Identification of the Interleukin-6 Polymorphism (-174) in the Saliva of Hemodialysis Patients

ABSTRACT

Background: Chronic Kidney Disease is prevalent in the general population and is associated with high morbidity and mortality and its pathogenic mechanisms are related to pro-inflammatory cytokines, such as Interleukin 6 (IL-6). It is known that polymorphisms associated with IL-6 can trigger a different immune response in the individual and therefore be a determining factor in the progression of the disease. The idea of using saliva as an analysis matrix for diagnostic methods suggests that the methodology may be viable due to the easy way collection of these fluids and the amount of information in saliva molecular constituents.
Aims: To identify the relationship between IL-6 polymorphism (-174) in dialysis patients using saliva.
Methodology:53individuals were assessed, divided into a test group: 27 on hemodialysis; and a control group: 26 healthy individuals. Saliva samples were collected, DNA was extracted, and genotyping was performed using Real Time-Polymerase Chain Reaction (RT-PCR). For statistical analysis, the $\chi^2$was performed on categorical data.
Results: The genotype frequency identified was 33.33% GC, 59.25% GG and 7.42% CC for the hemodialysis group and 19.23% GC, 50% GG and 30.77% CC for the healthy group(p=0.0806).
Conclusion: It was possible to verify the presence of the IL-6 (-174) polymorphism in saliva. Nonetheless, the predominance of GG was not significant, corroborating with other studies, that also indicate no relation between IL-6 Polymorphism and CKD. In this study,it was not possible to correlate hemodialysis patients with the polymorphism studied, but more studies about this subject are necessary, mainly in countries with diverse population, as Brazil.

Key words: *Interleukin-6; chronic kidney disease; polymorphism; IL-6 polymorphism*

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1. INTRODUCTION

Chronic Kidney Disease (CKD) is defined as an abnormality of the structure or function of the kidneys, present for more than three months with implications for the health of individuals, and therefore comprises a myriad of kidney diseases with a wide range of clinical and morphological characteristics [1].

CKD is considered a public health problem around the world [2]. According to the latest Brazilian Dialysis Census (2022), the increase in the prevalence of hemodialysis patients (758 patients per million) was significant. The incidence, although lower than in 2021, remained high - 224 patients per million - especially when compared to the estimates of the Latin American Society of Nephrology and the European Registry. The most frequent causes of CKD are hypertension, diabetes, glomerulonephritis, and polycystic kidney, among other less frequent ones [3].

CKD is progressive and irreversible, implying the limitation of glomerular filtration, causing uremia, and generating an accumulation of substances in the blood, which should have been filtered by the kidneys and subsequently excreted. Uremia causes immunodeficiency due to the increase in toxic substances in the bloodstream, so patients have a suppressed immune and humoral response [4]. In addition, it can cause various systemic changes such as cardiovascular alterations, anemia, hemostatic problems and lymphocytopenia [5,6]. Thus, CKD has a complicated interrelationship with other diseases [2].

The rate of progression of CKD varies between patients and is largely determined by genetic factors. Genetic mutations can result in disturbances in the function of the corresponding proteins, which will favor the development of kidney disease. One example is single nucleotide polymorphisms (SNPs) in genes that encode proteins with the ability to protect kidney tissue from permanent damage, and when present may be the basis of differences in susceptibility to disease progression between patients [7].

Koshino et al. showed that circulating levels of Interleukin-6 (IL-6) may be associated with a drop in renal function in patients with CKD and that the dosage of IL-6 in plasma and its changes over one year may be important in the prognosis for cardiovascular disease and progression of CKD in patients with type II diabetes at high cardiovascular risk. [8]. Therefore, this study aimed to identify IL-6 polymorphisms in saliva samples from patients with chronic kidney disease on hemodialysis.

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2. MATERIALS AND METHODS

The study was approved by the Santo Amaro University Research Ethics Committee - protocol number: 45478615.1.0000.0081

Fifty-three patients were selected, 26 were healthy and 27 undergoing hemodialysis at the Medirim Hemodialysis Sector in the municipality of Cariacica/ES- Brazil. All study participants were informed of the study's objectives and signed an informed consent form, which had previously been approved by the ethics committee.

