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Lack of Effects of the Genetic Polymorphisms of Interleukin-10 in Clinical Outcomes of COVID-19

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Abstract

Interleukin-10 (*IL-10*) gene polymorphisms have been associated with severity and outcomes in patients with respiratory and nonrespiratory viral infections. The aim of this study was to assess whether rs1800871 and rs1800872 polymorphisms of *IL-10* gene are associated with the clinical outcomes of COVID-19 in a Mexican population. Study subjects were 193 COVID-19 patients. The genotyping was carried out with real-time PCR and serum IL-10 levels were measured with enzyme-linked immunosorbent assay. Logistic regression analysis was used for analysis association with clinical outcomes. There was no evidence of an association between alleles, genotypes, or haplotypes frequencies between patient groups according to severity and outcomes. The rs1800871 and rs1800872 polymorphisms might not be genetic risk factors for severity and mortality for COVID-19 in Mexican mestizos patients from northwest Mexico.

Keywords: IL-10 gene, polymorphisms, severity, COVID-19, Mexican

Introduction

An equilibrium between proinflammatory and anti-inflammatory cytokines is required to achieve a stable and healthy immune response; however, in some infectious diseases an exacerbated synthesis of these can occur (7). This phenomenon, named ''storm cytokine,'' has been considered the main responsible immunopathological process of a more severe clinical course and cause of death in patients with severe acute respiratory syndrome coronavirus 2 (SARS-Cov-2)/COVID-19 (12). The first clinical and laboratory reports of COVID-19 showed an immunological disturbance, being the cytokines interleukin (IL)-2, IL-6, IL-7, IL-10, tumor necrosis factor (TNF)-α, granulocyte colony-stimulating factor (G-CSF), interferon gamma-induced protein 10 (IP-10), monocyte chemoattractant protein-1 (MCP-1), macrophage inflammatory protein 1A (MIP1A) associated with a worsening of the disease (4,6,15). In subsequent investigations, IL-10 was considered an excellent biomarker to predict unfavorable prognosis (8,15,16,29) and a risk factor for death (30). IL-10 is a cytokine secreted by most immune system cells in response to antigen; it regulates the inflammatory response inhibiting the synthesis of others cytokines, for example IL-2, IL-3, interferon-γ (IFN-γ), and granulocyte-macrophage colony-stimulating factor (GM-CSF) by T helper cells (21). Its involvement in several respiratory and nonrespiratory infections is very well documented (9,17,19).

Genetic variation in the *IL-10* gene is associated with risk of infection with influenza A/H3N2 (24), severity and fatality by influenza A(H1N1)pdm09 (7,19), severe rhinovirus bronchiolitis (10), predisposition to hepatitis C and tick-borne encephalitis viruses (2), and risk of HPV-associated cervical cancer development (17), in different populations.

In this study, we hypothesized that *IL-10* gene variation may be associated with COVID-19 severity considering IL-10 is a pivotal cytokine in antiviral action and inflammation regulation.

Materials and Methods

Subjects and definitions

Patients were recruited from Hospital General de Culiacan and Hospital Civil de Culiacan, between March 2020 and June 2020. We enrolled 193 hospitalized patients with laboratory-confirmed SARS-Cov-2 RNA detection, whose clinical data and whole blood samples were collected. The

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study was approved by the Research Ethical Committee of Hospital General de Culiacan ''Bernardo J Gastelum.'' A risk stratification system based on severity of disease for COVID-19 patients was adopted and with it, the patients were considered as critical and noncritical. The former includes moderate and severe clinical forms (9).

The inclusion criteria were the Mexican mestizo ethnic origin and adult age, and as exclusion criteria pediatric age patients, individuals without molecular test for SARS-Cov-2, family relationship, individuals with no clinical and laboratory data and those with IL-10 inhibitor treatment.

Cytokine quantification and genotyping

IL-10 levels were measured in serum samples, separated from the blood of hospitalized patients (range from 2 to 9 days, 7 days on average), using the Cytometric Bead Array-based assay Th1/Th2 Human Cytokine Kit II (Becton-Dickinson, San Jose, CA, USA), following the manufacturer's instructions.

