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Association between neutrophil recruitment and lung inflammation in type I hypersensitivity reaction

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Abstract. Type I hypersensitivity reactions can result in pulmonary inflammation via increasing the bronchospasm and mucous secretions in the airways of lungs; however, the participation of neutrophils in pulmonary inflammation mediated by type I hypersensitivity reactions are incompletely understood. The present study thus aimed to examine the association between neutrophil recruitment and lung inflammation in patients with type I hypersensitivity reactions. For this purpose, blood samples, that were obtained from 128 individuals with type I hypersensitivity reactions and 40 individuals who served as the controls, were analyzed to measure the levels of immunoglobulin E (IgE), basophils, neutrophils, platelet distribution width (PDW) and red blood cell distribution width (RDW). Moreover, sputum neutrophils were also measured in both the patients and the controls. In addition, consolidation areas were identified by specialist radiologist staff. The results revealed a significant increase in the levels of IgE and basophils corresponding to 91 and 79%, respectively, in patients with type I hypersensitivity reactions. The present study also observed a marked increase in the number of neutrophils in the blood and sputum of patients. Moreover, the results revealed that the levels of PDW and RDW were significantly increased in the patients. In addition, a marked association ($y=1.3061x+0.3546$) ($R^2=0.7067$) between the infiltrated neutrophils and the consolidation area in the lungs. On the whole, the present study demonstrates a notable association between neutrophil recruitment and lung inflammation in patients with type 1 hypersensitivity reactions; thus, neutrophils may prove useful as a therapeutic target for protection against pulmonary inflammation. Moreover, the presence of neutrophils in sputum may also be used as a biomarker of pulmonary inflammation mediated by type I hypersensitivity reactions.

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Introduction

The immune response has been shown to play an essential role in the protection of the human body against invading pathogens. However, an exaggerated immune response can result in inflammation that negatively affects the human body and this is known as hypersensitivity reactions (1). In fact, a variety of xenoantigens can be involved in hypersensitivity reactions. For instance, pollen, pollutants, food and medications have been observed to participate in type I hypersensitivity inductions (2). Exposure to the xenoantigens can lead to the development of anaphylaxis. Type I hypersensitivity reactions have been shown to be caused by the exaggerated secretion of immunoglobulin E (IgE) (3). This immune reaction can be induced by exposing the body to an antigen. The development of hypersensitivity diseases are indeed associated with various risk factors. These risk factors can be genetic or environmental factors (4-7). Presented antigens by antigen-presenting cells (APCs) can be recognized by T-cells that lead to the exaggerated production of IgE. Subsequently, IgE antibodies bind with their receptors on mast cells and eosinophils, and accordingly, release histamine and other mediators. Subsequently, these mediators induce high vasodilation that can lead to elevate mucous secretions in the lungs and bronchospasm (8,9). As a result, inflammatory reactions and lung edema in the pulmonary interstitial space can occur and may lead to severe pulmonary dysfunctions (8,10).

Platelets have been shown to play an essential role in pulmonary inflammation via activating neutrophil recruitment into lung tissue (10,11). It has demonstrated that platelet distribution width (PDW) may induce the activation of platelets and may be used as a marker of hypercoagulation and pulmonary inflammation in chronic obstructive pulmonary disease (COPD). Moreover, red blood cell distribution width (RDW) has also been used as a marker to predict the severity of COPD (12,13).

Consolidation is well-known as a pathogenic condition for lung tissue that can occur via the exudation of inflammatory cells and fluids into alveoli. The infiltration materials can be inflammatory cells, blood or inhaled water, and this condition

can lead to lung dysfunction (14). The hallmark of lung inflammation is the recruitment of pro-inflammatory immune cells into pulmonary inflamed sites. It has been shown that neutrophils are main immune cells that can be recruited to the site of pulmonary disease under various conditions. For instance, it has been found that neutrophils play an essential role in COVID-19-induced pulmonary infection (15), septic-induced lung infection (16,17) and systemic lung inflammation associated with acute pancreatitis (18,19). The infiltration of pro-inflammatory immune cells to lung tissue can lead to an increase in the viscosity of pulmonary mucus secretion, and can subsequently impair pulmonary gas exchange and cause systemic hypoxia (20). Thus, in the present study, neutrophils were measured as indicators of lung inflammation linked to hypersensitivity.

