Egyptian Journal of Medical Microbiology Volume 31 / No.3 / July 2022 117-124 Online ISSN: 2537-0979

ORIGINAL ARTICLE

Association of GATA3 gene polymorphism with allergic conjunctivitis and allergic rhinitis

Shimaa A. Ahmed[1], Rasha A. Reda Nasr[2], Mai A.O. Al Kaffas[1], Noha N. Salah El-Deen[2]*

[1]Department of Medical Microbiology and Immunology, Research Institute of Ophthalmology, Giza, Egypt
[2]Department of Medical Microbiology and Immunology, Faculty of Medicine, Ain Shams University, Cairo, Egypt

Key words:
Allergic conjunctivitis, allergic rhinitis, GATA3, SNP rs1269486, RFLP-PCR

Corresponding Author:
Noha Nagi Salah El-Deen. M.D. Department of Medical Microbiology and Immunology, Faculty of Medicine, Ain Shams University, Cairo, Egypt. Postal code: 11588
dr.noha_nagi@hotmail.com, noha.salaheldin@med.asu.edu.eg

ABSTRACT

Background: *The elaboration of allergic diseases as allergic conjunctivitis (AC) and allergic rhinitis (AR) is caused by the interaction between genetic predisposition and environmental factors. Trans-acting T-cell-specific transcription factor (GATA3) is a transcription factor that readjust the T helper (Th2) cell response. High expression of GATA3 in Th2 cells elevate Th2 differentiation, resulting in the release of large amounts of immunoglobulin E (IgE) from B-cells and eventually aggravating allergic disease.* ***Objective:*** *The present work aims to investigate the association between GATA3 single nucleotide polymorphism (SNPs) with allergic conjunctivitis and allergic rhinitis at locus rs1269486 in the promoter region using RFLP-PCR among a group of Egyptian patients.* ***Methodology:*** *Cross sectional study included a total of 60 participants; 20 AC patients, 20 AR patients and 20 healthy individuals. Peripheral blood samples were taken and DNA was extracted followed by RFLP-PCR to analyze GATA3 (rs1269486) SNPs.* ***Results:*** *A substantially higher prevalence of the homozygous rs1269486 GG genotype and G allele appeared in the AR patients and AC patients, compared to the control subjects (P-value 0.003, and 0.002 respectively).* ***Conclusion:*** *This study showed a highly significant association between GATA3 SNP at locus, rs1269486 in the promoter region in the studied Egyptian patients with allergic rhinitis and allergic conjunctivitis suggesting the role of GATA3 gene polymorphism in the pathogenesis of AC and AR.*

INTRODUCTION

Allergy is a hypersensitive inflammatory immunological response caused by immunoglobulin type E (IgE) antibodies to benign environmental stimuli. This is the underlying etiology of all allergic illness, although there is a significant variation between allergic asthma, allergic conjunctivitis (AC) and seasonal allergic rhinitis (AR) [1].

The World Allergy Organization (WAO) guesstimate of allergy prevalence of the whole population by country ranges between 10 - 40% [2]. Allergy rates are rising and almost 20% of the global popu1ation are now allergic to something [3]. AC and AR are greatly prevalent in Egypt and data on genetic factors of this disease among the Egyptian population is lacking [4].

The most prevalent phenotype of AC and AR is characterized by eosinophil dominated inflammation that is driven by Th2 cell. Trans-acting T-cell-specific transcription factor (GATA3) is the principal GATA-family member expressed by immune cells, and it can be found in T cells, natural killer (NK) cells, and CD1-restricted NKT cells in both developing and mature stages [5]. GATA3 is well known in immune cells for being a master regulator of T helper 2 cell development [6] which plays a ro1e in the recruitment and activation of IgE antibody-producing B cells, mast cells, and eosinophils. Th2 cells release IL-4, IL-5, IL-6, and IL-13 and control B cell and eosinophil responses [7].

GATA3 is located on chromosome 10 (10 p15) in T cells and consists of six exons. It acts as a transcription factor that control the Th2 response and suppresses IFNγ and Th1 cells [8]. Relation of GATA3 gene polymorphism and other allergic diseases is not yet widely studied [9]. The sing1e nucleotide polymorphism (SNP) 290G/A of the GATA3 gene was associated with pediatric asthma in Beijing area in China [10]. It was also found to be associated with allergic rhinitis in some studies at SNP rs1269486 in Chinese population [11] and in the Iranian population [12].

