Changes in Plamsa Concentrations of Hypoxanthine and Xanthine in Renal Vein as an Index of Delayed Kidney Allograft Function

Heshmatollah Shahbazian, Hayat Mombini, Ahmad Zand Moghaddam, Majid Jasemi, Mohammad Ali Hosseini, Payam Vaziri

Introduction: The aim of this study was to evaluate the plasma levels of hypoxanthine (HX) and xanthine in the renal vein blood samples for prediction of delayed graft function (DGF).

Materials and Methods: Two blood samples were taken from 47 kidney recipients, intraoperatively. The first sample was obtained from a peripheral vein before vascular anastomosis and the second from the allograft renal vein, 15 minutes after the anastomosis. Purine metabolites including xanthine and HX were measured and their associations with operative time, anastomosis time, frequency of clamping, urine output, and DGF were evaluated.

Results: The mean levels of xanthine and HX were 0.12 ± 0.10 mg/L and 0.37 ± 0.17 mg/L in the first plasma samples, respectively. Thirty patients (63%) had no significant changes in neither of their purine metabolite levels and 17 (37%) had higher levels of HX, but not xanthine, in their second samples. Only anastomosis time had a significant relation with the level of the metabolites (P = .04). Three patients (10%) with no changes in the metabolites and 5 (29.4%) with higher HX levels had DGF (P = .12). The anastomosis time and frequency of vascular clamping were higher and the urine output after the anastomosis was lower in the patients with DGF.

Conclusion: Cold ischemia in kidney transplantation causes a mild increase in the HX concentration indicative of short-term ischemia effects on the cell metabolism. But it cannot predict DGF. Anastomosis time, frequency of clamping, and urine output after the anastomosis are more sensitive indices.

Keywords: purines, xantine, hypoxanthine, kidney transplantation, delayed graft function

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INTRODUCTION

Delayed graft function (DGF) is one of the earliest complications after kidney transplantation and is generally defined as the need for dialysis within the first posttransplant week.[1] Its incidence vary from 5% in living-donor transplantation to 25% in cadaveric transplantation.[2] Delayed graft function can result in acute rejection within the early stages after transplantation impacting the long-term graft survival.[1] Thus, it is of utmost importance to find effective prevention and treatment measures and to introduce a sensitive and noninvasive method for its differentiation from other complications, especially acute rejection.

Although in some studies DGF has not shown any relation with increased duration of cold ischemia, its natural process and lower incidence

Department of Kidney Transplantation, Golestan Hospital, Jondishapour University of Medical Sciences, Ahwaz, Iran

Corresponding Author:
Heshmatollah Shahbazian, MD
Department of Kidney Transplant
Golestan Hospital
Ahwaz, Iran
Tel: +98 916 111 4595
Fax: +98 611 338 6257
E-mail: heshmatolahs@yahoo.com

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in transplantation from living donors (with shorter ischemia time) depict the role of ischemia and the similarity between the mechanism of DGF and acute tubular necrosis.[1] The main index of tissue ischemia is the cellular adenosine triphosphate reduction which can intervene the function of the Na/K pump of the cell and disrupt the intracellular electrolyte balance and cellular pH resulting in lysosomal activation, cell death, and kidney failure.[1-3] Another theory for DGF is free radical formation due to reperfusion.[1] According to this theory, mediator metabolites of adenosine triphosphate, such as hypoxanthine (HX), may result in cell damage during the ischemia period. Its oxidation to xanthine during the reperfusion period can also damage the cell by free radical formation.[4]

Measurement of the HX and xanthine levels following ischemia and reperfusion has been performed in animal models. Also, it has been studied in human models following myocardial infarction and in neonates following intrauterine hypoxic states.[5-7] We designed this study to evaluate the changes in the plasma levels of HX and xanthine in the blood samples from the renal vein following vascular anastomosis as a factor indicating DGF.

MATERIALS AND METHODS

In this cohort study, we evaluated the kidney recipients from living donors in Golestan hospital of Ahwaz between March 2004 and September 2005. The study was approved by the ethics committee of Ahwaz University of Medical Sciences.

Living donor nephrectomy was performed according to the standard method. The harvested kidney was put into a receiver containing frozen sterile saline and a preservation solution with the temperature of 0˚C to 4˚C was introduced into the kidney through an arterial cannula. The solution was injected within 20 minutes (50 mL/min). The ingredients of the preservation solution are mentioned in Table 1. After cooling, kidney transplantation was carried out. When the anastomosis was made, furosemide (3 mg/kg to 5 mg/kg), manitol (0.5 mg/kg), and normal saline (3 L to 4 L) were administered intravenously.

