Interleukin-10 (IL-10) 1082 promoter polymorphisms and plasma IL-10 levels in patients with bacterial sepsis

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Background. Interleukin-10 (IL-10) is a multifunctional cytokine which has been seen to play a relevant role in the pathogenesis of sepsis. We examined the association between a single nucleotide polymorphism (SNP) in IL-10 -1082G/A in patients with sepsis in Cali city.

Methods. A total of 100 patients with sepsis and 50 control subjects were enrolled in this study. Blood samples were collected from all patients in EDTA containing tubes. IL-10-1082G/A gene promoter polymorphism was analyzed by Sequence Specific Polymerase Chain Reaction (SS-PCR), while levels of serum IL-10 were measured by Enzyme Linked Immunoassay Assay (ELISA) in patients with sepsis and healthy controls.

Results. AA homozygous genotype was found more frequently in patients (32%), compared with controls (18%). AA homozygous patients showed an increased risk of developing infection by Gram-negative bacteria (OR = 2,875; 95% CI = 1.162-7.113; p = 0.020), and significantly high plasma levels of IL-10 (OR = 4.800, 95% CI 1.652–13.944; p = 0.002). AA homozygous patients high plasma IL-10 levels have greater risk of developing sepsis (63.6%; OR = 4,894; 95% CI: 1,337–17,909; p = 0.002). In this group, Afro-Colombian individuals were overrepresented among the sepsis patients with high plasma IL-10 levels (OR = 1.661; 95% CI: 1.408–1.959; p = 0.036).

Conclusion. Our study concluded that AA genotype of IL-10 -1082G/A polymorphism is a risk factor for high IL-10 production and development of sepsis by Gram negative bacteria, especially in Afro-Colombian patients.

Key words: Interleukin-10, single nucleotide polymorphism, SNPs, genotype, sepsis, septic shock.

INTRODUCTION

Interleukin-10 (IL-10) is a cytokine whose main function is to limit the inflammatory response. Infections are one of the factors that influence its production [1], and it has been seen to play a relevant role in the pathogenesis of sepsis [2]. In this sense, excessive production of Interleukin-10 has been related as an inducer of immunosuppression in bacterial sepsis and increased mortality [3].

On the other hand, it has been established that the risk of suffering septic shock is related to the increase in plasma concentrations of IL-10 [4, 5].

The production of this cytokine varies between individuals and is influenced by genetic characteristics, including single nucleotide polymorphisms (SNPs) within the IL-10 gene. Accumulating researches reported three SNPs presents in the promotor region of IL-10 (–1082, –819, and –592) that have been related to changes in IL-10 levels and in the susceptibility of sepsis [4, 6].

The SNP A/G-1082 is related with the outcome of sepsis [4, 7]. However, Shu *et al.* reported that may be associated with susceptibility to severe sepsis, but not to the evolution of severe sepsis [8].

The –1082A allele is related to susceptibility to the development of sepsis; the AA genotype being related to an increase in the production of IL-10 and, therefore, with immunosuppression induced by infections. The GG genotype has been associated with lower mortality in patients who develop sepsis [7]. Contradictory results have been found in other investigations, the –1082A allele is significantly associated with lower production of IL-10 induced due to the effect of lipopolysaccharide (LPS) of Gram negative bacteria and the –1082G allele is related to a higher IL-10 production and higher mortality in severe sepsis [9, 10].

Studies in this polymorphism are necessary to evaluate the precision of these data in different populations, since early diagnosis of the disease

and correct compliance with clinical support play a critical role in mortality. The objective of this study was to evaluate the -1082A/G polymorphism of the promoter region of the IL-10 gene in septic patients from a Intensive Care Units of Colombia.

MATERIALS AND METHODS

Patients

A total of 100 (44 females, and 56 males) samples derived from patients with sepsis from a Intensive Care Units of Colombia between March to November 2017.

