LIBYAN JOURNAL OF MEDICINE
2023, VOL. 18, 2156675
https://doi.org/10.1080/19932820.2022.2156675

Taylor & Francis
Taylor & Francis Group

ARTICLE

Association of vascular endothelial growth factor (VEGF) protein levels and gene polymorphism with the risk of chronic kidney disease

Yipin Liu[a,†], Kai Hong[b,†], Wenjuan Weng[a,†], Shuangyi Huang[a] and Tianbiao Zhou [a]

[a]Department of Nephrology, the Second Affiliated Hospital, Shantou University Medical College, Shantou, China; [b]Department of Clinical Laboratory, the Second Affiliated Hospital, Shantou University Medical College, Shantou, China

ABSTRACT

Vascular endothelial growth factor (VEGF) is a heparin-specific growth factor specific for vascular endothelial cells and induces angiogenesis via binding to vascular endothelial growth factor receptor (VEGFR). Chronic kidney disease (CKD), accompanied by microvascular disease, is recognized as an irreversible reduction of renal function. The effects of VEGF on CKD risk were evaluated in this study. 121 CKD patients and 50 healthy volunteers were evaluated in the current study. Data mining using the China Biological Medicine (CBM) and NCBI/PubMed databases, was performed and applicable investigations were pursued. Pooled mean differences (MD) and pooled odds ratios (OR), with corresponding confidence intervals (CIs), were calculated by meta-analysis. The levels of Scr, BUN and VEGF in the CKD group were significantly higher, when compared with the control group ($P < 0.01$). For the meta-analysis, thirteen articles and our current study were evaluated. VEGF levels was found to be associated with CKD risk ($P < 0.00001$). In the sub-group meta-analysis, we found that the pooled MD of VEGF levels was related to the early CKD group, although the difference was not notable. However, the meta-analysis itself indicated that the pooled MD of VEGF levels were in accordance with severe CKD group ($P < 0.00001$). Furthermore, VEGF +936C/T T allele was not associated with CKD risk ($P = 0.69$). VEGF levels are apparently associated with CKD risk, especially in more severe CKD. Gene polymorphism analysis indicates that the VEGF +936C/T T allele is not associated with CKD risk.

ARTICLE HISTORY
Received 18 August 2022
Accepted 6 December 2022

KEYWORDS
Chronic kidney disease (CKD); vascular endothelial growth factor (VEGF); +936C/T; gene polymorphism; meta-analysis

1. Introduction

Chronic kidney disease (CKD) is a medical condition whose diagnosis is established when (i) the glomerular filtration rates are lower than 60 ml/min/1.73 m^2, for more than 3 months, or (ii) the renal injury is greater than 3 months [1]. In China, the prevalence of CKD disease in adults, as well as the awareness of this condition, is 10% [2]. Renal injury is eventually confirmed based on a number of alterations, in the context of renal physiology, imaging examination and/or the levels of specific blood/urine biomarkers.

Distinct clinical manifestations may appear on different CKD stages. CKD stages 1 to 3 are regarded as early CKD, while stages 4–5 are typically considered as severe CKD, according to the National Kidney Foundation-Kidney Disease Outcomes Quality Initiative (NKF-KDOQI) [3]. Before stage 3 (early CKD), patients are usually asymptomatic or present some mild discomfort(s), such as backache, fatigue, nocturia, and others. A small number of CKD patients may also have metabolic acidosis, loss of appetite, and/or mild anemia. After CKD stage 3 (severe CKD), these symptoms become more evident and, upon entering the renal failure stage, it further aggravates. Additional complications may co-occur, and these could include gastrointestinal symptoms, hypertension, acid–acid balance disorder, severe hyperkalemia, anemia, heart failure, hyperparathyroidism, mineral bone metabolism abnormalities, CNS disorders, and even life-threatening. The outcome for patient with CKD is end-stage renal disease (ESRD), and patients with ESRD require more invasive treatments including hemodialysis, peritoneal dialysis and/or kidney transplantation.

