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RESEARCH ARTICLE

Comparison of serum uric acid assay by two different analyzers: Wet chemistry versus dry chemistry

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ABSTRACT

Background: Reference range of uric acid is narrow and at the cut-off of 7 mg/dl is defined as hyperuricemia, correct estimation of uric acid play an essential role in management of patient. The objective of this study was to find out the variation in serum uric acid values measured by two different analyzers, i.e., wet and dry chemistry.

Materials and method: Serum uric acid was measured in 227 blood samples received in clinical biochemistry laboratory for analysis of uric acid over a period of two months by two different instruments, i.e., wet chemistry instrument based on colorimetry method and dry chemistry instrument based on reflectance spectrophotometry.

Results: The mean difference of serum uric acid between two methods was 0.89 mg/dl which was statistically significant ($t = 12.92$, $p < 0.001$) and Pearson correlation coefficient was 0.9688. The Bland-Altman (BA) plot analysis showed the maximum difference between wet and dry chemistry from -1.15 mg/dl to 2.93 mg/dl, with a mean difference of 0.89 mg/dl. Samples were further categorized on the basis of gender, out of 227 patients enrolled 142 (63%) were males and 85 (37%) females. The BA plot demonstrated limit of agreement ranging from -1.55 to 3.57 mg/dl in female patients and -0.83 to 2.48 mg/dl in male patients.

Conclusion: Good correlation exists between wet and dry chemistry, however the two methods are not similar. While interpreting the laboratory findings of uric acid, the method used must be checked carefully and during the follow up period switching of the method for uric acid estimation should be avoided.

Keywords: dry chemistry, hyperuricemia, reflectance spectrophotometry, uric acid, uricase, wet chemistry

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INTRODUCTION

Uric acid, a nitrogenous compound is the end product of purine nucleosides catabolism. Approximately 400 mg of uric acid is synthesized daily, and another 300 mg is contributed from dietary sources [1]. Approximately 75% of uric acid is excreted by kidneys and the rest is eliminated through gastrointestinal tract. In plasma and synovial fluid uric acid exist in ionized form, i.e., urate with approximately 98% as monosodium urate [2]. Hyperuricemia is defined as elevated plasma uric acid concentration (> 7mg/dl in men and > 6 mg/dl in women) and occurs due to either increased formation or decreased excretion of uric acid [1]. Hyperuricemia may be classified as primary or secondary depending upon the cause like deficiency/overactivity of enzymes of purine catabolism, psoriasis, obesity, alcohol intake, purine-rich diet etc. While hypouricemia is defined as a state when plasma uric acid concentration is below 2 mg/dl occurs due to decreased production of urate or increased excretion of uric acid or both [1]. Conditions which lead to hypouricemia includes increased renal uric acid excretion, total parenteral hyperalimentation, hepatic cirrhosis, multiple myeloma, heavy metal toxicity etc. [2].

For the estimation of uric acid in body fluids commonly used techniques are

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phosphotungstic acid (PTA), uricase, and high pressure liquid chromatography (HPLC)-based methods [3]. PTA method is based on development of tungsten blue color at wavelength of 650 nm but it lacks specificity due to various factors causing interference. In uricase methods, the enzyme uricase is used as a single step or as the initial step to oxidize uric acid to produce allantoin, hydrogen peroxide and carbon dioxide. It also requires peroxidase along with an oxygen acceptor to produce a chromogen. Although a number of oxygen acceptors, e.g., 4-aminophenazone, 3-methyl-1-benzothiazoline hydrazone etc. are available but the choice depends upon the one with minimal interference to ensure good quality. The major interfering factors are ascorbic acid and bilirubin which are eliminated by using ascorbate oxidase and amino phenazone with a substituted phenol [4].

