Association of Blood and Urine Parameter Value Change With the Amount of Consumed Water Regime and Sample Sampling in Healthy Individuals

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Research

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Abstract

Background: Understanding the effect of pre-analytical factors is important for data quality of bio-specimens and health status. The study examines the effect of 9-days fluid intake and 2-time sampling on concentration changes of 7-Urine and 17-Blood variables.

Material and Method: SPSS software v23.0 applies to data processing. The group of 23 healthy subjects divide based on water intake and gender.

Results: A statistically significant difference($p<0.01$) between 1st/2nd sampling is confirmed for Freezing point depression, Sodium, Potassium, Creatinine Urea and Urate in Urine and Urea, Urate, Glucose, Hematocrit, Thrombocyte in Blood. The difference between water intake after 1st sampling is confirmed ($p<0.01$) for Freezing point depression, Sodium, Urate and($p<0.05$) for Potassium($p<0.05$), Chloride($p<0.05$), Creatinine($p<0.05$), Urate, Urea in Urine and Potassium($p<0.01$) and Chloride($p<0.05$) in Blood. Difference between gender exists for Urea($p<0.05$) in Urine after 2nd sampling and Urate($P<0.01$), Glucose($p<0.01/0.05$), Ht($p<0.01/0.05$) after 1st and 2nd sampling and MCHC($p<0.01$) after 2nd sampling in Blood samples.

Conclusion: Water intake increases blood and urine biomarker range after sampling.

Background

Sustainable effort must obtain for sensitivity, specificity, robustness and reproducibility of data. Pre-analytical factors influence sample quality; reproducibility, stability and false-positive results. [1, 2]. Pre-analytic variables include 3. categories, physiologic (age, gender, sex, time, season, altitude, menstruation, pregnancy, lifestyle like diet, caffeine, ethanol, smoking), specimens collection (overnight fasting, time of specimen collection, posture during sampling, exercise, water intake, anticoagulants-blood ratio, specimen handling and processing, added additives with anticoagulants), and influence or interference factors (drug metabolites, laboratory tests, collection tube) affecting variable range [3].

Nervous system maintains organism homeostasis in the inward environment answering to external stimulus [4, 5]. Water intake as pre-analytical variable contributes to biochemical processes and affects establishing reference range, disease diagnosis, prognosis and follow-up [6, 7]. Urine and Blood samples are the most commonly used in clinical practice reflecting state of metabolome and metabolic end product [6, 7]. Critical aspects of biomarker stability during clinical planning, sample collection, training, selection of sample preservation, buffers, shipping, logistics, method analysis and results in the presentation is known for the most utilized bio-specimens [8]. Studies analyzed conditions like storage time, temperature, freezing-thawing cycle on biomarker range and reproducibility [1–3]. Despite increasing sensitivity of methodology for determining biomarker range, discrepancies among variables exist in the literature due to sampling frequency, sample and pre-analytical factor interaction complexity

[9]. Fluid intake habits and the effect on Urine/Blood biomarkers of healthy participants lack in the literature.

A current study was done as a part of the PhD thesis aim to examine the effect of 7 and 9-day regime water intake in Test (T) and Control (C) group on 7-Blood (Freezing point depression_FPD, Potassium_K⁺, Sodium_Na⁺, Chloride_Cl⁻, Urea, Creatinine_Cr, Urate) and 17-Urine (Urea, Cr, Urat, Glucose_Glu, C reactive protein_CRP, Leucocyte_LE, Erythrocyte_ER, Hemoglobine_Hg, Hematocrit_Ht, Mean Corpuscular Volume_MCV, Mean Corpuscular Hemoglobin_MCH, Mean Corpuscular Hemoglobin Concentration_MCHC, MPV_Mean Platelet Volume, Trombocyte_TR, K⁺, Na⁺, Cl⁻) parameters in healthy subjects, during 2 sample collection, depending on gender and water intake level. Hypothesis postulate that water intake affects the reference range of Urine and Blood biomarkers.

Material And Methods

Human biological material dataset

Experiments were performed according to ethical standards and with the written consent of the blood donors. Permission from the National Medical Ethics Committee, number 82/07/14, is given.

