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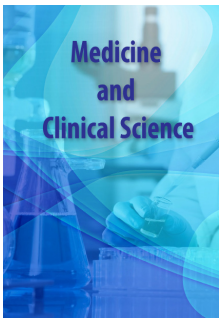

Effect of hyperuricemia on endothelium in kidney transplant patients

Lourdes Roca-Argente[1], Julio Hernández Jaras[1], Isabel Beneyto Castelló[1] and Celia Bañuls Morant[2]

[1]*Department of Nephrology, La Fe University and Polytechnic Hospital, Valencia, Spain*

[2]*Department of Endocrinology Investigation, Peset University Hospital, Valencia, Spain*

Correspondence

Lourdes Roca-Argente, MD

Departamento de Nefrología, Hospital Universitario y Politécnico La Fe, Bulevar Sur s/n, 46026 Valencia, Spain

Tel: +34 96124 4000.

E-mail: lroca_argente@hotmail.com

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Abstract

Background: Uric acid has been historically regarded as both a beneficial molecule and evolutionary factor for Homo sapiens and as a pathogenic molecule involved in the pathophysiology of some diseases. Nowadays, there is not any evidence-based explanation to justify the apparently causal role of serum uric acid in certain cardiovascular diseases such as hypertension in adults, chronic kidney disease, type II diabetes or metabolic syndrome. One pathophysiological mechanism proposed to explain the role of uric acid in cardiovascular diseases is the endothelial dysfunction.

Methods: We proposed an observational and prospective study of 38 first post-transplant patients to analyze the relationship between serum uric acid levels and markers of endothelial dysfunction and inflammation, such as VCAM, ICAM, IL10, CD40, TNFα, E-selectin, P-selectin and PCR.

Results: We observed an improvement in the endothelial markers VCAM and ICAM during the first year of transplant. Indeed, diabetic patients had higher levels of TNFα and serum uric acid one year after kidney transplant. Patients with cytomegalovirus infection presented higher levels of TNFα on the third month and serum uric acid at the twelfth month after kidney transplant. However, we were not able to demonstrate a positive correlation between serum uric acid and markers of endothelial dysfunction and inflammation within our sample.

Conclusions: Results show a relationship between uric acid and markers of endothelial dysfunction and inflammation in kidney transplant patients, although we were not able to identify whether uric acid was the cause.

Background

Uric acid is the end product of purine metabolism and it is catalyzed by enzyme Xanthine Oxidoreductase (XOR) activity. The purine degradation in uric acid by enzyme XOR is exclusive to humans and primates. Within the rest of mammals, it is the enzyme uricase the one that converts uric acid to allantoin, being the former more soluble, which facilitates its elimination [1]. Over Homo sapiens evolution, several mutations happened in the uricase precursor gen and inactivated it, which resulted in not being able to produce allantoin [2]. This evolution change has been for years related to survival functions, such as intelligence, due to the supposed brain stimulation properties of uric acid. In fact, in 1920 serum urate average was 3.5 mg/dl and in 1970 6-6.5 mg/dl.

Normal uric acid production on a healthy man with normal protein intake has been estimated to be 700 mg approximately, or 10 mg/kg of weight per day. The serum urate concentration is not constant throughout life. The average uricemia in children is 3-4 mg/dl and in puberty, it may increase to 1-2 mg/dl. On the contrary, women in fertile age present levels inferior to men's of similar age and menopause is associated to an uricemia increase close to 1 mg/dl. The uric acid execration pathway is mainly renal with a percentage higher than 95%. A small proportion is found on the gastric juice and less than 1% is eliminated through sweating. For that reason, patients with kidney failure and a decreased renal excretion present a higher prevalence of hyperuricemia.

Traditionally, we speak of asymptomatic hyperuricemia in clinical situations where serum urate concentration is high, but no symptoms or signs of monosodium urate crystal deposits are found. There is not an appropriate definition of asymptomatic hyperuricemia due to two primary reasons. First, the high prevalence of urate values within the two standard deviations of population average (5.5 ± 2.8 mg/dl) [3]. Second, it is believed that the negative effects of the serum urate high levels on cardiovascular events as well as the endothelium are produced in concentrations below saturation levels [4].

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The causes of hyperuricemia may be primary (over 90%) or secondary due to an increase of production (glycogenosis, enzyme deficiency such as hypoxanthine-guanine phosphoribosyl transferase or fructose aldolase or phosphoribosyl pyrophosphate synthetase hyperactivity, calorific over-intake, increase of cell turnover) or a decrease of its renal excretion (renal parenchymal damage, chronic lead poisoning, metabolic or respiratory acidosis, hyperthyroidism or hyperparathyroidism, ketosis).

