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Research Article

Development of Asthma Mouse Model By Dermal Sensitization

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Abstract

Background: Urbanization is often associated with the increased asthma prevalence in recent times. Asthma prevalence in Khartoum, Sudan has risen to 18.2% among children aged 13–14 years. Extensive research has been done on the prevalence and triggering factors of asthma, however, no experimental research using animal models has been done. Thus, this study aims to develop asthma phenotype in mice with TDI sensitization.

Methods: This study was a controlled experimental study in which 24 BALB/Lac mice were equally divided into control (G1) and treatment (G2) groups. G1 was treated with 25 μL 2:3 acetone olive oil (vehicle) applied dermally on days 1, 3, 7, and 10. G2 was treated with 25 μL of 0.3% TDI in acetone olive oil (AOO) applied on the same days. Autopsy and samples (blood, bronchoalveolar lavage fluid [BALF], and lung tissue) collection were performed on day 12. Results were analyzed using *t*-test on the SPSS.

Results: A statistically significant increase in neutrophils and eosinophils was observed in the blood of TDI-sensitized mice (G2) with a reduction in lymphocytes. In the TDI group, a significant increase was seen in the BALF, neutrophils, lymphocytes, and basophils; the increase in eosinophils and monocytes was nonsignificant. Besides, lung-related histopathological changes in the TDI group were hyperemia, leukocytic infiltration, thickening of bronchoalveolar walls, and damage of respiratory epithelium.

Conclusion: TDI-sensitized mice showed a significant increase in granulocyte count, especially neutrophils and eosinophils, both in the blood and BALF with inflammatory and allergic lung tissue changes. These changes confirmed the allergic responses and the development of asthma phenotype.

Keywords: asthma, mouse model, Sudan, TDI, dermal sensitization

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1. Introduction

The rising urbanization and industrialization in the recent decades has affected the prevalence of asthma prevalence, which is now considered the most common chronic respiratory problem with a prevalence of up to 15 – 20 % in some countries [1]. In Khartoum state (Sudan) asthma prevalence was found to be 18.2% amongst those aged 13–14 years [2]. This situation necessitates the need to investigate the causes and the possible further treatments of the disease. Therefore, an experimental animal model is a must for doing the initial trials. Animal models have been used to study atopic asthma [3]. Ovalbumin is often used to sensitize the animals [4]. Toluene di-isocyanate (TDI), which is used in many industries like paints and plastic industries, is the most prevalent agent in occupational asthma even at low concentrations and depending on the duration of exposure [5, 6], thus, it is used as a respiratory sensitizer in experimental mice [7]. Jeroen *et al.* (2004) succeeded in creating an experimental asthma mouse model using TDI. In their model, mice are dermally sensitized with TDI on the dorsum of their ears on days 1 and 8. On day 15, a respiratory challenge was applied through intranasal instillation of a low dose of TDI, then challenged with methacholine. 24 hrs later mice showed symptoms of hyperreactivity and bronchial obstruction compared to the control mice [8].

Rationale: Such an asthma model was needed in Sudan to study the triggers of asthma as well as the materials that suppress its incidence and exacerbation.

2. Materials and Methods

2.1. Animals (mice)

24 BALB/Lac mice [7] were used to develop asthma mouse model in Sudan. The mice were brought from the experimental animals' unit of the Central Laboratory of Veterinary Research (Soba) and kept in the animal house at the Faculty of Pharmacy, University of Khartoum. Animals were kept at room temperature with about 12 hrs light–dark cycle and fed ad-libitum with dietary pellets containing wheat flour, oil, milk powder, and dried poultry meat. The mice were left for about two months before starting the procedures.

2.2. Study design and procedures

The study was a controlled experimental study in which the 24 mice were divided into control (G1) and treatment (G2) groups of 12 mice each. G1 was treated with 25 μL 2:3 acetone olive oil (vehicle or adjuvant) applied dermally on the dorsum of each ear on days 1, 3, 7, and 10, according to the previous protocols. G2 was treated with 25 μL of 0.3% TDI in acetone olive oil (AOO) applied on the dorsum of each ear on days 1, 3, 7, and 10 (TDI is a known respiratory sensitizer associated with plastic industry). On day 10 after sensitization, both groups were induced with methacholine inhalation, which was followed by autopsy and samples collection on day 12.

2.3. Autopsy and samples collection

On day 12, the treated mice were anaesthetized by chloroform inhalation, then the areas of the neck, chest, and abdomen were dissected.

2.4. Blood sampling

1 ml of blood was taken from the inferior vena cava using 1 ml disposable syringe (Figure **1**), and 1 drop was spread for differential leukocyte count. Blood was centrifuged and serum was collected for immunoglobulins (IgE) and interleukins.

