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Case Report

Local report of cytokine landscape of SARS-CoV-2 infected patients in Chihuahua, Mexico

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Abstract

In December 2019, the first cases of a highly infectious disease causing severe acute respiratory syndrome were registered. In early 2020, Chinese authorities isolated and identified a new type of coronavirus, SARS-CoV-2. Currently, there are studies and controversies in the relationship of the progression of the disease with the cytokine release syndrome observed in infected patients. In this study, the levels of the cytokines IL-6, IL-8, IL-10, IL-1β, TNF-α and IL-12p70, were evaluated by Cytometric Bead Array methodology in 42 severely ill patients with Covid-19, admitted to the Hospital Central Universitario of the city of Chihuahua. Nevertheless limited, it was possible to assess the effect of ruxolitinib and dexamethasone treatments on the cytokine profile. Finally, the concentrations of the cytokines and the comorbidities that the patients presented at the time of admission were correlated. A significant increase in plasma levels of IL-6, IL-8, IL-10 and IL-1β was found in severely ill patients with Covid-19. Patients infected and treated with ruxolitinib exhibited a significant decrease in IL-6 and IL-10 levels five days after admission, thus improving their hyperinflammatory state. Cytokine levels in the Chihuahua population were found to be significantly higher in people with a high BMI and / or type 2 diabetes mellitus.

Keywords: Chihuahua; COVID-19; Mexico; SARS-CoV-2; Cytokine Storm

Introduction

One year have been passed, since a first reports of patients affected by severe illness and a pneumonia of unknown origin were described in the city of Wuhan, Hubei province, China [1,2]. Of the seven known human coronaviruses, four (HCoV-NL63, HCoV-229E. HCoV-OC43, and HCOV-HKU1) are less virulent, causing "common colds, however, the remaining three are highly pathogenic: Middle East respiratory syndrome (MERS)-Co-V, SARS-Co-V, and SARS-CoV-2 [3,4]. In the particular case of Mexico, the first case recorded occurred on February 27, 2020, a person, who had recently traveled to Italy, had mild symptoms. At the end of April, Mexico begun

phase 3 on Covid-19, since it had registered more than a thousand cases [5]. One month before to end of 2020 year, the number of cases reached 1 million and 114,297 deaths [6]. The Health Council in Chihuahua State (Secretaria de Salud del Estado de Chihuahua) confirmed the first case of Covid-19 on March 17, 2020. This occurred in a man with residence in Ciudad Juárez [5]. The virus continued to spread in 32 municipalities in the state. Among them, the highest numbers of infections have been reported in Ciudad Juarez and Chihuahua City among others. On November, Chihuahua reached 28,797 infected cases and 3,344 total deaths [5]. There is a misconception and controversy, about the concept of "cytokine storm" emerged at the beginning of the COVID-19 pandemic; a study conducted in Wuhan, China was published where the most critical patients had higher serum levels of cytokines, therefore the authors associated this response with the severity of the infection, effectively since the pathogenesis has been linked to the Cytokine Release Syndrome (CRS) and positive correlations have been found, especially with IL-6, IL-10, IL-1, IL-12, TNF-α, IL-8 and IFN-γ [7-11]. Is necessary, to clarify the concepts and their biological outcomes, according with Sinha, et al., a "cytokine storm" suggests, levels of secreted cytokines could be injurious to host cells. Is important to distinguish an regulate from a dysregulated inflammatory response in the pathophysiology of critical illness [11]. This work has been focused on determining the cytokine profile in Mexican patients infected by SARS-CoV-2, the samples were taken from patients seen at the Hospital Central Universitario located in Chihuahua, Mexico. Cytokine levels have been evaluated in Covid-19 patients at the moment of admission and five days later, in order to associate and compare them, with the main comorbidity's severity - associated in coronavirus disease, lastly, preliminary data of cytokine profile from patients receiving dexamethasone or Ruxolitinib are described.

Material and Methods

Ethics statement

This study was approved by Facultad de Medicina y Ciencias Biomedicas, Program Institutional Review Boards (CI-015-20) and Ethics Review Board from Secretaria de Salud del Estado de Chihuahua (SI-002-2020). Informed consent was obtained from all enrolled patients and healthcare workers.

Patients / Donors

Blood samples were collected from Hospital Central Universitario, a total of 42 individuals were included in this study. No statistical methods were used to predetermine sample size. Paired whole blood for was collected in sodium heparin-coated vacutainers and kept on gentle agitation until processing. All blood was processed on the day of collection. All plasma samples from a patient infected with SARS-CoV-2 were confirmed by PCR. Healthy Donor (HD) plasma was obtained from a person acquired months before COVID-19 pandemic. The samples were taken during the months of August to October 2020.