Subjects and methods

Design of study. The present study was performed on patients who suffered from type 1 hypersensitivity and aimed to study the link between infiltrated neutrophils and lung inflammation in type 1 hypersensitivity. The included patients attended the Al-Sader Teaching Hospital in Amarah, Maysan, Iraq during the period from October, 2022 to April, 2023. Ethics approval for the current study was obtained and authorized by the Ethics Committee of Al-Sader Teaching Hospital (Approval no. 2021-02). Written informed consent was obtained from all the participants.

Study subjects. The present study included 128 patients who were identified with type 1 hypersensitivity, as deemed by shortness of breath, wheezing, and high levels of IgE and basophils. In addition, 40 healthy individuals with no etiology of type 1 hypersensitivity served as the controls in the present study. Blood and sputum samples were collected from the patients and the controls to analyze serum IgE, blood neutrophils, basophil, PDW, RDW and sputum neutrophils. Moreover, the patients and controls were also subjected to chest radiographs in the unit of x-ray in Al-Sader Hospital by specialist radiologist staff and the existence or absence of consolidation were analyzed in a blinded manner.

The inclusion criteria for the study were as follows: i) Shortness of breath; ii) wheezing; iii) high IgE levels; iv) a high number of basophils; v) patients aged ≥18 years. However, the exclusion criteria were as follows: i) Patients who suffered from chronic illnesses; ii) patients who inhaled corticosteroids; iii) those who had pulmonary fibrosis; iv) patients with other diseases.

Measurement of IgE levels. A commercial kit (Human IgE ELISA kit, cat. no. 89-022-695, Thermo Fisher Scientific, Inc.;) was used to measure the levels of IgE in the serum of the patients and controls. This was performed as per the manufacturer's instructions.

Identification of sputum neutrophils. Sputum cells were identified as previously described (21). The induction of sputum was performed using 200 μg albuterol (Merck KGaA) that was administered to the study subjects 10 min prior through a metered-dose inhaler (MDI). The subjects were then nebulized with 3.5% saline using ultrasonic nebulizer at a rate of 3 ml/min for a total of 10 min. Deep cough was performed by subjects at intervals of 3 min for a total of 10 min and subjects were requested to perform mouth washing at each interval. In order to release infiltrated cells from pulmonary mucin, sputum samples were homogenized using 0.1% DTT, which contained 3% BSA (Merck KGaA) to protect the infiltrated cells. The mixture was then vortexed and placed on a tube rocker at room temperature for 10 min. The mixture was then subjected to filtration using a 48-μm nylon filter. Thereafter, centrifugation was performed to the filtrated mixture at 500 x g for 10 min at 4°C. Cold PBS (Thermo Fisher Scientific, Inc.) was added to the cell pellet and the cell pellet was then stained with Leishman stain (Micromaster Laboratories Pvt. Ltd.) as previously described (22). Briefly, for 5 min at room temperature, Leishman's stain was applied to the slides. Methanol, which was part of the dye preparation, was used to fix the cells. Before diluting the solution with an equivalent volume of pH 7.0 buffered water, the slides were kept for 2 to 3 min. Using a plastic Pasteur pipette, the buffered water was gradually added. These slides were then held at room temperature for 7 min. The surplus stain was then removed using buffered water. Once the slides had dried completely, neutrophils were identified under a light microscope (Olympus CX21, Olympus Corporation) using x100 high power fields.

Measurement of neutrophils, basophils, PDW and RDW in blood samples. After harvesting the blood samples from the patients and controls, CELL-DYN (Abbott Pharmaceutical Co. Ltd.) was used to count and classify white blood cells using MAPSS technology (https://www.gmi-inc.com/product/abbott-cell-dyn-3200-automated-hematology-analyzer/). The device also uses optical laser light scatter analysis to determine the PDW and RDW.

X-ray images analysis. According to regional protocols, all chest radiographs were obtained as digital radiographs in the X-ray Unit of Al-Sader Hospital. Specialist radiologist staff who were blinded to the patient data analyzed the existence or absence of consolidation. A homogeneous opacification that conceals the blood vessels and airway walls was described as consolidation.