Table 1. Ingredients of Preservation Solution

Ingredient	Value
Ringer lactate solution	1 L
Verapamil	20 mg
Glucose 50%	10 mL
Heparin	5000 U
Lidocaine 2%	5 mL
Bicarbonate	20 meq

Three blood samples were taken from each patient. These samples included Sample 1, obtained from a peripheral vein before the anastomosis for evaluating the baseline level of the metabolites; Sample 2, obtained from the allograft renal vein 15 minutes after the anastomosis; and Sample 3, obtained from a peripheral vein 15 minutes after the anastomosis. The blood samples were centrifuged and the plasma was mixed with an equal volume of 10% trichloroacetic acid. The resultant solution was again centrifuged and the supernatant liquid was used for injection into the high-performance liquid chromatography (HPLC) system to measure the plasma levels of xanthine and HX. To provide the appropriate conditions for HPLC, the ambulatory phase, the column (Eurosphere 100 C18, Knauer, Berlin, Germany), the detector (UV/Visable, Cecil Instruments, Cambridge, UK), sample injection, data analysis, and producing standard curves were tested and adjusted according to the instructions.[8-10]

According to the changes in the plasma levels of the purine metabolites (Sample 2 in comparison with Sample 1), the patients were divided into two groups: group 1, without and group 2, with increase in the metabolite levels. The criteria for selecting the patients of group 2 were (1) an increase in the plasma level of HX greater than 11 μmol/L (> 1.5 mg/L) or in the plasma level of xanthine greater than 2.7 μmol/L (> 0.38 mg/L) when the initial levels were normal, (2) a 3-fold increase in the plasma level of HX or xanthine, and (3) any increase in each metabolite to the levels greater than the accepted laboratory biases when the initial levels were higher than the normal range.[7,11] A necessary condition for attributing this increase to the graft was the higher level of the metabolites' concentrations in Sample 2 compared to Sample 3. If the levels of purine metabolites were higher in Sample 3, the patient would be excluded because that increment could be indicative of the decreased perfusion and ischemia in other tissues (eg, due to the decrease in the systemic blood pressure or severe bleeding). None of the patients met this criterion and thus, none of them was excluded.

The patients were followed up for 10 to 14 days postoperatively and any episode of DGF was recorded. Delayed graft function was defined as one of the following conditions: (1) need for dialysis within the first week after the operation,[4] (2) serum creatinine level of 2.5 mg/dL or more and the peak activity time of more than 6.5 minutes on renal isotope scan performed on day 5,[2] and (3) less than 10% decrement in the serum level of creatinine within the first 24 hours after the transplantation.[12]

During the follow-up period, monitoring of the fluid intake and output and daily laboratory tests including blood urea nitrogen, and serum creatinine levels were performed. Also, ultrasonography, Doppler ultrasonography, and renal scintigraphy were used to rule out other causes of the kidney allograft dysfunction (eg, acute tubular necrosis). Percutaneous needle biopsy was performed in patients if diagnosis could not be made by noninvasive methods. Other variables including age of the donor and the recipient, sex of the recipient, anastomosis time (the time period between the removal of the kidney from the cooling solution and removal of the vascular clamps), operative time, frequency of clamping, and urine output after the anastomosis were recorded.

The collected data were analyzed by Fisher exact test and chi-square test, for comparison of categorical variables; Mann-Whitney test, for comparison of nonparametric continuous variables; and Wilcoxon rank sum test, for evaluation of alterations in HX and xanthine levels. The SPSS software (Statistical Package for the Social Sciences, version 11.5, SPSS Inc, Chicago, Ill, USA) was used and *P* values less than .05 were considered significant.

RESULTS

Forty-seven patients were enrolled in this study, of whom 30 (63%) were men and 17 (37%) were women. The mean age of the patients was 34.8 ± 7.1 years (range, 16 to 62 years).

The mean levels of xanthine and HX were 0.12 ± 0.10 mg/L and 0.37 ± 0.17 mg/L in Sample 1, respectively. Thirty patients (63.8%) had no significant changes in their purine metabolite levels (group 1) and 17 (36.2%) had higher levels in their second samples (group 2). Table 2 demonstrates the mean changes in the level of the metabolites in each group. In group 1, no significant difference was seen in the level of HX and xanthine. In group 2, the significant change was attributed to the level of HX ($P < .001$), but, xanthine was not increased significantly ($P = .13$). Evaluation of other variables showed that only anastomosis time had a significant relation with the level of the metabolites (35.0 ± 7.3 minutes in group 1 versus 40.0 ± 7.9 minutes in group 2; $P = .04$).

Three patients (10%) in group 1 and 5 (29.4%) in group 2 had DGF ($P = .12$). The anastomosis time and frequency of vascular clamping were higher and the urine output after the anastomosis was lower in patients with DGF (Table 3).

Table 2. Concentrations of Purine Metabolites in Plasma Samples from Peripheral Vein Before Anastomosis (Sample 1) and from Allograft Renal Vein 15 Minutes After Anastomosis (Sample 2)*

Purine Metabolites	Sample 1	Sample 2	*P*
Group 1			
Xanthine	0.12 ± 0.14	0.20 ± 0.12	.14
HX	0.37 ± 0.35	0.63 ± 0.51	.24
Group 2			
Xanthine	0.11 ± 0.60	0.30 ± 0.07	.13
HX	0.36 ± 0.89	1.69 ± 1.22	< .001

*Values are means ± standard deviations. HX indicates hypoxanthine.