Isolation of Patient Plasma

Plasma samples were collected after centrifugation of whole blood at 400g for 10 min at room temperature (RT) without brake. The undiluted serum was then transferred to 15-ml polypropylene conical tubes, and aliquoted and stored at -80 °C for subsequent analysis.

Procedures

Plasma, cytokines were determined using BDTM Cytometric Bead Array (CBA) Human Inflammatory Cytokine KIT (551811), samples were analyzed in Attune NXT flow Cytometer, and data analysis was carried out with Microsoft Excel, GraphPad 9.0 and FlowJo.

Data and Statistical Analysis

A linear regression was applied and the equation of the line of each curve was obtained; the median fluorescence intensity (MFI) of each sample was interpolated and the concentration of each analyte (pg/mL) was obtained, from a standard curve developed for each cytokine. Statistical analysis, a comparison of the ranges with the Mann Whitney U test for each cytokine; a Kruskal-Wallis test to compare the mean range of the comorbidity's groups and a one-way ANOVA for non-parametric tests to assess the effect of treatments, plots developed in GraphPad Prism version 9.0.

Results and Discussion

In this study, we performed cytokine surveillance of admitted COVID-19 patients, with severe symptom at the Hospital Central in Chihuahua City. During May to September 2020, we collected plasma samples from 42 individuals, with SARS-CoV-2 infection confirmed by qPCR. Among those sampled, 14/42 (33%) are female and 28/42 (66%) males, and the median age is 65 (range 28–74). On the other hand, for healthy controls, plasma samples were collected from 18 random blood donors, were 9 are female and 9 males, and the median age is 40 (range 22–58). A blood sample was taken in a tube with EDTA, and was immediately processed to separate the plasma, in order to assay concentrations of IL-1b, IL-6, IL-8, IL-10, IL-12 and TNFa (Figure 1). As a general view of cytokine profile, it was found that the concentrations of interleukins IL-1b, IL-6, IL-8, and IL-10 from infected patients are elevated compared to healthy donors. Being observed significant for IL-6 and IL-8. Otherwise, TNF-α and IL-12p70 concentrations in COVID-19 infected patients do not show significant changes compared to healthy controls (Figure 1A-1F). The results obtained in this work are consistent

with other reports, however, it's important to highlight the amount (pg/ml) of measured cytokines using different approaches, the common results indicate levels of IL-6 in a range from 6-165 pg/ml [11], if the comparison is made with a healthy control seems to be high, however these levels in confrontation with sepsis or influeza [12] seem to be not as high as previously thought. When the patients were grouped by the comorbidities, ten of them presented no comorbidity; twelve, with a high BMI (overweight or obesity); four, with Type 2 Diabetes Mellitus (DM2); six, with Systemic Arterial Hypertension (SAH) and nine with both SAH and DM2 conditions (Figure 1G-1L). A significant increase of IL-6 was found in all comorbidity groups compared to the healthy donor; with an exception the groups with SAH and DM2 (Figure 1H). However, IL-8 levels showed significant differences in the groups without comorbidity (Figure 1I). No significant differences were found between the groups of the interleukins IL-1β, TNF-α and IL-12p70. Finally, IL-10, it was only observed a significant increase on the cytokine concentration within the group of high BMI compared to the healthy control (Figure 1J). In this study, significantly higher levels of pro-inflammatory cytokines associated with Covid-19 infection, IL-6, IL-8 and IL-1β, were found compared to the healthy control, although the quantified levels may suggest severity, it does not necessarily involve pathogenesis. While IL-6 levels are not as high as in other conditions, unfavorable results could be found. These levels could be related to lung injury, as a consequence of pneumonia caused by Covid-19 [11,13]. It is important to high point, the plasma levels of pro-inflammatory cytokines in the Chihuahua population were found to be higher in patients with overweight, obesity and with type 2 Diabetes Mellitus. One of the consequences of the acceptance of the term cytokine storm in Covid-19 has motivated the use of potent immunomodulatory therapies both in the setting of clinical trials and on a compassionate basis. These drugs, such as IL-6 inhibitors and high-dose corticosteroids, block pathways critical to host immune responses [11,14,15]. In Mexican hospitals, these strategies have been used, in here show the results obtained using dexamethasone and a JAK1 and JAK2 kinase inhibitor. Of a total of twenty COVID-19 patients who were treated with dexamethasone, unfortunately four departed. It was possible to follow up three, where it was found that there is a slight recovery in IL-6 levels, between admission and day 5, when compared with the healthy control (Figure 1M-1R). In the case of patients treated with Ruxolitinib, ten were treated with this drug, of which six received follow-up and two passed away. Results obtained, shows a reduced level of IL-6, IL-8, IL-10 and IL-12p70, between the day of admission and day 5, however, cytokine levels do not reach basal levels observed in healthy donors (Figure 1S-1X).