The pathogenesis of CKD is very complex, and many factors can be involved in its development [4–7]. In recent years, it has been reported that vascular endothelial growth factor (VEGF), also known as vascular permeability factor, can be potentially associated with CKD. In fact, a number of studies have found that VEGF is associated with glomerular sclerosis and renal interstitial fibrosis, and is also involved in the progression of CKD [8–10].

VEGF is a heparin-specific growth factor, specific for vascular endothelial cells, which is capable of inducing

CONTACT Tianbiao Zhou zhoutb@aliyun.com Department of Nephrology, the Second Affiliated Hospital, Shantou University Medical College, Shantou 515041, China
†The authors should be regarded as joint First Authors.

angiogenesis via binding to vascular endothelial growth factor receptor (VEGFR) [11]. Alterations on VEGF protein levels and differential gene polymorphisms have been reported and also associated with a number of diseases [12–16]. In this study, we analyzed VEGF protein levels in the sera of CDK and control patients, and further explored the relationship between VEGF levels and CKD. For this, we have used a meta-analysis method to assess the relationship between VEGF protein levels and gene polymorphisms against CKD risk.

2. Material and methods

2.1. Clinical study

2.1.1. Specimen collection

A total of 121 CKD patients (CKD group) were enrolled at the Department of Nephrology of the Second Affiliated Hospital of Shantou University Medical College, between June 2018 and March 2021. These patients included glomerulonephritis (n = 58), diabetes nephropathy (n = 32), hypertensive nephropathy (n = 12), lupus nephritis (n = 2) and obstructive nephropathy (n = 3), and others (n = 14). All patients were non dialysis patients. A healthy control group was also designed by recruiting 50 healthy volunteers from outpatient health checkups.

No significant difference was observed in gender and age when comparing these two groups (P > 0.05; Table 1). Informed consent forms were provided to all patients. Signed forms were reviewed and approved by the Ethics Committee of our institution.

Three milliliters (ml) of blood sample was drawn from each subject, and blood specimens were placed in clean, dry tubes. Samples were allowed to stand for 30 minutes at room temperature for proper coagulation. Specimens were then centrifuged at 3000 rpm for 5 minutes at room temperature, and 2 ml serum was transferred to another dry and clean tube. Serum were stored at −20C until further analysis.

2.2. Detection of serum creatinine (Scr) and blood urea nitrogen (BUN)

The determination of Scr levels in serum samples was performed by Picric acid method, using a Scr assay kit (Beckman Coulter., Co., USA). BUN levels in serum samples were quantified by urease-glutamate dehydrogenase method, using a urea assay kit (AUZ6163, Beckman Coulter., Co., USA). Estimated Scr and BUN levels were detected by Beckman Biochemical AU5400 System (Beckman, Co., USA).

2.3. Detection of VEGF in the serum

VEGF levels were detected according to the manufacturer's instructions, using an enzyme-linked immunosorbent assay (ELISA) kit (Abcam, USA). Samples were measured by absorbance at 450 nm, using an enzyme-labelling measuring instrument (Fermi automatic enzyme-linked immunosorber Co., Switzerland). The VEGF concentrations of the samples were calculated by extrapolating each absorbance values using a standard curve.

2.4. Meta-analysis

2.4.1. Search strategy

The retrieval strategy utilized the following keywords: chronic kidney disease, CKD, vascular endothelial growth factor, VEGF, end-stage renal disease, ESRD and renal failure. Data mining was performed using public databases (China Biological Medicine Database/CBM, and NCBI/PubMed, USA) available up to 1 May 2022. Screening was performed by reference to the literature for further compliance studies.

2.5. Inclusion and exclusion criteria

2.5.1. Inclusion criteria

(1) the investigation was related to a cohort study or case–control study; (2) the resulting outcome should be 'CKD' or 'ESRD'; (3) both case and control groups were present.

2.5.2. Exclusion criteria

(1) Review articles, editorials, and case reports; (2) Articles not providing detailed genotype data and/or VEGF levels; (3) Relationship between VEGF genotype/VEGF level and other diseases not related to CKD or ESRD.

