Relationships of Urinary VEGF/CR and IL-6/CR with Glomerular Pathological Injury in Asymptomatic Hematuria Patients

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Background: Interleukin-6 (IL-6) and vascular endothelial growth factor (VEGF) have important functions in injury and repair processes of glomerular intrinsic cells. A study was conducted to analyze the urinary VEGF/creatinine (CR) and IL-6/CR levels in simple hematuria patients after excluding the interference of creatinine. We aimed to investigate the function and relationships of the above indices in the glomerular pathological injury process, and to elaborate the values of urinary VEGF and IL-6 changes in the diagnosis of asymptomatic hematuria or hematuria with proteinuria.

Material/Methods: A total of 121 renal hematuria patients diagnosed by clinical and laboratory tests were included as research subjects. The midstream fresh morning urine was collected on the day renal biopsy was performed.

Results: The IL-6/CR value of the group III was significantly greater than in group I (Z=–2.478, P<0.05), with a statistically significant difference between these 2 groups. The VEGF/CR value of group III was significantly greater than in group II (P<0.01). Compared with group I, the VEGF/CR of group III was significantly greater (Z=–4.65, P<0.01), with a statistically significant difference.

Conclusions: The VEGF/CR and IL-6/CR values in simple hematuria patients were positively correlated with glomerular pathological injury scores. VEGF/CR and IL-6/CR might be used as biological diagnostic indicators in determining the extent of simple hematuria glomerular injury.

MeSH Keywords: Cytokine Receptor gp130 • Kidney Glomerulus • Vascular Endothelial Growth Factor A • Hematuria

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Background

Asymptomatic hematuria (AH) is a common kidney disease with insidious onset and high incidence. Previously, pathological changes were considered minor and the prognosis would be satisfactory; however, follow-up studies [1] have shown that the isolated microscopic hematuria is closely related to early chronic kidney disease (CKD). Renal hematuria (RH) is a common disease of the urinary system, and includes diseases with clinical microscopic hematuria (centrifuged urinary red blood cells >3/HP) or gross hematuria as the main characteristic; in this disease, no obvious edema and hypertension occur, with or without minor proteinuria (qualification <2+, quantitation <0.5 g/24 h), and the renal functions are normal [2]. The pathogenesis of RH is currently unclear. The immune damage that causes pathological changes in the glomerular basement membrane and the abnormalities of collagen fibres and chemical composition structures of the glomerular basement membrane might produce the hematuria [3]. The abnormalities of red blood cells (RBC) may have an important function in the formation of hematuria because of the changes in the charges on the RBC surface and the enhanced RBC deformability. Thus, RBC might easily pass through the basement membrane and form the hematuria. The etiology of the disease is complex, pathological types are varied, and attacks are recurrent. The cause of this syndrome is unknown, and current Western medicine treatment programs appear to lack effectiveness. Most of the available treatment options mainly focus on the symptoms, and on preventing and controlling infection and avoiding the use of kidney-damaging drugs.

Most cases of glomerulonephritis are immune-mediated inflammatory diseases. Generally, the immune mechanism is considered the original mechanism of glomerular diseases. Based on this mechanism, the inflammatory mediators (complements, cytokines, and reactive oxygen species) participate and finally lead to glomerular damage and clinical symptoms. Inflammatory mediators might affect the local renal hemodynamics by contracting or relaxing the blood vessels. These mediators could act on different cells, such as the glomerular cells and the interstitial tubular cells, and promote or inhibit cell proliferation, promote cellular autolysis and paracrine, promote the secretion of extracellular matrix (ECM) from cells, or inhibit the decomposition of ECM. Such mediators regulate inflammatory injury and related sclerotic changes. Previously, a series of important inflammatory mediators with pro-inflammatory functions was recognized and the important function of these mediators in nephritis pathogenesis was proven.