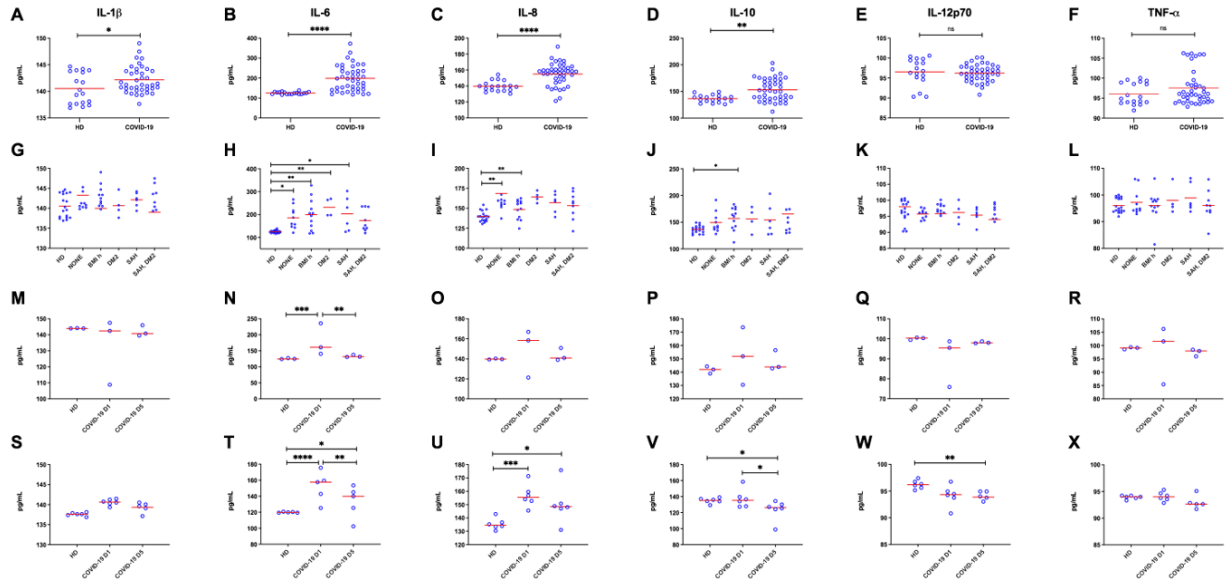

Figure 1: Cytokine profile in COVID-19 infected patients. Plasma samples were obtained from a Hospital Central Universitario, in Chihuahua Mexico cohort of 42 COVID-19-patients. (A-F) Plots show cytokine concentrations in healthy donors (HD, n=18) and COVID-19 patients (n = 42) for six cytokines measured. U de Mann Whitney value 79 to IL-6 with $P < 0.0001$; IL-8 = 120, $P < 0.0001$; IL-10 = 185, $P < 0.001$; IL-1β = 241, $P < 0.05$; TNF- α = 300, $P > 0.05$; IL-12p70 = 289.5, $P > 0.05$. (G-L) Plasma concentrations of IL-1b, IL-6, IL-8, IL-10, IL-12 and TNFa in patients with COVID-19, samples were grouped according to associated comorbidities, high body mass index (BMIh), diabetes mellitus 2 (DM2), syndrome hypertension (SAH). (M-R) Plasma concentrations of cytokines in patients with COVID-19 (n = 3), who received dexamethasone, samples were interrogated on day 1 (D1) and day 5 (D5). (S-X) Plasma concentrations of cytokines in patients with COVID-19 (n = 5), who received Ruxolitinib (JAKAVI), samples were interrogated on day 1 (D1) and day 5 (D5). Data are presented as scatter plots with red horizontal bars indicating the geometric mean levels. $P < 0.05$ = *, $P < 0.001$ = **, $P < 0.0001$ = ***, $P < 0.00001$ = **** significant.

Conclusions

The findings in this work show an increase in the plasma levels of the cytokines IL-6, IL-8, IL-10 and IL-1β; This increase is positively related to the severity of the Covid-19 infection and its decrease can improve the progression of the disease. However, when the cytokine levels obtained are compared with other disease such as influenza or sepsis, seems to be not higher as they are not higher as they appear. Ruxolitinib as a treatment for infection, compared to dexamethasone, can mitigate the hyperinflammatory state observed in patients who experience an elevation in the concentration of pro-inflammatory cytokines associated with Covid-19. Plasma levels of proinflammatory cytokines in the Mexican -Chihuahua population were found to be higher in patients with overweight or obesity and with type 2 diabetes mellitus.

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Conflict of Interest

Authors declare no conflict of interests.

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