Asymptomatic hyperuricemia is a biochemical alteration whose impact on cardiovascular diseases has been from many years subject of discussion [5].

On the one hand, today there is not any plausible biological mechanism to explain hyperuricemia development on cardiovascular diseases and the overlapping of other traditional risk factors as chronic kidney disease, diabetes mellitus, high blood pressure, smoking, sedentary habits and obesity in patients at high risk of cardiovascular events. Some of the physiopathological proposals to explain its mechanism are oxidative stress, activation of the renin- angiotensin-aldosterone system (RAAS) and endothelial dysfunction. Actually, the increasing relevance of the XOR enzyme's study over the last years is due to its affinity for producing reactive oxygen species as superoxide anion (O_2^-) and hydrogen peroxide (H_2O_2), all of which favor endothelial dysfunction. Accordingly, it is believed that the beneficial effect of hypouricemic drugs, such as allopurinol or febuxostat, is caused by XOR inhibition. According to this theory, hyperuricemia does not constitute *per se* a risk factor for the vascular endothelium and cardiovascular diseases, but a mere epiphenomenon.

On the other hand, there is research directly connecting hyperuricemia with the increase of cardiovascular events. An observational study of 7978 patients with high blood pressure followed over 20 years published in 1999 showed that serum urate baseline and follow-up levels were acute myocardial infarction independent risk factors, as well as the need of coronary revascularization independently from blood pressure control. These events were most frequent in patients with concomitant cardiovascular risk factors, i.e. diabetes mellitus, smoking, left ventricular hypertrophy, a history of cardiac disease and pulse wave alterations [6]. In 2001, a study published in Japan observed that the presence of hyperuricemia coincided with an increase of inflammation markers and the worst renal diagnosis histologically objectified [7]. In 2010 Dr. Goicoechea and her team conducted a prospective, randomized trial with a 20 month follow-up of 113 patients with a glomerular filtration rate of < 60 ml/min/1.73 m^2 (MDRD-4) and a serum urate average of 7.5 mg/dl. They observed that the glomerular filtration decrease was 3.3 ± 1.2 ml/min/1.73 m^2 in the control group and 1.3 ± 1.3 ml/min/1.73 m^2 in the group receiving allopurinol after 24 months (p = 0.018) [8].

Some of the factors involved in serum urate increase in transplant patients are decreased renal excretion, use of diuretics, male gender, time spent on dialysis before transplant, suboptimal donors [9], atherosclerosis in donor, cytomegalovirus infection and use of calcineurin inhibitors. Armstrong and her team observed hyperuricemia was prevalent in patients with functional kidney graft as well as an existing relationship between the ciclosporin dose and serum urate and the function of the kidney graft after a two-year follow-up [10]. On the other hand, the FAVORIT research analyzed uric acid levels of 3,512 subjects who underwent renal transplantation, and hyperuricemia did not appear to be related to the presence of cardiovascular events, mortality or renal transplant dysfunction [11].

Until now, there is scarce evidence to know the role of the asymptomatic hyperuricemia on the functional kidney graft. Following the study of the endothelium as a possible link between uric acid and cardiovascular diseases, several works quantifying endothelial dysfunction and inflammation markers on both healthy subjects and patients with CKD have been published, while limited by the lack of a normal range of markers. Results were inconclusive [12].

The endothelium is a single layer of continuous cells that limit the whole circulatory system. The normal endothelium is characterized by a vasodilator, thrombus-resistant and anti-inflammatory phenotype. The endothelium is first to be damaged in the development of an arteriosclerosis process and once the balance between contraction and relaxation, aggregation and anti-aggregation, thrombosis and fibrinolysis, smooth muscle cells proliferation and anti-proliferation is altered, it can lead to endothelial dysfunction.

The endothelium activation involves secretion of several substances, as vasodilators factors (nitric oxide, prostacyclin, hyperpolarizing factor derived from endothelium), vasoconstriction factors (endothelin, thromboxane, prostaglandin), platelet derived growth factors, cell adhesion molecules for specific receptors of circulating leukocytes (E-selectin, P-selectin, vascular cell adhesion molecule [VCAM], intercellular adhesion molecule [ICAM]), anti-inflammatory cytokine (interleukin-10 [IL-10]) and pro-inflammatory cytokine (tumor necrosis factor [TNFα), hemostatic and thrombotic factors (plasminogen activator inhibitor [PAI-1], tissue plasminogen-activator [TPA] and thrombomodulin).

