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ORIGINAL ARTICLE

IL-22, TNF-α, IL-17, and IL-8 in Allergic Rhinitis and Correlation with Disease Severity

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ABSTRACT

Background: *The inflammatory role of some cytokines in allergic rhinitis (AR) is not yet well illustrated. Understanding its role may form a target for novel therapeutics.* ***Objectives:*** *To assess the levels of IL-17, TNF-α, IL-8 and IL-22 in sera of AR patients and to correlate their levels with disease severity.* ***Methodology:*** *This case-control study included 26 AR patients and 26 healthy controls. Blood samples of all participants were tested for levels of total IgE, IL-17, TNF-α, IL-8 and IL-22 serum using ELISA method. Skin prick test (SPT) and Specific IgE (sIgE) for aeroallergens were done by immunoblot assay for all AR patients.* ***Results:*** *AR patients possessed higher serum levels of IL-17, IL22, TNF-α and IL-8 (P= 0.006, <0.00, 0.013 and<0.001 respectively) compared to the control group. There was a statistically significant relation between disease severity (evaluated through visual analogue score (VAS)) and TNF-α& IL-22 serum levels (P= 0.031& <0.001 respectively).* ***Conclusion:*** *IL-17, IL-22, TNF-α and IL-8 promote the inflammatory response in AR patients. TNF-α and IL-22 were directly correlated with AR severity.*

INTRODUCTION

Allergic Rhinitis (AR) is a medical condition manifested by nasal symptoms such as congestion, frequent sneezing, rhinorrhea, postnasal discharge, and itching. In addition, patients may present with other symptoms such as conjunctivitis, cough, and sinusitis [1]. AR may be complicated by other comorbid medical conditions like asthma, rhinosinusitis, nasal polyposis, and sleep disorders. This will affect quality of life, mental and learning capacities in children, work performance in adults which represents an economic burden [2]. AR burden and severity can be assessed by different scores such as, Total nasal symptoms score, Rhinoconjunctivitis Quality of Life Questionnaire [3].

AR is considered as an immunological response to allergens exposure leading to IgE-mediated inflammatory reactions in nasal mucosa[4]. T lymphocyte helper (Th) 2 is the principal cell implicated in allergic reactions. T-helper 17 cells that release IL-17, IL6 and IL-22 can also participate in pathogenesis and progression of allergic airway disease[5]. IL-17A is known as an inflammatory cytokine, affecting large scale of cells, and triggering the production of numerous cytokines and mucosal proteins genes [6].

IL-22 was initially detected in 2000, and it was believed that Th17 is the only source for it, however, a novel Th cell population (Th22) was described later. Recently, it has been reported that "IL-22" can be produced by Th22, Th1, Th2, Th17, innate lymphoid, and natural killers. IL-22 may be crucial in the occurrence of allergic asthma and AR but its role as inflammatory or anti-inflammatory agent is still under investigation [5].

Tumor necrosis factor α (TNF-α) is considered one of the inflammatory cytokines. Macrophages and monocytes are the main source for it. Previously conducted studies reported that some pulmonary diseases may be associated with expression of TNF-α [7].

Interleukin-8 (IL-8) is one of the chemotactic agents attracting several cells such as neutrophils, lymphocytes, basophils, and eosinophils to site of inflammation. Also, it activates migrating cells and may stimulate basophils to release histamine under some circumstances [8].

Type 2 inflammation is the major feature of AR but mixed endotypes/sub-endotypes are also proposed such as Th1/Th17 or Th2/Th17 endotype[9]. This interplay between different lymphocytes among mixed endotypes added layers to the complexity of the traditional Th1/Th2 balance paradigm of AR [10]. Additionally, T lymphocytes can show some degree of plasticity when they are triggered by certain re-polarizing signals [11]. For instance, Th17 and Th1 cells can produce IL-4 under certain circumstances[12]. Chemokines such as IL-8 seems to contribute to the pathophysiology of allergic diseases through its roles in some aspects of these mechanisms [9]. Therefore, it is essential to have a comprehensive view

of the interaction between chemokines and cytokines, of different Th lymphocytes, in the context of AR.

The underlying pathogenesis of AR including serum IL22, IL17, TNF-α and IL8 levels and their roles in AR is needed to be more investigated for better understanding of the disease markers and their potential use as therapeutic targets, so our goal was to evaluate the serum levels of IL22, IL17, TNF-α and IL8 among AR patients and to correlate their levels with the disease severity.

METHODOLOGY

Study Design and Setting:

This Case Control study has been carried out in Allergy and Immunology Unit at Faculty of Medicine, Zagazig University, Egypt, during the period (June 2021 - February 2022). Laboratory procedures were carried out at the Department of Microbiology and Immunology, Faculty of medicine, Zagazig University, Egypt.

Ethical approval:

The study has received approval from Zagazig University's research ethics committee (IRB number 9283-8-6-2021). All research participants provided their informed consent before participating. The study protocol complies with country rules regarding subject data confidentiality and integrity, and this study has been carried out according to the Helsinki Declarations. Informed consent was obtained from all individual participants included in the study.

