy. Although correlation found in our study was good and statistically significant, it was lesser than few other studies which employed intrasubject correlation.

CONCLUSION

The relationship found between serum and salivary lithium was statistically significant but not strong enough to allow saliva monitoring as a substitute of serum lithium estimation in patients on lithium carbonate therapy. Further studies are needed in this arena which employs intersubject as well as intrasubject correlation design, to establish salivary therapeutic monitoring as a viable option for patients on lithium carbonate therapy.

The relationship found between serum and urine lithium was not statistically significant therefore urine lithium estimation may not be a suitable alternative for monitoring lithium therapy.

SUMMARY

The present study is a cross sectional study to compare serum lithium, salivary lithium and urinary lithium in patients on lithium carbonate admitted in Department of Psychiatry of KLE'S Dr.Prabhakar Kore Hospital and Medical Research Centre, Belgaum.

Blood, saliva and urine samples were collected in the morning (12hrs after the last dose of lithium carbonate) before the patient consumed breakfast and the morning dose of lithium. Samples were collected under aseptic precautionary measures.

Estimation of serum, salivary and urine lithium was done using atomic absorption spectrophotometer. Data was entered into Microsoft excel and analysed using SPSS(version 17). Statistical tests of significance employed were Pearson's correlation coefficient,student 't' test and linear regression analysis.

Following are our observations:

1. There is a statistically significant correlation between serum and salivary lithium levels.($r= 0.695$, $p<0.001$)
2. More correlation in serum and salivary lithium levels was observed in females ($r =0.77$, $p=<0.001$) than in males ($r=0.665$, $p=<0.001$)
3. Correlation observed between serum lithium and urine lithium was not statistically significant.

Our study showed that there is a significant correlation between serum and salivary lithium levels and it calls for further studies to throw more light in this field of salivary lithium monitoring.

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Annexure I

Dr. K. K. SINGH
Scientist – F & Head
Division of Manpower Development

INDIAN COUNCIL OF MEDICAL RESEARCH
Ansari Nagar, New Delhi – 110029, India
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No.3/2/2010-2/PG-thesis-MPD-3
Dated: 06.08.2010

To,

Dr. Sindhu J Shetty,
Room No.122, New NRI Girls Hostel,
Nehru Nagar,
JN Medical College Campus,
Belgaum-590010.

Dear Dr. Shetty,

This is with reference to your application seeking financial assistance from the Council for MD/MS/DM/MCH dissertation thesis entitled **"A cross sectional study to campare serum lithium, salivary lithium and urinary lithium in patients on lithium carbonate".**

I am glad to inform you that Director General, ICMR, based on the recommendation of Expert Committee, has sanctioned a sum of Rs.25, 000/- (Twenty five thousand only) to you for providing an electronic and hard copy of your dissertation thesis to the Council.

I am enclosing herewith a copy of the detailed guidelines for this scheme and would appreciate that necessary information as per these guidelines may be provided to the undersigned enabling us to release the grant. This is to inform you that Rs.25,000/- will be disbursed to you in two installments. Initial amount of Rs.15,000/- after receipt of the **undertaking** as per the guidelines and remaining amount of Rs.10, 000/- on receipt of the electronic copy, hard copy and summary of work done of your dissertation thesis duly approved by the University/Institution along with one publication in a reputed Journal.

With best wishes,

Yours Sincerely,

(K.K.SINGH)

Copy to: Dr. P B Desai, Prof. & Head, Deptt. Of Biochemistry, J N Medical College, Belgaum-590010.

Annexure II

KLES
DR. PRABHAKAR KORE HOSPITAL
&
MEDICAL RESEARCH CENTRE
NEHRUNAGAR, BELGAUM-590010
KARNATAKA-INDIA

ಕೆ. ಎಲ್. ಇ ಸಂಸ್ಥೆಯ
ಡಾ. ಪ್ರಭಾಕರ ಕೋರೆ ಆಸ್ಪತ್ರೆ ಮತ್ತು
ವೈದ್ಯಕೀಯ ಸಂಶೋಧನಾ ಕೇಂದ್ರ
ನೆಹರುನಗರ, ಬೆಳಗಾವಿ–590010 ಕರ್ನಾಟಕ, ಇಂಡಿಯಾ

Phone : 0831-2473777 (16 Lines)
Fax : 0831-2470732
E-Mail : klehosp@satyam net.in
Website : http://www.kleshospital.org

REF. NO: KLES/PKHOSP/DCS/09-10/ 13867 DATE: 24/03/2010

To,

Dr. Sindhu J. Shetty
1 Year M.D. Bio-chemistry
Dept of Biochemistry
J.N.Medical College
Belgaum.

Sub: Permission to carry out Dissertation work.

1. Ref. to your application on above subject dt 24/02/2010 addressed to MD & CE of the hospital.

2. After perusal of protocol of study, review of literature, consent form and ethical clearance, you are permitted to carry out Dissertation work on "A cross sectional study to compare serum lithium, salivary lithium and urinary lithium in patients on lithium carbonate" in this hospital.

3. You will work under the guidance of Dr. N.M.Patil, Asso. Professor, Psychiatry and Dr. Anil Malleshappa, I/C Biochemistry Dept of the Hospital.

3. The hospital will not have any financial implications for your project.

Brig (Retd) Dr. Dinesh Prasad
Director – Clinical Services
for Medical Director &
Chief Executive.

Copy to:-

1.	Medical Director & CEO	-	Sir, for kind information.
2.	Dr. N.M.Patil Asso. Prof., Psychiatry	-	The candidate will work under your co-guidance and will have no financial implications.
3.	Dr. Anil Malleshappa I/C Bio-chemistry Lab	-	The candidate will work under your co-guidance and will have no financial implications.
4.	Dr. P.B. Desai Prof. & HOD Dept of Biochemistry		

ANNEXURE III

CONSENT FOR PARTICIPATION IN RESEARCH STUDY

Title: "**A cross sectional study to compare serum lithium, salivary lithium and Urinary lithium in patients on lithium carbonate".**

Principal Investigator: Dr. Sindhu.J.Shetty

Guide: Dr. P.B.Desai M.D.

We are requesting you to be a participant in the above said research at KLES Dr. Prabhakar Kore Hospital and MRC,Belgaum being conducted by Dr. Sindhu.J.Shetty, postgraduate student in the department of Biochemistry at J.N.Medical College, Belgaum.

I. **Research purpose:** Patients suffering from mood disorders are advised various medications. Lithium carbonate is one of the main drugs used. When the patient is on lithium therapy frequent serum lithium estimations are done because lithium can cause a lot of problems if levels are lower or higher than prescribed limits. Therefore for serum lithium estimation each time patient has to be pricked and blood has to be collected. I am trying to find out if salivary lithium or urinary lithium can be compared with serum lithium levels so that repeated pricks for drawing blood are avoided.

II.Procedures involved: If you agree to participate in this research you will be asked the relevant history and will be subjected to clinical examination. You will be requested to come in the fasting state the next day. You will be asked to give urine, saliva and blood. 10ml of blood will be collected by intravenous route by pricking a small blood vessel which may give rise to small amount of pain. Urine, saliva and blood will be collected from you and will be subjected to lithium estimation.

III. Risks and benefits: There are no risks involved in this procedure. If any complications arise during the procedure you will be treated in KLES Dr. Prabhakar Kore Hospital and MRC, Belgaum with the best of our knowledge and the availability of resources in the free hospital. There will be no compensation or payment for such medical treatment.

During the course of the study you will be informed of any significant new findings such as changes in the risks and benefits resulting from participation in the research.

IV. Privacy and confidentiality: The only people who will know that you are a research participant are members of the research team. No information provided by you or about you during the research will be disclosed to others without your written consent.

V. Institutional policy: Your participation in this study is voluntary, whether or not to participate will not affect your current or future relationship with the KLES Dr. Prabhakar Kore Hospital and MRC, Belgaum.

VI. Financial Incentives for participation: You will not receive any reimbursement for participation in the research.

VII. Authorization to Publish Results: When the results of the research are published or discussed in conferences no information will be disclosed that would disclose your identity. Any information obtained in connection with this study and that can be identified with you will remain confidential and will be disclosed only with your permission.

VIII. Consent Statement: To voluntarily take part in this study I must sign on the line below. If I choose to take part in this study I may withdraw at any time. I am not giving up any of my legal rights by signing this form. My signature below indicates that I have read or the contents of the entire consent form including the risks and benefits have been read to me this and all my questions have been answered. I will be given a copy of this consent form. If I have any questions about the study I can contact Dr.Sindhu.J.Shetty, Phone No 9886165868

and Dr.P.B.Desai $_{M.D.}$ Professor and HOD, Department of Biochemistry, Phone no. , Phone no.08312473777extension 1522.

If I have any questions about my rights as a research participant I may contact Dr. V.D.Patil, Principal and Chairman of JNMC Institutional Ethical Committee for Human Subjects Research, Phone No. 08312471530 at J.N. Medical College, Belgaum.

Signature or left thumb print of participant or legally authorized representative.

Participant's Name:	Participant's Signature or thumb print:
Experimenter's Name:	Experimenter's Signature:
Witness' Name:	Witness' Signature:
Guardian's Name:	Guardian's Signature or thumb print:
Date:	Place:

Annexure IV

PROFORMA

I. Patient Identification

Name: Age/Sex: I.P.No/O.P.No:

Address: Rural/urban :

Date of examination:

Occupation: Religion:

II. History:

Diagnosis:

Duration of illness:

Drug: Dose:

Duration of treatment:

Other drugs:

Diabetes: Hypertension:

Renal disease:

Personal history:

Bowel and bladder habits:

Menstrual history:

III. General Physical Examination:

Pallor: Weight (kg):

Icterus: Height :

Lymph nodes: Body mass index(BMI):

Temperature:

Pulse: Blood pressure:

Respiratory rate:

Oral cavity:

IV. Investigations:

Blood glucose estimation:

Renal function test :

- Blood urea
- serum creatinine

Thyroid function test:

Urine analysis:

Complete hemogram:

Lithium estmation:

- Serum lithium
- Salivary lithium
- Urinary lithium

ANNEXURE V

MASTER CHART

SERIAL NO	NAME	SEX	AGE	SERUM LI	SALIVA LI	URINE LI	DOSE
1	SU.	F	32	0.53	0.92	5.53	1200
2	DE.	F	18	0.48	0.96	5.84	800
3	SHI.	M	24	0.62	1.57	1.85	800
4	L.	F	18	0.64	0.84	6.73	800
5	KG.	M	40	0.34	0.95	7.46	800
6	B.	M	22	0.54	0.89	10	800
7	M.	M	36	0.68	1.8	12.5	800
8	R.	F	35	0.6	1.75	7.23	800
9	KU.	M	54	0.63	1.34	2.22	800
10	SG.	M	26	1.08	3.33	2.31	1000
11	MK.	M	18	0.64	1.37	6.9	800
12	V.	M	20	0.6	1.59	7	800
13	SD.	M	46	1.24	3.08	18.22	600
14	KS.	F	43	0.54	1.32	5.1	800

15	**KM.**	**F**	**40**	**0.6**	**2**	**15**	**800**
16	**SHV.**	**M**	**44**	**0.84**	**2.23**	**9**	**800**
17	**JD.**	**F**	**50**	**0.74**	**2.22**	**15.7**	**800**
18	**AS.**	**M**	**55**	**0.49**	**3.45**	**2.54**	**800**
19	**VDY.**	**F**	**24**	**0.74**	**2.49**	**8.51**	**800**
20	**SNT.**	**F**	**39**	**0.94**	**1.74**	**6.07**	**800**
21	**SJT.**	**F**	**20**	**0.73**	**1.69**	**2.27**	**800**
22	**AB.**	**M**	**37**	**0.67**	**1.61**	**11.5**	**800**
23	**SDL.**	**F**	**40**	**1.2**	**3.49**	**5.05**	**1000**
24	**SRT.**	**F**	**30**	**0.68**	**2.13**	**2.68**	**800**
25	**SNK.**	**M**	**48**	**0.78**	**1.49**	**14.8**	**800**
26	**MH.**	**M**	**25**	**1.04**	**2.29**	**5.25**	**800**
27	**KL.**	**M**	**35**	**0.66**	**0.96**	**2.21**	**800**
28	**NM.**	**M**	**41**	**0.9**	**2.5**	**4**	**800**
29	**VN.**	**M**	**28**	**0.6**	**3**	**9**	**800**
30	**SJA.**	**F**	**20**	**0.47**	**1.09**	**2.27**	**800**
31	**SRA.**	**F**	**30**	**0.68**	**1.18**	**2.68**	**800**
32	**SLG.**	**F**	**40**	**1.2**	**2.08**	**5.05**	**1000**
33	**MN.**	**M**	**35**	**0.99**	**2.86**	**14.6**	**800**
34	**SRD.**	**M**	**46**	**1.24**	**1.78**	**18.22**	**600**
35	**BLB.**	**M**	**35**	**0.53**	**0.88**	**10**	**800**

36	**RK.**	**M**	**52**	**0.87**	**2.77**	**1.17**	**800**
37	**SGM.**	**M**	**26**	**1.08**	**2.04**	**2.31**	**1000**
38	**ASK.**	**M**	**39**	**0.43**	**1.26**	**2.54**	**800**
39	**LX.**	**M**	**32**	**0.83**	**2.07**	**5.84**	**800**
40	**HN.**	**M**	**50**	**1.21**	**3.55**	**12.9**	**800**
41	**MT.**	**M**	**35**	**0.57**	**1.58**	**1.58**	**800**
42	**LM.**	**M**	**30**	**0.95**	**3.03**	**13.5**	**1000**
43	**NR.**	**M**	**28**	**0.38**	**0.99**	**1.31**	**800**
44	**MG.**	**M**	**35**	**0.63**	**1.89**	**7.4**	**1000**
46	**ST.**	**F**	**20**	**1.16**	**3.22**	**10.1**	**800**
45	**SKR.**	**M**	**48**	**0.45**	**0.87**	**14.8**	**800**
47	**RG.**	**M**	**28**	**0.42**	**0.94**	**4.9**	**600**
48	**SB.**	**M**	**50**	**1.17**	**2.55**	**3.4**	**800**
49	**GJW.**	**F**	**38**	**0.95**	**1.95**	**1.6**	**800**
50	**SNT.**	**F**	**50**	**0.84**	**2.26**	**9.6**	**800**

INTRODUCTION

Global burden of disease statistics indicate that 4 out of the 10 most important causes of disease worldwide are psychiatric in origin[1].In India the prevalence of major mental and behavioral disease is estimated to be 65/1000 population. Prevalence of mood disorders is estimated to be 16/1000 population[2].

Lithium carbonate is used in the treatment of bipolar disorders, major depressive disorders and schizoaffective disorder. Even though lithium was introduced in psychiatry in 1949 for treatment of mania[3] it was approved by FDA in the United states of America only in 1970 due to concerns about its safety[4]. Evidence for both the safety and the efficacy of lithium salts in the treatment of mania and the prevention of recurrent attacks of bipolar manic-depressive illness is both abundant and convincing[5,6].

When patients are being treated with lithium periodic measurement of lithium concentration in serum is an essential aspect of patient care[4]. This is important because the amount of lithium required to achieve a therapeutic response depends on concentration of ion in serum which in turn reflect its distribution throughout the body rather than on the actual amount given per day[7].

Lithium therapy is initiated in divided doses .once the patient is stabilized, single daily dose is sometimes convenient. In the presence of normal renal function, a total daily dose of 1,200 to 1,800 mg of lithium carbonate generally produces an antimanic serum concentration of 0.8 to 1.2meq/l. Maintenance levels of 0.6 to 1.0 meq/l can usually be attained with 900 to 1200 mg daily. A conservatively low dose is started, perhaps 300mg twice or three times daily, a serum concentration is obtained

after steady state is reached in 4 or 5 days and the dose is adjusted accordingly. During maintenance therapy, patients must be evaluated clinically and lithium levels determined periodically. Early in the treatment monthly visits are common later on if the patient is stable for extended period may be seen at intervals of 3, 4, or 6 months[4].

Knowledge of sampling interval (the time between last dose and the drawing of blood), the dose form, and the dosage schedule is vital in the interpretation of serum lithium levels. A twelve hour interval has been adopted as standard and has been defined as follows:

1. The blood should be drawn in the morning,12hrs(±30 minutes) after the last dose
2. A multiple-dose regimen should be used and
3. A steady –state condition should exist (skipped or extra doses within 4 or 5 days should be avoided)[4].

Lithium therapy is monitored using series of serum determinations of lithium which requires repeated drawing of blood samples. An alternative method of determining lithium level is to assay salivary gland secretions or urine which has several practical advantages such as patients are spared the discomfort of repeated venipunctures. This is all the more important because many patients are afraid of "blood loss". Moreover trained technicians and collection equipment like syringes, needles, sterile gauze etc are not needed thus reducing cost of the investigation. Another important finding is the stability of salivary lithium which is very similar to the stability of serum lithium[8].

Lithium is a metallic ion therefore it is not metabolised nor it is bound to plasma protein. Only the kidneys eliminate lithium. It is filtered by glomerulus and 80% is reabsorbed by proximal tubule but it is not reabsorbed by the distal tubule[9].

Therefore the present study was planned to explore the relationship between serum lithium, salivary lithium and urinary lithium, with the possibility of discovering alternate methods for monitoring patients who are on lithium therapy.

AIM AND OBJECTIVES

Aim:

Aim of the present study was to explore the relationship between serum lithium, salivary lithium and urinary lithium, with the possibility of discovering alternate methods for monitoring patients who are on lithium therapy

Objectives:

1. Comparing serum lithium, salivary lithium and urinary lithium in patients on lithium carbonate.

2. Evaluate the possibility of replacing serum with salivary or urine lithium estimation for monitoring of patients on lithium carbonate.

REVIEW OF LITERATURE

Lithium has an established use in three main indications: treatment of acute mania, prophylaxis of bipolar episodes and augmentation therapy for severe, refractory depression[10]. Although the psychopharmacological field of bipolar disorders has evolved rapidly during the last 10 years, lithium is still considered the 'gold standard' for these conditions and a first-choice mood stabilizer in recent guidelines[11-13]. Lithium and its augmentation by antidepressants, antipsychotics, and benzodiazepines had been the major approach for the management of bipolar disorder[14].

HISTORY

Lithium salts were used in the nineteenth century as a treatment of gout, sedative and as a putative anticonvulsant. Thereafter, lithium salts were unpopular until the late 1940s, when lithium chloride was employed as a salt substitute for cardiac and other chronically ill patients. This ill-advised use led to several reports of severe intoxication and death and to considerable notoriety concerning lithium salts within the medical profession[15].Cade, in Australia, while looking for toxic nitrogenous substances in the urine of mental patients for testing in guinea pigs, administered lithium salts to the animals in an attempt to increase the solubility of urates. Lithium carbonate made the animals lethargic, and in an inductive leap, Cade gave lithium carbonate to several agitated or manic psychiatric patients, reporting that this treatment seemed to have a specific effect in mania [3,16] .

Chemistry.

Lithium is the lightest of the alkali metals (group Ia); the salts of this monovalent cation share some characteristics with those of Na^+ and K^+. Li^+ is readily assayed in biological fluids and can be detected in brain tissue by magnetic resonance spectroscopy[17]. Traces of the ion occur normally in animal tissues, but it has no known physiological role. Lithium carbonate and lithium citrate currently are used therapeutically.

Pharmacological Properties

Therapeutic concentrations of lithium ion (Li^+) have almost no discernible psychotropic effects in normal individuals. It is not a sedative, depressant, or euphoriant, and this characteristic differentiates Li^+ from other psychotropic agents[15].The precise mechanism of action of Li^+ as a mood-stabilizing agent remains unknown, although many molecular and cellular actions of Li^+, as well as similarities of actions of other mood-stabilizing agents, including valproate, have been described.The main effect of lithium is probably to inhibit hydrolysis of inositol phosphate,therefore reducing the recycling of free inositol for synthesis of phophotidylinositides. These intracellular molecules are part of the transmembrane signaling system that is important in regulating intracellular calcium ion concentration which subsequently affects neurotransmitter release[18-20] .

Absorption

Water-soluble salts, such as chloride and sulphate,are rapidly and almost completely absorbed from the upper gastrointestinal tract, while the less soluble carbonate salt is absorbed more slowly[21].Absorption half-lives for standard- and

sustained release forms of lithium carbonate is 0.78 ± 0.05 hours and 3.73 ± 0.37 hours, respectively[22].

Distribution

Li^+ initially is distributed in the extracellular fluid, then gradually accumulates in various tissues; it does not bind appreciably to plasma proteins. The concentration gradient across plasma membranes is much smaller than those for Na^+ and K^+. The final volume of distribution (0.7 to 0.9 liter per kilogram) approaches that of total body water and is much lower than that of most other psychotropic agents, which are lipophilic and protein bound. Passage through the blood-brain barrier is slow, and when a steady state is achieved, the concentration of Li^+ in the cerebrospinal fluid and in brain tissue is about 40% to 50% of the concentration in plasma[15].

Metabolism and Excretion

Lithium is not subject to metabolic transformation and is almost exclusively excreted via the kidney as a free ion. Similarly to sodium, it is able to freely cross the glomerular membrane. Eighty percent of lithium is reabsorbed by passive diffusion in the proximal tubules. Its clearance varies from 0.6 to 2.4 L/h with high interindividual variability[21]. Both creatinine clearance and bodyweight are important factors in predicting lithium clearance[23].

The plasma half life of lithium is dependent on the Volume of distribution, clearance and duration of lithium therapy [21]. Mean plasma half life of lithium ranged from 16 to 30 hours in subjects with normal renal function[22, 24-28].

Approximately 95% of a single dose of Li^+ is eliminated in the urine. From one- to two-thirds of an acute dose is excreted during a 6- to 12-hour initial phase of

excretion, followed by slow excretion over the next 10 to 14 days. With repeated administration, Li^+ excretion increases during the first 5 to 6 days until a steady state is reached between ingestion and excretion. When therapy with Li^+ is stopped, there is a rapid phase of renal excretion followed by a slow 10- to 14-day phase. Since 80% of the filtered Li^+ is reabsorbed by the proximal renal tubules, clearance of Li^+ by the kidney is about 20% of that for creatinine, ranging between 15 and 30 ml per minute. This rate is somewhat lower in elderly patients (10 to 15 ml per minute). Less than 1% of ingested Li^+ leaves the human body in the feces, and 4% to 5% is secreted in sweat. Li^+ is secreted in saliva in concentrations about twice those in plasma, while its concentration in tears is about equal to that in plasma. Since the ion also is secreted in human milk, women receiving Li^+ should not breast-feed infants[15].

Influence of Intrinsic Factors on Lithium Pharmacokinetics

1. Age

   Elderly patients (usually aged>65 years) require lower doses than younger adults to achieve a desired steady state plasma concentration[23] .

2. Renal disease

   Renal clearance of lithium is decreased in patients with abnormal renal function therefore the risk of lithium intoxication in such patients are increased[21]. In severe renal insufficiency, the contraindication is definite and absolute[29].

3. Obesity

   Lithium clearance was significantly greater for obese subjects than for the control group. The steady-state volume of distribution for the obese group was also significantly less than that for the control group.

4. Pregnancy

Lithium ion passes through the placental barrier[30]. It is recommended to balance the expected benefit of lithium versus the fetal risk[31].Lithium clearance increases by 30–50% in last month of pregnancy and at the time of delivery, clearance of the drug falls to pre-pregnancy levels. Therefore doses must be adapted and therapeutic drug monitoring must be carried out more frequently[30] .

Management of Lithium Therapy

Lithium has narrow therapeutic range therefore its use must confirm some strict rules. Therapeutic monitoring is the basis for optimal use and dosing of lithium.

Lithium doses should be adjusted on the basis of the concentration in serum drawn preferably 12 hours(security interval 10–14 hours) after the last dose[29]. Serum concentrations at this time are in the'flat part' of the pharmacokinetic curve [32].In patients receiving once-daily administration, the serum concentration at 24 hours should serve as the control value.

Lithium efficacy is dose dependent and reliably correlates with serum concentrations. The optimal serum lithium concentration for preventing mania and depression in maintenance treatment is not well established[32]. Typically, it has been considered that lithium concentrations should be maintained between 0.6 and 1.0 mmol/L but some authors still favour 0.8–1.2 mmol/L [21].

Before starting treatment with lithium personal and familial medical history, especially with respect to thyroid function, previous heart disease and comedications has to be noted. Following investigations should be done before starting lithium-

creatinine clearance,blood TSH, free thyroxine, calcium, phosphorus,sugar and electrolytes; pregnancy test if appropriate;ECG in patients aged >40 years; and EEG in patients with a previous history of seizures. Suitable Contraceptive method should be started in female patients[21] .

Lithium therapy is initiated in divided doses. In the presence of normal kidney function, a total daily dose of 1,200 to 1,800 mg of lithium carbonate generally produces an antimanic serum concentration of 0.8 to 1.2 mEq/L. Maintenance levels of 0.6 to 1 mEq/L can usually be attained with 900 to 1,200 mg daily[4] . Sustained-release preparations can be given in a single dose and, to simplify therapeutic monitoring and are best taken at night[29] .

In case of sustained –release preparations slower increase in plasma concentrations and lower maximum plasma concentration result in benefits with respect to adverse effects such as tremor, upper gastrointestinal cramping and nausea, rashes, cognitive dulling, urinary frequency and neuromuscular slowing[33].

Measuring serum lithium concentration is usually recommended after 1 week of commencing Therapy[34]. As a general rule, blood samples should be taken 12 hours (10–14 hours) after the last drug intake. In case of sustained-release preparations, bearing in mind the later peak of serum lithium concentration,it has been advised that serum concentrations should be maintained within the upper range (0.8–1 mmol/L), rather than within the 0.6–0.8 mmol/L range recommended for conventional formulations[25].

The frequency of blood sampling is usually every 1–2 weeks until a desirable serum concentration is achieved, then every 2–3 months for the first 6 months of treatment, and at least every 6–12 months thereafter[35].

After any change in dosage, and when there has been intercurrent disease or any change in co-medication, serum lithium concentration should be checked and adjusted to the desired range, after steady state has been reached [34,35].

During maintenance therapy, patients must be evaluated clinically, lithium levels determined periodically, and appropriate laboratory tests performed at regular intervals. Monthly visits are common early in treatment if the clinical course is uncomplicated. Patients who have been stable for extended periods may be seen at intervals of 3, 4, or even 6 months[4].

Serum creatinine must be measured every 6–12 months in order to check renal function. Measurement of serum calcium every 6–12 months and of thyroid status (TSH initially, every 6 months for the first 3 years, then once a year) must be performed[21].

