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Factors affecting some cells and immune mediators in COVID-19 patients

Allaa Khalaf Bediwi[1], Shehab Ahmed Lafi[2], Hazim Ismael Ghazzay[2]

Department of Microbiology, College of Medicine, University of Anbar,Ramadi,Iraq;

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ABSTRACT

COVID-19 induces a hyper-inflammatory immune response in cells, such as neutrophils and monocytes, and the release of various cytokines. Bronchoconstriction is one of the complications after a COVID-19 infection and mediated by hypersensitivity-inducing components, including immunoglobulin E (IgE) and specific IgE. Thus, this study explored the effects of some factors on some blood cells (basophils, monocytes, and eosinophils) and some immune mediators in patients who had COVID-19 infection. The study included 163 adult convalescent patients who had COVID-19 and complained of bronchial spasm and 75 healthy volunteer participants as controls. The patients visited Ramadi and Fallujah Teaching Hospitals, Anbar Province, Iraq, from September 2022 to May 2023. Complete cell blood count, anti-COVID-19 immunoglobulin M, immunoglobulin G, and interleukin 5, total IgE, and IgE specific for COVID-19 antigen were measured for each participant. Gender, severity, history of vaccination, and smoking status were reported. The effects of these factors on serological parameters were determined through data analysis. Results revealed that patients with severe COVID-19 infections had higher basophil counts and total IgE, specific IgE, and IL5 levels than patients with mild infections and the control. No significant differences were found between males and females in all the studied serological parameters. The same observation was found between smokers and nonsmokers. Vaccination had no effect on the studied parameters except IgG.

Introduction

COVID-19 is an infectious contagious disease caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which was first diagnosed in Wuhan, China, in December 2019 and spread globally **[1]**. Many studies reported a clear relation between COVID-19 infection and hypersensitivity reactions, which are abnormal immunologic reactions caused by an allergen or antigen response. Furthermore, researchers found detectable immunoglobulin E (IgE) antibodies specific to bacteria and viruses in the sera of infected patients **[**2, 3, 4**]**. COVID-19 might shift immunity toward allergic inflammation based on elevated levels of mediators, such as IgE, eosinophils, mast cell tryptase, and cytokines linked to allergy post-infection [1, 2].

*Corresponding author at: Department of Microbiology, College of Medicine, University of Anbar,Ramadi,Iraq;
ORCID:https:// https://orcid.org/0000-0000-0000-0000 ,
Tel: +9647805776727
Email: khalafalaa1994@gmail.com

Indeed, in vitro studies have shown that the production of specific IgE antibodies for different viruses and the ability of IgE to suppress some viruses plays an important role in IgE or a specific IgE antibody expression against viruses during viral pathogenesis [3-6]. High levels of IgE are an advantage for protecting against SARS-CoV-2. However, the protective effects related to higher expression of IgE in asthmatic patients are the result of the activity of their humeral immune system rather than higher levels of IgE, as the increasing IgE expression can lead to exacerbation of various syndromes [5,6]. Regarding the cell immunity associated with COVID-19 infection, blood samples with eosinopenia are detected in a high percentage of patients with acute COVID-19 infections in severe and less severe cases. The normalization of eosinophil counts showed improvement in clinical status in other cases [7]. Eosinopenia is inversely related to inflammatory markers and can be associated with the severity of COVID-19 [8]. The relationship between COVID-19 and allergic inflammatory response requires

characterization [9]. Therefore, the aim of the present study is to study the factors affecting some cells and immune mediators in patients infected with COVID-19 in Anbar Governorate.

Materials and Methods

Patients and Control individuals

A total of 163 adult participants representing adult convalescent COVID-19 patients showing the symptoms of respiratory bronchial spasm and 75 healthy participants as controls were included. The patients were visiting Ramadi and Fallujah Teaching Hospitals in Anbar Province, Iraq. The study was performed from September 2022 to May 2023. A questionnaire was used for data collection. Adult vaccinated or unvaccinated male and female patients who had recovered from COVID-19 and who still had respiratory bronchospasm were selected. Patients with asthma, chronic obstructive pulmonary disease, history of allergic bronchitis, history of skin allergy, allergic sinusitis, connective tissue diseases and those taking steroid medications were excluded. Venous blood sample (5 ml) was collected from each individual through venipuncture with a 10 ml sterile plastic disposable syringe. Each blood sample was divided into two portions: 2 ml of the sample was drawn using an EDTA tube for complete blood count (CBC), and the second (3 ml) was allowed to clot at room temperature and then centrifuged at 3000 rpm for 15 min. The serum was collected, immediately stored in a sterile plastic white tube, and stored at −20 °C to be used further for immunological tests.

