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The Role of IL-33 Polymorphism during Asthma Disease Iraqi Patients

[1]Basima Q. Hasan AL-Saadi

[1]Institute of genetic engineering and biotechnology for post graduate studies / University of Baghdad

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Abstract: Genetic diversity has play a role in evolution of asthma, but effect of genetic influence may modify between various populations. Current study was designed to look for the association of SNP rs1929992T> C in IL-33 serum level in Iraqi population. The study is a case control study included 50 asthmatic patients and 30 healthy as a control. Blood sample was collected from each individual participate the study and DNA was extracted and genotyping was determined using High Resolution Malting Technique (HRM-PCR) for description of genetic variants,While serum sample was used to assess the level of IL-33 in two study groups using ELISA technique .Results of the current study revealed that there was no significant in allele frequencies in the rs1929992 IL-33 gene between asthma patients and control with particular P value of 0.22 for the rs1929992,however protective effect of T allele was recorded, as well as the asthmatic patient showed a higher level of the IL-33 in serum .As a conclusion the result indicate that the gene polymorphism have not shown any significant differences while the level of IL-33 was highly increased in asthmatic patients compare to control group.

Keywords:IL-33, rs1929992T> C polymorphism,HRM-PCR, ELISA.

Corresponding author: (Email: basima22000@yahoo.co.uk).

Introduction

Asthma is define as incurable chronic inflammatory a heterogeneous disease with many complications that lead to death in more severe disease, accompanied by expiratory airflow limitation and an overall decline in lung function (1,2). It is a major public health problem that affects approximately 300 million people worldwide. And about 455 thousand died world widely from disease., disease morbidity and mortality rates are increased in low- and middles income countries than in developed countries (3) according to estimates from the World Health Organization, there are currently 235 million people with asthma worldwide. While several genes have been associated with asthma in different largescale genetic studies(4), clinical reported claim that the severity of disease may triggered by many environmental factors such as exposure to allergens, air pollutants and bacterial and viral infections of respiratory tract.

Intrlukin-33 is a pleiotropic cytokine with critical roles in both type 2 and type 1 immunity, allergic and non-allergic inflammation, and regulatory responses(4) it is released by structural cells such as endothelial, epithelial, and fibroblast cells (5), role as alarmin when response to some tissue necrosis, injury, moreover it can activate a large number of cells such as basophils, DCs, macrophages Th2 cells,

epithelial cells and mesenchymal cells by binding to IL-33 receptors complex (6).

This interleukin plays a role in the pathogenesis of many diseases, and studies of the entire genome have implicated IL-33 in the pathogenesis of asthma (7). It is Stord as a full length protein (-31KDs) in the cell nucleus and act as transcription factors (8). Located on chromosome 9p24.1, the IL-33 gene contains one non-coding (exon 1) and seven coding exons (exons 2-8). (9,10), It is released into the extracellular environment and functions as an endogenous danger signal or alarmin and its amember of the IL-1 family of cytokines (7), clinical studies revealed that the serum level of this interleukin and its soluble form were indicators of asthma severity and progression as well as SNPs in the genes encoding IL-33 have been associated with allergic asthma (11) moreover, the induction of IL-33 expression by environmental or endogenous triggers suggests a wider role for the pathway during infection, inflammation and tissue damage (12).

As a for said, that the disease prevalence increase in Iraqi population recently, for this many studies focusing on prevalence of asthma in Iraq, Ali Jan *et al* (13), study on the role of IL-8 expression with asthma in adult Iraqi asthmatic patients, while Abdul Kareem and ALsaadi (14) assess IL-17 and its serum level in Iraqi asthmatic children, moreover, a study by Abbas (15) revealed that disease more prevent in adults 31-45 years old= 40%, as well as a study of AL-Qadhi and AL-Saadi (16) that searched on role of IL-4R (rs1805011) Gene Polymorphism on IL-4 Serum Level in Iraqi Allergic Asthma Patients and investigate the highest incidence of disease in age group 15-30 years =50%, and study conducted by Atta and Aoubaidy (17) whose suggest that IL-17A G197A is a candidate gene for asthma development in Iraqi patients , Astudy conducted by Goodi and AL-saadi (18) on the polymorphism of C>T (rs 13217795) of *FOXO3a* gene associated with the developing asthma in sample of Iraqi patients . concluded that, the T allele variant of *FOXO3a* gene (rs 13217795) polymorphism may be associated with increased susceptibility of the development of asthma in Iraqi patients, as well little is known about the susceptibility factors to the disease especially the genetic basis, therefore the aim of the present study was design to investigate the genetic analysis of IL-33 variations in Iraqi populations and to realize if the variants in this interleukin award to capable of being inherited in asthma.