IL-6 is a multifunctional cytokine produced by monocytes, fibroblasts, endothelial cells, mesangial cells, T lymphocytes, and other cells. Studies have shown that certain kidney diseases are closely related to IL-6 [4,5]. IL-6 is a cytokine secreted by the glomerular membrane system; it can stimulate the proliferation of mesangial cells and promote the release of inflammatory mediators, such as platelet-activating factor, thromboxane B2, and superoxide anion; thus, IL-6 has an important function in the immune pathological injury of glomerulonephritis [6]. VEGF is an intensive endothelial cell-specific mitogen and permeability factor that is widely involved in the formation of vascular basement membrane and angiogenesis [7]. VEGF is synthesized and secreted by numerous cells, including smooth muscle cells, lutein cells, adrenal cortex cells, and pituitary folliclestellate cells; the kidney primarily comprises glomerular visceral epithelial cells and podocytes [8]. In the kidney, VEGF is mainly expressed in the podocytes [9] and tubular epithelial cells, whereas the VEGF receptor is mainly expressed in the anterior-glomerular vessel, glomerulus, and peritubular capillary endothelial cells. Satchell [10] found that VEGF can reduce the electrical resistance of glomerular endothelial cells down to 12.5 Ω/cm², thereby increasing the permeability of endothelial cells. VEGF also exhibits a bradykinin effect that is similar to that exhibited by histamine. Such an effect could directly cause plasma extravasation and the phosphorylation of occluding protein serine or threonine residue, thereby resulting in degradation or conformational changes that occlude and increase vascular permeability. During embryonic development, the glomerular visceral and collecting duct epithelial cells exhibit high VEGF expression. VEGF is also expressed inside the glomerulus and the peritubular endothelial cells. Under certain physiological conditions, the kidney tissues of adult humans or animals (mice or rats) can express VEGF continuously in the foot process and in collecting duct epithelial cells, thereby regulating the permeability of the glomerular filtration membrane. The glomerular podocytes secrete VEGF and the VEGF receptors on the surface of glomerular endothelial cells. Thus, VEGF produced by the podocytes might bind to the glomerular endothelium through the paracrine, thereby participating in local regulation. The interaction of the 2 cell types function in tandem to maintain the integrity and normal functions of the basement membrane [11].

AH etiology is complicated and has various clinical manifestations, so an early diagnosis is difficult to establish. The lack of early diagnosis delays treatment and leads to the progressive development of kidney disease. Previously, a series of important inflammatory mediators that exhibits proinflammatory functions has been recognized, and its important functions in the pathogenesis of nephritis have been confirmed. VEGF and IL-6 have an important function in the injury and repair processes of glomerular intrinsic cells. In this study, the urinary VEGF and IL-6 levels of simple hematuria were detected and analyzed to investigate their relationship and functions in the pathological process of glomerular injury, showing the relevance of urinary VEGF and IL-6 changes and the value of early AH diagnosis.
Material and Methods

Subjects

A total of 121 patients diagnosed with primary renal hematuria in our hospital from September 2011 to April 2012 were selected. This study used the case-control method and divided the patients into groups according to the 24-h urine protein levels: the isolated hematuria group (group I) with proteinuria >150 mg; <1000 mg (group II) with proteinuria; and >1000 mg (group III). The exclusion criteria were: severe liver dysfunction, allergies, unexplainable fever, infection, and inflammation caused by trauma and surgery. This study was conducted in accordance with the Declaration of Helsinki and with approval from the Ethics Committee of Beidaihe Sanatorium. Written informed consent was obtained from all participants.

Detection of IL-6 and VEGF

Fresh morning midstream urine (2 mL) was collected on the day of renal biopsy and subsequently centrifuged to remove particles and polymers. An ELISA test was performed to detect the compounds. The IL-6 and VEGF detection agents were obtained from BlueGene Company, Shanghai, China (Lot No. J216.006.096). The samples of creatinine (CR) and 24-h urinary protein were collected before renal biopsy. If the qualitative test result showed more than “+++”, the 5-fold diluted 24-h urine protein was subjected to a quantitation test using an automatic biochemical analyzer (Olympus Chemistry Analyzer AU640 Olympus Optical Co., Ltd. 128, Shimotogari, Nagizumi-Chu, Sunto-Gun, Shizuoka, Japan). The reagents were from Randox (Crumlin, United Kingdom). The method was performed strictly according to the kit instructions.

Renal biopsy

The renal biopsy needle was positioned under the B-mode ultrasound imaging system for renal biopsy puncturing. The sample was then subjected to paraffin sectioning, followed by the hematoxylin and eosin, Marchand, periodic Schiff-methenamine silver, and periodic acid-Schiff staining strategies for light microscope observation (BX40 OLYMPUS optical microscope). The frozen section was subjected to direct immunofluorescence detection. All the antibodies were purchased from Dako Denmark A/S (Productionsvej 42 Glostrup). IgG, IgA, IgM, C3c, C4c, C1q, and fibrinogen serum for direct detection. The reagents were from Randox (Crumlin, United Kingdom). Written informed consent was obtained from all participants.

Clinical diagnostic criteria

At present, unified Western diagnostic criteria for adult RH are not available. Thus, this study adapted the diagnostic criteria of hematuria and glomerular hematuria in the ‘Clinical Practice Guidelines-Nephrology Volume’ proposed by the Chinese Medical Association. Among the 3 types of conventional urine examination, if the “RBC ≥3/HP” appeared twice, the condition is defined as hematuria. When urinary RBC showed abnormal sizes and shapes >70%, the condition is glomerular hematuria. The following conditions were excluded: urinary tract infection, benign prosstatic hyperplasia, hypertrophy and inflammation, renal ptosis, urinary stones, tumors, renal traumas, renal tuberculosis, female annex inflammations, pelvic inflammatory diseases, and other secondary diseases.