Participants

The present study included 26 AR patients and 26 healthy controls. The sample size was estimated by assuming the means of IL22 positive cells were 68.3± 7.24 vs 76.43±12.64 (controls vs cases) [4], at 80% power and 95% CI. The cases and controls were matched for age and sex. Family members of the patients were used to find healthy controls who attended the Outpatient Clinic for Internal Medicine. They didn't have any chronic diseases or allergies, weren't taking any drugs, and didn't have any health issues either mental or physical.

Allergic Rhinitis and its effect on Asthma guidelines were used to diagnose AR [13]. Our study included adult atopic AR patients with a positive family history of atopy and a positive result of SPT for at least one inhalant allergen. The exclusion criteria included patients with statin therapy, acute illnesses such as elevated body temperature, the two weeks after surgery, nasal inflammation without allergy, disorders of sleep, diabetics, obesity, hypothyroid, hypertension, smokers, and those with autoimmune disease. The patients were not under Immunotherapy, biologics, or systemic steroids. They were receiving intranasal steroids and antihistamines. Patients with AR have been subdivided into (2) groups in accordance with allergen sensitization and pattern of symptoms such as seasonal allergic rhinitis (SAR) and perennial allergic rhinitis (PAR). Additionally, AR individuals were subdivided according to association of asthma.

Evaluation of AR Severity

The score of VAS for evaluating the degree of nasal and non-nasal symptoms severity has been applied to detect the severity of AR. Patients with AR were asked to evaluate their overall symptoms both nasal and non-nasal on a measure (0 to 10 cm) as shown: Mild: 0–3; Moderate: 3.1–7; Severe: 7.1–10 [13].

Patients' work-up

Clinical evaluation

Each participant provided a detailed medical history of allergy, including atopy history in their families. In the clinic, a clinical evaluation (examination for; nose, ears, throat, and chest) and SPT to popular aeroallergens have been carried out. The included cases had positive SPT for at least one inhalant allergen, while included controls had negative SPT result. All subjects (cases and controls) had venous blood samples taken to test for serum total IgE, and serum level of IL22, IL 8, TNF-α, and IL 17. Moreover, AR cases were assessed for aeroallergens sIgE. All samples were collected from stable AR patients in absence of exacerbation.

Skin prick test (SPT)

European Academy of Allergy and Clinical Immunology's SPT Practical Guide (EAACI) has been used to conduct and interpret SPT [14]. A panel of standardized aero-allergen extracts (Omega Laboratories Ltd., Montreal, Canada) were used including: *Dermatophagoides pteronyssinus (D. pteronyssinus), Dermatophagoides farina (D. farina), mold mix (Penicillium notatum (P. notatum), Aspergillus niger (A. niger), Aspergillus fumigatus (A. fumigatus)*, and *Cladosporium*), feather mix (chicken, duck, goose), Timothy, Ragweed, Mugwort, cockroach mix (*P.ameriana* and *B.germania*) , cat, and dog hair. Negative standard (Glycerinated saline) and positive one (histamine dihydrochloride (10 mg/mL)) were used.

Sample Collection

A clean venipuncture was used to collect 10 mL of venous blood under completely aseptic conditions. Serum has been separated by centrifugation at 1000 g for 15 min then, kept at -20°C until further measurement of serum total IgE, SIgE of inhalant allergens, IL 17, IL22, TNF-α and IL 8 levels. Hemolyzed samples have been discarded. Repeated freezing and thawing were avoided.

Serum Levels of Total and Specific IgE

The total amount of IgE in the serum was quantified and the findings were reported in IU/mL. Up to 100 IU/mL of total IgE serum were deemed normal amounts by using enzyme-linked immunosorbent assay (ELISA) Kit **(Calbiotech Inc.) (1935 Cordell Ct., El Cajon, CA 92020, USA)**

The immunoblot technique was used following the manufacturer's policy (AllergyScreen® system, MEDIWISS Analytic GmbH, Moers, Germany) to measure the serum level of inhalant-specific IgE for 14 aeroallergens: *Dermatophagoides pteronyssinus* (*Der p*) , *Dermatophagoides farina* (*Der f*), *Penicillium notatum* (*P. notatum*), *Alternaria alternate* (*A. alternate*), *Aspergillus niger* (*A. niger*), *Aspergillus fumigatus* (*A. fumigatus*), *Candida albicans* (*C. albicans*), feather, birch, latex , mixed grasses, cat and dog epithelium, and cockroach. A fast scanner has been used to examine serum specific IgE. (Improvio Scanner System, Moers, Germany). If the process control on the 1st position of each strip has been coloured, the test was recorded to be legitimate. A positive result was recorded when sIgE level ≥ 0.35 IU/mL.

IL- 22 Serum level

According to the manufacturer's recommendations, human IL-22 was quantified using a widely available quantitative Human IL-22 ELISA Kit provided by Fine Test **(Optics Valley Biomedical Industrial Park, Wuhan, China)**. The results were presented in pg/mL.

IL-17 Serum level

Following to the manufacturer's recommendations, human IL17-A was quantified using a widely available ELISA kit (**Thermo Fisher Scientific**) **(Bender Med Systems GmbH, Vienna, Austria)**. The obtained results have been expressed in pg/mL.

