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The relationship between HIF-2 and chronic renal allograft nephropathy in renal transplantation recipients

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Abstract

Aim: We aim to examine the role of HIF-2α on chronic allograft nephropathy (CAN), which is a consequence of chronic inflammation and fibrosis in patients with renal transplantation.

Materials and Methods: The study was conducted between November 2019 and January 2021, patients who had a renal biopsy in our university in the last two years were enrolled in this study. Fifteen recipients with a diagnosis of chronic allograft nephropathy proven by biopsy, 15 recipients with an eGFR below 60 ml/dk for other reasons as non-CAN group. Also, fifteen patients with an eGFR above 60 ml/dk were enrolled in the study as a control group. Serum HIF-2α levels were analyzed by the immunoassay technique.

Results: HIF-2α was found higher in the CAN group compared to other groups. HIF-2α was significantly predictive in ROC analysis in identifying renal transplantation patients with CAN. The sensitivity and specificity of HIF-2α were 100% and 76% (cut off >0.2) with an area of under the ROC curve of 0.941 (95% CI 0. 850-1.000), $p < 0.001$).

Conclusion: Serum HIF-2α may be useful both as potential biomarkers for diagnosing CAN after kidney transplantation and may be a guide in determining treatment strategies.

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Introduction

Although kidney transplantation is the best renal replacement treatment, which increases patient survival and facilitates social rehabilitation of patients, the 10-year survival rate following allografts is <50% [1,2]. Alloantibodies are the main factor causing graft dysfunction. The term of "chronic allograft nephropathy" (CAN) has been included in the specific terminology in the Banff classification of renal allograft pathology and tubular atrophy and interstitial fibrosis are the main pathological features of CAN. However, it can also be caused by ischemia-reperfusion injury or early acute rejection, calcineurin inhibitor (CNI) nephrotoxicity, recurrent glomerular diseases, and allograft damage during BK virus (BKV) infection [3,4]. CAN, defined as renal allograft dysfunction occurring at least three months after transplantation, is a poorly understood process. Although clinical diagnosis is usually made by decreasing glomerular filtration rate (GFR), worsening hypertension and rising proteinuria, reliance on these clinical features may lead to delayed diagnosis and loss of allograft [5-7]. HIFs are heterodimeric transcription factors that mediate adaptive responses to low oxygen levels under hypoxic conditions and include HIF-α and HIF-β. HIFs are involved in angiogenesis and cellular regulation of vascular tone [8]. The two main HIF-α isoforms, HIF-1α and HIF-2α, are structurally and functionally similar, but their target genes are distinctly different. While HIF-1α is mainly expressed in the myocardium, HIF-2α shows its effects mostly endothelial. This suggests that HIF-2α has an effect on vascular function during development [9]. It has been shown the effects of HIF on renal fibrosis in a one study [10]. We present to examine the role of HIF-2α on CAN, which is a consequence of chronic inflammation and fibrosis.

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Materials and Methods

Study design

The study was conducted between November 2019 and January 2021, after the ethics committee approval (no:2021/191) was obtained at Erciyes University Medical Faculty Hospital. Among the patients who had kidney transplantation who admitted to the nephrology outpatient clinic for their routine controls, patients who had a renal biopsy in our university in the last two years were included in the study. 15 patients with a diagnosis of chronic allograft nephropathy proven by biopsy, fifteen patients with an eGFR below 60 ml/dk for other reasons as Non-CAN group. Non-CAN group with impaired renal function including 2 of the patients were due to CMV, 1 of them had BK nephropathy, 2 had previous disease recurrence and the remainder were acute rejection and diagnoses of all patients were made biopsy-proven. Also, 15 patients with an eGFR above 60 ml/dk were included in the study as a control group. After recording the patient's creatinine levels, calcium/phosphorus levels, proteinuria levels, body mass index, HIF-2α levels, and several biochemical parameters, comparisons were made among the 3 groups, and all parameters were evaluated in terms of correlation with the HIF-2α levels. As exclusion criteria were viral/bacterial infections, autoimmune diseases, diabetes mellitus, inflammatory diseases, malignancy, severe heart and respiratory diseases.