Material and methods

1-Subject characteristic and sampling

Subjects enrolled in current study includes eighty (80) Asthmatic patients and apparently healthy as a control. Fifty (50) Asthmatic patients (26 men and 24 female) aged between 16-50years, study conducted during period January – May 2022. Diagnosis of Asthma was detected by specialist physicians in respiratory and chest disease following the criteria that based on clinical, medical history and physical examination At Alzahra'a` center for Asthma and Allergy in Baghdad and total of (30) apparently healthy individuals (12 men and 18 female) aged 15-55 years were recruited as a control group in current study, they did not suffer from any respiratory tract infection during 4 weeks prior the study, and they did not have any family history of asthma, writing informed consents were obtained from both study

groups, asthmatic patients and apparently healthy individuals, moreover this study was approved by the Scientific Committee at the institute of genetic engineering and biotechnology / University of Baghdad.

All the information needed for current study were collected through questionnaire forma from each participant that's include age, sex, family history, lifestyle and duration of disease.

Five (5) ml of venous blood was collect from each of the study groups, asthmatic patients and apparently healthy individual using plastic disposable syringe, 3 ml of blood was kept in EDTA anticoagulant tubes and freezer at -20°C to be a source for DNA extraction and molecular study, While 2 ml of blood were collected Gel clot tubes and left for 30 min at room temperature(25c°) for clotting, then centrifuged for 10 min at 3000 rpm for serum separation and then serum was stored at -20 C° for detecting the level of IL-33 in serum of both study groups using ELISA Kits and determining the level of IL-33 in nanogram per liter (ng/l) by ELISA through Sunlong Kit (China). This kit was based on sandwich Enzyme linked immune sorbent assay technology. Procedure was conducted using the protocol according to the company's instructions (Sunlong, China).

2- DNA extraction

The DNA was extracted from the blood of asthmatic patient and apparently healthy control groups procedure was conducted using the protocol in EasyPure® Blood Genomic DNA Kit (Transgene Biotech, China) according to the company's instructions. DNA bands were visualized using UV light after electrophoresis in a 1% (w/v) agarose gel. Later on, DNA concentration and purity were deliberate by using Nanodrop (NanoDrop™ 2000/2000c Spectrophotometers, USA) The acceptable concentration and purity within 32-77 ng/µl and 1.8-2, respectively

3-Primer design and SNP choosing

First primers were designed depending on their reference sequence in the database of NCBI National Center of Biotechnology and matching the primers sequence for target study gene il-33 rs1929992T> C, the primers were used for IL-33 rs1929992T> C: represent in (Tables 1,2).

Table (1): primer designed in current study.

Gene	Primers	Sequence (5'------3')	Product size	Temp.
IL-33 rs1929992T> C	**Forward**	AAACACATTTTCCCCCCAAA	20	54
	Revers	AGCTCTTTTCTTTCATGGTCA	21	54

Table (2): Matching of the primer sequence using NCBI

IL—33 gene (rs1929992)
54 bp region
Homo sapiens chromosome 9 GRCh38
NCBI reference : NC_000009.12

61 ATCTTTTGGGAAAAAAACACATTTTCCCCCCAAATTTCAAT/CATTTTGACCATGAAAGA
>>>>>>>>>>>>>>>>>>>> *** <<<<<<<<<<<<

121
AAAGAGCTATTATATTTTTAAATATAATTCTAACACTTTATACATTTTAAGTAGTAGTTA
<<<<<<<<

4- High Resolution Melting -HRM analysis

The extracted DNA was amplified using Quantitative Real time PCR (qRT-PCR) using the (Qiagen Rotor gene Q-Real-time PCR system, Germany). To reveal the genetic variation in the IL-33 gene, one single nucleotide polymorphism (SNP) (IL-33 rs1929992) was selected to explore its association with the asthmatic patients problem, As HRM characterizes double-stranded PCR products based on their dissociation (melting) behavior as they transition from double-stranded DNA (dsDNA) to single-stranded DNA (ssDNA) with increasing temperature, program shown in Figure (3) When the dsDNA dissociates (or melts) into single strands, the dye is released, causing a change in fluorescence. The result is a melt curve profile characteristic of the amplicon. Even single-base changes such as SNPs (single nucleotide polymorphisms) can be readily identified (19).

