Lack of effect of the CD14 promoter gene C-159T polymorphism on nutritional status parameters in hemodialysis patients

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Authors' Contribution:
A Study Design
B Data Collection
C Statistical Analysis
D Data Interpretation
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Summary

Background: CD14 is a membrane glycoprotein that acts as a co-receptor for the detection of bacterial lipopolysaccharide (LPS). Mutual interaction between CD14 and LPS plays an important role in the innate immune system. Increased serum soluble CD14 levels have been described in hemodialysis (HD) patients, and linked to increased mortality risk, inflammation and protein-energy wasting. The expression of CD14 may be influenced by CD14 promoter gene C-159T polymorphism.

This study aimed to clarify the possible association between CD14 promoter gene C-159T polymorphism and nutritional status in hemodialysis patients.

Material/Methods: The study population consisted of 185 (104 males; 81 females) long-term HD patients treated in 5 dialysis centers. The control group consisted of 112 apparently healthy volunteers (32 males and 80 females). Nutritional status was assessed using a modified SGA scale, and anthropometric methods (BMI, WHR, waist, hip and mid-arm circumferences, biceps, triceps, subocular and subscapular skinfolds). Biochemical parameters evaluated included: CRP, albumin, creatinine, urea, cholesterol, triglycerides and TIBC. CD14 promoter gene C-159T polymorphism was determined by restriction fragment length polymorphism, after digestion of the PCR product with Hae III restriction endonuclease.

Results: Genotype and allele frequencies were similar to controls and compliant with Hardy-Weinberg equilibrium. No between-group differences were detected in measured variables with the exception of lower triglyceride levels in carriers of C allele in comparison to TT genotype.

Conclusions: CD14 promoter gene C-159T polymorphism does not seem to be associated with nutritional status parameters in HD patients. It does seem, however, to influence triglyceride blood levels.

key words: hemodialysis • CD14 promoter gene C-159T polymorphism • nutritional status • inflammation

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BACKGROUND

In recent years, it has been established that in patients maintained on chronic hemodialysis (HD) there is a strong association between markers of inflammation, nutritional status, cardiovascular disease and overall morbidity and mortality [1]. It was found that 30–60% of HD patients have evidence of activation of inflammation [2–4]. The stimuli for such a response have not been clearly defined, however, several have been proposed, such as reduced renal clearance of cytokines, accumulation of AGE's, occult inflammatory processes or infections, bioincompatibility of dialysis membranes and exposure to endotoxins, bacterial DNA fragments, and unspecified pro-inflammatory substances present in dialysis fluid. Nonetheless, activation of inflammation, even if low grade, is linked to malnutrition.

An important role in the innate immune system is played by a CD14 pattern-recognition receptor. CD14 is a receptor for endotoxin, and binds components of Gram-positive and Gram-negative bacteria. CD14-positive monocytes react to stimulation by increasing synthesis and release of cytokines [5]. CD14 protein is present both in soluble (sCD14) and membrane-bound forms.

Increased sCD14 levels have been described in HD patients [6–8] and linked to increased mortality risk, inflammation and protein-energy wasting [7,8]. In the promoter region of the CD14 gene polymorphism (dbSNP: rs2569190) was identified at position -159 designated according to the transcription start site that corresponds to -260 from the AUG start codon, which is associated with the intensity of CD14 gene product expression [9].

The aim of the present study was to investigate the distribution of CD14 promoter gene C-159T polymorphism in HD patients, and to determine whether this polymorphism in the CD14 gene is associated with indices of nutritional status.

MATERIAL AND METHODS

Subjects

The study population consisted of 185 Caucasian patients (81 females and 104 males) undergoing HD in 3 centers in Warsaw, 1 in Bydgoszcz and 1 in Bialystok, Poland. Patients had been maintained on chronic hemodialysis for at least 3 months before entering the study. Causes of CRF were as follows: uncertain (n=36), chronic glomerulonephritis histologically not examined (n=54), primary glomerulonephritis (9), chronic pyelonephritis/interstitial nephritis (n=31), various cystic kidney diseases (n=25), renal vascular diseases and ischemic renal diseases (n=16), amyloidosis (n=6) and other (n=8).

