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Original Article

Gender affects IL-23 serum levels in the hospitalized COVID-19 infected patients

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Abstract: Cytokine storm is a main complication in the hospitalized patients, who are infected with the novel coronavirus (COVID-19). The pro-inflammatory cytokines are the main causes of the cytokine storm, however, the roles played by IL-17A, IL-23 and CCL3 are yet to be clarified completely. This prospective study was aimed to explore serum levels of these cytokines in the hospitalized patients infected by COVID-19. Serum levels of IL-17A, IL-23 and CCL3 were measured in 30 COVID-19 infected patients in parallel with 30 healthy controls using ELISA technique. Although serum levels of IL-17A, IL-23 and CCL3 did not alter in the patients in comparison to healthy controls, male patients had higher serum levels of IL-23 than women. Hypertension, type 2 diabetes, lung involvement and age did not affect serum levels of IL-17A, IL-23 and CCL3 in the patients. It appears that IL-17A, IL-23 and CCL3 do not participate in the pro-inflammatory responses in Iranian hospitalized COVID-19 infected patients. However, the gender can be considered as a risk factor for production of more IL-23, which needs to be explored further.

Keywords: IL-17A, IL-23, CCL3, COVID-19, inflammation

Introduction

The recent pandemic coronavirus (COVID-19) is associated with increased morbidity and mortality [1]. The infection can induce wide ranges of symptoms and can affect several tissues, including the lungs, liver, kidney and cardiovascular system [1]. Lethal inflammation is a main mechanism used by the virus to induce the complications, which are triggered by cytokine storm [2]. The resoles played by some well-known innate immunity cytokines, such as IL-6 and tumor necrosis factor-alpha (TNF-α), in the pathogenesis of COVID-19 have been documented by several investigations [3, 4]. However, the roles of adaptive immunity in the pathogenesis of COVID-19 are yet to be clarified.

IL-17A is a main adaptive immune cytokine that is produced by T helper-17 (Th17) and plays key roles against bacteria and fungi infections. The cytokine is a main responsible molecule participates in the pathogenesis of pro-inflammatory-related disorders, including autoimmunity and type 4 hypersensitivity [5]. Therefore, the cytokine and other cytokine related to production of IL-17A can be considered as the risk factors for development of some COVID-19-related pro-inflammatory complications. This cytokine categorized as a pro-inflammatory molecule, because of its roles in the development of Th17 and activations of memory T cells, natural killer T cells, monocytes, and dendritic cells (DCs) [6]. Therefore, IL-17A and its related cytokine, IL-23, can be risk factors for the hospitalized patients, who are infected by COVID-19.

Additionally, CCL3, which is known also as macrophage inflammatory protein 1-alpha (MIP-1-alpha), is categorized as a chemokine; the cytokines participate in chemotaxis and activation

of immune cells. The chemokine is a main responsible molecule in the proinflammatory responses against viral infections [7, 8].

Due to the inflammatory-based functions of IL-17A, IL-23 and CCL3 during viral infections, this project was designed to explore the serum levels of the cytokines in the hospitalized COVID-19 infected patients. In this project the effects of gender, lung involvement and age on the serum levels of IL-17A, IL-23 and CCL3 have also been evaluated.

Material and methods

Subjects

This prospective study was performed on 30 hospitalized COVID-19 infected patients (12 males and 18 females) and 30 healthy controls (14 males and 16 females), which were matched regarding age (P = 0.939) and sex (P = 0.195) with the patients. Accordingly, the mean age of COVID-19 infected patients and healthy controls were 62.40 ± 3.733 and 57.50 ± 3.61 years old, respectively. The patients were selected from the Ali-Ibn Abi-Talib Hospital, Rafsanjan, Iran, with a positive quantitative PCR test for COVID-19. The patients with severe COVID-19 were entered to the study, who have the following symptoms: 1. Respiratory distress; over 20 breaths per minute, 2. Blood oxygen level less than 90%, 3. Need for intubation, and 4. More than 50% lung involvement) [9]. The patients with allergy, infectivity with other viruses and bacteria, autoimmunity, smoking, opium consuming and receiving immune suppressor drugs were excluded from the study. The blood samples were collected in pre-coated anti-coagulant agents (Ethylenediaminetetraacetic acid (EDTA)) tubes at the start of hospitalization and before starting the drug administration. The informed consent form was signed by the participants prior enter into the project and the project protocol was approved by the local ethical committee (IR.RUMS.REC.1399.015).