Genotyping was performed by real-time PCR using TaqMan SNP Genotyping Assays (Applied Biosystems; Thermo Fisher Scientific) and TaqMan Universal PCR Master Mix for the -819 C/T and -592 C/A single nucleotide polymorphisms (SNPs) of *IL-10* gene (rs1800871 and rs1800872, respectively). Automated genotype calling was carried out using the TaqMan Genotyper Software v1.3.

Statistical analysis

By means of direct counting, allele and genotype frequencies were obtained. Hardy–Weinberg equilibrium was carried out with the DeFinetti online program (http://ihg2.helmholtz-muenchen.de/cgi-bin/hw/hwa1.pl). Association between categorical variables was evaluated using chi-square test. Odds ratio (OR) with 95% confidence interval (CI) were calculated by binary logistic regression analysis. The haplotype analysis was carried out using SHEsis software (http://analysis.bio-x.cn) (25). To compare quantitative data between groups, we used the Mann–Whitney test. Values of $p < 0.05$ were considered statistically significant.

Results

Characteristics of the study population

Characteristics of the COVID-19 patients are given in Table 1. A total of 193 COVID-19 patients with a mean age of 55.1 years were recruited in this study, being significantly higher in critical and deceased groups ($p < 0.05$). Moreover, BMI and the frequency of tachypnea were higher in critical patients, whereas nephropathy was more prevalent in the deceased group. On the contrary, cough and headache were more prevalent in noncritical and survivor groups.

As expected, low oxymetry was more frequent in critical and deceased groups. Furthermore, mechanic ventilation and organic failure, critical category defining parameters, were more prevalent in the deceased group.

Genotype analysis

Observed genotype distributions at rs1800872 and rs1800871 polymorphic loci were in agreement with Hardy–Weinberg equilibrium expectations, in all cases. The comparisons between noncritical and critical and between survivor and deceased patients were not statistically significant (Table 2), including additional assessments with dominant and recessive genetic models (data not shown), as well as analysis of the four different haplotypes found (Table 3). Of interest, in a genotype-qualitative trait analysis of clinical variables seen in Table 1, a logistic regression model revealed that compared with patients with -819 CC and CT genotypes of rs1800871 polymorphism, those with TT genotype had a higher prevalence of myalgia (OR = 5.8, 95% CI = 1.730–19.640, $p = 0.004$), as well as obesity (OR = 4.9, 95% CI = 1.641–15.027, $p = 0.005$), whereas the CA and AA genotypes of rs1800872 polymorphism were less prevalent in obese subjects (OR = 0.368, 95% CI = 0.191–0.708, $p = 0.003$ and OR = 0.275, 95% CI = 0.078–0.962, $p = 0.043$, respectively) (data not shown). The above associations were confirmed with a haplotype analysis. In this sense, the CT haplotype was associated with myalgia (OR = 1.852, 95% CI = 1.048–3.274, $p = 0.03$), and AC and CT haplotypes with obesity (OR = 0.469, 95% CI = 0.283–0.777, $p = 0.002$, and OR = 1.827, 95% CI = 1.146–2.913, $p = 0.010$, respectively).

Discussion

The possibility of human genetic factors participation in SARS-Cov-2/COVID-19 susceptibility is very obvious. Not all exposed individuals to the virus become infected, and of those infected not all develop symptoms, and only a small fraction of patients develop a serious illness. However, it is no clear to what extent individual/familial/population genetic factors may explain or contribute to this dangerous epidemic. Cumulative experience with other epidemic coronaviruses and pandemic influenza has taught us that this genetic contribution can be minimal and, sometimes, contradictory (1,7,13,18,24).

Fortunately, recent progress in genetic predisposition of this variable response is increasing. SNPs and loss-of-function variants in *ABO*, *ACE2*, *ApoE*, *HLA*, *IFITM3*, *SLC6A20*, *LZTFL1*, *CCR9*, *FYCO1*, *CXCR6*, *XCR1*, *TLR7*, *TMEM189-UBE2V1*, and *TMPRSS2* genes were associated to rate and predisposition to infection, susceptibility and severity to COVID-19, mortality, and comorbidities such arterial hypertension, obesity, diabetes, and others in COVID-19 patients (1).

