How to Cite:
Al-Saeedi, E., Shukur, W., & Jaleel, S. Z. (2022). The role of IL-35 and IL-22 in patients with allergic rhinitis and their correlation with severity of disease in Najaf province. *International Journal of Health Sciences*, *6*(S4), 10751–10760. https://doi.org/10.53730/ijhs.v6nS4.11680

The role of IL-35 and IL-22 in patients with allergic rhinitis and their correlation with severity of disease in Najaf province

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***Abstract*---**Introduction: Allergic rhinitis (AR) is a non-infectious disorder of the upper respiratory system that is recognized by nasal obstruction, nasal itching, sneezing, and rhinorrhea induced by inhaled allergens and involves mucosal inflammation. Despite various medicines are available to manage AR but not all have been shown to be effective in all patients, and thus necessitating the development of innovative therapeutics. Objectives: This study was designed to measure the serum levels of IL-22 and IL-35 in Allergic Rhinitis patients and assessing their correlation with disease severity. Materials and Methods: This study included 48 patients with AR and 42 healthy controls. IL-35 and IL-22 were measured using ELAISA kits while the Allergic sensitization of patients was demonstrated by measuring IgE and Eosinophil count. Severity scores of AR patients were calculated using ARIA's recommendations for Total Nasal Symptoms Score (TNSS). The AR patients were divided into three groups based on these scores: mild (n=16), moderate (n=12), and severe (n=20). Results: Serum levels of IL-35 and IL-22 were compared between AR patients and control group: IL-22 was indicated as higher (P=0.0001) in AR patients while the levels of IL-35 detected an inverse result as being lower (P=0.037) in AR patients than control group. when compared the levels of IL-35 and IL-22 with TNSS it detected a non significant difference (P=0.6), (P=0.5) respectively, expect for little elevation among the severe group for both IL-35 and IL-22. Pearson correlation analysis revealed a strong positive correlation between IL-35 and IL-22 (P =0.001, r=0.5); while there was no correlation for IL-35 with IgE and Eosinophil (P=0.7, r =0.04) (P=0.6, r=0.07)

Manuscript submitted: 9 April 2022, Manuscript revised: 18 June 2022, Accepted for publication: 27 July 2022

respectively. Also, there was weak relation between IL-22 eosinophil (p=0.3, r=0.1) ; while a negative correlation was detected with total IgE (P=0.9, r=-0.004). Conclusion: It was found that the level of serum levels of IL-35 and IL-22 is associated with the presence of AR; where IL-22 was higher in AR patients than control, while IL-35 was the opposite. There was no correlation of both interleukins with the severity of symptoms in AR patients.

***Keywords*---**allergic rhinitis, correlation, severity disease.

Introduction

Allergic rhinitis (AR) is a global concern, and even more so given the disease's dramatic rise in recent years. It is the most prevalent allergic disease and it affects nearly 400 million people worldwide (Mirmoezzi, Yazdi and Gholami, 2017). In Europe, the prevalence of AR has steadily increased among Danish adults, over the last three decades, from 19% to 32%.1(Leth-Møller, Skaaby and Linneberg, 2020). The mechanism of action of AR is unknown at the moment. The available evidence suggests that inflammation has a contribution in Allergic Rhinitis. Numerous earlier investigations found a variety of risk factors for AR, including familial history, dust exposure history, pollen allergy history and medication allergy history (Eguiluz-Gracia *et al.*, 2020).

AR is triggered by a T-helper cell imbalance in which cytokines associated with TH-type 2 predominate while TH1-type cytokines are present at normal levels. Other subtype of T cells is regulatory T cells, which decrease both TH1 and TH2 cytokine expression. As a result, it has been postulated that AR also exhibits an imbalance of TH2 and Treg cells. IL-22 is unique in that, unlike other cytokines in the IL-10 family, It regulates the proliferation and barrier function of non-hematopoietic cells on mucosal surfaces, such as epithelial and stromal cells (Dudakov, Hanash, and van den Brink,2015). Consistent with mounting evidence that lung epithelial cells play a critical role in the development of allergic airway inflammation, recent studies have demonstrated the effect of IL-22 on allergic airway inflammation regulation (Ito *et al.*, 2017).