HD was performed 3 times a week using bicarbonate dialysate. Most of the patients were on antihypertensive drugs and phosphate binders. Dietary recommendations during the study were as follows: protein 0.8–1.0 g/kg of ideal body mass (with at least half of the protein being of high biologic value), 30% of calories derived from fat, and 62% of calories from carbohydrates.

The control group consisted of 94 apparently healthy volunteers (34 males, 60 females; aged 45.7±17.2 y).

Study protocol

Patients were eligible if they were between the ages of 18 and 90 years, had been receiving HD treatment for at least 3 months before entering the study, and agreed to participate in the study. Exclusion criteria included: recent acute inflammatory disease, history of allergy, diabetes mellitus, lupus erythematosus, Wegener's granulomatosis, colitis ulcerosa, Crohn's disease, history of juvenile rheumatoid arthritis, history of neoplastic disease, and treatment with steroids and immunosuppressive drugs.

Subjects were weighed in light clothing to the nearest 0.5 kg and their height recorded to the nearest 0.5 cm. Skinfold thickness (SFT) was measured using Harpenden skinfold calipers at the triceps, biceps, and subscapular sites, using defined anatomical landmarks. The mean of 3 measurements at each site was calculated. The waist and hip circumference were measured to the nearest 0.5 cm at the umbilicus and greater trochanter.

Nutritional status was assessed using the modified SGA scale (MIS) proposed by Kalantar-Zadeh and colleagues [10]. Each MIS component has 4 levels of severity from 0 (normal) to 3 (very severe). The sum of all 10 MIS components ranged from 0 to 30, denoting increasing degree of severity. Nutritional status was also evaluated by anthropometric methods, including triceps skinfold thickness, mid-arm circumference (MAC) and mid-arm muscle circumference (MAMC). MAMC was calculated as follows:

$$MAMC = MAC - (3.1415 \times TSF)$$

Skinfold measurement was performed by trained investigators (AS, TP, AP).

Laboratory analyses

Blood samples for analysis of CRP, albumin, creatinine, urea, cholesterol and triglycerides were taken before scheduled hemodialysis, as a part of routine protocol. Serum was separated after centrifugation and stored at −70°C until analyzed. Other laboratory and clinical data were extracted from medical records. Data corresponding closest to the date of anthropometric measurements were used. We allowed for a time span of 14 days before and after this encounter. All the routine laboratory parameters were measured routinely in local clinical laboratories.

Genomic DNA was isolated from blood, using a commercially available kit (NucleoSpin Blood, Macherey-Nagel, Germany). Genotyping was performed after amplification of the target sequence using specific primers (F 5'-GTGCCAACAGATGAGGTTCA -3'; R 5'-CGCAGCGGAAATCTTCATC-3'). The PCR products were digested with the restriction enzyme Hae III (Roche). Digestion reaction products were electrophoresed in polyacrylamide gel, silver stained and scanned.

The Ethics Committee of Central Clinical Hospital of the Ministry of Home Affairs approved the study protocol, and informed consent was obtained from all the patients and control subjects.

Table 1. Baseline characteristics of the chronic HD patients.

Age (years)	61.2±14.3
Male gender (%)	56.2
Dialysis vintage (years)	4.6±4.9
BMI (kg/m2)	24.1±4.64
Kt/V	1.34±0.27
Serum albumin (g/l)	4.0±0.60
Cholesterol (mg/ml)	172.4±44.6

Statistical analysis

Results are expressed as means ± standard deviations for normally distributed variables, or as median (interquartile range). Univariate statistical analysis was performed using Chi-square test, Fisher's exact test or Student's t-test as appropriate. Associations between variables were analyzed using Pearson's correlation coefficient or Spearman rank correlation, as appropriate. Bonferroni adjustments were made for multiple comparisons. Genotype distributions between the study groups were compared by 2X2 and 2X3 contingency table and chi-square analysis. Hardy-Weinberg Equilibrium test was performed using package STB-48:

sg110 in STATA [11]. STATA software, version 9.2 (Stata Corporation, College Station, TX, USA) was used for statistical computations.