There are reports that show association between SNPs of the *IL-10* gene with respiratory viral infectious diseases, a cytokine considered as a key molecule in COVID-19 pathogenesis. For this reason we proposed to investigate, for the first time, if rs1800871 (-819 C>T) and rs1800872 (-592 C>A) polymorphisms are associated with the clinical outcome in a group of Mexican patients with COVID-19. These polymorphisms, along with rs1800896 (-1082A>G) form a part of haplotypes group associated with levels of different IL-10 production (27).

Compared with noncritical and survivors patients, critical and deceased patients had higher serum levels of IL-10, being statistically significant in the noncritical versus critical comparison ($p = 0.04$). Our result agrees with several recent meta-analyses about the role of IL-10 in pro-inflammatory cytokine storm, which is higher in severe/critical COVID-19 patients than in mild cases and is associated with severity (11,28), but contrasts with Song's findings that discard a

Table 1. Demographic, Laboratory, and Clinical Characteristics of the COVID-19 Patients According to Disease Severity and Outcome

					Outcome		
	COVID-19 (n = *193*)	*Noncritical* (n = *102*)	*Critical* (n = *86*)	p	*Survivors* (n = *106*)	*Deceased* (n = *87*)	p
Age	55.1 ± 15.360	52.6 ± 14.61	60.51 ± 14.74	0.000	52.09 ± 14.53	60.56 ± 15.13	0.000
Gender (F%/M%)	78/115	40/62 (39.2/60.8%)	35/51 (40.7/59.3%)	0.836	43/63 (40.6/59.4%)	35/52 (40.2/59.8%)	0.962
IL-10 (pg/mL)	0 (0–2.64)	0 (0–0)	1.66 (0–3.28)	0.046	0.00 (0.00–1.35)	1.60 (0.00–3.47)	0.064
BMI	32.061 ± 6.226	30.724 ± 5.699	33.91 ± 6.78	0.021	31.2 ± 5.6	33.0 ± 6.8	0.160
Days hospitalized	10.617 ± 8.333	9.327 ± 4.922	11.296 ± 8.137	0.059	11.0 ± 8.8	10.2 ± 7.7	0.497
Dyspnea	138 (95.2%)	73 (93.6%)	62 (98.4%)	0.225	67 (91.8%)	71 (98.6%)	0.116
Cough	102 (78.5%)	60 (85.7%)	39 (68.4%)	0.019	56 (86.2%)	46 (70.8%)	0.033
Headache	72 (58.1%)	47 (67.1%)	24 (45.3%)	0.015	45 (69.2%)	27 (45.8%)	0.008
Fever	122 (86.5%)	68 (90.7%)	52 (82.5%)	0.158	63 (91.3%)	59 (81.9%)	0.104
Myalgia	50 (40.7%)	28 (41.8%)	20 (37.7%)	0.653	28 (43.8%)	22 (37.3%)	0.456
Odynophagia	21 (18.8%)	14 (22.6%)	6 (12.2%)	0.159	14 (23.7%)	7 (13.2%)	0.154
Rhinorrhea	12 (10.4%)	7 (11.5%)	5 (9.6%)	0.749	6 (10.5%)	6 (10.3%)	0.975
Diarrhea	18 (16.8%)	10 (17.5%)	8 (16.3%)	0.868	10 (20.0%)	8 (14.0%)	0.411
Chest pain	35 (29.4%)	23 (34.9%)	12 (23.1%)	0.165	22 (36.1%)	13 (22.4%)	0.102
Tachypnea	39 (27.1%)	14 (17.5%)	25 (40.3%)	0.003	19 (23.25%)	20 (32.3%)	0.224
Oximetry <94%	146 (78.5%)	66 (67.4%)	75 (90.4%)	0.000	71 (70.3%)	75 (88.2%)	0.003
Mechanic ventilation	72 (40.0%)	0 (0.0%)	71 (86.6%)	0.000	13 (12.9%)	59 (74.7%)	0.000
Organic failure	31 (21.7%)	0 (0.0%)	30 (46.2%)	0.000	6 (7.4%)	25 (40.3%)	0.000
Abnormalities on chest CT	84 (82.4%)	46 (79.3%)	35 (85.4%)	0.442	45 (76.3%)	39 (90.7%)	0.059
Arterial hypertension	101 (56.1%)	48 (50.5%)	51 (63.8%)	0.079	50 (50.5%)	51 (63.0%)	0.094
Diabetes mellitus	71 (39.7%)	36 (37.9%)	32 (40.5%)	0.725	41 (41.8%)	30 (37.0%)	0.514
Obesity	91 (48.7%)	47 (47.0%)	39 (47.6%)	0.940	53 (51.0%)	38 (45.8%)	0.481
Other comorbidities	18 (10.2%)	10 (10.8%)	8 (10.1%)	0.894	6 (6.3%)	12 (14.8%)	0.060
Nephropathy	14 (7.9%)	5 (5.3%)	8 (10.1%)	0.232	3 (3.1%)	11 (13.6%)	0.010