Scoring criteria

Scoring criteria of renal pathological injuries [12–14] were: 1) Glomerular scoring was performed; 2) Mesangial cell proliferation was scored as 0, 1, 2, and 3 points for none, mild, moderate, and severe proliferation, respectively; and 3) Matrix widening was scored as normal=0 points, mild changes without obvious effect towards the capillary loops=1 point, moderate changes in appearance and diffused widening with <50% stenosis and atresia of capillary loops=2 points, and severe changes such as appearance of diffused widening with >50% stenosis and atresia of capillary loops=3 points; 4) Hardening changes were scored as normal=0 points, focal segmental distribution with <30% glomerulosclerosis=1 point, condition with 30% to 60% showing glomerulosclerosis=2 points, and the condition with >60% glomerulosclerosis=3 points; 5) Crescent formation was scored as normal=0 points, the condition with <30% focal segmental distribution=1 point, the condition with 30% to 50% showing diffused segmental distribution=2 points, and the condition with >50% diffused focal distribution=3 points; 6) Basement membrane thickening was scored; 7) Saccular adhesion was scored using the scoring criteria in 5) and 6) as <30% lesions=1 point, 30% to 60% lesions=2 points, and >60% lesions=3 points.

Statistical analysis

The data are expressed as mean ± standard deviation. The differences of related indicators between the groups used the rank sum test. The correlation of urinary VEGF/CR, IL-6/CR, and glomerular pathological scores used the Pearson linear correlation test. SPSS 18.0 software was used for the statistical analysis, and P<0.05 was considered to be statistically significant.

Results

Comparison of VEGF/CR

The comparison between groups I and II revealed that Z=−1.018, but without statistical significance (P>0.05). The comparison...
between groups II and III revealed that Z=–3.675 (P<0.01). The VEGF/CR of group III was significantly higher than that of group II, and the difference was statistically significant. The comparison between groups I and III revealed that Z=–4.65 (P<0.01). The VEGF/CR of group III was significantly higher than that of group I, and the difference was statistically significant (Figure 1).

Comparison of IL-6/CR

The comparison between groups I and II revealed that Z=–1.465 (P>0.05), and the difference between the 2 groups had no statistical significance. The comparison between groups II and III revealed that Z=–0.852 (P>0.05), and the difference between the 2 groups had no statistical significance. The comparison between groups I and III revealed that Z=–2.478 (P<0.05). The IL-6/CR of group III was significantly higher than that of group I (Figure 2).

**Comparison of glomerular pathological scoring**

The comparison between groups I and II revealed that Z=–3.912 (P<0.01), and the difference was statistically significant. The comparison between groups I and III revealed that Z=–3.675 (P<0.01), and the difference was statistically significant. The comparison between groups II and III revealed that Z=–4.65 (P<0.01). The VEGF/CR of group III was significantly higher than that of group II, and the difference was statistically significant. The comparison between groups I and III revealed that Z=–2.478 (P<0.05). The IL-6/CR of group III was significantly higher than that of group I (Figure 2).
comparison between groups II and III revealed that \( P > 0.05 \), and the difference was statistically significant. The comparison between groups I and III revealed that \( Z = -5.446 \) (\( P < 0.01 \)), and the difference was statistically significant (Table 1).

### Levels of IL-6, VEGF

The urinary levels of IL-6 were observed among the 3 groups to investigate its function in RH. The urinary IL-6/Cr and glomerular damage score were positively correlated in groups I and II (\( R^2 = 0.629, P < 0.001 \); \( R^2 = 0.193, P = 0.002 \); \( R^2 = 0.053, P = 0.060 \)), whereas no correlation was observed in group III (\( R^2 = 0.053, P < 0.05 \)) (Figure 3).

The urinary levels of VEGF were determined among the 3 groups to investigate the VEGF function in RH. The urinary VEGF/Cr and glomerular damage score were positively correlated in group I (\( R^2 = 0.429, P = 0.001 \)), but were not correlated in groups II and III (\( R^2 = 0.002, P = 0.821 \); \( R^2 = 0.006, P = 0.548 \)) (Figure 1).

Comparison of IL-6/Cr level and VEGF/Cr level among the 3 groups showed a statistically significant difference, and a positive correlation (\( R^2 = 0.629, P < 0.05 \)) was observed among the groups.

### Discussion

We aimed to provide a basis for the clinical diagnosis of asymptomatic renal hematuria and to determine the correlation of IL-6/Cr and VEGF/Cr with glomerular injury in patients with asymptomatic renal hematuria and in patients with asymptomatic renal hematuria and proteinuria. The urinary protein clearance method [15] was used to remove the interference caused by CR.

