Journal of Medical Research and Health Sciences
Received 14 Dec 2020 | Revised 15 Feb 2021 | Accepted 25 Feb 2021 | Published Online 14 Mar 2021

DOI: https://doi.org/10.15520/
JMRHS 4 (3), 1111–1118 (2021)

ISSN (O) 2589-9031 | (P) 2589-9023

ORIGINAL RESEARCH ARTICLE

Flow cytometry-based profiling of immune cells among patients with bronchial asthma in Basrah, Iraq

Dr .Ihsan Edan A. Alsaimary [*1] | *Faleh H. Mezban*[2]

[1]University of Basrah, Collage of Medicine, Department of Microbiology, Basrah, Iraq.

Abstract

This study aimed to the assessment of the Flow cytometrric analysis in asthmatic patients of Basrah. This study estimated the total number of cells by the flow cytometry (257485 cell) ,the number of cells was as follows lymphocyte(27215) cells, T. lymphocyte (5639) cells, B lymphocyte (21893)cells, Monocyte (964) and Granulocyte (2269) .The high level of distributions was for lymphocyte 27.2% (B- lymphocyte 21.4% and on T-lymphocyte 5.6%) followed by granulocyte 2.3% and monocyte 1%.Percentages and intensity of IL 4,10 and 17 on T.lymphocyte IL 4 and 10 100%, while IL 17 record 45% . IL 4 100%, IL 10 90% and IL 17 40% on B-lymphocyte also distributions on neutrophil show IL 4 100% , IL 10 99% and IL 17 98% while on monocyte record IL 4, 10 100% and IL 17 93% . High level distribution on lymphocyte may be due to viral infection with asthmatic patients.Results of the present study indicate that the IL-4 ,IL10 and IL-17 associated with risk of Asthma.

Keywords: Flow cytometry, human bronchial asthma, immune cells

Copyright : © 2021 The Authors. Published by Publisher. This is an open access article under the CC BY-NC-ND license (https://creativecommons.org/licenses/by-nc-nd/4.0/).

1 | INTRODUCTION

Asthma is a chronic inflammatory disorder of the airways in which many cells and cellular elements play a role.It is a major health problem throughout the world, affecting an estimated more than 300 million people worldwide, of these 10-15% have severe asthma (Olin and Wechsler, 2014).

Individuals with asthma suffer from episodic airflow obstructions that are reversible either spontaneously or by treatment (Papaiwannou *et al*., 2014). These airflow obstructions results in asthma symptoms such as recurrent episodes of wheezing, chest tightness, breathlessness and coughing, particularly at night or in the early morning, airflow obstructions are a consequence of smooth muscle contraction, mucus secretion, airway edema, airway wall thick-

ening or a combination of these occurrences, characteristic pathophysiological features of asthma are airway hyperresponsiveness (AHR), inflammation and remodeling (Bel, 2013). Airway inflammation is persistent affecting all airways in special medium-sized bronchi. This pattern appears to be similar in all clinical forms of asthma, whether allergic or non-allergic at all ages and is characterized by the presence of activated mast cells, increased numbers of activated eosinophils, increased numbers of T cell receptor invariant natural killer T cells and Th2, which release mediators that contribute to symptoms (Cohn *et al*., 2004 ; Hamid and Tulic, 2009 ; Lambrecht and Hamida, 2015).

The development of asthma requires an interaction between the environment and genetic susceptibility (Murphy *et al*., 2010). Many factors can trigger an asthma attack, which include allergens, infections, exercise, cold, flu, sinusitis, coughing (Price *et al*., 2014 ; Tanday, 2014).

2 | MATERIALS AND METHODS

Samples

A total of (312) patients (149 males and 163 females) of various age groups were included in this Case –control study. The patient was examined, and diagnosed as asthma under supervision of the Physician.The study was carried out during a period from July 2018 to January 2020.

Control group

A total of (204) healthy individual (81 males and 123 females) without any features of asthma or any allergies to be compared with asthmatic patient in genetic and immunological studies.

