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Brief report

Interleukin (IL) -1 β, IL-6 and tumor necrosis factor in patients with seasonal flu

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ABSTRACT

Introduction. The role of tumor necrosis factor (TNF), interleukin (IL)-1β and IL-6 in the pathogenicity of seasonal flu is unknown.

Methods. We analyzed the profiles of these cytokines in 77 flu patients and 17 controls with non-flu respiratory infection, using molecular biology techniques (real-time polymerase chain reaction).

Results. Flu patients had lower monocyte counts (p=0.029) and a slightly lower median level of IL-6 (P=0.05) than the control group. Twenty-four flu patients (31.2%) had pneumonia; this group had higher C-reactive proteins (p=0.01) and monocyte levels (p=0.009). Pro-inflammatory cytokines levels did not rise in patients with pneumonia complicating seasonal influenza

Conclusion. IL-6 levels were lower in adults with influenza.

Keywords: Respiratory Tract Infections; Influenza, Human; Pneumonia

Interleucina (IL)-1 β, IL-6 e TNF en pacientes con gripe estacional

RESUMEN

Introducción. No se conoce el papel del factor de necrosis tumoral (TNF), interleucina (IL)-1β e IL-6 en la patogenicidad de la gripe estacional

Métodos. Se analizaron los perfiles de estas citoquinas en 77 pacientes con gripe y 17 controles con infección respiratoria sin gripe, utilizando técnicas de biología molecular

Resultados. Los pacientes con gripe tuvieron recuentos de monocitos más bajos (p = 0.029) y un nivel medio de IL-6 ligeramente menor (P = 0,05) que el grupo de control. Veinticuatro pacientes con gripe (31,2%) tenían neumonía; este grupo tenía proteínas C-reactivas más altas (p = 0,01) y niveles de monocitos (p = 0,009). Los niveles de citoquinas proinflamatorias no aumentaron en pacientes con neumonía que complica la gripe estacional

Conclusión. Los niveles de IL-6 fueron más bajos en adultos con gripe

Palabras clave: Infección del tracto respiratorio; gripe; neumonía

INTRODUCTION

Tumor necrosis factor (TNF), interleukin (IL)-1β and IL-6 are pro-inflammatory cytokines that play a role in inflammation, infection, and immune response. The cytokine profile during the flu season has been studied little, and the information available is contradictory. During the H1N1 pandemic of 2009, elevated levels of cytokines IL-6, IL-8, IL-1β and TNF-α were associated with greater flu severity. In particular, IL-1β and IL-6 were associated with increased mortality in patients with the virus. These cytokines are related to greater lung damage and have been considered biomarkers for infection severity [1-4]. On the other hand, in animal models, mice that were deficient in IL-6 and in the soluble IL-6 receptor (sIL-6R) died earlier after a sublethal dose of the influenza virus compared to mice with normal levels. The authors of this study concluded that IL-6 was necessary to overcome the viral flu infection by means of the production of neutrophils, which was associated with the clearing of the virus from the respiratory tract [5,6]. Other authors have observed greater propensity for lung damage due to viral infection in IL-6-deficient animals, increasing the probability of death [7]. In light of these discrepancies, we

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designed a pilot study to analyze the relation between IL-1β, IL-6 and TNF-α levels and severity of seasonal influenza in patients with confirmed flu virus infection, who needed hospital admission, during the 2015-16 winter flu season.

MATERIAL AND METHODS

Of the 231 patients diagnosed with the seasonal flu in patients during the 2015-16 winter flu season (34 December 2015 to 30 April 2016) by means of molecular biology techniques (real-time polymerase chain reaction (qPCR) samples were obtained by nasal lavage with the GeneXpert test, using the Xpert Flu and Xpert Flu/RSV XC (RS Cepheid) assays), 136 patients were excluded because they were children, pregnant women, or because they were not ultimately admitted. Thus, we initially included 95 patients, albeit we were only able to analyze 77 because samples were not available for 17. The control group was drawn from a pool of 21 age- and sex-matched patients admitted to the same unit during the same period, with symptoms of respiratory tract infection but whose PCR results were negative for the flu. Samples were obtained from 17 of these patients, who made up the final control group.

Samples were preserved at –70 ºC until they were thawed for an ELISA assay (ELISA Ready-SET-Go!, Affymetrix eBioscience) to determine the cytokine profile.

We expressed quantitative variables as medians and interquartile range (IQR), comparing between-group differences with the U-Mann Whitney test. The center's ethics committee approved our data recording procedures (number PI 2016/03), and the clinical investigation was in line with the principles of the Declaration of Helsinki.

Table 1 **Demographic, analytical, immunological and outcome variables in patients with influenza and control group**

