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AN EXPRESSION STUDY PROFILE OF PROINFLAMMATORY CYTOKINES IN ASTHMA PATIENT

Mustafa Mudhafar[1+]
H.A. Alsailawi[2]

[1]*Department of Chemistry, Faculty of Science and Mathematics, Universiti Pendidikan Sultan Idris, Perak, Malaysia.*
Email: 1988mohammed198819881988@gmail.com
[2]*Department of Biology, Faculty of Science and Mathematics, Universiti Pendidikan Sultan Idris, Perak, Malaysia.*

(+ Corresponding author)

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ABSTRACT

Over the past years, there has been a remarkable development in the diagnosis and control of asthma. However, despite the development, there are still problems facing people infected with it, classified as a chronic disease. Over 50 cytokines have been identified in asthma and COPD, but their role in the pathophysiology of these complex airway diseases is often unclear. Genetic studies have proved that multiple genes are involved in asthma and these genes code for cytokines. In the present work, the expression levels of Interleukin 4 and Interleukin 17 have been examined with real-time reverse transcriptase PCR. It was found that when compared with normal controls, the expression levels of these cytokines were increased to folds in asthmatic patients. As these cytokines along with other cytokines, interact and result in symptoms of asthma, regulation of these cytokines will provide therapy for asthma. It was found that the levels of these pro-inflammatory cytokines were elevated in the case of asthma patients as compared to controls. This gives evidence that therapy against these cytokines can assist in asthma treatment.

Contribution/ Originality: In the present work, the expression levels of Interleukin 4 and Interleukin 17 have been examined with real-time reverse transcriptase PCR. It was found that when compared with normal controls, the expression levels of these cytokines were increased to folds in asthmatic patients.

1. INTRODUCTION

Asthma is a ceaseless illness that influences people because of irritation of the air channels in the lungs (Bronchi) and stenosis, which lessens or anticipates the progression of air to these individuals, causing constant scenes of the brevity of breath joined by an alarm in the chest region and some different manifestations [1]. Asthma defined as a chronic inflammatory that is associated with allergic hyperresponsiveness, which leads to recurrences of symptoms such as wheezing, dyspnea, chest snugness, and hacking. It is a disorder localized to the lungs. Asthma coexists with other atopic diseases, particularly allergic rhinitis [2, 3].

Cytokines are little discharged proteins discharged by cells that specifically affect the connections and correspondences between cells. Cytokines are excess in their action, and comparable capacities can be invigorated by various cytokines. Cytokines can likewise act synergistically or unfairly. Specific provocative cytokines in the spinal string, dorsal root ganglion (DRG), harmed nerve, or skin are known to be related to agony practices and with irregular unconstrained exercises. Cytokines are transcendently delivered by assistant T cells(Th), and

macrophages Cytokines might be created in and by fringe nerve tissue during physiological procedures by inhabitant and selected macrophages pole cells, endothelial cells, Schwann cells [4-6].

It is a critical cytokine in the improvement of hypersensitive aggravation, with the acceptance of isotype and emission of IgE by Lymphocytes. - It Advances cell aggravation in the asthmatic lung-vascular cell bond atom (VCAM)- 1 on vascular endothelium the capacity to drive the separation of innocent T assistant sort 0 (TH0) lymphocytes into TH2 lymphocytes [7, 8].

Asthma has been connected to chromosome 5q31-33 through genome searches and screening of applicant qualities. This locus incorporates the conditions for the TH2 cytokines IL-4, IL-5, IL-9, and IL-13. Against IL-4: IL-4 adds to asthma pathophysiology by including Th2 cell separation and development, isotype, exchanging of B cells to IgE blend, redesigning collagen, and fibronectin creation [9, 10]. The adapted enemy of IL-4 monoclonal immune response pascolizumab is very much endured; it needs clinical adequacy in asthmatic patients. The present investigation is completed to comprehend the job of cytokines and their cooperation bringing about asthma. Study of these cytokines will give an approach to create hostile to asthmatic therapeutics.

2. MATERIALS AND METHODS

2.1. Materials

Sodium chloride (NaCl), Potassium chloride (KCl), Sodium phosphate dibasic (Na_2HPO_4), Potassium dihydrogen phosphate (KH_2PO_4), Ammonium chloride (NH_4Cl), Potassium bicarbonate ($KHCO_3$), Ethylenediaminetetraacetic acid (EDTA), Guanidinium thiocyanate, sodium citrate, N-laurosylsarcosine, Ethanol.

