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**ORIGINAL ARTICLE**

# Role of CD8+ T Cells in Bronchial Asthma: A Case-Control Study

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## ABSTRACT

**Keyword**: Asthma, CD8, eosinophils, indicator.

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**Background:** Individuals with asthma experience rapid deterioration in their lung function. Although it is commonly known that CD4+ T lymphocytes are essential for the development course of asthma, less is known about the function of CD8+ T cells which could contribute to the severity of asthma. **Purpose:** To evaluate the relation between CD8 levels and the severity of bronchial asthma, as well as to analyze the discriminative performance of CD8 in distinguishing between asthmatics that are under control and those that are not. **Methods:** This case-control study included 120 asthmatic individuals and 120 non-asthmatics. The diagnosis of asthma was accomplished in accordance with GINA recommendations[1]. The asthma control test (ACT), The spirometry test, and the CD8 level calculation were performed for each study participant. **Results:** CD8 (%) showed a significant variance between mild, moderate and severe persistent cases (p-value = 0.001). CD8 (%) was elevated in uncontrolled patients in contrast to well controlled. A significant positive correlation between CD8 (%) and Eosinophils in sputum (r = 0.94). Conversely, a significant negative correlation was observed between CD8(%) and the FEV1 (p-value = 0.001) (r = -0.66). **Conclusion:** Both the severity of asthma and level of control were associated with CD8%.

## INTRODUCTION

There are about 300 million people with asthma in the world, the most prevalent chronic lung illness. A history of respiratory symptoms, including coughing, tightness in the chest, wheezing, and shortness of breath, that change in severity and over time, as well as changing expiratory airflow limitation, are indicative of the disease. Furthermore, it presents as a multifaceted disease that is often marked by persistent inflammation of the airways[2].

The majority of studies on the function of T lymphocytes in asthma have concentrated on CD4+ T cells in relation to chronic airway inflammation, despite the fact that asthma is linked with a variety of clinical and cellular traits. The underlying inflammation associated with asthma may be exacerbated by the cytokines that CD4+ T cells generate, such as IL-4, IL-9, and IL-13[3].

The idea that pathogenic type 2 secretes CD4+ Th2 cells in the etiology of asthma was validated by numerous later investigations, which dominated asthma research and thought. Crucially,

several of these investigations did not specifically look at the existence or functionality of CD8+ T-lymphocytes. Furthermore, even though there were more CD8+ T-cells than CD4+ T-cells in numerous molecular investigations that found type 2 signatures in cells from asthmatics' airways, the precise cell origin of these cytokines was not identified. The identification of additional type 2 cytokine-secreting cells in the lung and the recognition of CD8+ T-cells as significant type 2 cytokine producers are relatively new[4].

In fact, CD8+ T-cells in human blood generate more IL-4 than CD4+ T-cells. In situ hybridization and immunohistochemistry on bronchial samples later verified this, demonstrating that CD4+ and CD8+ T-cells, as well as mast cells and eosinophils, generated IL-4 and IL-5 in the airways. Furthermore, human atopic illness is associated with CD8+ cells that secrete IL-4, -5 and -13 and are distinctive to the major house dust mite aeroallergen. These cells are thought to respond through antigen cross-presentation[5].

Despite routine inhaled corticosteroid treatment, CD8+ T-cells were found in the lamina propria of bronchial biopsies and were linked to airway hyperresponsiveness airway (AHR )as determined by methacholine challenge, while AHR did not correlate with CD4+ T-cells [6].

## PATIENTS AND METHODS

**Study design:** It is a case control study that was accomplished in Aswan university hospital in the period from February 2023 to August 2024. This study included 120 asthmatic patients diagnosed based on GINA guidelines 2022 with similar age and sex matched controls selected from the outpatient clinics at Aswan University Hospital.

**Exclusion criteria**: - participants with acquired immunodeficiency, autoimmune disorders, cardiovascular diseases, malignant tumours, beta-blocker-treated patients, patients with mental health conditions, patients taking ACE inhibitors or monoamine oxidase inhibitors, children younger than five years old, patients with severe uncontrolled asthma, and cases receiving immunotherapy.

Ethical consent: All participants provided written informed consent before beginning the study, which was authorized by the Aswan University Faculty of Medicine's ethical committee.

