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Diagnostic Values of Uric Acid and Pro-Inflammatory Cytokines for Renal Failure in Arab Adults

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ABSTRACT

The detection of risk factors and the early diagnosis of renal failure are vital for the success of preventive therapeutic interventions. This study aimed to determine the potential association between uric acid (UA) levels and pro-inflammatory markers in patients undergoing hemodialysis (HD). Moreover, their possible role in premature diagnosis of renal injury (RI) was also evaluated. 50 HD patients and 24 healthy volunteers were included. Uricase-PAP test was used to measure UA. Tumor necrosis factor (TNF-α), interleukin6 (IL-6), and C-reactive protein (CRP) were measured by multiplex ELISA assay. The usefulness of UA and Pro-inflammatory markers for timely detection of renal damage was measured using the receiver operating characteristic (ROC) curve. Patients had significantly higher plasma concentrations of UA, TNF-α, IL-6, and CRP relative to volunteers. A positive correlation was found in HD patients between UA and TNF-α, and CRP, but there was no significant correlation between UA and the inflammatory markers in healthy controls. The area under the ROC curve for serum CRA was smaller than that for UA, demonstrating that UA may serve as a superior biomarker for early detection of renal injury in comparison to TNF- α. Nevertheless, serum CRP was found to be greater than both TNF-α and IL-6, the latter of which had a non-significant area under the ROC curve. Taken together, serum UA, CRP, and TNF-α—but not IL-6—are potentially early indicators for the diagnosis of RI. Moreover, serum UA is significantly elevated in HD patients and positively associated with inflammatory markers.

Keywords: hemodialysis, inflammation, uric acid, chronic kidney disease.

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INTRODUCTION

Chronic kidney disease (CKD) is a crucial global communal health problem, and its incidence is enormously rising. Thus, detection of risk features and premature diagnosing of the disease is a critical for therapeutic interventions to shun development of renal failure [1].

Uric acid is produced in human by the action of xanthine oxidase, where it is the last product of purine catabolism [2]. UA is also documented as a simple risk factor of morbidity and mortality in heart diseases; it is incorporated in oxidative stress as well as inflammation [3]. A positive correlation between plasma UA and inflammatory markers was demonstrated in heart disease patients [4]. Moreover, positive correlation between UA and CRP was recorded in general population [5]. Additional cross-sectional population study revealed that uric acid had a positive association with IL-6, CRP, and TNF-α, and a negative one with nterleukin-1 beta (IL-1β) [6]. In a murine model of metabolic syndrome, it was demonstrated that elevated uric acid in plasma functions as mediator of pro-inflammatory endocrine imbalance in the adipose tissue; which is speculated to enhanced synthesis of monocyte chemotactic protein-1 (MCP-1) leading to decreased production of adiponectin [7]. Moreover, mortality in CDK patients was found to correlate with the levels of UA, intracellular adhesion molecule-1 (ICAM-1) and CRP [8]. Shahbazian et al also demonstrated that there exists a correlation between elevation UA levels and reduced renal function in 50% of subjects on dialysis [9].

Despite the fact, that HD patients frequently have elevated levels of serum pro-inflammatory cytokines, CRP, and UA [10], the potential role of these markers for early diagnosis of RI have not been sufficiently investigated.

Furthermore, the survey of the literature revealed few studies on connection between UA and markers of inflammation in HD patients. One such study showed a positive correlation between markers of inflammation and UA among Brazilian HD patients. It was suggested that UA may play a key role in observed inflammation and atherosclerosis in these patients [11]. Survey of literature also revealed that no such study was conducted in Arab patients and it is apparent that the ethnicity differences may play an important role in such analysis, such a study would provide valuable information.

Objectives

This study was designed to investigate the potential use of UA and pro-inflammatory markers as early diagnostic parameters for renal failure in Arab adults. Moreover, the possible association between uric acid (UA) levels and inflammatory markers in patients undergoing hemodialysis (HD) due to renal injury was also analyzed.

Materials and methods

Study population

This retrospective study enrolled 50 hemodialysis patients and 24 sex and age matched volunteers. The study setting was the outpatient clinic of King Khaled General Hospital located in Hail, Saudi Arabia. Demographic data including age, gender and clinicopathological features of the investigated patients and control subjects were collected.