2.2. Methods

The cells have been washed with ice-cold PBS once. Lyse cells particularly inset a culture dish by counting 1 ml of TRIZOL Reagent per 3.5 cm separate over the plate and scratching with cell scrubber. The cell of lysate passed several times through a pipette. Vortex totally. The whole of TRIZOL reagent included was based on the zone of the culture dish (1 ml per 10 cm2) and not on the number of cells appear, at that point, was Shakded enthusiastically and bring forth at room temperature for 2-3 minutes, included 0.2 ml of chloroform per 1 ml of TRIZOL Reagent. Test tubes have been caped securely, and vortexed tests excitedly for 15 seconds and brought forth them at room temperature for 2 to 3 minutes. Centrifuged the tests at 14,000 rpm for 15 minutes.

2.3. Sample Collection

Samples were collected from Global Hospital Normal Patient 5 male and five female and Asthma Patient 5 male and five female blood samples were collected and transported to the laboratory and kept at 4°C. RNA was isolated from the samples, and further experiment analysis was done.

3. RESULTS

Blood Samples were collected from hospitals of the healthy patient and Asthma patient and further RNA analysis was done.

Total RNA extraction was done by the conventional Trizol method from asthma and healthy patients. Agarose Gel Electrophoresis did RNA confirmation with a 1% agarose concentration. The Extracted total RNA was converted to cDNA by TAKARA cDNA Synthesis Kit. These conversions of RNA to cDNA was confirmed by running a PCR setup using cDNA as template and GAPDH forward and reverse primers. Figures 1 and 2 explain the bands of total RNA extracted from asthma and regular patients. Table 1 was qualitatively checked and quantitatively estimated by nanophotometer A260/A280.

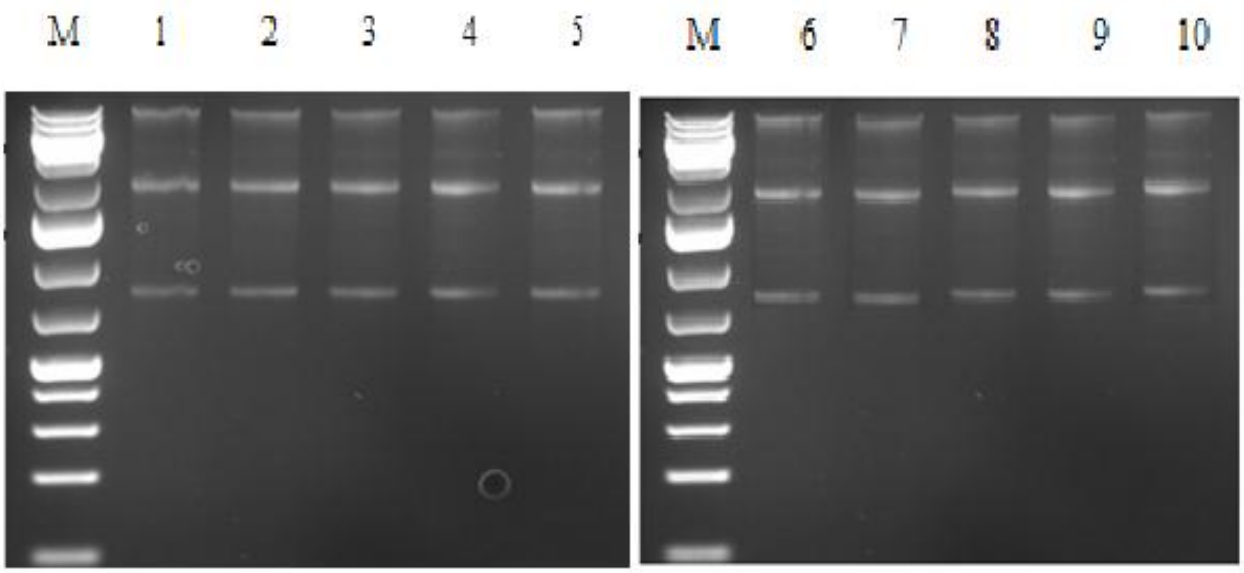

Figure-1. Gel Picture showing the bands of total RNA extracted from asthma patients, Lane M: Marker, and Lane 1-10: Isolated Total RNA from asthma patients.
Source: Dr. Reddy's Institute of Life Sciences (DRILS).