The instrument which uses uricase in a dry reagent form are also available in which multilayered film system consisting of spreading layer, reagent layer, support layer and scavenging layer. The chances of interference are low in dry method, but ascorbic acid still remains a significant interferant resulting in significantly low uric acid results in urine sample of individuals taking high doses of vitamin C. HPLC methods using ion-exchange or reversed-phase column are used to separate and quantify uric acid. But this method requires technical skill and is not cost effective as compared to enzymatic method. There are a number of conditions, for example acute gout, in which there is hyperuricemia which needs immediate attention for diagnosis and management of the patient. The reference range for uric acid by enzymatic method is 3.5 to 7.0 mg/dl for males and 2.6 to 6.0 mg/dl for females. As the range of uric acid is narrow and at the cut-off of 7 mg/dl condition is defined as hyperuricemia, correct estimation of uric acid play an essential role in management of patient [5]. The objective of this study is to find out the difference in the level of serum uric acid measured by two different methods, i.e., dry chemistry and traditional wet chemistry method.

MATERIALS AND METHOD

This comparative study was conducted in the department of biochemistry, King George's Medical University in Lucknow Uttar Pradesh, India. Ethical clearance was taken from Institutional Ethics Committee at King George's Medical University (Ref.code no 102ndECMIIMBBS-S/P1 dated 10.09.2020).

Study Design

In this study we randomly selected 227 blood samples received in clinical biochemistry laboratory for analysis of uric acid over a period of two months from January and February 2021. Blood samples received in clinical laboratory requested for uric acid estimation were centrifuged and serum was used for analysis of uric acid by two different instruments on same day.

Inclusion and Exclusion Criteria

Study participants are humans only.

Inclusion criteria

Serum samples analyzed in clinical biochemistry laboratories for uric acid requested by the clinician.

Exclusion criteria

Samples with quantity not sufficient**,** hemolyzed samples, lipemic, icteric, hemolyzed samples received for estimation of uric acid were excluded.

Measurement of Uric Acid

Serum uric acid was measured in 227 sample by two different instruments and values exceeding the measuring range was diluted as per the guidelines given in respective literature.

Instrument 1. Wet chemistry instrument on colorimetric method

Uric acid was estimated in serum by uricase enzymatic–colorimetric method (fully automated biochemistry analyzer–Selectra). To ensure quality, internal quality control (ELITROL L1 and L2) were used.

Instrument 2. Dry chemistry instrument on reflectance spectrophotometry

The VITROS uric acid slide and VITROS calibrator were used for uric acid estimation in each blood sample on VITROS 350 fully automated biochemistry analyzer. VITROS uric acid slide is a multilayered slide on which a drop of serum sample is evenly distributed on spreading layer. Uric acid from sample migrates to the reagent layer where in presence of uricase it is oxidized to allantoin and hydrogen peroxide. Hydrogen peroxide in presence of peroxidase oxidizes a leucon dye to generate a colored dye optical density of which is measured at 670 nm. Quality was ensured by internal quality control (Biorad L1 and L2).

Statistical Analysis

The statistical analysis of data was performed by using software package SPSS version 25 and Microsoft Excel 2019. Paired t-test was used to compare mean of serum uric acid estimated by wet and dry chemistry method. To study the relationship between serum uric acid level estimated by wet and dry chemistry method, Pearson correlation coefficient was applied. $p < 0.05$ was considered statistically significant, $p < 0.01$ as highly significant, and $p < 0.001$ as very highly significant. To assess the difference between uric acid levels estimated by two methods the Bland-Altman (BA) plot was used, using average difference ±1.96SD as the 95% limits of agreement.

RESULTS

In this study after excluding samples based on exclusion criteria, 227 samples received in clinical biochemistry laboratory for uric acid estimation were analyzed by two different methods.