Statistical analysis

Statistical analysis is done by using statistical package for social sciences(SPSS) software version 11, the chi square test, univariate and multivariate logistic regression analysis, the ANoVA analysis were applied for correlation between each study parameter, and the difference between two proportion by T- tests were used to assess the significance of difference between groups,P-Value less than 0.05 was considered as Statistically significance(S).P-value < 0.01 as highly significant(HS).and P-value > 0.001 as extremely significant(ES).

3 | RESULTS

Asthmatic patients

The present investigation applied a new modern technique (flow cytometry) to determine

1. A number of cells that have receptor for interleukins 4,10 and 17
2. Type of cells that have these receptor.
3. Compare the results in asthmatic patient with these for healthy persons.

The results, illustrate in table (3-6 ,3-7, 3-8 and 3-9) and in figures 123, 124, 125, 126, 127 and 128

The total account of cells measured by flow cytometry was 257.485 for asthmatic patient the number of the total lymphocyte was 27,215, T.lymphocyte (5,639) and B.lymphocyte(21,398) monocyte (964) and granulocytes(2,269) 20.7% of T. lymphocyte and (78.6%) of B. lymphocyte,(1%) monocyte and (2.3%) of granulocytes were have receptor of IL -4, IL-10 and IL -17.

From total number of cells(257,485) the percentage of cells that have receptors for IL- 4,IL- 10 and IL -17 as follows (27.2%) of lymphocytes ,(5.6%) T.lymphocyte (21.4%) B.lymphocytes ,(1%) monocytes and (2.3%)granulocytes

Other type of cell that mentioned as Q1,Q2,Q3,Q4,Q1-1,Q2-1,Q3-1 and Q4-1 recorded the following percentages as follows :

0.1% ,1.8% ,80.1% ,17.9% 0.6%, 31.5%,64.6% and 3.3%

Supplementary information The online version of this article (https://doi.org/) contains supplementary material, which is available to authorized users.

Corresponding Author: *Dr .Ihsan Edan A. Alsaimary*
University of Basrah, Collage of Medicine, Department of Microbiology, Basrah, Iraq.

According to statictical analysis there are significant differences between receptors of various interleukines (IL-4 , IL-10 ,and IL-17)present on the surface of various measured cells and between the interleukine receptors within the same cell of asthmatic patients (P<0.05).

And the figures illustrate dynamic of cell distribution within the cell community that stained with various stains and distributed according to computerized programs especially to specific instrument.

TABLE 1: illustratepercentages and numbers of various cells detected by Flow cytometry P < 0.05

Population	Event	Parent %	No. of cell from parent	Total %	No. of cell in Total
All Event	100.000			100.00	
Lymphocyte	27.215	27.2	7.402	27.2	7.003
T. lymph.	5.639	20.7	1.167	5.6	1.416
B.lymph.	21.398	78.6	16.818	21.4	5.510
Monocyte	964	1.0	9.64	1.0	2.574
Granulocyte	2.269	2.3	5.218	2.3	5.922
Q1	118	0.1	1.18	0.1	0.257
Q2	1.838	1.8	3.3	1.8	4.634
Q3	80.121	80.1	64.176	80.1	206.245
Q4	17.923	17.9	3.208	17.9	46.0898
Q1-1	579	0.6	3.36	0.6	1.544
Q2-1	31.524	31.5	9.930	31.5	8.110
Q3-1	64.632	64.1	41.752	64.6	166.02

TABLE 2: illustratepercentages and intensity of IL-4, IL-10 and IL-17 in various cells T.lymphocytes,
B.lymphocytes, Neutrophils and monocytes in asthmatic patients.