2.6. Data extraction and synthesis

Two researchers were selected to independently extracted data and information as the following: First author's last name, year of publication, serum VEGF levels

Table 1. Characteristics and laboratory data of the study groups.

Parameter	CKD group	Healthy group	P value
n	121	50	
Age (years)	59.42 ± 10.95	55.16 ± 9.32	0.194
Sex (M/F)	68/53	27/23	0.792
Scr (umol/L)	719.60 ± 353.18	79.29 ± 15.55	<0.01
BUN (mmol/L)	30.81 ± 19.28	5.17 ± 1.45	<0.01
VEGF (pg/ml)	1141.02 ± 1173.51	107.67 ± 32.48	<0.01

CKD: chronic kidney disease; VEGF: vascular endothelial growth factor; Scr: serum creatinine; BUN: blood urea nitrogen; M: male; F: female.

and VEGF genotyping data, for both groups. The allele frequency of the two groups was calculated accordingly. Allele frequency was counted for each group. The extracted data were further compared between the groups.

Severe renal dysfunction (eGFRcys < 15 ml/min/1.73 m2 or were undergoing dialysis)

2.7. Statistical analysis

Clinical study data were collected and analyzed using a statistical software package for the social science 13.0 (SPSS 13.0). Data were presented as mean plus or minus standard deviation (mean ± SD). Comparison between groups were performed by independent sample t-test. $P < 0.05$ was regarded as a cut-off for statistical significance.

Extracted data were analyzed in silico, using Cochrane Review Manager Version 5 software. If the heterogeneity test resulted in P-value >0.1, results were pooled using the fixed effects model fixed model statistics, otherwise, a random -effects model was applied. Confidence intervals (CI) at 95% were calculated. Mean differences (MD) were applied to present the results for continuous data, and odds ratios (OR) were applied to show the results for dichotomous data. A P-value <0.5 was considered statistically significant. The Egger regression asymmetry test [17] and Begg adjusted rank correlation test [18] were used to calculate publication bias ($P < 0.1$ as significance cut-off).

3. Results

3.1. Clinical studies

3.1.1. Comparison of serum VEGF levels between CDK and control patient groups

Compared with the control group, the levels of serum Scr and BUN in CKD group were significantly higher (Scr: 719.60 ± 353.18 vs 79.29 ± 15.55; BUN: 30.81 ± 19.28 vs 5.17 ± 1.45; all $P < 0.01$). Furthermore, VEGF levels in CKD group increased compared with those in the control group (1141.02 ± 1173.51 vs 107.67 ± 32.48; $P < 0.01$).

3.2. Meta-analysis

3.2.1. Search results

Our meta-analysis was performed using 13 articles [19–31], in addition to our current study, all describing a potential association between VEGF levels and CKD susceptibility. Altogether, this compiled data included 788 CKD patients and 602 controls. Five reports [19,22,24,27,31] have associated VEGF levels with early CKD susceptibility, while nine studies [21,22,24–31] and our current work were utilized for the meta-analysis associating VEGF levels with severe CKD susceptibility. Two other reports [32,33] were considered for the meta-analysis correlating a VEGF +936C/T gene polymorphism with CKD susceptibility.

3.3. Relationship between VEGF and CKD susceptibility

In the current meta-analysis, VEGF levels were generally associated with CKD susceptibility (MD = 169.39, 95% CI: 128.50–210.28, P < 0.00001; Figure 1). The heterogeneity test showed a p-value <0.00001. Thus, a random-effects model has been further applied.

In the sub-group meta-analysis, we have found that the pooled MD of VEGF levels were in favor to an early CKD group, although the difference was not significant (MD = 14.48, 95% CI: −2.17–31.12, P = 0.09; Figure 2). However, the meta-analysis indicated that the pooled MD of VEGF levels were still in favor to a severe CKD group (MD = 233.77, 95% CI: 176.77–290.16, P < 0.00001; Figure 2).