Biochemical Analyses

For preparation of hemodialysis (HD) the subjects fasted overnight. Blood samples were collected from the arterial

line of the hemodialysis immediately before the start of HD session. The serum was collected following standard procedure and kept frozen at -80 C until analyzed. Quantitation of the cytokines IL-6, TNF-α and inflammation marker CRP was carried out using the kit from R&D Systems (Minneapolis, MN, USA) in a Luminex® 100/200™System (Austin, TX, USA) following the instruction of the manufacturer. Serum uric acid was measured by standard analytical methods (Clauss technique and uricase enzymatic test).

Statistical Analysis

SPSS version 23 was used for analysis of collected data. The student t-test was used to compare the mean of the patients and control subjects and the data were expressed as mean ± SD. Pearson's (*r*) correlation coefficient was determined for testing of bivariate correlations. Area under the receiver operating characteristic (ROC) curve (AUC) was evaluated to determine the ability of the studied tests and formulae to discriminate the early renal impairment. The two-tailed p values less than 0.05 were considered significant. ROC was used to calculate; the specificity, sensitivity, positive predictive value (PPV), negative predictive value (NPV), positive likelihood ratio (PLR) and negative likelihood ratio (NLR).

Ethical statement

The research protocol received agreement by the ethical review committee of Hail University (Ethical No.2017/0123). Written informed consent was obtained from all the subjects for inclusion in this study.

Results

Demographic characteristics of the patients and controls

The present study was performed on a total of 50 hemodialysis patients from the outpatient's clinics at the King Khaled hospital, Hail, KSA including 21 males (42%) and 29 females (58%) with a mean age of 52.30 ±18.6 yrs. The second group consisted of 24 healthy individuals including 14 (58%) males and 10 (42%) females with mean age of 47.54 ± 8.47 yrs. The age of the two groups was comparable and no significant difference was observed (Table 1).

Table1. Demographic data of the patients and control

	Number	M/F ratio	Age (yrs)	>50/<50yrs
Hemodialysis	50	21/29	52.30 ±18.6	21/29
Control	24	14/10	47.54 ± 8.47	15/9
			p > 0.05 (NS)	

Serum uric acid and inflammatory factors in the patients and healthy individuals

A significant difference was detected in the level of serum uric acid in the patient groups and control. For hemodialysis patient group, serum uric acid was 6.39 ± 1.50 mg/dL while in the control the level was 3.91 ± 0.68 mg/dL (p < 0.001). For CRP, significant greater level was recorded in the hemodialysis group (1.40 ± 0.88 mg/ml) compared with the control group (0.77 ± 0.86 pg/ml, p < 0.05). For IL-6, no significant difference could be discovered (3.45 ± 2.38 pg/ml, p = 0.119). For TNF-α, hemodialysis group showed a significantly higher level (2.88 ± 1.85 pg/ml) than the control (1.68 ± 0.61 pg/ml, p = 0.003). Table 2 summarized the results.

Table 2. Serum uric acid and inflammatory factors in the patients and controls

	Uric acid	CRP	IL-6	TNF-α
Hemodialysis	6.39 ± 1.50	1.40 ± 0.88	3.45 ± 2.38	2.88 ± 1.85
Control	3.91 ± 0.68	0.77 ± 0.86	2.59 ± 1.72	1.68 ± 0.61
p-value	0.000	0.005	0.119	0.003

Effect of gender and age on serum uric acid and inflammatory factors in the patients and controls

In the hemodialysis patients, serum uric acid was significantly higher in males (6.88 ± 1.76 mg/dL) than in females (6.02 ± 1.18 mg/dL, p < 0.05), but for the inflammatory factors, no significant differences between males and females were observed. No significant association was found between gender and the inflammatory factors or uric acid among controls. For age, no significant difference was observed for patients or controls (Table 3).

Table 3. Effect of gender and age on serum uric acid and inflammatory factors in the patients and controls.