Table 1. Descriptive analysis of uric acid levels estimated by wet chemistry and dry chemistry method

Method	Mean (mg/dl)	Standard Deviation	Standard Deviation Error	Median	Minimum	Maximum	Range
Wet chemistry	6.27	3.57	0.24	5.56	1.07	20	18.93
Dry chemistry	5.38	2.90	0.19	4.9	1.1	17.5	16.4

Table 2. Comparison of serum uric acid estimated by wet and dry chemistry

	Paired Differences					t	Df	p-value
	Mean	SD	Std. Error Mean	95% Confidence Interval of the Difference				
				Lower	Upper			
Wet Chemistry - Dry Chemistry	0.8934	1.04	0.0691	0.7579	1.029	12.92	226	**<0.0001**

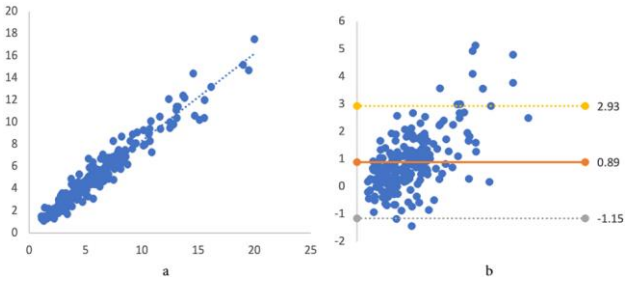

Figure 1. (a) Correlation between serum uric acid level estimated by wet and dry chemistry (r = 0.9688; p < 0.0001) & (b) The BA plot for the difference between wet and dry chemistry method (n = 227) (Source: Authors' own elaboration)

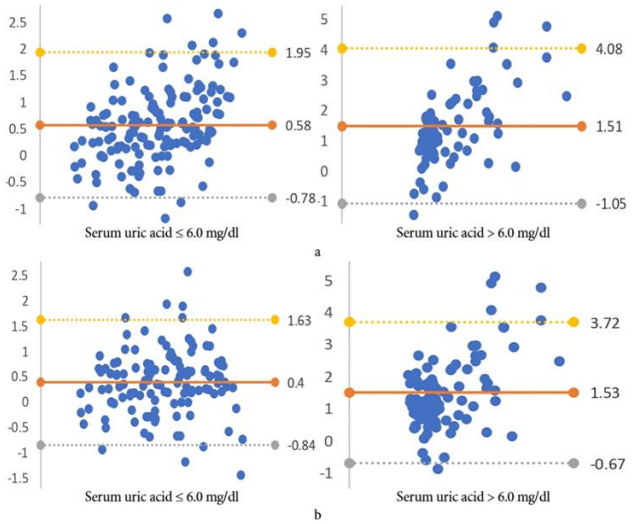

Figure 2. The BA plot for the difference between wet and dry chemistry method using cut-off 6.0 mg/dl on (a) dry chemistry method & (b) wet chemistry method (Source: Authors' own elaboration)

Descriptive analysis and comparison of serum uric acid estimated by two methods is summarized in **Table 1** and **Table 2**.

The correlation and BA plot analysis of two methods is shown in part a and part b in **Figure 1**.

All the 227 samples were categorized into two by using 6.0 mg/dl cut-off and the BA plot analysis is shown in part a and part b in **Figure 2**.

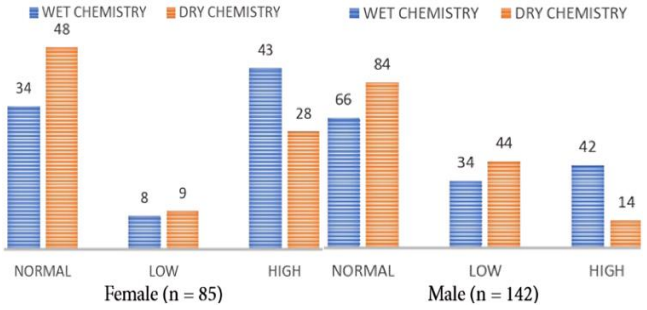

Figure 3. Gender based distribution into three groups (normal, high, & low levels of uric acid) according to reference range of wet chemistry (female: 2.6-6.0 mg/dl; male: 3.5-7.2 mg/dl) & dry chemistry method (female: 2.5-6.2 mg/dl; male: 3.5-8.5 mg/dl) (Source: Authors' own elaboration)