Values are expressed as mean ± standard deviation and *n* (%). IL-10 values are expressed as median with interquartile ranges. *p*-Value was obtained using χ^2 or Mann Whitney test.
BMI, body mass index; CT, computed tomography; IL-10, interleukin-10.

Table 2. Genotypes and Allele Frequencies of the Interleukin-10 Gene Polymorphisms in COVID-19 Patients According to Severity and Outcome

	Risk				*Outcome*			
Genotype	*Noncritical,* n *(%)*	*Critical,* n *(%)*	*OR (95% CI)*	p	*Survivors,* n *(%)*	*Deaths,* n *(%)*	*OR (95% CI)*	p
rs1800871								
CC	43 (42.2)	34 (39.5)	1.00 (Reference)		43 (40.6)	34 (39.1)	1.00 (Reference)	
CT	51 (50.0)	41 (47.7)	1.017 (0.553–1.870)	0.957	52 (49.1)	41 (47.1)	0.997 (0.543–1.832)	0.993
TT	8 (7.8)	11 (12.8)	1.739 (0.630–4.802)	0.286	11 (10.4)	12 (13.8)	1.380 (0.542–3.510)	0.499
Allele								
C	137 (67.2)	109 (63.4)	1.00 (Reference)		138 (65.1)	109 (62.6)	1.00 (Reference)	
T	67 (32.8)	63 (36.6)	1.182 (0.772–1.810)	0.442	74 (34.9)	65 (37.4)	1.112 (0.733–1.688)	0.618
rs1800872								
CC	49 (52.1)	38 (50.7)	1.00 (Reference)		51 (53.1)	41 (52.6)	1.00 (Reference)	
CA	40 (42.6)	29 (38.7)	0.935 (0.494–1.771)	0.836	39 (40.6)	30 (38.5)	0.957 (0.510–1.795)	0.891
AA	5 (5.3)	8 (10.7)	2.063 (0.625–6.816)	0.235	6 (6.3)	7 (9.0)	1.451 (0.453–4.654)	0.531
Allele								
C	138 (73.4)	105 (70)	1.00 (Reference)		141 (73.4)	112 (71.8)	1.00 (Reference)	
A	50 (26.6)	45 (30)	1.183 (0.735–1.904)	0.489	51 (26.6)	44 (28.2)	1.086 (0.676–1.744)	0.732

CI, confidence interval; OR, odds ratio.

role of IL-10 cytokine in the aggravation of inflammatory processes of COVID-19 (26). In addition, there is evidence that levels of IL-10 link with mortality exists (11,28), although it does not reach statistical difference ($p = 0.06$) among survivors versus deceased. The opposite results on the measurement of circulating cytokines can be the result of different issues such as time of sampling (''on admission,'' ''at early stage of disease,'' or ''on first day after admission before initiation of treatment''), type of sample (plasma or serum), technique of measure (bioassays, immunoassays, flow cytometry, and nanoparticle-modified aptamer), cutoff for each cytokine, and population/geographical region-based variability of cytokine expression (31).