		No	Uric acid	CRP	IL-6	TNF-α
Hemodialysis	M	29	6.88 ± 1.76*	1.43 ± 0.87	3.62 ± 2.27	2.97 ± 1.55
	F	21	6.02 ± 1.18	1.38 ± 0.89	3.33 ± 2.48	2.81 ± 2.06
Control	M	14	3.59 ± 0.68	0.91 ± 0.95	2.96 ± 2.03	1.68 ± 0.76
	F	10	3.86 ± 0.71	0.59 ± 0.74	2.08 ± 1.05	1.68 ± 0.36
Hemodialysis	> 50yrs	21	6.39 ± 1.73	1.54 ± 0.82	2.88 ± 1.91	1.68 ± 2.38
	< 50yrs	29	6.38 ± 1.35	1.31 ± 0.92	3.87 ± 2.62	2.92 ± 1.40
Control	> 50yrs	15	3.79 ± 0.71	0.73 ± 0.91	2.13 ± 0.98	1.78 ± 0.70
	< 50yrs	9	4.12 ± 0.61	0.85 ± 0.83	3.36 ± 2.39	1.52 ± 0.41

ROC analysis of the studied parameters

Table 4 presents the AUC for each of the studied parameters. Three parameters had significant AUCs including serum uric acid, TNF-α, and CRP. IL-6 had a non-significant area.

Table 4. Area under the ROC curve of the studied markers

	AUC	SE	p-value	95% C. I.	
Uric acid	0.948	0.026	0.000	0.898	0.999
TNF-α	0.719	0.070	0.002	0.582	0.855
CRP	0.858	0.049	0.000	0.762	0.954
IL-6	0.617	0.071	0.106	0.478	0.755

Diagnostic criteria of the studied markers

The optimal cut off value (which is the nearest point to the upper left corner of a ROC plot) that satisfies maximum sensitivity and maximum specificity was revealed. At a cut off value of 4.94 mg/dL for serum uric acid, a sensitivity and specificity of 90% and 95.83%, respectively were obtained. The DPR, PPV, NPV, PLR and NPR were 85.83%, 17.86, 21.58% and 0.10, respectively. For TNF-α, a cut off value of 2.05 pg/ml was obtained at which the sensitivity and specificity were 80% and 87.5%, respectively. The other diagnostic criteria are shown in table 5. For CRP, at a cut off value of 0.5 pg/ml, the sensitivity and specificity were 80% and 58.33%, respectively (Table 5& Figure 1 a, b, and c).

Table 5. Diagnostic criteria of uric acid and inflammatory markers in the hemodialysis patients against controls.

marker	cut	sensitivity	Specificity	DPR	PPV	NPV	PLR	NLR
UA	4.94	90	95.83	85.83	97.83	17.86	21.58	0.10
TNF-α	2.05	80	87.50	67.50	93.02	32.26	6.40	0.23
CRP	0.5	80	58.33	38.33	80.00	41.67	1.92	0.34

DPR: differential positive rate, PPV: positive predictive value, NPV: negative predictive value, PLR: positive likelihood ratio, NLR: negative: likelihood ratio.