Biochemical measurements

After 12 hours of fasting, biochemical parameters were measured with OLYMPUS 2700 (Olympus Diagnostics) and hemogram was measured with SIEMENS ADVIA 2120 (Siemens Healthcare Diagnostics). Serum HIF-2α levels were measured by immunoassay technique (Human HIF-2α ELISA kit; Elabscience Biotechnology Co., Ltd.). Serum samples were taken simultaneously with the biopsy.

Radiological examination

Renal ultrasonography was performed to evaluate the size, shape, echogenicity of kidney allograft, presence of stones, masses, renal artery stenosis, hydronephrosis, or lymphocele in patients.

Statistical analysis

Shapiro-Wilk's test were performed to assess the data normality. Levene test was applied to test variance homogeneity. One-way analysis of variance (ANOVA) and Kruskal-Wallis H test were used for continuous variables, while Pearson chi-square analysis was used for categorical variables for comparing the differences between groups. Tukey and Dunn-Bonferroni tests were applied for post-hoc comparisons. To identify the correlation between HIF-2α and age, proteinuria, transplantation period, hemoglobin, eGFR, CAXP and albumin variables, Pearson correlation analyses were conducted. To detect the predictive effect of HIF-2α on fibrosis in renal transplantation patients, receiver characteristic curve (ROC) analyses were applied. Youden index was used to detect the optimal cut-off value. The area under the ROC curves, sensitivity, specificity, negative and positive predictive value statistics were calculated with 95% confidence intervals.

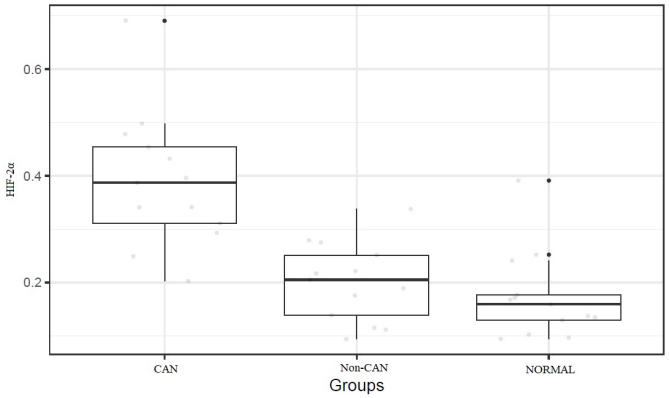

Figure 1. HIF-2α was higher in the CAN group compared to other groups.

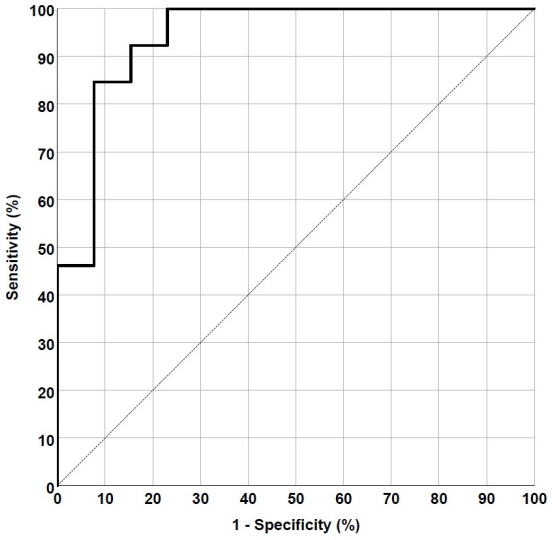

Figure 2. The sensitivity and specificity of HIF-2α were 100% and 76% (cut off >0.2).