RESULTS

The demographic and laboratory characteristics of HD patients are provided in Table 1.

The genotypic distribution and allele frequencies

The frequencies of the C and the T allele in HD subjects were 61.3% and 38.7%, respectively. Sixty-seven (36.0%) were homozygous for the C allele (CC), 89 were heterozygous (CT, 48.1%), 26 patients (14.1%) had the genotype TT, and in 3 cases (1.6%) it was not possible to determine genotype due to technical reasons. In the control group, frequencies of the C and the T allele were 55.9% and 44.2%, respectively. Twenty-eight patients (29.8%) had CC, 49 (52.1%) had CT, and 17 (18.1%) had TT genotype. Neither allele (P=0.225) nor genotype (P=0.460) frequencies differed between HD patients and controls. Allele and genotype frequencies did not show a significant departure from the Hardy–Weinberg Equilibrium in HD patients (P=0.630) or controls (P=0.580).

There were no differences in investigated biochemical (with the exception of triglycerides), clinical and anthropometrical parameters, between groups defined by the presence of

Table 2. Relationship between CD14 C allele presence and investigated parameters in HD patients. Results are given as Mean ±SD or median (interquartile range).

Genotype	CT/CC	TT	P
Kt/V	1.35±0.26	1.32±0.27	0.563
MIS (0-30)	7.1±4.2	7.3±4.7	0.804
BMI (kg/m2)	23.9±4.6	25.1±4.9	0.229
Waist circumference (cm)	89.7±13.05	93.1±10.1	0.211
Hip circumference (cm)	91.9±5.4	93.4±7.9	0.730
WHR	0.937±0.081	0.967±0.069	0.072
MAC (cm)	26.3±3.7	26.6±3.7	0.709
MAMC (cm)	22.1±3.2	21.8±2.8	0.569
Biceps skinfold (mm)	9.8±4.8	10.7±8.1	0.399
Triceps skinfold (mm)	13.2±6.1	15.3±8.2	0.119
Subocular skinfold (mm)	3.9±2.1	3.9±2.3	0.857
Subscapular skinfold (mm)	14.7±6.8	15.2±6.5	0.697
CRP (ng/ml)	6.0 (2.5–11.3)	6.0 (2.8–6.6)	0.967
Creatinine pre HD (mg/dl)	9.2±2.2	9.0±2.5	0.612
Serum cholesterol (mg/dl)	171.1±44.3	181.6±47.4	0.278
Triglicerides (mg/dl)	160.0±114.4	237.9±137.7	0.007*
Serum protein (g/l)	7.2±0.8	7.2±0.7	0.973
Serum albumin (g/l)	4.0±0.6	3.9±0.7	0.573
TIBC (mg/dl)	252.3±63.4	243.5±40.8	0.504

non-C allele status (TT) or C allele presence (CT, CC) in the CD14 promoter gene 159 site (Table 2). Interestingly, carriers of the C allele had lower triglyceride levels in comparison to TT genotype (P=0.007).

Due to technical reasons, IL-6 level determinations were possible only in 115 patients, and therefore are not given in Table I. Its concentration was 8.5 pg/ml (5.4–14.3; n=98) 8.2 pg/ml (5.2–11.2; n=17) in C allele (CT/CC) and non-C allele (TT) allele carriers, respectively (NS).

DISCUSSION

We found no association between CD14 C-159T polymorphism and indices of nutrition in patients on maintenance hemodialysis therapy. Genotype and allele frequencies in our study were not different from the control group, and were similar to previously described results from central Europe [12]. Additionally, allele and genotype frequencies did not show a significant departure from the Hardy–Weinberg Equilibrium.