The inclusion criteria were hemodialysis patients who agreed with the study objectives and signed a consent form. Patients who did not agree with the objectives of the study and who refused to sign the consent form were excluded from the study, as were pregnant and breastfeeding women and patients seropositive for HIV, hepatitis B (HBV), and C (HCV). For the control group, samples were collected from individuals who accompanied patients without a medical history of chronic renal disease.

Saliva samples were collected using the Salivette®tubes method (dry cotton swab in a plastic tube).The samples (containing at least 5mL) were placed in collection tubes, following all the manufacturer's guidelines. The samples were frozen at -20° C for subsequent IL-6 genotyping.

Genomic DNA was extracted using the QIAampDNA Kits extraction kit according to the manufacturer's instructions. Allelic discrimination assays were used to genotype the rs1800795 SNPs in the IL-6 gene (position -174), and amplification and reading were carried out using the Real-Time PCR technique(StepOne™ Real-Time PCR System - Applied Biosystems). The products were digested by 1U per reaction with 25 µl of NlaIII (CATGk) at 37° C to detect the G allele and the C allele. Three possible genotypes can be detected at position -174 in the IL-6 promoter gene, defined as high (G/G), medium (G/C) or low (C/C). The following primers was used to amplify the genomic DNA samples (Invitrogen Life Technologies) (-174): 5' -TTGTCAAGACATGCCAAGTGCT-3' (forward primer) and 5'-GCCTCAGAGACATCTCCAGTCC-3' (reverse primer).

2.1 Statistical Analysis

For statistical analysis, SPSS software version 13.0 was used (SPSS, Chicago, Ill). The χ^2 test was performed on categorical data.The significance level for all tests was set at 5%.

3. RESULTS

The hemodialysis group, five were female and 22 weremale. The initial cause of the disease was hypertensive nephrosclerosis in 22 (81.48%) patients and hypertensive diabetic

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nephropathy in five (18.52%) patients.From the heathy individuals 19 were female and seven were male.

The results obtained from the analysis of the distribution of IL-6 genotypes among healthy individuals were 50% for the GG genotype, 30.77% for CC and 19.23% for GC. The distribution among hemodialysis patients was 59.25% for GG, 33.33% for GC and 7.42% for CC(Table 1). No statistical difference was found in either group (*P*-value = 0.0806).

Table 1: Distribution of IL-6 genotypes in healthy individuals and those on hemodialysis

Genotype	Health		Hemodialysis		*P*-value
	n	%	*n*	%	
GG	13	50.00	16	59.25	0.0806
GC	05	19.23	09	33.33	
CC	08	30.77	02	7.42	

In terms of allele distribution, 59.6% of healthy individuals had the G allele and 40.4% had the C allele. 75.92% of hemodialysis patients had the G allele and 24.08% had the C allele(Table 2). No statistical difference was found (*P*-value = 0.0721).

Table 2: Distribution of IL-6 alleles in healthy individuals and on hemodialysis

Allele	Health		Hemodialysis		*P*-value
	n	%	*n*	%	
G	31	59.6	41	75.92	0.0721
C	21	40.4	13	24.08	

4. DISCUSSION

Some studies have linked genetic polymorphisms as a risk factor associated with CKD and different related pathologies [2]. According to the justification that genetic factors influence the susceptibility and progression of CKD [7], and the IL-6 single nucleotide polymorphism (SNP) is related to various diseases and complications related to CKD [9-11]. This study was designed to verify a possible relationship between the IL-6 polymorphism (-174 G/C) and chronic kidney disease.

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Saliva composition monitoring may be aneconomic, non-invasive, and easy tool to diagnose and clinically evaluate oral and systemic diseases. In fact, there is a relationship between CKD and saliva composition, which changes in association with an increase in urea, creatinine, calcium, sodium, potassium, phosphorus, bicarbonate and phosphate blood levels [12].Our group, in 2015 also developed a study with kidney patients and verified through saliva that hemodialysis patients showed higher changes in immunological and inflammatory components such as IgA, IgG, NO, and CRP levels [13]. Therefore, saliva may be an important tool for diagnosing and monitoring CKD, corroborating the results found in the present study.