Table (3): Thermal profile for HRM IL-33 (rs1929992).

Steps	Temperature c°	Duration sec.	Cycles
Enzyme activation	94	60	1
Denaturation	94	5	
Annealing	45	15	
Extention	72	20	40
HRM	60-95	0.1 /1 degree	

Extracted DNA was amplified in a 20 µl solution containing (10 µl of master mix, 1 µl of Forward Primer and Reverse Primer respectively, 3 µl of DNA template and completed with 5 µl of D.W), Table (4).

Table (4): Shows the components of HRM.

Components	Volume (µl)
Trans Start Tip master mix	10
Forward Primer	1
Reverse Primer	1
DNA	3
Distal Water	5
Total volume	20

Statistical analysis

The Statistical Analysis System SAS (20) program was used to detect the effect of difference factors in study parameters. Least significant difference –LSD test (Analysis of Variation ANOVA) and T-test was used to significant compare between means. Chi-square test was used to significant compare between percentage (0.05 and 0.01 probability). Estimate of Odd ratio and CI in this study. And to Estimate of correlation coefficient between variables.

Results and Discussion

The level of the IL-33 in the serum of asthmatic patients showed a highly significant elevation when compared with control groups. The mean level of IL33 in asthmatic patients was 30.72 ±5.16 (pg/ml) while the mean level in control groups were -15.29±0.09 (pg/ml) with (P value $P \leq 0.05$) as shown in Table (5) Figure (1).

The analysis of ROC revealed that elevated serum level of IL-33 occupied a AUC, which was 0.859 (p-value = 0.001). At a cut-off value of 16.4635 pg/ml for the serum level of the IL-33, and the sensitivity, and specificity are 70, 96% respective Table (6) Figure (2).

Table (5): Comparison between patients and control groups in IL-33 level.

Group	Mean ± SE of IL-33 (pg/ml)
Patients	30.72 ±5.16
Control	15.29 ±0.09
T-test	14.558 *
P-value	0.0381
*** (P≤0.05).**	

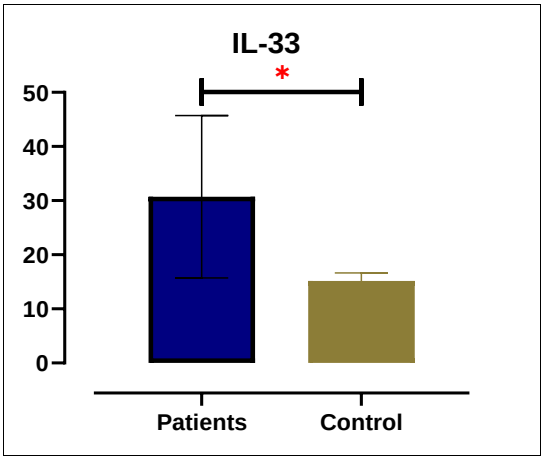

Figure (1): Show comparison of serum level between asthmatic patients and control groups in IL-33.

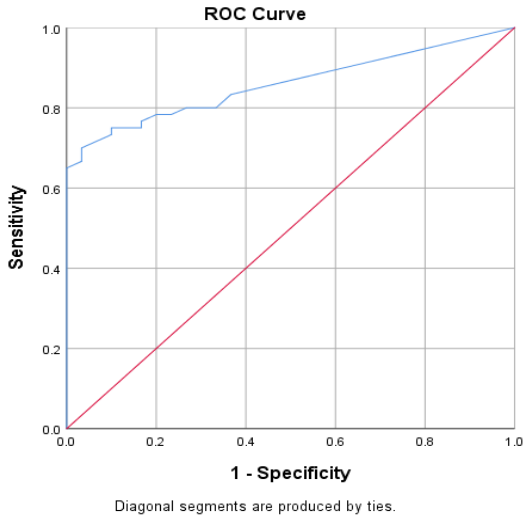

Figure (2): Represent Receiver operating characteristic (ROC) analysis of IL-33.