The aim of the current study is to investigate the association between GATA3 SNPs with AC and AR at locus rs1269486 in the promoter region using RFLP PCR among a group of Egyptian patients.

METHODOLOGY

Study design:

This cross sectional study design was conducted at the Allergy Lab, Research Institute of Ophthalmology and Ain Shams University during the period from September 2020 till June 2021.

Patients and controls:

A total of 60 participants were included in the present study. They were divided into 3 groups: 20 cases diagnosed as allergic conjunctivitis, 20 cases diagnosed as allergic rhinitis and 20 healthy subjects as control.

Criteria for inclusion of the present study were: patients who were clinically diagnosed allergic conjunctivitis and al1ergic rhinitis according to the Allergy guideline criteria [13, 14] with laboratory confirmed positive skin – prick test. Subjects receiving corticosteroid treatment and other immunosuppressant drugs or suffering from other allergic diseases such as asthma and allergic dermatitis were ruled out from the study.

Allergenic extracts:

Allergenic extracts used for diagnostic skin prick testing done for all participants were prepared in the laboratory of Research Institute of Ophthalmology (RIO) and the Allergy and Immunology Department of Ain Shams University.

***Allergens prepared in RIO Laboratory*:** Cat hair, dog hair, goat hair, rabbit hair, sheep wool, pollens of orange, pollens of palm, feather mix (duck, goose and chicken), house dust, nicotine and pollens of grass (mixed Bermuda and timothy).

***Allergens from Allergy and Immunology Department of Ain Shams University*:** Cockroach, mite mix (Dermatophagoides farina and Dermatophagoidespteronyssinus), mould mix (Penicillumnotatum, Cladosporiumherparum, Aspergillus fumigates and Alternaria alternate).

Sample collection:

Five (5ml) blood samples were collected in whole blood EDTA tube from each participant and samples were stored at -20°C until processing.

Genotyping of SNP rs1269486 of GATA3 using RFLP- PCR technology:

The SNP is in locus rs1269486 in the promoter area is schematically shown in *Figure1*.

Fig. 1: The promoter region contains the genotype rs1269486 (A/G) [12].

- ***DNA isolation and purification*:** They were done using the Gene JET™ Whole Blood Genomic DNA Purification Mini Kit following the guidance of Thermo Fisher Scientific genomic DNA extraction from blood Mini Handbook
- ***Genomic DNA amplification*:** A single-round PCR was carried out using master mix, water, template DNA, and the primer in *Table 1* [11,12].

Table 1: Sequence of Primers used in PCR

SNP name	Primer sequences	PCR product (bps)
GATA3 (rs1269486)	F = 5'AGG CTC GGG AAA GAG GTG ACA 3' R = 5'GGC TCC TGC CAA TTC ATT CG 3'	333 bp

- ***Preparation of PCR Mix*:** It was assembled into 50 µL volume in a thin walled 0.2 mL PCR tube and reagents were added in following order: 25 µL master mix, 1 µL of each primer, 13 µL water, and 10 µL template DNA. The tube was gently mixed by tapping and then centrifuged to settle tube contents.
- ***Amplification*:** Performed using PXE 0.2 thermal cycler (Thermo Electron Corporation©, USA) and PCR conditions were adjusted as the following; one initial step of Taq- Polymerase activation (95 °C for 15 min.), 35 cycles of amplification (95 °C for 30 sec., 50 °C for 30 sec., 72 °C for 30 sec) and a final elongation step (72 °C for 5 min).
- ***DNA fragmentation using restriction endonucleases*:** The length of enzyme recognition sites is usually 4 to 6 base pairs. BamHI binds to the 5'-GGATCC-3' recognition sequence and cleaves these sequences shortly after the 5'-guanine on both strands.
- ***Detection of DNA products*:** Gel electrophoresis was carried off and bands were visualized using UV trans illuminator as the following:
  1) If the DNA amplicon was restricted by Thermo Scientific Fast Digest BamHI, two bands could be detected at 208bp,125bp
  2) If the DNA amplicon was not restricted by Thermo Scientific Fast Digest BamHI, only one band could be detected at 333bp

Statistical analysis:

Continuous variables are expressed as mean and standard deviation, median, and interquartile range according to data distribution. The comparison between two groups regarding quantitative data and parametric distribution was done by independent t-test while with

non-parametric distribution was done by using Mann-Whitney test. The comparison between more than two groups regarding quantitative data and parametric distribution was done by using One Way ANOVA test.