Ethical Approval and Consent

The study was approved by the ethical approval committee at University of Anbar. Consent was obtained from each patient.

Complete Blood Count (CBC) Test

CBC tests were performed for blood specimens in EDTA tubes with an XN-350 Sysmex automated hematology analyzer and the results were reported.

Detection of IgM and IgG antibodies to the COVID-19 virus

The serum levels of IgM and IgG antibodies were quantitatively determined in the sera of patients and healthy individuals with a quantitative two-step sandwich enzyme immunoassay technique and the final fluorescence detection with VIDAS SARS-COV-2 IgG kit (Biomerieux, France).

Detection of Total IgE Antibodies

The serum levels of total IgE antibodies were quantitatively determined in the sera of patients and healthy individuals with an enzyme linked immunosorbent assay (ELISA) kit (DiaMetra UK).

Detection of Specific COVID-19 IgE Antibodies

Specific COVID-19 IgE Antibodies were qualitatively determined in the sera of patients and healthy subjects with a previously described method [10]. The IgE antibody specific for the COVID-19 antigen was identified through ELISA.

Preparation of COVID-19 Antigen Discs

Whatman filter paper No. 1 and a puncturing tool were used for preparing blank discs. The paper discs were kept in a sterile petri dish and sterilized with a UV light illuminator overnight. Sterility test was performed on each disc. The sterilized discs were immersed in a solution containing COVID-19 antigen (COVID-19 vaccine; Vero Cell) and inactivated COVID-19 antigen (Beijing Institute of Biological Products (China). The paper disks were dried in an incubator at 37 °C for 1 h. Dry discs were kept overnight in a refrigerator and in determining the specific IgE antibody on the next day.

Detection of the IgE Antibody Specific for the COVID-19 Antigen

A blank microtiter plate (96 wells) was employed. Wells 1–6 were used as controls. The other wells were used in testing specimens from patients and control sera: 1) Test discs were transferred and distributed on the microplate wells with sterile forceps. 2) Serum sample (50 µl) from each patient was added to a well. 3). The plate was covered with a plastic film, gently shaken for 30 s at 200 rpm with a microplate shaker, and incubated at room temperature for 90 min. 4) The wells were washed three times using a washing solution 5). An anti-IgE HRP conjugate (50 µl) was added to each well, and the plate was incubated overnight at room temperature. 6) The wells were

washed as in step 4. 7) An aliquot of a TMB substrate (100 µl) was added to each well, and the plate was incubated for 15 min at room temperature in the dark. After incubation, A stop solution (50 µl) was added to each well, and the solutions were mixed thoroughly. The paper disks were removed from the plate with sterile forceps, and then the optical density of each well was determined immediately with a microplate reader at 450 nm. The result was reported and plotted. The same procedures were performed for the control sera, and the results were reported and analyzed.

Results And Discussion

Factors affecting serological parameters in the study

Severity of COVID-19 infection in patients

Patients with severe COVID19 infection showed higher total and specific IgE levels ($P < 0.001$) and IL-5 level ($P < 0.0017$) than patients with mild infections and control individuals. This finding was in accordance with previous findings [11, 12]. This significant difference in the above parameters was due to the status of patients who were suffering from complicated COVID-19 infections leading to bronchoconstriction because of increases in total IgE and IL-5 levels and the release of the IgE antibody specific to COVID-19 antigens in atopic patients [10, 13]. This result was inconsistent with the findings [14, 15] (Table-1).

Table 1. Serological markers in the control and severely and mildly infected patients

Case of Patient	Total IgE	Specific IgE -ve	Specific IgE +ve	IL-5
Severe	188.0 ± 119.2	67%	33%	276.2± 223.5
Mild	92.38± 79.0	98%	2%	135.3± 137.5
Control Individuals	60.91± 34.9	-	-	62.67± 38.6
***P* Value**	< 0.0001 **	< 0.0001 **		< 0.0001 **

Vaccination of individuals

The proportion of vaccinated male patients (36; 69%) was higher than that of females (33; 49%). Significant difference ($P \leq 0.0230$) was found between them (Table-2).

Table 2. Vaccinated versus unvaccinated individuals

Vaccination	Male (N, %)	Female (N, %)	Chi-Square	Sig.	p-Value
Yes	36 (69%)	33 (49%)	5.167	*	0.0230
No	16 (31%)	35 (51%)			
Total	52 (100%)	68 (100%)			

The number of males that preferred taking a COVID-19 vaccine was significantly higher than that of females. Other studies revealed similar findings regarding COVID-19 vaccination [16]. No significant differences in the values of the studied serological parameters except IgG were found between the serum samples of the vaccinated and unvaccinated patients (Table-3).

