TOTAL AND SPECIFIC IgE IN SERA OF PATIENTS COMPLAINING BRONCHOCONSTRICTION POST-COVID-19 INFECTION

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Abstract. The link between infections and hypersensitivity reactions was revealed by many pioneers. Many researchers found that detectable IgE is specific to bacteria and viruses in sera of infected patients. Some patients undergo a post-COVID-19 bronchospasm due to a reaction to the viral antigens. This study aimed to reveal the association between COVID-19 infection and respiratory allergy in post-COVID-19 convalescent patients. The blood specimens were taken from each of the patients and control individuals. Complete cell blood count (CBC), anti-COVID-19 virus IgM, IgG, as well as IL-5, total IgE and IgE specific for COVID-19 antigen were done for each of patients and control individuals. The results: no significant difference was found in the mean number of the total WBCs, neutrophil and monocyte cells between patients and control individuals. Patients who were suffering from bronchoconstriction and showed high total and specific IgE revealed normal percentage of eosinophil and high basophil. The basophil ratio undergoes an increase in COVID-19 patients who were suffering from bronchoconstriction during and after post-COVID-19 infection. Furthermore, a positive correlation was found between IL-5, total IgE, COVID-19 antigen specific IgE and basophils in patients while a normal relation was found between parameters and control individuals. We can conclude that bronchospasm is one of the post-COVID-19 complications, due to a hypersensitivity reaction to the viral antigens. The production of COVID-19 virus antigen specific IgE has an important role in post-infection allergy as well as its relation to other factors like IL-5 in patients' sera.

Keywords: COVID-19, total IgE, specific IgE, bronchoconstriction.

List of Abbreviations

IgG – Gamma immunoglobulin
IgE – Epsilon immunoglobulin
Sars-Cov-2 – Severe Acute Respiratory Syndrome Coronavirus-2
COVID-19 – Coronavirus disease 19
CBC – Complete blood count
WBC – White blood cell
IL-5 – Interleukin-5
HIV – Human immunodeficiency virus
DAMPS – Damage associated material pathological substance
ELISA – Enzyme-linked immunosorbent assay
TMB – Tetramethylbenzidine
HRP – Horseradish peroxidase
OD – Optical density
Th2 – T-helper2

Introduction

Coronavirus disease 2019 (COVID-19) is an infectious contagious disease caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) that was first diagnosed in Wuhan, China, in December 2019 and is now Globally spread (Abedi *et al*., 2021). In general, the age and the inflammatory markers such as CRP, ESR, ferritin, D-dimer, and NLR showed higher medians in severe cases of COVID-19 compared to non-severe cases (Al-Humairi *et al*., 2022). But, many studies reported a clear relation between infection and hypersensitivity reactions, which is abnormal immunologic reaction due to allergen or antigen response (Kraft, 2000; Lafi, 2004; Dakhama *et al*., 2004). Furthermore, researchers found detectable IgE specific to bacteria and viruses in sera of infected patients (Dakhama *et al*., 2004; Khan *et al.*, 2008; Lafi, 2004). There is evidence that COVID-19 might shift immunity toward allergic inflammation based on elevated levels of mediators like immunoglobulin E (IgE), Eosinophils, Mast cell tryptase, and Cytokines linked to allergy post-infection (Hamad *et al.*, 2023). Indeed, *in vitro* studies have shown that the production of specific IgE for different viruses and the ability of IgE to suppress some viruses indicate an important role of IgE/or specific IgE ex-

pression against the virus in viral pathogenesis (Bergerson & Freeman, 2019; Klimek *et al*., 2020; Smith-Norowitz *et al.*, 2012; Tan *et al*., 2022). It seems that higher levels of IgE have 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 (Bergerson & Freeman, 2019; Tan *et al.*, 2022). On the other hand, 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, both in severe and less severe cases. Normalization of eosinophil counts showed improvement in clinical status in several of other cases (Jesenak *et al*., 2020). Eosinopenia is inversely related to inflammatory markers and can be associated with the severity of COVID-19 (Zhao *et al*., 2021). The relationship between COVID-19 and allergic inflammatory response requires more characterization (Oprea & Ferastraoaru, 2022). Therefore, the aim of the present study is the investigate this characterization in Covid-19 patients in Anbar Governorate, we devoted to reveal some allergic mediators in the sera of infected patients**.**

Materials and Methods

Patients and Control individuals

A total of 238 participants were enrolled in this study. They were sub-grouped into 163 participants representing COVID-19 post-infected patients who showed symptoms of respiratory bronchial spasm and 75 healthy participants who represent the control group. Patients with COVID-19 infection were attending Al-Ramadi Teaching Hospital and Fallujah Teaching Hospital in Ramadi Province, Iraq The study was done from September 2022 to May 2023. A questionnaire was used for data collection from patients and control individuals. The study included adult recovered convalescent COVID-19 patients from both genders who were vaccinated or not and still have respiratory bronchospasm. Excluded patients were known for asthma, chronic obstructive pulmonary disease, history of allergic bronchitis or any previous history of skin allergy, allergic sinusitis, connective tissue diseases and patients on steroid medication.