The inclusion criteria were: patients who are in the Intensive Care Unit with a diagnosis of bacterial sepsis, over 18 years of age and who signed the informed consent. Sepsis diagnosis was defined by standard criteria [11].

The patients included in the studies represented the following ethnic groups: Afro-Colombian population (for individuals of mixed Amerindian and black ancestry), Amerindian population (or Indigenous ethnic groups), and non-ethnic population (consisting of whites, mestizos and castizos).

Patients were excluded if they met with the following: infected with the human immunodeficiency virus (HIV), pregnancy, therapeutic use of cytokines, heparin or thrombolytics, antibiotics treatment prior to admission, and patients who did not survive more than 24 hours.

Fifty from individuals who were identified as at-risk patients who did not meet criteria for sepsis during the ICU stay and had no prior history of sepsis were enrolled as controls. This study was conducted in accordance with the Helsinki Declaration and approved by the hospital Medical Ethics Committee (Act No. 05-013). All patients (or their legal representatives) were sufficiently informed about goals and procedures. Samples and data were obtained after written informed consent was signed.

Determination of IL-10 in plasma

Venous blood samples for the determination of plasma levels of IL-10 were obtained at admission and at 48 hours after admission in the ICU.

Blood was collected by venipuncture into 15-ml sterile vacutainers either containing EDTA (10 mmol/L) as anticoagulant. The tubes were

centrifuged immediately for ten minutes at 3,000 rpm, and the plasma was stored in aliquots at -70°C. The remaining blood was used to DNA extraction.

The analysis of IL-10 was performed using an enzyme immunoassay with Human ELISA kit (Elabscience Biotechnology Inc. USA) following manufactures' instructions. The detection range was 7.81-500 pg/mL, with intra and interassay coefficients of variation below 5%. Readings were carried out in an automated optical Spectramax reader at 450 nm. The sensitivity of the assay was <2 pg/mL.

DNA extraction

Total genomic DNA was extracted from 2-ml blood of each sample using DNeasy Blood & Tissue Kit (Cat No. 69506 QIAGEN) following the protocol established by the commercial Company and then stored at -70°C. To assess the DNA quality after DNA extraction, the β -globin gene amplification was used in all extracted samples. The concentration of DNA in the samples was quantified by using a FOTO/Analyst® Investigator/FX Systems (FOTODYNE Incorporated).

Genotyping of the gene encoding IL-10 (-1082G/A) was carried out using the sequence Specific Polymerase Chain Reaction assay (SS-PCR), which consists of using the primers with the polymorphism -1082G for allele G and -1082A for allele A with the following amplification conditions: one cycle at 95°C for 5 min followed by 30 cycles at 95°C for 1 min, 60°C for 1 min, 72°C for 1 min and finally for 10 min extension at 72°C. The generic IL-10 primers 5'-TCT CGG TTT CTT CTC CAT CG-3', allele primer G 5'-ATA GGT TTT GAG GGG CAT GG-3' and allele primer A 5'-ATA GGT TTT GAGGGG CAT GA-3' - Amplification was carried out using the Bio-Rad thermocycler.

The electrophoresis of the PCR products was carried out on 2% agarose gel and visualized by Syber Green (Fisher Scientific Inc. Madrid). The presence or absence of fragments was visualized by means of a UV transilluminator. A product size of 161 bp was considered a positive amplification. Each of the primer mixes contained a pair of control primers that amplified a portion of the human β -globin gene.

Statistical analysis

Frequencies of the genotypes, alleles, and phenotypes were analyzed by Chi-square test.

Continuous variables are expressed as mean $\pm$ SD and categorical variables as frequencies and percentages (%). For non-normally distributed variables median and 25th–75th percentiles were used.

Regression analysis was employed to analyze the association between the studied SNPs and the risk of sepsis. The clinical impact for the predictor variables was calculated by odds ratio (OR) and 95% confidence intervals (CI). Homozygotes of the most frequent genotypes were used as a reference group. We plotted survival curves of patient groups with AA, AG and GG genotypes using the Kaplan-Meier method. We tested the Hardy-Weinberg equilibrium in the genotypes using the Chi-square test. P-values of less than 0.05 were considered statistically significant. SPSS® statistical package, version 26.0 (SPSS Inc., Chicago, IL, USA) for Windows® were used for statistical analyses.