IL-8 Serum level

Human IL-8 was quantified using a widely available ELISA Kit Picokine provided by **Boster Biological Technology (3942 B Valley Ave, Pleasanton, CA 94566) in** accordance with the company's recommendations and the results were presented in pg/mL.

TNF-α Serum level

Human TNF-α was quantified using a widely available quantitative ELISA Kit provided by **BT LAB (202 5／F 2 Bidg, Nanhu Dist, Jiaxing, Zhejiang, China)** regarding to the manufacturer's instructions and the obtained data were recorded in pg/mL.

Statistical Analysis

Version 26 of SPSS (Statistical Package for the Social Sciences) has been used for statistical analysis. We used the means and standard deviations of quantitative variables to characterize them. Description of categorical variables was shown through their ultimate frequencies and the chi square test, Monte Carlo tests and Fisher exact test were used for comparison. For systematic binary data, chi square for trend test has been used. Kolmogorov-Smirnov (distribution-type) and Levene (homogeneity of variances) tests have been used to validate presumptions for being used in parametric tests. Independent sample t tests (for normally distributed data) and Mann Whitney tests (for not normally distributed data) were also used to compare quantifiable data between two groups. Spearman rank and Pearson correlation coefficient were used, respectively, to measure the intensity and association of the correlation between two continuous variables. ROC curve was used to determine the best cutoff of certain quantitative parameters in diagnosis of certain health problem. The level statistical significance was set at $P<0.05$. A highly significant difference was present if $P\leq0.001$.

RESULTS

Baseline Characteristics

The mean age among cases was 29.19 ± 7.14 years while among controls were 26.23 ± 6.77 years. There were fifteen (57.7%) males in AR group while there were 14 (53.8%) males in the control group. All demographic and clinical data of the participants are presented in table 1.

Table 1: Baseline Characteristics of the Study Subjects

Characteristics	AR group (n=26)	Control Group(n=26)	*P*-value
Age, years	29.19 ± 7.14	26.23 ± 6.77	0.131
Male sex, n (%)	15 (57.7%)	14 (53.8%)	0.78
Duration, years; median, range	4.0 (3.0 – 7.0)		
SAR, n (%) **PAR, n (%)**	18 (69.2%) 8 (30.8%)		
Family history, n (%)	11 (42.3%)		
Associated asthma, n (%)	13 (50%)		
Total IgE (IU/mL); median, range	102 (14.3 – 1630)	13.95 (5.3 – 200)	<0.001
VAS, n (%) Mild Moderate Severe	 20 (76.7%) 3 (11.9%) 3 (11.9%)		
Severity of AR, n (%) Mild Intermittent Mild Persistent Moderate to Severe Persistent	 4 (15.4%) 16 (61.5%) 6 (23.1%)		

SAR, seasonal allergic rhinitis; PAR, perineal allergic rhinitis; VAS, visual analogue score

Pattern of Inhalant Allergens Sensitization among AR patients

There is statistically non-significant relation between type of AR and different aeroallergens sensitization as shown in table 2.

Table 2: Pattern of Aero-allergens Sensitization among AR Group

Allergen sIgE, N (%)	AR (n=26)	Seasonal AR (n=8)	Perennial AR (n=18)	*P*-value
Der p	20 (76.9%)	4 (50%)	16 (88.9%)	0.051
Der f	14 (53.8%)	4 (50%)	10 (55.6%)	>0.999
Cockroach	16 (61.5%)	5 (62.5%)	11 (61.1%)	>0.999
Dog Epithelium	14 (53.8%)	3 (37.5%)	11 (61.1%)	0.409
Mixed Grasses	9 (34.6%)	5 (62.5%)	4 (22.2%)	0.078
Birch	10 (38.5%)	2 (25%)	8 (44.4%)	0.42
C. albicans	6 (23.1%)	2 (25%)	4 (22.2%)	>0.999
A. fumigatus	4 (15.4%)	0 (0%)	4 (22.2%)	0.147
A. alternate	8 (30.8%)	3 (37.5%)	5 (27.8%)	0.667
Feather Mix	6 (23.1%)	2 (25%)	4 (22.2%)	>0.999
Latex	3 (11.5%)	1 (12.5%)	2 (11.1%)	>0.999
P. notatum	8 (30.8%)	4 (50%)	4 (22.2%)	0.197
A. niger	2 (7.7%)	0 (0%)	2 (11.1%)	0.268
Cat Epithelium	3 (11.5%)	0 (0%)	3 (16.7%)	0.529

Data presented as number and percentage N (%) of positive responses (above the positive cutoff >0.35 IU/ml), *p*-value for chi square test. *Der p*, *Dermatophagoides pteronyssinus*; *Der f*, *Dermatophagoides farina*; *P. notatum*, *Penicillium notatum*; *A. alternate*, *Alternaria alternate*; *A. niger*, *Aspergillus niger*; *A. fumigatus*, *Aspergillus fumigatus*; *C. albicans*, *Candida albicans*.

Cytokine levels among AR group and control group

In comparison to the control group, serum levels of IL-17(P= 0.006), IL-8 (p<0.001), TNF-α (P=0.013) and IL22 (<0.001) were significantly elevated more in patients with AR (Table 3).