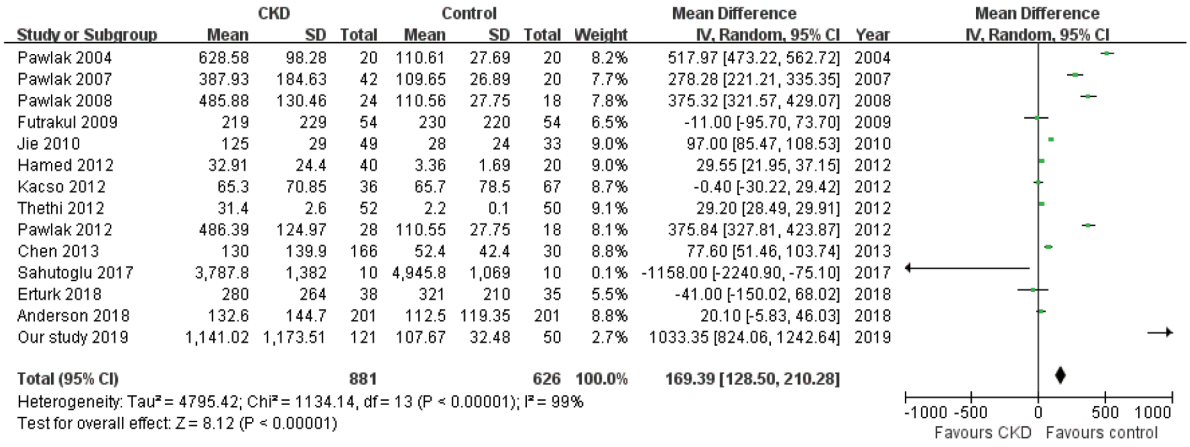

Figure 1. Association of vascular endothelial growth factor protein levels with CKD risk. CKD: chronic kidney disease; SD: standard deviation; Total: the total number of CKD group or control group; CI: confidence intervals; I^2: test the heterogeneity among recruited studies; df: degrees of freedom.