RESULTS

The mean age of patients with sepsis was 53 years (SD $\pm$ 18,880), SOFA index of 89 (SD $\pm$ 0.945) and 7 days (SD $\pm$ 4.874) of hospital stay. The mean age of the control group (89 females and 21 males) was 39.4 years (SD $\pm$ 11.95).

In this study, 68% of the patients presented sepsis and 32% septic shock. The most frequently isolated etiologic agent was Gram negative bacteria (55%; $p < 0.05$). The ICU mortality rate was 13%. Septic shock was more frequent among non-survivors than survivors (61.5% vs. 27.6%, $p < 0.05$). We do not find statistically significant differences between patient groups in sex, age, ethnicity coexisting diseases (hypertension, diabetes mellitus, coronary artery disease, chronic obstructive pulmonary disease [COPD], malignancy), primary site of infection, evolving organ dysfunction, and outcomes of sepsis.

Genotypes and alleles in IL-10 promoter polymorphism (–1082A/G). The frequencies of genotypes and alleles of IL-10 polymorphisms according to the characteristics of the patients are presented in Table 1. Analysis indicated a predominance of the A allele with a frequency of 85%, compared to the G allele (66%).

The AG heterozygous genotype was found more frequently in patients with sepsis (35%) and septic shock (16%), followed by the AA homozygous genotype with 22% and 11%, respectively.

Genotype frequencies in both patients and controls were in accordance with the Hardy-Weinberg equilibrium ($p > 0.05$). As shown in Table 2, frequency of AG (56%) and GG (26%) genotypes were found more frequently in healthy individuals. While that AA homozygous genotype was found more frequently in patients (32%), compared with controls (18%). Although GG genotype occurred only in control group with a frequency of 26%, no significant differences were found in the frequencies of genotypes between septic patients and the control group at this position ($p = 0.26$).

None of the genotypes was representative for patients with an ICU hospital stay of more than 7 days.

IL-10 serum level

The median plasma levels of IL-10 in patients with sepsis at admission and at 48 hours after admission in the ICU were 43.00 [26.5–72.5] pg/mL and 45 [30–69] pg/mL, respectively. In healthy individuals, median plasma levels of IL-10 was 8.00 [7.50–9.63] pg/mL values above these were considered high. Patients with high plasma levels of IL-10 represented 63%.

We found that 44.4% patients with genotype AG, 42.9% with AA genotypes and 11.1% GG genotypes showed high plasma IL-10 levels (Table 2). However, AA homozygous patients showed higher plasma IL-10 levels than patients with genotypes GG ($p = 0.002$) or AG ($p = 0.002$), and patients with genotypes AG showed higher plasma IL-10 levels than patients with genotypes GG ($p = 0.040$) (Figure 1).

On regression analysis, AA homozygous patients showed had a 2.875-fold increased risk of developing infection by Gram-negative bacteria (OR = 2.875; 95% CI = 1.162–7.113; $p = 0.020$), and showed significantly high plasma levels of IL-10 (OR = 4.800; 95% CI 1.652–13.944; $p = 0.002$).

In addition, we found that AA homozygous patients high plasma IL-10 levels have greater risk of developing sepsis (63.6%; OR = 4.894; 95% CI: 1,337–17.909; $p = 0.002$) (Tables 2 and 3). On the other hand, Afro-Colombian patients with AA genotype were overrepresented among the sepsis patients with high plasma IL-10 levels at admission in the ICU (OR = 1.661; 95% CI: 1.408–1.959; $p = 0.036$), and at 48 hours after admission (OR = 1.525; 95% CI: 0.1316–1.766; $p = 0.060$).