Approval of the study

A written consent was taken from all the participants in this study, the study was approved by the Approval Committee of University of Anbar according to the to the standards set by the latest revision of the Declaration of Helsinki and local legislative of the Approval Committee Guide lines, approval certificate was attached to the manuscript.

Specimens Collection

Five ml (5 ml) of venous blood specimens were taken from each individual included in this study, these were taken from both patients and control individuals via venipuncture using 10 ml sterile plastic disposable syringes. Each blood specimen was divided into two portions: two milliliters were drawn in an EDTA tube for complete blood count (CBC), the second portion was 3 ml which allowed to clot at room temperature, and then centrifuged at 3000 rpm for 15 min. The serum was taken and immediately stored in sterile plastic white tubes to be kept at -20 °C to be used further for immunological tests.

Complete Blood Count (CBC) Test

The test was done for blood specimens in EDTA tubes using (XN-350 Sysmex Automated Hematology Analyzer) and the results were reported.

Detection of IgM and IgG Antibodies to COVID-19 virus

Serum levels of IgM and IgG Abs were quantitatively determined in patients' serum and healthy control individuals using a quantitative 2-step sandwich enzyme immunoassay technique and the final fluorescence detection (ELFA) using a ready kit VIDAS® SARS-COV-2 IgG, Biomerieux, France.

Detection of Total IgE Antibodies

Serum levels of total IgE Abs were quantitatively determined in patient serum and healthy

control subjects using enzymatic linked immune-sorbent assay (ELISA) using a ready-to-use kit (DiaMetra).

Detection of Specific COVID-19 IgE Antibodies

Specific COVID-19 IgE Abs were qualitatively determined in patients serum and healthy control subjects following the method mentioned by Lafi (2004). An enzyme-linked immunosorbent assay method was used for the determination of IgE Specific for Covid-19 Antigen.

Preparation of Covid 19 Antigen Discs

Whatman filter paper No. 1, was used for the preparation of blank discs using a manual puncturer. The paper discs were kept in a sterile petri dish and sterilized with a UV light illuminator overnight. Sterility test was done for a sample of these discs to prove their sterility. Sterile discs were immersed with COVID-19 antigen (COVID-19 Vaccine (Vero Cell), Inactivated (Beijing Institute of Biological Products (China). The paper disks were allowed to dry in an incubator at 37C for one hour. Dry discs were kept overnight in the refrigerator to be used the next day for determination of Specific IgE.

Detection of IgE specific for Covid-19 Antigen

A blank Microtiter plate (96 wells) was employed for this purpose, wells from number 1 to number 6 were used as control wells. Other wells were used for test specimens from patients and control sera: 1) Test discs were transferred and distributed into the microplate wells using sterile forceps. 2) Fifty microliters (50 µl) of patient serum were added to their suitable wells. 3) The plate was covered with plastic film, gently shaken for 30 seconds at 200 rpm using microplates shaker and incubated at room temperature for 90 minutes. 4) Wells washed 3 times using washing solution. 5) Fifty microliter 50 µl of Anti IgE HRP conjugate was added to the wells, and the plate was incubated overnight at room temperature to react. 6) Washing as in step 4. 7) Aliquot of 100 µL of TMB substrate was added to each well. The plate was incubated for 15 min at room temperature in a dark place. Following incubation, 50 µL of stop solution was added to each well. The plate was mixed well. The paper disks were removed from the plate using sterile forceps then the optical density (OD) of each well was determined immediately, using a micro-plate reader at 450 nm. The result was reported and plotted. The same thing was done for control sera and the results were reported also.

Results

A – Hematology parameters in patients and control individuals

Regarding total white blood cells count, neutrophil and monocyte percentages, no significant difference was found in the mean number of these cells between patients and control individuals while significant difference was found in the number of basophils and lymphocytes (Fig. 1).

B – Serological parameters in patients and control individuals

Regarding total IgE, IL-5, IgM and IgG mean values, a significant difference was found between patients and control individuals except for IgM (Table 1).

C – Serological parameters in mild versus severe infections of COVID-19 in patients

Patients with severe COVID-19 infection showed higher total, specific IgE ($P < 0.003$) and IL5 ($P < 0017$) than patients with mild infections and control individuals.