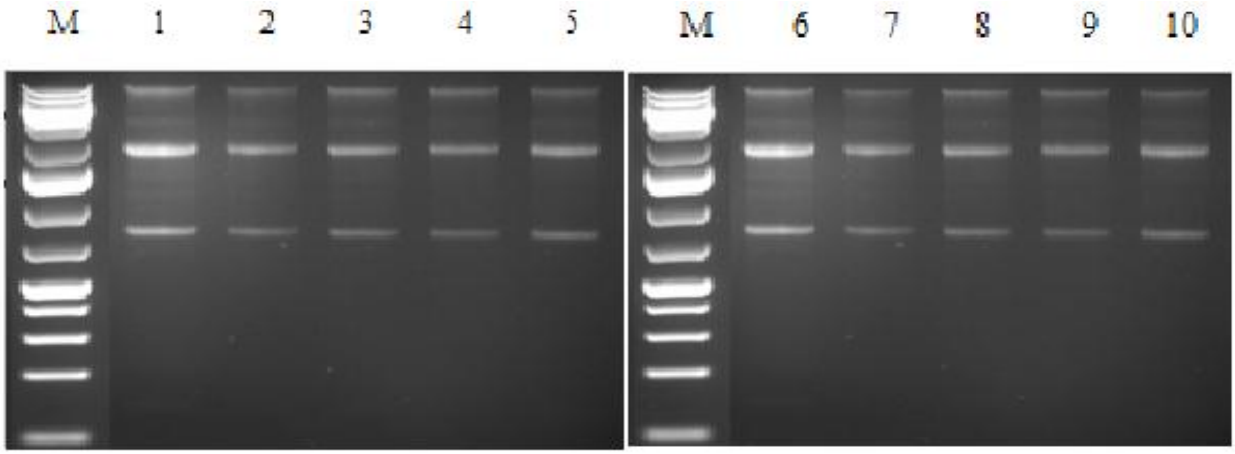

Figure-2. Gel Picture showing the bands of total RNA extracted from normal patients, Lane M: Marker and Lane 1-10: Isolated Total RNA from normal patients.
Source: Dr. Reddy's Institute of Life Sciences (DRILS).

Table-1. The extracted total RNA was qualitatively checked and quantitatively estimated by nanophotometer A260/A280.

Sl.No	Samples (Male)	A260/A280	Concentration (µg/µl)	Sl.No	Sample (Female)	Concentration (ug/ul)
1	Normal Patient 1	1.88	1.98	11	Normal Patient 6	1.98
2	Asthma Patient1	1.91	2.08	12	Asthma Patient 6	2.08
3	Normal Patient 2	1.86	0.99	13	Normal Patient 7	0.99
4	Asthma Patient 2	1.89	2.56	14	Asthma Patient 7	2.56
5	Normal Patient 3	1.93	0.78	15	Normal Patient 8	0.78
6	Asthma Patient 3	1.95	2.67	16	Asthma Patient 8	2.67
7	Normal Patient 4	1.86	1.99	17	Normal Patient 9	1.99
8	Asthma Patient 4	1.99	1.76	18	Asthma Patient 9	1.76
9	Normal Patient 5	1.89	2.05	19	Normal Patient 10	2.05
10	Asthma Patient 5	1.97	2.87	20	Asthma Patient 10	2.87

Source: Dr. Reddy's Institute of Life Sciences (DRILS), Hyderabad-India.

3.1. Conversion of RNA to cDNA

The extracted overall RNA changed into converted to cDNA with the aid of TAKARA cDNA Synthesis package. Those conversions of RNA to cDNA become shown with the support of jogging a PCR set up the use of cDNA as template and GAPDH forward and reverse primers. Gel image displaying amplified cDNA from RNA of allergies sufferers. It's miles obvious from the gel that cDNA got amplified, which in flip proves that RNA changed into effectively converted to cDNA. Figure 3 and Figure 4 are shown Gel picture amplified cDNA from RNA of asthma patients and healthy patients.