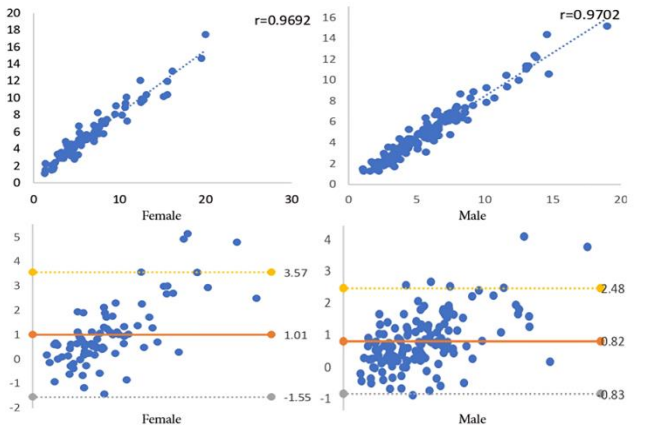

Figure 4. (a) Correlation between serum uric acid level estimated by wet and dry chemistry in female and male patients & (b) The BA plot for the difference between wet and dry chemistry method in female and male patients (Source: Authors' own elaboration)

All the male and female patients were categorized as normal, high and low serum uric acid levels in both wet and dry chemistry, as shown in **Figure 3**.

The correlation and BA plot analysis of both the methods in male and female patients is shown in part a and part b in **Figure 4**.

Table 3. Descriptive analysis of serum uric acid levels using cut-off 6.0 mg/dl

	Serum uric acid ≤ 6.0 mg/dl				Serum uric acid > 6.0 mg/dl			
	C-O applied on DC method		C-O applied on WC method		C-O applied on DC method		C-O applied on WC method	
	WC	DC	WC	DC	WC	DC	WC	DC
n	151	151	127	127	76	76	100	100
Mean	4.34	3.76	3.89	3.5	10.1	8.58	9.29	7.77
SD	1.57	1.29	1.27	1.23	3.32	2.52	3.24	2.64
SDE	0.13	0.11	0.11	0.11	0.38	0.29	0.32	0.26
Minimum	1.07	1.1	1.07	1.1	5.27	6.1	6.01	4.2
Maximum	8.11	6.0	5.89	6.7	20	17.5	20	17.5
Bias	0.58		0.40		1.51		1.53	
Lower LOA	-0.78		-0.83		-1.05		-0.67	
Upper LOA	1.95		1.63		4.08		3.72	

Note. C-O: Cut-off; WC: Wet chemistry; DC: Dry chemistry; SD: Standard deviation; SDE: Standard deviation error; & LOA: Limits of agreement

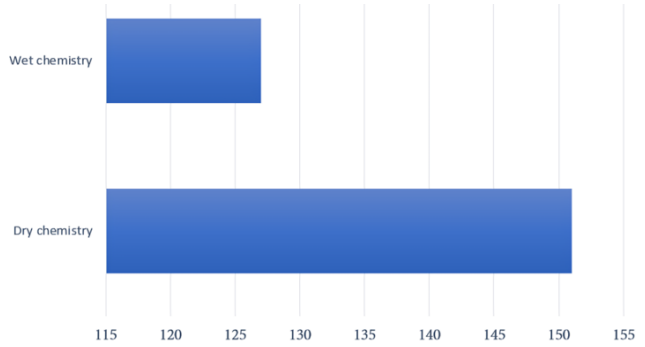

Figure 5. Number of patients having serum uric acid level ≤ 6.0 mg/dl when estimated by wet and dry chemistry (Source: Authors' own elaboration)

DISCUSSION

Mean serum uric acid levels estimated by wet and dry chemistry were 6.27 ± 3.57 mg/dl and 5.38 ± 2.90 mg/dl, respectively (**Table 1**). The mean difference of serum uric acid between two method was 0.8934 which was statistically significant, as shown in **Table 2** (t = 1 2.92, p < 0.001). The relationship between the two methods correlated well in this study, which was also statistically significant with Pearson correlation coefficient was 0.9688 (part a in **Figure 1**). To assess the average difference between two method BA plot analysis showed the maximum difference between wet and dry chemistry results varied from -1.15 mg/dl to 2.93 mg/dl, with a mean difference of 0.89 mg/dl (part b in **Figure 1**).