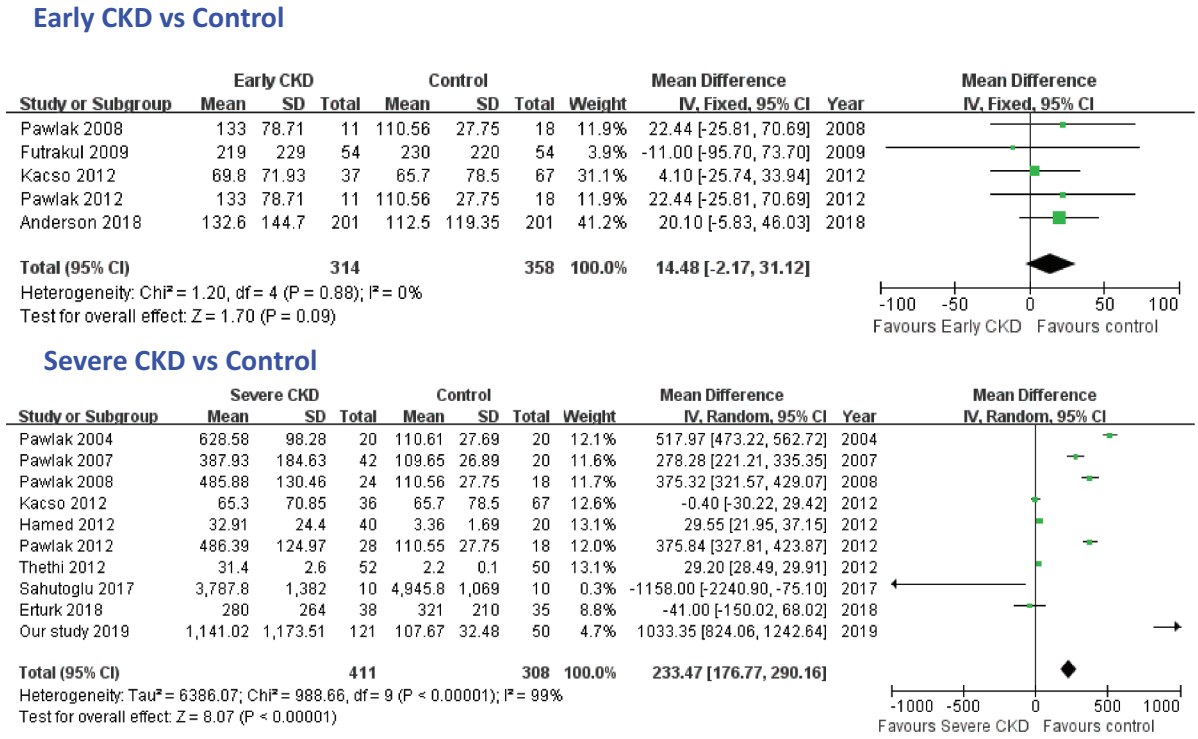

Figure 2. Association of vascular endothelial growth factor protein levels with early- or severe-CKD risk. CKD: chronic kidney disease; SD: standard deviation; Total: the total number of CKD group or control group; CI: confidence intervals; I2: test the heterogeneity among recruited studies; df: degrees of freedom.

3.4. Relationship between the VEGF +936C/T gene polymorphism and CKD susceptibility

In the current meta-analysis, VEGF +936C/TT allele was not found to be associated with CKD susceptibility (OR = 1.31, 95% CI: 0.34–5.05, P = 0.69; Figure 3).

3.5. Evaluation of publication bias

No significant publication bias associating VEGF levels with CKD susceptibility was detected (Begg P = 0.001, funnel plot was presented in Figure 4; Egger P = 0.001).

4. Discussion

In this clinical study, no statistical difference in baseline data was observed after comparing the CKD group with a healthy control group. Patients with CKD had a significant increase on both Scr and BUN levels when compared with healthy subjects. Interestingly, VEGF protein levels in CKD patients were also significantly higher than those in the control group. This suggests that VEGF might be closely related to the CKD pathogenesis. However, how VEGF participates in the pathogenesis of CKD is still unclear. Whether it is because the excretion of related factors decreases after the decline of renal function, or whether it is because VEGF is really activated and participates in the pathogenesis of CKD. This problem needs further research and confirmation in the future.

The data structure of a single center frequently has some limitations. Therefore, we conducted meta-analysis to better establish a correlation between VEGF and CKD. In this meta-analysis, fourteen studies

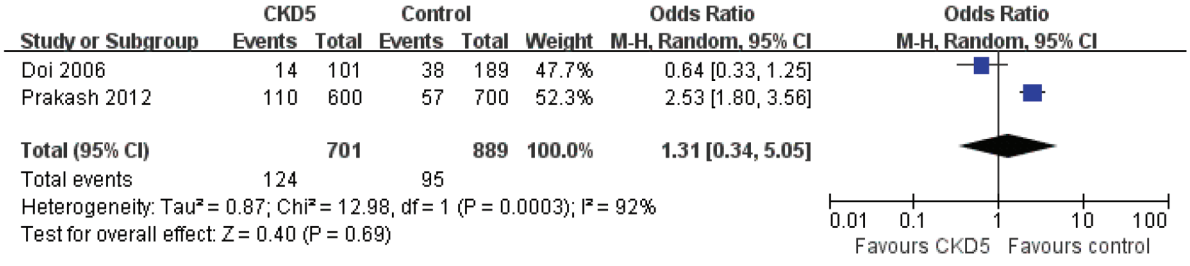

Figure 3. Association of the VEGF +936C/T gene polymorphism with CKD susceptibility. CKD: chronic kidney disease; SD: standard deviation; Total: the total number of CKD group or control group; CI: confidence intervals; I2: test the heterogeneity among recruited studies; df: degrees of freedom.