On the contrary, the comparison of alleles, genotypes, and haplotypes between noncritical and critical, and between both survivors and deceased patients were not statistically different, and therefore do not support a role in severity, neither an association with death. In this respect, we speculate that the higher IL-10 levels seen in critical patients could represent a compensatory strategy of the immune system, maybe by inhibiting the proinflammatory response, for reducing damage in patients infected with SARS-CoV-2 with obesity, diabetes, nephropathy, which are characterized by immune disturbances; which is not directly related to rs1800871 and rs1800872 genetic polymorphisms.

Unfortunately, there is no information available about the participation of these polymorphisms in the pathogenesis of COVID-19. Therefore, the following comparisons will be within the context of similar illnesses.

In Hong Kong, Chinese patients discarded the association of rs1800872 polymorphism with both susceptibility and severity for influenza A/H1N1pdm09 (13) and also for acute respiratory syndrome in India (7). The opposite effect was reported in Mexican population: rs1800872 seems to be associated with susceptibility to influenza severe disease, whereas rs1800871 had no effect (19).

In this type of studies, the contradictory results could be explained from immunogenetics and population genetics point of view. On the one hand, the differential human immune response against the different viruses, and the other, by the genetic structure of populations (18). Nevertheless, the differences in sample size could be a confounding factor generating spurious or false associations.

The significance of the interaction between myalgia and obesity in COVID-19 patients with polymorphisms of the IL-10 gene is unclear. For myalgia, we believed that such association may not be statistically valid because of minimal proportion of COVID-19 patients that reported it (50/193 of those with rs1800871 genotyped). However, its role in the inflammatory pain mechanisms is well documented, from regulation of neuroinflammatory processes in pain-relevant regions of the nervous system in several animals models to its influence as a candidate genetic factor for pain (20,22,23). Obesity and its different phenotypes are complex

Table 3. Frequency Distribution of Interleukin-10 Haplotypes (-819 C>T and -592 C>A) in a Mexican Population with COVID-19 According Myalgia and Obesity

Haplotype	*Myalgia*	*W-myalgia*	p	*OR (95% CI)*	*Obesity*	*W-obesity*	p	*OR (95% CI)*
AC	0.207	0.303	0.117	0.601 (0.317–1.140)	0.193	0.338	0.002	0.469 (0.283–0.777)
CC	0.339	0.409	0.299	0.742 (0.421–1.305)	0.400	0.385	0.777	1.066 (0.685–1.659)
CT	0.428	0.288	0.032	1.852 (1.048–3.274)	0.393	0.262	0.010	1.827 (1.146–2.913)
AT	0.025	0.000	0.066	—	0.013	0.015	0.895	0.883 (0.138–5.674)

W means without.

traits because some lifestyles and genetic factors influence it. Thus, its link with IL-10 does not have conclusive evidence owing to obesity showing considerable intrapopulation variability, that is, contradictory results were reported in Mexicans and Spaniards, despite the ancestry, whereas in African American individuals IL-10 was not associated with obesity (3,5,14).

Studies of the association between obesity and rs1800871 and rs1800872 SNPs are most scarce. Bassols *et al.* found a higher prevalence in patients with nonmorbid obesity with higher bone mass index and waist circumference (3), a similar finding as the present study.

It is possible that genotypes or haplotypes of *IL-10* gene can modulate the tissue-specific production of the IL-10 levels and this in turn can generate a differential clinical effect in the patients. To discard this hypothesis, it would be interesting to know the distribution of these genotypes and levels of IL-10 in lean and obese individuals with and without COVID-19. Future research could rule out or confirm these ideas.

Finally, our results do not support the hypothesis that IL-10 gene promoter polymorphisms (rs1800871 and rs1800872) may serve for assessing the evaluation of genetic risk for severity and mortality in COVID-19 patients in Mexico. However, we cannot exclude the participation of other cytokine genes in SARS-CoV-2/COVID-19 pathogenesis.

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