Ethical Considerations

Informed consents were obtained from patients or guardians before specimen collection. The work has been carried out after approval of Ain Shams University Ethics Committee and in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments in humans.

RESULTS

Demographic data and family history of the studied subjects (n = 60) are summarized in *Table 2*. Concerning age and sex, there was no statistically significant difference between the groups (*p*-value 0.329 and 0.819, respectively). Regarding the positive family history, there was a highly statistical significant difference between the studied groups (*p*-value 0.000).

Table 2: Demographic data and family history of the AC patients, AR patients and control subjects

		Allergic conjunctivitis group	Allergic rhinitis group	Control group	Test value	P-value
		No. = 20	No. = 20	No. = 20		
Age	**Mean ± SD**	27.95 ± 14.31	34.80 ± 13.89	32.00 ± 15.17	1.134•	0.329
	Range	10 – 55	12 – 59	11 – 60		
Sex	**Female**	9 (45.0%)	10 (50.0%)	11 (55.0%)	0.400*	0.819
	Male	11 (55.0%)	10 (50.0%)	9 (45.0%)		
Family history	**Negative**	10 (50.0%)	7 (35.0%)	20 (100.0%)	19.600*	0.000
	Positive	10 (50.0%)	13 (65.0%)	0 (0.0%)		

*: Chi-square test; •: One Way ANOVA test

Results of skin prick test are shown in *Table 3 and Figure 2*. There was no statistically significant difference among the two studied patients' groups regarding the number of positive reactions to skin prick test.

Table 3: Results of skin prick test in the 2 patients' groups

		Allergic conjunctivitis group	Allergic rhinitis group	Test value	P-value
		No. = 20	No. = 20		
No. of positive reactions to skin prick test	**Median (IQR)**	7.5 (6 – 9.5)	9 (6.5 – 10.5)	-0.749≠	0.454
	Range	5 – 14	4 – 14		
Allergens	**PALM**	12 (60.0%)	14 (70.0%)	0.440*	0.507
	Grass	10 (50.0%)	14 (70.0%)	1.667*	0.197
	Orange	16 (80.0%)	12 (60.0%)	1.905*	0.168
	Cat	6 (30.0%)	10 (50.0%)	1.667*	0.197
	Dog	10 (50.0%)	8 (40.0%)	0.404*	0.525
	Goat	12 (60.0%)	10 (50.0%)	0.404*	0.525
	Rabbit	11 (55.0%)	8 (40.0%)	0.902*	0.342
	Mite	10 (50.0%)	15 (75.0%)	2.667*	0.102
	Mould	13 (65.0%)	17 (85.0%)	2.133*	0.144
	Cockroach	15 (75.0%)	18 (90.0%)	1.558*	0.212
	House dust	14 (70.0%)	15 (75.0%)	0.125*	0.723
	Feather mix	13 (65.0%)	13 (65.0%)	0.000*	1.000
	Nicotine	10 (50.0%)	9 (45.0%)	0.100*	0.752
	Wool	11 (55.0%)	9 (45.0%)	0.400*	0.527
Duration (years)	**Median (IQR)**	5 (3 – 7)	4 (2 – 10)	-0.355≠	0.723
	Range	2 – 10	1 – 20		