Laboratory Monitoring

Lithium has been measured in virtually every body fluid[4]. Serum analysis is the most useful and is used in clinical practice[4,37]. Concentrations considered to be effective and acceptably safe are between 0.6 and 1.25 mEq per liter. The range of 0.9 to 1.1 mEq per liter is favored for treatment of acutely manic or hypomanic patients. Somewhat lower values (0.6 to 0.75 mEq per liter) are considered adequate and are safer for long-term use for prevention of recurrent manic-depressive illness. Some patients may not relapse at concentrations as low as 0.5 to 0.6 mEq per liter, and lower levels usually are better tolerated[15]. The concentration of lithium can be determined by flame emission photometry,atomic absorption spectrometry or electrochemically using an ion selective electrode[37].

A study done on 20 patients on lithium carbonate investigated the correlations between serum and mixed saliva and parotid fluid lithium levels. High correlations from 0.90 to 0.95 were found between serum and saliva and serum and parotid fluid. A reproducible constant relationship of 2.26±10% was noted between mixed saliva and serum level. The study recommended that to use saliva most effectively in determining lithium levels the ratio between saliva and serum lithium levels should be calculated for each individual and the obtained ratio should be used to calculate subsequent determination for that individual[7].

A study was done on ten patients receiving lithium carbonate for affective disorder. 24 samples of serum and saliva were collected from them. Correlation coefficient between serum and salivary lithium was +0.88($P<0.01$).the salivary and serum lithium ratio was 2.22±0.5. The study found good degree of stability for saliva lithium levels. A therapeutic range of saliva lithium between 1.5-3 mEq/L was suggested to adjust lithium dosage[8].

The above two studies found high correlation between serum and saliva lithium levels but there was a high individual variation of paired results .To circumvent this problem a study was done using a naturally occurring marker in saliva and serum along with serially paired samples in individual patients. Thirty synchronous samples of serum and saliva was collected from thirty patients on lithium carbonate and lithium estimation was corrected for potassium(natural marker) potassium . Three patients were monitored with a series of 7,6 and 8 paired results. Lithium was estimated using flame photometer. In 30 patients correlation coefficient was 0.71($P<0.001$). In series of paired samples correlation coefficient was 0.85($P<0.01$), 0.80($P<0.05$) and 0.63($P<0.1$).It was found that saliva levels variation

for fixed serum levels were unacceptably large. The study showed satisfactory correlation from zero through the therapeutic range of lithium but was sceptical about such a correlation in excessive levels of serum lithium[38].

A study was done on 95 patients in india who received lithium carbonate. 309 samples of serum and saliva were collected. Flame photometer was used for lithium estimation. A positive and highly significant correlation was found[39].

A study was done to evaluate the relationship among plasma,RBC and saliva lithium levels using atomic absorption spectrophotometry. 30 synchronous samples of blood and saliva was taken from 9 subjects. High correlation(r=0.569,P <0,001) was found between serum and salivary lithium levels. Correlation between RBC lithium and saliva was less compared to serum and saliva levels. The study found high interindividual and intraindividual correlation[40].

In 11 manic-depressive outpatients on chronic lithium therapy the relationship between serum and saliva lithium was studied. Trough serum and saliva lithium levels were measured using atomic absorption spectrometry every 3 or 4 weeks during clinic visits for a period of atleast 16 weeks. Clinical status of patient was rated according to level of mania. Intersubject analysis showed poor correlation(r=0.5) intrasubject correlation was strong(r=0.72-0.94). The study suggested that patient's saliva can be used to estimate serum lithium levels in clinically stabilised manic-depressive patients on prophylactic lithium therapy. In poorly stabilised patients serum concentration should be monitored[41].

Salivary and serum lithium concentrations were measured using atomic absorption spectrophotometry simultaneously in 118 manic-depressive patients. Lithium concentration in saliva was 2.24 ±0.35 times higher than in serum. An

equation to calculate serum lithium concentration from salivary measurements was derived: Li serum = 0.36 Li saliva + 0.13. Psychotropic drugs had no effect on the salivary:serum ratio. Eighteen patients were followed for several weeks. A significant correlation coefficient($P<0.05$) between salivary and serum lithium concentrations was found in thirteen of the eighteen patients studied[42].

140 synchronous samples of serum and saliva was collected from 28 patients undergoing lithium therapy and was estimated using flame photometer. The mean saliva/serum ratio was calculated from 120 synchronous samples from 24 patients was found to be 2.68. Regression line equation calculated for same population came out to be $Y=0.325+0.22X$. Predictive value of saliva lithium was tested by applying this regression equation and the population mean ratio on 20 samples from the next 4 patients. Prediction was also tried in 24 patients who had given more than 3 synchronous samples using individual mean saliva/serum ratio.

An individual's mean was calculated from the initial 3 synchronous samples and predictive value of saliva was tested on subsequent samples in the same patient by using his mean ratio. This method was found to be better than predicting on the basis of population figures[43].

The usefulness of salivary lithium values for monitoring long-term lithium prophylaxis was studied in 60 patients on lithium therapy. A total of 99 pairs of saliva and serum samples were obtained, and analysed using flame photometer. The correlation between serum and salivary lithium levels ($r = 0.73$) was found to be significant at the 1% level. The ratio of salivary serum levels to that of serum was found to range from 1.77 to 6.68. The ratio of 51% of the samples was between 3 and 3.99. The study suggested that it is important to identify the subgroup of patients who

show better correlation of salivary and serum lithium levels and use each individual's ratio to monitor only his or her lithium therapy[44].

The salivary composition and flow rate of 78 patients with primary affective disorders and of 49 healthy volunteers were examined. The former were divided into two groups: Group 1(n=57) patients receiving lithium carbonate and psychoactive drugs, and Group 2(n=21)patients receiving psychoactive drugs only. A significant correlation between salivary and serum lithium was found in patients on chronic lithium therapy. The use of saliva analysis for monitoring lithium dosage was recommended by this study[45].

Serum and saliva lithium levels were simultaneously determined using flame photometer in 14 prepubertal children being treated with lithium carbonate. Saliva and serum lithium levels were strongly correlated. The concentration of lithium in saliva was almost two times (1.82) that in serum. There was more variability in saliva levels than in serum levels. It was possible to predict serum levels from saliva levels using regression analysis. If dose was added to saliva as a second predictor variable, accuracy of the prediction was increased[46].

A study was been carried out to find out the reasons for the wide variations observed in the correlation between serum and saliva lithium levels. Serum/saliva lithium levels were monitored using flame photometer in 10 individuals on 6 occasions. The correlation coefficient (r) varied within individuals from 0.19 to 0.91 ($p < 0.025$) whereas when all the individuals were considered simultaneously, it was 0.59 ($p < 0.005$). It was concluded that prediction of serum lithium levels from salivary lithium levels would only be possible in those individuals showing good

correlation. The variability in this correlation could be mainly due to inter-individual variations in the particular sample of individuals studied[47].

In a study one tablet containing 755 mg of lithium tryptophanate (10.8 mEq of lithium) was administered to eight healthy volunteers. The main pharmacokinetic parameters for the group of subjects were estimated. Pharmacokinetic parameters (mean ± SD) from plasma and saliva were respectively: half life (t1/2) 17 ± 6 vs. 21.8 ± 14 h; mean residence time 23.7 ± 7.4 vs. 24.4 ± 15.3 h; total clearance 30.6 ± 9.3 vs. 28.6 ± 6.2 ml/h/kg; and apparent volume of distribution 0.71 ± 0.20 vs. 0.84 ± 0.37 L/kg. Although the mean pharmacokinetic parameters in plasma and saliva were similar, there was no significant correlation between the calculated parameters in the individual subject ($p > 0.05$). The study concluded that usefulness of monitoring salivary levels of lithium is questionable[48].

Within- and between-subject variability in serum and salivary lithium concentrations in nine psychiatric inpatients on stable drug regimens undergoing therapy was assessed using criteria for determining biologic variation. Estimation of lithium was done using flame photometer This allows separation of analytic from other measures of variance. There were marked differences in inter- and intra-subject variance for serum and salivary lithium concentrations for serum/salivary ratios. these variances were greater for salivary lithium than for serum concentrations. The results were used to assess analytical performance, the usefulness of the therapeutic range, and the reference change interval.the study found that despite greater variance for salivary concentrations, predicted serum concentrations from predetermined serum/saliva ratios were in good agreement with actual concentrations in most subjects[49].

A study was done to check if dialysis of saliva improves accuracy of saliva lithium determinations. Estimation of lithium was done using lithium sensitive electrode. Saliva has two major components: the aqueous and the mucopolysaccharide portions. Since Li is likely to distribute only in the aqueous fraction, saliva was dialysed through a 3000 Da filter to isolate the aqueous component and determine the Li level in it. Lithium levels in the dialyzed saliva agreed more closely with plasma levels ($r = 0.901$, $p < 0.001$) than did whole saliva ($r = 0.775$, $p = 0.012$). the study concluded that dialysis of saliva may contribute to more accurate saliva Li levels[50].

In a study lithium ions concentration in human serum and saliva was determined using dry-slide technology Vitros 250 Analyser (Ortho Clinical Diagnostic) and atomic absorption spectrometry Perkin Elmer 403 (AAS). lithium ions were analysed in 100 serum and saliva specimens of patients after oral administration of lithium carbonate (3 x 300 mg) Jadran, Galen Laboratory Rijeka. Saliva and blood were taken 2 and 12 hours after the last dose. At the same time lithium ions at samples of blood and saliva were determined with both methods which showed high level of correlation. The mean difference of lithium ions between saliva and serum was statistically significant for $p<0.05$ using t student test. saliva constant of elimination Kel = 0.02(-1)h and elimination half life (t(1/2)) was t(1/2)=34.6 h. For serum t(1/2)= 24 h which means that lithium ions elimination is slower from saliva than from serum. That is the reason why probably concentration at saliva is higher then at serum. Lithium elimination is two compartment pharmacokinetic model where important part of compartment are saliva and salivary glands. The study concluded at a certain point in medical treatment it could be expected to use controlled determination of lithium ions in saliva with serum as control[51].

The relationship between the plasma concentration, saliva concentration and urinary excretion rate of lithium was investigated in a study and the possibility of using the saliva concentration or the urinary excretion rate for monitoring dosage was considered. The results in the study support the idea that saliva concentration of lithium could be useful in monitoring dosage but, there are many difficulties to be overcome in using the urinary excretion rate of lithium due to inter-subject variation, intra-subject variation during the night and circadian variation in the renal excretion of lithium[52].

MATERIALS AND METHODS

Source of the data

The present study is a cross sectional study to compare serum lithium, salivary lithium and urinary lithium in patients on lithium carbonate.

Study period

The study was undertaken between December 2009 to March 2011.

SAMPLE SIZE

Sample size was determined to be 50.With α=0. 01 and β=0.2 sample size required to demonstrate correlation 'r'=0.6 minimum sample size of 33 is required.

STUDY POPULATION.

Consisted of in patients and out patients attending the Department of Psychiatry of KLE'S Dr. Prabhakar Kore Charitable Hospital and Medical Research Centre, Belgaum between the ages of 18-50 years who are on lithium carbonate for more than a week.

CRITERIA FOR SELECTION OF THE STUDY GROUP

Inclusion Criteria**:**

1) Patients who are on lithium carbonate therapy for at least a week.
2) Age: 18-50 years.
3) Renal function test within normal limits.
4) Urine analysis within normal limits.

Exclusion Criteria:

1) Factors affecting normal salivary secretion.
2) Pregnancy
3) Age <18years and >50 years
4) Dehydration
5) Drugs affecting lithium pharmacokinetics :Nonsteroidal anti-inflammatory drugs, Diuretics, angiotensin-converting enzyme inhibitors, angiotensin IIreceptor type -1 antagoinists, metranidazole, sodium bicarbonate, propranolol.

APPROVAL FROM THE AUTHORITIES:

Permission to conduct the study was obtained from all the concerned authorities viz.

1. Institutional ethics committee on human subjects research of Jawaharlal Nehru medical college, Belgaum.
2. Director –clinical services for medical director and chief executive of KLE'S Dr.Prabhakar Kore Hospital and Medical Research Centre, Belgaum.(Annexure II)
3. University science instrument centre (USIC), Shivaji university, Kolhapur.

OBTAINING INFORMED CONSENT

Informed consent was taken from all the participants in the study.(annexure III)

SCHEDULING:

This study was carried out for a period of 14 months. It was undertaken during December 2009 to March 2011.

PILOT STUDY

Pilot study was conducted after taking serum, saliva and urine from 3 patients to assess the feasibility of the study and to standardise methods of sample collection.

Patient information

A structured proforma was used to collect sociodemographic and clinical information about the study participants.(annexure IV)

COLLECTION OF SAMPLE

Patient preparation:

Blood, saliva and urine samples were collected in the morning (12hrs after the last dose of lithium carbonate) before the patient consumed breakfast and the morning dose of lithium. Samples were collected under aseptic precautionary measures.

Serum

5 ml of blood was collected in a plain non vacuum tube and was allowed to stand in room temperature till clot was formed. Serum was separated within one hour of venipuncture by centrifuging the tubes at 3,000 r.p.m for 10 minutes. Serum was pipetted out into a sterile eppendorf tubes.

Saliva

Saliva was collected in a sterile container after asking the patient to rinse mouth with water. Subjects were asked to collect saliva in the mouth and then were

asked to spit into the container till adequate amount was collected. Saliva was centrifuged to remove mucus.

Urine

Mid stream urine was collected in sterile container.

Sample collection was supervised by nursing staff. All the 3 samples were collected within 20 minutes.

All samples were stored in non vacuum sterile tubes at -20 ^{0}C till further analysis.

STATISTICAL TESTS USED

The following methods of statistical analysis have been used in this study. Data was entered in Microsoft excel and analyzed using SPSS (version 17).

Mean and standard deviation of serum lithium, salivary lithium and urinary lithium was computed and compared by using paired't' test. Pearson's correlation coefficient and Linear regression analysis was done to between three parameters was computed to know the strength of association between them was done.

MATERIAL USED IN THE STUDY

1. Non vacuum plain tube.
2. 5 ml Disposable syringe.
3. Tourniquet.
4. Sterile container.
5. Automated pipettes and tips.
6. Deionised water.

7. Microcentrifuge tubes.
8. Gloves.

INSTRUMENTS USED IN THE STUDY

1. Centrifuge machine.
2. Flame atomic absorption spectrophotometer (Perkin Elmer Analyst 300).

ESTIMATION OF LITHIUM

Lithium estimation in serum, saliva and urine was done using Atomic Absorption Spectrophotometer (Perkin Elmer Analyst 300). Permission to use the instrument for lithium analysis was obtained from head of University science instrument centre (USIC), Shivaji University, Kolhapur.

SAMPLE PREPARATION

Serum

Serum sample was diluted to 1:10 or 1:5 with deionized water(1, 2) . The dilution ratio was adjusted to ensure that concentrations fall within a suitable absorbance range.

Saliva

Saliva sample was diluted to 1:5 with deionized water . The dilution ratio was adjusted to ensure that concentrations fall within a suitable absorbance range.

Urine

Urine sample was diluted to 1:50 with deionized water(1, 2). The dilution ratio was adjusted to ensure that concentrations fall within a suitable absorbance range.

Samples were taken in batches of twenty for analysis in flame atomic absorption spectrophotometer.

PRINCIPLE OF FLAME ATOMIC ABSORPTION SPECTROPHOTOMETER

Every element has a specific number of electrons associated with its nucleus. The normal and most stable orbital configuration of an atom is known as the "ground state." If energy is applied to an atom, the energy will be absorbed and an outer electron will be promoted to a less stable configuration known as the "excited state." Since this state is unstable, the atom will immediately return to the "ground state," releasing light energy.

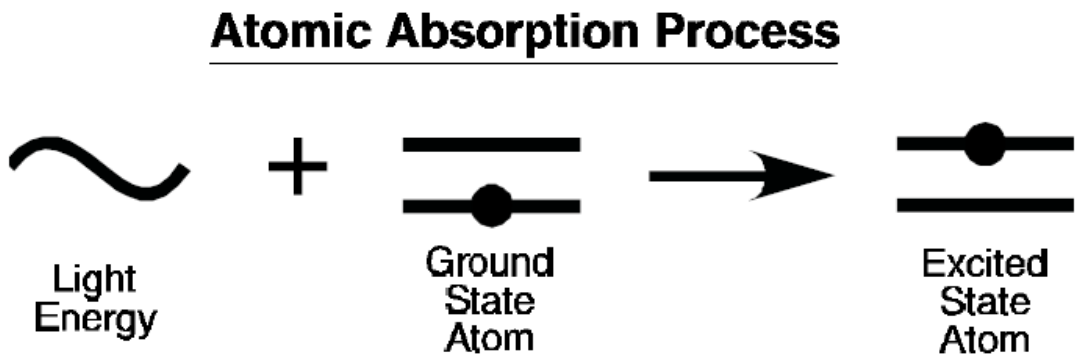


Figure 1: Atomic absorption process.

The "ground state" atom absorbs light energy of a specific wavelength as it enters the "excited state." As the number of atoms in the light path increases, the amount of light absorbed also increases. By measuring the amount of light absorbed, a quantitative determination of the amount of analyte can be made. The use of special light sources and careful selection of wavelengths allow the specific determination of individual elements.

ATOMIC ABSORPTION INSTRUMENTATION

There are five basic components of an atomic absorption instrument:

1. The light source that emits the spectrum of the element of interest
2. An "absorption cell" in which atoms of the sample are produced
3. A monochromator for light dispersion
4. A detector, which measures the light intensity and amplifies the signal.
5. A display that shows the reading after it has been processed by the instrument electronics[37].

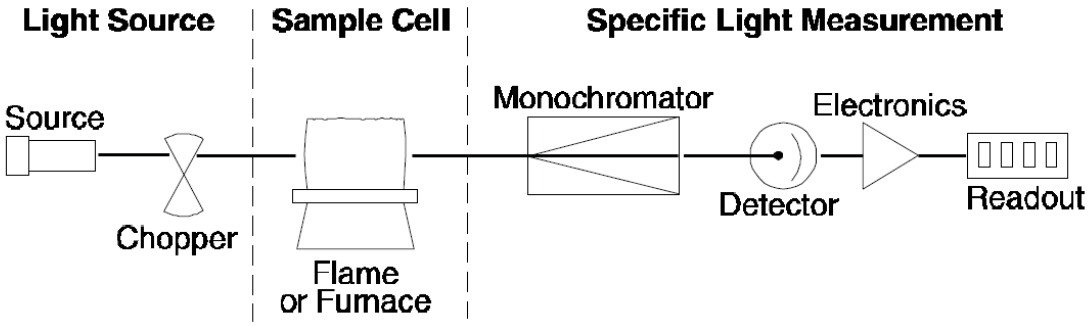


Figure 2: Schematic representation of flame Atomic absorption process.

PROCEDURE

Standard

Appropriate standards are prepared by diluting the lithium stock solution provided by the manufacturer.

Blank

Deionized water is used for blank solution.

ANALYSIS

Instrument was set in standard condition for lithium analysis.

Blank was aspirated first followed by a suitable standard and then the sample. Lithium standard was read after every five sample.

The readings were recorded in p.p.m. and then converted to meq/L.

PHOTOGRAPHS

Photograph 1: Flame atomic absorption spectrophotometer (Perkin Elmer Analyst 300)

Photograph 2: Investigator performing analysis of lithium on Flame atomic absorption spectrophotometer (Perkin Elmer Analyst 300).

RESULTS

A cross sectional study was done to compare serum lithium, salivary lithium and urinary lithium in patients on lithium carbonate to evaluate the possibility of replacing serum with salivary or urine lithium estimation for monitoring of patients on lithium carbonate.

The data obtained from the study was compiled, tabulated and subjected to statistical analysis. The results are presented here under the headings of the various parameters considered for the study.

Table 1:Distribution of age, serum lithium, salivary lithium, urine lithium and dose of lithium carbonate in 50 study subjects.

The mean age of study participants was 35.10±10.63. Their Mean serum lithium was 0.75±0.25 mEq/L, mean salivary lithium was 1.91±0.80 mEq/L and mean urine lithium was 7.16±4.84 mEq/L. Mean dosage of lithium carbonate prescribed to the subjects was 820±101.01 mg.

Table 2: :Distribution of Age, serum lithium, salivary lithium, urine lithium and dose of lithium carbonate in male and female.

The number of female participants were 18 and male participants were 32.The mean age of male participants was 36.50±10.50 years and that of female participants was 32.61±10.69 years. There was no statistically significant difference between the mean ages of males and females (p=0.22).

In males mean serum lithium was 0.75±0.2 mEq/L, mean salivary lithium was 1.91±0.80 mEq/L and mean urine lithium was 7.53±5.26 mEq/L. Mean dosage of lithium carbonate prescribed to the subjects was 806.25±94.82 mg.

In females mean serum lithium was 0.76±0.23 mEq/L, mean salivary lithium was 1.85±0.74 mEq/L and mean urine lithium was 6.50±4.06 mEq/L. Mean dosage of lithium carbonate prescribed to the subjects was 844.44±109.66 mg.

There was no statistically significant difference between the mean serum lithium (p=0.91) , salivary lithium(p=0.67), urine lithium(p=0.47) and dosages of lithium carbonate (p=0.2) between males and females.

Table 3:Correlation analysis of serum and saliva lithium levels

Correlation between serum lithium and salivary lithium was statistically significant (r=0.695, p <0.001). The serum lithium and salivary lithium was more strongly correlated in females (r=0.770, p <0.001) when compared to males (r=0.665, p <0.001).

Table 4(Graph 1):Linear regression analysis for serum and salivary lithium correlation.

Linear regression analysis for serum and salivary lithium was done with serum lithium as dependent variable, statistically significant correlation was found (r=0.695). The equation for calculating serum lithium from saliva lithium measurements was derived from the graph 1: Y=0.332+0.221X (Y=serum lithium concentration, X=salivary lithium concentration).

Table 4 (Graph 2): Linear regression analysis for serum and salivary lithium correlation for females

In females linear regression analysis for serum and salivary lithium was done with serum lithium as dependent variable, statistically significant correlation was found (r=0.770). The equation for calculating serum lithium from saliva lithium

measurements was derived from the graph 2:Y=0.355+0.271X(Y=serum lithium concentration, X=salivary lithium concentration).

Table 4 (Graph 3): Linear regression analysis for serum and salivary lithium correlation for males

In males linear regression analysis for serum and salivary lithium was done with serum lithium as dependent variable, statistically significant correlation was found (r=0.665). The equation for calculating serum lithium from saliva lithium measurements was derived from the graph 3: Y=0.259+0.204X(Y=serum lithium concentration, X=salivary lithium concentration).

Table 5:Correlation analysis of serum and urine lithium levels.

Correlation between serum lithium and urine lithium was not statistically significant (r=0.234, p =0.102). In males correlation between serum lithium and urine lithium was (r=0.319, p =0.08) in females (r=0.022, p =0.932).

Table 6(Graph 4):Linear regression analysis for serum and urine lithium correlation.

Linear regression analysis for serum and urine lithium was done with serum lithium as dependent variable. Correlation was found to be statistically not significant (r=0.234). Graph 4 showing serum versus urine lithium measurements shows a large deviation.

Table 1: Distribution of age, serum lithium, salivary lithium, urine lithium and dose of lithium carbonate in 50 study subjects.

Sl.No	Variable	Mean±S.D
1	Age (years)	35.10±10.63
2	Serum lithium (mEq/L)	0.75±0.25
3	Salivary lithium (mEq/L)	1.91±0.80
4	Urine lithium (mEq/L)	7.16±4.84
5	Dose of lithium carbonate (mg)	820±101.01

Table 2: Distribution of age, serum lithium, salivary lithium, urine lithium and dose of lithium carbonate in male and female.

Variable	Male (n=32)	Female (n=18)	t value	p value
Age	36.50±10.50	32.61±10.69	1.25	0.22
Serum lithium(mEq/L)	0.75±0.26	0.76±0.23	-0.12	0.91
Salivary lithium(mEq/L)	1.91±0.80	1.85±0.74	0.43	0.67
Urine lithium(mEq/L)	7.53±5.26	6.50±4.06	0.72	0.47
Dose of lithium carbonate (mg)	806.25±94.82	844.44±109.66	-1.29	0.20

Table 3:Correlation analysis of serum and saliva lithium levels.

	r	r^2	S.E	p value
Total study Subjects(n=50)	0.695	0.47	0.18	**<0.001***
Male (n=32)	0.665	0.42	0.20	**<0.001***
Female (n=18)	0.770	0.57	0.16	**<0.001***

*-statistically significant

Table 4:Linear regression analysis for serum and salivary lithium correlation.

	Unstandardized Coefficients		Standardized Coefficients	t	p value.	95.0% Confidence Interval for B	
	B	Std. Error	Beta			Lower Bound	Upper Bound
(Constant)	0.332	0.069		4.835	**<0.001***	0.194	0.470
Salivary Lithium	0.221	0.033	0.695	6.688	**<0.001***	0.155	0.288
Dependent Variable: Serum Lithium *-statistically significant							

Table 5: Correlation analysis of serum and urine lithium levels.

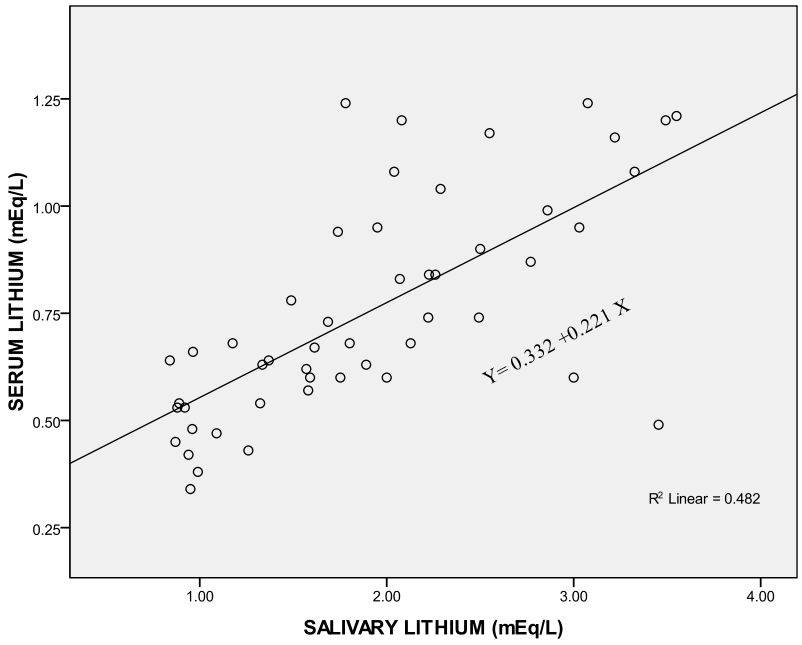
	r	r^2	S.E	p value
Total study subjects(n=50)	0.234	0.04	0.25	0.102
Male (n=32)	0.319	0.07	0.26	0.08
Female (n=18)	0.022	-0.06	0.25	0.932

Table 6:Linear regression analysis for serum and urine lithium .