T-lymphocytes		
Positive markers	Percentage	Intensity
IL17A	45 %	Dim
IL10	100 %	Dim to Moderate
IL4	100 %	Dim
B-lymphocytes		
Positive markers	Percentage	Intensity
IL17A	40%	Dim
IL10	90%	Dim
IL4	100 %	Dim to moderate
Neutrophils		
Positive markers	Percentage	Intensity
IL17A	98 %	Moderate to bright
IL10	99 %	Moderate
IL4	100 %	Moderate
Monocyte		
Positive markers	Percentage	Intensity
IL17A	93%	Dim to moderate
IL10	100%	Dim to moderate
IL4	100 %	Moderate

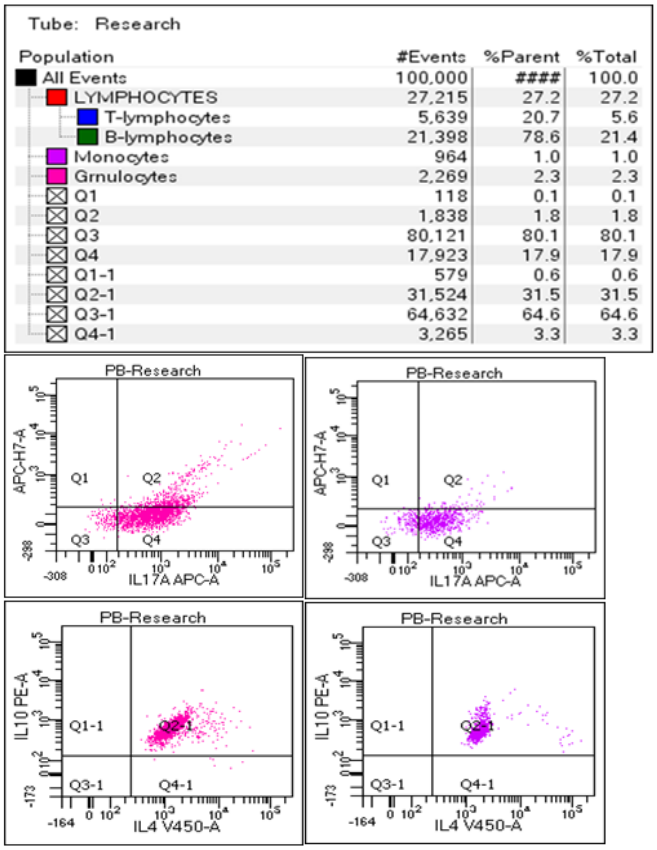

FIGURE 1: Distribution of cell group according to intensity and theirreceptors detectby flow cytometry.in asthmatic patient(M,N)

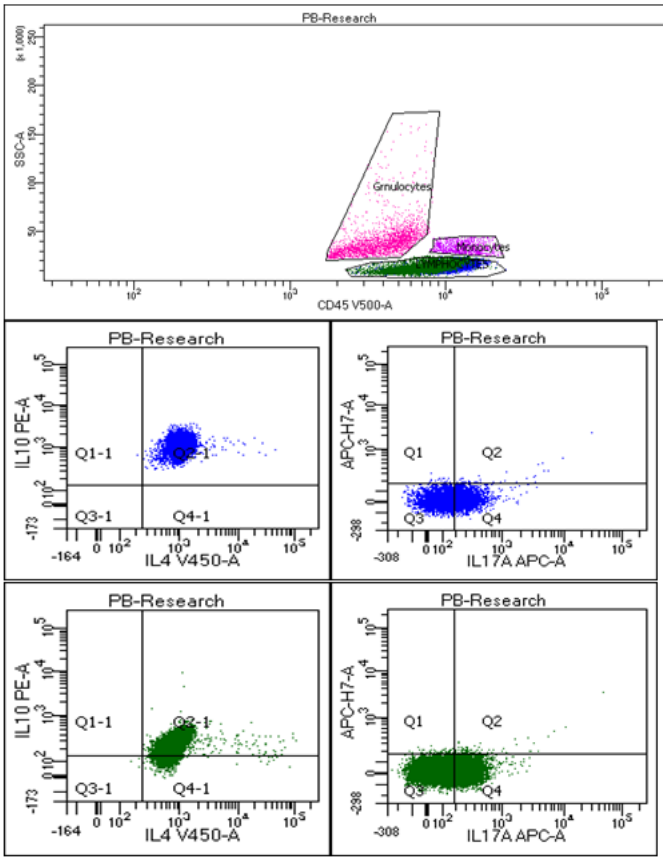

FIGURE 2: cont.(T,B Lymphocyte)