*: Chi- square test; ≠: Mann-Whitney test

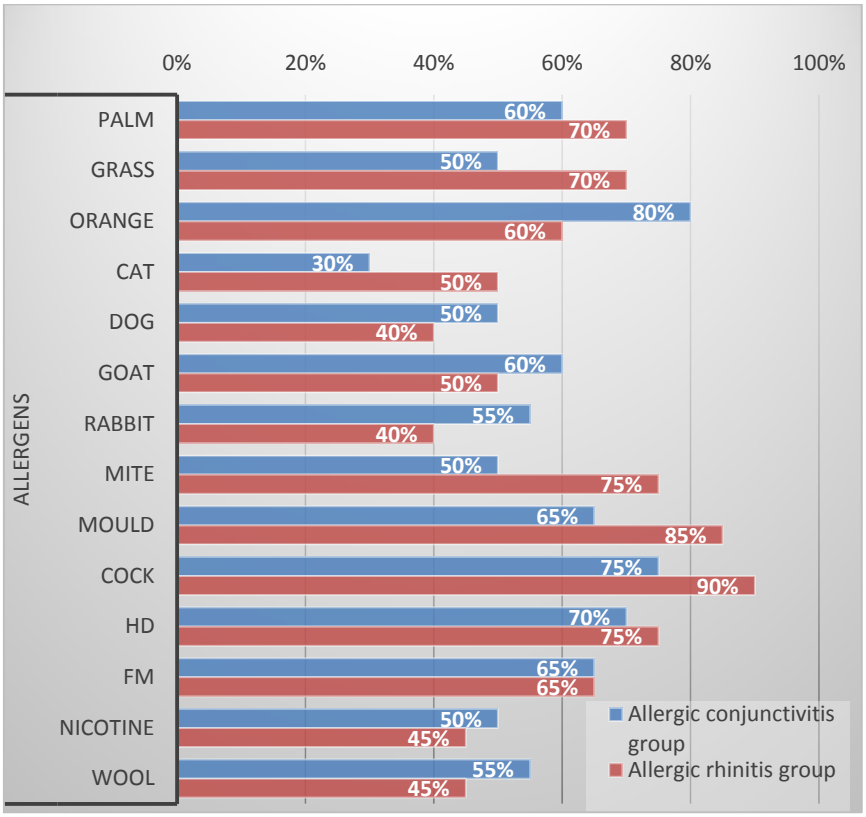

Fig. 2: Comparison between the allergic conjunctivitis and allergic rhinitis groups regarding skin prick test.

GATA3 (rs1269486) genotypes and allele frequencies:

Genotypes at locus rs1269486 were GG, GA and AA in both a1lergic conjunctivitis and control groups, whereas it was only GG and AG in a1lergic rhinitis group. Both patients' groups had substantially higher rates of genotype GG with *p*-value 0.003 and allele G with *p*-value 0.002 at rs1269486 than the control group as listed in *Table4.*

Table 4: Genotype and allele frequencies in patients' groups and controls for GATA3 (rs1269486)

Genotype/Allele	Allergic conjunctivitis group	Allergic rhinitis group	Control group	Test value	P-value	Sig.
GG	13 (65.0%)	16 (80.0%)	4 (20.0%)	16.331*	0.003	HS
AG	6 (30.0%)	4 (20.0%)	15 (75.0%)			
AA	1 (5.0%)	0 (0.0%)	1 (5.0%)			
G	32 (80.0%)	36 (90.0%)	23 (57.5%)	12.095*	0.002	HS
A	8 (20.0%)	4 (10.0%)	17 (42.5%)			

*: Chi-square test

There was a statistically significant increase in the percentage of patients with GG genotype in allergic conjunctivitis (65%) than in control group (20%) with *p*-value = 0.005 as shown in *Table 5*. The percentage of G allele as well was found higher in allergic conjunctivitis group (80%) than in control group (57.5%) with *p*-value = 0.033 as demonstrated in *Table 5*.

Table 5: Genotype and allele frequencies in allergic conjunctivitis patients' group and controls for GATA3 (rs1269486)

Genotype/Allele	Allergic conjunctivitis group	Control group	Test value	P-value	Sig.
GG	13 (65.0%)	4 (20.0%)	2.799	0.005	HS
AG	6 (30.0%)	15 (75.0%)	-	-	-
AA	1 (5.0%)	1 (5.0%)	-	-	-
G allele	32 (80.0%)	23 (57.5%)	2.132	0.033	S
A allele	8 (20.0%)	17 (42.5%)	-	-	-

*: Chi-square test

Table 6 demonstrates a statistically significant increase in the percentage of patients with GG genotype in a1lergic rhinitis group (80%) than in control group (20%) with *p*-value = 0.0006. The percentage of G allele was found also higher in a1lergic rhinitis group (90%) than in control group (57.5%) with *p*-value = 0.0021.

