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Evaluation of peripheral natural killer cells in asthma patients

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Abstract

Background : natural killer cell (NK) cells are activated in response to allergens in vivo. NK cells promote allergic sensitization, type-2 immune response, development of eosinophilic inflammation and airway hyperresponsiveness.

Material and methods: Peripheral blood natural killer (NK) level was analysed from 25 asthma patients and 13 control donors. Male and female patients with atopic asthma enrolled to the AL-Imam AL-Sadiq Teaching Hospital in Hilla City during the period from December 2023 to January 2024. 3 ml of venous blood from patients and healthy controls were collected by the physician, and the samples were used for immunological detection of pNK cell levels by the ELISA method.

Results: Depending on the data that appeared in the work, there is elevation level in pNK in patients with astma compared with its level in healthy persons.

Introduction

Asthma is a chronic airway disease, whose symptoms include wheezing, chest tightness, breathlessness, and coughing with variable and often reversible airway obstruction together with airway hyper-responsiveness , Asthma generally develops in childhood and is associated with sensitization of the airways to aeroallergens such as house dust mites, cockroaches, animal dander, fungi, and pollens. In most cases, this occurs through the selective expansion of T lymphocytes that secrete an array of largely Th2-type

cytokines. These cytokines drive the characteristic events of air-way inflammation central to disease pathophysiology in allergic asthma. Such events include: promotion of Th2 cell survival, B cell isotype switching to IgE synthesis, mast-cell differentiation and maturation, basophil recruitment, and eosinophil maturation

and survival (Holgate , 2011; Karimi and Forsythe, 2013). Allergic asthma arises as result of a complex interplay between stromal cells of the lung and cells of innate and adaptive immunity. Innate immunity contributes to initiation, maintenance and resolution of the allergic response. Some innate immune cells play a master regulatory role and some exert specific effector functions. Innate lymphoid cells belong to master regulators of allergic inflammation. The importance of innate lymphoid type-2 cells (ILC2s) has been highlighted in multiple original research and review articles. Less is known about other innate lymphoid cells, including NK cells (Gorska , 2017).

the most popular paradigm regarding asthma pathogenesis involves allergen-specific TH2 cells and adaptive immunity. TH2 cells orchestrate the inflammation seen in asthma by producing IL-4, IL-5, and IL-13,

which increase airway mucus production; increase the growth and differentiation of airway eosinophils, basophils, mast cells, and B cells producing IgE and TH2 cells; and directly induce the development of airway hyperreactivity (AHR), a cardinal feature of asthma (Umetsu et al. 2010).

Some evidence suggests that NK cells can promote allergic airway responses during sensitization and ongoing inflammation. In animal models, increased NK cells are observed in the lung following antigen challenge and depletion of the cells before immunization inhibits allergic airway inflammation. Moreover, in asthmatics, NK cell phenotype is altered and may contribute to the promotion of a pro-inflammatory Th2-type environment. Conversely, driving NK cells toward an IFN-γ-secreting phenotype can reduce features of the allergic airway response in animal modelsDespite high numbers of NK cells in the lung and their ability to generate a variety of immunomodulatory mediators, the potential of NK cells as therapeutic targets in allergic airway disease has been largely overlooked (Karimi and Forsythe , 2013).

The current study aims to investigate the association between the natural killer cell with asthma pathogenesis

Material and methods :

1-Study design and patient

This study was planned in the college of science for women at the University of Babylon, and the places where samples were collected were the AL-Imam AL-Sadiq Teaching Hospital in Hilla City during the period from The population of the study consisted of 28 subjects: 13 healthy persons (the control group), while the other group consisted of 25 specimens from asthma patients (the patient group). The patients and

controls were all between the ages of 25 and 46. The study population was divided into two age groups, which are 25–35, and 36–46 years old. Patients with other lung diseases like malignant disease were excluded from this study.

2-Specimens collection and methods

5 ml of venous blood is collected in a gel tube from patients and controls. Allow the specimens to clot for 10–20 minutes at room temperature, and then place them in a centrifuge for 20 minutes at 2000–3000 RPM. Then collect the supernatant without sediment to obtain the serum layer, which was used to assess the immune status by evaluating the peripheral blood level of natural killer cells by using a quantitative sandwich ELISA kit (Bioassay Technology Laboratory (BT LAB), Shanghai, China). The procedure was done according to the guidelines provided by the manufactured scientific institute. The NK concentration in serum was determined by plotting the absorbance of each sample against a standard curve of typical concentrations supplied by the kit.

