Roll of CD4+CD25 treg Cell in A Patient with Hay Fever

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Abstract
Background and Objective:CD4/CD25Tregs in patient with hay fever whose Th2 cells predominate plays a critical role in releasing cytokines that change the balance of Th1/Th2 to Th1 , which lessens the severity of the condition and produces the signs and symptoms of allergy. Materials and method: 40 patients and 40 controls' peripheral blood cells were taken, and using flow cytometry, the proportion of CD4+CD25 Treg cells was calculated. Results: As compared to healthy controls, cases of allergic rhinitis had lower mean CD4+CD25 concentrations (1.906 4.16 pg/ml vs. 1.906 4.16 pg/ml), and this difference was highly significant (p0.001)
Keywords: hay fever, Allergic rhinitis, CD4/CD25Treg cells, flow cytometry, Th lymphocyte

1. Introduction

A chronic inflammation of the nasal mucosa is referred to as hay fever or allergic rhinitis (AR). It is accomplished by CD4+ Th2 effector cells secreting IL4, IL5, and IL13 in response to environmental antigens (protein or glycoprotein substance) [1]. In the nasal mucosa, Th2 T cells proliferate and produce the cytokines that stimulate B cells to create immunoglobulin E. (IgE) . These immunoglobulin conjucated with mast cell by FcεRI Following the release of mediators like histamines and leukotrienes , symptoms like itching, smooth muscle spasm, arteriolar dilatation, increased vascular permeability, mucous secretion, and rhinorrhea occur [2]. It has been proven that regulatory T cells (Tregs) play a part in immunological tolerance.They also have a significant impact on hay fever [3]. 15% of CD4 T cells have the ability to express CD25, the IL2 growth factor receptor chain. By regulating the activation and expansion of auto-reactive T cells that have avoided thymic deletion, CD4+CD25+ Tregs prevent organ-specific autoimmunity, minimize allograft rejection, and maintain self-tolerance in healthy people [4, 5]. Individuals' CD4+CD25Tregs Limiting allergic inflammation in the airways and preventing incorrect Th2 reactions to allergens may be achieved by blocking the progression from the early activation stage to the differentiated Th2 location using Treg cells [6, 7]. Numerous pieces of evidence suggest that people with allergies have fewer or less-functional Tregs than individuals without allergies [8, 9].
According to some research, a decrease in CD4+CD25+ TRegs cell numbers may be linked to allergy illness, while others imply that individuals with (AR) do not have altered CD4+CD25+ Treg cell numbers or function. [10, 11, 12, 13]. The function of Treg cells in the pathogenesis of allergic rhinitis, however, is not well understood, and their part in the development of allergic disease has only recently been defined. The goal of the study was to comprehend the function of Treg cells in the pathophysiology of hay fever by studying the CD4+CD25+ Treg population during allergies. Additionally, it looked into whether the number of Treg cells in an individual was associated with how severe their case of hay fever was.

2. Material and Method

Design of the Study

This case-control study was done on 40 patients recruited from AlDiwaniaya Teaching Hospital and AL Hamzah General Hospital in Al-Qadisiyah governorate and 40 healthy as control subjects from the first of December 2021 to the end of January 2022. A case-control study was conducted on the following study groups from December 2021 to the end of March 2022. In this study, the patient's group is composed of (40) (21) of them are males and (19) females in the age range 10-63 years old. All that is listed in table 1. Patients in the hay fever group displayed at least six symptoms, including rhinorrhea, sneezing, nasal congestion, and nasal and ocular itching, according to the Total 5 Symptom Score (T5SS) [14].

Table (3-1): Demographic characteristics of patients with hay fever and control subjects.

Characteristic	Patients n = 40	Control n = 40	P
Age (years)			
Mean ±SD	31.33 ± 15.68	22.10 ± 7.60	0.079 † NS
Range	10 - 63 years	12- 34 years	
< 20, n (%)	12 (30.0 %)	16 (40.0 %)	0.377 ¥ NS
20-29, n (%)	10 (25.0 %)	12 (30.0 %)	
≥ 30, n (%)	18 (45.0 %)	12 (30.0%)	
Gender			
Male, n (%)	19 (47.5 %)	20 (50.0 %)	0.823 ¥ NS
Female, n (%)	21 (52.5 %)	20 (50.0 %)	
Male: female ratio	1: 1.11	1:1	
Residency			
Urban, n (%)	25 (62.5 %)	22 (55.0 %)	0.496 ¥ NS
Rural, n (%)	15 (37.5 %)	18 (45.0%)	

n: number of cases; SD: standard deviation; †: independent samples t-test; ¥: Chi-square test; NS: not significant at P > 0.05

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Specimen collection

3ml of peripheral blood were collected in K3-EDTA anticoagulated tube and transferred directly to the lab for Flow Cytometry assay procedure