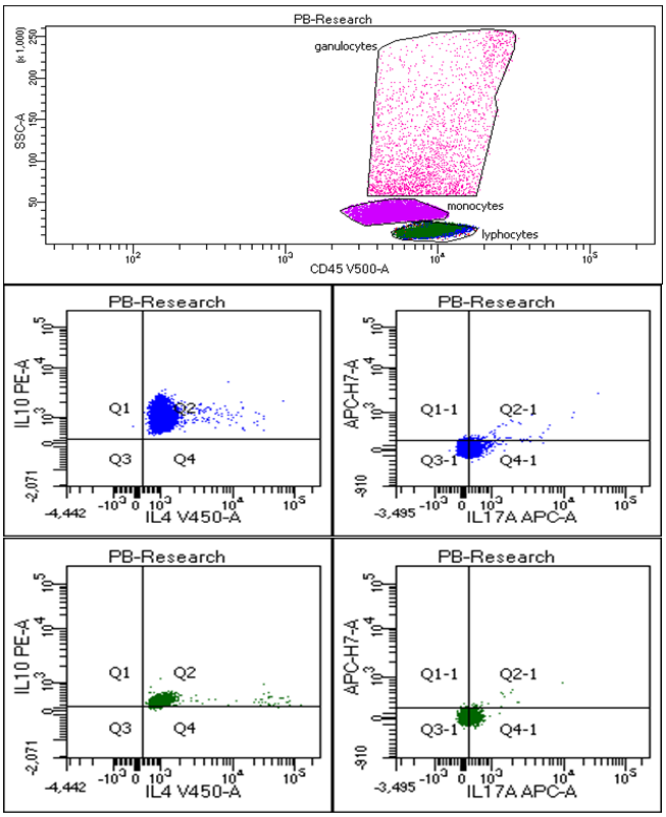

FIGURE 3: cont.other patients

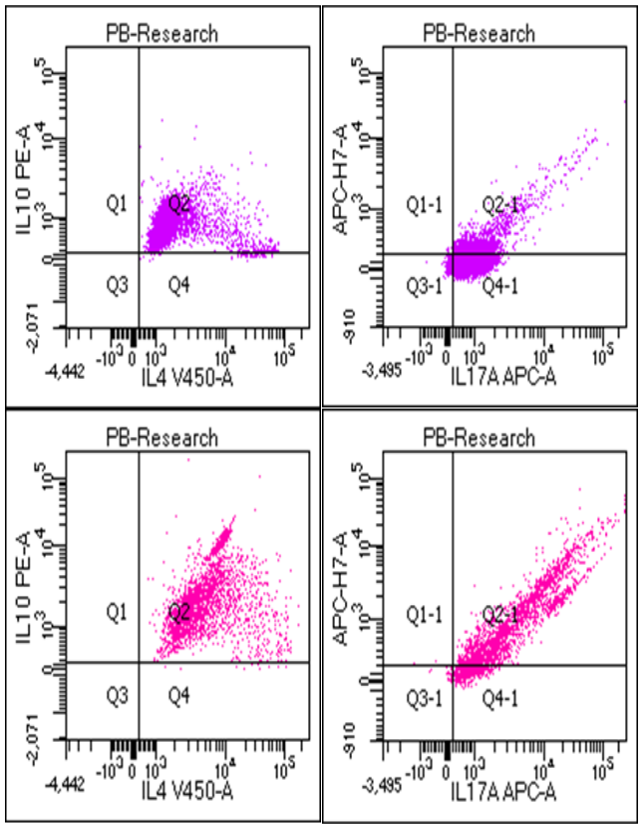

FIGURE 4: cont.

Control group

While in the healthy control the total number of cells estimated by the flow cytometry was (957,825) cells the number of cells were as follows leukocytes (99,035) cells lymphocytes (339), T. lympho-cytes (240) , B. lymphocytes (84) and granulocytes (26,543)

The percentages of receptors bearing cells from total number of cells were as follows (99, 0.3 , 0.2 , 9.1 and 96.5 %)for leukocytes, lymphocytes and granulocytes respectively

All the above the results show in table 3-8 and 3-9 and figures 124, 125, there are significant differences between the numbers of various cell and between the receptors bearing cells($P<0.05$)

TABLE 3: illustratepercentages and numbers of various cells in healthy person detected by Flowcytometry P < 0.05.