D – Correlation between the studied parameters in patients

Results of the Pearson test confirmed that IL-5 is highly directly correlated with total IgE titer in post-COVID-19 patients with respiratory manifestation. The relationship between IL-5 and IgE levels in patients recovering from COVID-19 with persistent respiratory illness and hypersensitivity symptoms was previously investigated.

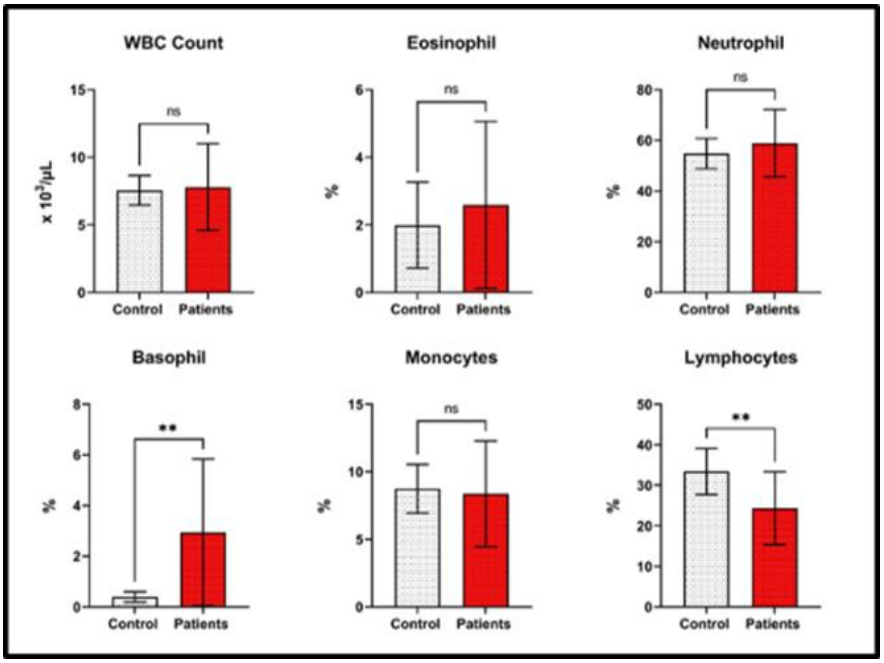

Fig. 1. Mean hematological parameters ± SD differences between control and COVID-19 post-infected cases

Table 1

Serological parameters between COVID-19 post-infected cases control

Parameter	Mean Value ± SD		SSig.	*p* Value
	Control	Patients		
IL-5	62.67 ± 38.6	225.0 ± 201.1	**	0.0017
IgG	0.29 ± 0.23	23.23 ± 10.8	**	<0.0001
IgE	60.91 ± 34.9	148.2 ± 114.3	**	0.0030
IgM	0.3571 ± 0.24	0.47 ± 0.22	NS	0.0838

Note: NS = non-significant. ** $p < 0.01$. SD = Standard Deviation

Table 2

Total and specific IgE & Il-5 in Sera of COVID-19 patients of different severity and control individuals

Case of patient	Total IgE	Specific IgE		IL-5
		-ve	+ve	
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	Negative		62.67 ± 38.6
P Value	< 0.0001 **	< 0.0001 **		< 0.0001 **

Note: ** $p < 0.01$. SD = Standard Deviation

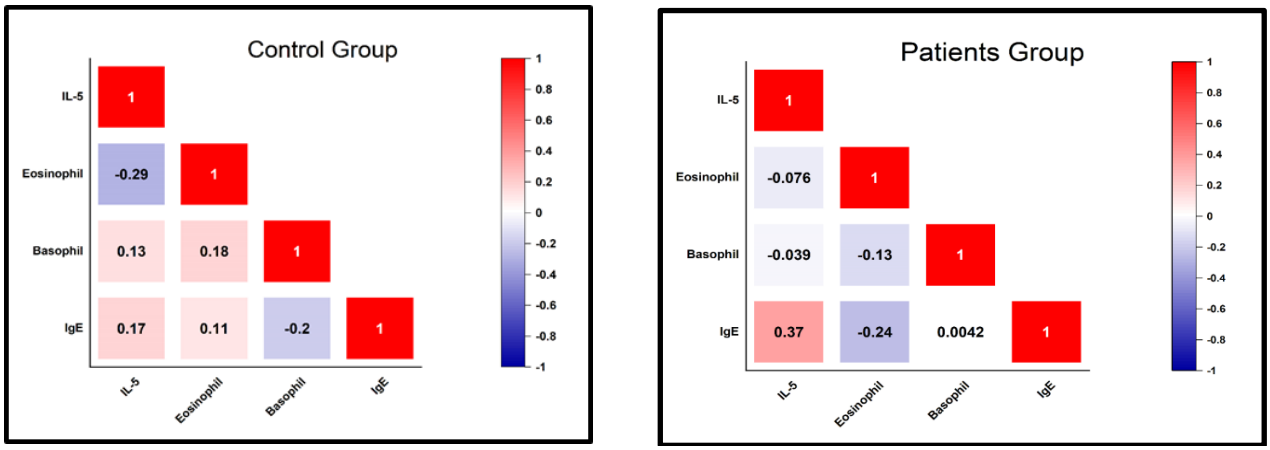

Fig. 2. A&B Correlation between the studied parameters in patients control individuals