IL-35 exerts a significant suppressive influence on immunological responses by increasing IL-35-inducible regulatory T (iTr35) cells (Egwuagu *et al.*, 2015). Additionally, IL-35 exhibit a strong inhibitory effect on CD4+ effector T cells, including Th1 and Th17 cells, via Treg cell proliferation and IL-10 production (Choi *et al.*, 2015). Numerous in vivo studies have established that IL-35 acts as an anti-inflammatory factor in a variety of aspects of the pathophysiology of an allergic rhinitis (AR) mice model. While Th2 cells and ILC2s create type 2 cytokines and perform a critical role in the pathogenesis of AR, B cells also play a role in the pathogenesis of AR by generating antigen- specific IgE. It was recently reported that IL-35 may block the function of Th2 cells, B cells that produce IgE and ILC2s (Shamji *et al.*, 2019).

Objectives

1. This study was designed to measure the serum levels of IL-35 and IL-22 in the AR patient group in comparing with the healthy control group and to assess if there's a significant difference between them.
2. finding out if there's a correlation between the severity scores of the AR group and IL-35 and IL-22.
3. Identifying the relationship between IgE and eosinophil count and the cytokines (IL-35 ,IL 22).

Material and Methods

Subjects

This is a case control study and was carried out from October 2020 to August 2021 including 48 Patients with allergic rhinitis who enrolled into in Nose-Ear and Throat Unit in AL-Sader medical city in Al-Najaf Governorate. Their ages ranged between (18 and 60) years, with a mean of (36.41± 13.02). Before testing, all patients underwent a thorough examination by an ENT specialist and were taken off histamines and systemic oral steroids for at least one week. We collected information on the patient's age, gender, occupation, residency, duration of disease, family history, history of chronic disease of comparable or other disorders, smoking and drug use. The control group consisted of 42 apparently healthy, non-allergic volunteers who did not have any family history of allergy. The mean age of the participants were (32.24 ± 9.687) years, with a range of (18-50) years. All AR patients were evaluated by a consultant ENT based on the British Society for Allergy and Clinical Immunology (BSACI) guidelines. AR was diagnosed based on the patient's history, symptoms, clinical examination, and total serum IgE and CBC levels. Healthy control subjects were chosen since they did not have a history of allergies or other chronic conditions.

Patients were excluded from the research:

1. If they had received immunosuppressive medication during the last month.
2. Had antihistamine treatment within the last 10 days.
3. Had an active infection.
4. Had another chronic condition such as nasal polyposis, chronic rhinosinusitis, or malignancy.

Both Allergic rhinitis and control groups were matched regarding age, sex and residency and there no differences in such factors. All patients underwent a clinical evaluation that included a medical history and physical examination, as well as a collection of approximately 5 ml of blood was taken from each patient via vein puncture and then collected in two groups of tubes, the first being an EDTA tube containing an anti-coagulant to evaluate complete blood count specifically to detect the Eosinophil cell count, and the second being a gel tube containing no anti-coagulant to be used as a simple tube of another tests such as Immunoglobulin E (IgE), Interleukin-22, and Interleukin-35 levels in human serum samples from both AR patients and controls using an enzyme-linked immunosorbent assay (ELISA).

Statistical analysis

First of all, the data of finding were tested for normality in addition to large samples (> 40) which emphasized normality of continuous variables. So t-test was applied for the difference of two means of continuous variable between cases and control. Application of ANOVA as a parametric test was applied for the difference among more than two continuous variables like cytokines, Eosinophil and Ig E data by three levels of severity. Pearson correlation with scatter dots was used to test the relation between the study variables. Chi square was applied for categorical variables association like sociodemographic characteristics of cases and control through using SPSS (Version 26) considering level of significance at $\alpha = 0.05$.