Table 3: AR Patients' Serum Cytokine Levels Compared to Those of the Control Group

Cytokine(ng/ml)	AR group	Control group	*P*-value
	Median (IQR)	**Median (IQR)**	
TNF-α	171.9 (56.13 – 385.2)	91.4 (71.88 – 114.65)	0.013
IL-22	128.27 (91.36– 192.5)	52.22 (42.78 – 110.57)	<0.001
IL-17	9.32 (6.48 – 15.33)	7.5 (5.48 – 9.33)	0.006
IL-8 (mean ± SD)	252.81 ± 67.9	161.04 ± 9.89	<0.001

Data presented as median (IQR) except IL-8 presented as mean ± SD.

Cytokine levels among Seasonal AR and Perineal AR

There was statistically insignificant difference between perennial AR (PAR) and seasonal AR (SAR) regarding serum TNF-α, IL-22, IL-17, and IL-8 (P= 0.846, 0.868, 0.824, and 0.284 respectively) (Fig 1).

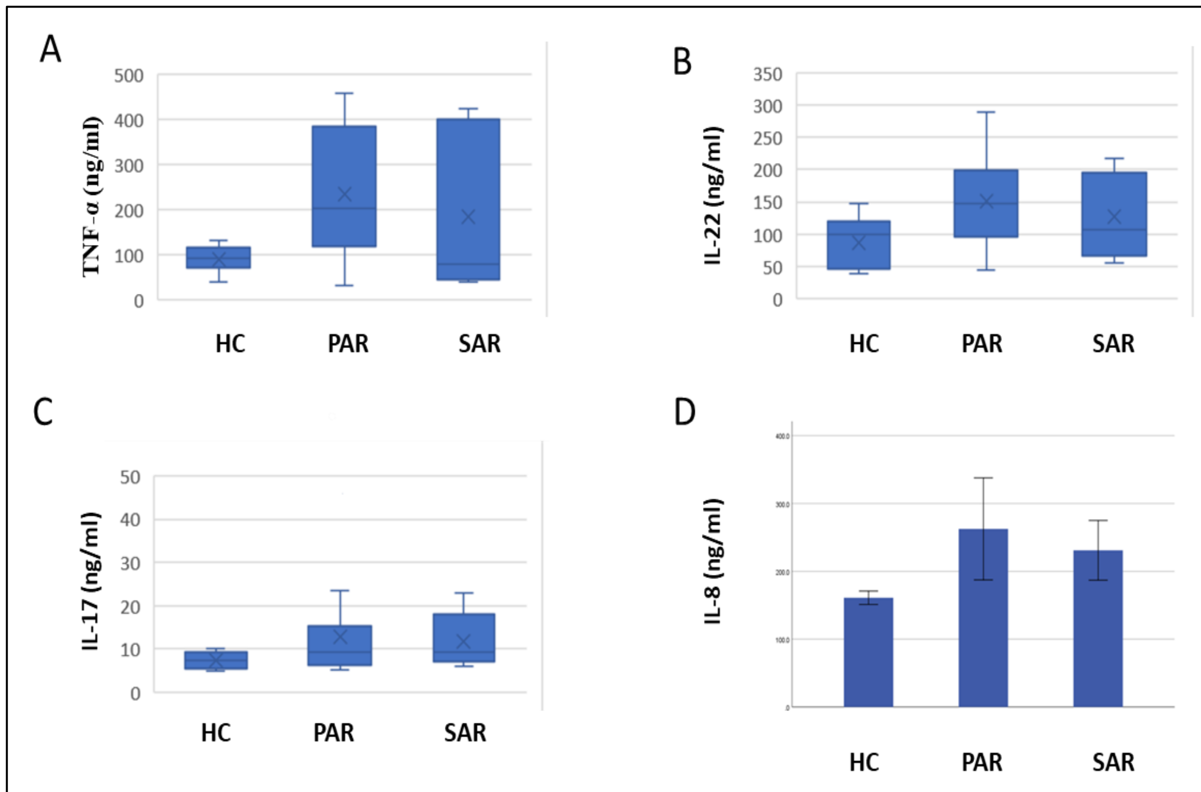

Fig.1 Serum levels of the different cytokines among the studied groups (healthy controls (HC), Perineal allergic rhinitis (PAR), and seasonal allergic rhinitis (SAR). (A) TNF-α, (B) IL-22, (C) IL-17, (D) IL-8.

Cytokine Levels of AR without Asthma and AR Associated with Asthma

Serum IL-22 was greater in AR individuals associated with asthma in comparison to AR without asthma (P=0.007). However, there was statistically insignificant variation between these 2 groups concerning serum levels of TNF-α, IL-17, and IL-8 (P= 0.72, 0.898, and 0.368 respectively) (Fig 2).

