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The association of interleukin-6 (−174 G/C) polymorphism with risk of chronic kidney disease and erythropoietin hyporesponsiveness

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ABSTRACT

Introduction: The plasma levels of the cytokine interleukin-6 (IL-6) have been reported to be associated with risk of chronic kidney disease (CKD) and erythropoietin (EPO) responsiveness. The G/C promoter polymorphism of IL-6 is associated with expression and levels of IL-6, so it may confer increased risk to CKD and modulate EPO responsiveness.

Objectives: This study aimed to examine the association of IL-6 G/C polymorphism with risk of CKD and EPO hyporesponsiveness.

Patients and Methods: A total of 40 haemodialysis patients on EPO therapy, and 30 age- and sex-matched apparently healthy volunteers as a control group were recruited for this study. Blood samples were collected from each participant and used for complete blood count (CBC) using automated haematology analyser and molecular analysis of IL-6 -174 G/C polymorphism using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP).

Results: The results showed that IL-6 GG genotype is the most frequent in patients (82.5%) followed by GC genotype (17.5%), while all subjects of the control group were found to have GG genotype only. There was a statistically significant association between the polymorphism and end-stage CKD ($P=0.02$). Only 10% of the patients were found to achieve the target haemoglobin level. Although all of them had GG genotype, no association was found between the polymorphism and the achievement of target haemoglobin level ($P=0.16$).

Conclusion: IL-6 G/C polymorphism is significantly associated with CKD, but not with EPO responsiveness in haemodialysis patients.

Implication for health policy/practice/research/medical education:
In a case-control study on 40 hemodialysis patients and 30 apparently healthy volunteers as a control group we found that IL-10 -174 G/C polymorphism is significantly associated with the risk of end-stage renal disease. Although all patients achieved target hemoglobin levels, had GG genotype, no statistically significant association was found between the polymorphism and erythropoietin responsiveness.

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Introduction

Chronic kidney disease (CKD) is a global health issue and major cause of death in all countries of the world (1,2). The mortality rate of CKD has increased dramatically during the last three decades (3). The high rate of cardiovascular mortality and morbidity due to CKD are expected to reach epidemic proportions over the coming decade (4).

Anemia is a frequent clinical consequence of CKD. Many protocols for the management of anaemia of CKD are recommended in recent years and has changed its definition (5,6). The Kidney Disease Improving Global Outcome (KDIGO) group defined anemia in patients with CKD as hemoglobin concentration less than 13.0 g/dL in adult males and less than 12.0 g/dL in adult females. Hemoglobin concentration should not be raised above13 g/dL because of the risk of cardiovascular diseases (7).

Although anaemia of CKD is multi-factorial, it is mainly caused by decreased erythrocyte production due

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to insufficient erythropoietin (EPO) secretion by diseased kidneys (8). Other contributing factors include impaired bone marrow response to EPO, inflammation, iron deficiency, increased hepcidin levels, shortened red blood cell lifespan and vitamin B12 or folic acid deficiencies (9).

The management protocol of anemia of CKD involves the administration of EPO. Recombinant human EPO (rhEPO) is used for the management of anaemia of CKD since the late 1980s, which leads to an important improvement in patients' quality of life, and reducing the blood transfusions. EPO therapy aims at maintaining a target hemoglobin level of 11-12 g/dL (10). However, resistance to rhEPO occurs frequently in patients with CKD and associated with patients' failure to achieve the target hemoglobin levels, increased hospitalisation and frequent blood transfusion. Common causes of EPO resistance in haemodialysis patients include malnutrition, iron deficiency, hyperparathyroidism, infection and inflammation (11-13).

The interleukin-6 (IL-6) is an essential cytokine produced by many types of cells in the human body and playing an important role in the pathogenesis of anaemia of chronic diseases. It causes down-regulation of the expression of a gene in late normoblasts and decreases hemoglobin synthesis. Furthermore, it modulates the expression of genes involved in iron homeostasis and reduces iron release from reticuloendothelial macrophages to erythroblasts (14).

Genetic polymorphisms and plasma levels of IL-6 have been reported to be correlated with end-stage renal failure, its related complications, and EPO hyporesponsiveness in hemodialysis patients with sufficient iron (15-18).

A polymorphism involving the substitution of cytosine (C) for guanine (G) at position -174, in the promoter region Il-6 gene has been identified and suggested to play a role in the control of transcription and thus serum levels of this cytokine (19).

Objectives

The present study aimed to examine the association of IL-6 -174 G/C polymorphism with the risk of CKD and EPO hyporesponsiveness in Sudanese hemodialysis patients.