Results

Table (1) General characteristics

Variables		Groups				*P value
		AR patients		Controls		
Gender	Female	No. 15	% 31.3	No. 20	% 47.6	0.1
	Male	33	68.8	22	52.4	
Age (years) (mean± SD)		36.4 ±13.02		32.24 ± 9.687		0.1
Residence	Urban	No. 29	% 60.4	No. 26	% 61.9	0.9
	Rural	19	36.9	16	38.1	
Family history	No	No. 7	% 14.6	No. 39	% 92.9	0.001
	Yes	41	85.4	3	7.1	
Smoking	No	No. 37	% 77.1	No. 31	% 73.8	0.7
	Yes	11	22.9	11	26.2	
Eosinophil (10^9/L) (mean ±SD)		4.97 ± 2.17		2.98 ± 1.55		0.001
Total Ig-E (IU/ml) (mean ± SD)		623.33 ± 414.51		256.20 ± 150.46		0.001

Table (2) Comparison between the studying parameters and TNSS of allergic rhinitis group

Parameters	Mild n=16	Moderate n=12	Severe n=20	P-value
Eosinophil(10^9/L)	4.50 ±0.71	5.05 ± 0.41	5.30 ± 0.44	0.5
Ig-E (IU/ml)	581.6 ±44.5	522.2 ±37.7	717.2 ± 136.5	0.3
IL-22 (ng/ml)	27.6± 4.34	22.23 ± 2.28	25.0 ± 2.68	0.5
IL-35 (ng/ml)	11.0 ± 1.97	11.27 ± 1.29	12.6 ± 1.17	0.6

Result of Biomarkers assessment in studying groups

Table (4) indicates a highly significant difference in levels of Interlukin-22 among AR patients and controls at a (P value =0.0001); the means of IL-22 are (25.20**±**13.15 ng/ml) in patients and (13.50 **±** 6.87 ng/ml) in controls; while the levels of Interlukin-35 we notice an inverse significant difference between patient and control groups in fact it declined significantly in patients with AR (11.76 **±** 6.02 ng/ml) versus (14.02 **±** 3.60 ng/ml) control group at a (P value = 0.037).

Table (3) Comparison of studying parameters (IL-22 and IL-35) between AR group and control group

Parameters	AR patient n=48 (mean ± SD)	Control n=42 (mean ± SD)	*P value
IL-22 (ng/ml)	25.20 ±13.15	13.50 ± 6.87	0.0001
IL-35 (ng/ml)	11.76 ± 6.02	14.02 ± 3.60	0.037

Table (4) Correlations of studying parameters

Studying parameters	Age (years)		Disease Duration (years)		Total IgE (IU/ml)		Eosinophil count (10^9/L)	
	r	p	r	p	r	p	r	p
IL-22 (ng/ml)	0.07	0.61	0.19	0.17	0.004	0.9	0.13	0.37
IL-35 (ng/ml)	0.09	0.52	0.216	0.14	0.04	0.77	0.07	0.63
Total IgE (IU/ml)	0.01	0.92	-0.1	0.46	-	-	0.04	0.75
Eosinophil count (10^9/L)	0.26	0.07	0.04	0.74	0.04	0.75	-	-

Disease duration (years)	-0.04	0.7	-	-	-	-	-	-

Discussion

The general characteristics of the AR patients and healthy controls are represented in Table (1). Male patients represent the higher frequency (68.8%) among the AR group at (P value=0.1) and this consistent with other studies like Agache *et al.*, (2021) and Delahoy *et al.*,(2021) .Otherwise, another studies like Pesut *et al.*, (2014); Who said In our study, Compared to male patients, female patients viewed AR as more threatening because they believed it to be more serious.

The Age was similarly distributed in healthy controls and AR patients and the current study's findings indicate that the majority of AR patients were in their fourth decade of life (30-39 years) with percent (29.2 %) at (P value=0.1) and this is in accordance with other studies like Li *et al.*, (2014) and Yonekura *et al.*, (2012) who said that the peak of prevalence of allergic rhinitis occur in the fourth decade and decreases gradually. Concerning the family history, the present study had shown a higher frequency with a significant difference (p value=0.001) and this finding had been proven previously by numerous studies like Digheari *et al.*, (2018). Regarding smoking habit, the findings of this study shows that AR was more prevalent among nonsmokers (77.1%) than active smokers (22.9%) at (P value=0.7). Our finding was in accordance with Zhou *et al.*,(2021) who reported that there is no link between active smoking and prevalence of AR in adults.