A cut-off of 6.0mg/dl was used and samples processed by wet and dry chemistry were categorized into two groups (serum uric acid level ≤ 6.0 mg/dl and > 6.0 mg/dl). Twenty-four patients had uric acid level below 6.0 mg/dl when estimated by dry chemistry however the level was > 6.0 mg/dl of these patients when estimated by wet chemistry, as shown in **Figure 5**. When samples were categorized into two groups on the basis of dry chemistry method out of 227 samples, serum uric acid level were ≤ 6.0 mg/dl in 151 samples and in 76 samples values were > 6.0 mg/dl. On the other hand, when 227 samples were categorized on the basis of wet chemistry method, serum uric acid level were ≤ 6.0 mg/dl in 127 samples and 100 samples were > 6.0 mg/dl. Mean serum uric acid level was higher and mean difference was statistically significant among all the groups when estimated by wet chemistry method as compared to dry chemistry also, as shown in **Table 3**. The BA plot analysis done for wet and dry chemistry in sample ≤ 6.0 mg/dl and > 6.0 mg/dl serum uric acid level is shown in part a and part b in **Figure 2**.

Samples were further categorized based on gender, out of 227 patients enrolled 142 (63%) were males and 85 (37%) females. As the reference range provided by the manufacturer in both methods were different for male and female, we divided the samples into three groups, i.e., normal, low and high level among male and female in both wet and dry chemistry method, as shown in **Figure 3**. When serum uric acid in female was estimated by dry chemistry method, 48 patients (56.5%) were categorized normal, 9 (10.6%) were in low level group and 28 (32.9%) were in high level group of serum uric acid while same samples were estimated by wet chemistry method number of female patients were 34 (40%), 8 (9.4%), and 41 (50.6%) in normal, low and high group, as shown in **Figure 3**. Similarly, among male 84 (59.1%) patients were categorized normal when estimated by dry chemistry and 66 (46.5%) as normal when estimated with wet chemistry. Serum uric acid levels were low in 44 (31%) and 34 (23.9%) patients when estimated with dry and wet chemistry, respectively. Number of male patients were 42 (29.6%) in high serum uric acid group when estimated by wet chemistry as compared to dry chemistry in which only 14 (9.9%) male patients were having higher serum uric acid level according to the reference range provided by the manufacturer. Mean serum uric acid level were higher in both female and male when estimated with wet chemistry method as compared to dry chemistry, as shown in **Table 4**. Mean difference of serum uric acid level

Table 4. Descriptive analysis of uric acid levels estimated by wet and dry chemistry method

	Female (n = 85)		Male (n = 142)	
	WC	DC	WC	DC
Mean	6.87	5.86	5.91	5.09
Standard deviation	4.12	3.17	3.15	2.70
Standard deviation error	0.45	0.34	0.26	0.23
Median	5.54	5.10	5.61	4.80
Minimum	1.28	1.10	1.07	1.30
Maximum	20.00	17.50	18.98	15.20

Note. WC: Wet chemistry & DC: Dry chemistry

was 1.011 among female and 0.8233 among male when estimated by wet and dry chemistry method which was statistically significant, as shown in **Table 5**. Both wet and dry chemistry method showed good correlation, as shown in part a in **Figure 4**. Further, BA plot demonstrated limit of agreement ranging from -1.55 to 3.57 in female patients and -0.83 to 2.48 in male patients, as shown in part b in **Figure 4**.