2.5. Bronchoalveolar lavage fluid (BALF) collection

Following the removal of the lymph nodes, the trachea was made free by removing the surrounding tissues, a piece of sewing thread was pulled underneath the trachea. Small incision (hole) was made by a scissor in the upper part of the trachea and a pink cannula (size 20) was inserted into the trachea to a point just before its bifurcation. Once inserted, the sewing thread was tied around the trachea (one node) fixing the cannula. Using 1 ml disposable syringe 1 ml normal saline was injected through the cannula into the lungs and then after 10–20 sec the fluid was withdrawn back into the syringe. This procedure was repeated two more times collecting BALF in a conical plastic tube (Figure **2**). It was used for differential leukocytes count and can be used for IgE and interleukins detection.

2.6. Isolation of the heart–lung complex

After completing the collection of BALF, the same syringe was used to inject 0.5–0.7 ml formaldehyde solution through the cannula into the lungs till they fully expand. The trachea was tied and closed using the sewing thread around it while withdrawing the cannula, another node in the sewing thread was made. The trachea was cut distal to the tied node and gently pulled removing the heart–lung complex from the thoracic cavity. The complex was kept in 10% formaldehyde (formalin) solution to be used for histopathology.

2.7. Statistical analysis

The obtained data from differential leukocytes count of blood and BALF were statistically analyzed using the independent *t*-test through the SPSS statistical program. Data were presented as mean and standard deviation (SD). $P < 0.05$ was considered statistically significant.

3. Results

3.1. Blood differential leukocytes count

Neutrophils were the most common in the two groups with the highest percentage (49.25%) in G2. Lymphocytes were next (44.5% in G1 and 41.5% in G2). Eosinophils were found to be 2.25% in G1 and 3% in G2 (Table 1). There were statistically significant increases in the percentages of circulating neutrophils ($P = 0.022$) and eosinophils ($P = 0.024$) in the TDI-sensitized mice (G2) compared to the control group (G1) with a reduction in circulating lymphocytes ($P = 0.010$) in G2 (Table 1; Figures 3 & 4).

3.2. BALF differential leukocytes count

Neutrophils, lymphocytes, and basophils showed significant increases in the TDI-treated group (G2) compared to the control (G1). Eosinophils showed an increase in the TDI group (G2) compared to the control (G1), but the difference was not statistically significant ($P = 0.14$). Moreover, monocytes

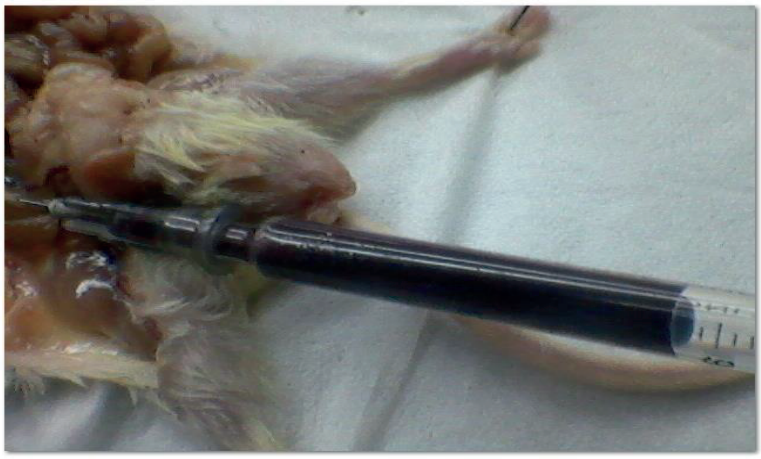

Figure 1: Blood sampling from the mouse inferior vena cava.

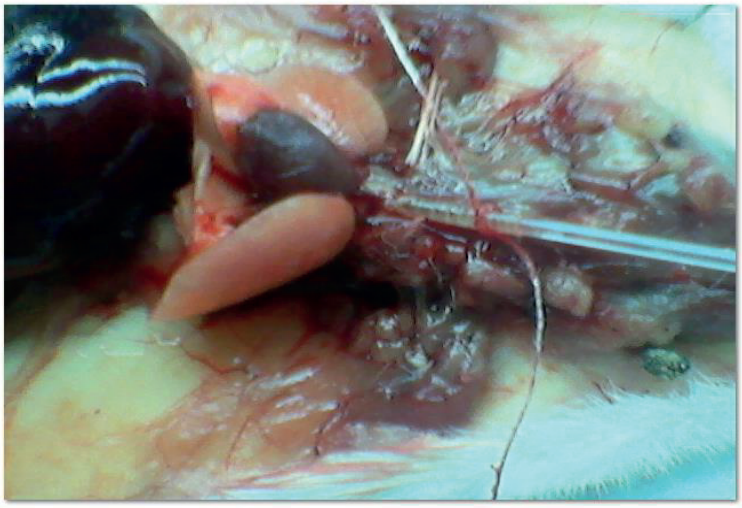

Figure 2: Bronchoalveolar lavage fluid (BALF) collection in mice.

showed statistically nonsignificant increase in G2 (P = 0.071; Table 1; Figures 3 & 4).