Statistical analysis. The data were analyzed using SigmaStat 10.0 software. The t-test and linear regression were used for statistical analysis. The data are presented as the mean values ± standard error of the mean. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Characteristics of patients with hypersensitivity type 1. The data from 128 patients with hypersensitivity type 1 were obtained. There were 71 males and 57 females with ages ranging from 18 to 53 years and a mean ± SE age of 33.039±0.844 years. There were 89.84% of hypersensitivity type 1 patients who suffered from shortness of breath. Additionally, 87.5% of patients with hypersensitivity type 1 were suffered from wheezing (Table I).

Table I. Characteristics of patients with hypersensitivity type 1.

Characteristics	Patients (n=128)
Age, years	
Mean ± SE	33.039±0.844)
Range	18-53
Sex (%)	
Male	55.47
Female	44.53
Shortness of breath (%)	
Yes	89.84
No	10.16
Wheezing (%)	
Yes	87.5
No	12.5

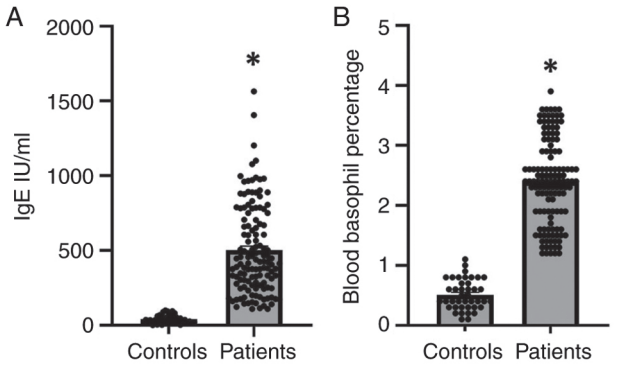

Figure 1. Measurement of the levels of (A) IgE and (B) basophils in patients with hypersensitivity reactions and healthy controls. Data represent the mean ± standard error of the mean. *P<0.05 vs. the controls. IgE, immunoglobulin E.

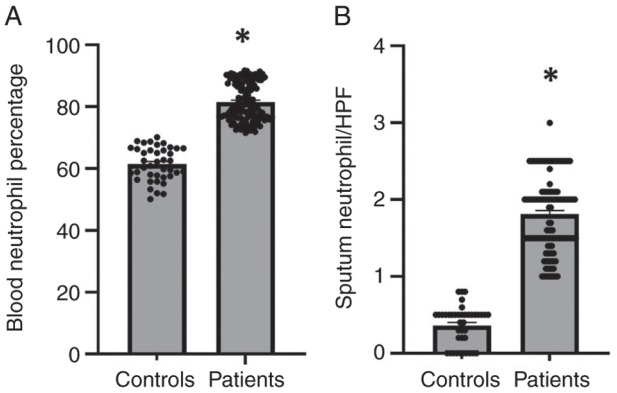

Figure 2. Estimation of neutrophils in the blood and sputum of patients with hypersensitivity reactions and healthy controls. (A) Percentage of neutrophils in blood samples. (B) Number of infiltrated neutrophils into sputum. Data represent the mean ± standard error of the mean. *P<0.05 vs. the controls.

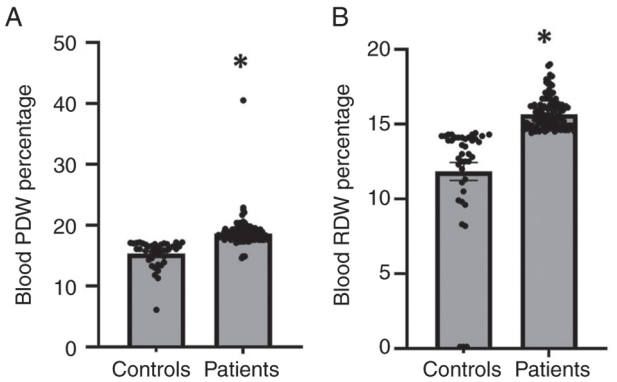

Figure 3. PDW and RDW in patients with hypersensitivity and healthy controls. (A) Percentage of PDW in blood samples. (B) Percentage of RDW in blood samples. Data represent the mean ± standard error of the mean. *P<0.05 vs. the controls. PDW, platelet distribution width; RDW, red blood cell distribution width.