Participants differ by Gender (Male_M;Female_F), the amount of drinking water, Blood and Urine parameter values. A total number of participants consist of 23. healthy participants (F = 9, M = 14). M (60.9%) were numerous in comparing to F (39.1%) participants. Mean water intake for 5. days, is recorded. An upward trend in the T group and downward in the C group exists. The T group drank more than 1800 ml with a mean water intake value of 2055 ml. The C group consumed less than 2000 ml, with a mean water intake value of 1846 ml. The number of M/F participants in T/C group varies. The 14 subjects in the T group [M:(9/23); F:(5/23)] represent 39.12% M and 21.74% F of the total participant. Meanwhile, 9 subjects in the C group [M:(5/23); F:(4/23)] represent 21.74% M and 17.4 % F-number of the total participant.

The regime of water intake for 23 healthy subjects included 5-days of controlled water consumption, 2-days of arbitrary higher water intake in T group before 1st sampling (7th -day of water intake), 2-days of desired lower water intake in T group before 2nd sampling (9th -day of water intake), while C group drank the same level of water during 1st and 2nd sampling. Of the total M/F participants, 7-Urine and 14 Blood (Urea, Creatinine, Urate, Glucose, CRP, LE, ER, Hg, Ht, MCV, MCH, MCHC, MPV, TR) variables tested for a change. On 11 male participants, 3 additional electrolytes (Na⁺, K⁺, CL⁻) test for a change.

Statistical analysis

IBM-SPSS software v23.0 applies for statistical data analysis. Descriptive statistics, Shapiro-Wilk normality test, parametric and non-parametric statistical tests such as Paired and Independent T-test, Wilcoxon and Mann-Whitney/Kruskal-Wallis test is employed. Results display mean (SD) and p-value. A statistically significant correlation was assumed when P < 0.05.

Results And Discussion

Urine and Blood characteristics are present in Table 1 and Table 2. Shapiro-Wilk test indicated the presence of normality ($P > 0.05$) for FPD, K^+ , Na^+ , Cl^- , Cr and Urate during 2nd sampling and Urea during 1st sampling in Urine samples and for Glucose, MPV during 2nd sampling, Urea, Urate, Ht, MPV during 1st sampling and K^+ during 1st and 2nd sampling in blood samples. All other variables showed an absence of normality ($P < 0.05$). All non-parametric data transform to normality using log transformation, except for CRP and additional electrolytes in the Blood. Based on this notion, the parametric and non-parametric test is employed.

Table 1
Urine parameter value during 1st and 2nd sampling

Table 1. Participant Urine parameter		Participants group			
		Control		Test	
		Mean	Standard Deviation	Mean	Standard Deviation
Freezing point depression (mK)_1st sampling	Female	852.75	591.82	479.40	240.33
	Male	1072.00	455.10	334.44	103.97
Freezing point depression (mK)_2nd sampling	Female	752.50	287.25	858.40	380.45
	Male	1000.20	362.34	1283.44	579.44
K (mmol/L)_1st sampling	Female	37.50	19.23	27.40	13.72
	Male	59.20	28.58	17.56	9.61
K (mmol/L)_2nd sampling	Female	23.75	8.46	38.20	18.09
	Male	51.40	19.45	39.00	17.20
Na (mmol/L)_1st sampling	Female	82.25	65.51	38.40	15.42
	Male	99.80	48.02	23.89	8.67
Na (mmol/L)_2nd sampling	Female	103.50	57.74	68.00	25.09
	Male	101.00	61.56	84.56	47.67
Cl (mmol/L)_1st sampling	Female	77.50	64.92	49.40	18.69
	Male	140.80	73.65	33.56	13.00
Cl (mmol/L)_2nd sampling	Female	82.25	50.41	52.20	19.92
	Male	115.60	77.35	78.89	61.76
Urea (mmol/L)_1st sampling	Female	188.50	145.42	111.40	70.96
	Male	210.80	85.51	80.11	44.90
Urea (mmol/L)_2nd sampling	Female	144.50	58.03	216.00	126.16
	Male	212.00	70.25	370.22	188.24
Cr (mmol/L)_1st sampling	Female	13.08	13.43	4.26	4.23
	Male	12.06	9.09	2.84	1.68
Cr (mmol/L)_2nd sampling	Female	8.25	1.63	9.68	5.54
	Male	8.88	4.40	15.07	8.59

