Section: Biochemistry

Original Research Article

ESTABLISHMENT OF REFERENCE RANGE OF URIC ACID IN SALIVA

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Received : 23/03/2023
Received in revised form : 30/04/2023
Accepted : 11/05/2023

Keywords:
CLSI guideline, Reference range, salivary uric acid.

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DOI: 10.47009/jamp.2023.5.3.160

Source of Support: Nil,
Conflict of Interest: None declared

Int J Acad Med Pharm
2023; 5 (3); 773-778

Abstract

Background: The reference range of uric acid in the serum has already been established and widely used in health care management. Salivary diagnostics have even been identified as potential substitutes for serum protein biomarkers. Salivary bioscience technologies are widely applied for diagnosing systemic health status. The purpose of this study is to establish the reference range of uric acid in saliva as per Clinical and Laboratory Standard Institute (CLSI) document EP- 28A3C. It is a pilot approach to establish the reference range of uric acid in salivary sample. The objective is to establish the reference range of uric acid in saliva as per Clinical and Laboratory Standard Institute (CLSI) document EP- 28A3C. **Materials and Methods:** A cross sectional study, in which Sample were collected from subjects who visited phlebotomy section for regular health check-up in Father Muller Medical College Hospital. The duration was two-month period, 2019 June-July, and analysis was done at Clinical Biochemistry Laboratory. Total number of 121 subjects including 70 women and 51 men, within the age limit of 18-60 years were included in the study. Subjects with the presence of any systemic disease such as gout, diabetes, hypertension, hepatitis, kidney failure, and metabolic dysfunction, alcohol consumption, smoking, the consumption of drugs that increase uric acid like antidiuretics, immunosuppressive drugs, non-steroid anti-inflammatory drugs, systemic or topical corticosteroids, thyroid disease, and a history of surgery or trauma in the past month, pregnancy, those with the presence of active carious lesions, sign of periodontitis, faulty dental restoration, patients who suffer from xerostomia were excluded from the study. **Result:** Based on CLSI guidelines this study established a reference range for uric acid in saliva as 0.29-6.11 mg/dl. **Conclusion:** This study demonstrated the effectiveness of saliva as a diagnostic tool to measure uric acid by establishing reference range as 0.29-6.11 mg/dl. A moderate correlation between salivary uric acid and serum uric acid was determined. Studies in a greater number of samples are needed to validate this approach.

INTRODUCTION

The standard definition of a reference range for a particular measurement is defined as the prediction interval between which 95% of values of a reference group fall into, in such a way that 2.5% of the time a sample value will be less than the lower limit of this interval, and 2.5% of the time it will be larger than the upper limit of this interval, whatever the distribution of these values.[1] So many comparative

studies were done using salivary uric acid, such as there is a current study that provides evidence that there is a relationship between salivary uric acid and hippocampal response to psychosocial stress. They conclude that there is uric acid's influence on emotion-related neural systems.[2]

Salivary uric acid is used as a noninvasive biomarker of metabolic syndrome, more research works are going on to validate this approach. Several studies demonstrate that salivary uric acid is elevated in patients with metabolic syndrome and correlates with several cardio metabolic risk factors including blood pressure, triglyceride levels, HDL and fasting blood glucose. Also found that the relationship between salivary uric acid and metabolic syndrome was stronger in females than in males.[3]

To determine a reference interval, the reference interval for a biochemical analyte is usually the central interval of values bounded by the reference limit values at certain designated percentiles. That is, the reference interval refers to that interval set of values observed in the reference sample group or predicted for the reference population, defined by a specific percentage, all you need to test many healthy people, the reference population.[4]

A reference range is a set of values that includes upper and lower limits of a lab test based on a group of healthy people. The values in between those limits may depend on such factors as age, sex, and specimen type (blood, urine, spinal fluid) and can also be influenced by circumstantial situations such as fasting and exercise. These intervals are thought of as "normal ranges or limits. "Reference intervals are the most common decision support tool used for interpretation of numerical pathology reports. As laboratory results may be interpreted by comparison with these intervals, the quality of the reference intervals can play as large a role in result interpretation as the quality of the result itself. The key data source for setting a reference interval is the reference interval study performed according to the Clinical and Laboratory Standards Institute (CLSI) and the International Federation of Clinical Chemistry (IFCC) recommendations. Ideally this should be performed by the laboratory establishing the reference interval. However, data from one study is rarely sufficient and, in any case, should be compared with data from other sources. Other such data sources include peer-reviewed literature, posters and meeting abstracts, manufacturer's information, and unpublished data from other laboratories. Data mining studies, for example using the Bhattacharya method to extract information from large patient result databases, may also provide valuable information.[5]