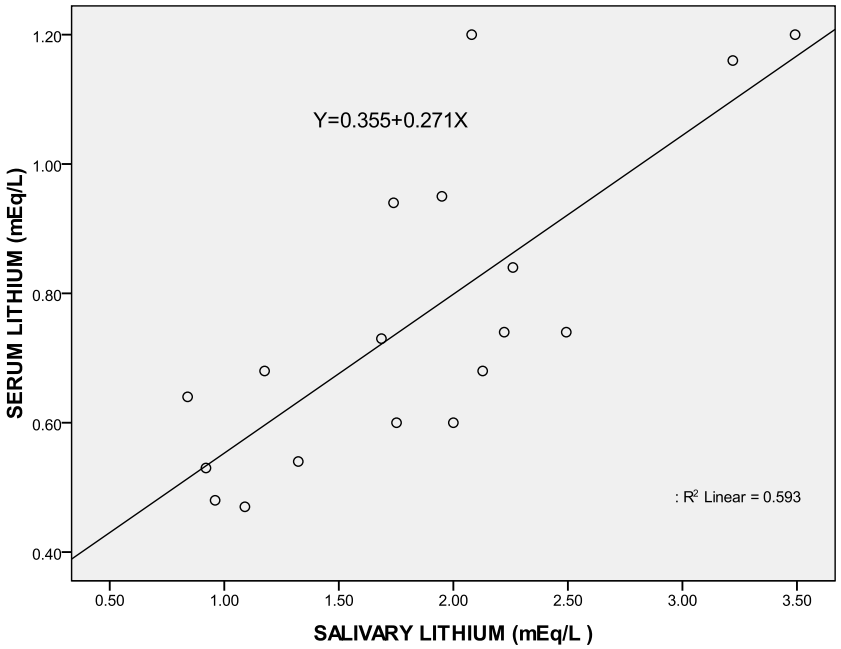
Model	Unstandardized Coefficients		Standardized Coefficients	t	p value
	B	Std. Error	Beta		
(Constant)	0.668	0.064		10.459	**<0.001***
Urine Lithium	0.012	0.007	0.234	1.667	0.102
Dependent Variable: Serum Lithium *-statistically significant					

GRAPHS

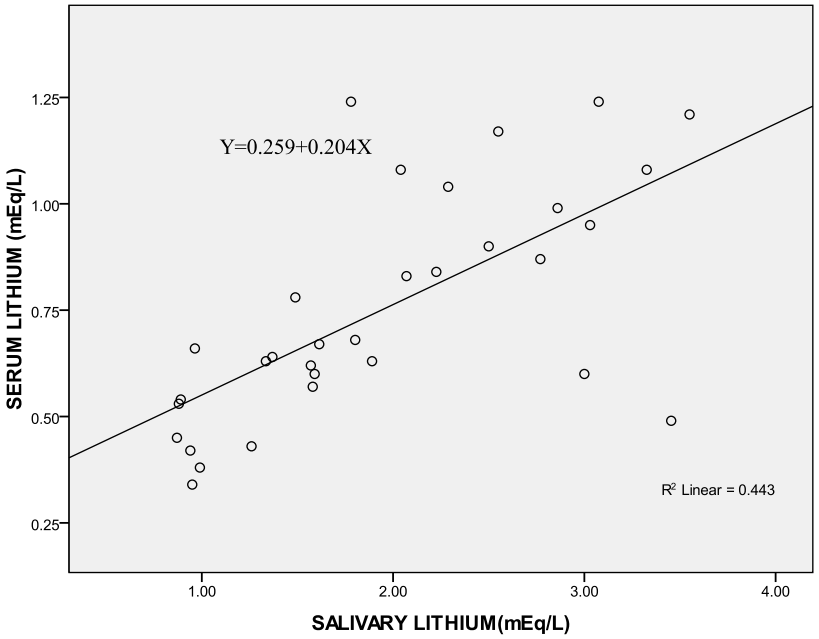
Graph 1: Linear regression analysis of serum versus salivary lithium of 50 study participants



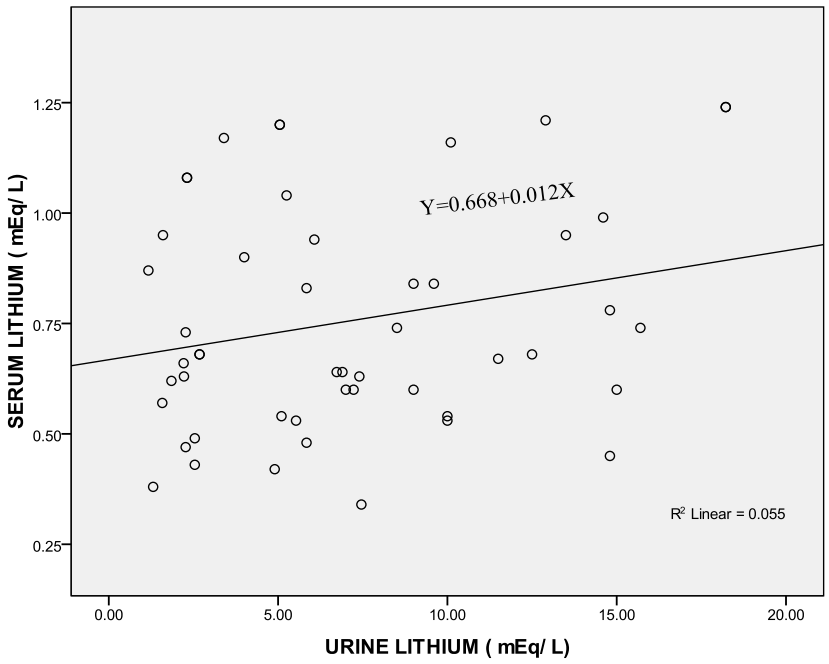
Graph 2: Linear regression analysis of serum versus salivary lithium in female participants.



Graph 3: Linear regression analysis of serum versus salivary lithium in male participants.



Graph 4: Linear regression analysis of serum versus urine lithium of 50 study participants



DISCUSSION

Lithium carbonate is used in the treatment of psychiatric and non psychiatric disorders[53]. Lithium has an established use in treatment of acute mania, prophylaxis of bipolar episodes and augmentation therapy for severe, refractory depression[10]. The difference between toxic and therapeutic dose of lithium carbonate is narrow. Lithium therapy must be guided by monitoring plasma concentration it has been considered that lithium concentrations should be maintained between 0.6 and 1.0 mmol/L[21]. At plasma concentrations above 1.5mmol/L is associated with signs of intoxication mainly affecting gastrointestinal and neurological system. Frank toxicity is associated with plasma concentration greater than 2mmol/L which is an acute medical emergency. Therefore when patients are on lithium strict monitoring of serum levels are done since the amount of lithium required to achieve a therapeutic response depends on concentration of ion in serum which reflects its distribution throughout the body rather than on actual amount required per day[7] .

The frequency of blood sampling for lithium monitoring is usually every 1–2 weeks until a desirable serum concentration is achieved, then every 2–3 months for the first 6 months of treatment, and at least every 6–12 months thereafter[35]. These series of lithium monitoring requires repeated drawing of blood samples.

To monitor health status, disease onset and progression, and treatment outcome noninvasively is a most desirable goal in the health care delivery and health research. There

are three prerequisites necessary to reach this goal

1. A non-invasive method for collecting biological samples.

2. Specific biomarkers associated with health or disease.

3. A technology platform to rapidly discriminate the biomarkers[54].

Diagnostic tests based on fluid generally use blood and urine and less frequently the esoteric fluids such as saliva, sweat, and tears. Saliva's popularity has suffered because it lacks "the drama of blood, the sincerity of sweat and the emotional appeal of tears.'" Sweat and tears, however, are difficult to obtain in sufficient quantities for routine testing, and urine will always lack the charisma of the other fluids. Saliva, by default, therefore becomes the most favoured alternative to blood[55].

Most molecules present in blood or urine can also be detected in salivary secretions. Their concentrations in saliva are usually one tenth to one thousandth of those in blood.

Although highly sensitive methods of detection are required, technical advances have made this feasible. Studies of the correlation between concentrations in blood and saliva have

found examples of excellent concordance (ethanol, cortisol, theophylline, and antibodies to HIV)[55].

Lithium prophylaxis of bipolar disorder is prolonged with the duration of treatment extending over several years. The narrow therapeutic index of lithium combined with long duration of treatment necessitates that adequate blood levels be maintained[44]. The use of saliva or urine collection versus blood collection has many advantages like ease of collection, safety, acceptance by the patient and cost effectiveness.

Totally valid comparisons could not be done between our study and other studies in literature due to paucity of researches devoted to finding correlation between serum, salivary and urine lithium. Also there was a wide variation with respect to age group, study design methodologies employed and instruments used. However a sincere attempt is being made to compare and discuss to the extent possible and permissible.

In the present study all the study subjects were on lithium carbonate (Tablet. Lithosun SR, Sun pharmacy). The mean dose was 820±101.01mg/day (range 600-1200mg). Dose of lithium carbonate was in the range of 900-1800mg/day(eskalith,smith kline & French)in study done by Rosman.A.W et al(41);300-1800mg/day in study done by Ben-Aryeh. H et al[42], mean 1050mg/day with range 500-1600mg/day in study done by Mckeage.M.J[56].

Estimation of lithium can be done by flame emission photometry, atomic absorption spectrometry and ion selective electrode[37]. Atomic absorption spectrophotometer is superior to colorimetric and flame emission spectrophotometric methods for measuring lithium in serum and urine because of its relative lack of susceptibility to interfering substances[57]. Therefore in the present study lithium estimation was done by using atomic absorption spectrometry. Flame emission photometry was used for lithium determination in some studies [7, 8, 38, 39, 42-44, 46, 47, 49]. Atomic absorption spectrometry was used in lithium estimation in earlier studies [40, 41, 52, 56]. Ion selective electrode was used in lithium estimation in study done by El-Mallakh RS et al[50].

Saliva lithium concentration was more than serum lithium concentration. The mean ratio of salivary lithium to serum lithium was 2.57±0.91. Similar results were

observed in previous studies[7, 8, 41, 43, 47].Higher ratio of 3.64±1.04 was reported by Khare CB et al[44]. while Weller EB et al[46] found lower ratio 1.82±0.29 when compared to the present study. The reason behind getting a higher saliva : serum lithium ratio might be that we employed unstimulated saliva for estimation. Similar technique was used by Khare C B et al[44] . Unstimulated saliva was preferred over stimulated saliva in this study as this unstimulated flow, is what is secreted by the salivary glands in normal physiological conditions. Lithium in unstimulated saliva was found to be directly proportional to serum concentration. lithium in stimulated saliva tends to be a negative function of flow rate[58].

Mean serum lithium was 0.75±0.25 mEq/L in the present study. Lower mean serum lithium was found to be by Sankaranarayanan A et al(0.59±0.19 mEq/L) and by Khare CB et al 0.65±0.24 mEq/L[44, 47] .Higher levels were found by Weller EB et al(1±0.2 mEq/L) and by Rosman AW et al(0.91±0.27 mEq/L)[41, 46] .These differences can be attributed to differences in pharmacological preparations, dosage and duration of lithium therapy.

Mean salivary lithium was 1.91±0.80 mEq/L in the present study. Salivary lithium concentration was found to be 1.56 mEq/L by Neu C et al[7] , 1.37±0.97 mEq/L[47] by Sankaranarayanan A et al[47] which was lesser than the present study. Higher saliva lithium was found by some other studies. The slight differences which were found can be ascribed to differences in saliva collection technique and dosage of lithium.

In the present study Correlation between serum lithium and salivary lithium was statistically significant (r=0.695, p <0.001). Similar correlations were found in studies done by Sankaranarayanan A et al[47] (r=0.59, p <0.005), Khare CB et al[44] (r=0.73) and Nataraj G et al [43] (r=0.71, p <0.001)[43, 44, 47] . Higher correlations were found in other studies [7, 8, 45, 50] . Lower correlations were found in studies done by Prakash,R.S et al (r=0.41, p<0.001) and Rosman AW et al(r=0.5) [39, 41].

The linear regression equation derived was: **Serum lithium = 0.332 + 0.221 X Salivary lithium**. This equation was utilized to calculate the serum lithium levels from salivary lithium levels measured. The correlation between the calculated and the measured serum lithium was highly significant(r=0.695, p<0.001).Our results support the assumption of several previous reports[7, 8, 42] that recommend salivary lithium measurements for monitoring serum lithium. Other finding in studies done by Sims A et al[38] and Mathew RJ et al[40] dispute the usefulness of salivary lithium for monitoring probably because of methodological differences in saliva collection and patient selection.

Several factors have been found to contribute to variability of salivary lithium concentrations. These include blood ion concentration, stimulation of salivary glands and aspects of lithium administration such as dosage and duration of treatment. Another potential variable can be mucopolysaccharide content. El Mallakh RS et al[50] found that when all mucinous material is removed from the saliva by filtration the agreement between the lithium levels of ultrafilterate and plasma is stronger than when saliva is centrifuged and unfiltered supernatant is measured.

More correlation was found in females($r=0.770$, $p<0.001$) than in males($r=0.665$, $p<0.001$).Exact reason for this finding is not known.

Correlation of ($r=0.234$, $p= 0.102$) was found between serum lithium and urine lithium levels which had no clinical or statistical significance. There is scarcity of literature demonstrating any correlation between serum and urine lithium levels. In a study done by Kyroudis A et al [52] , a good intra -subject correlation between urinary excretion rate and plasma concentration was found. Intersubject variability might have accounted for a statistically insignificant correlation in our study which used inter-subject design. It can be deduced that there are many difficulties to be overcome in using the urinary excretion rate of lithium due to inter subject variation, intra-subject variation during the night and circadian variation in the renal excretion of lithium.

Rosman AW et al[41]suggest that one must use the linear regression equation derived from intrasubject not intersubject data. These intrasubject estimates of serum lithium concentrations can be used safely in the clinically stabilized manic patient on prophylactic lithium therapy. Although correlation found in our study was good and statistically significant, it was lesser than few other studies which employed intrasubject correlation.

CONCLUSION

The relationship found between serum and salivary lithium was statistically significant but not strong enough to allow saliva monitoring as a substitute of serum lithium estimation in patients on lithium carbonate therapy. Further studies are needed in this arena which employs intersubject as well as intrasubject correlation design, to establish salivary therapeutic monitoring as a viable option for patients on lithium carbonate therapy.

The relationship found between serum and urine lithium was not statistically significant therefore urine lithium estimation may not be a suitable alternative for monitoring lithium therapy.

SUMMARY

The present study is a cross sectional study to compare serum lithium, salivary lithium and urinary lithium in patients on lithium carbonate admitted in Department of Psychiatry of KLE'S Dr.Prabhakar Kore Hospital and Medical Research Centre, Belgaum.

Blood, saliva and urine samples were collected in the morning (12hrs after the last dose of lithium carbonate) before the patient consumed breakfast and the morning dose of lithium. Samples were collected under aseptic precautionary measures.

Estimation of serum, salivary and urine lithium was done using atomic absorption spectrophotometer. Data was entered into Microsoft excel and analysed using SPSS(version 17). Statistical tests of significance employed were Pearson's correlation coefficient,student 't' test and linear regression analysis.

Following are our observations:

1. There is a statistically significant correlation between serum and salivary lithium levels.($r= 0.695$, $p<0.001$)
2. More correlation in serum and salivary lithium levels was observed in females ($r =0.77$, $p=<0.001$) than in males ($r=0.665$, $p=<0.001$)
3. Correlation observed between serum lithium and urine lithium was not statistically significant.

Our study showed that there is a significant correlation between serum and salivary lithium levels and it calls for further studies to throw more light in this field of salivary lithium monitoring.

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Annexure I

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No.3/2/2010-2/PG-thesis-MPD-3
Dated: 06.08.2010

To,

Dr. Sindhu J Shetty,
Room No.122, New NRI Girls Hostel,
Nehru Nagar,
JN Medical College Campus,
Belgaum-590010.

Dear Dr. Shetty,

This is with reference to your application seeking financial assistance from the Council for MD/MS/DM/MCH dissertation thesis entitled **"A cross sectional study to campare serum lithium, salivary lithium and urinary lithium in patients on lithium carbonate".**

I am glad to inform you that Director General, ICMR, based on the recommendation of Expert Committee, has sanctioned a sum of Rs.25, 000/- (Twenty five thousand only) to you for providing an electronic and hard copy of your dissertation thesis to the Council.

I am enclosing herewith a copy of the detailed guidelines for this scheme and would appreciate that necessary information as per these guidelines may be provided to the undersigned enabling us to release the grant. This is to inform you that Rs.25,000/- will be disbursed to you in two installments. Initial amount of Rs.15,000/- after receipt of the **undertaking** as per the guidelines and remaining amount of Rs.10, 000/- on receipt of the electronic copy, hard copy and summary of work done of your dissertation thesis duly approved by the University/Institution along with one publication in a reputed Journal.

With best wishes,

Yours Sincerely,

(K.K.SINGH)

Copy to: Dr. P B Desai, Prof. & Head, Deptt. Of Biochemistry, J N Medical College, Belgaum-590010.

Annexure II

KLES
DR. PRABHAKAR KORE HOSPITAL
&
MEDICAL RESEARCH CENTRE
NEHRUNAGAR, BELGAUM-590010
KARNATAKA-INDIA

ಕೆ. ಎಲ್. ಇ ಸಂಸ್ಥೆಯ
ಡಾ. ಪ್ರಭಾಕರ ಕೋರೆ ಆಸ್ಪತ್ರೆ ಮತ್ತು
ವೈದ್ಯಕೀಯ ಸಂಶೋಧನಾ ಕೇಂದ್ರ
ನೆಹರುನಗರ, ಬೆಳಗಾವಿ–590010 ಕರ್ನಾಟಕ, ಇಂಡಿಯಾ

Phone : 0831-2473777 (16 Lines)
Fax : 0831-2470732
E-Mail : klehosp@satyam net.in
Website : http://www.kleshospital.org

REF. NO: KLES/PKHOSP/DCS/09-10/ 13867

DATE: 24/03/2010

To,

Dr. Sindhu J. Shetty
1 Year M.D. Bio-chemistry
Dept of Biochemistry
J.N.Medical College
Belgaum.

Sub: Permission to carry out Dissertation work.

1. Ref. to your application on above subject dt 24/02/2010 addressed to MD & CE of the hospital.

2. After perusal of protocol of study, review of literature, consent form and ethical clearance, you are permitted to carry out Dissertation work on "A cross sectional study to compare serum lithium, salivary lithium and urinary lithium in patients on lithium carbonate" in this hospital.

3. You will work under the guidance of Dr. N.M.Patil, Asso. Professor, Psychiatry and Dr. Anil Malleshappa, I/C Biochemistry Dept of the Hospital.

3. The hospital will not have any financial implications for your project.

Brig (Retd) Dr. Dinesh Prasad
Director – Clinical Services
for Medical Director &
Chief Executive.

Copy to:-

1.	Medical Director & CEO	-	Sir, for kind information.
2.	Dr. N.M.Patil Asso. Prof., Psychiatry	-	The candidate will work under your co-guidance and will have no financial implications.
3.	Dr. Anil Malleshappa I/C Bio-chemistry Lab	-	The candidate will work under your co-guidance and will have no financial implications.
4.	Dr. P.B. Desai Prof. & HOD Dept of Biochemistry		

ANNEXURE III

CONSENT FOR PARTICIPATION IN RESEARCH STUDY

Title: "**A cross sectional study to compare serum lithium, salivary lithium and Urinary lithium in patients on lithium carbonate".**

Principal Investigator: Dr. Sindhu.J.Shetty

Guide: Dr. P.B.Desai M.D.

We are requesting you to be a participant in the above said research at KLES Dr. Prabhakar Kore Hospital and MRC,Belgaum being conducted by Dr. Sindhu.J.Shetty, postgraduate student in the department of Biochemistry at J.N.Medical College, Belgaum.

I. **Research purpose:** Patients suffering from mood disorders are advised various medications. Lithium carbonate is one of the main drugs used. When the patient is on lithium therapy frequent serum lithium estimations are done because lithium can cause a lot of problems if levels are lower or higher than prescribed limits. Therefore for serum lithium estimation each time patient has to be pricked and blood has to be collected. I am trying to find out if salivary lithium or urinary lithium can be compared with serum lithium levels so that repeated pricks for drawing blood are avoided.

II.Procedures involved: If you agree to participate in this research you will be asked the relevant history and will be subjected to clinical examination. You will be requested to come in the fasting state the next day. You will be asked to give urine, saliva and blood. 10ml of blood will be collected by intravenous route by pricking a

small blood vessel which may give rise to small amount of pain. Urine, saliva and blood will be collected from you and will be subjected to lithium estimation.

III. Risks and benefits: There are no risks involved in this procedure. If any complications arise during the procedure you will be treated in KLES Dr. Prabhakar Kore Hospital and MRC, Belgaum with the best of our knowledge and the availability of resources in the free hospital. There will be no compensation or payment for such medical treatment.

During the course of the study you will be informed of any significant new findings such as changes in the risks and benefits resulting from participation in the research.

IV. Privacy and confidentiality: The only people who will know that you are a research participant are members of the research team. No information provided by you or about you during the research will be disclosed to others without your written consent.

V. Institutional policy: Your participation in this study is voluntary, whether or not to participate will not affect your current or future relationship with the KLES Dr. Prabhakar Kore Hospital and MRC, Belgaum.

VI. Financial Incentives for participation: You will not receive any reimbursement for participation in the research.

VII. Authorization to Publish Results: When the results of the research are published or discussed in conferences no information will be disclosed that would disclose your identity. Any information obtained in connection with this study and

that can be identified with you will remain confidential and will be disclosed only with your permission.

VIII. Consent Statement: To voluntarily take part in this study I must sign on the line below. If I choose to take part in this study I may withdraw at any time. I am not giving up any of my legal rights by signing this form. My signature below indicates that I have read or the contents of the entire consent form including the risks and benefits have been read to me this and all my questions have been answered. I will be given a copy of this consent form. If I have any questions about the study I can contact Dr.Sindhu.J.Shetty, Phone No 9886165868 and Dr.P.B.Desai M.D. Professor and HOD, Department of Biochemistry, Phone no. , Phone no.08312473777extension 1522.

If I have any questions about my rights as a research participant I may contact Dr. V.D.Patil, Principal and Chairman of JNMC Institutional Ethical Committee for Human Subjects Research, Phone No. 08312471530 at J.N. Medical College, Belgaum.

Signature or left thumb print of participant or legally authorized representative.

Participant's Name: Participant's Signature or thumb print:

Experimenter's Name: Experimenter's Signature:

Witness' Name: Witness' Signature:

Guardian's Name: Guardian's Signature or thumb print:

Date: Place:

Annexure IV

PROFORMA

I. Patient Identification

Name: Age/Sex: I.P.No/O.P.No:

Address: Rural/urban :

Date of examination:

Occupation: Religion:

II. History:

Diagnosis:

Duration of illness:

Drug: Dose:

Duration of treatment:

Other drugs:

Diabetes: Hypertension:

Renal disease:

Personal history:

Bowel and bladder habits:

Menstrual history:

III. General Physical Examination:

Pallor: Weight (kg):

Icterus: Height :

Lymph nodes: Body mass index(BMI):

Temperature:

Pulse: Blood pressure:

Respiratory rate:

Oral cavity:

IV. Investigations:

Blood glucose estimation:

Renal function test :

- Blood urea
- serum creatinine

Thyroid function test:

Urine analysis:

Complete hemogram:

Lithium estmation:

- Serum lithium
- Salivary lithium
- Urinary lithium

ANNEXURE V

MASTER CHART

SERIAL NO	NAME	SEX	AGE	SERUM LI	SALIVA LI	URINE LI	DOSE
1	SU.	F	32	0.53	0.92	5.53	1200
2	DE.	F	18	0.48	0.96	5.84	800
3	SHI.	M	24	0.62	1.57	1.85	800
4	L.	F	18	0.64	0.84	6.73	800
5	KG.	M	40	0.34	0.95	7.46	800
6	B.	M	22	0.54	0.89	10	800
7	M.	M	36	0.68	1.8	12.5	800
8	R.	F	35	0.6	1.75	7.23	800
9	KU.	M	54	0.63	1.34	2.22	800
10	SG.	M	26	1.08	3.33	2.31	1000
11	MK.	M	18	0.64	1.37	6.9	800

12	**V.**	**M**	**20**	**0.6**	**1.59**	**7**	**800**
13	**SD.**	**M**	**46**	**1.24**	**3.08**	**18.22**	**600**
14	**KS.**	**F**	**43**	**0.54**	**1.32**	**5.1**	**800**
15	**KM.**	**F**	**40**	**0.6**	**2**	**15**	**800**
16	**SHV.**	**M**	**44**	**0.84**	**2.23**	**9**	**800**
17	**JD.**	**F**	**50**	**0.74**	**2.22**	**15.7**	**800**
18	**AS.**	**M**	**55**	**0.49**	**3.45**	**2.54**	**800**
19	**VDY.**	**F**	**24**	**0.74**	**2.49**	**8.51**	**800**
20	**SNT.**	**F**	**39**	**0.94**	**1.74**	**6.07**	**800**
21	**SJT.**	**F**	**20**	**0.73**	**1.69**	**2.27**	**800**
22	**AB.**	**M**	**37**	**0.67**	**1.61**	**11.5**	**800**
23	**SDL.**	**F**	**40**	**1.2**	**3.49**	**5.05**	**1000**
24	**SRT.**	**F**	**30**	**0.68**	**2.13**	**2.68**	**800**
25	**SNK.**	**M**	**48**	**0.78**	**1.49**	**14.8**	**800**
26	**MH.**	**M**	**25**	**1.04**	**2.29**	**5.25**	**800**
27	**KL.**	**M**	**35**	**0.66**	**0.96**	**2.21**	**800**

28	**NM.**	**M**	**41**	**0.9**	**2.5**	**4**	**800**
29	**VN.**	**M**	**28**	**0.6**	**3**	**9**	**800**
30	**SJA.**	**F**	**20**	**0.47**	**1.09**	**2.27**	**800**
31	**SRA.**	**F**	**30**	**0.68**	**1.18**	**2.68**	**800**
32	**SLG.**	**F**	**40**	**1.2**	**2.08**	**5.05**	**1000**
33	**MN.**	**M**	**35**	**0.99**	**2.86**	**14.6**	**800**
34	**SRD.**	**M**	**46**	**1.24**	**1.78**	**18.22**	**600**
35	**BLB.**	**M**	**35**	**0.53**	**0.88**	**10**	**800**
36	**RK.**	**M**	**52**	**0.87**	**2.77**	**1.17**	**800**
37	**SGM.**	**M**	**26**	**1.08**	**2.04**	**2.31**	**1000**
38	**ASK.**	**M**	**39**	**0.43**	**1.26**	**2.54**	**800**
39	**LX.**	**M**	**32**	**0.83**	**2.07**	**5.84**	**800**
40	**HN.**	**M**	**50**	**1.21**	**3.55**	**12.9**	**800**
41	**MT.**	**M**	**35**	**0.57**	**1.58**	**1.58**	**800**
42	**LM.**	**M**	**30**	**0.95**	**3.03**	**13.5**	**1000**
43	**NR.**	**M**	**28**	**0.38**	**0.99**	**1.31**	**800**

44	**MG.**	**M**	**35**	**0.63**	**1.89**	**7.4**	**1000**
46	**ST.**	**F**	**20**	**1.16**	**3.22**	**10.1**	**800**
45	**SKR.**	**M**	**48**	**0.45**	**0.87**	**14.8**	**800**
47	**RG.**	**M**	**28**	**0.42**	**0.94**	**4.9**	**600**
48	**SB.**	**M**	**50**	**1.17**	**2.55**	**3.4**	**800**
49	**GJW.**	**F**	**38**	**0.95**	**1.95**	**1.6**	**800**
50	**SNT.**	**F**	**50**	**0.84**	**2.26**	**9.6**	**800**

INTRODUCTION

Global burden of disease statistics indicate that 4 out of the 10 most important causes of disease worldwide are psychiatric in origin[1].In India the prevalence of major mental and behavioral disease is estimated to be 65/1000 population. Prevalence of mood disorders is estimated to be 16/1000 population[2].