Table 3. Serological parameters in COVID-19 post-infected cases with respect to vaccination

Parameter	Mean value ± SD		Sig.	p-Value
	None Vaccinated	Vaccinated		
IL-5	222.7 ± 194.4	226.7 ± 207.4	NS	0. 9141
IgM	0.5333 ± 0.2519	0.5203 ± 0.237	NS	0. 7724
IgG	18.48 ± 10.81	26.74 ± 9.328	**	<0.0001
IgE	142.5 ± 118.1	152.4 ± 112.0	NS	0. 6400

Consistent results were obtained for specific IgE (Figure 1). However, a study [17] showed that COVID-19 vaccines induce immediate hypersensitivity reactions in vaccinated individuals. Our findings were in accordance with those of another study [18], which revealed no significant difference in IgE result between the samples obtained from vaccinated and unvaccinated individuals. Moreover, no correlation was found between the specific IgE results after COVID-19 infection and vaccination status [19].

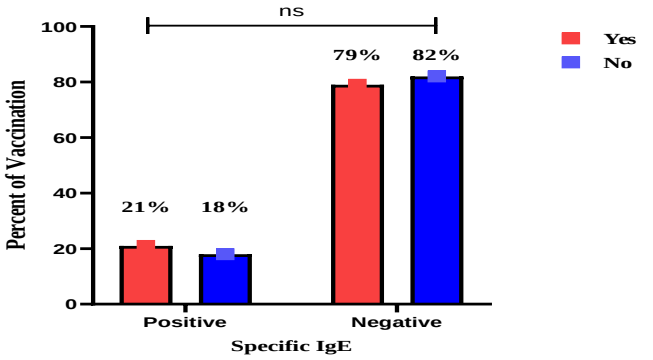

Figure 1. Specific IgE results in vaccinated and unvaccinated individuals.

Smoking habit

A total of 36 male patients (69%) were smokers, whereas only 3 females (4%) (Table 4). This difference might be due to the social habits in our community.

Table 4. Smoking habit in males and females

Smoking	Male (N, %)	Female (N, %)	Chi-Square	Sig.	p-Value
Smoker	36 (69%)	3 (4%)	56.44	**	<0.0001
Non-Smoker	16 (31%)	65 (96%)			
Total	52 (100%)	68 (100%)			

No significant differences in the values of the studied serological parameters were found. The mean values of IL-5, total IgE, and specific IgE are shown in Table 4 and Figure 2. This indicated that smoking habit does not appear to affect post –COVID immunological recovery and function. The virulence and inflammatory effects of COVID19 may override more subtle smoking induced effects [20].

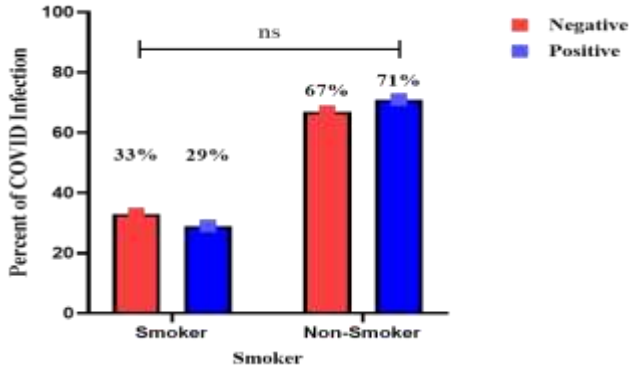

Figure 2. Effect of smoking on the specific IgE results of the studied individuals.

The present findings are not in accordance with the fact that exposure to tobacco smoke can influence innate immunity toward a pattern of respiratory diseases achieved by Th2 type and atopy and increase the risk of IgE-mediated allergen sensitization. It can also cause a worsening in symptoms and severity of asthma and rhinitis [21]. Smokers have higher IgE serum titers than nonsmokers [11, 20, 22].

Gender effect on the serological parameters of the studied individuals

Table-5 shows the results of the serological parameters in males and females. No significant differences in all the values of the studied serological parameters were found. The same observation was found in the results of specific IgE in males and females (Table-6).

