Is Hif-1α Expression Important in Proliferative and Nonproliferative Glomerulopathy in Terms of Prognosis?

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ABSTRACT

The most effective factors in chronic kidney failure are kidney’s oxygen delivery and distribution. This study aimed to investigate the effects of hypoxia-inducible-factor 1α (HIF-1α) and its target genes glucose transporters (GLUT-2) and vascular endothelial growth factor (VEGF) expression, which are directly proportional with proliferative and nonproliferative glomerulopathy, in hypoxia in treatment and prognosis. A total of 78 patients (24 focal and segmental glomerulosclerosis (FSGS), 34 membrandoloproliferative glomerulonephritis (MPGN), 20 amyloidosis) with at least 2 years follow-up between January 1996-January 2006, were evaluated. The patients were allocated to five groups as negative or positive response to treatment in the first 3 months, negative or positive response to treatment after 3 months and finally CRF development within 12 months. HIF-1α, GLUT-2 and VEGF immunohistochemical studies were made from renal needle biopsy paraffin blocks and the expressions were classified. Increased tubular HIF-1α expression affected the treatment positively in the first 3 months but had a negative effect thereafter. Increased tubular and glomerular VEGF expression affected the treatment positively in the first 3 months and negatively thereafter. Increased peritubular capillary VEGF expression affected the treatment positively throughout the follow-up time intervals. Increased tubular GLUT-2 expression affected the treatment negatively after 3 months. While the increase in tubular HIF-1α and GLUT-2 expressions increased the risk of CRF development within 12 months, increased peritubular capillary VEGF expression decreased the risk of CRF development. Early period HIF-1α and VEGF expressions have protective effects but late period HIF-1α and GLUT-2 expressions increase CRF development within 12 months. The positive effects of HIF-1α genes on renal diseases in the early period must be supported with more extensive studies.

Key words: Hypoxia-inducible factor (HIF-1α), glomerulopathy


INTRODUCTION

The majority of chronic renal diseases inevitably progress to the final stage of renal failure. Although the etiological factors of chronic renal diseases are different, all renal diseases progress in a similar way and nephron destruction, glomerulosclerosis and tubulointerstitial fibrosis are seen in all of them. These findings are based on an imbalance of the oxygen and its distribution in the kidneys. Recent studies have revealed that chronic hypoxia is the most important factor in the course of end-stage renal disease. Therefore, to prevent the course of chronic renal disease, new treatment agents should be found and put into use to protect the kidneys from hypoxia.

Increased cellular oxygen requirement and decreased local oxygen support expose the proximal tubule epithelium and the peripheral part of the medulla to hypoxic-ischaemic injury. As in all organs, the expression of hypoxia-inducible-factor 1α (HIF-1α) increases particularly in the kidneys in the event of hypoxia. HIF-1α is an important mediator with a key role in oxygen homeostasis in cells. This system has an effect in the kidneys on Vascular Endothelial Growth Factor (VEGF), Glucose transporters (GLUT), vasomotor regulation, matrix metalloproteases and transforming growth factor as well as apoptosis and Erythropoietin (EPO) production which regulate target genes.

In several studies, HIF-1α activation has been proven to have a protective effect in various renal disease models. In experimental animal models, HIF-1α activation signal was shown in renal epithelial cells of chronic renal failure patients with diabetic and IgA nephropathy. It has been suggested that HIF-1α activation causes the development of fibrosis in the kidneys.
The effects of HIF-1α in renal diseases has been examined more in the acute phase and there has not been much research on the effects in chronic stages. This study aimed to determine the relationship between the course of chronic renal failure, early and late stage treatment responses of HIF-1α and its target GLUT-2 and VEGF immunohistochemical expressions in chronic renal diseases.

MATERIALS AND METHODS

General characteristics of the patients and follow-up: The study comprised 78 cases of at least 2 years follow-up, whose kidney biopsies were examined in our clinic between January 1996 and January 2006. Of 78 biopsies, 24 were diagnosed with FSGS, 34 with MPGN and 20 with amyloidosis. Within this period, clinical and laboratory monitoring was made on all the patients.

At the time of biopsy and at 3, 6, 12, 18 and 24 months following the biopsy, creatine (mg dL⁻¹), creatine clearance (mL min⁻¹) and proteinuria (mg 24 h⁻¹) values of the patients were examined. The patients were evaluated according to a positive (showing a fall in creatine, creatine clearance and proteinuria and remaining stable) or negative (showing an increase in creatine, creatine clearance and proteinuria) response in the first 3 months and at the following intervals to the treatment which had been administered.

During follow-up, patients with creatine clearance falling below 50 mL min⁻¹ were accepted as Chronic Renal Failure (CRF). The patients were examined in 2 groups as those who showed the development of CRF in the first 12 months and those who did not.

Immunohistochemical examination: For immunohistochemical examination, 3 µm thickness slices were cut from all the samples, placed on poly-L-lysine slides and deparaffinised. Together with positive and negative checks, HIF-1α (clone: H1 alpha 67, monoclonal Mouse, 1/25 dilution, Lab Vision, Neomarkers), GLUT-2 (monoclonal Mouse, 1/30 dilution, R and D Systems) and VEGF (monoclonal Mouse, 1/50 dilution, Dako) antibodies were applied. The standard avidin-biotin-peroxidase method was used.