3.3. Lung histopathological findings in the TDI (G2) and control (G1) groups

Positive inflammatory changes were observed in the histopathological lung preparation of TDI-sensitized group of mice (G2). The inflammatory changes include hyperemia, infiltration with leukocytes, thickening of bronchoalveolar walls, and damage of respiratory epithelium surrounding the airways. No inflammatory changes were noticed in the lungs of the control group (G1) that received only the vehicle AOO (Figure **5**). Lung histopathological examination of TDI-sensitized mouse showed damaged respiratory epithelium with numerous goblet cells, markedly thickened respiratory membrane, and mononuclear infiltration in peri-bronchial area.

4. Discussion

The pulmonary inflammatory response in our model in response to sensitization was evident

TABLE 1: Differential leukocytes count in blood and BALF.

Leukocyte	Group	Blood			BALF		
		Mean %	SD	P-value	Mean %	SD	P-value
Neutrophils	G1	45.75	0.96	0.022	44.57	2.07	0.00
	G2	49.25	2.06		55.86	3.89	
Lymphocytes	G1	44.50	1.00	0.01	37.14	3.18	0.00
	G2	41.50	1.29		45.29	2.29	
Monocytes	G1	6.25	0.50	0.207	5.29	2.29	0.07
	G2	6.75	0.50		7.00	0.00	
Eosinophils	G1	2.25	0.50	0.024	1.43	1.13	0.14
	G2	3.00	0.00		2.143	0.38	
Basophils	G1	0.25	0.50	0.537	0.286	0.488	0.028
	G2	0.50	0.58		1.00	0.577	

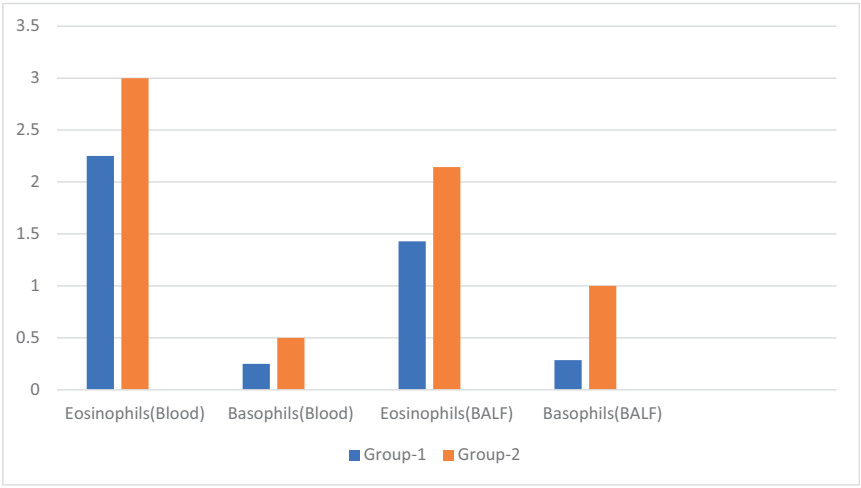

Figure 3: The effect of TDI sensitization on eosinophils and basophils in the blood and BALF of BALB/c mice.

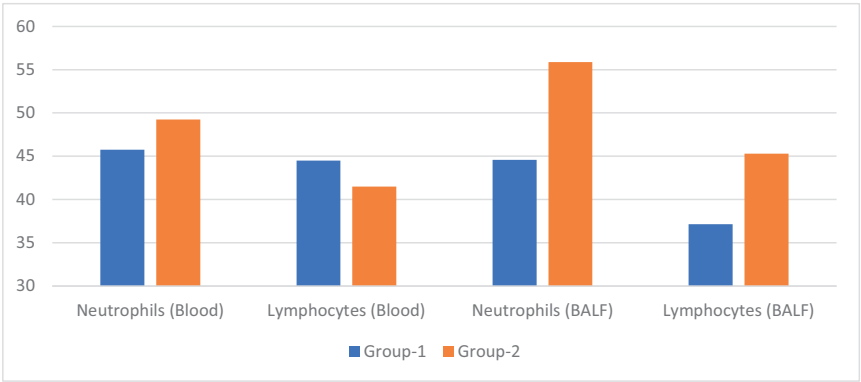

Figure 4: The effect of TDI sensitization on neutrophils and lymphocytes on the blood and BALF of BALB/c mice.