Table 1

Characteristics of septic patients according to genotype of IL-10 promoter polymorphism (−1082 A/G)

Characteristic	Total (n = 100)	AG/GG n = 68 n (%)	AA n = 32 n (%)	OR (95%CI)	P-value
Gender					
Female	25 (40.3)	31 (44.1)	11 (34.4)	0.625 (0.261–1.495)	0.427
Male	37 (59.7)	37 (54.4)	21 (65.6)		
Ethnicity					
non-ethnic	41	29 (42.6)	12 (37.5)	0.807(0.34–1.19)	0.625
Afro-Colombian	40	30 (44.1)	10 (31.3)		
Amerindian	19	9 (13.2)	10 (31.3)		
Primary site of infection					
Lung	32	25 (37.8)	7 (21.9)	0.482 (0.182–1.273)	0.136
Urinary tract	19	15 (22.1)	4 (12.5)	0.505 (0.153–1.666)	0.256
Abdomen	25	15 (22.1)	10 (21.3)	1.606(0.626–4.120)	0.322
Soft tissue infections	5	3 (4.4)	2 (6.3)	1.444 (0.229–9.102)	0.694
Unknown foci	6	3 (4.4)	3 (9.4)	2.241 (0.427–11.777)	0.330
Coexisting diseases					
Hypertension	42	26 (38.2)	16 (50)	1.615 (0.692–3.773)	0.266
Diabetes mellitus	29	16 (23.5)	13 (40.6)	2.224 (0.903–5.474)	0.079
Coronary artery disease	15	12 (17.6)	3 (9.4)	0.483 (0.126–1.848)	0.280
Malignancy	3	3 (4.4)	0	1.046 (0.994–1.101)	0.228
COPD	21	14 (20.6)	7 (21.9)	1.080 (0.388–3.006)	0.883
Liver disease	12	9 (13.2)	3 (9.4)	0.678 (0.171–2.696)	0.579
Neurological disease	8	3 (4.4)	5 (15.6)	4.012 (0.895–17.983)	0.054
Evolving organ dysfunction					
Acute renal failure	15	8 (11.8)	7 (21.9)	2.100 (0.688–6.414)	0.187
Respiratory failure	11	9 (13.2)	2 (6.3)	0.437 (0.089–2.152)	0.298
Haematological process	11	7 (10.3)	4 (12.5)	3.095 (0.650–14.746)	0.139
Microbes					
Gram-positive bacteria	9	9 (13.2)	0	1,153 (1.00 1.265)	0,031
Gram-negative bacteria	55	32 (47.1)	23 (71.2)	2.875 (1.162–7.113)	0.02
Unidentified bacteria	49	29 (42.6)	12 (37.5)	0.807 (0.341–1.911)	0.625
Outcomes					
Sepsis	68	46 (67.6)	22 (32.4)	0.853 (0.349–2.083)	0.727
Shock septic	32	19 (59.4)	11 (34.4)		
Non-survivors	13	8 (11.2)	5 (40.6)	1.389 (0.416–4.640)	0.592
Survivors	87	60 (88.2)	27 (84.4)	1.239 (0.582–2.638)	0.592

COPD = chronic obstructive pulmonary disease; *significant difference, $P < 0.05$; ^w Only in survivors.

Table 2

Distribution of IL-10 promoter polymorphism (−1082 A/G) in patients with sepsis and septic shock, with IL-10 – high, non-survivors, with length of ICU stay >7 days and healthy controls

Variables	Sepsis/septic shock n = 68/32 (%)	Non-survivors n (%)	IL-10 – High admission n (%)	IL-10 – High at 48 hrs. n (%)	Stay>7 days n (%)	Healthy controls (n = 50) n (%)
Allele						
G	46/19 (65)	7 (53.8)	35 (55.6) ¹	40 (58.8)	32 (50.8)	42 (84)
A	57/29 (86)	10 (76.9)	56 (88.9)	61 (89.7)	38 (60.3)	36 (72)
Genotype						
AA	22/11 (33)	5 (38.1)	27 (42.9) ²	27 (39.7) ³	22 (34.9)	9 (18)
AG	35/16 (51)	4 (30.8)	28 (44.4)	33 (48.5)	29 (46)	28 (56)
GG	11/3 (14)	3 (23.1)	7 (11.1)	7 (10.3)	11 (17.5)	13 (26)