Population	Event	Parent %	No. of cell from parent	Total %	No. of cell in Total
All Event	100.000			100.00	
Cells	99.256	99.3	98.56	99.3	951.120
Leukocyte	99.035	99.8	98.83	99.0	948.246
All. lymphocytes	339	0.3	101.7	0.3	2.873
T. lymph.	240	70.8	205.32	0.2	1.915
B.lymph.	84	24.8	20.832	0.1	0.957
Granulocyte	26.543	97.5	94.129	96.5	924.301
Total No.	957.825				
P. value		S	S	S	S

TABLE 4: illustratepercentages and intensity of IL-4, IL-10 and IL-17 in various cells T.lymphocyte, B.lymphocytes, Neutrophils in control patients.

T-lymphocytes		
Positive markers	**Percentage**	**Intensity**
IL17A	**Negative**	
IL10	**Negative**	
IL4	**100 %**	**Dim**
B-lymphocytes		
Positive markers	**Percentage**	**Intensity**
IL17A	**Negative**	
IL10	**Negative**	
IL4	**99%**	**Dim to moderate**
Neutrophils		
Positive markers	**Percentage**	**Intensity**
IL17A	**Negative**	
IL10	**Negative**	
IL4	**80%**	**Heterogeneous**

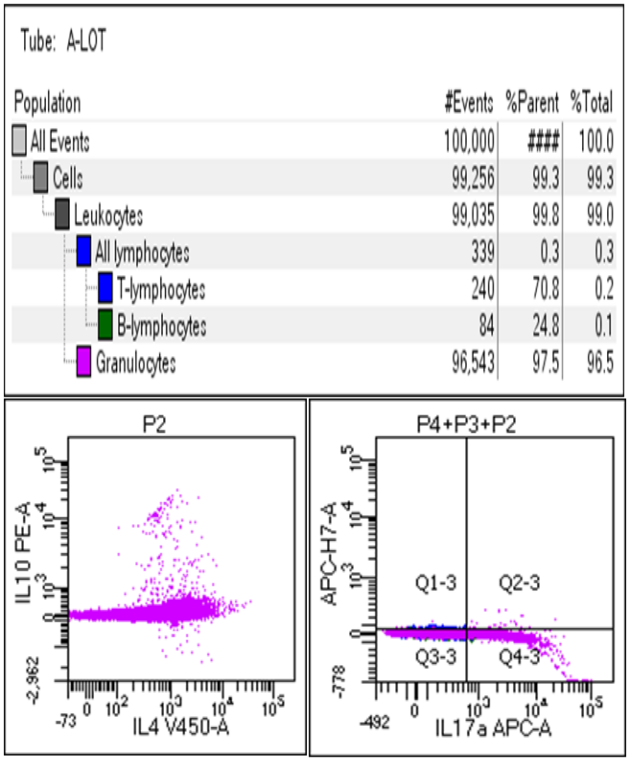

FIGURE 5: Distribution of cellgroup according to intensity and their receptors by flow cytometry in controlgroups.