Table 3. Factors Influencing Graft Function Within the First Week of Transplantation*

Factors	DGF (n = 8)	No DGF (n = 39)	*P*
Anastomosis time, min	42.0 ± 6.9	33.0 ± 7.2	.002
Clamping > 1 time	6 (75)	6 (15.4)	.002
Urine output < 200 mL at 1st day	4 (50)	4 (10.3)	.02

*Values in parentheses are percents.

DISCUSSION

The present study shows that any changes in the level of the purine metabolites following kidney transplantation are associated with the duration of the anastomosis and subsequently warm ischemia. Several studies have shown the relation between high amounts of xanthine and HX in the plasma following ischemia. Poulsen and colleagues designed a study to evaluate the effects of oxygen 100% in cardiopulmonary resuscitation of newborn pigs and showed an increase in the plasma level of HX, xanthine, and uric acid following generalized warm ischemia.[11] Saugstad and Aasen noticed that warm ischemia resulted in a significant increase in the level of HX.[13] Van Wylen and associates showed that brain ischemia and anoxia caused a 30-fold increase in the plasma level of HX and xanthine of cerebrospinal fluid in mice.[14] The level of xanthine did not significantly increase after ischemia in our study. This could be due to one of these reasons; first, the abovementioned studies have been performed in animal models. It can be speculated that the mechanism of purine metabolism is not similar in human beings and animals and the response may be different in humans. Second, it is possible that the pattern of xanthine increment does not follow a linear pattern in relation to the duration of ischemia. Third, our study was performed on cooled grafts. This may decrease the cellular metabolism rate while none of the previous studies has been performed on models with cold ischemia. Fourth, in the abovementioned studies, the periods of the tissue ischemia were significantly higher than those in our study. Fifth, reverse metabolic pathways may have been activated, metabolizing the HX formed in the peripheral parts. Eklund and colleagues evaluated the level of HX in the transitional tissue of rats' kidneys following warm ischemia and reperfusion using microdialysis method. They measured the HX level in two different groups of the rats with the warm ischemia duration of 20 and 40 minutes. In this study, although HX increased significantly during the 20-minute ischemia period, it became normal after reperfusion.[5] The 20-minute period in this study is approximately similar to the time of sampling from the renal vein in our study.

Ischemic damage and reperfusion have been accepted as two important etiologies of DGF in kidney transplants. In a study performed on 3365 patients between 1990 and 1998, Sola and associates showed that DGF had a significant relation with ischemia.[15] In another study performed by Ojo and colleagues on 37 216 cadaveric kidney transplants, it was shown that the increase in the duration of the ischemia had the strongest relation with DGF and by each 6-hour increase in the duration of the cold ischemia, the risk of this complication increased up to 23%.[16]

In 2002, Mota and colleagues evaluated the risk factors and their effects on DGF.[17] A significant relation was found between the duration of the ischemia and DGF. Abreu and coworkers evaluated the predisposing factors of the DGF in 100 patients. They showed that the most important risk factors were the age of the recipient, donor–recipient relation, and the duration of cold ischemia.[18]

In a study on liver transplantation, Net and colleagues divided 30 rats into 2 groups. One group underwent 10 minutes of warm ischemia and this time was about 40 minutes in the other group. All rats underwent reperfusion, afterwards. It was shown that a significant association existed between the tissue xanthine level and ischemia period, DGF, and graft survival. They suggested that the xanthine level of the tissue might be an appropriate indicator for the prediction of the graft survival.[19]

In our study, no significant relation was found between the HX level of the blood and DGF. Although the HX increment was significantly associated with the increase in the anastomosis time, both these variables have been mildly increased, which can be a sign of transient ischemia, especially because no change was seen in the xanthine levels of the 2 groups. We showed that among many risk factors affecting the DGF, anastomosis time, ischemia, and frequency of clamping had the essential role. The deleterious effects of frequent vascular clamping on the kidney parenchyma during the surgeries involving the vascular parts is well known.[20] Thus, it seems that the factor resulting in DGF in our study is not the mild ischemia and increased HX level, but it is due to the manipulations on the tissues and vasculature, accumulation of the inflammatory cells, and free radical formation because of the frequent clamping and damage to the parenchyma of the kidney.

CONCLUSION

During the phase of cold ischemia of the transplantation (with live donor) a mild increase in the level of HX is seen which can be due to the short-time ischemic defects on the cell metabolism. Thus, it can not be a good predictor of DGF. Our findings depicted that anastomosis time, frequency of clamping, and vascular manipulations were better indices for the prediction of this delay in the function of the transplanted kidney.

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