¹OR = 0.64, 95% CI 0.49–0.85; $p = 0.004$ ²OR = 4.800, 95% CI 1.652–13.944; $p = 0.002$ ³OR = 1.40, 95% CI 1.10–1.79; $p = 0.016$

Figure 1. Plasma interleukin-10 levels according to the genotype of IL-10 promoter polymorphism (–1082 A/G).

Table 3

Association analysis of genotypes in IL-10 promoter polymorphism (–1082A/G) with high plasma levels of IL-10 and the development of sepsis and ethnicity

Patients	Genotype			
	AA n = 22	GG/AG n = 78	OR	p
IL-10 – High admission				
Sepsis	14 (63.6)	44 (56.4)	4.894 (1.337–17.909)	0.010
Sepsis – Non-ethnic	8 (36.4)	55 (70.5)	2.545 (0.511–12.689)	0.241
Sepsis – Afro-Colombian	7 (31.8)	56 (71.8)	1.661 (1.408–1.959)	0.036
Sepsis – Amerindian	7 (31.8)	56 (71.8)	4.500 (0.531–38.122)	0.135
IL-10- High at 48 hrs.				
Sepsis	18 (81.8)	50 (64.1)	2.520 (0.776–8.185)	0.116
Sepsis – Non-ethnic	10 (45.5)	58 (74.4)	0.931 (0.290–2.990)	0.904
Sepsis – Afro-Colombian	7 (31.8)	61 (78.2)	1.525 (0.1316–1.766)	0.060
Sepsis – Amerindian	7 (31.8)	61 (78.2)	3.557 (0.419–30.218)	0.218

Patients with septic shock did not show a significant association with genotypes, ethnic group, or plasma levels of IL-10.

On the other hand, individuals with G allele showed lower plasma IL-10 levels than those with A allele (OR = 0.64; 95% CI: 0.49–0.85; $p = 0.004$).

Kaplan-Meier analysis did not show differences in mortality between different genotypes ($p = 0.281$) (Figure 2).

DISCUSSION

Sepsis is a complex immune response due to invasive infection, in which a prolonged and profound state of immunosuppression occurs. Hence, increased anti-inflammatory cytokines (eg IL-10) is one of the main features during immunosuppression in sepsis [12].

Previous studies showed that polymorphism in the regulatory region of the IL-10 gene may influence

its expression, and susceptibility to developing sepsis [5, 6, 9, 13, 14].

In the present study, we found that patients with AA homozygous in IL-10 promoter polymorphism (–1082A/G) are associated with high plasma levels of IL-10. Kang *et al.* reported that the poly

ADP-ribose polymerase 1 (PARP-1) acts in the regulation of IL-10 production in an 1082G>A alleles-dependent, which determines individual susceptibility to sepsis [15]. Schaaf *et al.* demonstrated in their studies, over-expression of IL10 induce immunosuppression in bacterial sepsis [10].

Figure 2. Kaplan-Meier curves showing the cumulative proportion of surviving patients according to the presence of AA vs. other genotypes (AG or GG) of IL-10 promoter polymorphism (–1082 A/G).

We hypothesize that elevated levels of IL-10 detected in AA homozygous individuals could induce a state of functional immunoparalysis, triggering an uncontrollable infection, as suggested by Abe *et al.* [16].

Our results indicate that AA homozygous patients had a higher risk of being infected with Gram negative bacteria (OR = 2.875; 95% CI: 1.162–7.113; $p = 0.02$), and the most common source was lung, as has been observed in a number of other studies [17–19]. Steinhauser *et al.* demonstrated in their study with murine models that the septic response impairs lung innate immunity to Gram-negative bacilli, and this effect is mediated primarily by increase in IL-10 levels [20].