Urat (mmol/L)_1st sampling	Female	2.18	1.71	0.96	0.55
	Male	2.02	0.86	0.78	0.32
Urat (mmol/L)_2nd sampling	Female	1.78	0.75	1.74	0.78
	Male	1.82	0.53	2.74	1.24

Table 2
Blood parameter value during 1st and 2nd sampling

Table 2. Participant blood parameter		Participants group			
		Control		Test	
		Mean	Standard Deviation	Mean	Standard Deviation
Urea (mmol/L)_1st sampling	Female	3.65	1.68	3.74	0.86
	Male	4.26	1.40	3.77	1.13
Urea (mmol/L)_2nd sampling	Female	3.60	0.82	3.82	0.68
	Male	4.32	1.43	4.51	1.67
Cr (μmol/L)_1st sampling	Female	72.25	9.54	61.80	4.60
	Male	67.00	6.08	72.44	10.50
Cr (μmol/L)_2nd sampling	Female	71.25	7.85	63.80	4.38
	Male	64.80	5.45	74.11	10.40
Urat (μmol/L)_1st sampling	Female	245.50	18.63	263.60	32.53
	Male	329.80	38.23	280.00	21.17
Urat (μmol/L)_2nd sampling	Female	259.00	14.70	287.20	20.19
	Male	332.20	58.98	310.22	42.42
Glucose (mmol/L)_1st sampling	Female	4.05	0.26	4.20	0.16
	Male	4.84	0.79	4.62	0.48
Glucose (mmol/L)_2nd sampling	Female	4.25	0.24	4.46	0.30
	Male	4.92	0.57	4.67	0.45
CRP (mg/L)_1st sampling	Female	1.00	0.00	0.80	0.84
	Male	2.80	2.59	0.67	0.50
CRP (mg/L)_2nd sampling	Female	1	1	1	2
	Male	3	3	1	1
LE (10 ⁹ /L)_1st sampling	Female	6.45	0.62	6.00	0.97
	Male	5.86	1.71	6.86	1.70
LE (10 ⁹ /L)_2nd sampling	Female	6.8	1.1	6.6	2.1
	Male	6.0	1.4	6.6	1.3

Table 2. Participant blood parameter		Participants group			
		Control		Test	
		Mean	Standard Deviation	Mean	Standard Deviation
ER (10 ⁹ /L)_1st sampling	Female	4.48	0.36	4.64	0.18
	Male	4.90	0.32	4.80	0.34
ER (10 ⁹ /L)_2nd sampling	Female	4.5	0.4	4.7	0.3
	Male	4.9	0.4	4.8	0.4
Hg (g/L)_1st sampling	Female	132.00	6.78	135.20	8.14
	Male	144.20	14.97	141.56	11.06
Hg (g/L)_2nd sampling	Female	130	8	138	9
	Male	143	14	139	12
Ht_1st sampling	Female	0.39	0.01	0.40	0.02
	Male	0.43	0.04	0.42	0.03
Ht_2nd sampling	Female	0.39	0.02	0.41	0.02
	Male	0.43	0.03	0.42	0.03
MCV (fl)_1st sampling	Female	88.25	6.18	86.80	3.03
	Male	88.00	6.20	88.33	4.95
MCV (fl)_2nd sampling	Female	88	5	87	3
	Male	89	7	88	5
MCH (pg)_1st sampling	Female	29.50	2.08	29.00	1.00
	Male	29.60	2.51	29.56	2.24
MCH (pg)_2nd sampling	Female	30	2	30	1
	Male	29	2	29	2
MCHC (g/L)_1st sampling	Female	335.00	8.21	337.20	6.02
	Male	334.40	5.90	334.11	8.10
MCHC (g/L)_2nd sampling	Female	333	9	341	5
	Male	328	8	331	10
MPV (fl)_1st sampling	Female	8.75	.50	8.40	0.89