Discussion

Regarding the above findings, our explanation for these findings was attributed to the inclusion criteria of patients. Patients included in this study were at the convalescent stage, and the majority of parameters reverted to normal values. Patients who were suffering from bronchoconstriction and showed high Total and Specific IgE revealed normal Eosinophil and high Basophil percentages. This was in accordance with the study done by (Yan *et al.*, 2021; Tan *et al.*, 2021; Xie *et al.,* 2021; Hamad *et al.*, 2023), who mentioned the normal or subnormal mean counts in eosinophils in the blood of COVID-19 patients complicating bronchoconstriction post-infection. These sequalae may be due to the effect of COVID-19 virus on eosinophils leading to a reduction of their numbers in patients (Alaaluah *et al.*, 2022; Zhao *et al.*, 2021). The basophil ratio undergoes an increase in COVID-19 patients who were suffering from bronchoconstriction during and after post-COVID-19 infection. This might be attributed to the viral antigens and metabolites leading to the stimulation of Th2 and other Damage Associated Material Pathological Substance (DAMPS) leading to the total and specific IgE reactions in the respiratory tract of infected patients particularly severe infections (Fig. 1) (Farmani *et al.*, 2021). Increased Lymphocyte ratio in patients was due to the nature of viral infection which always increases lymphocyte counts in infected patients except HIV (Delves *et al.*, 2017). This was different from the finding of (Kong *et al.*, 2020; Codd *et al.*, 2021) who found lymphocytopenia in COVID patients. This was attributed to the difference in the severity of disease in studied patients. The pioneers reported the above results in severe COVID-19 patients (Fig. 1). This was attributed to the effect of COVID-19 infection on the immune system of patients leading to the release of the above factors in patients. No significant results of IgM in the studied individuals were due to this controlled study being done on adult convalescent individuals only. Thus IgM undergo a decrease in sera of the patients at this time. Sera from normal control individuals showed negative results for specific IgE for COVID-19 antigen (Table 1). This was in accordance with the findings of (Farmani *et al.*, 2021; Hamad *et al.*, 2023). 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 due to an increase of Total IgE and IL-5 as well as the release of Specific IgE to COVID-19 antigens in atopic patients (Delves *et al.*, 2017). This result was agreed with the findings (Hamad *et al.*, 2023; Farmani *et al.*, 2021; Khan *et al.*, 2008; Zhang *et al.*, 2020) (Table 2). The result was in agreement with a previous study that showed a significant positive correlation between serum IL-5 and IgE levels in post-COVID patients (Hamad *et al.*, 2023; Iqbal *et al.*, 2022). The significant correlation indicates that in COVID-19 survivors with respiratory hypersensitivity, higher

IL-5 levels are associated with increased IgE, likely due to IL-5's role in promoting eosinophilic inflammation. On the other hand, results confirmed the inverse negative correlation between eosinophil and IgE. As eosinophil counts increased, IgE levels decreased, contrary to the typical positive association seen in other allergic conditions. It might suggest several potential mechanisms including IgE-independent eosinophilic inflammation (Gebremeskel *et al.*, 2021), IgE-mediated effects without eosinophilia (Fialho *et al.*, 2022), and dysregulation of type 2 cytokines linking eosinophils and IgE (MacCann *et al*., 2023) (Fig. 2A). In healthy control, the correlation between parameters was always within balance state, no significant correlation relationship was found between the parameters (Fig. 2B). This was similar to many findings of researchers who found no significant correlation between IL-5 levels and eosinophil counts, basophil counts, or IgE levels (Kandikattu *et al.*, 2019; Madhugiri *et al.*, 2023; Wilson *et al*., 2011). This suggests that under normal physiological conditions, IL-5 and eosinophil/basophil levels do not directly regulate IgE and allergic responses (Stone *et al.*, 2010).

Conclusion

One of the post-COVID-19 complications is bronchospasm due to a hypersensitivity reaction to the viral antigens. IgE is one of the most important components of sensitivity. Also, the production of specific IgE for different viruses and its important role in post-infection allergy as well as the increase of other related factors like IL-5 in sera of patients.

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