Saliva, an important physiologic fluid, containing a highly complex mixture of substances is rapidly gaining popularity as a diagnostic tool. The use of saliva has provided a substantial addition to the diagnostic armamentarium as an investigative tool for disease processes and disorders. Its advantages as a diagnostic tool include its ease of procurement and the correlation between many parameters in the serum.[6] The reference range of uric acid in the serum has been established and is used to compare the different types of clinical studies. At present there is no reference range established for uric acid in saliva. In this pilot study reference range of uric acid in the saliva was established as per the guidelines issued by Clinical and Laboratory Standards Institute (CLSI) as per Clinical and Laboratory Standard Institute (CLSI) document EP-28A3C.[7]

MATERIALS AND METHODS

This present study was a cross sectional study done in Father Muller Medical College Hospital, Kankanady, Mangalore over a period of 3 months. A convenience sample was composed of 121 (by CLSI guideline) healthy subjects selected from those who came for regular health checkup. A priori sampling was used for the well-defined exclusion and partitioning criteria before the selection of the reference individuals.[7] Healthy subjects in the age 18-60 years were included as study participants. Presence of any systemic disease such as gout, diabetes, hypertension, hepatitis, kidney failure, metabolic dysfunction, alcohol consumption, smoking, consumption of drugs that increases uric acid (antidiuretics, immunosuppressive drugs, non-steroid anti-inflammatory drugs, systemic or topical corticosteroids), thyroid disease, and history of surgery or trauma in the past month, pregnancy were excluded. Those with the presence of active carious lesions, sign of periodontitis, faulty dental restoration, patients who suffer from xerostomia, and other diseases that could affect in conducting the study were also excluded.

The study was done after getting permission from the institutional ethics committee. A questionnaire covering information on age, sex, systemic disease, daily medication, and various oral symptoms was filled out for everyone. Dietary regimen and oral hygiene habits were also noted along with any associated symptoms of xerostomia. All subjects signed an informed consent form before the study procedures. Then, after detailed examination of study participants, whole unstimulated saliva (fasting) was collected in the resting condition by drooling method.[8]

Study population and recruitment: Sample were collected from subjects who visited phlebotomy section for regular health check-up in Medical College Hospital over a period of two months**.** The subjects were given the following questionnaire and asked for detailed clinical history to recruit for the study based on the inclusion and exclusion criteria.

ISSN (O): 2687-5365; ISSN (P): 2753-6556

Table 1: The Questionnaire:[7]

Do you consider yourself to be healthy?	yes/no
Do you exercise regularly?	yes/no
Have you been sick recently?	yes/no
Are you taking any prescribed medication?	yes/no
Do you have high blood pressure?	yes/no
Do you use tobacco?	yes/no
Do you drink alcoholic beverages?	yes/no
Have you been hospitalized recently?	yes/no
Any inherited health disorders in family?	yes/no
Have you taken antidiuretics or any pain relievers recently?	yes/no
Have you taken any cold or allergy medicine recently?	yes/no
Are you taking diet pills? Have you taken any antacids recently?	yes/no yes/no
Does your mouth feel dry?	yes/no
Do you use toothpaste daily?	yes/no
Do you use dental floss daily?	yes/no
Do you use mouthwash daily?	yes/no
For women Are you on hormone replacement therapy?	 yes/no
Are you pregnant?	yes/no
Are you breastfeeding?	yes/no
Are you using oral or implant contraceptives?	yes/no

Saliva sample collection: Resting drooling (minimal oral movements) was used to collect whole mouth saliva (fasting) from the oral cavity.[9,10] Participants were asked to sit comfortably in an upright position and tilt their heads down slightly to pool saliva in the mouth. The first expectoration was discarded to eliminate unwanted substance contaminating the sample that may cause analytical inaccuracy.[11] Then samples were collected according to the procedure. After three times rinsing of mouth, 5 ml of unstimulated whole saliva were collected. Centrifugation done at 3000 rpm for 15 minutes, the samples then immediately stored at -20^{0}C. 2 ml of blood sample from antecubital vein under aseptic conditions was also collected at the same time from the subject to measure serum uric acid levels, centrifugation at 3000 rpm for 15 minutes was done and the supernatant was aspirated, stored at -20^{0}C and the levels of serum and salivary uric acid was measured using enzymatic colorimetric test in Roche/Hitachi MODULAR Cobas 6000 fully automated auto-analyzers.[12]

Statistical Analysis: Data obtained was analysed by using SPSS software. Mean, standard deviation, median, range, interquartile range, 95% confidence intervals (CI) were used to summarize the analytic output. Prior to the establishment of the reference intervals the data were tested for normal distribution and outliers were excluded.[13]
Medians, 2.5th, 5th, 95th, 97.5th percentile for salivary uric acid was calculated in accordance with the CLSI EP28-A3c guideline using a non-parametric analysis. The conventional 95th percentile reference limits were determined by calculating the rank numbers for the 2.5th and 97.5th percentiles and the range was established.