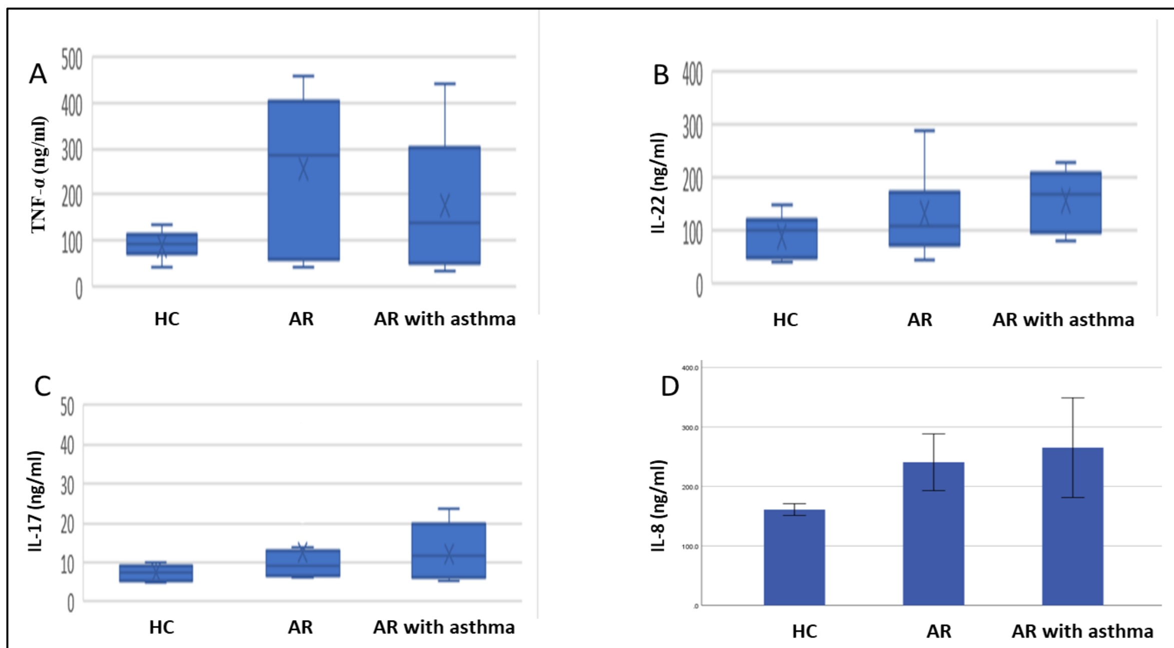

Fig.2 Serum levels of different cytokines among the studied groups (healthy controls (HC), AR without asthma, and AR with asthma). (A) TNF-α, (B) IL-22, (C) IL-17 (D) IL-8.

Potential Predictive Value of the Studied Cytokines in Diagnosis of AR

The best cutoff of TNF-α in diagnosis of AR was ≥101.15 with area under curve 0.7 (95% CI; 0.534 – 0.866), sensitivity 69.2% and specificity 61.5% (*P*=0.013) while, the best cutoff of IL-22 in diagnosis of AR was ≥105.32 with area under curve 0.743 (95% CI; 0.609 – 0.876), sensitivity 65.4% and specificity 61.5% (*P*=0.003). The best cutoff of IL-17 in diagnosis of AR was ≥8.1 with area under curve 0.721 (95% CI; 0.583 – 0.86), sensitivity 65.4% and specificity 65.4% (*P*=0.006) while, the best cutoff of IL-8 in diagnosis of AR was ≥175.7 with area under curve 0.956 (95% CI; 0.881 – 1), sensitivity 96.2% and specificity 92.3% (*P*<0.001) (Fig 3).