Table 6: Genotype and allele frequencies in allergic rhinitis patients' group and controls for GATA3 (rs1269486)

Genotype/Allele	Allergic rhinitis group	Control group	Test value	P-value	Sig.
GG	16 (80.0%)	4 (20.0%)	3.414	0.0006	HS
AG	4 (20.0%)	15 (75.0%)	-	-	-
AA	0 (0.0%)	1 (5.0%)	-	-	-
G allele	36 (90.0%)	23 (57.5%)	3.074	0.0021	HS
A allele	4 (10.0%)	17 (42.5%)	-	-	-

*: Chi-square test

No significant difference was found in the genotype and allele frequencies between allergic conjunctivitis group and the allergic rhinitis group (*p*-value = 0.425, *p*-value = 0. 210, respectively as shown in *Table 7*.

Table 7: Comparison between allergic conjunctivitis group and allergic rhinitis group regarding genotype and its allele frequencies

Genotype/Allele	Allergic conjunctivitis group	Allergic rhinitis group	Test value	P-value
	No. = 20	No. = 20		
GG	13 (65.0%)	16 (80.0%)	1.710*	0.425
AG	6 (30.0%)	4 (20.0%)		
AA	1 (5.0%)	0 (0.0%)		
G	32 (80.0%)	36 (90.0%)	1.569*	0.210
A	8 (20.0%)	4 (10.0%)		

*: Chi-square test

DISCUSSION

Genome-wide association studies, in particular, have identified single nucleotide polymorphisms (SNPs) in a large number of genes that are linked to higher rates of developing allergies [15]. Being an endemic problem with undetermined prevalence, an urgent need for genetic based studies do exist.

In order to identify a potential genetic factor involved in the pathogenesis of both AC and AR, this study investigated the association of GATA SNP at locus, rs1269486 in the promoter region with AC and AR.

In the present study, a1lergic conjunctivitis patients' age ranged from 10 to 55 which was in accordance with Han et al. [11] who reported that age range was from 4 to 78 years. Moreover, the age range in our study for a1lergic rhinitis patients was 12 to 59 which was comparable to Shirkani et al. [12] who reported that age range was from 15 to 60 years and to Zhang et al. [16] in which the age ranged between 8-61 years.

Allergic diseases affect both males and females [17]. Allergic rhinitis group was 50% male and 50 % female.

A slight preponderance of male patients (55%) in allergic conjunctivitis group was observed which agrees with Charani & Sailaja [18] and Nagrale et al. [19] who reported 57.4% and 82.5% male preponderance respectively, while ***Almaliotis et al.*** [20] reported a slight female preponderance (58.19%) among allergic conjunctivitis patients. This male predominance may be linked to lifestyles being more engaged in outdoor activities to a greater extent than females in our society.

In the current study it was found that about 10 (50%) of allergic conjunctivitis patients and 13 (65%) of allergic rhinitis patients had positive family history of allergy. In accordance, Kosrirukvongs et al. [21] reported that 66% of allergic conjunctivitis patients had positive family history of atopy, as well as ***Shirkani et al.*** [12] who demonstrated that 50% of allergic rhinitis patients had positive family history of allergy. This similarity might be due to that the main underlying factor for atopy, which is the genetic basis, the main point of investigation of this study.

Skin prick testing (SPT) is a re1iable test for diagnosing IgE-mediated allergic disease in patients suffering from rhino-conjunctivitis, asthma, urticaria, atopic eczema and suspected drug allergies. It provides proof for sensitization and can aid in the confirmation of a type I allergy diagnosis [22]. It is the gold standard method in diagnosis of allergic diseases because it is rapid, cheap and has good reproducibility and high sensitivity [23]. As regard SPT pattern of sensitization in this study, the number of positive reactions of skin prick test ranged from (5 to 14) in allergic conjunctivitis and (4 to 14) in allergic rhinitis. This is similar with Rasool et al. [24] who reported that 100% of nasobronchial allergy patients had multiple sensitizations to aeroallergens. Haggag [25] also reported that 58 (93.5%) out of 62 patients with allergic conjunctivitis revealed po1ysensitization pattern. Similar findings were reported in two successive studies done in RIO by Haggag and Hamed [26] and Mahran et al. [27]. In our opinion and based on other researches; allergies are caused by combined group of allergens which trigger the cytokines pathway more effectively than one type. Po1ysensitization might be the result of genetic factors or environmental factors which favour growth and vegetation of specific plant species such as grass and weeds with simi1ar survival conditions. It might also be due to cross-reactivity which reflects the presence of common allergenic epitopes in different but botanically close plant species [28].