Flow cytometry analysis

1. Add 100 µl of the cell suspension [or whole blood] into as many test tubes as required.
2. Mouse immunoglobin G1 isotype control conjugated to FITC (IgG1-FITC), mouse anti-human CD25 coupled to phycoerythrin (CD25-PE), and mouse immunoglobin G1 isotype control attached to PE (IgG1-PE) CD4-FITC, or mouse anti-human CD4, is a fluorescent dye (IgG1-PE) After thoroughly combining, incubate at 4°C for at least 30 minutes without any direct light.
3. Centrifuge the cells at 300-400 g for 5 minutes at 4°C after washing them with 2 cc of cold (4°C) PBS/BSA. Throw away the excess liquid. (Add 2 ml of freshly made erythrocyte red blood cell lysing buffer to the blood solution and thoroughly mix. 10 minutes of room temperature incubation is required. Discard the supernatant after centrifuging for five minutes at 300-400 x g at room temperature. Centrifuge at 300-400 x g for 5 minutes at room temperature after washing with 2 cc of PBS/BSA. Discard the supernatant. Proceed to step 4 now.
4. Cells are resuspended in 200 l of cold (4°C) PBS.
5. Data collection using flow cytometry. Within 24 hours, analyze fixed cells 24 hours.

Statistical Analysis

Statistical research was done using the SPSS v 0.26 program. It was determined if the data distribution was normal using the Kolmogorov-Smirnov test.The information was shown as either means with standard deviations or medians with 25% and 75% IQR (SD). To compare two independent groups, non-parametric Mann-Whitney U or Student's t-tests were used as needed. Correlation was evaluated using the Spearman's correlation test. The significance level is considered when a P value is less than (≤ 0.05).

3. Results

The mean concentration of CD4 was significantly decreased in cases with allergic rhinitis (28.47 ± 9.06 pg/ml) as compared to healthy controls (34.83 ± 5.50 pg/ml) (p=0.040). Also, the mean concentration level of CD25 was lower in cases with allergic rhinitis compared to healthy controls (1.906± 4.16 pg/ml) vs. (1.906± 4.16 pg/ml), and the difference was highly significant (p<0.001), also CD4CD25 significantly decrease in the patient(5.16 ± 1.97pg/ml)vs. (12.50 ± 0.377) in healthy control subject

Table (2): Mean levels of CD4 and CD25 in allergic rhinitis patients with healthy control

Markers	Cases – Control Comparison		
	allergic rhinitis patients n = 40	Healthy control n = 40	P
CD4			
Mean± SD	28.47 ± 9.06	34.83 ± 5.50	P= 0.040 † S
Range	3.50 – 46.00	30.10-43.50	
CD25			
Mean± SD	5.81 ± 1.85	13.17 ± 0.211	P= 0.001 † S
Range	0.81 – 9.90	12.90-13.60	
CD4/CD25			
Mean± SD	5.16 ± 1.97	12.50 ± 0.377	P= 0.001 † S
Range	0.54 – 10.00	12.00-13.10	

n: number of cases; SD: standard deviation; †: independent samples t-test; HS: Highly significant at P ≤ 0.001.