Table 2. Participant blood parameter		Participants group			
		Control		Test	
		Mean	Standard Deviation	Mean	Standard Deviation
	Male	9.00	1.22	7.78	0.97
MPV (fl)_2nd sampling	Female	9	1	8	1
	Male	9	1	8	1
TR (10 ⁹ /L)_1st sampling	Female	239.75	26.70	251.60	51.57
	Male	261.80	31.07	264.00	52.69
TR (10 ⁹ /L)_2nd sampling	Female	259	38	269	50
	Male	255	36	275	39
K (mmol/L)_1st sampling	Male	4.4	0.2	4.0	0.2
K (mmol/L)_2nd sampling	Male	4.2	0.2	4.1	0.2
Na (μmol/L)_1st sampling	Male	137	2	134	5
Na (μmol/L)_2nd sampling	Male	137	2	136	5
Cl (μmol/L)_1st sampling	Male	104	1	100	4
Cl (μmol/L)_2nd sampling	Male	102	1	101	4

Paired T-test revealed a statistically significant difference ($P < 0.01$, 95% CI), between 1st and 2nd sampling for FPD, Na^+ , K^+ , Cr, Urea, Urate in Urine and Urea, Urate, Glu, Ht, TR in Blood. Wilcoxon T-test shows the absence of statistically significant difference ($P > 0.05$, $\alpha = 0.05$, 95% CI) for CRP in Blood. Results are confirmed with Mann-Whitney and Kruskal-Wallis test. The difference between T/C group confirm independent T-test for FPD ($P < 0.01$), K^+ ($P < 0.05$), Na^+ ($P < 0.01$), Cl^- ($P < 0.05$), Cr ($P < 0.05$), Urate ($P < 0.01$) and Urea ($P < 0.05$) in Urine after 1st sampling and K^+ ($P < 0.01$) and Cl^- ($P < 0.01$) after 1st sampling in Blood. Figure 1. shows the difference between test and control group for 1st and 2nd sampling in Blood and Urine. Gender difference confirmed for Urea ($P < 0.05$) variable after 2nd sampling in Urine and for Urate ($P < 0.01/0.05$), Glu ($P < 0.01/0.05$) and Ht ($P < 0.01/0.05$) after 1st and 2nd sampling and MCHC ($P < 0.05$) during 2nd sampling in Blood.

The amount of water intake, diseases, usage of drugs and profession type can lead to electrolyte misbalance resulting in quality and interpretation, further prognosis, diagnosis and patient follow-up [10, 11]. In this study, the percentage difference between genders exists for Urea (52.22%) after 2nd sampling in Urine. In Blood for Urate (1st:15.16%; 2nd:14.5%), Glu (1st:13.63%; 2nd:8.7%), Ht (1st and 2nd:7.23%)

during 1st and 2nd sampling and MCHC (2.1%) during 2nd sampling. Males have higher values in comparing to females. Sex hormones affect gender differences [12–15]. Female sex hormones (Estrogen) regulate the activity of glucose and urate transporters (ABCG2 and SLC2A9), having different transporter expression (transcription, post-translational modification), localization and activity [12, 13]. Male sex hormones (Testosterone) affect MCHC and Ht level through the increase of erythropoietin, reduction of ferritin and hepcidin [14]. Testosterone influence protein metabolism and the urea cycle [15].