Table (6): Show Receiver Operating Characteristic curve of IL-33.

Parameters	AUC	P value	The best cut off	Sensitivity	Specificity
IL-33	0.859	0.001	16.4635	0.70	0.96

The current study revealed that there was a significant increase in the serum level of IL-33 in asthmatic patients when compare to the control group. Findings of the present study are in line with study conducted in Egyptian patients concluded that higher serum IL-33 levels were strongly associated with asthma severity in Egyptian adults(21), moreover similar study included meta-analysis that included 15 studies recruiting 633 asthmatic patients and 379 control, concluded that serum IL-33 levels in asthmatic patients is higher than in control groups comparison to controls(22).

While study of Gasiuniene *et al*. (23) did not show any a significant correlation between severity of asthma and serum IL-33 levels, in this manner Il-33 play a crucial role in the pathogenesis of asthma that the levels of these innate cytokines were strongly positively correlated, and were co-expressed in monocytes in peripheral blood mononuclear cells. And the IL-33 level may be related to inflammatory activity in chronic airway inflammatory disease (24).

Determining Genotype and allele frequencies of IL-33 gene polymorphism (rs1929992T> C).

The extract DNA samples of the study groups were genotyped of Il-33 gene (rs1929992) detection was realized by using HRMPCR technique. The process output of the thermocycler of the two genotypes were shown in Figure (3). T and the resulting of HRM analysis is shown in Figure (4).

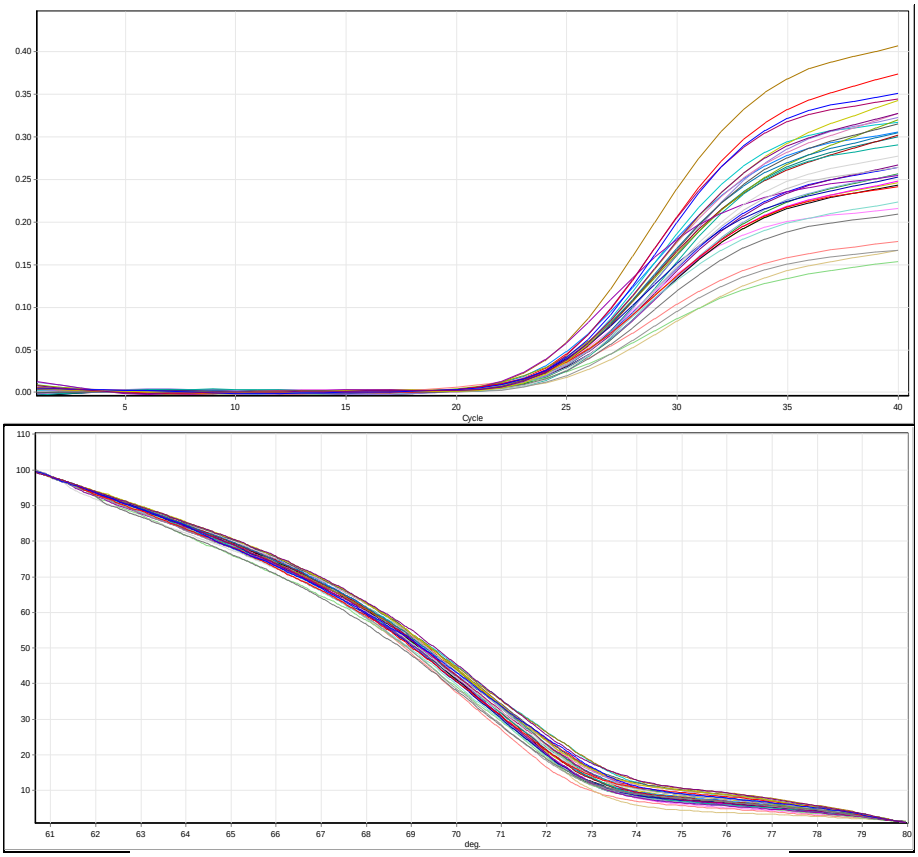

Figure (3): Show the HRM process output for genotyping.