Lithium carbonate is used in the treatment of bipolar disorders, major depressive disorders and schizoaffective disorder. Even though lithium was introduced in psychiatry in 1949 for treatment of mania[3] it was approved by FDA in the United States of America only in 1970 due to concerns about its safety[4]. Evidence for both the safety and the efficacy of lithium salts in the treatment of mania and the prevention of recurrent attacks of bipolar manic-depressive illness is both abundant and convincing[5,6].

When patients are being treated with lithium, periodic measurement of lithium concentration in serum is an essential aspect of patient care[4]. This is important because the amount of lithium required to achieve a therapeutic response depends on concentration of the ion in serum which in turn reflect its distribution throughout the body rather than on the actual amount given per day[7].

Lithium therapy is initiated in divided doses. Once the patient is stabilized, single daily dose is sometimes convenient. In the presence of normal renal function, a total daily dose of 1,200 to 1,800 mg of lithium carbonate generally produces an antimanic serum concentration of 0.8 to 1.2 mEq/l. Maintenance levels of 0.6 to 1.0 mEq/l can usually be attained with 900 to 1200 mg daily. A conservatively low dose is started, perhaps 300mg twice or three times daily, a serum concentration is obtained

after a steady state is reached in 4 or 5 days and the dose is adjusted accordingly. During maintenance therapy, patients must be evaluated clinically and lithium levels determined periodically. Early in the treatment, monthly visits are common later on if the patient is stable for extended period may be seen at intervals of 3, 4 or 6 months[4].

Knowledge of sampling interval (the time between last dose and the drawing of blood), the dose form, and the dosage schedule is vital in the interpretation of serum lithium levels. A twelve hour interval has been adopted as standard and has been defined as follows:

1. The blood should be drawn in the morning,12hrs(±30 minutes) after the last dose.
2. A multiple-dose regimen should be used .
3. A steady –state condition should exist (skipped or extra doses within 4 or 5 days should be avoided)[4].

Lithium therapy is monitored using series of serum determinations of lithium which requires repeated drawing of blood samples. An alternative method of determining lithium level is to assay salivary gland secretions or urine which has several practical advantages such as patients are spared the discomfort of repeated venipunctures. This is all the more important because many patients are afraid of "blood loss". Moreover trained technicians and collection equipment like syringes, needles, sterile gauze etc are not needed thus reducing cost of the investigation. Another important finding is the stability of salivary lithium which is very similar to the stability of serum lithium[8].

Lithium is a metallic ion therefore it is not metabolised nor it is bound to plasma protein. Only the kidneys eliminate lithium. It is filtered by glomerulus and 80% is reabsorbed by proximal tubule but it is not reabsorbed by the distal tubule[9].

Therefore the present study was planned to explore the relationship between serum lithium, salivary lithium and urinary lithium, with the possibility of discovering alternate methods for monitoring patients who are on lithium therapy.

AIMS AND OBJECTIVES

Aim:

Aim of the present study was to explore the relationship between serum lithium, salivary lithium and urinary lithium, with the possibility of discovering alternate methods for monitoring patients who are on lithium therapy

Objectives:

1. Comparing serum lithium, salivary lithium and urinary lithium in patients on lithium carbonate.

2. Evaluate the possibility of replacing serum with salivary or urine lithium estimation for monitoring of patients on lithium carbonate.

REVIEW OF LITERATURE

Lithium has an established use in three main indications: treatment of acute mania, prophylaxis of bipolar episodes and augmentation therapy for severe, refractory depression[10]. Although the psychopharmacological field of bipolar disorders has evolved rapidly during the last 10 years, lithium is still considered the 'gold standard' for these conditions and a first-choice mood stabilizer in recent guidelines[11-13]. Lithium and its augmentation by antidepressants, antipsychotics, and benzodiazepines had been the major approach for the management of bipolar disorder[14].

HISTORY

Lithium salts were used in the nineteenth century as a treatment of gout, sedative and as a putative anticonvulsant. Thereafter, lithium salts were unpopular until the late 1940s, when lithium chloride was employed as a salt substitute for cardiac and other chronically ill patients. This ill-advised use led to several reports of severe intoxication and death and to considerable notoriety concerning lithium salts within the medical profession[15]. Cade, in Australia, while looking for toxic nitrogenous substances in the urine of mental patients for testing in guinea pigs, administered lithium salts to the animals in an attempt to increase the solubility of urates. Lithium carbonate made the animals lethargic, and in an inductive leap, Cade gave lithium carbonate to several agitated or manic psychiatric patients, reporting that this treatment seemed to have a specific effect in mania [3,16] .

Chemistry.

Lithium is the lightest of the alkali metals (group Ia); the salts of this monovalent cation share some characteristics with those of Na^+ and K^+. Li^+ is readily assayed in biological fluids and can be detected in brain tissue by magnetic resonance spectroscopy[17]. Traces of the ion occur normally in animal tissues, but it has no known physiological role. Lithium carbonate and lithium citrate currently are used therapeutically.

Pharmacological Properties

Therapeutic concentrations of lithium ion (Li^+) have almost no discernible psychotropic effects in normal individuals. It is not a sedative, depressant, or euphoriant, and this characteristic differentiates Li^+ from other psychotropic agents[15].The precise mechanism of action of Li^+ as a mood-stabilizing agent remains unknown, although many molecular and cellular actions of Li^+, as well as similarities of actions of other mood-stabilizing agents, including valproate, have been described. The main effect of lithium is probably to inhibit hydrolysis of inositol phosphate, therefore reducing the recycling of free inositol for synthesis of phophotidylinositides. These intracellular molecules are part of the transmembrane signaling system that is important in regulating intracellular calcium ion concentration which subsequently affects neurotransmitter release[18-20] .

Absorption

Water-soluble salts, such as chloride and sulphate, are rapidly and almost completely absorbed from the upper gastrointestinal tract, while the less soluble carbonate salt is absorbed more slowly[21]. Absorption half-lives for standard and

sustained release forms of lithium carbonate is 0.78 ± 0.05 hours and 3.73 ± 0.37 hours, respectively[22].

Distribution

Li^+ initially is distributed in the extracellular fluid, then gradually accumulates in various tissues; it does not bind appreciably to plasma proteins. The concentration gradient across plasma membranes is much smaller than those for Na^+ and K^+. The final volume of distribution (0.7 to 0.9 liter per kilogram) approaches that of total body water and is much lower than that of most other psychotropic agents, which are lipophilic and protein bound. Passage through the blood-brain barrier is slow, and when a steady state is achieved, the concentration of Li^+ in the cerebrospinal fluid and in brain tissue is about 40% to 50% of the concentration in plasma[15].

Metabolism and Excretion

Lithium is not subject to metabolic transformation and is almost exclusively excreted via the kidney as a free ion. Similarly to sodium, it is able to freely cross the glomerular membrane. Eighty percent of lithium is reabsorbed by passive diffusion in the proximal tubules. Its clearance varies from 0.6 to 2.4 L/h with high interindividual variability[21]. Both creatinine clearance and bodyweight are important factors in predicting lithium clearance[23].

The plasma half life of lithium is dependent on the Volume of distribution, clearance and duration of lithium therapy [21]. Mean plasma half life of lithium ranged from 16 to 30 hours in subjects with normal renal function[22, 24-28].

Approximately 95% of a single dose of Li^+ is eliminated in the urine. From one to two-thirds of an acute dose is excreted during a 6 to 12-hour initial phase of

excretion, followed by slow excretion over the next 10 to 14 days. With repeated administration, Li^+ excretion increases during the first 5 to 6 days until a steady state is reached between ingestion and excretion. When therapy with Li^+ is stopped, there is a rapid phase of renal excretion followed by a slow 10 to 14 day phase. Since 80% of the filtered Li^+ is reabsorbed by the proximal renal tubules, clearance of Li^+ by the kidney is about 20% of that for creatinine, ranging between 15 and 30 ml per minute. This rate is somewhat lower in elderly patients (10 to 15 ml per minute). Less than 1% of ingested Li^+ leaves the human body in the feces, and 4% to 5% is secreted in sweat. Li^+ is secreted in saliva in concentrations about twice those in plasma, while its concentration in tears is about equal to that in plasma. Since the ion also is secreted in human milk, women receiving Li^+ should not breast-feed infants[15].

Influence of Intrinsic Factors on Lithium Pharmacokinetics

1. Age

   Elderly patients (usually aged>65 years) require lower doses than younger adults to achieve a desired steady state plasma concentration[23] .

2. Renal disease

   Renal clearance of lithium is decreased in patients with abnormal renal function therefore the risk of lithium intoxication in such patients are increased[21]. In severe renal insufficiency, the contraindication is definite and absolute[29].

3. Obesity

   Lithium clearance was significantly greater for obese subjects than for the control group. The steady-state volume of distribution for the obese group was also significantly less than that for the control group.

4. Pregnancy

Lithium ion passes through the placental barrier[30]. It is recommended to balance the expected benefit of lithium versus the fetal risk[31].Lithium clearance increases by 30–50% in last month of pregnancy and at the time of delivery, clearance of the drug falls to pre-pregnancy levels. Therefore doses must be adapted and therapeutic drug monitoring must be carried out more frequently[30] .

Management of Lithium Therapy

Lithium has narrow therapeutic range therefore its use must confirm to some strict rules. Therapeutic monitoring is the basis for optimal use and dosing of lithium.

Lithium doses should be adjusted on the basis of the concentration in serum drawn preferably 12 hours(security interval 10–14 hours) after the last dose[29]. Serum concentrations at this time are in the 'flat part' of the pharmacokinetic curve [32].In patients receiving once-daily administration, the serum concentration at 24 hours should serve as the control value.

Lithium efficacy is dose dependent and reliably correlates with serum concentrations. The optimal serum lithium concentration for preventing mania and depression in maintenance treatment is not well established[32]. Typically, it has been considered that lithium concentrations should be maintained between 0.6 and 1.0 mmol/L but some authors still favour 0.8–1.2 mmol/L [21].

Before starting treatment with lithium personal and familial medical history, especially with respect to thyroid function, previous heart disease and co-medications has to be noted. Following investigations should be done before starting lithium-

creatinine clearance, blood TSH, free thyroxine, calcium, phosphorus, sugar and electrolytes; pregnancy test if appropriate; ECG in patients aged >40 years; and EEG in patients with a previous history of seizures. Suitable Contraceptive method should be started in female patients[21] .

Lithium therapy is initiated in divided doses. In the presence of normal kidney function, a total daily dose of 1,200 to 1,800 mg of lithium carbonate generally produces an antimanic serum concentration of 0.8 to 1.2 mEq/l. Maintenance levels of 0.6 to 1 mEq/l can usually be attained with 900 to 1,200 mg daily[4] . Sustained-release preparations can be given in a single dose and, to simplify therapeutic monitoring and are best taken at night[29].

In case of sustained-release preparations slower increase in plasma concentrations and lower maximum plasma concentration result in benefits with respect to adverse effects such as tremor, upper gastrointestinal cramping and nausea, rashes, cognitive dulling, urinary frequency and neuromuscular slowing[33].

Measuring serum lithium concentration is usually recommended after 1 week of commencing therapy[34]. As a general rule, blood samples should be taken 12 hours (10–14 hours) after the last drug intake. In case of sustained-release preparations, bearing in mind the later peak of serum lithium concentration,it has been advised that serum concentrations should be maintained within the upper range (0.8–1 mmol/L), rather than within the 0.6–0.8mmol/L range recommended for conventional formulations[25].

The frequency of blood sampling is usually every 1–2 weeks until a desirable serum concentration is achieved, then every 2–3 months for the first 6 months of treatment, and at least every 6–12 months thereafter[35].

After any change in dosage, and when there has been intercurrent disease or any change in co-medication, serum lithium concentration should be checked and adjusted to the desired range, after steady state has been reached [34,35].

During maintenance therapy, patients must be evaluated clinically, lithium levels determined periodically and appropriate laboratory tests performed at regular intervals. Monthly visits are common early in treatment if the clinical course is uncomplicated. Patients who have been stable for extended periods may be seen at intervals of 3, 4 or even 6 months[4].

Serum creatinine must be measured every 6–12 months in order to check renal function. Measurement of serum calcium every 6–12 months and of thyroid status (TSH initially, every 6 months for the first 3 years, then once a year) must be performed[21].

Laboratory Monitoring

Lithium has been measured in virtually every body fluid[4]. Serum analysis is the most useful and is used in clinical practice[4,37]. Concentrations considered to be effective and acceptably safe are between 0.6 and 1.25 mEq per liter. The range of 0.9 to 1.1 mEq per liter is favored for treatment of acutely manic or hypomanic patients. Somewhat lower values (0.6 to 0.75 mEq/l) are considered adequate and are safer for long-term use for prevention of recurrent manic-depressive illness. Some patients may not relapse at concentrations as low as 0.5 to 0.6 mEq/l, and lower levels usually are better tolerated[15]. The concentration of lithium can be determined by flame emission photometry, atomic absorption spectrometry or electrochemically using an ion selective electrode[37].

A study done on 20 patients on lithium carbonate investigated the correlations between serum and mixed saliva and parotid fluid lithium levels. High correlations from 0.90 to 0.95 were found between serum and saliva and serum and parotid fluid. A reproducible constant relationship of 2.26±10% was noted between mixed saliva and serum level. The study recommended that to use saliva most effectively in determining lithium levels the ratio between saliva and serum lithium levels should be calculated for each individual and the obtained ratio should be used to calculate subsequent determination for that individual[7].

A study was done on ten patients receiving lithium carbonate for affective disorder. 24 samples of serum and saliva were collected from them. Correlation coefficient between serum and salivary lithium was +0.88($P<0.01$).The salivary and serum lithium ratio was 2.22±0.5. The study found good degree of stability for saliva lithium levels. A therapeutic range of saliva lithium between 1.5-3 mEq/l was suggested to adjust lithium dosage[8].

The above two studies found high correlation between serum and saliva lithium levels but there was a high individual variation of paired results .To circumvent this problem a study was done using a naturally occurring marker in saliva and serum along with serially paired samples in individual patients. Thirty synchronous samples of serum and saliva was collected from thirty patients on lithium carbonate and lithium estimation was corrected for potassium (natural marker) potassium. Three patients were monitored with a series of 7, 6 and 8 paired results. Lithium was estimated using flame photometer. In 30 patients correlation coefficient was 0.71($P<0.001$). In series of paired samples correlation coefficient was 0.85($P<0.01$), 0.80($P<0.05$) and 0.63($P<0.1$).It was found that saliva levels variation

for fixed serum levels were unacceptably large. The study showed satisfactory correlation from zero through the therapeutic range of lithium but was sceptical about such a correlation in excessive levels of serum lithium[38].

A study was done on 95 patients in India who received lithium carbonate. 309 samples of serum and saliva were collected. Flame photometer was used for lithium estimation. A positive and highly significant correlation was found[39].

A study was done to evaluate the relationship among plasma, RBC and saliva lithium levels using atomic absorption spectrophotometry. 30 synchronous samples of blood and saliva was taken from 9 subjects. High correlation(r=0.569,P <0,001) was found between serum and salivary lithium levels. Correlation between RBC lithium and saliva was less compared to serum and saliva levels. The study found high interindividual and intraindividual correlation[40].

In 11 manic-depressive outpatients on chronic lithium therapy the relationship between serum and saliva lithium was studied. Trough serum and saliva lithium levels were measured using atomic absorption spectrometry every 3 or 4 weeks during clinic visits for a period of atleast 16 weeks. Clinical status of patient was rated according to level of mania. Intersubject analysis showed poor correlation(r=0.5) intrasubject correlation was strong(r=0.72-0.94). The study suggested that patient's saliva can be used to estimate serum lithium levels in clinically stabilised manic-depressive patients on prophylactic lithium therapy. In poorly stabilised patients serum concentration should be monitored[41].

Salivary and serum lithium concentrations were measured using atomic absorption spectrophotometry simultaneously in 118 manic-depressive patients. Lithium concentration in saliva was 2.24 ±0.35 times higher than in serum. An

equation to calculate serum lithium concentration from salivary measurements was derived: Li serum = 0.36 Li saliva + 0.13. Psychotropic drugs had no effect on the salivary:serum ratio. Eighteen patients were followed for several weeks. A significant correlation coefficient($P<0.05$) between salivary and serum lithium concentrations was found in thirteen of the eighteen patients studied[42].

140 synchronous samples of serum and saliva was collected from 28 patients undergoing lithium therapy and was estimated using flame photometer. The mean saliva/serum ratio was calculated from 120 synchronous samples from 24 patients was found to be 2.68. Regression line equation calculated for same population came out to be $Y=0.325+0.22X$. Predictive value of saliva lithium was tested by applying this regression equation and the population mean ratio on 20 samples from the next 4 patients. Prediction was also tried in 24 patients who had given more than 3 synchronous samples using individual mean saliva/serum ratio.

An individual's mean was calculated from the initial 3 synchronous samples and predictive value of saliva was tested on subsequent samples in the same patient by using his mean ratio. This method was found to be better than predicting on the basis of population figures[43].

The usefulness of salivary lithium values for monitoring long-term lithium prophylaxis was studied in 60 patients on lithium therapy. A total of 99 pairs of saliva and serum samples were obtained and analysed using flame photometer. The correlation between serum and salivary lithium levels ($r = 0.73$) was found to be significant at the 1% level. The ratio of salivary serum levels to that of serum was found to range from 1.77 to 6.68. The ratio of 51% of the samples was between 3 and 3.99. The study suggested that it is important to identify the subgroup of patients who

show better correlation of salivary and serum lithium levels and use each individual's ratio to monitor only his or her lithium therapy[44].

The salivary composition and flow rate of 78 patients with primary affective disorders and of 49 healthy volunteers were examined. The former were divided into two groups: Group 1(n=57) patients receiving lithium carbonate and psychoactive drugs, and Group 2(n=21)patients receiving psychoactive drugs only. A significant correlation between salivary and serum lithium was found in patients on chronic lithium therapy. The use of saliva analysis for monitoring lithium dosage was recommended by this study[45].

Serum and saliva lithium levels were simultaneously determined using flame photometer in 14 prepubertal children being treated with lithium carbonate. Saliva and serum lithium levels were strongly correlated. The concentration of lithium in saliva was almost two times (1.82) that in serum. There was more variability in saliva levels than in serum levels. It was possible to predict serum levels from saliva levels using regression analysis. If dose was added to saliva as a second predictor variable, accuracy of the prediction was increased[46].

A study was been carried out to find out the reasons for the wide variations observed in the correlation between serum and saliva lithium levels. Serum/saliva lithium levels were monitored using flame photometer in 10 individuals on 6 occasions. The correlation coefficient (r) varied within individuals from 0.19 to 0.91 ($p < 0.025$) whereas when all the individuals were considered simultaneously, it was 0.59 ($p < 0.005$). It was concluded that prediction of serum lithium levels from salivary lithium levels would only be possible in those individuals showing good

correlation. The variability in this correlation could be mainly due to inter-individual variations in the particular sample of individuals studied[47].

In a study one tablet containing 755 mg of lithium tryptophanate (10.8 mEq of lithium) was administered to eight healthy volunteers. The main pharmacokinetic parameters for the group of subjects were estimated. Pharmacokinetic parameters (mean ± SD) from plasma and saliva were respectively: half life (t1/2) 17 ± 6 vs. 21.8 ± 14 h; mean residence time 23.7 ± 7.4 vs. 24.4 ± 15.3 h; total clearance 30.6 ± 9.3 vs. 28.6 ± 6.2 ml/h/kg; and apparent volume of distribution 0.71 ± 0.20 vs. 0.84 ± 0.37 L/kg. Although the mean pharmacokinetic parameters in plasma and saliva were similar, there was no significant correlation between the calculated parameters in the individual subject ($p > 0.05$). The study concluded that usefulness of monitoring salivary levels of lithium is questionable[48].

Within and between subject variability in serum and salivary lithium concentrations in nine psychiatric inpatients on stable drug regimens undergoing therapy was assessed using criteria for determining biologic variation. Estimation of lithium was done using flame photometer this allows separation of analytic from other measures of variance. There were marked differences in inter- and intra-subject variance for serum and salivary lithium concentrations for serum/salivary ratios. These variances were greater for salivary lithium than for serum concentrations. The results were used to assess analytical performance, the usefulness of the therapeutic range, and the reference change interval. The study found that despite greater variance for salivary concentrations, predicted serum concentrations from predetermined serum/saliva ratios were in good agreement with actual concentrations in most subjects[49].

A study was done to check if dialysis of saliva improves accuracy of saliva lithium determinations. Estimation of lithium was done using lithium sensitive electrode. Saliva has two major components: the aqueous and the mucopolysaccharide portions. Since Li is likely to distribute only in the aqueous fraction, saliva was dialysed through a 3000 Da filter to isolate the aqueous component and determine the Li level in it. Lithium levels in the dialyzed saliva agreed more closely with plasma levels ($r = 0.901$, $p < 0.001$) than did whole saliva ($r = 0.775$, $p = 0.012$). The study concluded that dialysis of saliva may contribute to more accurate saliva Li levels[50].

In a study lithium ions concentration in human serum and saliva was determined using dry-slide technology Vitros 250 Analyser (Ortho Clinical Diagnostic) and atomic absorption spectrometry Perkin Elmer 403 (AAS). Lithium ions were analysed in 100 serum and saliva specimens of patients after oral administration of lithium carbonate (3 x 300 mg) Jadran, Galen Laboratory Rijeka. Saliva and blood were taken 2 and 12 hours after the last dose. At the same time lithium ions at samples of blood and saliva were determined with both methods which showed high level of correlation. The mean difference of lithium ions between saliva and serum was statistically significant for $p<0.05$ using t student test. Saliva constant of elimination Kel = 0.02(-1)h and elimination half life (t(1/2)) was t(1/2)=34.6 h. For serum t(1/2)= 24 h which means that lithium ions elimination is slower from saliva than from serum. That is the reason why probably concentration at saliva is higher than at serum. Lithium elimination is two compartment pharmacokinetic model where important part of compartment are saliva and salivary glands. The study concluded at a certain point in medical treatment it could be expected to use controlled determination of lithium ions in saliva with serum as control[51].

The relationship between the plasma concentration, saliva concentration and urinary excretion rate of lithium was investigated in a study and the possibility of using the saliva concentration or the urinary excretion rate for monitoring dosage was considered. The results in the study support the idea that saliva concentration of lithium could be useful in monitoring dosage but, there are many difficulties to be overcome in using the urinary excretion rate of lithium due to inter-subject variation, intra-subject variation during the night and circadian variation in the renal excretion of lithium[52].

MATERIALS AND METHODS

Source of the data

The present study is a cross sectional study to compare serum lithium, salivary lithium and urinary lithium in patients on lithium carbonate.

Study period

The study was undertaken between December 2009 to March 2011.

SAMPLE SIZE

Sample size was determined to be 50.With α=0. 01 and β=0.2 sample size required to demonstrate correlation 'r'=0.6 minimum sample size of 33 is required.

STUDY POPULATION.

Consisted of in patients of the Department of Psychiatry of KLE'S Dr. Prabhakar Kore Charitable Hospital and Medical Research Centre, Belgaum between the ages of 18-50 years who are on lithium carbonate for more than a week.

CRITERIA FOR SELECTION OF THE STUDY GROUP

Inclusion Criteria**:**

1) Patients who are on lithium carbonate therapy for at least a week.
2) Age: 18-50 years.
3) Renal function test within normal limits.
4) Urine analysis within normal limits.

Exclusion Criteria:

1) Factors affecting normal salivary secretion.
2) Pregnancy
3) Age <18years and >50 years
4) Dehydration
5) Drugs affecting lithium pharmacokinetics :Nonsteroidal anti-inflammatory drugs,Diuretics, angiotensin-converting enzyme inhibitors, angiotensin II receptor type-1 antagoinists, metranidazole, sodium bicarbonate, propranolol.

APPROVAL FROM THE AUTHORITIES:

Permission to conduct the study was obtained from all the concerned authorities viz.

1. Institutional ethics committee on human subjects research of Jawaharlal Nehru medical college, Belgaum.
2. Director –clinical services for medical director and chief executive of KLE'S Dr.Prabhakar Kore Hospital and Medical Research Centre, Belgaum.(Annexure II)
3. University science instrument centre (USIC), Shivaji university, Kolhapur.

OBTAINING INFORMED CONSENT

Informed consent was taken from all the participants in the study. (Annexure III)

SCHEDULING:

This study was carried out for a period of 14 months. It was undertaken during December 2009 to March 2011.

PILOT STUDY

Pilot study was conducted after taking serum, saliva and urine from 3 patients to assess the feasibility of the study and to standardise methods of sample collection.

Patient information

A structured proforma was used to collect sociodemographic and clinical information about the study participants. (Annexure IV)

COLLECTION OF SAMPLE

Patient preparation:

Blood, saliva and urine samples were collected in the morning (12hrs after the last dose of lithium carbonate) before the patient consumed breakfast and the morning dose of lithium. Samples were collected under aseptic precautionary measures.

Serum

5 ml of blood was collected in a plain non vacuum tube and was allowed to stand in room temperature till clot was formed. Serum was separated within one hour of venipuncture by centrifuging the tubes at 3,000 r.p.m for 10 minutes. Serum was pipetted out into a sterile eppendorf tubes.