The present study revealed that Eosinophil count was significantly higher in AR group with mean (4.97 ± 2.17 10^9/L) than controls with mean (2.98 ± 1.55 10^9/L) at (P value=0.001) and this finding was consistent with other studies like (Sharma *et al.*, 2019) in India. Also this study reported a highly significant difference in Total IgE in AR group with mean (623.3 ± 414.5 IU/ml) than controls (256.20 ± 150.46 IU/ml) at (P value=0.001). this result was in agreement with Turki *et al.*, (2020) in Iraq.

The results of our study indicate that IL-22 levels are significantly higher in patients with AR than in healthy controls (P value=0.001), thus there is a remarkable difference between them. This finding is consistent with He *et al.*,(2021), who reported that patients with allergic rhinitis and/or allergic asthma had significantly higher levels of Interleukin-22 than healthy individuals in the most of clinical trials. The findings of this investigation corroborate anti-inflammatory properties of IL-22 in persistent allergic airway disorders. It's probable that IL-22 plays a role in these disorders, but the results of both experimental and clinical research are inconclusive (Tamasauskiene *et al.*, 2021). However, the results of research on the local concentration of IL-22 have been disputed. For example, Shahsavan *et al.*,(2019) in Iran discovered that patients with persistent allergic rhinitis had higher levels of IL-22 gene expression in their nasal mucosa than healthy persons. There may be discrepancies in results

between studies because of the limited sample sizes and varied methodologies employed in determining the IL-22 concentrations.

The current finding, Despite the fact that the difference was not statistically significant, it demonstrated an inverse significant difference (P value=0.037), indicated that the serum level of IL-35 was decreased in individuals with allergic rhinitis than in controls. This result was nearly identical with a recent study in Japan achieved by Kouzaki *et al.*,(2021) and Huang *et al.*,(2021) in china who found that the levels of IL-35 in serum were decreased in AR patients than in control group. Also, this result in agreed with Liu *et al.*,(2021), who found patients with allergic rhinitis had lower levels of IL-35 and a higher proportion of ITR35, while ILC2 and type II cytokines were elevated.

However, IL-35 Is a newly discovered anti-inflammatory cytokine belong to IL-12 family according to study's findings, and is responsible for many immune system responses as it acts as a negative regulatory protein by limiting the growth of T lymphocyte and other effector function. Moreover, the current study identified a negative correlation between IL-22 and TNSS which was a non significant difference (P value=0.5) except for a little elevation in levels of IL-22 among the mild group but it's still non-significant, this result was supported by Degirmenci *et al.*,(2018) and Tamasauskiene *et al.*,(2021). The present study, additionally found that when make a comparison between the level of IL-35 and disease severity, the level of IL-35 tend be elevated among patients in severe group despite being statistically non-significant at (P value=0.6), this findings was in agreement with Degirmenci *et al.*,(2018).

This study indicate that serum levels of IL-22 have a negative correlation with Eosinophil count (r=0.1) and nonsignificant at (p value=0.3) in patient with AR. However, serum IL-22 levels may not accurately reflect local levels of IL-22 in respiratory system, which may explain why certain studies are contradictory in Ovalbumin induce allergy in mice and bronchoalveolar washing and lung tissue and decreased infiltration of leukocyte, according to several experimental studies (Besnard *et al.*, 2011). Additionally, there was no correlation between IL-22 and Total IgE level in serum (r=-0.004) (P value=0.9). Also, we did not find any correlation with Age (P value=0.6, r=0.07) and Disease duration (P value=0.4, r=0.1).There is no correlation between the levels of IL-35 and Eosinophil count (r=0.07) at (p value=0.6); this finding was comparable with a recent study by Zeng *et al.*,(2021) in china who found that IL-35 protein levels were negatively correlated with eosinophil count (p> 0.01). Additionally, the current study is the first that found a strong positive correlation (r=0.5) with a significant difference (p value=0.001) between the levels of IL-35 and IL-22 in serum. While, there is no correlation for IL-35 with Age (r=0.09 and P value=0.5) and with diseases duration (r=0.2 and P value=0.1).

Conclusion

The current study indicates that there was a highly significant difference in mean of Interleukin 22, but Interleukin-35 shows an inverse significant difference in AR patients than in healthy controls. Also, there were a strong positive correlation between Interleukin-22 and Interleukin-35. While, this study shows no significant

difference when comparing the studying parameters levels with severity score (TNSS) of AR.