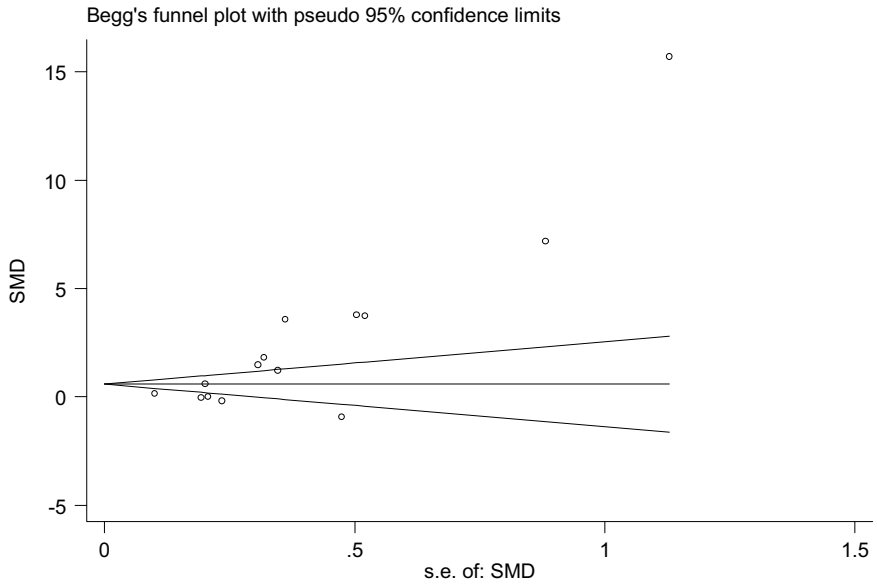

Figure 4. Publication bias was evaluated for association of vascular endothelial growth factor protein levels with chronic kidney disease risk. SMD, standardized mean difference; SE, standard error.

(including our study) were included and the results clearly indicated that VEGF levels are possibly associated with CKD susceptibility, especially in more advanced conditions. We reviewed these reports and verified that one particular study reported that low levels of VEGF were associated with CKD susceptibility [26], while seven studies (including our own study) indicated that higher levels of VEGF were associated with CKD susceptibility [20,22,23,29–31] indicated that high levels of VEGF were associated with CKD susceptibility. However, six other studies did not find any correlation between VEGF with CKD risk [19,21,24,25,27,28]. We also conducted an evaluation of publication bias, and found that there was significant variation of published data intended to associate VEGF levels with CKD susceptibility. This suggests that our results might not be conclusive. A higher number of standardized studies should be conducted to confirm this relationship.

We have also utilized the meta-analysis approach to investigate the relationship between *VEGF* gene polymorphism and CKD. In this study, only VEGF +936C/T polymorphism was included to assess any potential association with CKD susceptibility. Our results indicated that the VEGF +936C/TT allele was not associated with CKD risk. However, this meta-analysis only included two specific studies and, therefore, the sample size was small. Further clinical studies are still needed to confirm the current results in the near future.

Based on our knowledge, no other meta-analysis has attempted to establish an association between VEGF protein levels/gene polymorphisms and the pathogenesis of CKD. In the current study, VEGF levels were found to be associated with CKD susceptibility. Nevertheless, some limitations should be pointed out in this study. In the clinical side, the sample size was small and then study was not conclusive. Second, the sample size for the meta-analysis correlating VEGF +936C/T polymorphism and CKD risk was small and, therefore, still preliminary. Of note, the heterogeneity among the studies establishing a relationship between VEGF levels and CKD susceptibility was relevant. This heterogeneity could be associated with variations on the equipment and detection kits used in the current work. More studies are still required to strengthen the promising correlation that we have here defined, in the context of CKD. Furthermore, more studies such as genomewide association study (GWAS) should be performed to confirm the correlation between VEGF +936C/T polymorphism and CKD risk in the future.

5. Conclusion

In the current study, the VEGF levels in CKD group were increased compared with those in a healthy control group. Furthermore, the increased levels of VEGF were associated with CKD susceptibility, especially in a more severe CKD condition, but VEGF +936C/T T allele was not associated with CKD susceptibility. More in-depth studies should be conducted to confirm our current observations.

Acknowledgements

We would like to express our special appreciation for all patients participating in this study, and the authors would like to gratefully acknowledge the most helpful comments on this paper received from Professor Hong Qian, Karolinska Institutet.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

This study was supported by the Guangdong Medical Science and Technology Research Fund Project (no. B2020017).