**All the following data were collected from participated subjects in this study:**

1. **History taking**: -including age, sex, family history of asthma, allergies, medications, and the present symptoms: including nocturnal cough, wheezing, recurrent chest tightness, dyspnea, sleep disturbances, and recurrent chest infections.
2. **Clinical examination**: All participants had a thorough general and chest examination, and the severity of their asthma attacks was assessed using the recent guidelines.
3. **Standard spirometry:** - was completed using the WinsproPRo PFT machine for every patient. The following metrics were measured: FEV1/FVC%, Forced Vital Capacity (FVC), and Forced Expiratory Volume in First Second (FEV1). Spirometry was conducted prior to and subsequently repeated after a short-acting β2-agonist (SABA) has been inhaled. An elevation in FEV1 exceeding 200 ml and/or 12% above the pre-bronchodilator FEV1 at the time of evaluation was deemed diagnostic.
4. **The Asthma Control Test (ACT)** consists of five inquiries intended to assess night-time and day-time symptoms, frequency of beta-2 agonist uses as life-saving medications, and limitations on everyday tasks. A score of 25 points indicates "complete control," while a score between twenty and twenty-four points suggests "partial control," and a score below twenty points signifies "uncontrolled" asthma.

5. **Complete blood count:** - 5 ml venous blood samples were taken by vacutainer and put in tubes (Becton Dickinson Vacuum) containing ethylene diaminetetraacetic acid and gently mixed. (Sysmex XE-21N, Kobe, Japan) was used to measure neutrophils, lymphocytes, and eosinophils percentage within 1-2 hours after blood specimen collection.
6. **Sputum analysis**

   a-Sputum induction

   After the patients washed their lips with water, they were told to use a nebuliser to inhale a 3% saline solution at room temperature. After that, they were instructed to cough deeply every three minutes. Prior to further processing, the sputum samples were kept at 4°C for a maximum of two hours.

   b-Sputum homogenisation

   A phosphate-buffered saline (PBS) solution containing 10 mmol/L of dithiothreitol was mixed with a fraction of the samples with a fixed ratio 1:1

   c-Sample filtration& counting

   Filtration through a 48-um nylon mesh was used to remove mucus and debris. Total cell count (TCC) is performed manually using a hemocytometer and cell viability is determined by the trypan blue

   d-Centrifugation, staining and counts

   Centrifugation was done at 400xg for 10 minutes. Then sediments were stained using Giemsa stain for differential cell count. An eosinophil percentage of 3% or more of the total cell count is indicative of airway eosinophilia.

7. **Total Serum IgE Measurment:**

   Total serum IgE was detected by sandwich ELISA commercially available kit (Biocheck, Biokit, South San Francisco, CA 94080) in compliance with the guidelines provided by the manufacturer. The IgE antibody-coated micro titer wells were incubated for 30 minutes at room temperature with zero buffer after the serum samples have been added. IgE antibody labelled with horseradish peroxidase is then added to the well after it has been cleaned to eliminate any remaining test specimen. Following a 30 min incubation at room temperature, the wells were washed to get rid of unbound, labelled antibodies. After adding a tetramethylbenzidine reagent solution and letting it sit at room temperature for 20 min, a blue colour started to appear. When stop solution was added, the colour development was changed, turned yellow, and was measured by spectrophotometer at 450 nm. The test sample's colour intensity was directly correlated with the IgE content. Serum total IgE levels were measured as IU/ml with a limit of sensitivity (5.0 IU/ml).

8. **Counting of CD8 positive cells by Flowcytometry:** The CD8 positive cells were characterised using multiparametric flowcytometric analysis. The cells were labelled with three monoclonal antibodies: Rabbit anti-human CD8 as primary monoclonal antibodies, conjugated to mouse secondary antibody that was labelled with Alex: Fluor 488 fluorescent dye. In 5mL Wasserman tube, 50uL of binding buffer was added 50uL of heparinised blood, labelled with 5 uL of Human anti-CDS (CDS Monoclonal Antibody (SP16), cat no: MAS-14548 (**Invitrogen, ThermoFisher Scientific, USA**) for 20 minutes, then the cells washed once with phosphate buffer saline (PBS), and SuL of Fluorescein isothiocyanate (FITC) secondary antibody (IgG H&L chain) was added. The cells were incubated in the dark for 15 minutes. At the end of the incubation, 200uL of lysis buffer was added to lyse the RBCs, then the cells examined by Flowcytometry. After

sample processing, Flow cytometry was used to analyze the data, and monoclonal antibody labeling was used to gate the cells. **The Beckman Coulter Navios EX software: SM: BE14548 software version: Navios EX**. was used to analyse flow cytometry data. The histograms of logarithmic dot plots present the lymphocyte population and gating was performed on the CD8 positive cells. Parametric analysis as seen in flowcytometric images (CD8) showed the percentage of positive cells calculated from the total lymphocytes (figure 1).