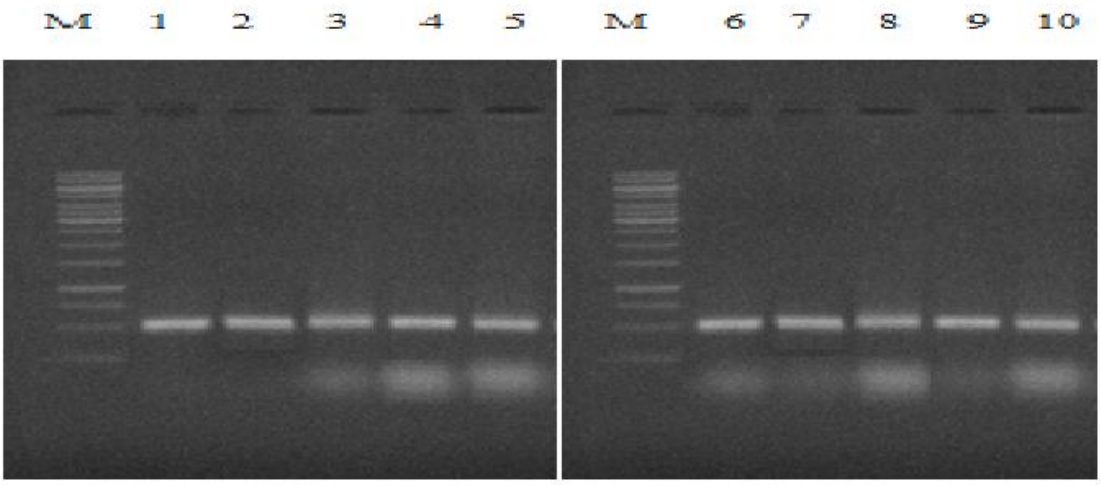

Figure-3. Gel picture showing amplified cDNA from RNA of asthma patients.
Source: Dr. Reddy's Institute of Life Sciences (DRILS).

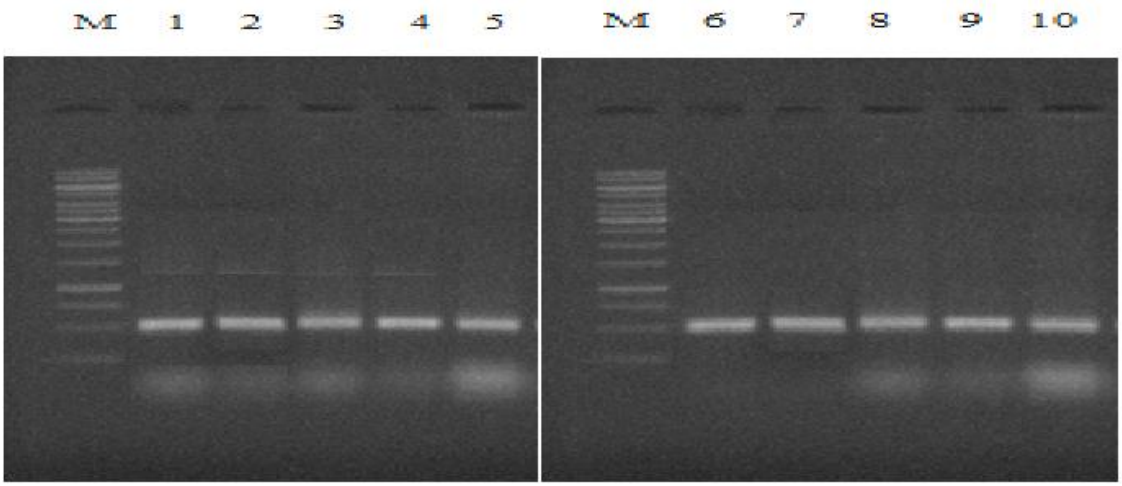

Figure-4. Gel picture showing amplified cDNA from RNA of normal patients.
Source: Dr. Reddy's Institute of Life Sciences (DRILS).

3.2. Quantitative Estimation of cDNA

After the presence of RNA and conversion into cDNA was confirmed, cDNA was estimated quantitatively to know its concentration and to know the requirement of any dilutions of cDNA to attain the appropriate level for RTPCR reaction. Table 2 is shown RNA present in asthma and normal patients and the concentration of cDNA.