Results

There were no radiological abnormality including, echogenicity of kidney allograft, presence of stones, masses, renal artery stenosis, hydronephrosis, or lymphocele in abdominal ultrasonography with renal transplantation patients. Baseline characteristics of patients are shown in Table 1. The mean age of the patients was 42.92±14.37 years in CAN group, 40.54±13.46 years in the Non-CAN group, and 44.08 ±49.35 years in the normal renal function group. While Hemoglobin and eGFR were higher in the normal group compared to the other groups, proteinuria and CaXP were higher in the CAN group. Also, HIF-2α was higher in the CAN group compared to other groups (Figure 1). Correlation analysis results were summarized in Table 2 in terms of HIF-2α and other variables among the three groups. HIF-2α levels were positively correlated with proteinuria in CAN group (r: 0.452, p:0.041) and negatively correlated with hemoglobin in normal group (r: -0.452, p: 0.043). HIF-2α levels were significantly predictive in ROC analysis in identifying renal transplantation patients with CAN group. The sensitivity and specificity

Table 1. Comparison of the distribution of the demographic and clinical variables among the study groups.

Variables	Groups			p
	CAN (n=13)	Non-CAN (n=13)	Normal (n=13)	
Age (years)	42.92 ± 14.37	40.54 ± 13.46	44.08 ± 9.35	0.767
Gender (male)	8(61.5)	5(38.5)	6(46.2)	0.488
Proteinuri (g/day)	0.97(0.87-2.10)^{a}	0.41(0.17-1.10)^{b}	0.11(0.09-0.15)^{b}	<0.001
Transplantion duration (month)	100.85 ± 76.32	80.08 ± 59.27	84.23 ± 41.50	0.656
Hemoglobin (g /dl)	10.67 ± 2.07^{a}	11.24 ± 1.29^{ab}	12.84 ± 1.82^{b}	0.009
eGFR (ml/min/1.73 m^2)	38.69 ± 17.28^{a}	48.05 ± 26.92^{a}	76.85 ± 20.53^{b}	<0.001
CAXP (mg^2/dL^2)	38.54 ± 8.16^{a}	30.15 ± 6.71^{b}	28.54 ± 5.71^{b}	0.002
Albumin (g/L)	4.07 ± 0.51	4.17 ± 0.39	4.36 ± 0.30	0.195
Potassium (mmol/L)	4.62 ± 0.95	4.27 ± 0.51	4.10 ± 0.40	0.137
Alanine aminotransferase (u/L)	16.0(9.0-20.0)	22.0(14.0-37.0)	14.0(11.0-18.0)	0.397
White blood cell (x 10^9/liter)	8260(6270-8630)^{a}	7770(7200-9170)^{a}	9630(9000-12800)^{b}	0.040
HIF-2α	0.390 ± 0.126^{a}	0.201 ± 0.074^{b}	0.173±0.082^{b}	<0.001

Values are expressed as median(1st-3rd quartiles). Different superscripts in the same row indicate a statistically significant difference between groups.

Table 2. Correlation analysis results between HIF-2α and clinical variables.

Groups	Age	Proteinuria	Duration of Tx	Hb	eGFR	CaXP	Alb
CAN							
r	-0.255	0.459	-0.172	-0.125	0.109	0.002	-0.320
p	0.400	0.041	0.575	0.684	0.723	0.994	0.171
Non-CAN							
r	-0.347	0.472	0.049	-0.058	-0.175	-0.048	-0.204
p	0.246	0.104	0.874	0.851	0.566	0.875	0.503
Normal							
r	0.163	0.163	0.270	-0.452	-0.246	0.292	0.304
p	0.596	0.596	0.373	0.043	0.419	0.332	0.313

r: Pearson correlation coefficient.

Table 3. Diagnostic measures calculated for HIF-2 (> 0.2 cut-off value) in identifying fibrosis in renal transplantation patients.

Diagnostic measure	Estimate	95% CI
AUC	0.941*	0.850-1.000
SEN	1.000	0.753-1.000
SPE	0.769	0.462-0.950
PPV	0.813	0.544-0.960
NPV	1.000	0.692-1.000

*p<0.001. AUC: Area under curve; SEN: Sensitivity, SPE: Specificity, PPV: Positive predictive value, NPV: Negative predictive value, CI: Confidence interval.

of HIF-2α were 100% and 76% (cut off >0.2) (Figure 2) with an area of under the ROC curve of 0.941 (95% CI 0. 850-1.000), $p < 0.001$) (Table 3).