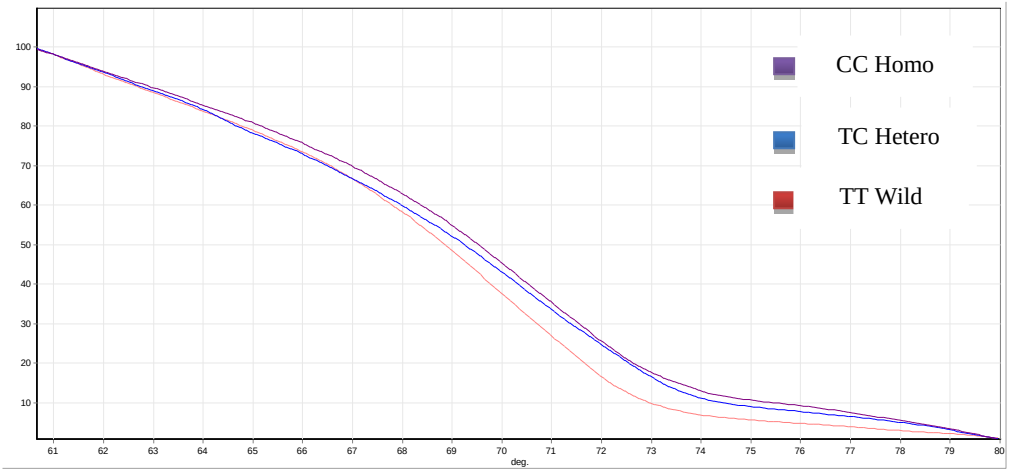

Figure (4): Represent resulting output fo genotying of Il-33 gene (rs1929992).

Table (7): Comparison of the Genotype and Allele Frequencies of *IL33* gene polymorphism (rs1929992 T>C) between Patients Group and Control group

polymorphsim rs1929992	Frequencies (%)		P value	Odd ratio (95% CI)
	Control groups (30)	Patients groups (50)		
Codominant				
TT	43.3% (13)	38.0% (19)	----------	1.00 (Reference)
TC	46.7% (14)	38.0% (19)	0.88	0.92 (0.3-2.4)
CC	10.0% (3)	24.0% (12)	0.17 NS	2.7 (o.6-11.6)
Dominant				
TT	43.3% (13)	38.0% (19)	------	1.00 (Reference)
TC+CC	56.7% (17)	62.0% (31)	0.63	1.2(0.4-3.1)
Recessive				
TT+TC	90.0% (27)	76.0% (38)	----------	1.00 (Reference)
CC	10.0% (3)	24.0% (12)	0.13 NS	2.8 (7.0-11.0)
Alleles				
T	66.7% (40)	57.0% (57)	------	1.00 (Reference)
C	33.3% (20)	43.0% (43)	0.22 NS	1.5 (0.7-2.9)

NS = Non significant

The genotype frequencies for the polymorphism of IL-33 (rs1929992 T>C) in asthma patients and control subjects show no Significant differences in the frequency of IL-33 alleles. The frequency of the T allele in asthma patients and controls was 57.0%(n=57) and 66.7 % (n=40) respectively. While the frequency of the C allele in asthma patients and controls was 43.0% (n=43) and 33.3% (n=20) respectively with odd ratio 1.5 and (p-value= 0.22) that may explain that T allele may be play as protective factors for this disease while C allele may act as a risk factor. Allocation of alleles frequency and genotype between asthmatic patients and apparently healthy groups of IL-33 gene rs1929992 T>C was appear in Table (7). Genotyping frequencies of asthmatic groups reveals that TT wild type was 38.0% (19), heterozygous was 38.0%(19) represent the TC alleles while the homozygous mutant CC showed 24.0% (12), in addition the genotype TT (wild type) in control patients were 43.3% (13), heterozygous TC were 46.7% (14) while mutant genotype CC were 10.0% (3). According to the result of the current