Saliva

Saliva was collected in a sterile container after asking the patient to rinse mouth with water. Subjects were asked to collect saliva in the mouth and then were

asked to spit into the container till adequate amount was collected. Saliva was centrifuged to remove mucus.

Urine

Mid stream urine was collected in sterile container.

Sample collection was supervised by nursing staff. All the 3 samples were collected within 20 minutes.

All samples were stored in non vacuum sterile tubes at -20 ^{0}C till further analysis.

STATISTICAL TESTS USED

The following methods of statistical analysis have been used in this study. Data was entered in Microsoft excel and analyzed using SPSS (version 17).

Mean and standard deviation of serum lithium, salivary lithium and urinary lithium was computed and compared by using paired't' test. Pearson's correlation coefficient and Linear regression analysis was done between three parameters and computed to know the strength of association between them.

MATERIAL USED IN THE STUDY

1. Non vacuum plain tube.
2. 5 ml disposable syringe.
3. Tourniquet.
4. Sterile container.
5. Automated pipettes and tips.
6. Deionised water.

7. Microcentrifuge tubes.

8. Gloves.

INSTRUMENTS USED IN THE STUDY

1. Centrifuge machine.

2. Flame atomic absorption spectrophotometer (Perkin Elmer Analyst 300).

ESTIMATION OF LITHIUM

Lithium estimation in serum, saliva and urine was done using Atomic Absorption Spectrophotometer (Perkin Elmer Analyst 300). Permission to use the instrument for lithium analysis was obtained from head of University science instrument centre (USIC), Shivaji University, Kolhapur.

SAMPLE PREPARATION

Serum

Serum sample was diluted to 1:10 or 1:5 with deionized water[1, 2] . The dilution ratio was adjusted to ensure that concentrations fall within a suitable absorbance range.

Saliva

Saliva sample was diluted to 1:5 with deionized water . The dilution ratio was adjusted to ensure that concentrations fall within a suitable absorbance range.

Urine

Urine sample was diluted to 1:50 with deionized water[1, 2]. The dilution ratio was adjusted to ensure that concentrations fall within a suitable absorbance range.

Samples were taken in batches of twenty for analysis in flame atomic absorption spectrophotometer.

PRINCIPLE OF FLAME ATOMIC ABSORPTION SPECTROPHOTOMETER

Every element has a specific number of electrons associated with its nucleus. The normal and most stable orbital configuration of an atom is known as the "ground state." If energy is applied to an atom, the energy will be absorbed and an outer electron will be promoted to a less stable configuration known as the "excited state." Since this state is unstable, the atom will immediately return to the "ground state," releasing light energy.

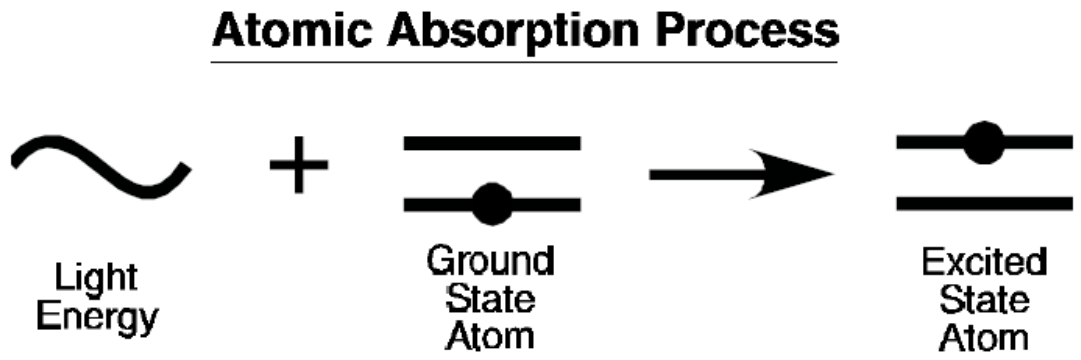


Figure 1: Atomic absorption process.

The "ground state" atom absorbs light energy of a specific wavelength as it enters the "excited state." As the number of atoms in the light path increases, the amount of light absorbed also increases. By measuring the amount of light absorbed, a quantitative determination of the amount of analyte can be made. The use of special light sources and careful selection of wavelengths allow the specific determination of individual elements.

ATOMIC ABSORPTION INSTRUMENTATION

There are five basic components of an atomic absorption instrument:

1. The light source that emits the spectrum of the element of interest
2. An "absorption cell" in which atoms of the sample are produced
3. A monochromator for light dispersion
4. A detector, which measures the light intensity and amplifies the signal.
5. A display that shows the reading after it has been processed by the instrument electronics[37].

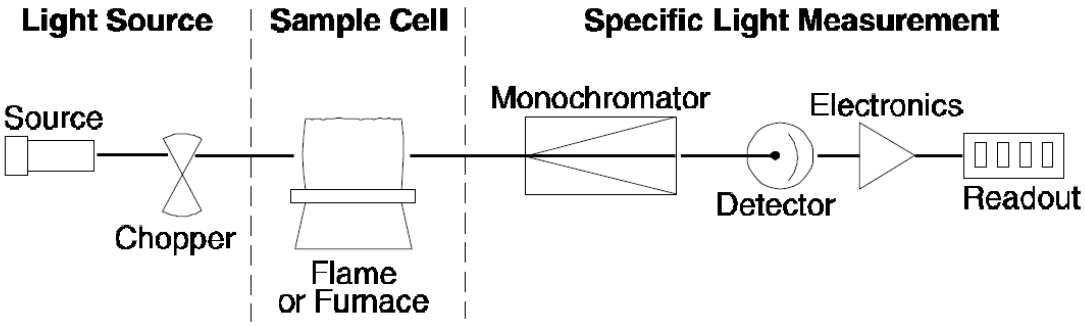


Figure 2: Schematic representation of flame Atomic absorption process.

PROCEDURE

Standard

Appropriate standards are prepared by diluting the lithium stock solution provided by the manufacturer.

Blank

Deionized water is used for blank solution.

ANALYSIS

Instrument was set in standard condition for lithium analysis.

Blank was aspirated first followed by a suitable standard and then the sample. Lithium standard was read after every five sample.

The readings were recorded in p.p.m. and then converted to mEq/l.

PHOTOGRAPHS

Photograph 1: Flame atomic absorption spectrophotometer (Perkin Elmer Analyst 300)

Photograph 2: Investigator performing analysis of lithium on Flame atomic absorption spectrophotometer (Perkin Elmer Analyst 300).

RESULTS

A cross sectional study was done to compare serum lithium, salivary lithium and urinary lithium in patients on lithium carbonate to evaluate the possibility of replacing serum with salivary or urine lithium estimation for monitoring of patients on lithium carbonate.

The data obtained from the study was compiled, tabulated and subjected to statistical analysis. The results are presented here under the headings of the various parameters considered for the study.

Table 1:Distribution of age, serum lithium, salivary lithium, urine lithium and dose of lithium carbonate in 50 study subjects.

The mean age of study participants was 35.10±10.63. Their Mean serum lithium was 0.75±0.25 mEq/l, mean salivary lithium was 1.91±0.80 mEq/l and mean urine lithium was 7.16±4.84 mEq/l. Mean dosage of lithium carbonate prescribed to the subjects was 820±101.01 mg.

Table 2: :Distribution of age, serum lithium, salivary lithium, urine lithium and dose of lithium carbonate in male and female.

The number of female participants was 18 and male participants were 32.The mean age of male participants was 36.50±10.50 years and that of female participants was 32.61±10.69 years. There was no statistically significant difference between the mean ages of males and females (p=0.22).

In males mean serum lithium was 0.75±0.26 mEq/l, mean salivary lithium was 1.91±0.80 mEq/l and mean urine lithium was 7.53±5.26 mEq/l. Mean dosage of lithium carbonate prescribed to the subjects was 806.25±94.82mg.

In females mean serum lithium was 0.76±0.23 mEq/l, mean salivary lithium was 1.85±0.74 mEq/l and mean urine lithium was 6.50±4.06 mEq/l. Mean dosage of lithium carbonate prescribed to the subjects was 844.44±109.66 mg.

There was no statistically significant difference between the mean serum lithium (p=0.91), salivary lithium(p=0.67), urine lithium(p=0.47) and dosages of lithium carbonate (p=0.2) between males and females.

Table 3:Correlation analysis of serum and saliva lithium levels

Correlation between serum lithium and salivary lithium was statistically significant (r=0.695, p <0.001). The serum lithium was more strongly correlated with salivary lithium in females (r=0.770, p <0.001) when compared to males (r=0.665, p <0.001).

Table 4(Graph 1): Linear regression analysis for serum and salivary lithium correlation.

Linear regression analysis for serum and salivary lithium was done with serum lithium as dependent variable, statistically significant correlation was found (r=0.695). The equation for calculating serum lithium from saliva lithium measurements was derived from the graph 1: Y=0.332+0.221X (Y=serum lithium concentration, X=salivary lithium concentration).

Table 4 (Graph 2): Linear regression analysis for serum and salivary lithium correlation for females

In females linear regression analysis for serum and salivary lithium was done with serum lithium as dependent variable, statistically significant correlation was found (r=0.770). The equation for calculating serum lithium from saliva lithium

measurements was derived from the graph 2:Y=0.355+0.271X(Y=serum lithium concentration, X=salivary lithium concentration).

Table 4 (Graph 3): Linear regression analysis for serum and salivary lithium correlation for males

In males linear regression analysis for serum and salivary lithium was done with serum lithium as dependent variable, statistically significant correlation was found (r=0.665). The equation for calculating serum lithium from saliva lithium measurements was derived from the graph 3: Y=0.259+0.204X(Y=serum lithium concentration, X=salivary lithium concentration).

Table 5:Correlation analysis of serum and urine lithium levels.

Correlation between serum lithium and urine lithium was not statistically significant (r=0.234, p =0.102). In males correlation between serum lithium and urine lithium was (r=0.319, p =0.08) in females (r=0.022, p =0.932).

Table 6(Graph 4):Linear regression analysis for serum and urine lithium correlation.

Linear regression analysis for serum and urine lithium was done with serum lithium as dependent variable. Correlation was found to be statistically not significant (r=0.234). Graph 4 showing serum versus urine lithium measurements shows a large deviation.

Table 1: Distribution of age, serum lithium, salivary lithium, urine lithium and dose of lithium carbonate in 50 study subjects.

Sl.No	Variable	Mean±S.D
1	Age (years)	35.10±10.63
2	Serum lithium (mEq/l)	0.75±0.25
3	Salivary lithium (mEq/l)	1.91±0.80
4	Urine lithium (mEq/l)	7.16±4.84
5	Dose of lithium carbonate (mg)	820±101.01

Table 2: Distribution of age, serum lithium, salivary lithium, urine lithium and dose of lithium carbonate in male and female.

Variable	Male (n=32)	Female (n=18)	t value	p value
Age	36.50±10.50	32.61±10.69	1.25	0.22
Serum lithium(mEq/l)	0.75±0.26	0.76±0.23	-0.12	0.91
Salivary lithium(mEq/l)	1.91±0.80	1.85±0.74	0.43	0.67
Urine lithium(mEq/l)	7.53±5.26	6.50±4.06	0.72	0.47
Dose of lithium carbonate (mg)	806.25±94.82	844.44±109.66	-1.29	0.20

Table 3:Correlation analysis of serum and saliva lithium levels.

	r	r^2	S.E	p value
Total study Subjects(n=50)	0.695	0.47	0.18	**<0.001***
Male (n=32)	0.665	0.42	0.20	**<0.001***
Female (n=18)	0.770	0.57	0.16	**<0.001***

*-statistically significant

Table 4:Linear regression analysis for serum and salivary lithium correlation.

	Unstandardized Coefficients		Standardized Coefficients	t	p value.	95.0% Confidence Interval for B	
	B	Std. Error	Beta			Lower Bound	Upper Bound
(Constant)	0.332	0.069		4.835	**<0.001***	0.194	0.470
Salivary Lithium	0.221	0.033	0.695	6.688	**<0.001***	0.155	0.288
Dependent Variable: Serum Lithium *-statistically significant							

Table 5: Correlation analysis of serum and urine lithium levels.

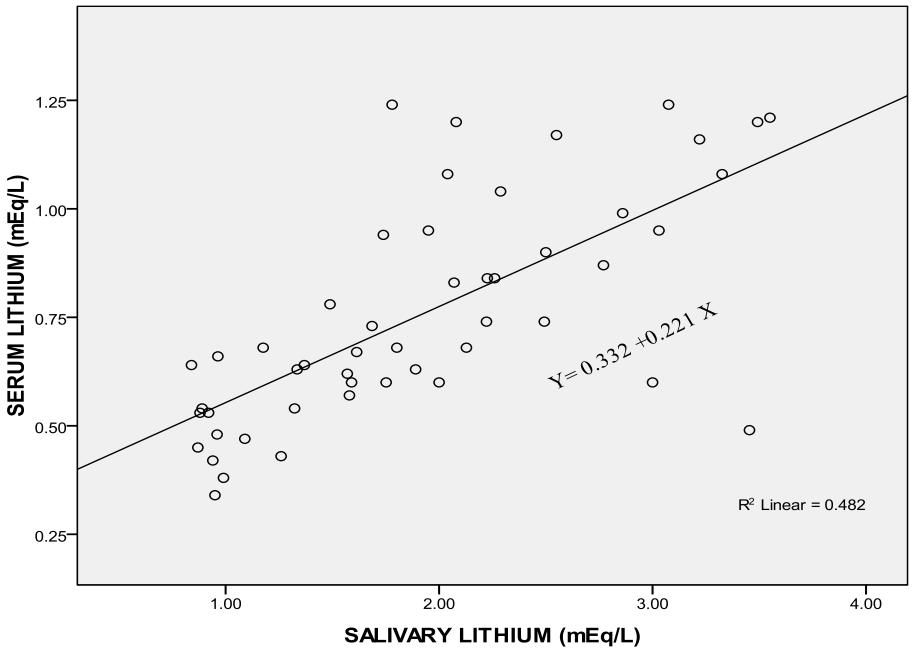
	r	r^2	S.E	p value
Total study subjects(n=50)	0.234	0.04	0.25	0.102
Male (n=32)	0.319	0.07	0.26	0.08
Female (n=18)	0.022	-0.06	0.25	0.932

Table 6:Linear regression analysis for serum and urine lithium .

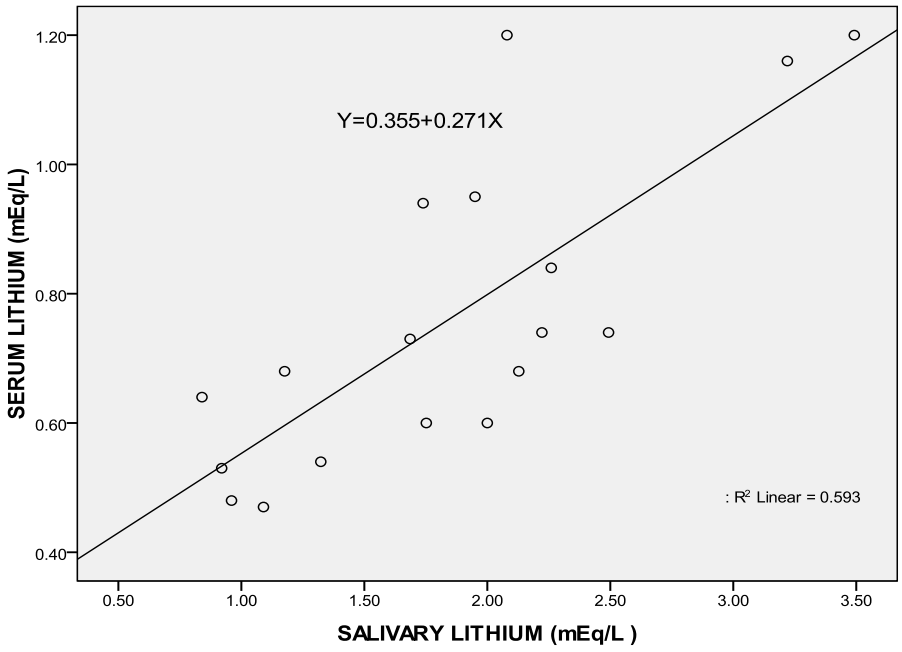
Model	Unstandardized Coefficients		Standardized Coefficients	t	p value
	B	Std. Error	Beta		
(Constant)	0.668	0.064		10.459	**<0.001***
Urine Lithium	0.012	0.007	0.234	1.667	0.102
Dependent Variable: Serum Lithium *-statistically significant					

GRAPHS

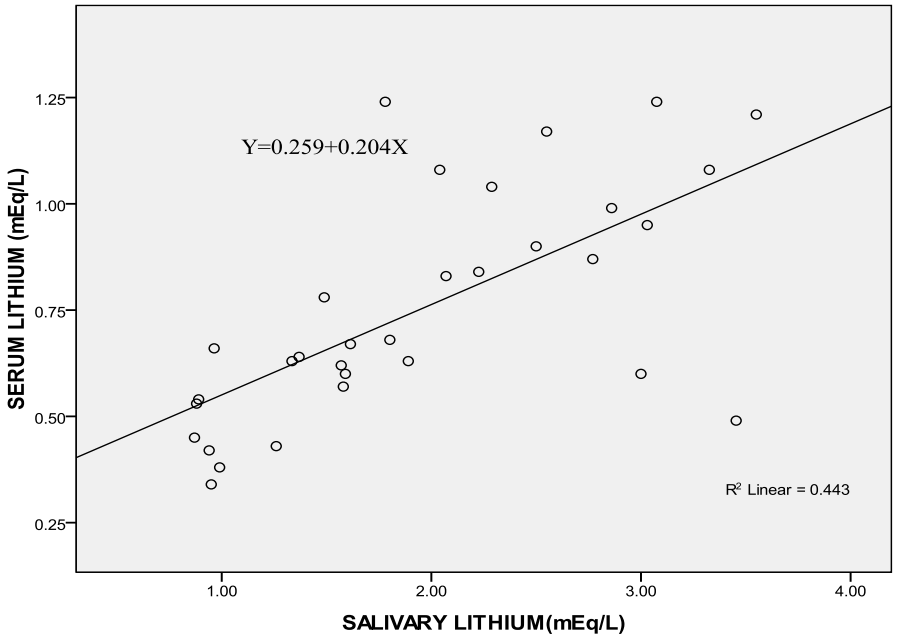
Graph 1: Linear regression analysis of serum versus salivary lithium of 50 study participants



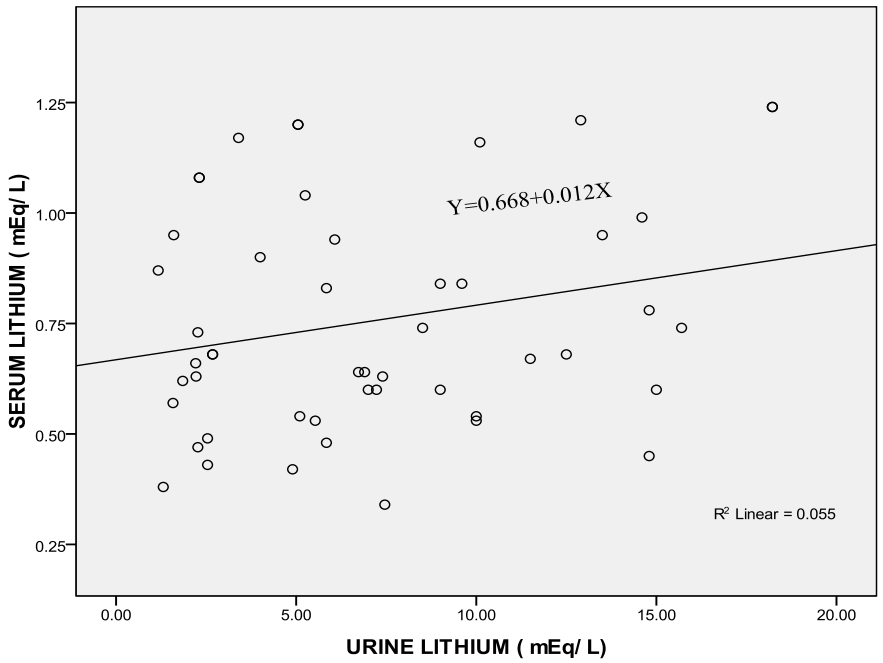
Graph 2: Linear regression analysis of serum versus salivary lithium in female participants.



Graph 3: Linear regression analysis of serum versus salivary lithium in male participants.



Graph 4: Linear regression analysis of serum versus urine lithium of 50 study participants



DISCUSSION

Lithium carbonate is used in the treatment of psychiatric and non psychiatric disorders[53]. Lithium has an established use in treatment of acute mania, prophylaxis of bipolar episodes and augmentation therapy for severe, refractory depression[10]. The difference between toxic and therapeutic dose of lithium carbonate is narrow. Lithium therapy must be guided by monitoring plasma concentration it has been considered that lithium concentrations should be maintained between 0.6 and 1.0 mmol/L[21]. At plasma concentrations above 1.5mmol/L is associated with signs of intoxication mainly affecting gastrointestinal and neurological system. Frank toxicity is associated with plasma concentration greater than 2mmol/L which is an acute medical emergency. Therefore when patients are on lithium strict monitoring of serum levels are done since the amount of lithium required to achieve a therapeutic response depends on concentration of ion in serum which reflects its distribution throughout the body rather than on actual amount required per day[7] .

The frequency of blood sampling for lithium monitoring is usually every 1–2 weeks until a desirable serum concentration is achieved, then every 2–3 months for the first 6 months of treatment, and at least every 6–12 months thereafter[35]. These series of lithium monitoring requires repeated drawing of blood samples.

The desirable goal in the health care delivery and health research is to monitor health status, disease onset, disease progression, and treatment outcome noninvasively. There are three prerequisites necessary to reach this goal

1. A non-invasive method for collecting biological samples.
2. Specific biomarkers associated with health or disease.
3. A technology platform to rapidly discriminate the biomarkers[54].

Diagnostic tests based on fluid generally use blood and urine and less frequently the esoteric fluids such as saliva, sweat, and tears. Saliva's popularity has suffered because it lacks "the drama of blood, the sincerity of sweat and the emotional appeal of tears.'" Sweat and tears, however, are difficult to obtain in sufficient quantities for routine testing, and urine will always lack the charisma of the other fluids. Saliva, by default, therefore becomes the most favoured alternative to blood[55].

Most molecules present in blood or urine can also be detected in salivary secretions. Their concentrations in saliva are usually one tenth to one thousandth of those in blood.

Although highly sensitive methods of detection are required, technical advances have made this feasible. Studies of the correlation between concentrations in blood and saliva have found examples of excellent concordance (ethanol, cortisol, theophylline, and antibodies to HIV)[55].

Lithium prophylaxis of bipolar disorder is prolonged with the duration of treatment extending over several years. The narrow therapeutic index of lithium combined with long duration of treatment necessitates that adequate blood levels be maintained[44]. The use of saliva or urine collection versus blood collection has many advantages like ease of collection, safety, acceptance by the patient and cost effectiveness.

Totally valid comparisons could not be done between our study and other studies in literature due to paucity of researches devoted to finding correlation between serum, salivary and urine lithium. Also there was a wide variation with respect to age group, study design methodologies employed and instruments used.

However a sincere attempt is being made to compare and discuss to the extent possible and permissible.

In the present study all the study subjects were on lithium carbonate (Tablet. Lithosun SR, Sun pharmacy). The mean dose was 820±101.01mg/day (range 600-1200mg). Dose of lithium carbonate was in the range of 900-1800mg/day (eskalith, smith kline & French)in study done by Rosman .A.W et al(41);300-1800mg/day in study done by Ben-Aryeh. H et al[42], mean 1050mg/day with range 500-1600mg/day in study done by Mckeage.M.J[56].

Estimation of lithium can be done by flame emission photometry, atomic absorption spectrometry and ion selective electrode[37]. Atomic absorption spectrophotometer is superior to colorimetric and flame emission spectrophotometric methods for measuring lithium in serum and urine because of its relative lack of susceptibility to interfering substances[57]. Therefore in the present study lithium estimation was done by using atomic absorption spectrometry. Flame emission photometry was used for lithium determination in some studies [7, 8, 38, 39, 42-44, 46, 47, 49]. Atomic absorption spectrometry was used in lithium estimation in earlier studies [40, 41, 52, 56]. Ion selective electrode was used in lithium estimation in study done by El-Mallakh RS et al[50].

Saliva lithium concentration was more than serum lithium concentration. The mean ratio of salivary lithium to serum lithium was 2.57±0.91. Similar results were observed in previous studies[7, 8, 41, 43, 47].Higher ratio of 3.64±1.04 was reported by Khare CB et al[44]. while Weller EB et al[46] found lower ratio 1.82±0.29 when compared to the present study. The reason behind getting a higher saliva : serum lithium ratio might be that we employed unstimulated saliva for estimation. Similar

technique was used by Khare C B et al[44] . Unstimulated saliva was preferred over stimulated saliva in this study as this unstimulated flow, is what is secreted by the salivary glands in normal physiological conditions. Lithium in unstimulated saliva was found to be directly proportional to serum concentration. Lithium in stimulated saliva tends to be a negative function of flow rate[58].

Mean serum lithium was 0.75±0.25 mEq/l in the present study. Lower mean serum lithium was found to be by Sankaranarayanan A et al(0.59±0.19 mEq/l) and by Khare CB et al 0.65±0.24 mEq/l[44, 47] .Higher levels were found by Weller EB et al(1±0.2 mEq/l) and by Rosman AW et al(0.91±0.27 mEq/l)[41, 46] .These differences can be attributed to differences in pharmacological preparations, dosage and duration of lithium therapy.

Mean salivary lithium was 1.91±0.80 mEq/l in the present study. Salivary lithium concentration was 1.56 mEq/l by Neu C et al[7], 1.37±0.97 mEq/l[47] by Sankaranarayanan A et al[47] which was lesser than the present study. Higher saliva lithium was found by some other studies. The slight differences which were found can be ascribed to differences in saliva collection technique and dosage of lithium.

In the present study Correlation between serum lithium and salivary lithium was statistically significant ($r=0.695$, $p<0.001$). Similar correlations were found in studies done by Sankaranarayanan A et al[47] ($r=0.59$, $p<0.005$), Khare CB et al[44] ($r=0.73$) and Nataraj G et al [43] ($r=0.71$, $p<0.001$) . Higher correlations were found in other studies [7, 8, 45, 50] . Lower correlations were found in studies done by Prakash,R.S et al ($r=0.41$, $p<0.001$) and Rosman AW et al($r=0.5$) [39, 41].