Among the methods available for estimation of serum uric acid, the uricase method is commonly used in laboratories. Even though the principle is the same in laboratories variation in results may occur due to different techniques/equipment used for estimation of the analyte [6]. In our study though both methods, i.e., wet and dry chemistry showed good correlation but the mean difference of serum uric acid between two method was statistically significant. Serum uric acid levels when estimated with wet chemistry method were higher as compared to dry chemistry method. This may be due to differences in technique as the dry chemistry microslide contain a spreading layer which reduces interference due to certain analytes like endogenous bilirubin, reduced glutathione, lipids etc. Cut-off for defining hyperuricemia remain under debate till date due to factors like ethnicity, gender, and the methods used for estimation of serum uric acid level. It was suggested stratification by age ranges and sex along with 97.5th percentile as threshold for hyperuricemia [7]. Researchers observed that a revision of uric acid upper normal limit needs to be redefine due to pathophysiological role of uric acid in human diseases as proposed in [8]. It was observed a threshold value < 6.0 mg/dL (< 360 µmol/L) seems to better identify true "healthy subjects" and should reasonably be considered for all subjects and similar cut-off was also proposed in [9]. While it was suggested that hyperuricemia should be defined using a statistical approach of upper decision limit selection (corresponding to the gender- and population-specific 66th percentile of data range) upon which an international consensus should exist as an expression of evidence and expert opinion [10]. In past, serum uric acid below 6.0 mg/dl was proposed to define healthy subjects, and same cutoff was used in this study which revealed that the number of patients classified as healthy subjects (≤ 6.0 mg/dl) and with elevated uric acid level (> 6.0 mg/dl) were different in wet and dry chemistry method. Limited studies on the uric acid reference range are available on Indian population. A reference range of 3.5 to 8.7 mg/dl in male and 2.5-6.9 mg/dl in female was published in healthy Assamese people [11]. Due to scarcity of data majority of laboratories use the reference range provided by the manufacturer which again varies from method to method and technique to technique. In this study also the two methods (wet and dry) compared had separate serum uric acid levels for male and female patient though the difference for lower cut-off was only 0.1 mg/dl among female and no difference was observed in lower cut-off among male. But for upper cut-off difference was 0.2 mg/dl in female and 1.3 mg/dl in male between wet and dry chemistry reference range. Due to this difference greater number of patients were categorized as normal in both male (59.1% vs. 46.5%) and female (56.5% vs. 40%) group in dry chemistry method as compared to wet chemistry. While number of patients categorized as high level of serum uric acid were less among both male (9.9% vs. 29.6%) and female (32.9% vs. 50.6%) when estimated by dry chemistry as compared to wet chemistry method.

A few limitations of our study include lack of availability of patients history, drug history, provisional diagnosis, and data related to variable age groups. Also, we have not included the healthy population in our study.

CONCLUSION

Currently all the methods available for estimation of serum uric acid have their own advantages and disadvantages. Similarly, high precision, easy to work and no use of water are advantages of dry chemistry over wet chemistry. Our study shows that though good correlation exists between wet and dry chemistry, the two methods are not similar. While reporting the results of serum uric acid laboratory should specify the method used for its estimation.

Table 5. Comparison of serum uric acid estimated by wet and dry chemistry among female and male

	Paired differences					t	ff	p
	Mean	SD	SEM	95% CI of the difference				
				Lower	Upper			
WC-DC (female)	1.0110	1.31	0.14	0.7285	1.2930	7	84	< 0.0001
WC-DC (male)	0.8233	0.84	0.07	0.6847	0.9620	11.64	141	< 0.0001

Note. CI: Confidence interval; SD: Standard deviation; & SEM: Standard error mean

Also, we must avoid to switch methods, especially while managing patients with hyperuricemia or hypouricemia. Due to the difference in reference range used in wet and dry chemistry, patients were categorized differently as normal, low or with elevated uric acid level among both male and female. A prospective study on sufficient sample size including healthy population may be conducted in future which will also help in establishing the reference range of methods available in our laboratory.

Author contributions: GM: preparation of manuscript and data collection & **KS:** concept of idea and study design, statistical analysis, and critical revision of the article. Both authors have agreed with the results and conclusions.

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Ethics declaration: The authors stated that the study was approved by the Institutional Review Board at King George's Medical University on 10 September 2020 with approval number 102ndECMIIBMBBS-S/P1.

Declaration of interest: No conflict of interest is declared by the authors.

Data sharing statement: Data supporting the findings and conclusions are available upon request from the corresponding author.

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