Patients and Methods

Study design

This was an analytical case-control study, conducted at Al-Neelain academy teaching hospital, Khartoum, Sudan, from April 2017 to April 2018. It included 40 patients with CKD on regular hemodialysis and EPO therapy and iron supplement for at least three months, and 30 apparently healthy volunteers, matched in age and gender with patents, as a control group. Patients with a known cause of EPO hyporesponsiveness such as infections, inflammation, aluminium toxicity, iron deficiency, folate deficiency and hyperparathyroidism were excluded from the study.

Blood sample collection

A blood sample was collected from each participant by the standard venipuncture procedure and transferred into two EDTA blood containers, one for complete blood count, and the other for molecular analysis.

Complete blood count (CBC)

CBC was performed immediately after the sample collection using an automated hematology analyzer (Sysmex KX 21N, Japan).

Molecular analysis

DNA extraction

Genomic DNA was extracted from peripheral leucocytes using the simple salting-out method and stored frozen (-20°C) until the polymerase chain reaction (PCR) was performed.

Detection of IL-6 -174G/C polymorphism

PCR reaction mixture (20 µL) was prepared for each sample, containing 1 µL of each of the forward (5′-ATGACTTCAGCTTTACTCTT-3′) and reverse (5′-ATAAATCTTTGTTG GAGGGT-3) primers (MACROGEN, KOREA), 2 µL genomic DNA, 4µl ready-to-load master mix (SOLIS BIODYNE, ESTONIA), and 12 µL distilled water.

The thermocycling conditions included a denaturation step at 95°C for six minutes followed by 35 cycles of denaturation at 95°C for 45 seconds, annealing at 58°C for 60 seconds and extension at 72°C for 60 seconds and then final extension step at 72°C for 10 minutes. The size of the PCR fragment amplified was 244 bp. The amplified fragments were subjected to restriction digestion using the restriction enzyme "NlaIII". The restriction fragments were separated on 2% agarose gel and visualised under UV transilluminator. Restriction digestion resulted in two fragments (200 and 44 bp).

Ethical issues

The study was in accordance with the Declaration of Helsinki and its later amendments. This paper was extracted from the M.Sc. thesis of Noha Alnair Abdelrhman at Al Neelain University, Sudan. An approval was obtained from the scientific research board of faculty of medical laboratory sciences (Ref#3/2017), Al-Neelain University. Informed consent was taken from all participants before sample collection. Data was kept confidentially and used only for the study.

Data collection and analysis

Patients' data were collected using a structured interview questionnaire and medical files and analyzed using SPSS, version 21. Quantitative variables were represented as mean+SD and independent 2-samples *t* test was used to compare means of quantitative variables in two groups. Qualitative variables were represented as frequency and percentage. The association between qualitative variables was tested by Fisher's exact test.

Results

A total of 40 patients with end-stage chronic renal failure and 30 apparently healthy volunteers, as a control group, were recruited for this study. The mean age of patients was 46.2 years and that of the control group was 41.1 years. Around 21 (52%) patients were males and 19 (48%) females, while 14 (47%) of the controls were males and 16 (53%) females.

Results showed that all patients were anaemic. Anaemia was normocytic normochromic in 32 (80%) and microcytic hypochromic in 8 (20%); none were found to have macrocytic anemia.

According to hemoglobin levels, 14 (35%) patients were found to have mild anaemia, 18 (45%) had moderate anemia and 8 (20%) had severe anemia (Table 1).

The results revealed a significantly decreased hemoglobin level in patients compared to the control group (9.47 ± 1.75 g/dL and 13.60 ± 1.30 g/dL respectively, $P < 0.001$).

Ten out of 40 patients (25%) reached the target hemoglobin level (11-12 g/dL), while 30 (75%) did not. Six (60%) of the patients who achieved the target hemoglobin level were males and four (40%) were females. We found a statistically significant association between gender and the achievement of target hemoglobin level ($P < 00.001$). Comparison of the mean age in patients who reached the target hemoglobin level and those who did not, showed no statistically significant difference (54 ± 13.8 g/dL and 50.4 ± 14.7 g/dL years respectively, $P = 00.62$).

The IL-6 -174 G/G genotype was the most frequent in hemodialysis patients, followed by G/C genotype. All controls were found to have G/G genotype; the C/C genotype was absent in both study groups. The IL-6 G/C polymorphic genotypes were found statistically significant with the risk of ESRD ($P = 0.02$; Table 2).

The frequency of G allele was 0.91 in patients and 1.0 in the control group and that of C allele was 0.09 in the patients and 0.00 in the control group.

The mean hemoglobin level was significantly lower in patients with G/C genotype compared to those with G/G genotype (6.8 ± 0.7 g/dL and 10.5 ± 1.5 g/dL respectively, $P < 0.001$); although all the patients who achieved the target hemoglobin level were found to have G/G genotype, the association between IL-6 G/C genotypic variants and achievement of target hemoglobin level was not statistically significant ($P = 0.16$).