The results of this study indicate that the susceptibility of the patient to infection should be an important factor to be considered, especially in patients with the AA genotype in IL-10 promoter polymorphism (–1082A/G). While individuals with the G allele is associated with lower IL-10 production being a protective factor. Contrary, Stanilova *et al.* reported that carriage of the G allele is associated with higher IL-10 production and increased mortality in severe sepsis [7]. Several studies indicate that GG genotype is associated with increased IL-10 production [4, 7].

The discrepancy between study findings could be due to differences in factors such as ethnicity. The meta-analysis performed by Pan *et al.* reported that IL-10 polymorphisms are associated with susceptibility to sepsis but in Asian populations, because the results in Caucasian populations were inconclusive [13]. Our data show that AA homozygous patients with high plasma IL-10 levels have greater risk of developing sepsis (OR = 4.894; 95% CI: 1.337–17.909; $p = 0.002$), especially Afro-Colombian individuals (OR = 1.661; 95% CI: 1.408–1.959; $p = 0.036$).

As suggested by Pan *et al.* [13], it appears that IL-10 promoter polymorphism (–1082A/G) has a race-specific effect in the association between sepsis susceptibility in different ethnicities.

Limitations

Our sample size is relatively small and may influence the analysis of multivariate logistic model. The determination of other genetic polymorphisms in the IL-10 gene and in other cytokines (such as tumor necrosis factor, IL-1, IL-6) might be relevant for to establish the critical state of the patient [21].

On the other hand, no data was provided on the treatments and the response to treatment over time.

CONCLUSION

An interesting finding was the association between IL-10 promoter polymorphism (–1082A/G) and plasma levels of IL-10.

The present study showed that plasma level of IL-10 is significantly higher in sepsis patients with AA homozygous genotype, especially in Afro-Colombian individuals. This would indicate that these individuals are more susceptible to dysregulation of IL-10 and enhanced immunopathology infection; however, this polymorphism was not related to the survival of septic patients.

Introducere. IL-10 este o citokină multifuncțională cu rol cheie în patogeniza sepsisului. Am examinat asocierea dintre un polimorfism (SNP) al IL-10 –1082G/A la pacienții cu sepsis din Cali.

Metode. Au fost recrutați 100 de pacienți cu sepsis și 50 de martori sănătoși. Nivelurile IL-10 au fost măsurate prin ELISA, iar polimorfismul IL-10 prin tehnica SS-PCR.

Rezultate. Genotipul homozigot AA a fost găsit mai frecvent la pacienți, comparativ cu martori sănătoși (32% versus 18%). Prezența acestui genotip s-a asociat cu dezvoltarea infecțiilor cu bacterii Gram negative (OR = 2 875; 95% CI = 1 162–7 113; $p = 0.020$) și cu niveluri crescute ale IL-10 (OR = 4 800, 95% CI 1 652–13 944; $p = 0.002$). Pacienții homozigoți AA și cu nivelurile crescute IL-10 au avut un risc mai mare să dezvolte sepsis (63,6%; OR = 4 894; 95% CI: 1 337–17 909; $p = 0.002$). În acest grup, pacienții afro-columbieni au fost mai degrabă reprezentați printre pacienții cu sepsis și niveluri crescute ale IL-10 (OR = 1 661; 95% CI: 1 408–1 959; $p = 0.036$).

Concluzii. Genotipul AA IL-10 –1082 G/A este un factor de risc pentru prezența unor niveluri ridicate de IL-10 și pentru dezvoltarea sepsisului cu bacterii Gram-negative mai ales la pacienții afro-columbieni.

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Acknowledgments: The authors thank the Directorate General for Research (DGI) of Universidad Santiago de Cali [DGI-COCCEIN No.450-621717-005] for funding this study.

Conflict of interest disclosure: The authors have no conflict to interests to declare.

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Received 2nd July 2020