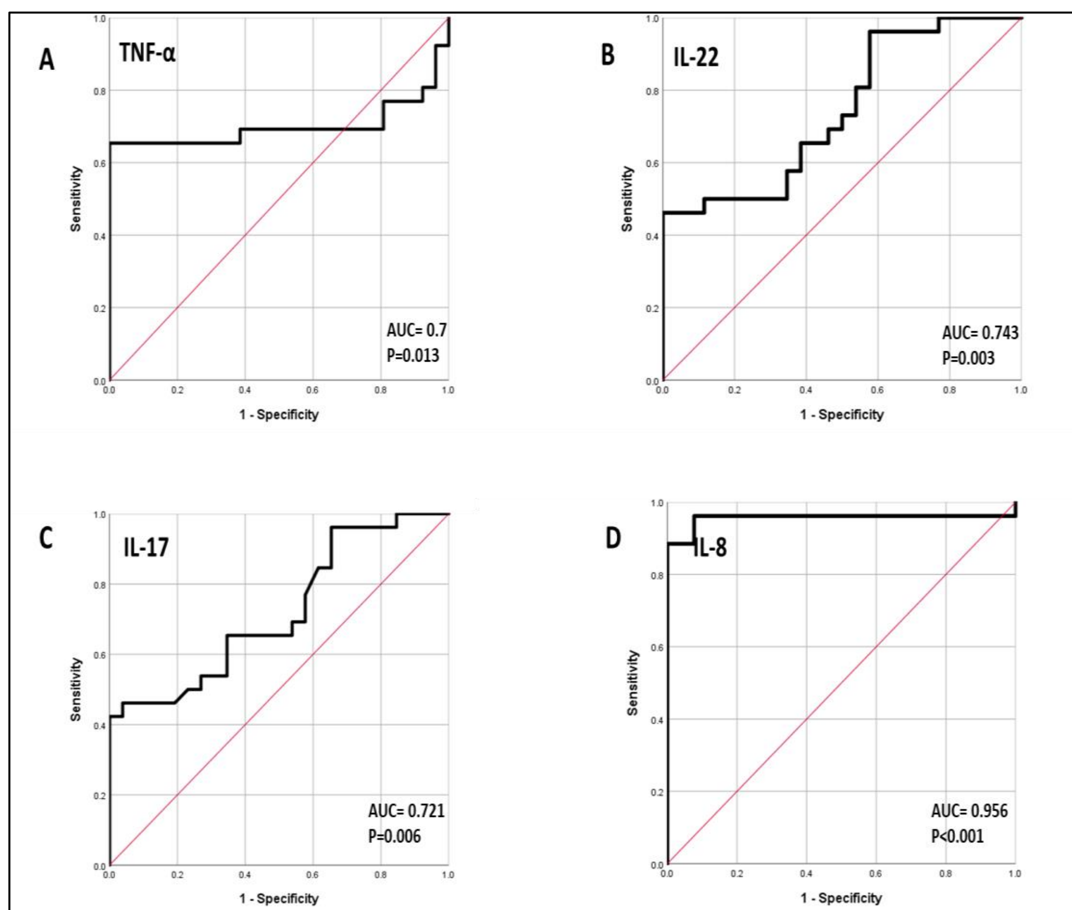

Fig.3 Roc curves for potential predictive value of the studied cytokines in diagnosing AR. (A) TNF-α, (B) IL-22, (C) IL-17, (D) IL-8

Correlation between Age, Total IgE, duration of disease, and Cytokine Levels among AR Patients Group

A statistically significant positive correlation was detected between TNF-α, and *A. fumigates* (r =1, p <0.001). IL-22 showed a significant negative correlation with gender (higher in males) (r =-0.462, p =0.018). There was statistically significant negative correlation between level of IL-17 in serum and both latex and feather mix (r =-1, *p*<0.001 r =-0.892, *p*= 0.042 respectively). *A. niger* showed negative correlation with all the studied cytokines, IL-22, IL-17, TNF-α and IL-8 (r = -1, *p*<0.001). (Supplementary Table 1). There was a statistically significant association between IL-8 and disease duration (r=0.48, *p*=0.013) (Fig 4).

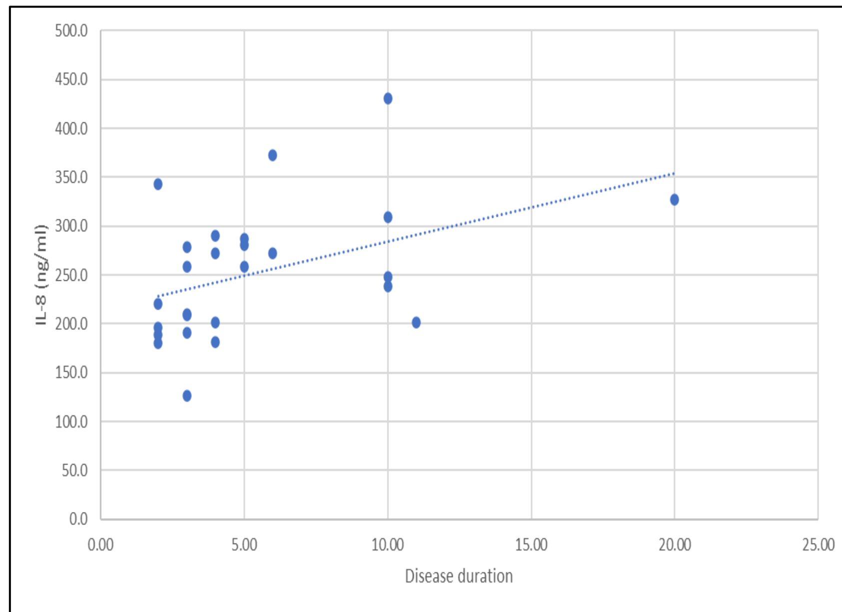

Fig.4 Scatter dot showing significant positive correlation between IL-8 and AR duration.

Correlation between Cytokine Levels and Severity of AR

On assessing the correlation between the different cytokines and the severity of AR, there was a statistically significant correlation between VAS and TNF-α, IL-22 (Fig 5).