Immunoreactivity evaluation: HIF-1α in the tubules (THIF-1α) was classified as 0, no staining, 1, less than 50% stain in the tubule epithelium and 2, more than 50% staining (Fig. 1). GLUT-2 in the tubules (TGLUT) was classified as 0, no stain, 1, stain only in the proximal tubule epithelium, 2, diffuse staining in the proximal tubule epithelium, focal in the distal tubule epithelium and 3, diffuse staining in proximal and distal tubule epithelium (Fig. 2). VEGF in the glomeruli (GVEGF) was classified as 0, no staining, 1, less than 50% staining in the glomeruli, 2, more than 50% staining in the glomeruli and cytoplasmic staining in the basal membrane and podocytes (Fig. 3). Interstitial VEGF (IVEGF) was
classified as 0, no staining, 1, less than 50% interstitial infiltration, 2, more than 50% staining. Peritubular capillary VEGF (PTCVEGF) was classified as 0, no staining, 1, less than 50% staining of the peritubular capillaries, 2, more than 50% staining.

The data of the groupings were compared using the Chi-square test. In the comparison of the immunohistochemical parameters with each other, the Pearson correlation test was used.

RESULTS
A total of 78 (24 FSGS, 34 MPGN, 20 amyloidosis) cases were evaluated comprising 42 (33.8%) males and 36 (46.2%) females. Mean follow-up following the biopsy was 24.2 ± 13 months. The relationships between the immunohistochemical parameters and treatment response of the cases are given in Table 1.

Increased THIF-1α and TVEGF expression had a positive effect on treatment response in the early stage (p=0.12, p<0.001) and a negative effect in the late stage (p<0.001, p= 0.01). Increased expression of TGLUT-2 and IVEGF had no effect on treatment response in the early stage (p = 0.11, p = 0.2) and showed a negative effect in the late stage (p<0.001, p = 0.01). Increased GVEGF expression had a positive effect on treatment response in the early stage (p = 0.015) and no effect in the late stage (p = 0.33). A positive effect on treatment response was seen in both the early and late stage by increased PTCVEGF expression (p = 0.035, p<0.001).

The immunohistochemical expressions of the renal biopsy materials were compared with cases progressing to CRF within one year (Table 2).

As THIF-1α, TGLUT and IVEGF expressions increased, so the development of CRF increased (p = 0.028, p<0.001, p = 0.04). However, in contrast, as PTCVEGF expression increased, the risk of CRF development decreased (p = 0.001).

DISCUSSION
In experimental studies, HIF-1α expression was induced in rats in which glomerulonephritis and ischemic acute renal failure had been created. The development of glomerulonephritis and tubulointerstitial injury in the acute phase was determined to be at a much lower rate in the groups in which HIF-1α was induced.

In experimental studies, it has been shown that HIF-1α activation results in epithelial cells changing to mesenchymal cells which causes the development of renal fibrosis. However, the role of HIF-1α in chronic conditions is not as yet fully understood.

In the current study, an increase in THIF-1α expression of the biopsy material of the chronic renal disease patients was shown to have a positive effect on treatment response within the first 3 months. However, in the period after the first 3 months, THIF-1α expression was determined to have no effect on treatment response. At the same time, THIF-1α was determined to have increased in CRF development within the first year.

Table 1: The effects of immunohistochemical parameters on short and long-term treatment

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THIF-1α: Tubular expression HIF-1α, TGLUT: Tubular expression GLUT, TVEGF: Tubular express VEGF, GVEGF: Glomerule VEGF, IVEGF: Interstitial expression VEGF, PTCVEGF: Peritubular capillary expression VEGF

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GLUT-2 is one of the target genes of HIF which is a glucose transporter facilitated by high affinity and low capacity in the cell membrane. In a study by Heiling et al., fibronectin and collagen expression in the glomeruli was increased by the high amount of glucose formed in the cell with increased GLUT-1 expression in rat mesangial cell cultures. Similarly, the results of the current study showed a significant relationship between increased GLUT-2 expression and the degree of fibrosis and CRF development.

By inducing neovascularisation, tubular and interstitial VEGF expressed chronically, while increasing blood flow, causes an inflammatory response showing permanence in the kidneys. The resulting fibroblast proliferation causes fibrosis and glomerulosclerosis. In contrast to literature, in the results of the current study, no statistically significant parallel was observed in TVEGF and IVEGF with the development of CRF. An increase in PTCVEGF expression with reduced development of CRF was found to be statistically significant.

In conclusion, while the induction of HIF-1α in renal diseases affects treatment response positively in the early period, it causes the development of fibrosis in long-term. HIF-1α and GLUT-2 expression increase in CRF development within the first year. According to these findings, it must be kept in mind that besides the renal protective effect of HIF-1α, there may be side effects in chronic periods. The results of this study showed that in the near future, rather than as a therapeutic agent in the treatment of renal diseases, attention to the anticipated indication of HIF-1α and treatment duration will be a statistically significant requirement.

REFERENCES

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