Levels of IgE and basophils in patient with hypersensitivity and the controls. It is well known that IgE has been used as a common indicator for the diagnosis of type 1 hypersensitivity (4). Moreover, basophils have been established to play a critical role in type 1 hypersensitivity (23). In the present study, type 1 hypersensitivity was identified in patients and healthy controls based on the measurement of the levels of IgE and basophils (Fig. 1). The results revealed that the levels of IgE were significantly increased by 7-fold in the serum of patients with type 1 hypersensitivity compared to the controls (Fig. 1A). In addition, the results demonstrated that the levels of basophils were substantially elevated in the blood of patients with hypersensitivity reactions as compared to the controls (Fig. 1B). Accordingly, elevated levels of IgE and basophils may explain the induction of type 1 hypersensitivity reactions.

Estimation of neutrophils in the blood and sputum of patients with hypersensitivity and the controls. Infiltrated neutrophils have been shown to play an essential role in the inflammatory response (24). The present study first examined the percentage of neutrophils in blood of patients with hypersensitivity and the controls (Fig. 2). The results revealed that the neutrophil percentage was significantly elevated (P<0.01) in the blood of patients with hypersensitivity compared with the controls (Fig. 2A). Furthermore, we explored the infiltration of neutrophil into lung tissue through examining the number of neutrophils in the sputum of patients and control groups (Fig. 2B). It was also observed that the number of infiltrated neutrophils was substantially increased by 5-fold in the sputum of patients compared with the control group (Fig. 2B). Consistently, the high levels of neutrophils could explain the lung inflammation and exudates into the pulmonary airways that result in shortness of breath in patients with hypersensitivity reactions.

PDW and RDW in patients with hypersensitivity and the controls. Subsequently, the present study examined the PDW and RDW in patients with hypersensitivity and the controls (Fig. 3). The results of statistical analysis revealed that the PDW was substantially increased in patients with hypersensitivity compared with the controls (Fig. 3A). Moreover, the results demonstrated that the RDW was markedly elevated in patients with hypersensitivity compared with the controls (Fig. 3B). Thus, an increased PDW and RDW may indicate,