The differences in IL-6/Cr, VEGF/Cr, and glomerular pathological scores between the AH group and the hematuria with proteinuria group were statistically significant. IL-6/Cr, VEGF/Cr, and glomerular pathological scores in the AH group were positively related. Increased IL-6/Cr was related with glomerular injury [16], so IL-6/Cr might be involved in the occurrence and development of glomerular disease [17]. Under pathological conditions, IL-6 may act as a proinflammatory cytokine, which promotes the excessive activation and amplification of T and B cells, accelerates cellular apoptosis, promotes the destruction of pancreatic B cells, and promotes kidney mesangial proliferation [18]. In addition, IL-6 may bind the IL-6 receptor of the mesangial cell surface and stimulate the proliferation of mesangial cells and matrix [19], forming a vicious cycle in the autocrine regulation pathway and resulting in renal pathological immune injury, inflammatory changes, and hardening. A previous study [20] showed that IL-6 might also induce the expression of adhesion molecules, procoagulant factors, and adhesive inflammatory cells in vascular endothelial cells. Such expression promotes the formation of vascular thrombosis, increases capillary permeability, and induces changes in glomerular capillaries. Thus, the glomerular filtration rate and the urinary protein excretion are increased. This result was consistent with our findings. In the course of diseases such as glomerular lesion and glomerulonephritis, the glomerulus highly expresses VEGF to maintain the glomerular structure. The glomerular podocytes may secrete VEGF, and the surface of glomerular endothelial cells showed the expression of VEGF receptors. VEGF could reduce the anion number of the glomerular basement membrane and induce the small window formation of glomerular endothelial cells, thereby simultaneously affecting the charge and mechanical barriers of the glomerular basement membrane and regulating the glomerular permeability [11]. VEGF could induce morphological changes in endothelial cells, such as the formation and aggregation of plasma membrane vesicles and vesicle-like bodies and the regulation of the proteinuria formation, by affecting the cellular signal transduction and intracellular transportation. The clear expression of VEGF in the mesangial region could promote

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**Figure 3.** Correlation scatter diagram of IL-6/Cr and VEGF/Cr. (A) Group I; (B) Group II; (C) Group III.
the protein extravasation outside this region. Thus, increase in the vascular extracellular matrix would result in a subsequent increase in glomerular volume [21]. The repair of capillaries is necessary for glomerulonephritis healing [22], and VEGF is the essential factor that affects the growth and proliferation of glomeruli and peritubular endothelial cells. The presence of VEGF and its expression inside the renal tubule might have an important function in the injury and repair of simple renal hematuria. The increase in the level of urinary protein would aggravate glomerular injury and stimulate secretion of IL-6 and VEGF by renal intrinsic cells to repair glomerular injury. The levels of IL-6 and VEGF would also increase. These phenomena were observed in our study.

IL-6 was positively correlated with VEGF ($R^2=0.483$, $P=0.009$ in group I, $R^2=0.274$, $P=0.012$ in group II, and $R^2=0.348$, $P=0.065$ in group III). IL-6 and VEGF might co-affect glomerular pathological injuries. Previous studies have confirmed that IL-6 can increase the expression of VEGF [23]. Research has also proved that IL-6 can directly induce the expression of VEGF through the specific DNA sequence of VEGF gene promoter and a specific element of the 5c untranslated region (Sc2UTR) [24]. In our study, the levels of VEGF and IL-6 were positively correlated, which was consistent with the results of extensive previous research [25–27].

In our study, the difference between the levels of IL-6/CR and VEGF/CR with the glomerular pathological injuries in group III was not statistically significant, suggesting the lack of correlation. The lack of correlation might be related to the source of urinary protein. The urinary protein could originate from glomerular, tubular, physiological, and overflow proteinuria. In case of severe glomerular injury, the glomerular tubules are involved. When the renal tubules are damaged or dysfunctional, the reabsorption function of proximal tubule towards normally filtrated proteins is triggered, resulting in the increase of urinary protein. When the urinary protein originated from the renal tubules, the relationship of IL-6/CR and VEGF/CR with glomerular injury scoring might be inhibited. The levels of IL-6/CR and VEGF/CR levels could not accurately reflect the relationship between IL-6/CR and VEGF/CR and the glomerular pathological scores. Furthermore, such a relationship might also be related to the other cytokines inside the samples. The specific cause of these phenomena requires further research and discussion.

Conclusions

The urinary levels of IL-6/CR and VEGF/CR in AH and/or the proteinuria patients could reflect glomerular pathological injuries to some extent. This study provides new data for the further exploration of the underlying mechanism and early warning signs of adult renal hematuria. In vitro and in vivo experiments are required to further reveal the relationships between RH, IL-6/CR, and VEGF/CR.

Conflict of interest

The authors have no financial or commercial interests in this work.

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