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Table-2. After the presence of RNA and conversion into cDNA was confirmed.

Sl. No	Sample (Male)	Concentration (µg/µl)	Sl. No	Sample (Female)	Concentration (µg/µl)
1	Normal Patient 1	1.98	11	Normal Patient 6	1.88
2	Asthma Patient 1	1.67	12	Asthma Patient 6	2.56
3	Normal Patient 2	2.09	13	Normal Patient 7	2.35
4	Asthma Patient 2	1.88	14	Asthma Patient 7	1.87
5	Normal Patient 3	2.56	15	Normal Patient 8	2.16
6	Asthma Patient 3	2.35	16	Asthma Patient 8	1.88
7	Normal Patient 4	1.87	17	Normal Patient 9	1.99
8	Asthma Patient 4	2.16	18	Asthma Patient 9	1.67
9	Normal Patient 5	1.88	19	Normal Patient 10	2.09
10	Asthma Patient 5	1.99	20	Asthma Patient 10	1.88

Source: Dr. Reddy's Institute of Life Sciences (DRILS).

Dilution of each cDNA sample to get the required concentration to proceed for Real-Time PCR, Each sample is diluted by adding 100µl of Rnase Free Water. The volume which corresponds to 100 ng concentration is taken from each sample for RTPCR.

3.3. RTPCR Analysis

Cycle number vs. fluorescence is provided in the below graph.

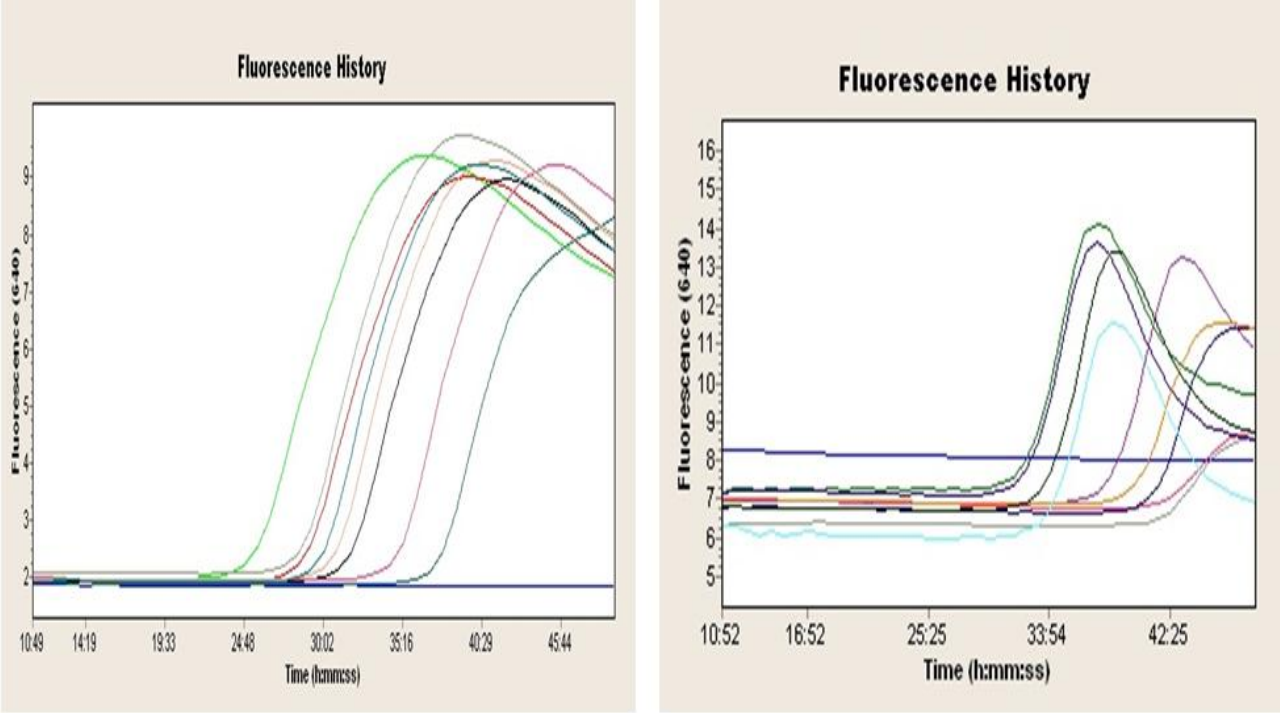

Figure-5. RTPCR Analysis (Cycle number vs. fluorescence is provided in the below graph).
Source: Dr. Reddy's Institute of Life Sciences (DRILS) Hyderabad-India.