Recommendations

Further studies along with larger sample size and Nasal lavage to identify the role of these Interleukins and find out if there's varying in levels between local and systemic concentration. Study of Interleukin-22 and Interleukin-35 gene polymorphism or their association with another markers or Cells in AR patients.

Limitations of the study

The timing of sample collection was encountered through the pandemic of COVID 19 which creates a lot of difficulties in progressing this project, one of them was the admitting of patients for taking sample from them.

Authors' contribution

AE, and SW were the principal investigators of the study. AE and SW were included in preparing the concept and design. SW and JS revisited the manuscript and critically evaluated the intellectual contents. All authors participated in preparing the final draft of the manuscript, revised the manuscript and critically evaluated the intellectual contents. All authors have read and approved the content of the manuscript and confirmed the accuracy or integrity of any part of the work.

Ethical Consideration

Ethical issues (including plagiarism, data fabrication, double publication) have been completely observed by the authors. All participants (patients and controls) were recruited from Al-Najaf province. They were instructed and convinced about the study's purpose and testing procedures, and their consent was obtained.

Conflicts of interest

The authors declare that they have no conflicts of interest.

Funding/support

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

References

Agache I, Akdis CA, Akdis M, Canonica GW, Casale T, Chivato T, *et al.* EAACI Biologicals Guidelines-Recommendations for severe asthma. Allergy. 2021 Jan;76(1):14-44.

Al-Digheari A, Mahboub B, Tarraf H, Yucel T, Annesi-Maesano I, Doble A, et al. The clinical burden of allergic rhinitis in five Middle Eastern countries: results of the SNAPSHOT program. Allergy Asthma Clin Immunol. 2018;14:63.

Aprianto, D. R., Parenrengi, M. A., Utomo, B., Fauzi, A. A., & Subagyo, E. A. (2022). Autograft and implant cranioplasty in pediatric patients. *International Journal of Health & Medical Sciences*, *5*(1), 129-136. https://doi.org/10.21744/ijhms.v5n1.1852

Bayrak Degirmenci P, Aksun S, Altin Z, Bilgir F, Arslan IB, Colak H, et al. Allergic Rhinitis and Its Relationship with IL-10, IL-17, TGF-*β*, IFN-γ, IL 22, and IL-35. *Dis Markers*. 2018;2018:9131432.

Besnard AG, Sabat R, Dumoutier L, Renauld JC, Willart M, Lambrecht B, et al. Dual Role of IL-22 in allergic airway inflammation and its cross-talk with IL-17A. Am J Respir Crit Care Med. 2011;183:1153-1163.

Choi J, Leung PS, Bowlus C, Gershwin ME. IL-35 and autoimmunity: a comprehensive perspective. Clin Rev Allergy Immunol. 2015 Dec;49(3):327-32.

Delahoy MJ, Ujamaa D, Whitaker M, O'Halloran A, Anglin O, Burns E, *et al*. Hospitalizations Associated with COVID-19 Among Children and Adolescents - COVID-NET, 14 States, March 1, 2020-August 14, 2021. MMWR Morb Mortal Wkly Rep. 2021 Sep 10;70(36):1255-1260.

Dudakov JA, Hanash AM, van den Brink MR. Interleukin-22: immunobiology and pathology. *Annu Rev Immunol*. 2015;33:747-785.

Eguiluz-Gracia I, Mathioudakis AG, Bartel S, Vijverberg SJH, Fuertes E, Comberiati P, *et al*. The need for clean air: The way air pollution and climate change affect allergic rhinitis and asthma. Allergy. 2020 Sep;75(9):2170-2184.

Egwuagu CE, Yu CR, Sun L, Wang R. Interleukin 35: Critical regulator of immunity and lymphocyte-mediated diseases. Cytokine Growth Factor Rev. 2015;26(5):587-93.

He R, Chen Y, Chen X, Yuan B. Mechanism of miR-181a-5p in Regulatory T/T-Helper 17 Immune Imbalance and Asthma Development in Mice with Allergic Rhinitis. Int Arch Allergy Immunol. 2022;183:375-88.

Huang L, Zhao M, Luo Q, Liang K, Iu CL. The role and correlation of IL-35 and type II intrinsic lymphocytes in children with allergic rhinitis. Cell Mol Biol. 2021;67:127-131.