Evaluation of IL-17A, IL-23 and CCL3 serum levels

Serum levels of IL-17A, IL-23 and CCL3 were measured using commercial kits from Karmania Pars gene Company, Kerman, Iran. Briefly, 100 mL serum in parallel with the standards was added to the pre-coated plates and after 1 hour incubation, the vials were washed and then detection antibodies were added. After 1 hour incubation, the plates were washed and then HRP-avidin were added and incubated for 30 minutes. After 5 times washing, the substrate was added and after 15 minutes the reactions were stopped using stopping solution. The optical densities (ODs) of the vials were measured using a BMP ELISA reader.

COVID-19 RNA extraction and real-time PCR condition

COVID-19 RNA was purified using a commercial kit (Symbiolab Co., Mashhad, Iran). The extracted viral-RNA was evaluated using a one-step COVID-19 detection kit (Pishtaz Teb Co., Tehran, Iran). The kit has evaluated 3 genes of COVID-19, including nucleocapsid (N), RNA-dependent RNA polymerase (RdRp) and envelope (E) genes, simultaneously.

Evaluation of blood oxygen, blood urea nitrogen, creatinine and white blood cell counts

Blood oxygen (BO) saturation was evaluated with a portable pulse-oximeter (BCI 3301@, Rockwood Drive, Waukesha, WI, USA). Blood urea nitrogen (BUN) and creatinine (Cr) were measured using the commercial kits from Man Company (Tehran, Iran) in the BS-400 Mindray instrument and also white blood cell (WBC) counts were determined employing a BS-5800 Mindray Coulter Counter (Shenzhen, China).

Statistical analysis

Using SPSS software version 16 (Kolmogorov Smirnov test) showed normal distribution of data. So, the parametric tests were used for data analysis. Thus, the differences between healthy controls and COVID-19 infected patients, men and women, the patients with and without patchy consolidations and ground glass opacities and the patients with and without type 2 diabetes were explored using independent student t test. The Pearson correlation test was used to calculate correlations among serum levels of IL-17A, IL-23 and CCL3 with BO, BUN, Cr, WBC counts and age were explored in the patients suffering from COVID-19.

Results

The results showed that serum levels of IL-17A (P = 0.650), IL-23 (P = 0.414) and CCL3 (P =

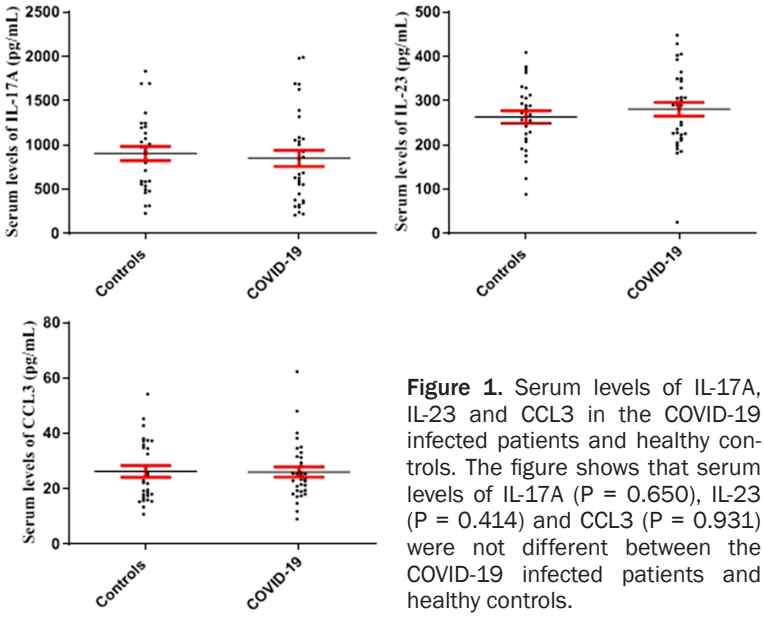

Figure 1. Serum levels of IL-17A, IL-23 and CCL3 in the COVID-19 infected patients and healthy controls. The figure shows that serum levels of IL-17A (P = 0.650), IL-23 (P = 0.414) and CCL3 (P = 0.931) were not different between the COVID-19 infected patients and healthy controls.