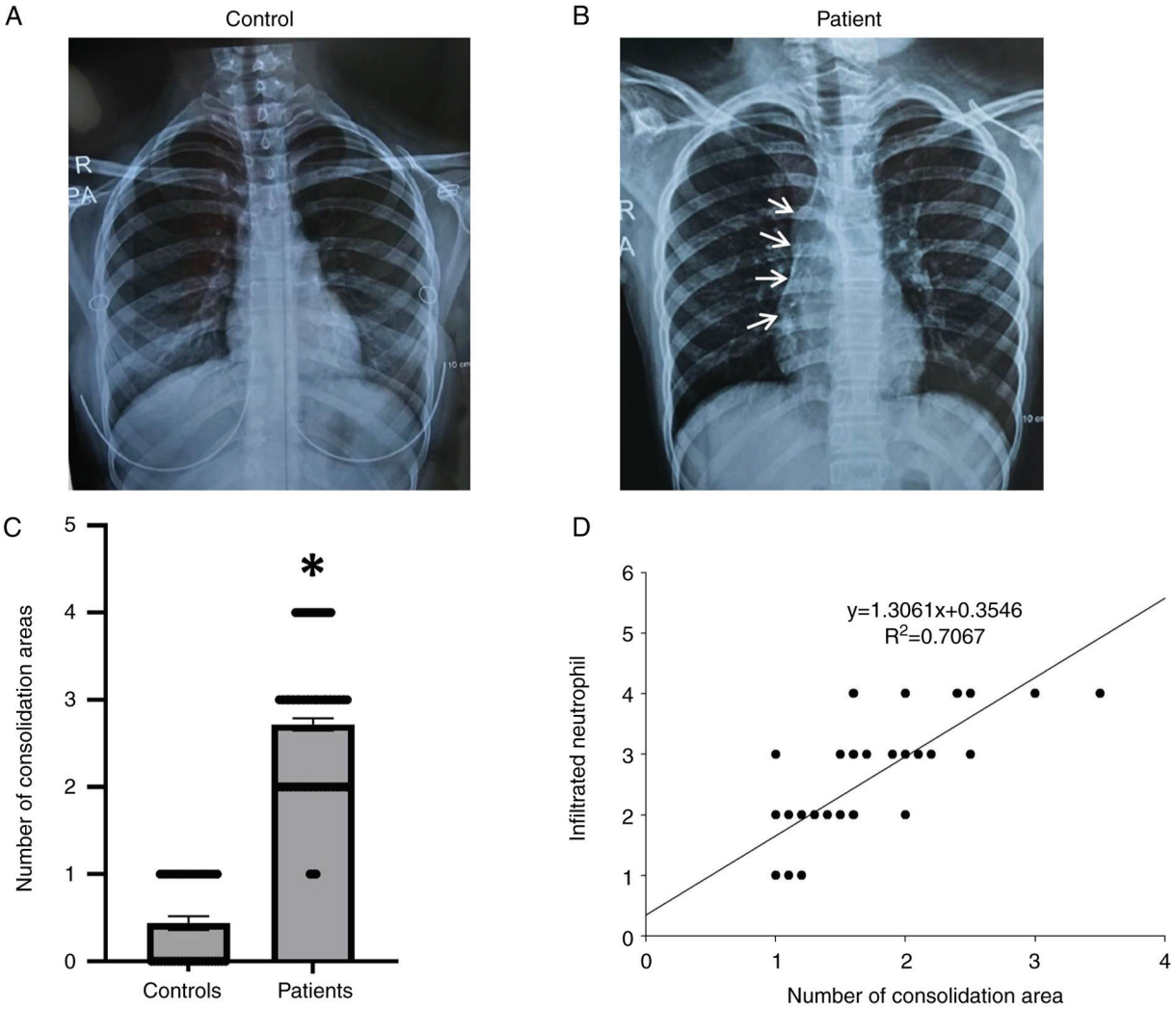

Figure 4. Lung consolidation and neutrophil navigation. X-ray images of the lungs in (A) a patient from the control group, and (B) a patient with hypersensitivity (consolidation areas are indicated by white arrows). (C) Consolidation area in the lungs of controls and patients with hypersensitivity. (D) Association between consolidation area and sputum neutrophil as represented by (y=1.3061x+0.3546) (R^2=0.7067). Data represent the mean ± standard error of the mean. *P<0.05 vs. the controls.

at least partly, the inflammation and disease severity in patients with hypersensitivity reactions.

Neutrophil navigation and lung inflammation in patients with hypersensitivity. Consolidation is a pathogenic condition that affects lung functions. The present study examined the area of consolidation in the lung tissue of patients with hypersensitivity and healthy controls using X-rays (Fig. 4A-C). The results indicated that the consolidation area was substantially increased by 70% in the lung tissues of patients with hypersensitivity reactions compared with the controls (Fig. 4A-C). Subsequently, the present study also examined the association between infiltrated neutrophils and lung inflammation in patients with hypersensitivity reactions. The results revealed a significant association (P<0.001) between neutrophil recruitment and lung inflammation with respect to the consolidation area in the lung tissues of patients with hypersensitivity reactions (Fig. 4D). Therefore, the association between infiltrated neutrophils and consolidation areas may reflect the levels of inflammation and secreted fluids in the lungs of patients with hypersensitivity reactions.

Discussion

Neutrophil infiltration has been demonstrated to be linked to lung inflammation. In the present study, the results demonstrated that the number of neutrophils was substantially increased in the blood and sputum of patients with hypersensitivity reactions. Moreover, the RDW and PDW were also elevated. Furthermore, the consolidation area was increased in the lungs of patients with hypersensitivity reactions. Therefore, targeting neutrophil navigation may decrease lung inflammation in patients with hypersensitivity reactions.

The immune system is crucial for maintaining bodily health and protecting against pathogen invasion. However, the same mechanism can also lead to heightened inflammatory and immunological reactions, which have adverse effects and are known as hypersensitive reactions (1). Antigen-presenting cells (APCs) pass allergens on to T-cells during the sensitization stage of hypersensitivity type I. The T-cells will signal once the B-cells have been induced to produce IgE antibodies, which bind to the Fc receptors on mast cells and basophils (8). The present study demonstrated that the serum levels of IgE

were significantly increased (P<0.001) in patients with hypersensitivity reactions (Fig. 1A). Moreover, the results of the present study revealed a marked increase in the levels of basophil in patients with hypersensitivity reactions (Fig. 1B). These results are in line with data form a recent study demonstrating that IgE antibody mediates type 1 hypersensitivity (25). In fact, the binding of IgE antibodies to mast cells and basophils can lead to the stimulation of degranulation and produce mediators, such as histamine, proteolytic enzymes, cytokines and platelet-activating factors. Subsequently, the produced mediators can cause inflammation in the lung tissue and this could explain the link between increased levels of IgE and lung inflammation in patients with hypersensitivity reactions.