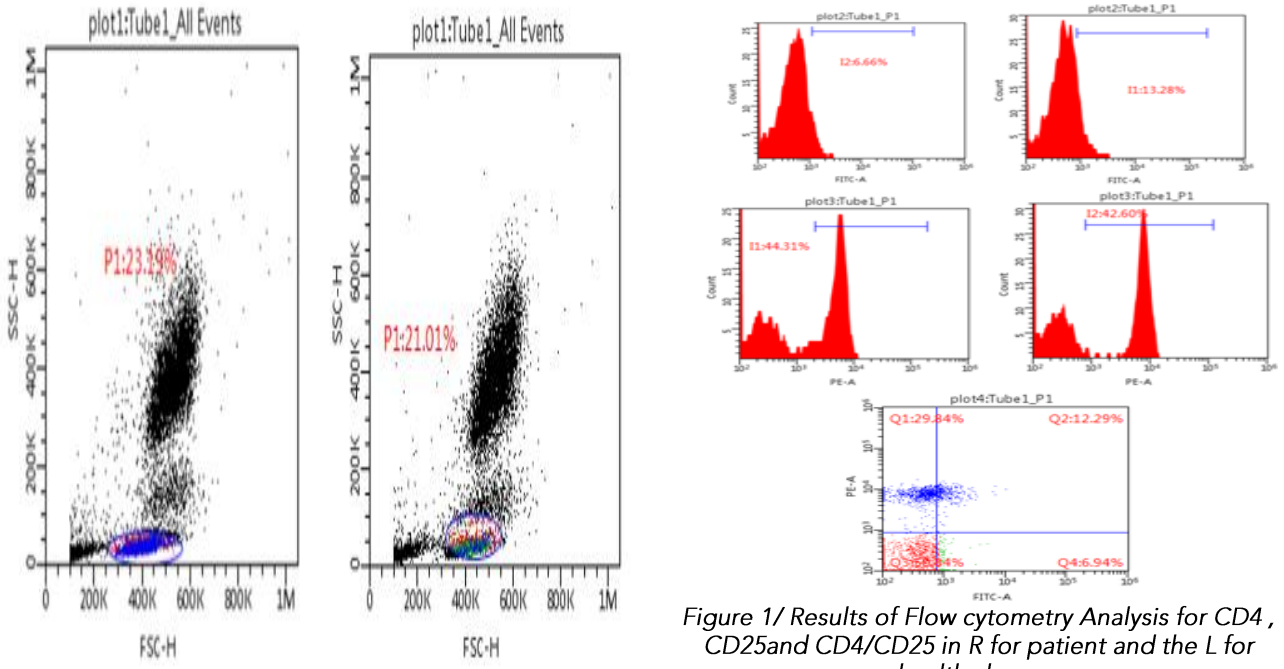

Figure 1/ Results of Flow cytometry Analysis for CD4 , CD25and CD4/CD25 in R for patient and the L for healthy human

4. Discussion

In healthy humans, the immune system is regulated by T regulatory cells (Treg), a subtype of T cells that works to restrict the inflammatory response to lessen a reaction to the body's tissues or harmless antigens. Treg cells are recognized by their surface markers CD4 and CD25 as well as the transcription factor. P3 Forkhead Box (FoxP3) [15]. According to one theory, regulatory T lymphocyte-based mechanisms called Treg cells inhibit IgE reactions to allergens in healthy persons by suppressing Th2 reactions [16]. It has been observed that a Guinea pig with allergic rhinitis significantly declined the relative abundance of CD4+ CD25+ Foxp3+ Treg cells, and an increase in the proportion of CD4 +CD25+ Foxp3+ Treg cells inhibits allergic reactions by increasing the production of IL10. In actuality, the allergic reaction is exacerbated by declining Treg populations[17]; individuals with hay fever have been demonstrated to have fewer Treg cells [18]. Treg cells significantly regulate allergic reactions, especially hay fever, and limit the progression of autoimmune illness [19].

As far as we know, the association between CD marker expression in allergic rhinitis is not well understood. One of the transcription factors in the forkhead/winged-helix family is FOXP3 [20]. Acute allergic inflammatory illness can be brought on by FOXP3 dysregulation in humans [21]. To keep CD4+CD25+Treg cells functioning and the immune system in a self-stabilizing condition, FOXP3 gene expression must be expected. According to a study, people with hay fever have lower levels of CD4+CD25+Tregs due to decreased FOXP3 expression [22]. SNPs in FOXP3 are hypothesized to cause immunological tolerance disorders by reducing the number of CD4+CD25+Tregs or altering their activity [23].

The current method of diagnosis uses functional analysis to determine any probable thymic abnormalities by counting and counting the number of circulating T-lymphocytes[24]. Analysis using flow cytometry T-cell subsets may seem like enough for a lone screening test. Where the present study revealed that the level of immune markers, CD4 became significantly decreased among cases with allergic rhinitis (28.47 ± 9.06 pg/ml) when compared with healthy controls (34.83 ± 5.50 pg/ml), also the mean levels of CD25 became significantly decreased among cases with allergic rhinitis (5.81 ± 1.85 pg/ml) when compared with healthy controls (13.17 ± 0.211 pg/ml), these results indicate a significant association between Treg cells (CD4 and CD25) and allergic rhinitis ($p<0.001$). The present research hypothesizes that allergic type 2 immunity developed in the nasal mucosa and airways as a consequence of hay fever exacerbations' decreased CD4+CD25+Treg cell numbers' ability to suppress T helper 2 responses. continuation of the current results Fewer CD4+CD25+Treg cells have been linked to the development of allergic rhinitis and hay fever, respectively, according to research by Shaoqing et al. (2018) and Zhou et al. (2021). These investigations also showed that as compared to healthy controls, individuals with allergic rhinitis had substantially reduced CD4 and CD25 expression [25]. The present results are consistent with past findings in individuals with allergic asthma [27]. The frequency and functional relevance of CD4+CD25+Treg cells in peripheral blood mononuclear cells from allergic asthma patients were examined by Shi et al. (2011) [28]. Using flow cytometry, the proportion of CD4+CD25+Treg cells and their function in the peripheral blood mononuclear cells of asthmatic patients were decreased. The researchers came to the conclusion that CD4+ CD25+Tregs are critical for maintaining self-tolerance and are related with mild to severe asthma. In order to prevent unnecessary T helper 2 responses in allergic diseases, CD4+CD25+Treg cells are crucial. The results of other studies conflict with the ones we currently have. Han et al. (2010) showed that allergy sufferers' CD4+CD25+Treg cell numbers and effectiveness were unaffected [29]. A cohort of asthmatic patients studied by Hoffmann et al. (2007) similarly shown that CD4+CD25+Treg cell counts were similar in allergic and healthy control individuals [30]. These discrepancies could be caused by the different studies' participants' varying ages and illness statuses. The suppressive effect of CD4+CD25+ Treg cells may also be influenced by a number of factors, including the kind of allergen, exposure to allergens, and individual allergy status, according to various reports [31].

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