Discussion

We conclude that HIF-2α levels were increased in CAN group compared to other groups in this study. HIF-2α levels were significantly useful markers foreseeing of CAN in patients with a kidney transplant. The presence of biochemical markers that are precursors of progressive fibrosis and decreased kidney function has the potential to guide immunosuppression therapy. At a minimum, the early diagnosis of patients with progressive fibrosis will allow a review of their immunosuppression treatment and concomitant medications [12]. HIF is an important molecule in regulating the adaptive response to hypoxia and [13] has been associated with poor outcomes in many types of cancer [14]. In the presence of hypoxia, HIF activation represents an elegant bioenergetics adaptation that allows cells to reduce toxic reactive oxygen species by reprogramming cellular oxidative metabolic mechanisms. Tubular cells have been shown to up-regulate HIF expression under hypoxic conditions, inducing a variety of adaptive responses. While HIF-2α in peritubular fibroblasts and endothelial cells, HIF-1α is induced in papillary interstitial cells and tubules [15]. Haase et al. [16] also showed that HIF plays a role in tubulointerstitial fibrosis in mice. HIF can induce fibrogenic changes by activation of connective tissue growth factor in mice, whereas hypoxia in human proximal tubular cells can inhibit this growth factor synthesis [17-19]. Prevention and management of chronic renal allograft rejection are one of the major challenges faced by

transplant nephrologists [20,21]. Findings regarding the time dependence of various factors suggest that different prevention and treatment strategies for chronic nephropathy may be effective, in part based on time after transplantation [22]. For example, in the first year, attention may often be directed towards preventing rejection, and treatment in among stable patients may focus on limiting exposure to calcineurin inhibitors in later years. Chronic allograft damages are an important cause of allograft loss in the first year in these patients. It has been shown that pathological changes in the kidney graft precede functional changes. Several studies have shown that approximately 50% of allografts with stable kidney function develop interstitial fibrosis and as well as tubular atrophy in the early years after transplantation [23,24]. In some previous studies, when HIF-2α expression was induced at the onset of CKD, it played a major profibrotic role, but conversely, HIF-2α activation at a later stage of CKD resulted in functional protection by protecting the kidney from the progression of renal fibrosis [25-27]. So far, it has been investigated the effects of HIFs on kidney fibrosis. However, its effectiveness in this regard has not been sufficiently evaluated yet [28]. In conclusion, we concluded that HIF-2α closely associated with in the pathogenesis of upregulation and CAN and is an indicator of the fibrotic process. Serum HIF-2α may be usefulness for detecting CAN after kidney transplantation and it can be also a guide in determining these treatment strategies. This result should be confirmed in clinical studies with a large population.

Ethics approval

Ethics committee approval (no:2021/191) was obtained at Erciyes University Medical Faculty Hospital.