3-Principle of the test:

This kit is an Enzyme-Linked Immunosorbent Assay (ELISA). This plate has been pre-coated with Human NK antibody. NK present in the sample is added and binds to antibodies coated on the wells. Then biotinylated Human NK Antibody is added and binds to NK in the sample.

Then Streptavidin-HRP is added and binds to the biotinylated NK antibody. After incubation un bound Streptavidin-HRP is washed away during a washing step. Substrate solution is then added and color develops in proportion to the amount of Human NK. The reaction is

terminated by addition of acidic stop solution and absorbance is measured at 450 nm.

4. Assay Procedure:

1. All reagents, standard solutions, and samples were prepared as instructed. Bringing all reagents to room temperature before use. The assay was performed at room temperature

2. The number of strips required for the assay was determined. Insert the strips in the frames for use. The unused strips should be stored at 2–8 °C.

3. A 50 µl of standard solution were added to the wells. Note. Don't add biotinylated antibody to the standard solution because the standard solution contains biotinylated antibody.

4. A 40 µl of sample were added to wells, and then 10 µl of anti-NK antibody was added to sample wells, and then 50 µl of streptavidin-HRP was added to sample wells and standard wells (not a blank control well). Mix well. Cover the plate with a sealer. Incubate for 60 minutes at 37°C.

5. Remove the sealer and wash the plate five times with a wash buffer. Soak wells with 300 µl wash buffer for 30 seconds to 1 minute for the wash. For automated washing, aspirate or decant each well and wash five times with a wash buffer. Blot the plate onto paper towels or other absorbent material.

6. A 50 µl of substrate solution A were added to each well, and then 50 µl of substrate solution B were added to each well. The plate was incubated and covered with a new sealer for 10 minutes at 37°C in the dark.

7. A 50 µl of stop solution were added to each well; the blue color will change into yellow immediately.

The optical density (OD value) of each well was determined immediately using a microplate reader set to 450 nm within 10 minutes after adding the stop solution

5- typical data

To determine the concentration of NK cells in serum need to standard curve the optical density at 450 nm (the primary wavelength) of the prepared typical concentration of NK as provided by the supplied company.

Table 1-The absorbance of standard concentrations at 450 nm

Absorbance	Concentration (nmol/ l)
0.014	**0**
0.0850	**15**
0.219	**30**
0.494	**60**
0.973	**120**
1.584	**240**
2.38	**480**