## RESULTS

The results were evaluated using the Statistical Package for Social Sciences (SPSS-model 25) software.

**Table 1: Comparison of CD8% between the groups under study**

| Variables | BA group (N=120) | Control group (N=120) | Test | P-value |
|---|---|---|---|---|
| **CD8 (%)** | | | **14.32** | **0.001*** |
| **Mean± SD** | **27.25±1.5** | **26.8±0.40** | | |

**Test: - independent t test *: Significant p-value**

**Figure 1**

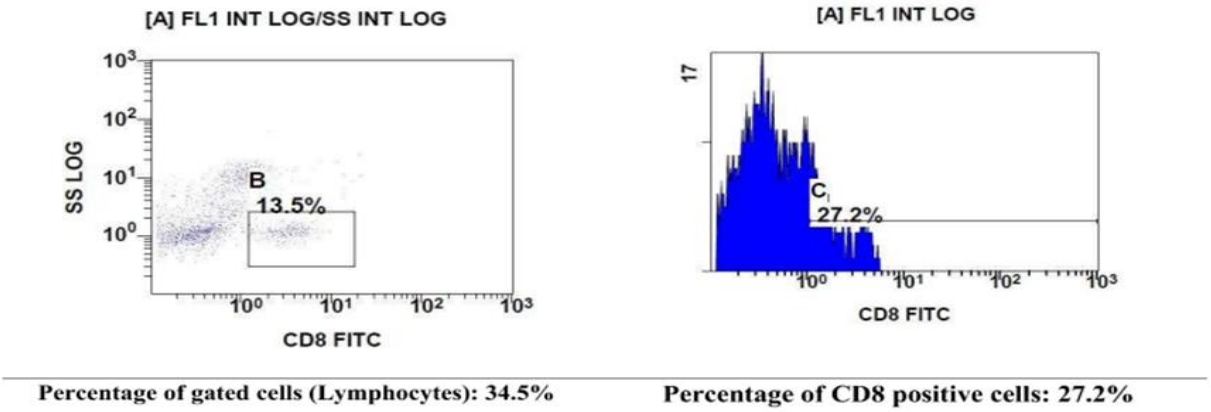


**Table 2:** Descriptive analysis of CD8% regarding degree of asthma severity

| **Degree of asthma severity** | **Mild Asthma N=19** | **Mild Persistent N=41** | **Moderate Persistent N=39** | **Severe Persistent N=21** | **Test** | **P-value** |
|---|---|---|---|---|---|---|
| **CD8 (%)** | | | | | **6.68** | **0.001*** |
| Mean± SD | 26.7±1.05 | 26.9±1.05 | 27.3±1.1 | 28.1±2.7 | | |

Test: -One way ANOVA test *: Significant p-value

According to table 2 (mild, moderate, and severe asthmatic cases differed significantly in CD8%)

**Table 3:** Comparisons of CD8 regarding asthma control level

| **Degree of asthma severity** | **Controlled** | **Partially controlled** | **Uncontrolled** | **Test** | **P-value** |
|---|---|---|---|---|---|
| **CD8 (%)** | | | | **8.94** | **0.001*** |
| Mean± SD | 25.9±0.95 | 26.5±1.05 | 27.5±1.3 | | |

Test: -one-way ANOVA test *: Significant p-value

Table 3 showed a statistically significant elevation in CD8 (%) in uncontrolled patients in contrast to partially controlled and well controlled patients.

**Table 4:** Correlation between CD8, pulmonary function test and laboratory data

| **Bronchial asthma group** | **CD8** | |
|---|---|---|
| | **r** | **p-value** |
| **Age** | -0.14 | 0.217 |
| **FVC%** | -0.06 | 0.602 |
| **FEV1%** | **-0.66** | **0.001*** |
| **TLC** | **-0.77** | **0.001*** |

| | | |
|---|---|---|
| **FEV1/FVC** | **-0.81** | **0.001*** |
| **Neutrophils in blood** | 0.03 | 0.77 |
| **Lymphocytes in blood** | 0.09 | 0.94 |
| **Eosinophils in blood** | **0.849** | **0.002*** |
| **Eosinophil in sputum** | **0.94** | **0.001*** |

r: Pearson correlation

Tab 4 presents a significant positive strong correlation (p-value < 0.05) between CD8 and eosinophils in blood (r = 0.84), as well as eosinophils in sputum (r = 0.94). Conversely, a considerable moderate inverse relationship is observed between CD8% and the FEV1 (r = -0.66) and TLC (r = -0.77) and a significant strong negative correlation between CD% and FEV1/FVC (r =-0.81) within the cases.