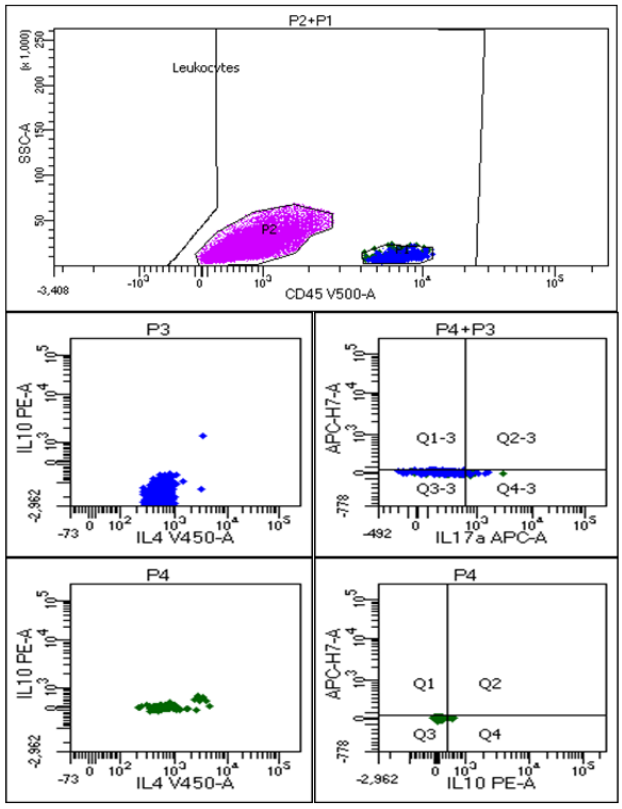

FIGURE 6: cont. to control

4 | DISCUSSION

Flow cytometry is a laboratory method that provides identifying cells in solution, used for evaluating pe-ripheral blood, bone marrow, and other body fluids. It is used for identification and quantification of the cells of immune system and also characterized hematological malignancies. Also by measuring the cell size.In this study show in patients total number of cells estimated by flow cytometry (257485 cell) the number of cells were as follows lymphocyte(27215) cells T. lymphocyte (5639) cells, B lympho-cyte (21893)cells, Monocyte (964) and Granulocyte (2269) high level distributions on lymphocyte 27.2%(B-lymphocyte 21.4% and on T-lymphocyte 5.6%) followed by granulocyte 2.3% and monocyte 1%. Percentages and intensity of IL 4,10 and 17 on T.lymphocyte IL 4 and 10 100% while IL 17 record 45% . IL 4 100%, IL 10 90% and IL 17 40% ON B- lymphocyte also distributions on neutrophil show IL 4 100% , IL 10 99% and IL 17 98% While on monocyte record IL 4, 10 100% and IL 17 93% . high level distribution on lymphocyte may due to viral infection with asthmatic patients and previous study agree with this study A El-Kelany,et al. (2019).

Here a flow cytometric method that allows detec-tion of collagen-I content in lung myofibroblasts is described. This novel method included mechani-cally and enzymatically disaggregated solid tissue in which cells are stained with a viability dye and then fixed prior to permeabilization and antibody staining. There are several advantages to this approach. Sam-ple preparation is a critical step in flow cytometry, specifically for a solid tissue such as the lungs with a high degree of cellular heterogeneity tightly bound together by abundant extracellular matrix. Experi-ments in mouse models are costly and it is desir-able to obtain the maximum amount of high quality data from each experiment with as little variability introduced by technical steps as possible.

Process-ing of lungs from a large group of mice (+10) for flow cytometry can be time consuming. (Anderson,et al.,2009) It is challenging to finish endpoint mea-surements in the live animal, harvest and process the lung tissue into single cell suspension, perform stain-ing for specific markers, and acquire flow cytometric data on the samples within a normal eight to ten hour workday. Often, experiments include multiple groups and are run over several days. The novel method described here may allow high-throughput or batch flow cytometric acquisition of all mice lungs from a specific experiment in a single run. This would be helpful to reduce variability in data due to batch effects and reduce the workload on days of organ harvest. In our experience, the mild fixa-tion with paraformaldehyde prevented disaggregated lung cells from fragmenting during the staining pro-cedure and maintained light scatter properties. Lung cells comprise a very heterogeneous population and processing of unfixed cells resulted in increased cell death and fragmentation of cells. Another advantage of staining with a fixable live/dead dye that is also suitable for cryopreservation is that it allows easy shipping of frozen samples to other laboratories in collaborative studies.(Beck,et al.,2013) Fluorescent cell barcoding is a powerful technique allowing high-throughput flow cytometric acquisition. Clin-ical studies on pathophysiological mechanisms of asthma bronchiale require methods of investigation that are simple, reproducible and rapid in respect of analysing relevant inflammatory cells and mecha-nisms. The article presents two new flow cytometric methods enabling on single-cell level the determi-nation of expression of intracellular cytokines in T-cells and of basic proteins in eosinophilic granulo-cytes. Both techniques of examination can be per-formed either with whole blood or with cells from bronchoalveolar lavage without preceding cell sepa-ration techniques. First results reveal interesting new aspects with regard to the expression of IFN-gamma, IL-2, IL-4 and IL-5 in T-cells, and of ECP and EPO in eosinophils in bronchial asthma.The validity of this flow cytometric determination was tested by morphological analysis of flow-sorted cellular subsets.