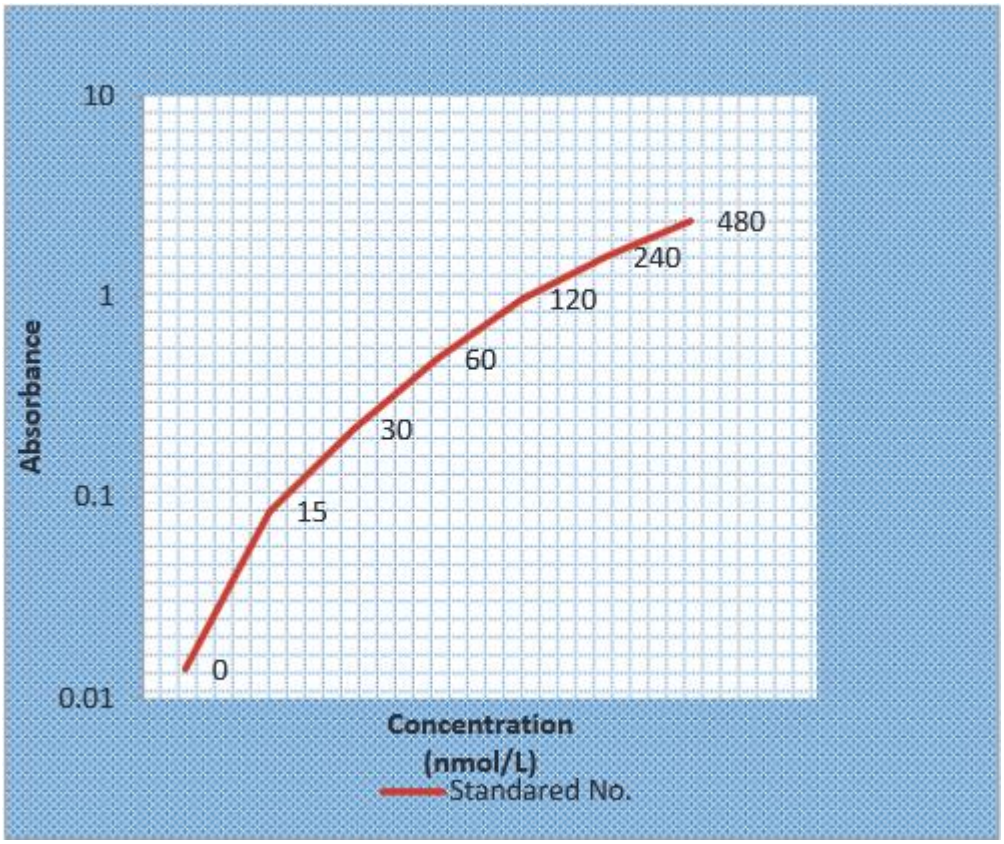

Figure 1. The standard concentration curve of NK in kit

Results and discussion

The statistical analysis of the data indicated that there is an elevation level in the NK concentrations between age groups of patients compared with controls, it reach the degree of significance $p<0.05$. The highest

concentration of NK cells in patients was noted in 36-46 age group ,it was 113.514± 40.321 compared with 25-35 age group and control , the concentrations were 98.322 ± 49.871 and 81.9856 ± 17.5037 respectively.

Table (2): The concentration of Natural killer in patients according to age groups.

Age group	Patient Mean ± Std. Deviation	Control Mean ± Std. Deviation	P value
25-35	98.322±49.871	74.926 ± 28.9946	0.352*
36-46	113.514±40.321	81.985 ± 17.503	
Total	105.920± 45.1	78.466± 23.254	

Natural killer (NK) cells are large granular lymphocytes of the innate immune system that were originally defined by their capacity to lyse target cells and produce interferon-γ without prior activation.Various subtypes of NK cells and specific NK derived cytokines/chemokines contribute to the promotion/cessation of allergic sensitization. Little is known about the role of regulatory NK cells in atopy and in relation to disease severity asthma. (Hossny et al. 2017).

T_H2 cells play a pivotal role in the pathogenesis of human asthma. Natural killer (NK) cells are divide into NK1 and NK2 subsets (Beasley *et al.* 2000). wei et al (2005), revealed that the NK2 cell subset is involved in the pathogenesis of asthma, they found that the ratio of IL-4^+CD56^+ NK2 cells in PBMCs of 8 asthmatic patients were higher than the ratio of IFN-γ^+CD56^+ NK1 cells

Liu et al (2023) showed that an imbalanced Th1/Th2 immunity in airways of asthma with acute upper respiratory viral infections. Upregulated peripheral CD3 -CD56 +CD16 + NK cells play a crucial role in biased

Th1 immunity of airways in asthma during the acute phase of viral infections. The anti-viral Th1 immunity by targeting NK cells may be a possible therapeutic option for virus-induced asthma exacerbation.

Conclusion: The high level of natural killer cells in asthma patients indicates the involvement of these cells in the pathology of this disease.

References :

Beasley R. *et al.*(2000). Prevalence and etiology of asthma. J Allergy Clin Immunol.13(5):45-59

Gorska M. M.(2017). NK cells in asthma. Curr Opin Allergy Clin Immunol. 17(1): 50–54.

Holgate, S. T. (2011a). The sentinelrole of the airway epithelium in asthma pathogenesis.Immunol. Rev.242, 205–219. doi:10.1111/j.1600-065X.2011.01030.x

Hossny,E,M., ElOwaidy, R.H., Neama L. and Hassanin E.A.(2017). Regulatory natural killer cell expression in atopic childhood asthma. Egypt J Pediatr Allergy Immunol .15(2):57-62

Karimi K.and Forsythe P.(2013)Natural killer cells in asthma. Frontiers in immunology , 4(159):1-8.

Liu M., Zhang Y., Hu Y. et al (2023). Upregulated peripheral CD3 - CD56 + CD16 + natural killer cell correlates with Th1/Th2 imbalanve in asthma patients during acute upper respiratory viral infections. Research sequare,https://creativecommons.org/licenses/by/4.0

Umetsu, D T. MD and DeKruyff, R. H. (2010). Current perspectives Natural killer T cells are important in the pathogenesis of asthma: The many pathways to asthma. J ALLERGY CLIN IMMUNOL, 12(5).

Wei H., Zhang J., Xiao W. et al. (2005). Involvement of human natural killer cells in asthma pathogenesis: Natural killer 2 cells in type 2 cytokinepredominance.