It is considered that neutrophils contribute to pulmonary inflammation (26). Moreover, increased levels of pulmonary neutrophils, which can emit a variety of pro-inflammatory cytokines and chemokines (27), as well as proteases that contribute to the emphysema development, are a defining characteristic of lung inflammation (28,29). A non-invasive approach for determining the amount of neutrophils in the airway spaces is induced sputum (30-32). In the present study, it was observed that the levels of infiltrated neutrophils were markedly elevated in the sputum of patients with hypersensitivity reactions. A previous study found that neutrophils play a critical role in IgE-mediated hypersensitivity reactions (33), and these findings were indeed in line with the results of the present study. Infiltrated neutrophils to the lung tissue can release reactive oxygen species that subsequently damage pulmonary tissue. Moreover, infiltrated neutrophils have been shown to produce neutrophil extracellular traps (NETs), which can lead to an increase the viscosity of pulmonary mucus secretion and can subsequently impair pulmonary gas exchange and cause systemic hypoxia (18,24). Moreover, neutrophils have been shown to play an essential role in various lung infections. For instance, neutrophils have been demonstrated to resist the invading pathogens in the lungs. However, it has been suggested that neutrophils may also contribute to exuberate pulmonary inflammation by producing different proteases, as well as expelling web-like structures known as NETs (17,19). The accumulation of NETs in the pulmonary airways causes lung tissue damage and increases mucus viscosity, leading to shortness of breath. In fact, since neutrophils are suspected to play an essential role in pathophysiology (34,35), the measurement of infiltrated neutrophils can be easily performed in the target organ using a non-invasive method (4,5); thus, measuring induced sputum neutrophils fulfills some of the ideal criteria for a biomarker of lung inflammation linked to hypersensitivity reactions. Future studies are required however, in order to examine the potential utility of this biomarker in hypersensitivity in further detail. It should be mentioned that PDW and RDW have been documented to be connected to lung inflammation. The present study found that the PDW and RDW were substantially increased in patients with hypersensitivity reactions. Moreover, the findings of the present study revealed that consolidation area was significantly increased in patients with hypersensitivity reactions. Notably, the present study also demonstrated a substantial association between infiltrated neutrophils in the sputum and the consolidation area in the lung tissue, which could explain the pulmonary inflammation in patients with hypersensitivity reactions.

In conclusion, the results of the present study demonstrate a substantial association between neutrophil navigation into lung airways and lung inflammation in patients with hypersensitivity reactions. In addition, the findings presented herein reveal that consolidation areas are strongly linked to neutrophil infiltration. In fact, targeting the navigation of neutrophils may reduce the inflammation and the viscosity of mucus secretion in the pulmonary airways and may subsequently enhance pulmonary gas exchange in patients with hypersensitivity reactions. This strategy may indeed have potential implications in the clinical management of such patients. However, the present study has certain limitations which should be mentioned. The authors were not able to obtain consent from patients to illustrate the infiltration of neutrophils in the lung tissues using hematoxylin and eosin staining. Moreover, neutrophils are essential immune cells for protecting the body against pathogens, and targeting them results in disorders in the immune response in real clinical practice. Thus, future studies are required to explore the exact role of neutrophils in hypersensitivity reactions. Additionally, further studies are also warranted to determine the signaling mechanisms involved in the navigation of neutrophils into lung tissue in patients with hypersensitivity reactions.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

KIJ and RM conceived the study. RM, AA, MCM and KIJ were involved in the study methodology. RM and AA were involved in the investigative aspects of the study. RM, AA, MCM and KIJ were involved in data analysis. RM, AA, MCM and KIJ were involved in the writing and preparation of the original draft of the manuscript, and in the writing, review and editing of the manuscript. RM, AA and MCM were involved in preparation of the figures. RM supervised the study. RM and AAL were involved in project administration. All authors have read and approved the final manuscript. RM and AA confirm the authenticity of all the raw data.

Ethics approval and consent to participate

The Maysan Health Department/Al-Sadr Teaching Hospital in the province of Maysan, Iraq was the authorizing committee that approved the present study. Written informed consent was obtained from all the participants (patients and controls).

Patient consent for publication

Written informed consent was obtained from the participants (patients and controls) for the publication of the X-ray images depicted in Fig. 4.

Competing interests

The authors declare that they have no competing interests.

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