In an animal model of ovalbumin-induced asthma, this new method correlated very well with the differential counts based on cytospins. Flow cytometric determination of the cellular composition of BAL fluid in mouse models of asthma is a rapid and easy method that can replace differential cell counts based on morphology. These observations suggest that flow cytometric analysis for intracellular ECP and mitogen-induced cytokine production reflects the activation of T cells in bronchial mucosa, and is useful for monitoring airway inflammation in bronchial asthma.(Amelink,et al., 2013)

5 | REFERENCES

1. Alsaimary ,Ihsan Edan A. Alsaimary, Hussein N. AlDhaheri, Murtadha M. ALMusafer. Assessment of Immunomolecular expression and prognostic role of TLR7 .Journal of Medical Research and Health Sciences among patients with Prostatitis . 3 (11), 1105-1109 (2020) DOI: https://doi.org/10.15520/jmrhs.v3i11.278

2. ALMusafer, Murtadha M. Hussein N. AlDhaheri Dr.Ihsan Edan A. AlSaimary.Clinical study of patients with prostatitis in Basrah and Missan governments: a case–control study. Journal of Medical Research and Health Sciences. 3 (11), 1110-1115 (2020). DOI: https://doi.org/10.15520/jmrhs.v3i11.279

3. AlSaimary ,Ihsan E., Hussein N. AlDhaheri, Murtadha M. ALMusafer. Molecular Gene Expression of Toll-Like Receptors 4 & 10 in Cellular Subsets of Human Peripheral Blood among Patients with Prostatitis: Conventional, Real Time Pcr and DNA Sequencing Techniques . International Journal of Medical Science and Clinical Invention 7(11): 5095- 5102 2020. DOI:10.18535/ijmsci/v7i11.06

4. AlDhaheri, Hussein N., Ihsan E. AlSaimary and Murtadha M. ALMusafer. PREVALENCE, INCIDENCE ESTIMATION, RISK FACTORS OF PROSTATITIS IN SOUTHERN IRAQ: CASE-CONTROL OBSERVATIONAL STUDY. EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH. 2020,7(12), 47-55

5. AlSaimary, Ihsan E Hussein N AlDhaheri and Murtadha M ALMusafer. Immunomolecular Expression of TLR1 and TLR2 Genes Related With Prostatitis. Journal of Biotechnology &Bioinformatics Research . Volume 2(4): 1-4

6. AlDhaheri Hussein N. , Ihsan E. A. Alsaimary , Murtadha M. ALMusafer . Flow Cytometric detection of Toll Like receptors on blood cells among patients with prostatitis. Journal of Medical Research and Health Sciences . 3 (11), 1116-1121 (2020) DOI: https://doi.org/10.15520/jmrhs.v3i11.279

7.Alyasin, S., Karimi, M.H., Amin, R., Babaei, M. and Darougar, S.(2013). Interleukin-17 gene expression and serum levels in children with severe asthma. Iranian journal of immunology, 10(3):177-185.

8.Amelink, M.; De Nijs, S.B.; De Groot, J.C.; Van Tilburg, P.M.; Van Spiegel, P.I.; Krouwels, F.H.; Lutter, R.; Zwinderman, A.H.; Weersink, E.J.; Ten Brinke, A.; Sterk, P.J. and Bel, E.H.(2013). Three phenotypes of adult-onset asthma Allergy: 68(5): 674-680.