## DISCUSSION

Among the most prevalent long-term inflammatory diseases affecting the respiratory system globally is bronchial asthma. Bronchial asthma is categorized as either atopic or non-atopic depending on whether atopy exists or not, which is defined as a positive skin prick test 3 mm or Immunoglobulin E (IgE) specific antibodies that are generated against common allergens[7].

There are currently few epidemiologic reports of severe asthma. Approximately ten percent of asthmatics go on to acquire severe asthma, according to the European Network for Understanding Mechanisms of Severe Asthma[8]. People with severe asthma are typically older and diagnosed with the condition later in life. Additionally, a high blood leukocyte count, primarily of neutrophils and eosinophils, and poor lung function are characteristics of severe asthma. Additionally, for those who suffer from severe asthma, their quality of life is greatly affected[8].

In this research, we summarized that there was a significant elevation in CD8+ T cells in the asthmatic group comparable to controls (27.25±1.5% vs 26.8±0.40%, p-value < 0.001) and CD8% showed a significant variance between mild, moderate and severe persistent cases (p-value < 0.001) .in addition to that, it was elevated in uncontrolled patients when compared to partially controlled and well controlled patients

This aligns with research by ***Hamzaoui et al.***,[9] who studied CD28 expression in CD8+ cells in induced sputum. CD8+CD28− cells were found to be more expanded and exhibited lower levels of IFN-γ in severe asthmatics than in mild asthmatics**.**

In a study by ***O'Sullivan et al.***[10] involved postmortem peri bronchial region samples, the proportion of CD8+CD25+ T cells and perforin expression was elevated in patients who had died from asthma more than in asthmatic patients who had died of unrelated causes or in individuals who had died without a history of lung diseases (control groups) .

***Lee et al.***[11] reported that asthmatic patients' peripheral blood had more IL-6Ralphhigh CD8+ T cells than healthy individuals, supporting our findings of elevated CD8+ levels in asthmatic patients. ***Wang et al.***[12] *showed* that Asthmatics had higher levels of peripheral blood Tc2 and Tc17 cells than non-asthmatics, which also complements our findings of elevated CD8+ cells**.** ***Eusebio et al.***[13] found a higher proportion of CD8+CD28-T cells in adult allergic asthmatics' peripheral blood when compared to controls.

Our research indicated a significant positive correlation between CD8 and Eosinophils % (p-value = 0.002) (r = 0.84), as well as eosinophils in sputum (r = 0.94). Conversely, a significant negative correlation was observed between CD8 and the FEV1 (p-value = 0.001) (r = -0.66) and TLC (r = -0.77) and FEV1/FVC (r =-0.81) among the asthmatic group.

Concur with our findings, a ***Noble et al*** [14]study, involving asthmatic individuals who were monitored for 14 years revealed a strong correlation between the quantity of CD8+ T-cells in airway biopsies and the deterioration in lung function (FEV1). Likewise, ***Dakhama et al*** [15]showed that the number of CD8+ T cells in the bronchoalveolar lavage fluid that expressed the high-affinity receptor for leukotriene B4 (BLT1) and produced IL-13 was linked with the degree of airflow restriction (FEV1 and FEF25–75).

***Enomoto et al.***[16] exhibited that CD8+ T cell percentages associated with peak expiratory flow values in asthmatics. Furthermore ***Stanciu et al.***[17] demonstrated IL-4 production in peripheral blood CD8+ T cells from patients with atopic asthma, supporting the inflammatory role of CD8+ T cells**.**

These results indicated how CD8 cells may predict the course of asthma, which implies that preventing the faster deterioration of lung function in asthmatic patients may need focusing on certain aspects of inflammation.

Study limitations: -

This work has certain limitations, such as sample size and lack of evaluation of other types of T cells.

## CONCLUSION

Both the severity of asthma and level of control were associated with CD8%. Moreover, CD8 cells may predict the course of asthma, which implies that preventing the faster deterioration of lung function in asthmatic patients may need focusing on certain aspects of inflammation. accordingly, CD8% may be considered as a reliable indicator for monitoring asthma severity

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