Findings of the present study reveal that frequencies of genotype GG and allele G at rs1269486 in the allergic conjunctivitis patients (65% and 80%, respectively) and allergic rhinitis patients (80% and 90%, respectively) were significantly higher than those in the control group (4% and 23%, respectively) and the frequencies of genotype GA and allele A at rs1269486 in the patient group were significantly lower than those in the control group (30.0%, 20.0% in AC group and 20.0%,10% in AR group). These findings show that genotype GG and allele G are highly associated with patients with allergic conjunctivitis and allergic rhinitis.

Results of this study are in consistence with Zhang et al. [11] who showed 82.6% of allergic rhinitis patient and 58.9% in the control group had genotype GG, meanwhile the frequency of allele G in allergic rhinitis was 86.05%, compared to 78.6% in control group**.** Shirkani et al. [12] also reported that 73.25% of their allergic rhinitis patients had genotype GG, compared to 18.6% in the control group. The frequency of allele G in allergic rhinitis was 91.3%, while it was 58.8% in control group [12.] Slight differences in outcomes could be due to differences in races and the small sample size of subjects in the studied patients' groups.

CONCLUSIONS

In conclusion, this study showed off a highly significant association between GATA3 SNP at locus, rs1269486 in the promoter region in the studied Egyptian patients with allergic rhinitis and allergic conjunctivitis suggesting the role of GATA3 gene polymorphism in the pathogenesis of AC and AR. This study recommends the use of a larger size sample that includes cases and control groups and comparing other types of allergies.

This manuscript has not been previously published and is not under consideration in the same or substantially similar form in any other reviewed media. I have contributed sufficiently to the project to be included as author. To the best of my knowledge, no conflict of interest, financial or others exist. All authors have participated in the concept and design, analysis, and interpretation of data, drafting and revising of the manuscript, and that they have approved the manuscript as submitted.

Author contributions

All authors have made substantial contributions to conception and design of the study. SAA collected the samples. SAA, NNS and MAK did the experiments. SAA, MAK and NNS performed the statistical analysis. MAK and NNS wrote the first draft of the manuscript. RAN and NNS provided critical suggestions on study design and manuscript writing. All authors contributed to the revision of the manuscript and approved the final version.

Declaration of interest and Funding information:
The authors report no conflicts of interest.

Acknowledgments: None.