study genotype frequencies of asthma patient's and control analysis manifest that the TT genotype and T wild type allele were accept as reference. Result revealed that odds ratio for the CC genotype was 2.7 and P-value =0.17. More over TC genotype had an odds ratio of 0.92 with (P-value=0.88). The TC+ CC genotype had an odd ration 1.2 and (p value=0.63) indicating that homo mutant genotype CC was at a higher risk of asthma than the wild-type TT. While the odd ratio of CC genotype vis. TT+TC was higher 2.8 with (P-value=0.13). IL-33 drives type 2 responses by inducing signalling through its receptor IL-1RL1 in several immune and structural cells, thereby leading to type 2 cytokine and chemokine production. IL-1RL1 gene transcript encodes different isoforms generated through alternative splicing. Its soluble isoform, IL-1RL1-a or sST2, acts as a decoy receptor by sequestering IL-33, thereby inhibiting IL1RL1-b/IL-33 signaling (3).

This study undertake to set the correlation between the IL-33 rs1929992 and the risk of asthma disease, the result of current study incompatible with study by Rabea (20) in Egyptian patients that concluded SNP rs1929992 of IL-33 gene is not associated with risk of bronchial asthma.

The relation between the genotyping and serum level of Interlukin-33 shown in Table (8), that reveals a significant increase in the serum level of IL-33 in all the three genotypes TT,TC,CC, 29.48±4.07, 32.51±4.71 and 30.44±4.83 respectively compare to control groups this cytokine in It's mainly expressed by epithelial and endothelial cells. This expression is upregulated by pro-inflammatory stimulation, thus has an important role in infla;mmatory responses, such as asthma. No previous study has been conducted the relation between the genotyping of this cytokine and its level in serum

Table (8): Association between IL -33 genotying and its serum level in asthma patients.

IL33		Group		
		Patient	Control	
		Mean±SE	Mean±SE	p-value
SNP	Wild TT	29.48±4.07	15.30±.17	0.01**
	Hetero TC	32.51±4.71	15.33±.11	0.005**
	Mutant CC	30.44±4.83	15.00±.20	0.02*

*** p>0.05 , ** p>0.0l**

Further supporting the role for IL-33 in the disease. A several GWA studies have identified multiple SNPs in IL-33 to be associated with asthma(5).

Limitation of this study due to few number of subject involved, moreover, the distribution of il-33polymorphism is different in statistical terms among the controls and patients, but it should be assumptive that these differences have a limited extent and might not be so

significant in clinical and epidemiological terms.