by the significant increase in granulocytes in the TDI-sensitized group both in blood and BALF. Neutrophils and eosinophils were more prominent. Many asthma models had used mice

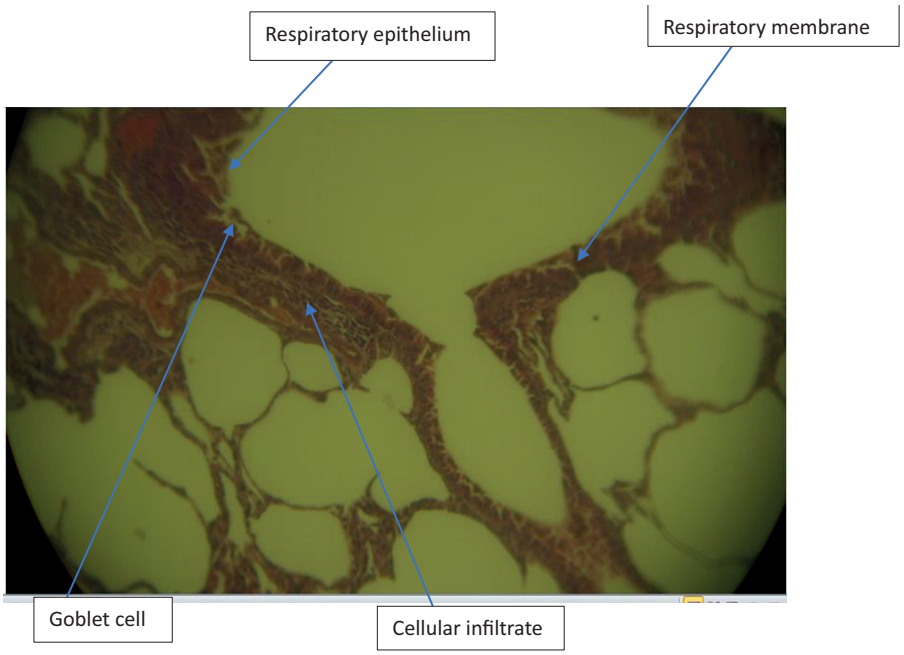

Figure 5: Light microscopy of lung histopathology of TDI-sensitized mouse.

and TDI sensitization both dermally and through inhalation. The inflammatory responses in those models were mainly increases in granulocytes both in blood and BALF; the same results were obtained in our model. Jung and Park's findings help explain our results that in TDI-sensitized models, neutrophils chemotactic activity increases 10 mins after provocation [9]. The tissue changes in those model as in ours were lung tissues hyperemia, infiltration with leukocytes, thickening of bronchoalveolar walls, and damage of respiratory epithelium surrounding the airways [10–15]. The part which was done by all aforementioned models and not done by ours due to technical difficulties were the measurement of serum IgE and lymphocytes subpopulations. The significant increase in lymphocytes count in BALF in our model was supported by Seigo Okada *et al.* [16]. In most models, the allergic response in sensitized mice was associated with increase in granulocytes rather than in agranulocytes [17]. This result was evident in our model where lymphocytes count in the blood decreased.

5. Conclusion

It is concluded that mice sensitization with TDI resulted in significant increase in granulocytes count specially neutrophils and eosinophils both in blood and BALF. Sensitization resulted in inflammatory changes which include hyperemia, infiltration with leukocytes, thickening of bronchoalveolar walls, and damage of respiratory epithelium surrounding the airways. The obtained asthma mouse model can be used in a wide range of asthma research. All these changes confirmed the allergic changes and the development of asthma phenotype. The obtained results have provided the minimal requirement for the development of mouse asthma model, because serum and BALF IgE, interleukins, and lymphocytes subpopulations of auricular lymph nodes homogenate have not been investigated

yet which could be considered as limitations, and these investigations are recommended in future trials.

Declarations

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Ethical Considerations

An ethical approval was issued by the Faculty of Medicine, National Ribat University.

Competing Interests

None.

Availability of Data and Material

Data presented in this article or any additional information shall be available upon request to the corresponding author.

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None.

Abbreviations and Symbols

AOO: Acetone olive oil

BALF: Bronchoalveolar lavage fluid

SD: Standard deviation

TDI: Toluene di-isocyanate

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