REFERENCES

1. Rayna DJ, Joseph NI, McGarry D, Jhaveri D, Sher T, Hostoffer R. Epidemiology of allergic diseases. Allergy and Asthma: The Basics to Best Practices. Cha. Springer International Publishing 2019; 31-51.
2. Pawankar R, Canonica GW, ST Holgate ST, Lockey RF, Blaiss M**.** The WAO White Book on Allergy 2013.
3. Palmares J, Delgado L, Cidade M, Quadrado MJ, Filipe HP. Allergic conjunctivitis: a national cross-sectional study of clinical characteristics and quality of life. Eur. J. Ophthalmol. 2010;20(2):257-264.
4. Al-Digheari A, Mahboub B, Tarraf H, 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
5. Samson S I, Richard O, Tavian M, Ranson T, Vosshenrich C A, Colucci F, Di Santo JP. GATA-3 promotes maturation, IFN-γ production, and liver-specific homing of NK cells. Immunity 2003;19(5): 701-711.
6. Ho I C, Tai T S, Pai SY.GATA3 and the T-cell lineage: essential functions before and after T-helper-2-cell differentiation. Nat. Rev. Immunol 2009; 9(2): 125-135.
7. Deo S S, Mistry KJ, Kakade A M, Niphadkar P V. Role played by Th2 type cytokines in IgE mediated allergy and asthma. Lung India 2010, 27(2), 66.
8. Yang C,Li C, Luo , Luo Y, Liu Y. Expression of transcription factors T-bet and GATA-3 and relationship to the numbers of eosinophils in rat model of allergic rhinitis.Clin Otolaryngol 2011; 25 (23):1090-3.
9. Laulajainen-Hongisto A, Lyly A, Hanif T.et al. Genomics of asthma, allergy and chronic rhinosinusitis: novel concepts and relevance in airway mucosa. Clin. Transl. Allergy 2020; 10(1):1-17
10. Xin C, Donggang X, Dongqun X, Jinfeng W, Minji Z, Wenliang F**.**A kind of markand primer detecting childhood asthma.Granted patent for intervintion 2015; CN103397088B
11. Zhang L,Wang X, Han D, Duan H, Zhao Y. . Association of single nucleotide polymorphisms in GATA-3 with allergic rhinitis. Acta oto-laryngol 2009;129(2);190-194.
12. Shirkani A, Mansouri A, Abbaszadegan M R, Faridhosseini R, Jabbari Azad F, Gholamin M. GATA3 Gene Polymorphisms Associated with Allergic Rhinitis in an Iranian Population. Rep. Biochem. Mol. Biol 2017; 5(2): 97–102.
13. Bielory L, Delgado L, Katelaris CH, Leonardi A,Rosario N, Vichyanoud P.Diagnosis and management of allergic conjunctivitisAnn. Allergy Asthma Immunol 2020 ;124, 118–134.
14. Small P, Keith PK, Kim H.Allergic rhinitis. Allergy Asthma Clin. Immunol. 2018; 14: 51 .
15. Gilles S, Akdis C, Lauener R, Schmid-Grendelmeier P, Bieber T, Schäppi G, Traidl-Hoffmann C**.** The role of environmental factors in allergy: A critical reappraisal. Exp. Dermatol 2018; 27(11): 1193-1200.
16. Han JY, Lee H, Chung J L, Kim Y J, Kim J Y, Tchah H. Multiple Allergen Simultaneous Test-Immunoblot Assay for Immunoglobulin E Detection in Patients with Isolated Allergic Conjunctivitis. J. Clin. Med 2021;10(5): 960.
17. Kahol P, Thakur M, Gupta A, Saini KS. Prevalence, morbidity and treatment seeking behavior for allergic conjunctivitis in children in a North Indian community. Clin. Epidemiology Glob 2018, 7(2), 239 – 245
18. Charani M , Sailaja M**.** Prevalence and risk factors of allergic conjunctivitis in a semi urban set up. Indian J Clin Exp Ophthalmol; 7(1): 83-86.
19. Nagrale P**.** Study of Clinical Features and Management of Vernal Keratoconjunctivitis. J. Clin. Med. Res 2017;05: 15754-15759. 10.18535/jmscr/v5i1.86.
20. Almaliotis D, Michailopoulos P, Gioulekas D, Giouleka P, Papakosta D, Siempis T, Karampatakis V. Allergic conjunctivitis and the most common allergens in Northern Greece. World Allergy Organ J 2013 ;6(1):12.
21. Kosrirukvongs P, Prabhasawat P, Srivannaboon S, Pornpanich K**.**Topical ketorolac tromethamine in the reduction of adverse effects of laser in situ keratomileusis. J Med Assoc Thai 2001 ;84(6):804-10
22. Heinzerling L, Mari A, Bergmann KC, Bresciani M, Burbach G, Darsow U, Lockey R.The skin prick test–European standards. Clin Transl Allergy 2013; 3(1): 1-10.
23. Kim H, Tse K, Levin L, Bernstein D, Reponen T, LeMasters G, Horner A A. House dust bioactivities predict skin prick test reactivity for children with high risk of allergy. Journal of allergy and clinical immunology 2012; 129(6): 1529-1537.
24. Rasool R, Gull A, Duri MY, Qadri Q , Ashraf T,Shafia S, Zafar AS. POLYSENSITIZATION TO AEROALLERGENS IN PATIENTS WITH NASOBRONCHIAL ALLERGY IN KASHMIR VALLEY. Int J Educ Res 2017 ; 7 (5): 7- 16
25. Haggag M. A comparative study between local and commercial allergens in diagnosis of allergic conjunctivitis. M. D. thesis, Faculty of Medicine Cairo University.2002

26. Haggag M, Hamed A. Comparison of the efficacy of local nasal and subcutaneous immunotherapy using natural allergen extracts in treatment of allergic rhinitis. Egypt. J. Med. Microbiol 2008, 17(4): 627-635
27. Mahran M, Haggag M , Shouman A. Involvement of Interleukin-16 (IL-16) in Allergic Conjunctivitis. Aust. j. basic appl. sci. 2009; 3(4):3266-3272.
28. Fereidouni M, Farid Hossini R, Assarehzadegan MA, Jabberi Azad F, Varasteh A**.** skin prick test reactivity to common aeroallergens among allergic rhinitis patients in Mashhad,Iran. allergo et immunopathol 2009; 37: 73-79.