Drinking more water improves kidney function and clearance of toxins by glomerular filtration, tubular secretion, and activation of various degradative metabolic pathways [17]. Results indicate that water intake influence Urine [FPD (86.5%), K⁺ (81.7%), Na⁺ (104.1%), Cl⁻ (97.37%), Urea (75.34%), Cr (116.45%), Urat (89.65%)] and Blood [K⁺ (9.5%), Cl⁻ (3.92%)] during 1st sampling. There is a link between water intake and homeostatic mechanisms to maintain water balance and health outcomes. Urine osmolality depends on cations, Na⁺, K⁺, NH₄⁺, anions and Urea, whereas FPD enables estimation of urine osmolality [18, 19]. Freezing point depression as a colligative property depends on the molality of the solute [20]. Renal Cr excretion level depends on the glomerular filtration rate, proximal tubular secretion and OCT-2 transporter [21, 22]. Higher water intake after 3. days causes a decrease of uric acid, up-regulation of GLUT9 and URAT1 and down-regulation of ABCG2 and OAT1, while after 7. days affect NPT1 down-regulation in hyperuricemia mice [23]. Urate level depends on transport proteins (URAT1 and GLUT9), uricase inactivation and possible change of the intestinal microbiota [23]. Production of concentrated Urine requires interactions among the nephron segments and vasculature in the kidney medulla [24]. Arginine vasopressin (AVP) is a key molecule in water homeostasis. Increase water intake, decrease AVP, reduce risk of renal and metabolic diseases and improve health outcome [24]. Vasopressin regulates urea transport acutely by increasing UT-A1 phosphorylation and the apical plasma-membrane accumulation of UT-A1 through two cAMP-dependent pathways [25]. Glut9 plays a major role in urate homeostasis by its dual role in urate handling in the kidney and uptake in the liver [26]. Small water intake can lead to dehydration, activation of the renin-angiotensin system (RAS) through angiotensin receptors and subsequent activation of signalling molecules, protein kinase C, reactive oxygen species, MAP kinase pathway mediated with angiotensin (27). Prolactin, aldosterone and antidiuretic hormone, influence water metabolism and electrolyte balance [28–37].

Results show higher concentration percentage increase in variables from Urine [FDP (68%), Na⁺ (62.96%), K⁺ (21.88%), Cr (65.2%), Urat (69.2%), Urea (96.27%)] in comparing to Blood [Urea (10.5%), Urate (7.1%), Glu (2.2%), Ht (2.43%), TR (3.9%)] between 1st and 2nd sampling of 7th and 9th day of water intake. Studies indicate that daily water intake in healthy Japanese adults' decrease blood pressure, Ht, Urine gravity and a rise in body temperature [38]. Lower and steady daily water intake increase Cr, Cortisol, Urea, Uric acid, Na⁺, Hg; decrease CRP, and have no change in Ht, active rennin, aldosterone and plasma osmolality [39–42].

Ex vivo cellular injury, disintegration, cellular granule release and protease activation causes alterations of cell release in vitro after sampling [1]. Hydration biomarkers in 24h urine correlate with daily total fluid intake volume in sedentary adults in free-living conditions [43]. Literature findings follow the results of the

study. Explain differences in concentration changes of healthy participants, due to water intake and sample sampling.

Conclusions

Information about the water regime during 7–9 days and gender in healthy participants positively impact further clinical studies on disease patients. Water intake changes Blood and Urine biomarker concentration. Advantage of the study is clinical reproducibility, applicability, fast and precise insight into physiological changes. Results can be a reference point for protocol standardization and quality control check. Lead the improvement of healthcare service. The disadvantage is the number of participants, absence of additional information (habits, demography and genetic analysis). Future work should emphasize disadvantages by comparing health/disease states to draw clinically applicable conclusions.

List Of Abbreviations

Arginine vasopressin (AVP), Chloride_ Cl^- , Control_C, Creatinine_Cr, C reactive protein_CRP, Erythrocyte_ER, Female_F, Freezing point depression_FPD, Glucose_Glu, Hematocrit_Ht, Hemoglobine_Hg, Leucocyte_LE, Male_M, Mean Corpuscular Volume_MCV, Mean Corpuscular Hemoglobin_MCH, Mean Corpuscular Hemoglobin Concentration_MCHC, MPV_Mean Platelet Volume, Potassium_ K^+ , Sodium_ Na^+ , Test_T, Trombocyte_TR,

Declarations

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Availability of data materials

Data are available in Table 1 and 2.

Ethical approval and consent to participate

Experiments were performed according to ethical standards and with the written consent of the sample donors. Permission from the National Medical Ethics Committee, number 82/07/14, is given.

Consent for publication

"Not applicable"

Competing interest

The author, a PhD student, Snežana Jovičić, declare no competing interest.

Author's contribution

Author (Snežana Jovičić, PhD student at the Faculty of Biology, University of Belgrade, Serbia), originated data collection, analysis, interpretation and writing. The author made all the effort, accuracy, integrity and quality. Author approves the final version of the presented manuscript for submission. Author, confirms that this work is original and has not been published elsewhere, nor is it currently under consideration for publication elsewhere.

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Figures

Figure 1

Differences between Test and Control group in Urine (A1, A2) and Blood (B) sample