Table 5. Serological parameters in males and females

Parameter	Mean Value ± SD		Sig.	p-Value
	Male	Female		
IL-5	224.2 ± 213.0	199.8 ± 183.6	NS	0.2329
IgM	0.531 ± 0.234	0.52 ± 0.26	NS	0.7956
IgG	23.6 ± 11.03	22.7 ± 10.5	NS	0.6650
IgE	145.6 ± 116.8	139.7 ± 111.4	NS	0.4818

Table 6. Specific IgE in males and females

Specific IgE	Male (N, %)	Female (N, %)	Chi-Square	Sig.	p-Value
-ve	42 (81%)	54 (79%)	0.03394	NS	0.8538
+ ve	10 (19%)	14 (21%)			
Total	52 (100%)	68 (100%)			

The present findings are consistent with the results of previous studies [23, 24], which found no statistically significant differences between males and females in terms of IL-5, IgM, IgG, and IgE levels. However, the findings varied from those obtained by one study [25].

Conclusion

The number of vaccinated male smokers was larger than that of vaccinated female worker. Basophil counts and IgE levels were higher in patients with severe COVID-19, and vaccinated COVID-19 patients had higher eosinophil and basophil levels than unvaccinated patients. Regarding studied immune factors, no significant differences were found between smoking and nonsmoking COVID-19 patients. IgE is one of the most important components of sensitivity and production of specific IgE antibodies for COVID-19 antigens, playing an important role in post-infection allergies and activating mast cells and basophils.

Acknowledgments

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Conflict of Interest

The authors declare that they have no conflict of interest.

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العوامل المؤثرة على بعض الخلايا والوسائط المناعية لدى مرضى كوفيد -19

الاء خلف بديوي، شهاب احمد لافي، حازم اسماعيل غزاي

. كلية الطب، جامعة الانبار، الأنبار، العراق

Email: khalafalaa1994@gmail.com

الخلاصة:

في مرض كوفيد-19، هناك استجابة مناعية شديدة الالتهاب تثيرها الخلايا، مثل العدلات وخلايا الدم الوحيدة، وتتضمن إطلاق الحركيات الخلوية المختلفة. يعد تضيق القصبات الهوائية أحد مضاعفات ما بعد عدوى كوفيد -19 بسبب مكونات فرط الحساسية مثل الضدIgE الكلي و IgE النوعي. اجريت هذه الدراسة للكشف عن تأثير بعض العوامل على بعض خلايا الدم القعدة، خلايا الدم الحامضية وخلايا الدم العدلة وبعض العوامل المناعية لدى المرضى بعد الإصابة بفيروس كورونا. شملت الدراسة 163 من مرضى كوفيد-19 البالغين المتعافين من كلا الجنسين الذين يشكون من تشنج القصبات الهوائية و75 مشاركًا سليمًا كمجموعة سيطرة. كان المرضى يترددون على مستشفيات الرمادي والفلوجة التعليمية، محافظة الأنبار، العراق خلال الفترة من ايلول 2022 إلى ايار 2023. فحص الدم الكامل للخلايا(CBC) ، الأجسام المضادة لفيروس كوفيد-IgM19 ، IgG، بالإضافة إلى الحركي الخلوي الخامسIL-5 ، IgEالكلي، وتم إجراء تحليل IgE النوعي بمستضد كوفيد-19 لكل فرد في الدراسة. تم الإبلاغ عن الجنس والشدة وتاريخ التطعيم والتدخين. وقد تم تحليل البيانات للكشف عن تأثير هذه العوامل على المعايير المصلية. بينت النتائج ان المرضى الذين يعانون من عدوى شديدة بكوفيد-19 ارتفاعًا في أعداد خلايا الدم القعدة والضد IgE الاجمالي والضد E النوعي. والحركي الخلوي الخامس (IL5) في المرضى الذين يعانون من التهابات خفيفة والسيطرة عليها. لم يتم العثور على فروق ذات دلالة إحصائية بين الجنسين في جميع المعايير المصلية المدروسة. وقد تم العثور على نفس الملاحظة بين الأفراد المدخنين وغير المدخنين. ليس للتطعيم أي تأثير على المؤشرات المدروسة باستثناء.IgG . أظهر المرضى الذين يعانون من عدوى شديدة بكوفيد-19 ارتفاعًا في مستوى الضد IgE الاجمالي والضد E النوعي. والحركي الخلوي الخامس (IL5). لا يوجد اختلاف بين الجنسين في قيم المعلمات المصلية المدروسة. ليس للتطعيم أي تأثير على المؤشرات المدروسة باستثناء الضد IgG.

الكلمات المفتاحية: كوفيد-19، الضد IgE النوعي، تضيق القصبات الهوائية. فرط الحساسية.