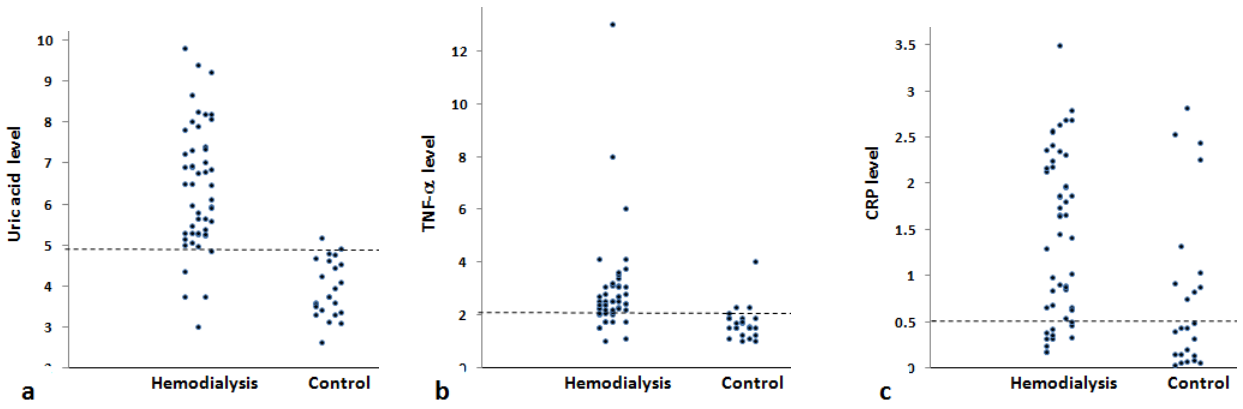

Figure 1. Scatter diagram showing the individual values of serum uric acid (a), TNF-α (b) and CRP (c). The dotted line represents the cut off value with respect to the control group.

Correlation between the studied parameters

Positive correlation was observed in the hemodialysis patients between uric acid and TNF-α ($r= 0.367$, $p < 0.01$), CRP ($r= 0.425$, $p < 0.01$) and IL-6 ($r= 0.468$, $p < 0.01$. No significant correlation could be detected between uric acid and the inflammatory factors in the controls. Table 6 & Figurer 2 (A-C).

Table 6. Correlation between serum uric acid and inflammatory factors in hemodialysis patients

		TNF-α	CRP	IL-6
Hemodialysis	Uric acid	0.367**	0.425**	0.468**
	TNF-α	1	0.251	0.172
	CRP		1	0.143
Control	Uric acid	0.131	0.326	0.128
	TNF-α	1	0.265	-0.125
	CRP		1	0.035

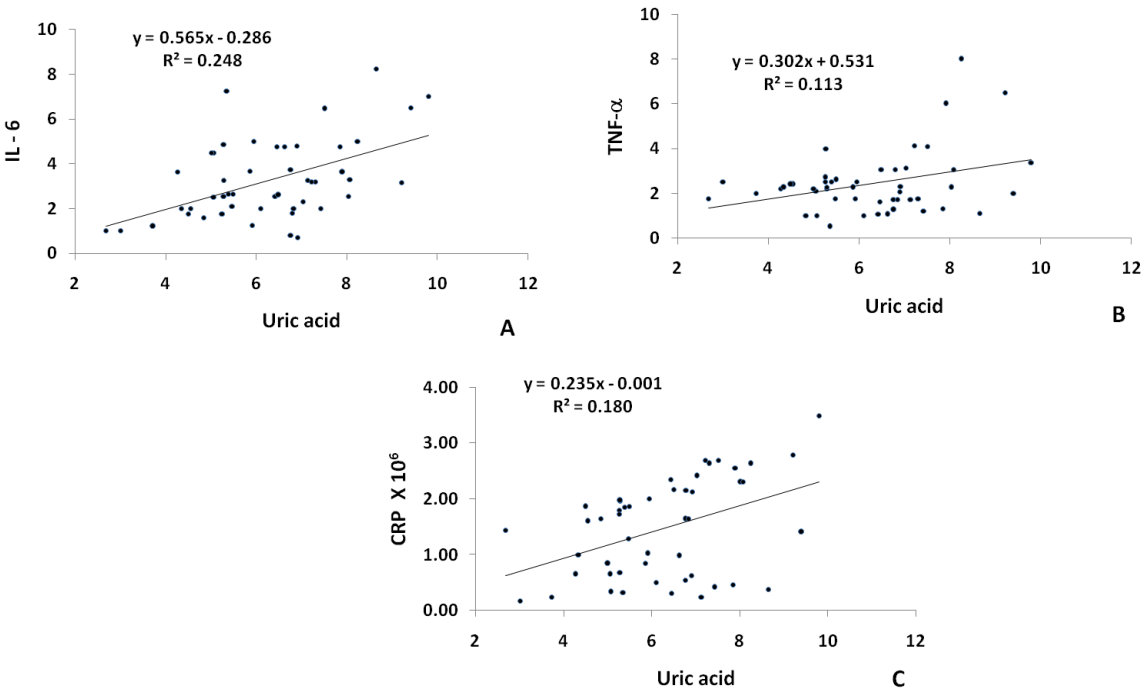

Figure 2. Correlation between uric acid and IL-6 (r= 0.468, p < 0.01, A), TNF-α (r= 0.367, p < 0.01, B) and CRP (r= 0.425, p < 0.01, C).