RESULTS

The present study was a pilot approach to establish the reference range of uric acid in the saliva, by collecting samples from healthy individuals. Reference range was established by following non-parametric method given by CLSI guidelines. Outliers were excluded by plotting a Box Whisker plot. The 'n' values were sorted in ascending order of magnitude and the Median is found out by plotting a histogram. The percentiles were calculated by using formula 0.025(n+1) and 0.975(n+1). The lower limit of RI is equal to 2.5th percentile and the upper limit is equal to 97.5th percentile with confidence interval of 95%.

Table 2: Class Interval and Frequency Distribution of 121 Salivary Uric Acid Values

Class interval	Frequency
0.2-1	21
1.1-1.9	38
2-2.8	22
2.9-3.7	11
3.8-4.6	11
4.7-5.5	11
5.6-6.4	5
6.5-7.3	2

Table 3: Rank Order of Salivary Uric Acid Value of 121 Healthy Subjects

Salivary Uric Acid Value	Frequency	Rank Order
0.23	1	1
0.26	1	2
0.29	1	3
0.3	1	4
0.42	1	5
0.49	1	6
0.7	2	7-8
0.79	1	9
0.8	1	10
0.85	2	11-12
0.86	1	13

ISSN (O): 2687-5365; ISSN (P): 2753-6556

0.9	4	14-17
0.95	1	18
0.97	1	19
1	3	20-22
1.09	1	23
1.1	2	24-25
1.2	4	26-29
1.3	3	30-32
1.35	1	33
1.4	2	34-35
1.45	1	36
1.5	3	37-39
1.56	1	40
1.58	2	41-42
1.6	3	43-45
1.66	1	46
1.68	1	47
1.7	2	48-49
1.74	1	50
1.79	1	51
1.8	1	52
1.81	1	53
1.86	1	54
1.9	4	55-58
1.93	1	59
1.95	1	60
2	2	61-62
2.04	1	63
2.07	1	64
2.14	1	65
2.17	1	66
2.2	2	67-68
2.27	1	69
2.39	1	70
2.5	3	71-73
2.6	2	74-75
2.62	1	76
2.7	2	77-78
2.78	1	79
2.8	3	80-82
3	1	83
3.16	1	84
3.2	1	85
3.37	1	86
3.39	1	87
3.46	1	88
3.47	1	89
3.6	2	90-91
3.7	1	92
3.73	1	93
3.8	1	94
3.9	1	95
4.01	1	96
4.04	1	97
4.1	1	98
4.2	2	99-100
4.4	2	101-102
4.6	1	103
4.7	1	104
4.76	1	105
4.8	1	106
4.86	1	107
4.9	2	108-109
5.1	1	110
5.21	1	111
5.28	1	112
5.42	1	113
5.45	1	114
5.6	1	115
5.7	1	116
5.94	1	117
6.1	1	118
6.11	1	119

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6.7	1	120
7	1	121

Non-parametric determination of reference interval:

Calculation of rank numbers of percentiles

Lower: 0.025(n+1)

0.025(121+1) =3.05(i.e., Rank #3)

Upper: 0.975(n+1)

0.975(121+1) =118.95(i.e., Rank #119)

Original value corresponding to these rank numbers.

Lower limit (2.5-percentile): 0.29 mg/dl

Upper limit (97.5-percentile): 6.11mg/dl

Table 4: 90% Confidence Intervals for Lower and Upper 95% Reference Limits

Analyte	Lower reference limit	Upper reference limit
Rank numbers (table 1)	#1 and #7	(121+1) -7= #115 and (121+1)-1= #121
Salivary uric acid (mg/dl)	0.29 [0.23 to 0.7]	6.11 [5.6 to 7]

Table 5: Descriptive Data of Serum and Salivary Uric Acid before Excluding Outliers

Analyte	Mean	Median	SD	Variance	Minimum	Maximum	Range	Interquartile range
Salivary uric acid	2.7255	2.02	1.965	3.865	0.23	11.70	11.47	2.47
Serum uric acid	5.2505	5.20	1.212	1.469	2.91	10.07	7.16	1.76

Table 6: Descriptive Data of Salivary Uric Acid after Excluding Outliers

Salivary uric acid	Mean	Median	SD	Minimum	Maximum	Range	Interquartile range
	2.53	2	1.6118	0.23	7	6.87	2.3

Table 7: Correlation between Serum and Salivary Uric Acid (n=121)

		Serum	Saliva
Serum	Correlation coefficient	1.000	0.384**
Saliva	Correlation coefficient	0.384**	1.000