The linear regression equation derived was: **Serum lithium = 0.332 + 0.221 X Salivary lithium**. This equation was utilized to calculate the serum lithium levels from salivary lithium levels measured. The correlation between the calculated and the measured serum lithium was highly significant($r=0.695$, $p<0.001$).Our results support the assumption of several previous reports[7, 8, 42] that recommend salivary lithium measurements for monitoring serum lithium. Other finding in studies done by Sims A et al[38] and Mathew RJ et al[40] dispute the usefulness of salivary lithium for monitoring probably because of methodological differences in saliva collection and patient selection.

Several factors have been found to contribute to the variability of salivary lithium concentrations. These include blood ion concentration, stimulation of salivary glands and aspects of lithium administration such as dosage and duration of treatment. Another potential variable can be mucopolysaccharide content. El Mallakh RS et al[50] found that when all mucinous material is removed from the saliva by filtration the agreement between the lithium levels of ultrafilterate and plasma is stronger than when saliva is centrifuged and unfiltered supernatant is measured.

More correlation was found in females($r=0.770$, $p<0.001$) than in males($r=0.665$, $p<0.001$).Exact reason for this finding is not known.

Correlation of ($r=0.234$, $p= 0.102$) was found between serum lithium and urine lithium levels which had no clinical or statistical significance. There is scarcity of literature demonstrating any correlation between serum and urine lithium levels. In a study done by Kyroudis A et al [52] , a good intra -subject correlation between urinary excretion rate and plasma concentration was found. Intersubject variability might have accounted for a statistically insignificant correlation in our study which used

inter-subject design. It can be deduced that there are many difficulties to be overcome in using the urinary excretion rate of lithium due to inter subject variation, intra-subject variation during the night and circadian variation in the renal excretion of lithium.

Rosman AW et al[41]suggest that one must use the linear regression equation derived from intrasubject not intersubject data. These intrasubject estimates of serum lithium concentrations can be used safely in the clinically stabilized manic patient on prophylactic lithium therapy. Although correlation found in our study was good and statistically significant, it was lesser than few other studies which employed intrasubject correlation.

CONCLUSION

The relationship found between serum and salivary lithium was statistically significant but not strong enough to allow saliva monitoring as a substitute of serum lithium estimation in patients on lithium carbonate therapy. Further studies are needed in this arena which employs intersubject as well as intrasubject correlation design, to establish salivary therapeutic monitoring as a viable option for patients on lithium carbonate therapy.

The relationship found between serum and urine lithium was not statistically significant therefore urine lithium estimation may not be a suitable alternative for monitoring lithium therapy.

SUMMARY

The present study is a cross sectional study to compare serum lithium, salivary lithium and urinary lithium in patients on lithium carbonate admitted in Department of Psychiatry of KLE'S Dr.Prabhakar Kore Hospital and Medical Research Centre, Belgaum.

Blood, saliva and urine samples were collected in the morning (12hrs after the last dose of lithium carbonate) before the patient consumed breakfast and the morning dose of lithium. Samples were collected under aseptic precautionary measures.

Estimation of serum, salivary and urine lithium was done using atomic absorption spectrophotometer. Data was entered into Microsoft excel and analysed using SPSS(version 17). Statistical tests of significance employed were Pearson's correlation coefficient,student 't' test and linear regression analysis.

Following are our observations:

1. There is a statistically significant correlation between serum and salivary lithium levels.($r= 0.695$, $p<0.001$)
2. More correlation in serum and salivary lithium levels was observed in females ($r =0.77$, $p=<0.001$) than in males ($r=0.665$, $p=<0.001$)
3. Correlation observed between serum lithium and urine lithium was not statistically significant.

Our study showed that there is a significant correlation between serum and salivary lithium levels and it calls for further studies to throw more light in this field of salivary lithium monitoring.

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ANNEXURE I

Dr. K. K. SINGH
Scientist – F & Head
Division of Manpower Development

INDIAN COUNCIL OF MEDICAL RESEARCH
Ansari Nagar, New Delhi – 110029, India
Phone: (Off.) 26589753; (Res.) 26266317
Gram: Scientific, Fax: 26588662
E.Mail: singhkeshari@yahoo.com

No.3/2/2010-2/PG-thesis-MPD-3
Dated: 06.08.2010

To,

Dr. Sindhu J Shetty,
Room No.122, New NRI Girls Hostel,
Nehru Nagar,
JN Medical College Campus,
Belgaum-590010.

Dear Dr. Shetty,

This is with reference to your application seeking financial assistance from the Council for MD/MS/DM/MCH dissertation thesis entitled **"A cross sectional study to campare serum lithium, salivary lithium and urinary lithium in patients on lithium carbonate".**

I am glad to inform you that Director General, ICMR, based on the recommendation of Expert Committee, has sanctioned a sum of Rs.25, 000/- (Twenty five thousand only) to you for providing an electronic and hard copy of your dissertation thesis to the Council.

I am enclosing herewith a copy of the detailed guidelines for this scheme and would appreciate that necessary information as per these guidelines may be provided to the undersigned enabling us to release the grant. This is to inform you that Rs.25,000/- will be disbursed to you in two installments. Initial amount of Rs.15,000/- after receipt of the **undertaking** as per the guidelines and remaining amount of Rs.10, 000/- on receipt of the electronic copy, hard copy and summary of work done of your dissertation thesis duly approved by the University/Institution along with one publication in a reputed Journal.

With best wishes,

Yours Sincerely,

(K.K.SINGH)

Copy to: Dr. P B Desai, Prof. & Head, Deptt. Of Biochemistry, J N Medical College, Belgaum-590010.

ANNEXURE II

KLES
DR. PRABHAKAR KORE HOSPITAL
&
MEDICAL RESEARCH CENTRE
NEHRUNAGAR, BELGAUM-590010
KARNATAKA-INDIA

ಕೆ. ಎಲ್. ಇ ಸಂಸ್ಥೆಯ
ಡಾ. ಪ್ರಭಾಕರ ಕೋರೆ ಆಸ್ಪತ್ರೆ ಮತ್ತು
ವೈದ್ಯಕೀಯ ಸಂಶೋಧನಾ ಕೇಂದ್ರ
ನೆಹರುನಗರ, ಬೆಳಗಾವಿ–590010 ಕರ್ನಾಟಕ, ಇಂಡಿಯಾ

Phone : 0831-2473777 (16 Lines)
Fax : 0831-2470732
E-Mail : klehosp@satyam net.in
Website : http://www.kleshospital.org

REF. NO: KLES/PKHOSP/DCS/09-10/ 13867

DATE: 24/03/2010

To,

Dr. Sindhu J. Shetty
1 Year M.D. Bio-chemistry
Dept of Biochemistry
J.N.Medical College
Belgaum.

Sub: Permission to carry out Dissertation work.

1. Ref. to your application on above subject dt 24/02/2010 addressed to MD & CE of the hospital.

2. After perusal of protocol of study, review of literature, consent form and ethical clearance, you are permitted to carry out Dissertation work on "A cross sectional study to compare serum lithium, salivary lithium and urinary lithium in patients on lithium carbonate" in this hospital.

3. You will work under the guidance of Dr. N.M.Patil, Asso. Professor, Psychiatry and Dr. Anil Malleshappa, I/C Biochemistry Dept of the Hospital.

3. The hospital will not have any financial implications for your project.

Brig (Retd) Dr. Dinesh Prasad
Director – Clinical Services
for Medical Director &
Chief Executive.

Copy to:-

1.	Medical Director & CEO	-	Sir, for kind information.
2.	Dr. N.M.Patil Asso. Prof., Psychiatry	-	The candidate will work under your co-guidance and will have no financial implications.
3.	Dr. Anil Malleshappa I/C Bio-chemistry Lab	-	The candidate will work under your co-guidance and will have no financial implications.
4.	Dr. P.B. Desai Prof. & HOD Dept of Biochemistry		

ANNEXURE III

CONSENT FOR PARTICIPATION IN RESEARCH STUDY

Title: "**A cross sectional study to compare serum lithium, salivary lithium and Urinary lithium in patients on lithium carbonate".**

Principal Investigator: Dr. Sindhu.J.Shetty

Guide: Dr. P.B.Desai M.D.

We are requesting you to be a participant in the above said research at KLES Dr. Prabhakar Kore Hospital and MRC,Belgaum being conducted by Dr. Sindhu.J.Shetty, postgraduate student in the department of Biochemistry at J.N.Medical College, Belgaum.

I. Research purpose: Patients suffering from mood disorders are advised various medications. Lithium carbonate is one of the main drugs used. When the patient is on lithium therapy frequent serum lithium estimations are done because lithium can cause a lot of problems if levels are lower or higher than prescribed limits. Therefore for serum lithium estimation each time patient has to be pricked and blood has to be collected. I am trying to find out if salivary lithium or urinary lithium can be compared with serum lithium levels so that repeated pricks for drawing blood are avoided.

II. Procedures involved: If you agree to participate in this research you will be asked the relevant history and will be subjected to clinical examination. You will be requested to come in the fasting state the next day. You will be asked to give urine, saliva and blood. 10ml of blood will be collected by intravenous route by pricking a

small blood vessel which may give rise to small amount of pain. Urine, saliva and blood will be collected from you and will be subjected to lithium estimation.

III. Risks and benefits: There are no risks involved in this procedure. If any complications arise during the procedure you will be treated in KLES Dr. Prabhakar Kore Hospital and MRC, Belgaum with the best of our knowledge and the availability of resources in the free hospital. There will be no compensation or payment for such medical treatment.

During the course of the study you will be informed of any significant new findings such as changes in the risks and benefits resulting from participation in the research.

IV. Privacy and confidentiality: The only people who will know that you are a research participant are members of the research team. No information provided by you or about you during the research will be disclosed to others without your written consent.

V. Institutional policy: Your participation in this study is voluntary, whether or not to participate will not affect your current or future relationship with the KLES Dr. Prabhakar Kore Hospital and MRC, Belgaum.

VI. Financial Incentives for participation: You will not receive any reimbursement for participation in the research.

VII. Authorization to Publish Results: When the results of the research are published or discussed in conferences no information will be disclosed that would disclose your identity. Any information obtained in connection with this study and

that can be identified with you will remain confidential and will be disclosed only with your permission.

VIII. Consent Statement: To voluntarily take part in this study I must sign on the line below. If I choose to take part in this study I may withdraw at any time. I am not giving up any of my legal rights by signing this form. My signature below indicates that I have read or the contents of the entire consent form including the risks and benefits have been read to me this and all my questions have been answered. I will be given a copy of this consent form. If I have any questions about the study I can contact Dr.Sindhu.J.Shetty, Phone No 9886165868 and Dr.P.B.Desai M.D. Professor and HOD, Department of Biochemistry, Phone no. , Phone no.08312473777extension 1522.

If I have any questions about my rights as a research participant I may contact Dr. V.D.Patil, Principal and Chairman of JNMC Institutional Ethical Committee for Human Subjects Research, Phone No. 08312471530 at J.N. Medical College, Belgaum.

Signature or left thumb print of participant or legally authorized representative.

Participant's Name:	Participant's Signature or thumb print:
Experimenter's Name:	Experimenter's Signature:
Witness' Name:	Witness' Signature:
Guardian's Name:	Guardian's Signature or thumb print:
Date:	Place:

ANNEXURE IV

PROFORMA

I. Patient Identification

Name: Age/Sex: I.P.No/O.P.No:

Address: Rural/urban :

Date of examination:

Occupation: Religion:

II. History:

Diagnosis:

Duration of illness:

Drug: Dose:

Duration of treatment:

Other drugs:

Diabetes: Hypertension:

Renal disease:

Personal history:

Bowel and bladder habits:

Menstrual history:

III. General Physical Examination:

Pallor:	Weight (kg):
Icterus:	Height :
Lymph nodes:	Body mass index(BMI):
Temperature:	
Pulse:	Blood pressure:
Respiratory rate:	
Oral cavity:	

IV. Investigations:

Blood glucose estimation:

Renal function test :

- Blood urea
- serum creatinine

Thyroid function test:

Urine analysis:

Complete hemogram:

Lithium estimation:

- Serum lithium
- Salivary lithium
- Urinary lithium

ANNEXURE V

MASTER CHART

SERIAL NO	NAME	SEX	AGE	SERUM LI	SALIVA LI	URINE LI	DOSE
1	SU.	F	32	0.53	0.92	5.53	1200
2	DE.	F	18	0.48	0.96	5.84	800
3	SHI.	M	24	0.62	1.57	1.85	800
4	L.	F	18	0.64	0.84	6.73	800
5	KG.	M	40	0.34	0.95	7.46	800
6	B.	M	22	0.54	0.89	10	800
7	M.	M	36	0.68	1.8	12.5	800
8	R.	F	35	0.6	1.75	7.23	800
9	KU.	M	54	0.63	1.34	2.22	800
10	SG.	M	26	1.08	3.33	2.31	1000
11	MK.	M	18	0.64	1.37	6.9	800
12	V.	M	20	0.6	1.59	7	800
13	SD.	M	46	1.24	3.08	18.22	600
14	KS.	F	43	0.54	1.32	5.1	800
15	KM.	F	40	0.6	2	15	800
16	SHV.	M	44	0.84	2.23	9	800
17	JD.	F	50	0.74	2.22	15.7	800
18	AS.	M	55	0.49	3.45	2.54	800
19	VDY.	F	24	0.74	2.49	8.51	800
20	SNT.	F	39	0.94	1.74	6.07	800
21	SJT.	F	20	0.73	1.69	2.27	800

22	AB.	M	37	0.67	1.61	11.5	800
23	SDL.	F	40	1.2	3.49	5.05	1000
24	SRT.	F	30	0.68	2.13	2.68	800
25	SNK.	M	48	0.78	1.49	14.8	800
26	MH.	M	25	1.04	2.29	5.25	800
27	KL.	M	35	0.66	0.96	2.21	800
28	NM.	M	41	0.9	2.5	4	800
29	VN.	M	28	0.6	3	9	800
30	SJA.	F	20	0.47	1.09	2.27	800
31	SRA.	F	30	0.68	1.18	2.68	800
32	SLG.	F	40	1.2	2.08	5.05	1000
33	MN.	M	35	0.99	2.86	14.6	800
34	SRD.	M	46	1.24	1.78	18.22	600
35	BLB.	M	35	0.53	0.88	10	800
36	RK.	M	52	0.87	2.77	1.17	800
37	SGM.	M	26	1.08	2.04	2.31	1000
38	ASK.	M	39	0.43	1.26	2.54	800
39	LX.	M	32	0.83	2.07	5.84	800
40	HN.	M	50	1.21	3.55	12.9	800
41	MT.	M	35	0.57	1.58	1.58	800
42	LM.	M	30	0.95	3.03	13.5	1000
43	NR.	M	28	0.38	0.99	1.31	800
44	MG.	M	35	0.63	1.89	7.4	1000
46	ST.	F	20	1.16	3.22	10.1	800
45	SKR.	M	48	0.45	0.87	14.8	800

47	RG.	M	28	0.42	0.94	4.9	600
48	SB.	M	50	1.17	2.55	3.4	800
49	GJW.	F	38	0.95	1.95	1.6	800
50	SNT.	F	50	0.84	2.26	9.6	800

INTRODUCTION

Global burden of disease statistics indicate that 4 out of the 10 most important causes of disease worldwide are psychiatric in origin[1].In India the prevalence of major mental and behavioral disease is estimated to be 65/1000 population. Prevalence of mood disorders is estimated to be 16/1000 population[2].

Lithium carbonate is used in the treatment of bipolar disorders, major depressive disorders and schizoaffective disorder. Even though lithium was introduced in psychiatry in 1949 for treatment of mania[3] it was approved by FDA in the United States of America only in 1970 due to concerns about its safety[4]. Evidence for both the safety and the efficacy of lithium salts in the treatment of mania and the prevention of recurrent attacks of bipolar manic-depressive illness is both abundant and convincing[5,6].

When patients are being treated with lithium, periodic measurement of lithium concentration in serum is an essential aspect of patient care[4]. This is important because the amount of lithium required to achieve a therapeutic response depends on concentration of the ion in serum which in turn reflect its distribution throughout the body rather than on the actual amount given per day[7].

Lithium therapy is initiated in divided doses. Once the patient is stabilized, single daily dose is sometimes convenient. In the presence of normal renal function, a total daily dose of 1,200 to 1,800 mg of lithium carbonate generally produces an antimanic serum concentration of 0.8 to 1.2 mEq/l. Maintenance levels of 0.6 to 1.0 mEq/l can usually be attained with 900 to 1200 mg daily. A conservatively low dose is started, perhaps 300mg twice or three times daily, a serum concentration is obtained

after a steady state is reached in 4 or 5 days and the dose is adjusted accordingly. During maintenance therapy, patients must be evaluated clinically and lithium levels determined periodically. Early in the treatment, monthly visits are common later on if the patient is stable for extended period may be seen at intervals of 3, 4 or 6 months[4].

Knowledge of sampling interval (the time between last dose and the drawing of blood), the dose form, and the dosage schedule is vital in the interpretation of serum lithium levels. A twelve hour interval has been adopted as standard and has been defined as follows:

1. The blood should be drawn in the morning,12hrs(±30 minutes) after the last dose.
2. A multiple-dose regimen should be used .
3. A steady –state condition should exist (skipped or extra doses within 4 or 5 days should be avoided)[4].

Lithium therapy is monitored using series of serum determinations of lithium which requires repeated drawing of blood samples. An alternative method of determining lithium level is to assay salivary gland secretions or urine which has several practical advantages such as patients are spared the discomfort of repeated venipunctures. This is all the more important because many patients are afraid of "blood loss". Moreover trained technicians and collection equipment like syringes, needles, sterile gauze etc are not needed thus reducing cost of the investigation. Another important finding is the stability of salivary lithium which is very similar to the stability of serum lithium[8].

Lithium is a metallic ion therefore it is not metabolised nor it is bound to plasma protein. Only the kidneys eliminate lithium. It is filtered by glomerulus and 80% is reabsorbed by proximal tubule but it is not reabsorbed by the distal tubule[9].

Therefore the present study was planned to explore the relationship between serum lithium, salivary lithium and urinary lithium, with the possibility of discovering alternate methods for monitoring patients who are on lithium therapy.

AIMS AND OBJECTIVES

Aim:

Aim of the present study was to explore the relationship between serum lithium, salivary lithium and urinary lithium, with the possibility of discovering alternate methods for monitoring patients who are on lithium therapy

Objectives:

1. Comparing serum lithium, salivary lithium and urinary lithium in patients on lithium carbonate.

2. Evaluate the possibility of replacing serum with salivary or urine lithium estimation for monitoring of patients on lithium carbonate.

REVIEW OF LITERATURE

Lithium has an established use in three main indications: treatment of acute mania, prophylaxis of bipolar episodes and augmentation therapy for severe, refractory depression[10]. Although the psychopharmacological field of bipolar disorders has evolved rapidly during the last 10 years, lithium is still considered the 'gold standard' for these conditions and a first-choice mood stabilizer in recent guidelines[11-13]. Lithium and its augmentation by antidepressants, antipsychotics, and benzodiazepines had been the major approach for the management of bipolar disorder[14].

HISTORY

Lithium salts were used in the nineteenth century as a treatment of gout, sedative and as a putative anticonvulsant. Thereafter, lithium salts were unpopular until the late 1940s, when lithium chloride was employed as a salt substitute for cardiac and other chronically ill patients. This ill-advised use led to several reports of severe intoxication and death and to considerable notoriety concerning lithium salts within the medical profession[15]. Cade, in Australia, while looking for toxic nitrogenous substances in the urine of mental patients for testing in guinea pigs, administered lithium salts to the animals in an attempt to increase the solubility of urates. Lithium carbonate made the animals lethargic, and in an inductive leap, Cade gave lithium carbonate to several agitated or manic psychiatric patients, reporting that this treatment seemed to have a specific effect in mania [3,16] .

Chemistry.

Lithium is the lightest of the alkali metals (group Ia); the salts of this monovalent cation share some characteristics with those of Na^+ and K^+. Li^+ is readily assayed in biological fluids and can be detected in brain tissue by magnetic resonance spectroscopy[17]. Traces of the ion occur normally in animal tissues, but it has no known physiological role. Lithium carbonate and lithium citrate currently are used therapeutically.

Pharmacological Properties

Therapeutic concentrations of lithium ion (Li^+) have almost no discernible psychotropic effects in normal individuals. It is not a sedative, depressant, or euphoriant, and this characteristic differentiates Li^+ from other psychotropic agents[15].The precise mechanism of action of Li^+ as a mood-stabilizing agent remains unknown, although many molecular and cellular actions of Li^+, as well as similarities of actions of other mood-stabilizing agents, including valproate, have been described. The main effect of lithium is probably to inhibit hydrolysis of inositol phosphate, therefore reducing the recycling of free inositol for synthesis of phophotidylinositides. These intracellular molecules are part of the transmembrane signaling system that is important in regulating intracellular calcium ion concentration which subsequently affects neurotransmitter release[18-20] .

Absorption

Water-soluble salts, such as chloride and sulphate, are rapidly and almost completely absorbed from the upper gastrointestinal tract, while the less soluble carbonate salt is absorbed more slowly[21]. Absorption half-lives for standard and

sustained release forms of lithium carbonate is 0.78 ± 0.05 hours and 3.73 ± 0.37 hours, respectively[22].

Distribution

Li^+ initially is distributed in the extracellular fluid, then gradually accumulates in various tissues; it does not bind appreciably to plasma proteins. The concentration gradient across plasma membranes is much smaller than those for Na^+ and K^+. The final volume of distribution (0.7 to 0.9 liter per kilogram) approaches that of total body water and is much lower than that of most other psychotropic agents, which are lipophilic and protein bound. Passage through the blood-brain barrier is slow, and when a steady state is achieved, the concentration of Li^+ in the cerebrospinal fluid and in brain tissue is about 40% to 50% of the concentration in plasma[15].

Metabolism and Excretion

Lithium is not subject to metabolic transformation and is almost exclusively excreted via the kidney as a free ion. Similarly to sodium, it is able to freely cross the glomerular membrane. Eighty percent of lithium is reabsorbed by passive diffusion in the proximal tubules. Its clearance varies from 0.6 to 2.4 L/h with high interindividual variability[21]. Both creatinine clearance and bodyweight are important factors in predicting lithium clearance[23].

The plasma half life of lithium is dependent on the Volume of distribution, clearance and duration of lithium therapy [21]. Mean plasma half life of lithium ranged from 16 to 30 hours in subjects with normal renal function[22, 24-28].

Approximately 95% of a single dose of Li^+ is eliminated in the urine. From one to two-thirds of an acute dose is excreted during a 6 to 12-hour initial phase of

excretion, followed by slow excretion over the next 10 to 14 days. With repeated administration, Li^+ excretion increases during the first 5 to 6 days until a steady state is reached between ingestion and excretion. When therapy with Li^+ is stopped, there is a rapid phase of renal excretion followed by a slow 10 to 14 day phase. Since 80% of the filtered Li^+ is reabsorbed by the proximal renal tubules, clearance of Li^+ by the kidney is about 20% of that for creatinine, ranging between 15 and 30 ml per minute. This rate is somewhat lower in elderly patients (10 to 15 ml per minute). Less than 1% of ingested Li^+ leaves the human body in the feces, and 4% to 5% is secreted in sweat. Li^+ is secreted in saliva in concentrations about twice those in plasma, while its concentration in tears is about equal to that in plasma. Since the ion also is secreted in human milk, women receiving Li^+ should not breast-feed infants[15].

Influence of Intrinsic Factors on Lithium Pharmacokinetics

1. Age

   Elderly patients (usually aged>65 years) require lower doses than younger adults to achieve a desired steady state plasma concentration[23] .

2. Renal disease

   Renal clearance of lithium is decreased in patients with abnormal renal function therefore the risk of lithium intoxication in such patients are increased[21]. In severe renal insufficiency, the contraindication is definite and absolute[29].

3. Obesity

   Lithium clearance was significantly greater for obese subjects than for the control group. The steady-state volume of distribution for the obese group was also significantly less than that for the control group.

4. Pregnancy

Lithium ion passes through the placental barrier[30]. It is recommended to balance the expected benefit of lithium versus the fetal risk[31].Lithium clearance increases by 30–50% in last month of pregnancy and at the time of delivery, clearance of the drug falls to pre-pregnancy levels. Therefore doses must be adapted and therapeutic drug monitoring must be carried out more frequently[30] .

Management of Lithium Therapy

Lithium has narrow therapeutic range therefore its use must confirm to some strict rules. Therapeutic monitoring is the basis for optimal use and dosing of lithium.

Lithium doses should be adjusted on the basis of the concentration in serum drawn preferably 12 hours(security interval 10–14 hours) after the last dose[29]. Serum concentrations at this time are in the 'flat part' of the pharmacokinetic curve [32].In patients receiving once-daily administration, the serum concentration at 24 hours should serve as the control value.

Lithium efficacy is dose dependent and reliably correlates with serum concentrations. The optimal serum lithium concentration for preventing mania and depression in maintenance treatment is not well established[32]. Typically, it has been considered that lithium concentrations should be maintained between 0.6 and 1.0 mmol/L but some authors still favour 0.8–1.2 mmol/L [21].

Before starting treatment with lithium personal and familial medical history, especially with respect to thyroid function, previous heart disease and co-medications has to be noted. Following investigations should be done before starting lithium-

creatinine clearance, blood TSH, free thyroxine, calcium, phosphorus, sugar and electrolytes; pregnancy test if appropriate; ECG in patients aged >40 years; and EEG in patients with a previous history of seizures. Suitable Contraceptive method should be started in female patients[21] .

Lithium therapy is initiated in divided doses. In the presence of normal kidney function, a total daily dose of 1,200 to 1,800 mg of lithium carbonate generally produces an antimanic serum concentration of 0.8 to 1.2 mEq/l. Maintenance levels of 0.6 to 1 mEq/l can usually be attained with 900 to 1,200 mg daily[4] . Sustained-release preparations can be given in a single dose and, to simplify therapeutic monitoring and are best taken at night[29].

In case of sustained-release preparations slower increase in plasma concentrations and lower maximum plasma concentration result in benefits with respect to adverse effects such as tremor, upper gastrointestinal cramping and nausea, rashes, cognitive dulling, urinary frequency and neuromuscular slowing[33].

Measuring serum lithium concentration is usually recommended after 1 week of commencing therapy[34]. As a general rule, blood samples should be taken 12 hours (10–14 hours) after the last drug intake. In case of sustained-release preparations, bearing in mind the later peak of serum lithium concentration,it has been advised that serum concentrations should be maintained within the upper range (0.8–1 mmol/L), rather than within the 0.6–0.8mmol/L range recommended for conventional formulations[25].

The frequency of blood sampling is usually every 1–2 weeks until a desirable serum concentration is achieved, then every 2–3 months for the first 6 months of treatment, and at least every 6–12 months thereafter[35].