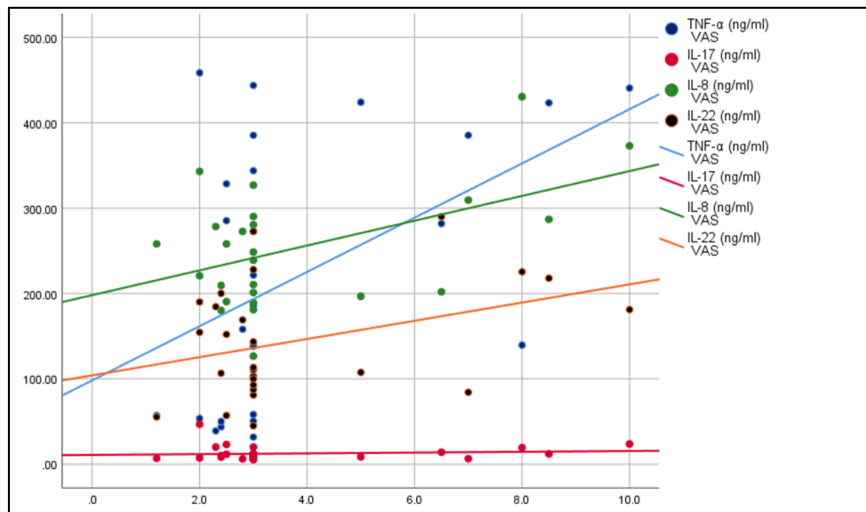

Fig.5 Scatter matrix showing the correlation between VAS and the studied cytokines (r for TNF=0.424 [*p*=0.031], IL-22=0.661 [*p*<0.001], IL-17 =0.099 [*p*=0.629] and IL-8=0.224 [*p*=0.271])

DISCUSSION

In the present study, we evaluate the cytokines of different Th subsets among AR patients for a better immunological characterization of the diseases. AR patients were found to have higher serum levels of IL-17, IL22, TNF-α and IL-8 compared to the control group. There was a statistically significant relation between disease severity and TNF-α& IL-22 serum levels. The potential predictive value of the studied cytokines in diagnosing AR was assessed with IL-8 showed higher sensitivity 96.2% and specificity 92.3% (P<0.001).

The current study found that serum IL-8 was significantly greater in AR patients compared to the control group. Similarly, Chai et al [15] revealed that AR patients expressed elevated levels of IL-8. To our knowledge, there are no researches available that discuss serum TNF-α levels in patients with AR where we indicated, in the current study, higher serum levels of TNF-α among AR group.

IL-17 and IL-22 were assessed in the current study to determine Th17 mediated inflammation in AR patients. A significant high level of serum IL-17 in AR patient group in comparison to the control one was found. In line with the mentioned finding, IL-17 in serum has been detected to be upregulated in patients with AR [4, 16-19]. In contrast, another study demonstrated no significant variation in serum IL-17 between AR individuals and controls [15,20].

Regarding serum levels of IL-22 in the current study, there was a significant variation between AR and control groups. Our findings concurred with other studies where serum level of IL-22 was higher among patients than controls [4,5,17,21]. On the other side, no changes in IL-22 level were detected by Andersson et al [22] between asthma group and the control group. There is controversial data about the possible anti-inflammatory or inflammatory role of IL-22 in AR [5, 23, 24]. Besnard et al [25] presented that IL-22 has double effect in allergic airway inflammation where it is essential for the occurance of inflammation, however the lack of IL-22, during continuous allergen exposure, could upregulate the allergic inflammation. Nakagome et al [26]., on the other hand, hypothesised that by reducing eosinophilic airway inflammation, early in an immune reaction, IL-22 may have immunosuppressive impact. These divergent roles of IL-22 among different studies can be explained by different ways where the co-expression of IL-17A, makes IL-22 able to evoke chemokine release, neutrophil recruitment, and airway inflammation. On the other hand, in lack of IL-17A, IL-22 expression provides tissue-protective effects by enhancing the integrity of the barrier of the epithelium [27]. Moreover, the relatively limited study sample sizes and various conditions and methodologies used to measure the amount of IL-22 can explain discrepancies between studies.

In our study, there was insignificant variation in serum IL-17, IL-22, between SAR and PAR patients. This is consistent with the findings published by a previous study where it showed when both SAR and PAR patients are experiencing symptoms, Th17 cells were active [17].

In the current study, we detected insignificant differences between the serum levels of IL 17, IL8, TNF-α among patients of AR only and patients with AR associated with comorbid asthma. However, our study reported higher levels of serum IL-22 among AR patients associated with comorbid asthma compared to AR patients only. Tamasauskiene and Sitkauskien [28] showed elevated levels of IL-22 in serum among AR patients only when compared with AR patients associated with asthma but with insignificant variation between both groups.

The present study showed no correlation between levels of IL-17, IL-22, TNF-α and IL-8 and total IgE in serum. This study's findings are supported by earlier research that reported levels of IL-22 in serum did not correlate with total IgE. Some studies indicated that there was a positive correlation between total IgE and IL-17A and IL-22+cell amounts in serum [4, 29]. Tamasauskiene and his coworkers [5] showed that levels of IL-17 in serum were positively correlated with total IgE. However, Makihara et al [30] reported no correlation between IL-17A+ cells and total IgE.