Discussion

The findings of this study demonstrated high concentrations of serum UA, CRP, TNF-α, and IL-6 in patients undergoing hemodialysis in comparison to the control group, and significant correlation between serum UA and inflammatory markers among these patients. Notably, the association stays significant following correction for a number of factors likely disturbing either serum UA or inflammatory markers concentrations, namely, age, sex, or drugs use.

These results support the hypothesis suggesting a positive correlation between elevation of serum UA and the possibility of occurring inflammation in different diseases and in general population. It has been demonstrated that UA is correlated with oxidative stress and inflammation in many cardiovascular disorders [3]. Furthermore, several studies found that high serum UA may be involved in impairment of glomerular capsule and renal tubules and thus, development of microalbuminuria [12-13]. On the other hand, active inflammatory infiltrate point toward tubule-interstitial injury following by interstitial fibrosis, indicating the implication of inflammatory markers in the progress of CKD [14]. A significant correlation was also found between the high serum levels of TNF-α, and IL-6 in serum with prognostic death among chronic renal diseases patients [15]. It was also demonstrated that expression of IL-6, IL1, CRP, and TNF-α can be induced by UA, suggesting additional vital indirect pathway of UA-induced renal impairment [16]. Stimulation of inflammation by elevated uric acid might occur through very complex pathways including angiotensin II, mitogen-activated protein kinases (p38 and ERK 1/2), transcription factor NF-κB, , Toll-like receptor (TLR) 2 and 4, (pro)renin receptor, and COX-2 [17-19].

In line with these results, few works had elucidated a relationship between elevations of plasma UA and inflammatory markers in patients with chronic kidney disease, e.g.; Suliman's et al showed that plasma uric acid is positively related to CRP and ICAM-1 among hemodialytic patients [20], and Guarda et al found positive associations between elevated levels UA in serum and increase of urinary proinflammatory cytokines in type 2 diabetic patients [21].

However, there are contradictory results concerning the beneficial effect of UA-reducing treatment on the progression of CKD; while, a meta-analysis work showed that UA -lowering treatment might improve renal impairment in CKD patients [22], a recent study revealed that UA–lowering with allopurinol hasn't ameliorated kidney injures in patients with asymptomatic hyperuricemia and stage 3 CKD [23]. It is difficult to explain the inconsistency in these data, but this may be partially attributed to ethnic and additional environmental factors.

As premature identification of renal dysfunction is crucial for decreasing morbidity and mortality, and in the light of increased concentrations of UA and other inflammatory molecules in HD patients obtained in the present study, the ROC curve analysis was used to assess the diagnostic value of these markers in identifying early renal impairment. It is generally accepted that ROC curve, utilized to estimate the best possible cut-off level of a laboratory test, is a specific and convincing measure of diagnostic performance [24]. In this investigation, it was established that, regardless of age or six, serum UA was superior biomarker than that of CRP, and TNF-α for early detection of RI. Nevertheless, serum CRP was found to be more diagnostically efficient than TNF-α., and IL-6 has non-significant diagnostic value.

In consonance with the current study, serum UA has also been reported to have prognostic importance for premature atherosclerosis in patients with chronic kidney disease [25]. Other study showed that high UA

level in serum has significant prognostic value for endothelial injury in CKD patients [26]. Nevertheless, other study demonstrated that serum UA has no prognostic value for kidney disease development [27].

Conclusion

Taken together, this study revealed that HD-patients have significantly higher serum UA, CRP, TNF-α, and IL-6 compared with healthy controls, and serum UA was positively correlated with the investigated inflammatory biomarkers in these patients. UA, CRP, and TNF-α, can be used for the premature diagnosis of kidney impairment. However, further randomized controlled trials will provide important information on the potential benefit of UA-lowering drugs in ameliorating renal impairment among CKD patients.

Limitations

Unfortunately, the present study has some limitations. Firstly, both the number of patients and healthy controls engaged were reasonably small. Additionally, it was not possible to determine the effect of UA reducing drugs on serum inflammatory markers analyzed in this study.

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Conflicts of interest

The author states no conflict of interest.

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