A study carried out in a population without any heart, liver or kidney disease progression observed that adhesion molecules of endothelial synthesis such as ICAM, P-selectin and E-selectin showed changes depending on age and gender. Men presented higher levels in comparison with women of reproductive age [13-15]. Another inflammation marker addressed in this study is TNFα, which is produced in adipocytes, macrophages and endothelial cells and its expression is directly correlated to obesity and insulin resistance [16]. It has been described that the culture of human adipocytes with TNFα decreases the mRNA expression of GLUT4, which is a hyperglycemia mechanism [17]. There is research conducted with murine models connecting the cytomegalovirus infection with endothelial dysfunction and increase of TNFα in kidney graft [18-21] and uric acid in animals with inoculation in the cytomegalovirus infection central nervous system.

The present study, including a kidney transplant population during the first year after transplantation, is the first to research a potential connection between endothelial markers (specifically, the inflammatory cytokines IL-10 and TNFα and the adhesion molecules VCAM-1, ICAM-1, P-selectine and E-selectine), other inflammation markers not catalyzed in the endothelium (such as C-reactive protein and CD40) and renal function and uricemia (as a quantitative variable), and it notably approaches common medical practices in research centers as performed daily.

Methods

This is an observational, longitudinal, prospective, analytical and single center institution study of 38 subjects recruited between December 2016 and July 2017. The inclusion criteria were being older than 18 years old and having had a recent transplantation (less than 3 months). The exclusion criteria were: rejecting participation in the study, low adherence, accompanying inflammatory chronic, neoplastic or rheumatoid diseases, receiving other drugs, such as anti-epileptic drugs, that might alter uricemia levels, participation in other trial, hospital admission in the previous 30 days and limited life of kidney graft.

The main objective was researching the relationship between endothelial dysfunction and inflammation markers and renal function and uric acid levels in a sample of patients with functional kidney graft after three and twelve months since renal transplantation. The secondary objective was analyzing the influence of endothelial markers within the study sample according to age, gender, DM presence, ACE inhibitor or ARA therapy and cytomegalovirus infection.

The quantification of endothelial dysfunction and inflammation markers in serum was achieved with xMap Technology by Luminex 200 (Austin, Texas). Measurements were carried out in duplicate, after 3 and 12 months after intervention, and coefficients of variation intra- and inter-trial were <10% and <15%, respectively. Statistical analysis was conducted with SPSS software, version 22 for Windows. For both parametric and non- parametric tests employed in the data analysis values of P lower than 0.05 were considered as significant.

Results

Out of 60 transplanted patients, 40 met the inclusion criteria. Out of these patients, one died due to an infection and another was excluded from the study due to premature loss of kidney graft without completing follow-up. 68.4% were males with an average age of 50.68 years old. Average time spent on dialysis before the kidney transplant was 34 months. The most frequent type of donor were corpses (71.1%) of an average age of 49.8 years old. 34.2% were smokers, 10.5% (4 patients) were diabetic and 3 subjects developed post-transplant diabetes in the next year after follow-up. More than half presented a history of high blood pressure complications, out of which only seven followed ACE inhibitor or ARA therapy other than Losartan. Only 10.5% (4 patients) followed a hypouricemiant therapy (specifically allopurinol) before transplantation. Four subjects presented cytomegalovirus infection. The uric acid average after three months since transplant was 6.64 ± 1.03 mg/dl and 6.64 ± 1.23 mg/dl after twelve months. The serum creatinine average was 1.71 ± 1.15 and 1.58 ± 0.56 mg/dl after three and twelve months since transplant, respectively.

Among the endothelial dysfunction and inflammation markers addressed, an improvement of VCAM ($p < 0.005$) and ICAM ($p < 0.0001$) a year after renal transplantation was observed regardless of renal function and uricemia levels. Uric acid levels were positively correlated with creatinine levels in a significant way ($p = 0.006$ after three months and $p = 0.010$ after twelve months). However, a correlation between P-selectin, E-selectin, TNFα, IL10 and PCR and uric acid was not observed. Women presented lower uric acid levels (6 ± 1.16 vs 6.9 ± 1.16 mg/dl; $p = 0.026$) and PCR (6 ± 1.16 v 6.9 ± 1.16 mg/dl; $p = 0.026$) at the end of follow-up. Diabetic patients (seven in total) presented increased uric acid levels ($p < 0.011$) after three months since transplantation. By the end of follow-up, this subpopulation presented increased TNFα levels after twelve months since transplantation ($p < 0.033$). In the case of patients following ACE inhibitor or ARA therapy, they presented a significant decrease of P-selectin within three months since post-transplant ($p < 0.029$). Subjects with cytomegalovirus infection during the first six months presented higher TNFα levels within three months from follow-up ($p < 0.005$) and serum urate levels within twelve months from follow-up ($p < 0.04$).