9.Anand, K.S.S.; Ravi, G.N. and Narasimhaswamy, K.N. (2016). Study of pulmonary function test in allergic rhinitis patients of Sullia, Dakshina Kannada. Journal of Evolution of Medical and Dental Sciences.; 5(79):5867-5870..

10.Anderson, S. D.; Charlton, B.; Weiler, J. M.; Nichols, S.; Spector, S. L.; Pearlman, D. S., and Group, A. S. (2009). Comparison of mannitol and methacholine to predict exercise-induced bronchoconstriction and a clinical diagnosis of asthma. Respir. Research, 10(1), 4.

11.Beck, I.; Jochner, S.; Gilles, S.; McIntyre, M.; Buters, J. T. M.; Schmidt-Weber, C.; Behrendt, H.; Ring, J.; Menzel, A. and Traidl-Hoffmann, C. (2013). High environmental ozone levels lead to enhanced allergenicity of birch pollen. PloS. One, 8(11), 80147.

12.Bedada, G.B.; Heinrich, J.; Gotschi, T.; Downs, S.H.;Forsberg, B. and Jarvis, D.(2007). Urban back-

ground particulate matter and allergic sensitization in adults of ECRHS II. Int. J .Hyg. Environ Health. ;210(6):691-700.

13.Beers, M.H. and Berkow, R. (1999). Bacteremia and septic shock. The Merck manual of diagnosis and therapy, 17th edition. Merck Research Laboratories, Whitehouse Station, New Jersey, pp.1143-1147.

14.Behrendt, H.; Kasche, A.; Von Eschenbach, C. E.; Risse, U.; Huss-Marp, J. and Ring, J. (2001). Secretion of proinflammatory eicosanoid-like substances precedes allergen release from pollen grains in the initiation of allergic sensitization. International Archives of Allergy and Immunology, 124(1–3), 121–125.

15.Bel, E. H. (2004). Clinical phenotypes of asthma. Current Opinion in Pulmonary Medicine, 10(1), 44–50.

16.Bel, E. H.; Sousa, A.; Fleming, L.; Bush, A.; Chung, K. F.; Versnel, J.; Wagener, A. H.; Wagers, S. S.; Sterk, P. J. and Compton, C. H. (2011). Unbiased Biomarkers for the Prediction of Respiratory Disease Outcome Consortium CG. Diagnosis and definition of severe refractory asthma: an international consensus statement from the Innovative Medicine Initiative (IMI). Thorax, 66(10), 910–917.

17.Chen, Z. and O'Shea, J.J.(2008). Regulation of IL-17 production in human lymphocytes. Cytokine.; 41(2):71-78.

18.Chernoff, A.E.; Granowitz, E.V.; Shapiro, L.(1995). A randomized, controlled trial of IL-10 in humans. Inhibition of inflammatory cytokine production and immune responses. J. Immunol.; 154: 5492–5499.

19.Cho, S.H., Stanciu, L.A., Holgate, S.T. and Johnston, S.L.(2005). Increased interleukin-4, interleukin-5, and interferon-γ in airway CD4+ and CD8+ T cells in atopic asthma. American journal of respiratory and critical care medicine.; 171(3):224-230.

20.Chung, K.F.; Wenzel, S.E.; Brozek, J.L.; Bush, A.; Castro, M.; Sterk, P.J.; Adcock, I.M.; Bateman, E.D.; Bel, E.H.; Bleecker, E.R.; Boulet, L.P.; Brightling, C.; Chanez, P.; Dahlen, S.E.; Djukanovic, R.; Frey, U.; Gaga, M.; Gibson, P.; Hamid, Q.; Jajour, N.N.; Mauad, T.; Sorkness, R.L. and Teague, W.G.(2014). International ERS/ATS guidelines on definition, evaluation and treatment of severe asthma. Eur. Respir. J.: 43(2): 343-373.

How to cite this article: D.I.E.A.A. Flow cytometry-based profiling of immune cells among patients with bronchial asthma in Basrah, Iraq. Journal of Medical Research and Health Sciences. 2021;1111−1118. https://doi.org/xx.xxxx/xxx.xx