Abbreviations

VEGF	vascular endothelial growth factor
CKD	chronic kidney disease
Scr	serum creatinine
BUN	blood urea nitrogen

Data availability statement

All data generated of this study are included in the published article. The data used in this study are available on request from the corresponding author.

Ethics approval

This study was approved by the ethics committee of the Second Affiliated Hospital, Shantou University Medical College. Written informed consent were given to all the participants prior to the collection of samples.

Informed consent

All authors consent to the submission of this manuscript.

ORCID

Tianbiao Zhou http://orcid.org/0000-0001-8065-1644

References

[1] Santana Machado T, Cerini C, Burtey S. Emerging roles of aryl hydrocarbon receptors in the altered clearance of drugs during chronic kidney disease. Toxins (Basel). 2019;11(4):209.
[2] Yang M, Zou Y, Lu T, et al. Revised equations to estimate glomerular filtration rate from serum creatinine and cystatin C in China. Kidney Blood Press Res. 2019;44(4):1–12.
[3] Levey AS, Coresh J, Bolton K. et al. K/DOQI clinical practice guidelines for chronic kidney disease: evaluation, classification, and stratification. Am J Kidney Dis. 2002;39(2 Suppl 1):S1–266.
[4] Annaldas S, Saifi M, Khurana A, et al. Nimbolide ameliorates unilateral ureteral obstruction-induced renal fibrosis by inhibition of TGF-β and EMT/Slug signalling. Mol Immunol. 2019;112:247–255.
[5] George C, Yako Y, Okpechi I, et al. An African perspective on the genetic risk of chronic kidney disease: a systematic review. BMC Med Gene. 2018;19(1):187.
[6] Khodeir SA, Okda HI, Abdalal HM. Clinical significance of fibroblast growth factor-23 and soluble alpha klotho in different stages of chronic kidney disease. Saudi J Kidney Dis Transpl. 2019;30(1):108–118.
[7] Suassuna PGA, de Paula RB, Sanders-Pinheiro H, et al. Fibroblast growth factor 21 in chronic kidney disease. J Nephrol. 2019;32(3):365–377.
[8] Yang W, Wang J, Shi L, et al. Podocyte injury and overexpression of vascular endothelial growth factor and transforming growth factor-beta 1 in adriamycin-induced nephropathy in rats. Cytokine. 2012;59(2):370–376.
[9] Sato W, Kosugi T, Zhang L, et al. The pivotal role of VEGF on glomerular macrophage infiltration in advanced diabetic nephropathy. Lab Invest. 2008;88 (9):949–961. DOI:10.1038/labinvest.2008.60
[10] Hasegawa S, Nakano T, Torisu K, et al. Vascular endothelial growth factor-C ameliorates renal interstitial fibrosis through lymphangiogenesis in mouse unilateral ureteral obstruction. Lab Invest. 2017;97 (12):1439–1452.
[11] Melincovici C, Bosca A, Susman S, et al. Vascular endothelial growth factor (VEGF) - key factor in normal and pathological angiogenesis. Rom J Morphol Embryol. 2018;59(2):455–467.
[12] Boon-Peng H, Mat Jusoh JA, Marshall CR, et al. Rare copy number variants identified suggest the regulating pathways in hypertension-related left ventricular hypertrophy. PLoS ONE. 2016;11(3):e0148755.
[13] Hollox EJ, Hoh BP. Human gene copy number variation and infectious disease. Hum Genet. 2014;133 (10):1217–1233.
[14] Asanghanwa M, Gorus FK, Weets I, et al. Clinical and biological characteristics of diabetic patients under age 40 in Cameroon: relation to autoantibody status and comparison with Belgian patients. Diabetes Res Clin Pract. 2014;103(1):97–105. DOI:10.1016/j.diabres.2013.11.013
[15] Mbunwe E, Van der Auwera BJ, Vermeulen I, et al. HLA-A*24 is an independent predictor of 5-year progression to diabetes in autoantibody-positive first-degree relatives of type 1 diabetic patients. Diabetes. 2013;62 (4):1345–1350. DOI:10.2337/db12-0747
[16] Zhou T, Li H, Zhong H, et al. Relationship between transforming growth factor-β1 and type 2 diabetic nephropathy risk in Chinese population. BMC Med Gene. 2018;19(1):201.
[17] Egger M, Davey Smith G, Schneider M, et al. Bias in meta-analysis detected by a simple, graphical test. BMJ. 1997;315(7109):629–634.
[18] Begg CB, Mazumdar M. Operating characteristics of a rank correlation test for publication bias. Biometrics. 1994;50(4):1088–1101.
[19] Futrakul N, Futrakul P. A mildly altered vascular homeostasis in early stage of CKD. Ren Fail. 2009;31 (7):538–543.
[20] Jie KE, Zaikova MA, Bergevoet MW, et al. Progenitor cells and vascular function are impaired in patients with chronic kidney disease. Nephrol Dialysis Transplantation. 2010;25(6):1875–1882. official publication of the European Dialysis and Transplant Association - European Renal Association. DOI:10.1093/ndt/gfp749
[21] Thethi I, Bansal V, Khan H, et al. Assessment of levels of vascular endothelial growth factor in patients with ESRD and its possible role in cardiovascular morbidity and mortality. Clin Appl Thromb Hemost. 2012;18 (5):534–537.