Ito T, Hirose K, Saku A, Kono K, Takatori H, Tamachi T, *et al*. IL-22 induces Reg3γ and inhibits allergic inflammation in house dust mite–induced asthma models. J Exp Med. 2017 Oct 2;214(10):3037-50.

Kouzaki H, Arai Y, Nakamura K, et al. Anti-inflammatory roles of interleukin-35 in the pathogenesis of Japanese cedar pollinosis. Asia Pac Allergy. 2021;11:e34.

Leth-Møller KB, Skaaby T, Linneberg A. Allergic rhinitis and allergic sensitisation are still increasing among Danish adults. Allergy. 2020;75(3):660-8.

Li CW, Chen DH DH, Zhong JT, et al. Epidemiological characterization and risk factors of allergic rhinitis in the general population in Guangzhou City in china. PLoS One. 2014;9:e114950.

Liu W, Zeng Q, Wen Y, Tang Y, Yan S, Li Y, et al. Inhibited interleukin 35 expression and interleukin 35-induced regulatory T cells promote type II innate lymphoid cell response in allergic rhinitis. *Ann Allergy Asthma Immunol*. 2021;126:152-161.

Mirmoezzi MS, Yazdi MS, Gholami OM. Comparative study on the efficacy of mometasone and fluticasone nasal sprays for treatment of allergic rhinitis. Int J Pharm Pharm Sci. 2017;9(3):211-4.

Pesut D, Raskovic S, Tomic-Spiric V, Bulajic M, Bogic M, Bursuc B, Peric-Popadic A. Gender differences revealed by the Brief Illness Perception Questionnaire in allergic rhinitis. Clin Respir J. 2014 Jul;8(3):364-8.

Shahsavan S, Pirayesh A, Samani OZ, Shirzad H, Zamani MA, Amani S, et al. The relationship between IL-17A and IL-22 expression and clinical severity in patients with moderate/severe persistent allergic rhinitis. *Am J Otolaryngol*. 2019;40:173-178.

Shamji MH, Layhadi JA, Achkova D, Kouser L, Perera-Webb A, Couto-Francisco NC, Parkin RV, Matsuoka T, Scadding G, Ashton-Rickardt PG, Durham SR. Role of IL-35 in sublingual allergen immunotherapy. J Allergy Clin Immunol. 2019 Mar 1;143(3):1131-42.

Sharma M, Khaitan T, Raman S, Jain R, Kabiraj A. Determination of Serum IgE and Eosinophils as a Diagnostic Indicator in Allergic Rhinitis. Indian J Otolaryngol Head Neck Surg. 2019 Nov;71(Suppl 3):1957-1961.

Suryasa, I. W., Rodríguez-Gámez, M., & Koldoris, T. (2021). Get vaccinated when it is your turn and follow the local guidelines. *International Journal of Health Sciences*, *5*(3), x-xv. https://doi.org/10.53730/ijhs.v5n3.2938

Tamasauskiene L, Gasiuniene E, Sitkauskiene B. Translation, adaption and validation of the total nasal symptom score (TNSS) for Lithuanian population. *Health Quality Life Outcomes*. 2021;19:54.

Turki SG, Ad'hiah AH, Brakhas SA, Atiyah MR. Allergen profile of rhinitis and asthma among Iraqi patients. *Clin Epidem Global Health*. 2020;8:637-42.

Yonekura S, Okamoto Y, Horiguchi S, Sakurai D, Chazono H, Hanazawa T, et al. Effects of aging on the natural history of seasonal allergic rhinitis in middle-aged subjects in South chiba, Japan. Int Arch Allergy Immunol. 2012;157:73-80.

Zeng Q, Zeng Y, Tang Y, Liu W, Sun C. Effect of IL-35 on apoptosis, adhesion, migration, and activation of eosinophils in allergic rhinitis. Pediatr Allergy Immunol. 2022;33:e13717.

Zhou Y, Chen J, Dong Y, Shen J, Tian M, Yang Y, et al. Maternal tobacco exposure during pregnancy and allergic rhinitis in offspring: A systematic review and meta-analysis. *Med*. 2021;100:e26986.