	Influenza group (N= 77)	Control group (N=17)	P value	Influenza and pneumonia (N=24)	Influenza without pneumonia (N=53)	P value
Demographic variables						
Age, median (IQR)	67.9 (50.5-79)	75 (53-82.5)	0.231	66.5 (50-74.5)	68 (56-82)	0.363
Sex, male, n (%)	38 (49.4)	8 (47.1)	0.99	10 (41.7)	28 (52.8)	0.567
Analytical variables						
Hemoglobin (g/dL), median (IQR)	11.8 (9.8-13.1)	10.2 (8.7-12.7)	0.128	12.6 /11.8-14.2)	13.1 (11.8-14.7)	0.779
White blood cell (x10^3/µL), median (IQR)	5.9 (4.1-7.9)	6.9 (5.1-8.9)	0.128	7.9 (5.1-12.5)	7.9 (6.0-11.1)	0.996
Neutrophils (x10^3/µL), median (IQR)	4.4 (2.7-6.4)	5.6 (2.9-7.1)	0.241	6.5 (4.0-10.4)	6.3 (4.4-8.5)	0.565
Lymphocytes (x10^3/µL), median (IQR)	0.594 (0.421-0.891)	0.722 (0.437-1.24)	0.119	899 (510-1440)	875 (665-1180)	0.635
Monocytes (x10^3/µL), median (IQR)	0.412 (0.207-0.585)	0.610 (0.373-0.870)	0.029	438 (215-820)	750 (470-960)	0.009
Platelets (x10^3/µL), median (IQR)	158 (113-204)	151 (140-177)	0.976	203 (162-263)	204 (154-287)	0.849
Creatinine (mg/dL), median (IQR)	0.96 (0.79-1.27)	0.97 (0.78-1.44)	0.980	0.80 (0.73-1.21)	0.99 (0.86-1.36)	0.093
Glomerular filtration rate (mL/min/1.73 m^2), median (IQR)	77.0 (53.7-90)	67.0 (54.0-84.4)	0.496	0.90 (0.83-110)	0.85 (0.66-0.92)	0.496
Sodium (mEq/L), median (IQR)	138 (135-140)	137 (135-1419	0.704	138 (135-140)	137 (133-140)	0.525
Potassium (mEq/L), median (IQR)	4.1. (3.7-4.4)	4.2 (4.0-4.6)	0.115	4.0 (3.65-4.9)	4.2 (3.9-4.4)	0.217
ASL (U/l), median (IQR)	24.0 (19.0-39.0)	19.0 (15.0-43.0)	0.210	44 (23-72)	37(16-44)	0.813
ALT (U/l), median (IQR)	21.0 (15.0-33.0)	18 (10.0-43.0)	0.331	39 (20-42)	28 (15-39)	0.408
C- reactive protein (mg/dL), median (IQR)	5.5 (2.2-9.4)	8.4 (2.5-12.4)	0.438	7.6 (4.7-15.9)	5.1 (2.1-7.5)	0.011
Lactate (mmol/L), median (IQR) (n=105)	1.50 (1.10-2.10)	1.50 (1.15-2.15)	0.694	2.2 (1.3-2.76)	2.4 (1.7-3.0)	0.131
Immunological variables						
TNF-α (pg/mL) median (IQR)	50.2 (47.1-63.4)	54.0 (48.7-203)	0.112	49.3 (45.6-63.4)	50.3 (47.1-63.4)	0.344
IL-1β (pg/mL), median (IQR)	19.5 (15.3-20.1)	15.9 (14.9-19.2)	0.902	17.6 (15.2-26.8)	19.6 (15.3-26.6)	0725
IL-6 (pg/mL), median (IQR)	40.9 (32.8-94.4)	50.9 (32.9-65.6)	0.046	40.0 (32.0-94.4)	42.2 (33.7-88.6)	0.664
Outcome variables						
Died, n (%)	0 (0)	2 (11.8)	0.031	0 (0)	0 (0)	

IQR: interquartile range; AST aspartate amino transferase; ALT: alanine amino transferase; IL: interleukin

RESULTS

Table 1 shows the differences between the 77 patients with flu infection and the 17 patients with non-flu respiratory infection. There were no differences in epidemiological aspects, although the flu patients did have a lower count of monocytes ($0.412 \times 10^3/\mu L$ vs. $0.610 \times 10^3/\mu L$; p=0.029) and a slightly lower median level of IL-6 (40.9 pg/mL vs. 50.9 pg/mL; p=0.046) than the control group. Moreover, none of the patients with flu died, while 2 (11.8%) in the control group did.

We also evaluated the immunological and analytical parameters of flu patients in order to test their association with pneumonia, considered a severe form of the flu. Of the 77 patients with flu, 19 (24.7%) had unilobar, and 5 (6.5%) multilobar, infiltrates. We observed higher C-reactive protein (CRP) (p=0.01) and monocyte levels (p=0.009) in patients with pneumonia. Cytokine levels were not related to the presence of radiological infiltrates during the flu season.

DISCUSSION

The lower IL-6 values in our cases are contrary to the results found by García-Ramírez et al. [8], carried out during the H1N1 pandemic. Those authors observed higher IL-6 levels in flu patients compared to the controls with H1N1-negative respiratory tract infections. Moreover, they investigated the IL-6 polymorphism and the IL-6 receptor (IL-6R), finding that the heterozygotic genotype for IL-6rs1818879 (with GA mutation) was related to flu severity. Higher IL-6 values have been found in more severe cases of the flu during the H1N1 pandemic [2,3,4]. This could explain why our cases, which were all moderate flu episodes and did not cause any deaths, showed more moderate IL-6 values that were even below the levels seen in controls–two of whom did die from their non-flu respiratory infections.

Flu severity, as manifested by flu-derived pneumonia, was associated with higher PCR values and lower monocyte counts. The latter may be because of a weaker activation of the phagocytic functions in these patients, monocytes are not related to a defense against viral infections [9,10]. The presence of pneumonia was not related with IL-6, IL-1β or TNF-α values.

Limitations of this study include the small number of cases and controls and the missing cytokines data for several cases. However, this is an exploratory study of the possible role of cytokines in the pathogenesis of the flu and in its severity. In light of the observed results, there is a need for larger studies with a broader focus, including determination of IL-8, IL-10 and others in addition to IL-1β, IL-6 and TNF-α. At present, little is known about the role of cytokines in patients with seasonal flu.

In summary, in our study, IL-6 levels were significantly lower in adults with influenza compared with other common community respiratory infections. Pro-inflammatory cytokines levels did not rise in patients with pneumonia complicating seasonal influenza.

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CONFLICT OF INTEREST

The authors declare that have no conflict of interest.

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