A predominance of the GG genotype was found for both healthy patients and hemodialysis patients. A literature review with meta-analysis conducted by Feng et al. showed that genotypes containing the G allele (GG and GC) are related to higher circulating levels of IL-6 and greater inflammatory amplitude. Despite this, the study concludes that the IL-6 polymorphism does not influence the progression of CKD [14], corroborating the results found in our study, in which there was no statistical difference between the groups analyzed. A study like ours, carried out in the portuguese population, analyzed two different polymorphisms related to CKD. The authors concluded that the CC genotype is the least frequent in the IL-6 promoter region (rs 1800795) and IL-6 levels were increased in patients with end-stage renal disease and associated with an increased risk of cardiovascular disease (15).

About the alleles analysis, it was possible to verify a predominance of the G allele (59.6%) in the healthy group. In the hemodialysis group, the Gallele (75.9%) was also higher in comparison with the C allele (24.08%). In the meta-analysis conducted by Feng et al, the authors found that most studies show that the population carrying the G allele in the IL-6 -174G/C polymorphism has higher levels of IL-6, with the G allele being related to an exacerbated inflammatory response. Although in our study G allele was found to be considerably higher than C allele, in dialysis group, it was not possible to establish statistical difference.

Other studies have also linked the G allele with an increased inflammatory response, such as that by Lorente et al, in which the authors linked the presence of the G allele with an increased inflammatory response in patients with sepsis. That is, patients with the GG and GC genotypes had higher circulating levels of IL-6. The same authors associated the allele with a worse prognosis and increased mortality in sepsis patients [16].

While some authors associate the G allele with increased levels of IL-6 and, consequently, deterioration of the clinical picture and greater disease susceptibility [10,17], others place the C allele as a determinant of a worse prognosis or increased risk [18,19]. This is probably due to the genetic variability of the different populations analyzed since genetics varies from one population to another. The population analyzed in our study was unable to establish a statistical

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difference in the comparison between the C allele and the G allele, indicating that there is no relationship between the polymorphism and CKD in this population.

The patients analyzed by our study showed underlying diseases as cause of CKD. Hypertensive nephrosclerosis (81.48%) was the most frequent cause identified, followed by hypertensive diabetic nephropathy (18.52%). Studies show that IL-6 polymorphism has a significant influence on diabetes, which acts as one of the main etiological factors of CKD [9,10,20]and can be considered an important biomarker for treatment management. There are also studies linking the risk of cardiovascular disease with IL-6 polymorphism. In 2015, through a cohort study, Spoto et al concluded that the functional polymorphism of IL-6 (-174G/C) was associated with a history of cardiovascular disease and implies a high risk of cardiovascular disease in patients with CKD[21]. Hypertension, the underlying disease of CKD, may also be related to an increased risk in the presence of higher IL-6 levels. Some studies have tried to establish this relationship with the risk of hypertension. However, there are still no consistent conclusions [22,23].

5. CONCLUSION

Given the results obtained, it was possible to verify the presence of the IL-6 polymorphism (-174) in saliva. We had found no statistical differences between the analyzed groups, which made impossible to determine relation between IL-6 polymorphism and hemodialysis patients, both in the analysis of genotypes and alleles.

CONSENT AND ETHICAL APPROVAL

The participants were informed about the purpose and methodology of the study and signed a consent form that had been previously approved by theEthics Committee (45478615.1.0000.0081).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

Authors' contributions

This work was carried out in collaboration among all authors. Authors DP and YJK Conceptualization, Formal Analysis and Project Administration authors LDR, LATRB, LP. Investigation authors. Methodology authors LDR, LATRB, LP. Writing – Original Draft. authors LDR, DP, YJK, WRS: Writing– Review and Editing. All authors read and approved the final manuscript.

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