After any change in dosage, and when there has been intercurrent disease or any change in co-medication, serum lithium concentration should be checked and adjusted to the desired range, after steady state has been reached [34,35].

During maintenance therapy, patients must be evaluated clinically, lithium levels determined periodically and appropriate laboratory tests performed at regular intervals. Monthly visits are common early in treatment if the clinical course is uncomplicated. Patients who have been stable for extended periods may be seen at intervals of 3, 4 or even 6 months[4].

Serum creatinine must be measured every 6–12 months in order to check renal function. Measurement of serum calcium every 6–12 months and of thyroid status (TSH initially, every 6 months for the first 3 years, then once a year) must be performed[21].

Laboratory Monitoring

Lithium has been measured in virtually every body fluid[4]. Serum analysis is the most useful and is used in clinical practice[4,37]. Concentrations considered to be effective and acceptably safe are between 0.6 and 1.25 mEq per liter. The range of 0.9 to 1.1 mEq per liter is favored for treatment of acutely manic or hypomanic patients. Somewhat lower values (0.6 to 0.75 mEq/l) are considered adequate and are safer for long-term use for prevention of recurrent manic-depressive illness. Some patients may not relapse at concentrations as low as 0.5 to 0.6 mEq/l, and lower levels usually are better tolerated[15]. The concentration of lithium can be determined by flame emission photometry, atomic absorption spectrometry or electrochemically using an ion selective electrode[37].

A study done on 20 patients on lithium carbonate investigated the correlations between serum and mixed saliva and parotid fluid lithium levels. High correlations from 0.90 to 0.95 were found between serum and saliva and serum and parotid fluid. A reproducible constant relationship of 2.26±10% was noted between mixed saliva and serum level. The study recommended that to use saliva most effectively in determining lithium levels the ratio between saliva and serum lithium levels should be calculated for each individual and the obtained ratio should be used to calculate subsequent determination for that individual[7].

A study was done on ten patients receiving lithium carbonate for affective disorder. 24 samples of serum and saliva were collected from them. Correlation coefficient between serum and salivary lithium was +0.88($P<0.01$).The salivary and serum lithium ratio was 2.22±0.5. The study found good degree of stability for saliva lithium levels. A therapeutic range of saliva lithium between 1.5-3 mEq/l was suggested to adjust lithium dosage[8].

The above two studies found high correlation between serum and saliva lithium levels but there was a high individual variation of paired results .To circumvent this problem a study was done using a naturally occurring marker in saliva and serum along with serially paired samples in individual patients. Thirty synchronous samples of serum and saliva was collected from thirty patients on lithium carbonate and lithium estimation was corrected for potassium (natural marker) potassium. Three patients were monitored with a series of 7, 6 and 8 paired results. Lithium was estimated using flame photometer. In 30 patients correlation coefficient was 0.71($P<0.001$). In series of paired samples correlation coefficient was 0.85($P<0.01$), 0.80($P<0.05$) and 0.63($P<0.1$).It was found that saliva levels variation

for fixed serum levels were unacceptably large. The study showed satisfactory correlation from zero through the therapeutic range of lithium but was sceptical about such a correlation in excessive levels of serum lithium[38].

A study was done on 95 patients in India who received lithium carbonate. 309 samples of serum and saliva were collected. Flame photometer was used for lithium estimation. A positive and highly significant correlation was found[39].

A study was done to evaluate the relationship among plasma, RBC and saliva lithium levels using atomic absorption spectrophotometry. 30 synchronous samples of blood and saliva was taken from 9 subjects. High correlation(r=0.569,P <0,001) was found between serum and salivary lithium levels. Correlation between RBC lithium and saliva was less compared to serum and saliva levels. The study found high interindividual and intraindividual correlation[40].

In 11 manic-depressive outpatients on chronic lithium therapy the relationship between serum and saliva lithium was studied. Trough serum and saliva lithium levels were measured using atomic absorption spectrometry every 3 or 4 weeks during clinic visits for a period of atleast 16 weeks. Clinical status of patient was rated according to level of mania. Intersubject analysis showed poor correlation(r=0.5) intrasubject correlation was strong(r=0.72-0.94). The study suggested that patient's saliva can be used to estimate serum lithium levels in clinically stabilised manic-depressive patients on prophylactic lithium therapy. In poorly stabilised patients serum concentration should be monitored[41].

Salivary and serum lithium concentrations were measured using atomic absorption spectrophotometry simultaneously in 118 manic-depressive patients. Lithium concentration in saliva was 2.24 ±0.35 times higher than in serum. An

equation to calculate serum lithium concentration from salivary measurements was derived: Li serum = 0.36 Li saliva + 0.13. Psychotropic drugs had no effect on the salivary:serum ratio. Eighteen patients were followed for several weeks. A significant correlation coefficient($P<0.05$) between salivary and serum lithium concentrations was found in thirteen of the eighteen patients studied[42].

140 synchronous samples of serum and saliva was collected from 28 patients undergoing lithium therapy and was estimated using flame photometer. The mean saliva/serum ratio was calculated from 120 synchronous samples from 24 patients was found to be 2.68. Regression line equation calculated for same population came out to be $Y=0.325+0.22X$. Predictive value of saliva lithium was tested by applying this regression equation and the population mean ratio on 20 samples from the next 4 patients. Prediction was also tried in 24 patients who had given more than 3 synchronous samples using individual mean saliva/serum ratio.

An individual's mean was calculated from the initial 3 synchronous samples and predictive value of saliva was tested on subsequent samples in the same patient by using his mean ratio. This method was found to be better than predicting on the basis of population figures[43].

The usefulness of salivary lithium values for monitoring long-term lithium prophylaxis was studied in 60 patients on lithium therapy. A total of 99 pairs of saliva and serum samples were obtained and analysed using flame photometer. The correlation between serum and salivary lithium levels ($r = 0.73$) was found to be significant at the 1% level. The ratio of salivary serum levels to that of serum was found to range from 1.77 to 6.68. The ratio of 51% of the samples was between 3 and 3.99. The study suggested that it is important to identify the subgroup of patients who

show better correlation of salivary and serum lithium levels and use each individual's ratio to monitor only his or her lithium therapy[44].

The salivary composition and flow rate of 78 patients with primary affective disorders and of 49 healthy volunteers were examined. The former were divided into two groups: Group 1(n=57) patients receiving lithium carbonate and psychoactive drugs, and Group 2(n=21)patients receiving psychoactive drugs only. A significant correlation between salivary and serum lithium was found in patients on chronic lithium therapy. The use of saliva analysis for monitoring lithium dosage was recommended by this study[45].

Serum and saliva lithium levels were simultaneously determined using flame photometer in 14 prepubertal children being treated with lithium carbonate. Saliva and serum lithium levels were strongly correlated. The concentration of lithium in saliva was almost two times (1.82) that in serum. There was more variability in saliva levels than in serum levels. It was possible to predict serum levels from saliva levels using regression analysis. If dose was added to saliva as a second predictor variable, accuracy of the prediction was increased[46].

A study was been carried out to find out the reasons for the wide variations observed in the correlation between serum and saliva lithium levels. Serum/saliva lithium levels were monitored using flame photometer in 10 individuals on 6 occasions. The correlation coefficient (r) varied within individuals from 0.19 to 0.91 ($p < 0.025$) whereas when all the individuals were considered simultaneously, it was 0.59 ($p < 0.005$). It was concluded that prediction of serum lithium levels from salivary lithium levels would only be possible in those individuals showing good

correlation. The variability in this correlation could be mainly due to inter-individual variations in the particular sample of individuals studied[47].

In a study one tablet containing 755 mg of lithium tryptophanate (10.8 mEq of lithium) was administered to eight healthy volunteers. The main pharmacokinetic parameters for the group of subjects were estimated. Pharmacokinetic parameters (mean ± SD) from plasma and saliva were respectively: half life (t1/2) 17 ± 6 vs. 21.8 ± 14 h; mean residence time 23.7 ± 7.4 vs. 24.4 ± 15.3 h; total clearance 30.6 ± 9.3 vs. 28.6 ± 6.2 ml/h/kg; and apparent volume of distribution 0.71 ± 0.20 vs. 0.84 ± 0.37 L/kg. Although the mean pharmacokinetic parameters in plasma and saliva were similar, there was no significant correlation between the calculated parameters in the individual subject ($p > 0.05$). The study concluded that usefulness of monitoring salivary levels of lithium is questionable[48].

Within and between subject variability in serum and salivary lithium concentrations in nine psychiatric inpatients on stable drug regimens undergoing therapy was assessed using criteria for determining biologic variation. Estimation of lithium was done using flame photometer this allows separation of analytic from other measures of variance. There were marked differences in inter- and intra-subject variance for serum and salivary lithium concentrations for serum/salivary ratios. These variances were greater for salivary lithium than for serum concentrations. The results were used to assess analytical performance, the usefulness of the therapeutic range, and the reference change interval. The study found that despite greater variance for salivary concentrations, predicted serum concentrations from predetermined serum/saliva ratios were in good agreement with actual concentrations in most subjects[49].

A study was done to check if dialysis of saliva improves accuracy of saliva lithium determinations. Estimation of lithium was done using lithium sensitive electrode. Saliva has two major components: the aqueous and the mucopolysaccharide portions. Since Li is likely to distribute only in the aqueous fraction, saliva was dialysed through a 3000 Da filter to isolate the aqueous component and determine the Li level in it. Lithium levels in the dialyzed saliva agreed more closely with plasma levels ($r = 0.901$, $p < 0.001$) than did whole saliva ($r = 0.775$, $p = 0.012$). The study concluded that dialysis of saliva may contribute to more accurate saliva Li levels[50].

In a study lithium ions concentration in human serum and saliva was determined using dry-slide technology Vitros 250 Analyser (Ortho Clinical Diagnostic) and atomic absorption spectrometry Perkin Elmer 403 (AAS). Lithium ions were analysed in 100 serum and saliva specimens of patients after oral administration of lithium carbonate (3 x 300 mg) Jadran, Galen Laboratory Rijeka. Saliva and blood were taken 2 and 12 hours after the last dose. At the same time lithium ions at samples of blood and saliva were determined with both methods which showed high level of correlation. The mean difference of lithium ions between saliva and serum was statistically significant for $p<0.05$ using t student test. Saliva constant of elimination Kel = 0.02(-1)h and elimination half life (t(1/2)) was t(1/2)=34.6 h. For serum t(1/2)= 24 h which means that lithium ions elimination is slower from saliva than from serum. That is the reason why probably concentration at saliva is higher than at serum. Lithium elimination is two compartment pharmacokinetic model where important part of compartment are saliva and salivary glands. The study concluded at a certain point in medical treatment it could be expected to use controlled determination of lithium ions in saliva with serum as control[51].

The relationship between the plasma concentration, saliva concentration and urinary excretion rate of lithium was investigated in a study and the possibility of using the saliva concentration or the urinary excretion rate for monitoring dosage was considered. The results in the study support the idea that saliva concentration of lithium could be useful in monitoring dosage but, there are many difficulties to be overcome in using the urinary excretion rate of lithium due to inter-subject variation, intra-subject variation during the night and circadian variation in the renal excretion of lithium[52].

MATERIALS AND METHODS

Source of the data

The present study is a cross sectional study to compare serum lithium, salivary lithium and urinary lithium in patients on lithium carbonate.

Study period

The study was undertaken between December 2009 to March 2011.

SAMPLE SIZE

Sample size was determined to be 50.With α=0. 01 and β=0.2 sample size required to demonstrate correlation 'r'=0.6 minimum sample size of 33 is required.

STUDY POPULATION.

Consisted of in patients of the Department of Psychiatry of KLE'S Dr. Prabhakar Kore Charitable Hospital and Medical Research Centre, Belgaum between the ages of 18-50 years who are on lithium carbonate for more than a week.

CRITERIA FOR SELECTION OF THE STUDY GROUP

Inclusion Criteria**:**

1) Patients who are on lithium carbonate therapy for at least a week.
2) Age: 18-50 years.
3) Renal function test within normal limits.
4) Urine analysis within normal limits.

Exclusion Criteria:

1) Factors affecting normal salivary secretion.
2) Pregnancy
3) Age <18years and >50 years
4) Dehydration
5) Drugs affecting lithium pharmacokinetics :Nonsteroidal anti-inflammatory drugs,Diuretics, angiotensin-converting enzyme inhibitors, angiotensin II receptor type-1 antagoinists, metranidazole, sodium bicarbonate, propranolol.

APPROVAL FROM THE AUTHORITIES:

Permission to conduct the study was obtained from all the concerned authorities viz.

1. Institutional ethics committee on human subjects research of Jawaharlal Nehru medical college, Belgaum.
2. Director –clinical services for medical director and chief executive of KLE'S Dr.Prabhakar Kore Hospital and Medical Research Centre, Belgaum.(Annexure II)
3. University science instrument centre (USIC), Shivaji university, Kolhapur.

OBTAINING INFORMED CONSENT

Informed consent was taken from all the participants in the study. (Annexure III)

SCHEDULING:

This study was carried out for a period of 14 months. It was undertaken during December 2009 to March 2011.

PILOT STUDY

Pilot study was conducted after taking serum, saliva and urine from 3 patients to assess the feasibility of the study and to standardise methods of sample collection.

Patient information

A structured proforma was used to collect sociodemographic and clinical information about the study participants. (Annexure IV)

COLLECTION OF SAMPLE

Patient preparation:

Blood, saliva and urine samples were collected in the morning (12hrs after the last dose of lithium carbonate) before the patient consumed breakfast and the morning dose of lithium. Samples were collected under aseptic precautionary measures.

Serum

5 ml of blood was collected in a plain non vacuum tube and was allowed to stand in room temperature till clot was formed. Serum was separated within one hour of venipuncture by centrifuging the tubes at 3,000 r.p.m for 10 minutes. Serum was pipetted out into a sterile eppendorf tubes.

Saliva

Saliva was collected in a sterile container after asking the patient to rinse mouth with water. Subjects were asked to collect saliva in the mouth and then were

asked to spit into the container till adequate amount was collected. Saliva was centrifuged to remove mucus.

Urine

Mid stream urine was collected in sterile container.

Sample collection was supervised by nursing staff. All the 3 samples were collected within 20 minutes.

All samples were stored in non vacuum sterile tubes at -20 ^{0}C till further analysis.

STATISTICAL TESTS USED

The following methods of statistical analysis have been used in this study. Data was entered in Microsoft excel and analyzed using SPSS (version 17).

Mean and standard deviation of serum lithium, salivary lithium and urinary lithium was computed and compared by using paired't' test. Pearson's correlation coefficient and Linear regression analysis was done between three parameters and computed to know the strength of association between them.

MATERIAL USED IN THE STUDY

1. Non vacuum plain tube.
2. 5 ml disposable syringe.
3. Tourniquet.
4. Sterile container.
5. Automated pipettes and tips.
6. Deionised water.

7. Microcentrifuge tubes.

8. Gloves.

INSTRUMENTS USED IN THE STUDY

1. Centrifuge machine.

2. Flame atomic absorption spectrophotometer (Perkin Elmer Analyst 300).

ESTIMATION OF LITHIUM

Lithium estimation in serum, saliva and urine was done using Atomic Absorption Spectrophotometer (Perkin Elmer Analyst 300). Permission to use the instrument for lithium analysis was obtained from head of University science instrument centre (USIC), Shivaji University, Kolhapur.

SAMPLE PREPARATION

Serum

Serum sample was diluted to 1:10 or 1:5 with deionized water[1, 2] . The dilution ratio was adjusted to ensure that concentrations fall within a suitable absorbance range.

Saliva

Saliva sample was diluted to 1:5 with deionized water . The dilution ratio was adjusted to ensure that concentrations fall within a suitable absorbance range.

Urine

Urine sample was diluted to 1:50 with deionized water[1, 2]. The dilution ratio was adjusted to ensure that concentrations fall within a suitable absorbance range.

Samples were taken in batches of twenty for analysis in flame atomic absorption spectrophotometer.

PRINCIPLE OF FLAME ATOMIC ABSORPTION SPECTROPHOTOMETER

Every element has a specific number of electrons associated with its nucleus. The normal and most stable orbital configuration of an atom is known as the "ground state." If energy is applied to an atom, the energy will be absorbed and an outer electron will be promoted to a less stable configuration known as the "excited state." Since this state is unstable, the atom will immediately return to the "ground state," releasing light energy.

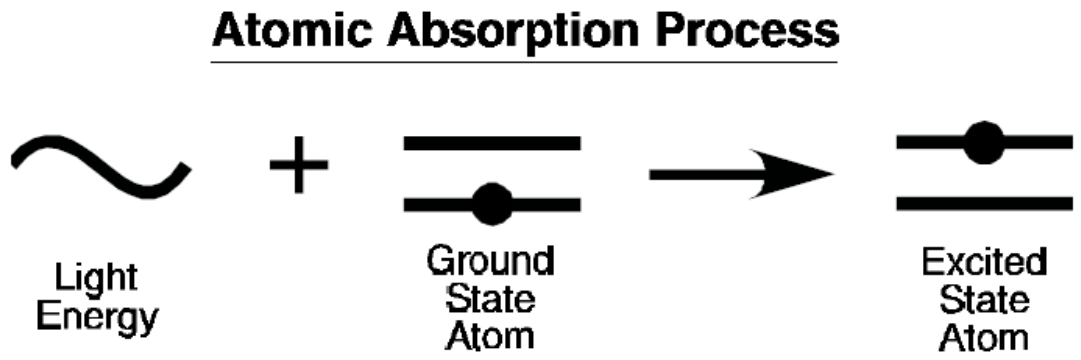


Figure 1: Atomic absorption process.

The "ground state" atom absorbs light energy of a specific wavelength as it enters the "excited state." As the number of atoms in the light path increases, the amount of light absorbed also increases. By measuring the amount of light absorbed, a quantitative determination of the amount of analyte can be made. The use of special light sources and careful selection of wavelengths allow the specific determination of individual elements.

ATOMIC ABSORPTION INSTRUMENTATION

There are five basic components of an atomic absorption instrument:

1. The light source that emits the spectrum of the element of interest
2. An "absorption cell" in which atoms of the sample are produced
3. A monochromator for light dispersion
4. A detector, which measures the light intensity and amplifies the signal.
5. A display that shows the reading after it has been processed by the instrument electronics[37].

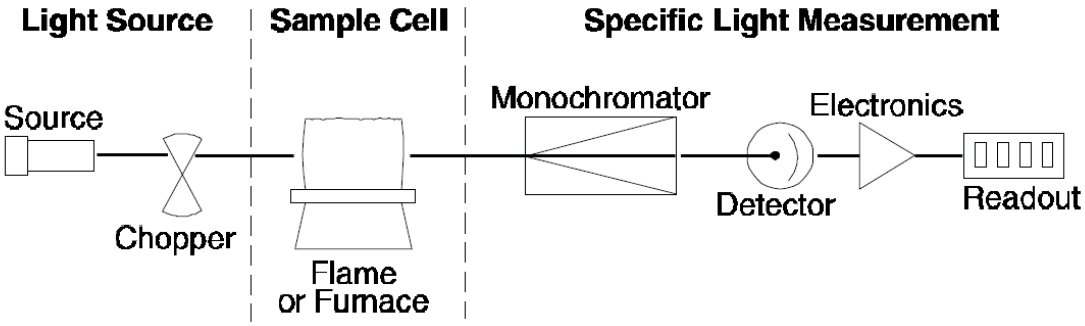


Figure 2: Schematic representation of flame Atomic absorption process.

PROCEDURE

Standard

Appropriate standards are prepared by diluting the lithium stock solution provided by the manufacturer.

Blank

Deionized water is used for blank solution.

ANALYSIS

Instrument was set in standard condition for lithium analysis.

Blank was aspirated first followed by a suitable standard and then the sample. Lithium standard was read after every five sample.

The readings were recorded in p.p.m. and then converted to mEq/l.

PHOTOGRAPHS

Photograph 1: Flame atomic absorption spectrophotometer (Perkin Elmer Analyst 300)

Photograph 2: Investigator performing analysis of lithium on Flame atomic absorption spectrophotometer (Perkin Elmer Analyst 300).

RESULTS

A cross sectional study was done to compare serum lithium, salivary lithium and urinary lithium in patients on lithium carbonate to evaluate the possibility of replacing serum with salivary or urine lithium estimation for monitoring of patients on lithium carbonate.

The data obtained from the study was compiled, tabulated and subjected to statistical analysis. The results are presented here under the headings of the various parameters considered for the study.

Table 1:Distribution of age, serum lithium, salivary lithium, urine lithium and dose of lithium carbonate in 50 study subjects.

The mean age of study participants was 35.10±10.63. Their Mean serum lithium was 0.75±0.25 mEq/l, mean salivary lithium was 1.91±0.80 mEq/l and mean urine lithium was 7.16±4.84 mEq/l. Mean dosage of lithium carbonate prescribed to the subjects was 820±101.01 mg.

Table 2: :Distribution of age, serum lithium, salivary lithium, urine lithium and dose of lithium carbonate in male and female.

The number of female participants was 18 and male participants were 32.The mean age of male participants was 36.50±10.50 years and that of female participants was 32.61±10.69 years. There was no statistically significant difference between the mean ages of males and females (p=0.22).

In males mean serum lithium was 0.75±0.26 mEq/l, mean salivary lithium was 1.91±0.80 mEq/l and mean urine lithium was 7.53±5.26 mEq/l. Mean dosage of lithium carbonate prescribed to the subjects was 806.25±94.82mg.

In females mean serum lithium was 0.76±0.23 mEq/l, mean salivary lithium was 1.85±0.74 mEq/l and mean urine lithium was 6.50±4.06 mEq/l. Mean dosage of lithium carbonate prescribed to the subjects was 844.44±109.66 mg.

There was no statistically significant difference between the mean serum lithium (p=0.91), salivary lithium(p=0.67), urine lithium(p=0.47) and dosages of lithium carbonate (p=0.2) between males and females.

Table 3:Correlation analysis of serum and saliva lithium levels

Correlation between serum lithium and salivary lithium was statistically significant (r=0.695, p <0.001). The serum lithium was more strongly correlated with salivary lithium in females (r=0.770, p <0.001) when compared to males (r=0.665, p <0.001).

Table 4(Graph 1): Linear regression analysis for serum and salivary lithium correlation.

Linear regression analysis for serum and salivary lithium was done with serum lithium as dependent variable, statistically significant correlation was found (r=0.695). The equation for calculating serum lithium from saliva lithium measurements was derived from the graph 1: Y=0.332+0.221X (Y=serum lithium concentration, X=salivary lithium concentration).

Table 4 (Graph 2): Linear regression analysis for serum and salivary lithium correlation for females

In females linear regression analysis for serum and salivary lithium was done with serum lithium as dependent variable, statistically significant correlation was found (r=0.770). The equation for calculating serum lithium from saliva lithium

measurements was derived from the graph 2:Y=0.355+0.271X(Y=serum lithium concentration, X=salivary lithium concentration).

Table 4 (Graph 3): Linear regression analysis for serum and salivary lithium correlation for males

In males linear regression analysis for serum and salivary lithium was done with serum lithium as dependent variable, statistically significant correlation was found (r=0.665). The equation for calculating serum lithium from saliva lithium measurements was derived from the graph 3: Y=0.259+0.204X(Y=serum lithium concentration, X=salivary lithium concentration).

Table 5:Correlation analysis of serum and urine lithium levels.

Correlation between serum lithium and urine lithium was not statistically significant (r=0.234, p =0.102). In males correlation between serum lithium and urine lithium was (r=0.319, p =0.08) in females (r=0.022, p =0.932).

Table 6(Graph 4):Linear regression analysis for serum and urine lithium correlation.

Linear regression analysis for serum and urine lithium was done with serum lithium as dependent variable. Correlation was found to be statistically not significant (r=0.234). Graph 4 showing serum versus urine lithium measurements shows a large deviation.

Table 1: Distribution of age, serum lithium, salivary lithium, urine lithium and dose of lithium carbonate in 50 study subjects.

Sl.No	Variable	Mean±S.D
1	Age (years)	35.10±10.63
2	Serum lithium (mEq/l)	0.75±0.25
3	Salivary lithium (mEq/l)	1.91±0.80
4	Urine lithium (mEq/l)	7.16±4.84
5	Dose of lithium carbonate (mg)	820±101.01

Table 2: Distribution of age, serum lithium, salivary lithium, urine lithium and dose of lithium carbonate in male and female.

Variable	Male (n=32)	Female (n=18)	t value	p value
Age	36.50±10.50	32.61±10.69	1.25	0.22
Serum lithium(mEq/l)	0.75±0.26	0.76±0.23	-0.12	0.91
Salivary lithium(mEq/l)	1.91±0.80	1.85±0.74	0.43	0.67
Urine lithium(mEq/l)	7.53±5.26	6.50±4.06	0.72	0.47
Dose of lithium carbonate (mg)	806.25±94.82	844.44±109.66	-1.29	0.20

Table 3:Correlation analysis of serum and saliva lithium levels.

	r	r^2	S.E	p value
Total study Subjects(n=50)	0.695	0.47	0.18	**<0.001***
Male (n=32)	0.665	0.42	0.20	**<0.001***
Female (n=18)	0.770	0.57	0.16	**<0.001***

*-statistically significant

Table 4:Linear regression analysis for serum and salivary lithium correlation.

	Unstandardized Coefficients		Standardized Coefficients	t	p value.	95.0% Confidence Interval for B	
	B	Std. Error	Beta			Lower Bound	Upper Bound
(Constant)	0.332	0.069		4.835	**<0.001***	0.194	0.470
Salivary Lithium	0.221	0.033	0.695	6.688	**<0.001***	0.155	0.288
Dependent Variable: Serum Lithium *-statistically significant							

Table 5: Correlation analysis of serum and urine lithium levels.