[22] Pawlak K, Ulazka B, Mysliwiec M, et al. Vascular endothelial growth factor and uPA/suPAR system in early and advanced chronic kidney disease patients: a new link between angiogenesis and hyperfibrinolysis? Transl Res. 2012;160(5):346–354.
[23] Chen YT, Cheng BC, Ko SF, et al. Value and level of circulating endothelial progenitor cells, angiogenesis factors and mononuclear cell apoptosis in patients with chronic kidney disease. Clin Exp Nephrol. 2013;17(1):83–91. DOI:10.1007/s10157-012-0664-9
[24] Kacso IM, Kacso G. Endothelial cell-selective adhesion molecule in diabetic nephropathy. Eur J Clin Invest. 2012;42(11):1227–1234.
[25] Hamed EA, El-Abaseri TB, Mohamed AO, et al. Hypoxia and oxidative stress markers in pediatric patients undergoing hemodialysis: cross section study. BMC Nephrol. 2012;13(1):136.
[26] Sahutoglu T, Sakaci T, Hasbal NB, et al. Serum VEGF-C levels as a candidate biomarker of hypervolemia in chronic kidney disease. Medicine. 2017;96(18):e6543. DOI:10.1097/MD.0000000000006543
[27] Anderson CE, Hamm LL, Batuman G, et al. The association of angiogenic factors and chronic kidney disease. BMC Nephrol. 2018;19(1):117. DOI:10.1186/s12882-018-0909-2
[28] Erturk I, Yesildal F, Acar R, et al. Vascular endothelial growth factor and soluble vascular endothelial growth factor receptor-1 in patients with end-stage renal disease. Associations with laboratory findings, comorbidities, and medications. Saudi Med J. 2018;39(6):586–591.
[29] Pawlak K, Pawlak D, Mysliwiec M. Possible new role of monocyte chemoattractant protein-1 in hemodialysis patients with cardiovascular disease. Am J Nephrol. 2004;24(6):635–640.
[30] Pawlak K, Pawlak D, Mysliwiec M. Impaired renal function and duration of dialysis therapy are associated with oxidative stress and proatherogenic cytokine levels in patients with end-stage renal disease. Clin Biochem. 2007;40(1–2):81–85.
[31] Pawlak K, Mysliwiec M, Pawlak D. Oxidative stress, phosphate and creatinine levels are independently associated with vascular endothelial growth factor levels in patients with chronic renal failure. Cytokine. 2008;43(1):98–101.
[32] Doi K, Noiri E, Nakao A, et al. Functional polymorphisms in the vascular endothelial growth factor gene are associated with development of end-stage renal disease in males. J Am Soc Nephrol. 2006;17(3):823–830.
[33] Prakash S, Prasad N, Sharma RK, et al. Vascular endothelial growth factor gene polymorphisms in North Indian patients with end stage renal disease. Cytokine. 2012;58(2):261–266.