Table 1. Characteristics of study subjects

Variable	Patients	Control
Age (years)		
Mean	46.1	41.1
SD	1.4	1.3
Gender		
Male	21 (52%)	14 (47%)
Female	19 (48%)	16 (53%)
Anaemia		
Yes	40 (100%)	0(0%)
No	0 (0%)	30(100%)
Type of anaemia		
Normocytic normochromic	32 (80%)	
Microcytic hypochromic	8 (20%)	
Severity of anaemia		
Severe	8 (20%)	
Moderate	18 (45%)	
Mild	14 (35%)	

Table 2. Genotype distribution among patients and control groups

Genotype	Study groups		*P* value*
	Patients	Control	
G/G	33 (82.5%)	30 (100%)	
G/C	7 (17.5%)	0 (0%)	0.02
Total	40 (100%)	30 (100%)	

* Significant at ≤ 0.05.

Discussion

Anemia of CKD is mainly caused by reduced production of renal EPO–which an important hormone for the control of erythropoiesis. Therefore, the treatment of anaemia of CKD includes administration of rhEPO or erythropoiesis-stimulating agents (20), which have been used for more than three decades ago to improve the management of anemia and reducing blood transfusions. Nevertheless, some patients show low response to EPO therapy (21). Identification of the factors that affect patients' response to EPO can further improve the management of the anaemia of CKD and will have a profound effect on the safety and financial cost of EPO therapy (22).

This study aims at examining the association of IL6 -174 G/C polymorphism with CKD and responsiveness to EPO therapy in haemodialysis patients.

The results showed that all patients were anaemic; most of the patients (80%) had normocytic normochromic anemia and the remaining (20%) had microcytic hypochromic anaemia. According to hemoglobin levels, 14 (35%) patients were found to have mild anaemia, 18 (45%) had moderate anemia and 8 (20%) had severe

anaemia. This study agrees with two studies by Schmidt & Dalton (23) and Cases et al, both reported that anemia in patients with CKD is commonly normocytic normochromic (23, 24).

Only ten out of 40 patients (25%) reached the target hemoglobin level. This indicates a low response to EPO therapy in the majority of our patients.

Results of this study revealed a statistically significant association between gender and the achievement of the target hemoglobin level, since approximately two-thirds of the patients who reached the target hemoglobin level were males. This may be because women in the reproductive age are usually at an increased risk of developing anaemia (25), and most of the females enrolled in this study were in this age period.

In this study, age was found to have no significant effect on the achievement of target hemoglobin levels in hemodialysis patients, as no statistically significant difference was found in the mean age of patients who achieved the level, compared to those did not. However, all patients enrolled in this study were adults (>20 years). This may not properly reflect the actual effect of age; for this purpose, further studies should be conducted to include patients from different age groups.

In the current study, the IL-6 -174 G/G genotype was most common (82.5%) in patients with CKD, followed by G/C genotype (17.5%). While all subjects of the control group were found to have G/G genotype; CC genotype was absent in both study groups. The association between the polymorphism and risk of end-stage chronic CKD was statistically significant (P=0.02). The frequency of G allele was higher in the control group, while that of C allele was higher in the patients. Previous studies concerned with the association of this polymorphism and risk of CKD showed controversial results and this attributed to regional and ethnic differences (26). As IL-6 (-174G/C) polymorphism is located in the promoter region and thus associated with the blood levels of IL-6, this supported the finding of Ng et al and Barreto et al, who reported that "levels of IL-6 are associated with risk of ESRD" (27, 28). Regarding alleles frequencies, our finding is consistent with that of Ranganath et al, who reported a significantly higher frequency of C allele in patients with ESRD than in the control group (29). On the other hand, our results disagree with studies by Buckham et al, who reported no association between IL-6 polymorphism and ESRD (30).

Although the mean hemoglobin level was significantly lower in patients with G/C genotype compared to those with G/G genotype and none of the patients with G/C genotype reached the target hemoglobin level, no statistically significant association was found between genotypes and achievement of target hemoglobin level (P=0.16). This finding indicated that IL-6 -174 G/C polymorphism not effecting the EPO responsiveness in hemodialysis patients independently. Low-EPO response in this study's subjects could be due to the presence of other causes of EPO resistance; this needs further investigation.

Conclusion

In conclusion, IL-6 -174 G/C polymorphism is associated with CKD, but not with EPO hyporesponsiveness in hemodialysis patients.

Limitations of the study

A limitation of this study was the small sample size; we recommended further studies conducted in the future with large sample size to well verify the association of the studied polymorphism with risk of CKD among Sudanese population.

Authors' contribution

NE and EW conducted the study. NE participated in the data collection, practical work, and draft writing. EW participated in the design of the study, description of the methodology, data analysis, and review of the final manuscript.

Conflicts of interest

The authors report no conflict of interest

Ethical considerations

Ethical issues including plagiarism, double publication, and redundancy have been completely observed by the authors.

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