Asthma Patients and Control Patients were examined for the expression levels of IL 4 using Real-Time Quantitative PCR. The results showed that Proinflammatory Cytokine IL 4 expression was considerably decreased in regular patients as compared to Asthma Patients. Figure 5 is shown the RT-PCR analysis graph.

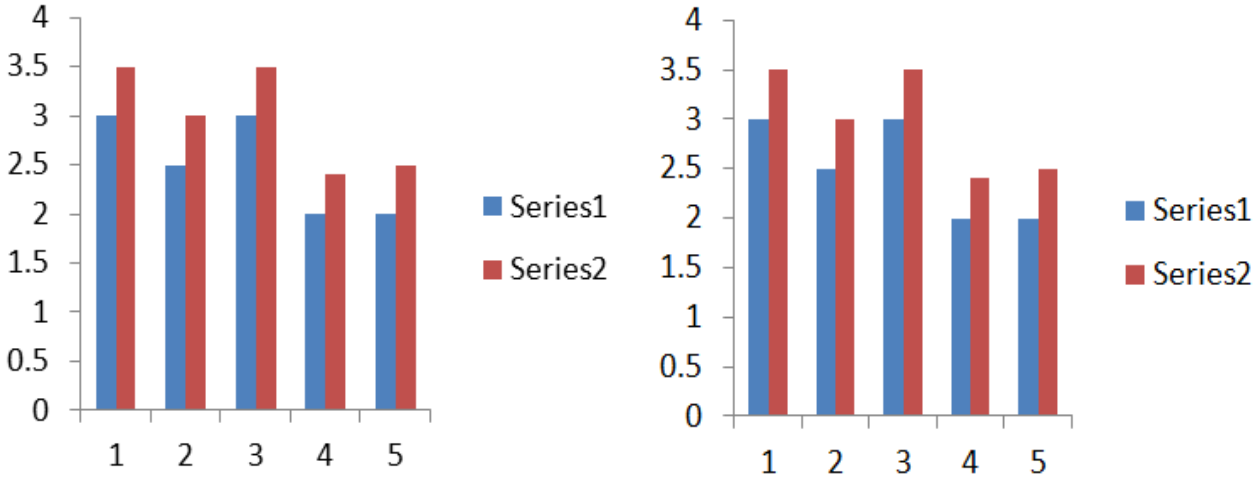

Figure-6. Levels of IL 4 Asthma Patients and Control Patients (Series 1 – Normal Patients, Series 2 – Asthma Patients).
Source: Dr. Reddy's Institute of Life Sciences (DRILS).

Quantitative Real-Time PCR. The results showed in Table 3 that IL-4 levels were considerably increased in healthy patients when compared to Asthma patients.

Table-3. IL-4 levels for asthma patients and control patient.

Sl. No	Sample (Male)	Ct values	Sl. No	Sample (Female)	Ct values
1	Normal Patient 1	11.56	11	Normal Patient 6	13.78
2	Asthma Patient 1	13.69	12	Asthma Patient 6	15.66
3	Normal Patient 2	12.46	13	Normal Patient 7	12.88
4	Asthma Patient 2	14.56	14	Asthma Patient 7	14.99
5	Normal Patient 3	13.59	15	Normal Patient 8	11.56
6	Asthma Patient 3	14.99	16	Asthma Patient 8	13.69
7	Normal Patient 4	11.97	17	Normal Patient 9	12.46
8	Asthma Patient 4	13.97	18	Asthma Patient 9	14.56
9	Normal Patient 5	15.69	19	Normal Patient 10	13.59
10	Asthma Patient 5	18.96	20	Asthma Patient 10	14.99

S.No	Sample	CT values mean ± SD
1	Normal Patients	12.94±1.26
2	Asthma Patients	15.72±1.52

Source: Dr. Reddy's Institute of Life Sciences (DRILS).