Table 1. Serum levels of IL-17A, IL-23 and CCL3 in the men versus women patients infected with COVID-19 and various statuses of hypertension and type 2 diabetes

Variables			Mean ± SE	*P* value
Gender	IL-17A	Men	775.68 ± 152.45	0.523
		Women	901.26 ± 119.48	
	IL-23	Men	314.21 ± 32.47	0.050
		Women	253.69 ± 13.16	
	CCL3	Men	29.11 ± 3.44	0.242
		Women	24.48 ± 2.18	
Hypertension	IL-17A	Yes	850.00 ± 183.94	0.990
		No	846.48 ± 104.35	
	IL-23	Yes	235.08 ± 14.70	0.168
		No	290.34 ± 17.99	
	CCL3	Yes	25.50 ± 2.11	0.889
		No	26.18 ± 2.21	
Type 2 diabetes	IL-17A	Yes	491.55 ± 149.52	0.147
		No	894.50 ± 98.10	
	IL-23	Yes	286.75 ± 53.62	0.879
		No	279.41 ± 16.23	
	CCL3	Yes	22.06 ± 5.00	0.428
		No	26.61 ± 1.98	

0.931) were not different between the COVID-19 infected patients and healthy controls. Accordingly, **Figure 1** illustrates the raw concentrations of the cytokines (**Figure 1**).

Serum levels of IL-23 (P = 0.050) were higher in the COVID-19 infected men patients when compared to women. However, serum levels of IL-17A (P = 0.523) and CCL3 (P = 0.242) were not significantly different in the men and women COVID-19 infected patients. **Table 1** illustrates the data in details.

Statistical analysis showed that hypertension and type 2 diabetes did not change the serum levels of IL-17A (P = 0.523), IL-23 (P = 0.523) and CCL3 (P = 0.242) in the COVID-19 infected patients (**Table 1**).

The statistical analysis showed that serum levels of IL-23 were significantly higher in the men when compared to women patients. However, hypertension and type 2 diabetes did not change serum levels of IL-17A, IL-23 and CCL3 in the patients. Serum levels of IL-17A (P = 0.645), IL-23 (P = 0.357) and CCL3 (P = 0.472) were not different between men when compared to women in the healthy controls.

Serum levels of IL-17A (P = 0.229), IL-23 (P = 0.877) and CCL3 (P = 0.920) in the patients with and without patchy consolidations and ground glass opacities, by using CT scan technique, were not different significantly (**Figure 2**).

Table 2 illustrates the correlations among serum levels of IL-17A, IL-23 and CCL3 with BO, BUN, Cr, age and WBC counts in the COVID-19 infected patients. Data analysis revealed that there were no significant correlations between serum levels of IL-17A, IL-23 and CCL3 with BO, BUN, Cr, age and WBC counts.

Discussion

COVID-19 is a virus containing RNA can be recognized by several innate immune cell recep-

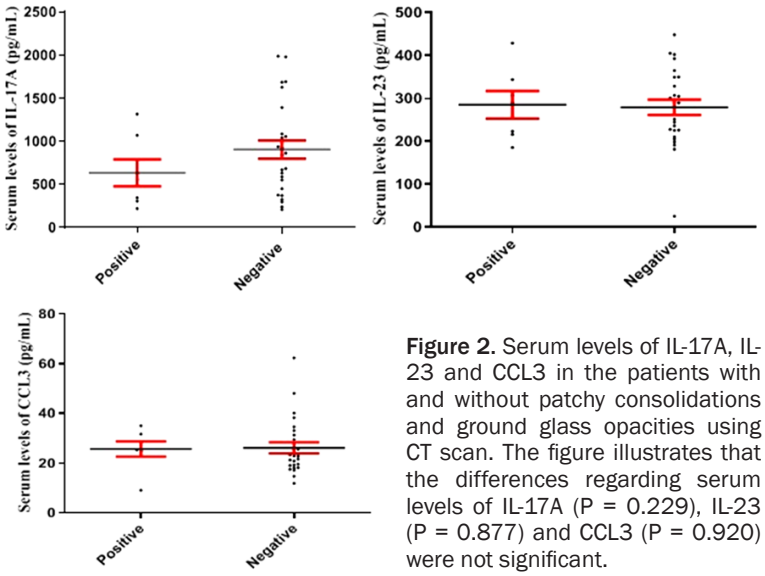

Figure 2. Serum levels of IL-17A, IL-23 and CCL3 in the patients with and without patchy consolidations and ground glass opacities using CT scan. The figure illustrates that the differences regarding serum levels of IL-17A (P = 0.229), IL-23 (P = 0.877) and CCL3 (P = 0.920) were not significant.