To the best of the researches' information, this present research is the first Egyptian one to assess the correlation between serum levels of IL-17, IL-22, TNF-α and IL-8 with sIgE of different aeroallergens, where we reported positive correlation that was statistically significant between serum levels of TNF-α and sIgE of Aspergillus fumigates, significant negative correlation between serum levels of IL-17 and sIgE of both latex and feather mix. Specific IgE of aspergillus niger showed negative correlation with all the studied cytokines TNF-α, IL-22, IL-17, IL-8. Tang et al [4] and Shahsavan et al [21] showed a relation between serum levels of IL-22 and sIgE of house dust mites. The current study reported statistically significant correlation between VAS and TNF-α& IL-22 serum levels. This agrees with Shahsavan et al [4] and Zhu et al [31] who reported that IL-22 levels in serum were positively correlated with the severity of asthma and AR. According to Farfariello et al., [29] patients with severe AR and asthma had greater levels of IL-22 than those with moderate AR and asthma. However, Bayrak Degirmenci et al [17] demonstrated no correlation between the severity of the symptoms in AR patients and levels of IL-22 in serum. While IL-22 level in serum and the Rhinoconjunctivitis Quality of Life Questionnaire (RQLQ) were found to be negatively correlated by Tamasauskiene et al. [5] Nasal lavage IL-22 is discovered to have negative correlation with serum IL-22. This result implies that the local level of cytokines is not always accurately reflected by serum cytokine measurements. The severity of the illnesses could be the cause of this inadequacy. The majority of patients in our research have mild AR. The disparities among different studies might be attributed to small research populations, various IL-22 investigational methods, and the application of different methods for determining degree of severity of symptoms.

Some contradictory findings have been obtained revealing the relations between severity of AR and IL-17. Some studies showed that levels of IL-17A in serum were related to severity of AR [18, 32, 33]. Conversely, others reported that TNSS did not correlate with levels of IL-17A in serum in patients with AR patients [13]. Shashavan et al [4] demonstrated a statistical correlation between TNSS and serum amounts and production of IL-22 and IL-17A locally.

Limitations:

Assessing levels of cytokines in nasal lavage would be more informative than the serum levels, but due to

technical issues, we estimated the serum levels. Our study did not consider the possible risk factor that can alter the levels of cytokines such as vitamin D deficiency. Cause -effect relationship between cytokines level and disease severity couldn't be assessed by this case control study where longitudinal follow up one is needed to clarify changes in cytokine level through the course of disease. Moreover, measuring Th-cells and other immune cells in blood should be taken into consideration.

CONCLUSION

Our research described the immune responses in AR patients. It showed a possible role for TNF-α, IL-17, IL-8, and IL-22 in pathogenesis of AR patients. The studied cytokines showed some diagnostic potential for AR patients where IL-8 showed the higher predictive value in diagnosing AR. TNF-α, and IL-22 showed direct correlation with AR severity so they can be used as objective indicators to evaluate AR severity.

Abbreviation List:
Allergic rhinitis (AR), Skin prick test (SPT), visual analogue score (VAS), Tumor necrosis factor α (TNF-α), Interleukin (IL), seasonal allergic rhinitis (SAR), perennial allergic rhinitis (PAR), European Academy of Allergy and Clinical Immunology's SPT Practical Guide (EAACI), enzyme-linked immunosorbent assay (ELISA), SPSS (Statistical Package for the Social Sciences).

Data availability statement: Data available from the corresponding author upon reasonable request.
Author Contributions: All authors contributed to the study conception and design, material preparation, data collection and analysis. All authors read and approved the final manuscript.
Competing Interests: The authors have no relevant financial or non-financial interests to disclose.

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Supplementary Table 1: Correlation Analysis between Age, Gender, Total IgE, duration of disease, and Cytokine Levels among AR Patients

	TNF-α		IL-22		IL-17		IL-8	
	r	*P*-value	r	*P*-value	r	*P*-value	r	*P*-value
Age	0.092	0.564	0.085	0.68	0.062	0.765	0.224	0.271
Total IgE	-0.017	0.934	0.042	0.843	-0.089	0.667	0.003	0.989
Disease duration	-0.189	0.356	0.203	0.319	-0.188	0.357	0.48	0.013
Der p	-0.136	0.537	0.14	0.556	-0.391	0.089	-0.038	0.872
Der f	-0.059	0.84	0.409	0.146	-0.159	0.588	0.007	0.982
Cockroach	0.121	0.565	0.032	0.905	-0.267	0.318	-0.097	0.72
Dog Epithelium	0.097	0.742	-0.024	0.935	0.251	0.387	-0.152	0.605
Mixed Grasses	0.31	0.417	-0.583	0.099	-0.15	0.7	0.417	0.265
Birch	-0.018	0.96	0.128	0.725	-0.091	0.802	0.28	0.434
C. albicans	0.348	0.499	0.086	0.872	0.771	0.072	-0.429	0.397
A. fumigatus	1	<0.001	0	>0.999	0.4	0.6	0.4	0.6
A. alternate	0.214	0.61	-0.524	0.183	0.071	0.867	-0.476	0.233
Feather Mix	-0.429	0.397	0.143	0.787	-0.892	0.042	-0.371	0.468
Latex	0.5	0.667	0.5	0.667	-1	<0.001	-0.5	0.667
P. notatum	-0.024	0.955	0.167	0.693	-0.695	0.056	-0.31	0.456
A. niger	-1	<0.001	-1	<0.001	-1	<0.001	-1	<0.001
Cat epithelium	-0.5	0.667	0.5	0.667	0.5	0.667	-0.5	0.667

Abbreviations: *Der p*, *Dermatophagoides pteronyssinus*; *Der f*, *Dermatophagoides farina*; *P. notatum, Penicillium notatum*; *A. alternate, Alternaria alternate*; *A. niger, Aspergillus niger*; *A. fumigatus, Aspergillus fumigatus*; *C. albicans, Candida albicans*.