4. DISCUSSION

Asthma is a convoluted disorder including numerous individual incendiary cells, cytokines, and chemokines that final product in necessary changes, updating and accordingly in the side effects and manifestations of asthma. Asthma is portrayed by utilizing bronchoconstriction, bronchial hyperresponsiveness, a diminished reaction to ß-adrenergic merchants and contamination [11]. The irritation is set apart by aspiratory aviation route eosinophilia, increased breathed out nitric oxide, and the declaration of exact Lymphocyte cytokines together with interleukin-4 (IL-4), IL-5, IL-9 and IL-13 [12]. Sputum from patients with bronchial asthma is portrayed by methods for tight spirals of bodily fluid that begin from the little bronchioles and by way for low bodily fluid fittings that are basic of bronchopulmonary aspergillosis. Bronchial checks and bronchoconstriction lead to dyspnea, wheezing, and chest hack.

Notwithstanding persistent aggravation, bronchial asthma is described by method for auxiliary contrasts in the aviation routes that together are alluded to as aviation route rebuilding. Aviation route upgrading in the vast and

little aviation routes incorporates subepithelial thickening, epithelial denudation with cup Mobilephone metaplasia, quickened aviation route pure bulk, bronchial organ extension, angiogenesis and changes in the extracellular grid parts. This renovating is thinking of being started by disturbance of the aviation routes [13].

Complex interactions between aviation route irritation, necessary alterations, and aviation route hyperresponsiveness happen in mice sharpened by method for constant attention to allergens. Patients who grandstand control aviation route hyperresponsiveness with steady wind stream issues may have critical aviation route narrowing notwithstanding maximal treatment; segments of aviation route rebuilding are conceivable to add to aviation route hyperresponsiveness, wind current confinement, and aviation route narrowing [14].

Cytokines assume a crucial job in arranging the eternal irritation of asthma and COPD by selecting, initiating, and advancing the endurance of numerous fiery cells in the respiratory tract. More than 50 cytokines have now been distinguished in asthma and COPD. However, their job in the pathophysiology of these perplexing aviation route sicknesses is regularly misty. Cytokines are grouped into lymphokines (cytokines that are emitted by White blood cells and control safe reactions), proinflammatory (cytokines that enhance and propagate the fiery procedure), development factors (cytokines that advance cell endurance and result in auxiliary changes in the aviation routes), chemokines (cytokines that are chemotactic for incendiary cells), and calming (cytokines that contrarily tweak the provocative reaction), albeit a significant number of these capacities may cover [15, 16].

In the present study, serum levels of IL-4 mRNAs were evaluated using RT-PCR analysis with a sample size of ten asthma patients and ten controls, and it is statistically significant. It was found that the levels of these pro-inflammatory cytokines were elevated in the case of asthma patients as compared to controls. This gives evidence that therapy against these cytokines can assist in asthma treatment.

5. CONCLUSION

Chemokines anti-inflammatory cytokines, although many of these functions may overlap. Serum levels of IL-4 mRNAs were evaluated using RT-PCR analysis with a sample size of five asthma patients and five controls. It was found that the levels of these pro-inflammatory cytokines were elevated in the case of asthma patients as compared to controls. This gives evidence that therapy against these cytokines can assist in asthma treatment. Asthma is an unpredictable sickness, including numerous unmistakable provocative cells, cytokines, and chemokines that outcome in auxiliary changes, updating, and in this way in the signs and side effects and manifestations of asthma. The disease is set apart with the guide of pneumonic aviation route eosinophilia, broadened breathed out nitric oxide, and the declaration of one of a kind T-cytokines together with IL-4, IL-5, IL-9, and IL-thirteen Sputum from patients with asthma is described through tight spirals of bodily fluid that begin from the little bronchioles and through low bodily fluid fittings that are standard of bronchopulmonary Aspergillosis. Over 50 cytokines have been identified in asthma and COPD. However, their position in the pathophysiology of these complicated airway illnesses is frequently unclear. Two Cytokines are categorized into lymphokines proinflammatory cytokines increase factors.

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Competing Interests: The authors declare that they have no competing interests.
Acknowledgement: Both authors contributed equally to the conception and design of the study.

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