Table 2. Correlation between serum levels of IL-17A, IL-23 and CCL3 with blood oxygen (BO), blood urea nitrogen (BUN), creatinine (Cr), white blood cell (WBC) counts and age

Variables		IL-17	IL-23	CC-L3
BO	Pearson Correlation	-0.104	0.343	0.090
	P value	0.702	0.193	0.740
BUN	Pearson Correlation	0.105	-0.029	0.065
	P value	0.699	0.914	0.812
Cr	Pearson Correlation	0.195	0.053	0.398
	P value	0.454	0.840	0.113
WBC	Pearson Correlation	0.037	0.074	0.119
	P value	0.862	0.730	0.580
Age	Pearson Correlation	-0.408	-0.215	-0.322
	P value	0.074	0.362	0.167

tors, which are the main source of IL-23 [10]. The results demonstrated that, although IL-23 serum levels were not changed between patients and healthy controls, it was significantly higher in men when compared to women COVID-19 infected patients. Previous studies demonstrated that male patients had higher inflammatory molecules and higher rates of hospitalization when compared to females [11]. Due to the results that revealed that men and women were not different regarding the serum levels of IL-23, it may be concluded that male patients may show more inflammatory responses in dependent of IL-23 in the COVID-19 infected patients. Accordingly, it appears that gender may be a potential risk factor for COVID-19 pathogenesis. Increased expression of innate immunity receptors on the immune cells of women in comparison to men COVID-19 infected patients have been reported by Conti and colleagues [12]. Butchi et al., also showed that myeloid differentiation primary response 88 (MYD88) pathway, which is an important pathway for the expression of IL-23 [13], is a critical pathway to induce early innate immune responses during Coronavirus encephalomyelitis [14]. The roles played by IL-23 in the pathogenesis of COVID-19 have also been demonstrated by Schön and colleagues [15]. IL-23 plays several roles in immune responses, including activation and maintenance of T helper 17 (Th17) cells, activation of natural killer cells (NK cells) and proliferation of memory T cells [16, 17]. Due to our results, IL-17A were not different between men and women patients. It appears that increased IL-23 in the men did not affect Th17 functions and it may induce some pro-inflammatory responses independent of IL-17A. Although some investigations proved the potential roles played by IL-17A in the pathogenesis of COVID-19 [18, 19], our results demonstrated that the cytokine serum levels were not increased in the COVID-19 infected patients. Additionally, age and severity of lung involvement have not also affected serum levels of IL-17A in our patients, and it may be related to different ethnic population. However, due to our results, it may be hypothesized that IL-23/IL-17A axis do not play key roles in the pathogenesis of COVID-19 in the Iranian patients.

CCL3 serum levels did not change also between the groups and also did not change between men and women and the patients with and without patchy consolidations and ground glass opacities. Therefore, it appears that CCL3 does not involve in immune responses against COVID-19.

Due to the fact that serum levels of IL-17A, IL-23 and CCL3 did not change between COVID-19 infected patients with and without type 2 diabetes, it may be concluded that type 2 diabetes

has no effects on the serum levels of the cytokines and this project has evaluated the effect of infection with COVID-19 on the cytokine serum levels only.

Previous studies served age as a risk factor for COVID-19 mortality [20], and our results revealed that age had no significant correlation with serum levels of IL-17A, IL-23 and CCL3. Thus, it seems that age-related mortality is independent of IL-17A, IL-23 and CCL3 serum levels.

Conclusion

It appears that IL-17A, IL-23 and CCL3 do not participate in the pro-inflammatory responses in Iranian hospitalized COVID-19 infected patients. However, the gender can be considered as a risk factor for production of more IL-23, which needs to be explored further.

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Disclosure of conflict of interest

None.

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