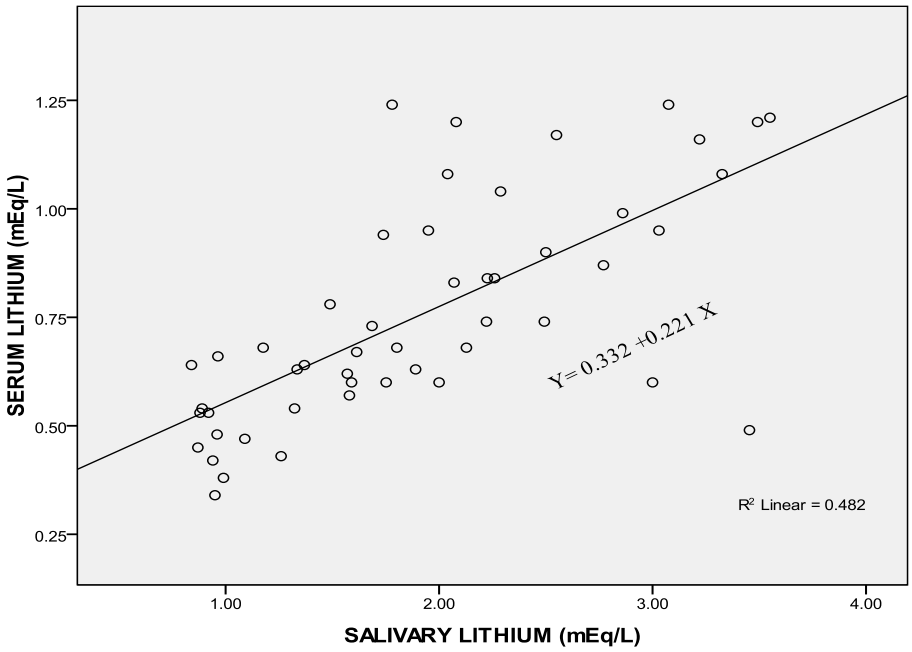
	r	r^2	S.E	p value
Total study subjects(n=50)	0.234	0.04	0.25	0.102
Male (n=32)	0.319	0.07	0.26	0.08
Female (n=18)	0.022	-0.06	0.25	0.932

Table 6:Linear regression analysis for serum and urine lithium .

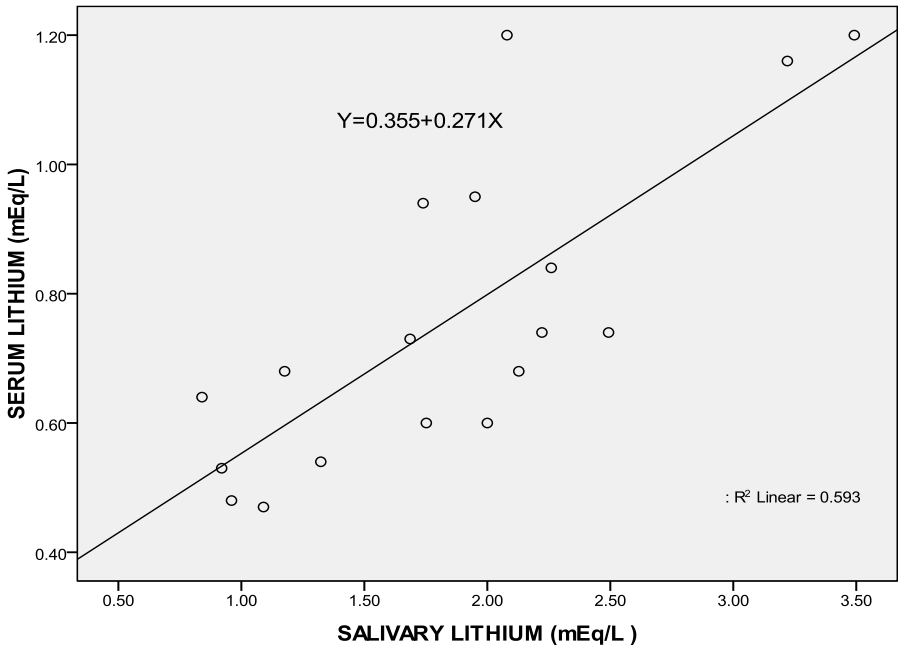
Model	Unstandardized Coefficients		Standardized Coefficients	t	p value
	B	Std. Error	Beta		
(Constant)	0.668	0.064		10.459	**<0.001***
Urine Lithium	0.012	0.007	0.234	1.667	0.102
Dependent Variable: Serum Lithium *-statistically significant					

GRAPHS

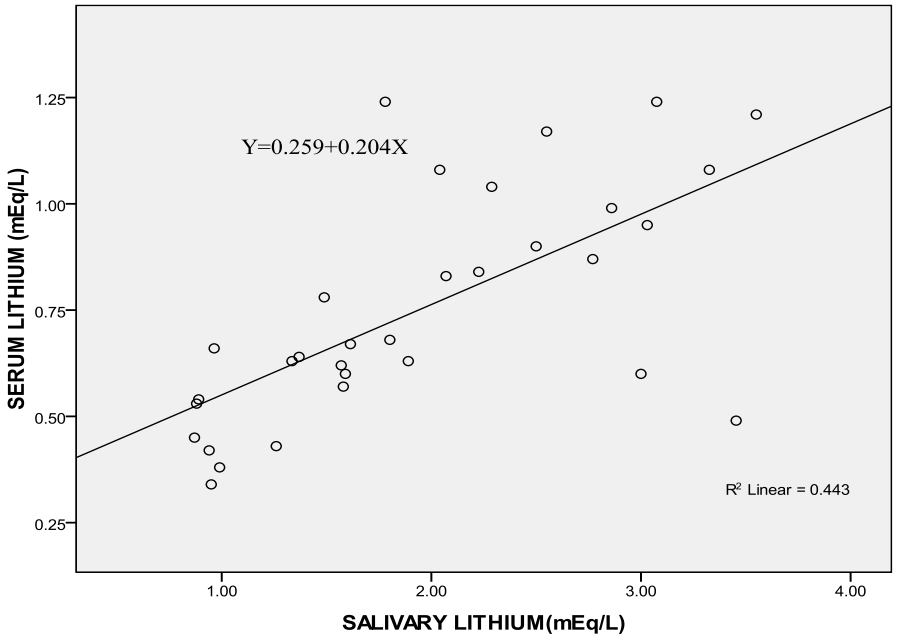
Graph 1: Linear regression analysis of serum versus salivary lithium of 50 study participants



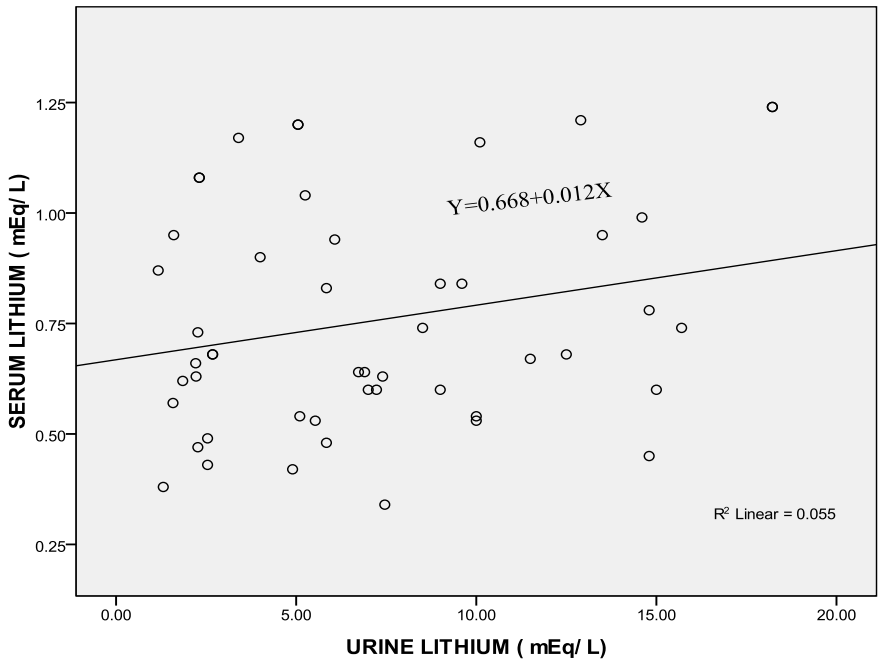
Graph 2: Linear regression analysis of serum versus salivary lithium in female participants.



Graph 3: Linear regression analysis of serum versus salivary lithium in male participants.



Graph 4: Linear regression analysis of serum versus urine lithium of 50 study participants



DISCUSSION

Lithium carbonate is used in the treatment of psychiatric and non psychiatric disorders[53]. Lithium has an established use in treatment of acute mania, prophylaxis of bipolar episodes and augmentation therapy for severe, refractory depression[10]. The difference between toxic and therapeutic dose of lithium carbonate is narrow. Lithium therapy must be guided by monitoring plasma concentration it has been considered that lithium concentrations should be maintained between 0.6 and 1.0 mmol/L[21]. At plasma concentrations above 1.5mmol/L is associated with signs of intoxication mainly affecting gastrointestinal and neurological system. Frank toxicity is associated with plasma concentration greater than 2mmol/L which is an acute medical emergency. Therefore when patients are on lithium strict monitoring of serum levels are done since the amount of lithium required to achieve a therapeutic response depends on concentration of ion in serum which reflects its distribution throughout the body rather than on actual amount required per day[7] .

The frequency of blood sampling for lithium monitoring is usually every 1–2 weeks until a desirable serum concentration is achieved, then every 2–3 months for the first 6 months of treatment, and at least every 6–12 months thereafter[35]. These series of lithium monitoring requires repeated drawing of blood samples.

The desirable goal in the health care delivery and health research is to monitor health status, disease onset, disease progression, and treatment outcome noninvasively. There are three prerequisites necessary to reach this goal

1. A non-invasive method for collecting biological samples.
2. Specific biomarkers associated with health or disease.
3. A technology platform to rapidly discriminate the biomarkers[54].

Diagnostic tests based on fluid generally use blood and urine and less frequently the esoteric fluids such as saliva, sweat, and tears. Saliva's popularity has suffered because it lacks "the drama of blood, the sincerity of sweat and the emotional appeal of tears.'" Sweat and tears, however, are difficult to obtain in sufficient quantities for routine testing, and urine will always lack the charisma of the other fluids. Saliva, by default, therefore becomes the most favoured alternative to blood[55].

Most molecules present in blood or urine can also be detected in salivary secretions. Their concentrations in saliva are usually one tenth to one thousandth of those in blood.

Although highly sensitive methods of detection are required, technical advances have made this feasible. Studies of the correlation between concentrations in blood and saliva have found examples of excellent concordance (ethanol, cortisol, theophylline, and antibodies to HIV)[55].

Lithium prophylaxis of bipolar disorder is prolonged with the duration of treatment extending over several years. The narrow therapeutic index of lithium combined with long duration of treatment necessitates that adequate blood levels be maintained[44]. The use of saliva or urine collection versus blood collection has many advantages like ease of collection, safety, acceptance by the patient and cost effectiveness.

Totally valid comparisons could not be done between our study and other studies in literature due to paucity of researches devoted to finding correlation between serum, salivary and urine lithium. Also there was a wide variation with respect to age group, study design methodologies employed and instruments used.

However a sincere attempt is being made to compare and discuss to the extent possible and permissible.

In the present study all the study subjects were on lithium carbonate (Tablet. Lithosun SR, Sun pharmacy). The mean dose was 820±101.01mg/day (range 600-1200mg). Dose of lithium carbonate was in the range of 900-1800mg/day (eskalith, smith kline & French)in study done by Rosman .A.W et al(41);300-1800mg/day in study done by Ben-Aryeh. H et al[42], mean 1050mg/day with range 500-1600mg/day in study done by Mckeage.M.J[56].

Estimation of lithium can be done by flame emission photometry, atomic absorption spectrometry and ion selective electrode[37]. Atomic absorption spectrophotometer is superior to colorimetric and flame emission spectrophotometric methods for measuring lithium in serum and urine because of its relative lack of susceptibility to interfering substances[57]. Therefore in the present study lithium estimation was done by using atomic absorption spectrometry. Flame emission photometry was used for lithium determination in some studies [7, 8, 38, 39, 42-44, 46, 47, 49]. Atomic absorption spectrometry was used in lithium estimation in earlier studies [40, 41, 52, 56]. Ion selective electrode was used in lithium estimation in study done by El-Mallakh RS et al[50].

Saliva lithium concentration was more than serum lithium concentration. The mean ratio of salivary lithium to serum lithium was 2.57±0.91. Similar results were observed in previous studies[7, 8, 41, 43, 47].Higher ratio of 3.64±1.04 was reported by Khare CB et al[44]. while Weller EB et al[46] found lower ratio 1.82±0.29 when compared to the present study. The reason behind getting a higher saliva : serum lithium ratio might be that we employed unstimulated saliva for estimation. Similar

technique was used by Khare C B et al[44] . Unstimulated saliva was preferred over stimulated saliva in this study as this unstimulated flow, is what is secreted by the salivary glands in normal physiological conditions. Lithium in unstimulated saliva was found to be directly proportional to serum concentration. Lithium in stimulated saliva tends to be a negative function of flow rate[58].

Mean serum lithium was 0.75±0.25 mEq/l in the present study. Lower mean serum lithium was found to be by Sankaranarayanan A et al(0.59±0.19 mEq/l) and by Khare CB et al 0.65±0.24 mEq/l[44, 47] .Higher levels were found by Weller EB et al(1±0.2 mEq/l) and by Rosman AW et al(0.91±0.27 mEq/l)[41, 46] .These differences can be attributed to differences in pharmacological preparations, dosage and duration of lithium therapy.

Mean salivary lithium was 1.91±0.80 mEq/l in the present study. Salivary lithium concentration was 1.56 mEq/l by Neu C et al[7], 1.37±0.97 mEq/l[47] by Sankaranarayanan A et al[47] which was lesser than the present study. Higher saliva lithium was found by some other studies. The slight differences which were found can be ascribed to differences in saliva collection technique and dosage of lithium.

In the present study Correlation between serum lithium and salivary lithium was statistically significant ($r=0.695$, $p<0.001$). Similar correlations were found in studies done by Sankaranarayanan A et al[47] ($r=0.59$, $p<0.005$), Khare CB et al[44] ($r=0.73$) and Nataraj G et al [43] ($r=0.71$, $p<0.001$) . Higher correlations were found in other studies [7, 8, 45, 50] . Lower correlations were found in studies done by Prakash,R.S et al ($r=0.41$, $p<0.001$) and Rosman AW et al($r=0.5$) [39, 41].

The linear regression equation derived was: **Serum lithium = 0.332 + 0.221 X Salivary lithium**. This equation was utilized to calculate the serum lithium levels from salivary lithium levels measured. The correlation between the calculated and the measured serum lithium was highly significant($r=0.695$, $p<0.001$).Our results support the assumption of several previous reports[7, 8, 42] that recommend salivary lithium measurements for monitoring serum lithium. Other finding in studies done by Sims A et al[38] and Mathew RJ et al[40] dispute the usefulness of salivary lithium for monitoring probably because of methodological differences in saliva collection and patient selection.

Several factors have been found to contribute to the variability of salivary lithium concentrations. These include blood ion concentration, stimulation of salivary glands and aspects of lithium administration such as dosage and duration of treatment. Another potential variable can be mucopolysaccharide content. El Mallakh RS et al[50] found that when all mucinous material is removed from the saliva by filtration the agreement between the lithium levels of ultrafilterate and plasma is stronger than when saliva is centrifuged and unfiltered supernatant is measured.

More correlation was found in females($r=0.770$, $p<0.001$) than in males($r=0.665$, $p<0.001$).Exact reason for this finding is not known.

Correlation of ($r=0.234$, $p= 0.102$) was found between serum lithium and urine lithium levels which had no clinical or statistical significance. There is scarcity of literature demonstrating any correlation between serum and urine lithium levels. In a study done by Kyroudis A et al [52] , a good intra -subject correlation between urinary excretion rate and plasma concentration was found. Intersubject variability might have accounted for a statistically insignificant correlation in our study which used

inter-subject design. It can be deduced that there are many difficulties to be overcome in using the urinary excretion rate of lithium due to inter subject variation, intra-subject variation during the night and circadian variation in the renal excretion of lithium.

Rosman AW et al[41]suggest that one must use the linear regression equation derived from intrasubject not intersubject data. These intrasubject estimates of serum lithium concentrations can be used safely in the clinically stabilized manic patient on prophylactic lithium therapy. Although correlation found in our study was good and statistically significant, it was lesser than few other studies which employed intrasubject correlation.

CONCLUSION

The relationship found between serum and salivary lithium was statistically significant but not strong enough to allow saliva monitoring as a substitute of serum lithium estimation in patients on lithium carbonate therapy. Further studies are needed in this arena which employs intersubject as well as intrasubject correlation design, to establish salivary therapeutic monitoring as a viable option for patients on lithium carbonate therapy.

The relationship found between serum and urine lithium was not statistically significant therefore urine lithium estimation may not be a suitable alternative for monitoring lithium therapy.

SUMMARY

The present study is a cross sectional study to compare serum lithium, salivary lithium and urinary lithium in patients on lithium carbonate admitted in Department of Psychiatry of KLE'S Dr.Prabhakar Kore Hospital and Medical Research Centre, Belgaum.

Blood, saliva and urine samples were collected in the morning (12hrs after the last dose of lithium carbonate) before the patient consumed breakfast and the morning dose of lithium. Samples were collected under aseptic precautionary measures.

Estimation of serum, salivary and urine lithium was done using atomic absorption spectrophotometer. Data was entered into Microsoft excel and analysed using SPSS(version 17). Statistical tests of significance employed were Pearson's correlation coefficient,student 't' test and linear regression analysis.

Following are our observations:

1. There is a statistically significant correlation between serum and salivary lithium levels.($r= 0.695$, $p<0.001$)
2. More correlation in serum and salivary lithium levels was observed in females ($r =0.77$, $p=<0.001$) than in males ($r=0.665$, $p=<0.001$)
3. Correlation observed between serum lithium and urine lithium was not statistically significant.

Our study showed that there is a significant correlation between serum and salivary lithium levels and it calls for further studies to throw more light in this field of salivary lithium monitoring.

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ANNEXURE I

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No.3/2/2010-2/PG-thesis-MPD-3
Dated: 06.08.2010

This is with reference to your application seeking financial assistance from the Council for MD/MS/DM/MCH dissertation thesis entitled **"A cross sectional study to campare serum lithium, salivary lithium and urinary lithium in patients on lithium carbonate".**

I am glad to inform you that Director General, ICMR, based on the recommendation of Expert Committee, has sanctioned a sum of Rs.25, 000/- (Twenty five thousand only) to you for providing an electronic and hard copy of your dissertation thesis to the Council.

I am enclosing herewith a copy of the detailed guidelines for this scheme and would appreciate that necessary information as per these guidelines may be provided to the undersigned enabling us to release the grant. This is to inform you that Rs.25,000/- will be disbursed to you in two installments. Initial amount of Rs.15,000/- after receipt of the **undertaking** as per the guidelines and remaining amount of Rs.10, 000/- on receipt of the electronic copy, hard copy and summary of work done of your dissertation thesis duly approved by the University/Institution along with one publication in a reputed Journal.

With best wishes,

Yours Sincerely,

(K.K.SINGH)

ANNEXURE II

KLES
DR. PRABHAKAR KORE HOSPITAL
&
MEDICAL RESEARCH CENTRE
NEHRUNAGAR, BELGAUM-590010
KARNATAKA-INDIA

ಕೆ. ಎಲ್. ಇ ಸಂಸ್ಥೆಯ
ಡಾ. ಪ್ರಭಾಕರ ಕೋರೆ ಆಸ್ಪತ್ರೆ ಮತ್ತು
ವೈದ್ಯಕೀಯ ಸಂಶೋಧನಾ ಕೇಂದ್ರ
ನೆಹರುನಗರ, ಬೆಳಗಾವಿ–590010 ಕರ್ನಾಟಕ, ಇಂಡಿಯಾ

Phone : 0831-2473777 (16 Lines)
Fax : 0831-2470732
E-Mail : klehosp@satyam net.in
Website : http://www.kleshospital.org

REF. NO: KLES/PKHOSP/DCS/09-10/ 13867

DATE: 24/03/2010

Sub: Permission to carry out Dissertation work.

1. Ref. to your application on above subject dt 24/02/2010 addressed to MD & CE of the hospital.

2. After perusal of protocol of study, review of literature, consent form and ethical clearance, you are permitted to carry out Dissertation work on "A cross sectional study to compare serum lithium, salivary lithium and urinary lithium in patients on lithium carbonate" in this hospital.

3. You will work under the guidance of Professor, Psychiatry and I/C Biochemistry Dept of the Hospital.

3. The hospital will not have any financial implications for your project.

Brig (Retd) Dr. Dinesh Prasad
Director – Clinical Services
for Medical Director &
Chief Executive.

ANNEXURE III

CONSENT FOR PARTICIPATION IN RESEARCH STUDY

Title: "**A cross sectional study to compare serum lithium, salivary lithium and Urinary lithium in patients on lithium carbonate".**

We are requesting you to be a participant in the above said research at KLES Dr. Prabhakar Kore Hospital and MRC,Belgaum.

I. Research purpose: Patients suffering from mood disorders are advised various medications. Lithium carbonate is one of the main drugs used. When the patient is on lithium therapy frequent serum lithium estimations are done because lithium can cause a lot of problems if levels are lower or higher than prescribed limits. Therefore for serum lithium estimation each time patient has to be pricked and blood has to be collected. I am trying to find out if salivary lithium or urinary lithium can be compared with serum lithium levels so that repeated pricks for drawing blood are avoided.

II. Procedures involved: If you agree to participate in this research you will be asked the relevant history and will be subjected to clinical examination. You will be requested to come in the fasting state the next day. You will be asked to give urine, saliva and blood. 10ml of blood will be collected by intravenous route by pricking a small blood vessel which may give rise to small amount of pain. Urine, saliva and blood will be collected from you and will be subjected to lithium estimation.

III. Risks and benefits: There are no risks involved in this procedure. If any complications arise during the procedure you will be treated in KLES Dr. Prabhakar Kore Hospital and MRC, Belgaum with the best of our knowledge and the availability

of resources in the free hospital. There will be no compensation or payment for such medical treatment.

During the course of the study you will be informed of any significant new findings such as changes in the risks and benefits resulting from participation in the research.

IV. Privacy and confidentiality: The only people who will know that you are a research participant are members of the research team. No information provided by you or about you during the research will be disclosed to others without your written consent.

V. Institutional policy: Your participation in this study is voluntary, whether or not to participate will not affect your current or future relationship with the KLES Dr. Prabhakar Kore Hospital and MRC, Belgaum.

VI. Financial Incentives for participation: You will not receive any reimbursement for participation in the research.

VII. Authorization to Publish Results: When the results of the research are published or discussed in conferences no information will be disclosed that would disclose your identity. Any information obtained in connection with this study and that can be identified with you will remain confidential and will be disclosed only with your permission.

VIII. Consent Statement: To voluntarily take part in this study I must sign on the line below. If I choose to take part in this study I may withdraw at any time. I am not giving up any of my legal rights by signing this form. My signature below indicates that I have read or the contents of the entire consent form including the risks and

benefits have been read to me this and all my questions have been answered. I will be given a copy of this consent form.If I have any questions about the study I can contact the experimenter.

If I have any questions about my rights as a research participant I may contact Principal and Chairman of JNMC Institutional Ethical Committee for Human Subjects Research.

Participant's Name: Participant's Signature or thumb print:

Experimenter's Name and phone no: Experimenter's Signature:

Witness' Name: Witness' Signature:

Guardian's Name: Guardian's Signature or thumb print:

Date: Place:

ANNEXURE IV

PROFORMA

I. Patient Identification

Name: Age/Sex: I.P.No/O.P.No:

Address: Rural/urban :

Date of examination:

Occupation: Religion:

II. History:

Diagnosis:

Duration of illness:

Drug: Dose:

Duration of treatment:

Other drugs:

Diabetes: Hypertension:

Renal disease:

Personal history:

Bowel and bladder habits:

Menstrual history:

III. General Physical Examination:

Pallor:	Weight (kg):
Icterus:	Height :
Lymph nodes:	Body mass index(BMI):
Temperature:	
Pulse:	Blood pressure:
Respiratory rate:	
Oral cavity:	

IV. Investigations:

Blood glucose estimation:

Renal function test :

- Blood urea
- serum creatinine

Thyroid function test:

Urine analysis:

Complete hemogram:

Lithium estimation:

- Serum lithium
- Salivary lithium
- Urinary lithium

ANNEXURE V

MASTER CHART

SERIAL NO	NAME	SEX	AGE	SERUM LI	SALIVA LI	URINE LI	DOSE
1	SU.	F	32	0.53	0.92	5.53	1200
2	DE.	F	18	0.48	0.96	5.84	800
3	SHI.	M	24	0.62	1.57	1.85	800
4	L.	F	18	0.64	0.84	6.73	800
5	KG.	M	40	0.34	0.95	7.46	800
6	B.	M	22	0.54	0.89	10	800
7	M.	M	36	0.68	1.8	12.5	800
8	R.	F	35	0.6	1.75	7.23	800
9	KU.	M	54	0.63	1.34	2.22	800
10	SG.	M	26	1.08	3.33	2.31	1000
11	MK.	M	18	0.64	1.37	6.9	800
12	V.	M	20	0.6	1.59	7	800
13	SD.	M	46	1.24	3.08	18.22	600
14	KS.	F	43	0.54	1.32	5.1	800
15	KM.	F	40	0.6	2	15	800
16	SHV.	M	44	0.84	2.23	9	800
17	JD.	F	50	0.74	2.22	15.7	800
18	AS.	M	55	0.49	3.45	2.54	800
19	VDY.	F	24	0.74	2.49	8.51	800
20	SNT.	F	39	0.94	1.74	6.07	800
21	SJT.	F	20	0.73	1.69	2.27	800

22	AB.	M	37	0.67	1.61	11.5	800
23	SDL.	F	40	1.2	3.49	5.05	1000
24	SRT.	F	30	0.68	2.13	2.68	800
25	SNK.	M	48	0.78	1.49	14.8	800
26	MH.	M	25	1.04	2.29	5.25	800
27	KL.	M	35	0.66	0.96	2.21	800
28	NM.	M	41	0.9	2.5	4	800
29	VN.	M	28	0.6	3	9	800
30	SJA.	F	20	0.47	1.09	2.27	800
31	SRA.	F	30	0.68	1.18	2.68	800
32	SLG.	F	40	1.2	2.08	5.05	1000
33	MN.	M	35	0.99	2.86	14.6	800
34	SRD.	M	46	1.24	1.78	18.22	600
35	BLB.	M	35	0.53	0.88	10	800
36	RK.	M	52	0.87	2.77	1.17	800
37	SGM.	M	26	1.08	2.04	2.31	1000
38	ASK.	M	39	0.43	1.26	2.54	800
39	LX.	M	32	0.83	2.07	5.84	800
40	HN.	M	50	1.21	3.55	12.9	800
41	MT.	M	35	0.57	1.58	1.58	800
42	LM.	M	30	0.95	3.03	13.5	1000
43	NR.	M	28	0.38	0.99	1.31	800
44	MG.	M	35	0.63	1.89	7.4	1000
46	ST.	F	20	1.16	3.22	10.1	800
45	SKR.	M	48	0.45	0.87	14.8	800

47	RG.	M	28	0.42	0.94	4.9	600
48	SB.	M	50	1.17	2.55	3.4	800
49	GJW.	F	38	0.95	1.95	1.6	800
50	SNT.	F	50	0.84	2.26	9.6	800