In recent papers, groups from the USA and Sweden have show in 2 separate cohorts that sCD14 levels correlate positively with levels of several inflammatory markers, and independently predict mortality in HD patients [7,8]. There is a growing body of evidence that inflammatory response in chronic HD patients is at least in part induced by cytokines. The simultaneous combination of malnutrition and inflammation has been referred to as 'malnutrition-inflammation complex syndrome' (MICS) [13] or "malnutrition-inflammation-atherosclerosis" syndrome [14]. MICS appears to play a central role in poor clinical outcome, including the high rate of mortality and hospitalization and diminished quality of life seen in dialysis patients. MICS is also believed to be the underlying condition of the phenomenon known as "reverse epidemiology" of cardiovascular risks in these patients, where a low, and not a high, BMI or serum cholesterol is associated with poor dialysis outcome

Several putative factors were proposed as triggers of this reaction. From previous studies it appears, however, that endotoxin is one of the most active. Lipopolysaccharide (LPS), after interacting with LPS-binding protein, binds to CD14, TLR4 and MD4-2, making lipopolysaccharide-recognition complex [2,3]. This complex activates nuclear factor-κB, which moves into the nucleus and induces the transcriptional activation of inflammatory and immune-response genes, including tumor necrosis factor-α (TNF-α) [5]. In addition, caspase-1 is activated, resulting in the activation of the pro-inflammatory cytokine IL-1β [15]. CD14 is upregulated by bacterial stimuli, IFN-γ and TNF-α, and is downregulated by Th2-type cytokines such as IL-4 [16]. Low concentrations of LPS induce inflammatory genes, including IL-10, IL-12 p35, TNF-α, and IRF-1 through CD14 and TLR4-dependent pathways in macrophages [17]. Pretreatment of healthy subjects with a CD14-specific monoclonal antibody prior to LPS attenuates LPS-induced fever, clinical symptoms, and leukocyte activation and degranulation, and inhibits release of TNF-alpha, IL-6 and IL-10, and delays the release of sTNFR(I) and IL-1ra [18]. Wang et al. reported that anti-CD14 antibody can alleviate the progression of acute necrotizing pancreatitis in mice [19]. Sharif et al. found that, in mice lacking either TLR4 or CD14 receptors,

the severity of acute experimental pancreatitis was ameliorated [20]. CD14-deficient mice are resistant to shock induced by either gram-negative bacteria or LPS [21].

A CD14 promoter gene C-159T polymorphism influences plasma sCD14 level [9]. TT homozygotes appear to have significantly higher sCD14 serum levels, and higher CD14 density on the surface of monocytes than do carriers of both the CC and CT genotypes [9,22], although some authors dispute this [23]. The CD14-260 polymorphism is also associated with IL-1 beta levels, and higher values were found in C homozygotes. No association was found between the CD14-260 genotypes or the TNFα-308 – CD14-260 genotypes and the leukocyte TNF-α and IL-1β synthesis capacity upon endotoxin stimulation [24].

In clinical reports, CD14 promoter gene C-159T polymorphism was reported to be associated with decreased kidney function [25], progression of IgA nephropathy [26], carotid artery disease in HD patients [27], coronary heart disease [28], septic shock susceptibility and mortality [29], and may affect susceptibility to allergic asthma [12]. Although Rahman et al. found that soluble CD14 receptor expression was associated with severity of acute pancreatitis, C-260T CD14 genotype was not [30].

We have found significant association of CD14 C-159T polymorphism with serum triglyceride levels, but this must be interpreted with caution. Carriers of C allele (low-expressing) had lower triglyceride levels. Similar observations have been made in Asian populations [31,32]. This finding was not confirmed, however, by a group from Europe in a population closely related to ours ethnically [33]. It should be mentioned, however, that age of our subjects was more similar (mean 61.2) to the report by Shin et al. [31] (mean 64.8 years) than to Hubacek's [33] paper (mean 48.9 years).

There are several limitations of our study that merit consideration. Firstly, and most important, is the small sample size of the study, which therefore may be regarded rather as hypothesis-generating. Secondly, our patients were relatively well nourished, and this might have obscured a weak relationship with genotype CD14 C-159T polymorphism. While our study focused on the impact of only 1 polymorphism, a combination of different polymorphisms, as well as environmental factors, very likely might have influenced the investigated parameters. Therefore, more studies in larger populations are required.

CONCLUSIONS

In conclusion, the results of this study indicate that CD14 promoter gene C-159T polymorphism does not seem to be associated with nutritional status parameters in HD patients. It may, however, influence triglyceride blood levels. The possible influence of other genetic polymorphisms on nutritional status and inflammatory response in chronic kidney disease patients should be investigated.

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