**Correlation is significant at the 0.01 level.

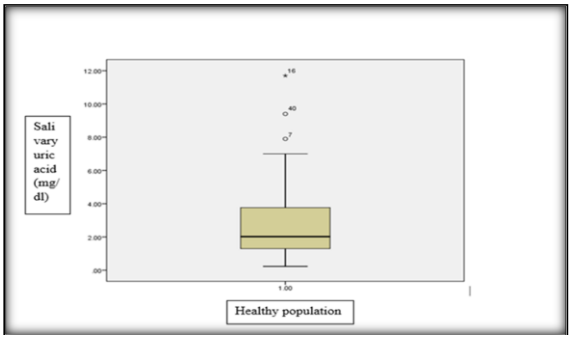

Figure 1: Box and Whisker Plot of 124 Salivary Uric Acid

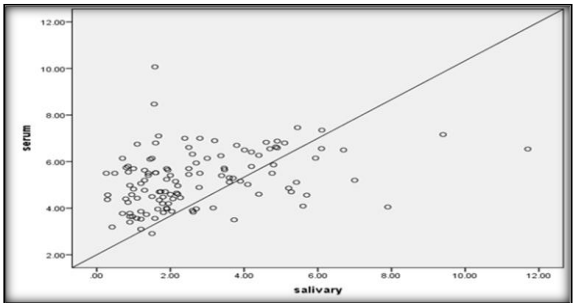

Figure 2: Scatter Plot between Serum and Salivary Uric Acid

Only a moderate correlation is observed between salivary and serum uric acid (P<0.01; significant=0.4).

DISCUSSION

In the present study, we determined the concentration of uric acid in the saliva of homogenous (fasting, unstimulated, passive drooling) subjects to establish reference interval. Lower reference limit 90% confidence interval: 0.29 [0.23 to 0.7] mg/dl Upper reference limit 90% confidence interval: 6.11 [5.6 to 7] mg/dl. Our study found a slight difference in the lower limit of reference range of salivary uric acid than the serum uric acid. The mean level of salivary UA (mean=2.53) was significantly lower than the mean of serum UA (mean=5.24) because of the low concentration of uric acid in saliva compared with blood. However, a previous Study by Bakhtiari et al, on assessment of uric acid Level in the saliva of patients with oral lichen planus, showed that OLP was associated with decreased UA levels in saliva. The mean level of salivary UA was significantly lower in the patients with OLP (2.10 ± 0.19 mg/dL)

ISSN (O): 2687-5365; ISSN (P): 2753-6556

in comparison with the control group (4.80 ± 0.29 mg/dL; $p < 0.001$).[14]

A similar study by Khozeimeh et al., in saliva of patients with halitosis, showed a 20% increase in uric acid concentration in halitosis group when compared to control group with a significance of 0.05 ($P< 0.05$). The mean concentration in halitosis group was 3.19 ± 1.12 mg/dl and 2.27 ± 1.18 mg/dl in the control group. In most patients with halitosis, uric acid concentration was between 4 and 5 mg/dl and between 1 and 2 mg/dl in the control group.[15] Our present study shows a moderate correlation between salivary and serum uric acid with a significance of 0.01 ($P < 0.01$).

In concordance with the previous study by Das et al., in 2013 among healthy Assamese population by establishing reference range of serum uric acid as 2.6–8.2mg/dL and the mean uric acid was 5.5 ± 1.4mg/dl.[16] Our study shows uric acid mean as 5.25 and SD as 1.2 in serum. It is the first study to establish uric acid in saliva. So, this can be used to find uric acid in saliva and can be used for comparing with the serum uric acid values.

The main criticism that can be levelled against most studies on saliva is that in our experimental design, we did not control the variables that can affect salivary flow rate or composition. These include the source of saliva from different glands, degree of hydration of the subject, the nature of the stimulus, duration of stimulation and the different time of day.

Limitations of the Study

Gender based RI is not established, so further studies are needed to establish the RI of uric acid in the saliva for male and female separately by taking a greater number of sample size. Further investigation on the relationship between serum and salivary uric acid will be a useful asset for clinical utility because Saliva shows positive correlation between many parameters in the serum. So, if future studies show a strong positive correlation between serum and salivary uric acid then saliva can be used as a diagnostic tool for the determination of uric acid.

CONCLUSION

In this study, reference interval of uric acid in the saliva is established as 0.29-6.11mg/dl by using non-parametric method as per Clinical and Laboratory Standard Institute (CLSI) document EP-28A3C. A moderate correlation is found between salivary and serum uric acid. Like any other analyte, reference range of salivary uric acid also depends on age, gender, and source, time of collection and method of collection. Larger sample sizes are required for better decision making.

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ISSN (O): 2687-5365; ISSN (P): 2753-6556