References

1. Lamb KE, Lodhi S and Meier-Kriesche HU: Long-term renal allograft survival in the United States: A critical reappraisal. Am J Transplant 11: 450-462, 2011.
2. Gatault P, Bertrand D, Büchler M, et al: Eight-year results of the Spiesser study, a randomized trial comparing de novo sirolimus and cyclosporine in renal trans- plantation. Transpl Int 29: 41-50, 2016.
3. Morath C, Zeier M. Chronic allograft injury. In: Johnson RJ, Feehally, J, Floege J (eds.). Comprehensive Clinical Nephrology. 5th ed. Philadelphia: Elsevier: Saunders; 2015: 1214-1221.
4. Bos EM, Leuvenink HG, van Goor H, Ploeg RJ (2007) Kidney grafts from brain dead donors: Inferior quality or opportunity for improvement? Kidney Int 72:797–805.
5. Monaco AP, Burke JF Jr, Ferguson RM, et al. Current thinking on chronic renal allograft rejection: issues, concerns, and recommendations from a 1997 roundtable discussion. Am J Kidney Dis 1999; 33:150.
6. Chapman JR, O'Connell PJ, Nankivell BJ. Chronic renal allograft dysfunction. J Am Soc Nephrol 2005; 16:3015.
7. Yakupoglu U, Baranowska-Daca E, Rosen D, et al. Post-transplant nephrotic syndrome: A comprehensive clinicopathologic study. Kidney Int 2004; 65:2360.
8. Bishop T, Ratcliffe PJ. HIF hydroxylase pathways in cardiovascular physiology and medicine. Circ Res. 2015;117(1):65-79.
9. Chen L, Endler A, Shibasaki F. Hypoxia and angiogenesis: regulation of hypoxia-inducible factors via novel binding factors. Exp Mol Med. 2009;41(12):849.
10. Kong KH, Oh HJ, Lim BJ, et al. Selective tubular activation of hypoxia-inducible factor-2α has dual effects on renal fibrosis. Sci Rep. 2017 Sep 12;7(1):11351.
11. Haas M, Loupy A, Lefaucheur C, et al. The Banff 2017 Kidney Meeting Report: Revised diagnostic criteria for chronic active T cell-mediated rejection, antibody-mediated rejection, and prospects for integrative endpoints for next-generation clinical trials. Am J Transplant. 2018 Feb;18(2):293-307.
12. O'Connell PJ, Zhang W, Menon MC, et al. Biopsy transcriptome expression profiling to identify kidney transplants at risk of chronic injury: a multicentre, prospective study. Lancet. 2016 Sep 3;388(10048):983-93.
13. Haase, V. H. Hypoxia-inducible factors in the kidney. Am J Physiol Renal Physiol 291, F271–281. (2006).
14. Cancer Genome Atlas Research Network. Comprehensive molecular characterization of clear cell renal cell carcinoma. Nature. 2013; 499:43–9.
15. Nangaku, M. & Eckardt, K. U. Hypoxia and the HIF system in kidney disease. J. Mol. Med. 85, 1325–1330 (2007).
16. Haase, v. H. Oxygen regulates epithelial-to- mesenchymal transition: insights into molecular mechanisms and relevance to disease. Kidney Int. 76, 492–499 (2009).
17. Kimura, K. et al. Stable expression of HIF-1α in tubular epithelial cells promotes interstitial fibrosis. Am. J. Physiol. Renal Physiol. 295, F1023–F1029 (2008).
18. Higgins, D. F. et al. Hypoxic induction of Ctgf is directly mediated by Hif-1. Am. J. Physiol. Renal Physiol. 287, F1223–F1232 (2004).
19. Kroening, S., Neubauer, E., Wessel, J., Wiesener, M. & Goppelt-Struebe, M. Hypoxia interferes with connective tissue growth factor (CTGF) gene expression in human proximal tubular cell lines. Nephrol. Dial. Transplant. 24, 3319–3325 (2009).
20. Joosten SA, Sijpkens YW, van Kooten C, Paul LC. Chronic renal allograft rejection: pathophysiologic considerations. Kidney Int 2005; 68:1.
21. Pascual M, Theruvath T, Kawai T, et al. Strategies to improve long-term outcomes after renal transplantation. N Engl J Med 2002; 346:580.
22. Nankivell BJ, Borrows RJ, Fung CL, et al. The natural history of chronic allograft nephropathy. N Engl J Med 2003; 349:2326.
23. Yilmaz S, Tomlanovich S, Mathew T, et al. Protocol core needle biopsy and histologic Chronic Allograft Damage Index (CADI) as surrogate end point for long-term graft survival in multicenter studies. J Am Soc Nephrol. 2003;14:773–779.
24. Baboolal K, Jones GA, Janezic A, Griffiths DR, Jurewicz WA. Molecular and structural consequences of early renal allograft injury. Kidney Int. 2002;61:686–696.
25. Semenza GL. Defining the role of hypoxia-inducible factor 1 in cancer biology and therapeutics. Oncogene. 2010;29(5):625–634.
26. Schito L, Semenza GL. Hypoxia-inducible factors: master regulators of cancer progression. Trends Cancer. 2016;2(12):758–770.
27. Semenza GL. Hypoxia-inducible factors: mediators of cancer progression and targets for cancer therapy. Trends Pharmacol Sci. 2012;33(4):207–214.
28. Kong KH, Oh HJ, Lim BJ, et al. Selective tubular activation of hypoxia-inducible factor-2α has dual effects on renal fibrosis. Sci Rep. 2017 Sep 12;7(1):11351.