References

1. Global Initiative for Asthma (2019). GINA 2019. Global strategy for asthma management and prevetion.
2. Global Initiative for Asthma (2020). Global initiative for asthma: Global strategy for asthma management and prevention (Updated 2020). Revue Francaise d'Allergologie et d'Immunologie Clinique. https://doi.org/10.1016/S0335-7457(96)80056-6.
3. Jayalatha, A.K.S.;. Hesse A,B L.; Ketelaar, M.E.;. Koppelman, G.H. and Nawijn,M.C. (2021) The central role of IL-33/IL-1RL1 pathway in asthma: From pathogenesis to intervention Pharmacology and Therapeutic, 225, 107847
4. Cayrol, C. and Girard, J-P. (2022). Interleukin-33 (IL-33): A critical review of its biology and the mechanisms involved in its release as a potent extracellular cytokine. cytokine, 156: 155891
5. El-Husseini, Z. W.; Gosens, R.; Dekker, F. and Koppelman, G. H. (2020). The genetics of asthma and the promise of genomics-guided drug target discovery. The Lancet Respiratory Medicine, 8(10): 1045-1056
6. Rank, M. A.; Kobayashi, T.; Kozaki, H.; Bartemes, K. R.; Squillace, D. L. and Kita, H. (2009). IL-33-activated dendritic cells induce an atypical TH2-type response. The Journal of Allergy and Clinical Immunology, 123(5): 1047-1054
7. Canoğlu, K.; Oğuzhan, O.; Dilaver T.; İsmail, Y.; Soner, Y.; Tayfun, C., *et al*. (2021). Evaluation of serum interleukin-33 and gene polymorphisms in patients with bronchial asthma . Gulhane Mededical Journal 2021;63:104-9
8. Gordon, E. D.; Palandra, J.; Wesolowska-Andersen, A.; Ringel, L.; Rios, C. L.; LachowiczScroggins, M. E., *et al*. (2016). IL1RL1 asthma risk variants regulate airway type 2 inflammation. JCI Insight, 113(31): 8765-70
9. Ferreira, M. A.; Vonk, J. M.; Baurecht, H.; Marenholz, I.; Tian, C.; Hoffman, J. D., *et al*. (2017). Shared genetic origin of asthma, hay fever and eczema elucidates allergic disease biology. Nature Genetics, 94(12): 1752-1757
10. Smith, D.; Helgason, H.; Sulem, P.; Bjornsdottir, U. S.; Lim, A. C.; Sveinbjornsson, G., *et al*. (2017). A rare IL33 loss-of-function mutation reduces blood eosinophil counts and protects from asthma. PLoS Genetic 13(3).
11. Watanabe, M.; Nakamoto, K.; Inui, T.; Sada, M.; Honda, K.; Tamura, M., *et al*. (2018). Serum sST2 levels predict severe exacerbation of asthma. Respiratory Research, 19: 169.
12. Lloyd, C.M. (2010): IL-33 family members and asthma-bridging innate and adaptive immune responses. Current Opinion in Immunology, 22: 800-806,
13. Ali Jan, M.F.; Al-Saadi, B.Q.H.; Al-Khafaji, H.M.A. and Al-Saedi, M.K.A. (2021). Primer and Probe Designing to Detect SNP rs 4073 in Interleukin-8 Gene in Iraqi Patients with Bronchial Asthma. Journal of Applied Sciences and Nanotechnology, 1(3): 51-57.
14. Abdul Kareem, Z.T. and AL-Saadi, B.Q.H. (2020). Determine gene expression of IL-17 in Iraqi Child Asthmatic Patients Iraqi Journal of Biotechnology, December 2020, 19(3): 21-32.
15. Abbas, Y. (2020). Evaluation the Role of Tumor Necrosis Factor alpha, Interleukin1Beta and Interleukin-17F Gene Expression in some Iraqi Patients with Asthma. M.Sc. Thesis. Genetic Engineering and Biotechnology Institute for Postgraduate Studies. University of Baghdad.
16. AL-Qadhi, I.Y. and AL-Saadi B.Q. (2022). Impact of IL-4R (rs1805011) Gene Polymorphism on IL-4 Serum Level in Iraqi Allergic Asthma Patients. Iraqi Journal of Biotechnology, 21(2) : 358-369.
17. Atta, R. Z. and. Aloubaidy, R.M. (2022) Genetic polymorphism of asthma in iraq, Iraqi Journal of Agricultural Sciences, 53(2): 288-296.
18. Goodi,G.A. and ALssadi ,B.Q.H.(2018) Polymorphism of *FOXO3a* Gene and Its Association with Incidence of Asthma in Iraqi Patients, Iraqi Journal of Biotechnology ,17(3):67-77
19. Druml, B. and Cichna-Markl, M. (2014). High resolution melting (HRM) analysis of DNA–Its role and potential in food analysis. Food chemistry, 158: 245-254.
20. SAS, (2018). Statistical Analysis System, User's Guide. Statistical. Version 9.6th ed. SAS. Inst. Inc. Cary. N.C. USA.

21. Rabea, R.A.; El-Gamal,R.; Fahmy, E.M.; Taha, H.K.E.; Bakr, A.O.; Zaki, M. E., *et al*. (2021) Serum interleukin 33 levels and single nucleotide polymorphism rs1929992 in Egyptian patients with chronic asthma. The Egyptian Journal of Immunology. 28 (4): 264–271.
22. Li, R;, Yang, G.; Yang, R.; Peng, X. and Li, J. (2015). Interleukin-33 and receptor ST2 as indicators in patients with asthma: a meta-analysis. International Journal of Clinical Experimental Medicine, 8(9): 14935-43.
23. Gasiuniene, E.J. and Zemeckiene, Z. (2019). Elevated levels of interleukin-33 are associated with allergic and eosinophilic asthma. Scandanavian Journal of Immunology, 89(5): e12724.
24. Kim, M. H.; Kwon, J. W.; Hahn, J. H.; Kim, M.; Chang, H. S.; Park, J. S., *et al*. (2022). Circulating IL-32 and IL-33 levels in patients with asthma and COPD: a retrospective cross-sectional study. Journal of Thoracic Disease, 14(6): 2437.