Discussion

Hyperuricemia is one of the most common complications in kidney transplant patients. Under the hyperuricemia definition used in other studies [10], 52.6% and 50% of subjects from sample had high serum urate levels within 3 and 12 months from transplantation, respectively. It has been confirmed an improvement of some endothelial dysfunction markers (VCAM, ICAM) through the first year regardless of renal function and uric acid levels, reaffirming the known benefits of renal transplantation for patients with an advanced CKD. However, while no correlation between uric acid levels and inflammatory patterns was observed, it was so with renal function patterns. This would indirectly favor the widely accepted theory on the beneficial effect of allopurinol, that is, its anti-inflammatory effect is not related to the decrease of uric acid values, but to XO inhibition [11]. This means that uric acid does not constitute per se a risk factor for the vascular endothelium and cardiovascular diseases, but a mere epiphenomenon. The study results were expected according to the observations published in the FAVORIT research [12]. After analyzing the uric acid levels of 3512 subjects who underwent renal transplantation, hyperuricemia did not appear to be related to the presence of cardiovascular events, mortality or renal transplant dysfunction.

Today there is not a normal range of endothelial dysfunction and inflammation markers. Gender is a determinant factor in the renal function progression related to age. In this sample women presented lower uric acid and PCR levels, without observing any other differences in the rest of the endothelial and inflammation reviewed markers. On the other hand, the notably increased PCR in males must be carefully read taking into account the low specificity of the mentioned acute inflammation reactant protein and the presence of uncontrolled confounders in this observational study.

With regard to the results obtained from diabetic patients (4 at the start of the study and 7 by the first follow-up year), there are many studies researching the correlation between insulin resistance with hyperuricemia and insulin resistance with TNFα as an endothelial dysfunction marker with contradictory results in some cases [16-17]. It has been described that the culture of human adipocytes with TNFα decreases the mRNA expression of GLUT4 and it is a hyperglycemia mechanism [17]. Therefore, the results obtained from this study are clinically relevant in spite of the limited sample, since they might reconsider the need of new endothelial dysfunction markers in the diabetic population with a functional renal graft beyond the known glycemic control standards and above all, because they might serve as a starting point to the development of further ambitious research in the field of diabetes and renal transplantation.

Patients following therapy with RAAS inhibitors, such as AECI and ARA II, presented lower P-selectin levels 3 months after transplantation, losing significance by the end of follow-up, without observing any relationship with serum urate. P-selectin is always expressed, and it is stored in platelet cytoplasmic granules and endothelial cells. The study results would favor the known beneficial effects of AECI or ARA II on the endothelium [22-23]. It must be stressed that no patient received losartan, which presents uricosuric properties that set it apart from other from its group.

Finally, it was noted that four patients with cytomegalovirus infection during the first six months were characterized by presenting higher TNFα levels within three months and uric acid within twelve months from renal transplantation. There

is research conducted with murine models connecting the cytomegalovirus infection with endothelial dysfunction and increase of TNFα in kidney graft [18-19]. A study carried out with 1,468 aged Latin American patients found that subjects with higher antibody titers against cytomegalovirus infection presented a higher incidence of mortality, regardless of age, gender and underlying pathology. On this same study, the Sobel test suggested that the relationship between cytomegalovirus infection and cardiovascular mortality was partially mediated by TNFα and IL-6 [20]. Another study conducted in murine models with inoculation in the cytomegalovirus infection central nervous system observed significantly increased transient signs of neurological deterioration coinciding with uric acid concentrations in infected animal tissues compared with control animals. Histology revealed signs of focal ischemia in cytomegalovirus infected animals with changes in ischemia cells [21]. These results would indicate that uric acid might be a sensitive marker of the resulting endothelial dysfunction secondary to the cytomegalovirus infection.

In conclusion, these observations lead to suggest TNFα and serum urate as potential markers of endothelial dysfunction and inflammation secondary to cytomegalovirus infection in renal transplant patients, being TNFα more premature than serum urate. While these observations must be carefully read due to research limitations, this is the first study of this nature and being aware of their clinical relevance, we identify a need of further research and more ambitious trials to address with an evidence-based approach the role of TNFα and serum urate in population with renal transplant and cytomegalovirus infection.

Author's contribution

- **Conception and design:** Lourdes Roca-Argente, Julio Hernández Jaras, Isabel Beneyto Castelló
- **Administrative support:** Lourdes Roca-Argente, Julio Hernández Jaras, Isabel Beneyto Castelló
- **Provision of study materials or patients:** Lourdes Roca-Argente, Julio Hernández Jaras, Isabel Beneyto Castelló
- **Collection and assembly of data:** Lourdes Roca-Argente, Julio Hernández Jaras, Isabel Beneyto Castelló, Bañuls Morant
- **Data analysis and interpretation:** Lourdes Roca-Argente, Julio Hernández Jaras, Isabel Beneyto Castelló, Celia Bañuls Morant
- **Manuscript writing:** All authors
- **Final approval of manuscript:** All authors

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