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Study Of Some Electrolytes And Tumour Necrosis Factor Alpha (TNF-α) Gene Polymorphism In Patients With Allergic Rhinitis In Babylon Province In Iraq.

A Thesis

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بسم الله الرحمن الرحيم

﴿ وَقُل رَّبِّ زِدْنِي عِلْمًا﴾

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Supervisor Certification

We certify thesis entitled ″ Study the effect of electrolyte imbalance and tumour necrosis factor alpha (TNF-α) gene polymorphsim in Iraqi patient with allergic rhinitis in Babylon Province ″ was prepared under our supervision at the department of biochemistry ,College of medicine ,university of Babylon ,in partial requirement for degree of master in clinical biochemistry.

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Dedication

To my amazing husband and lovely children, who provided inspiration, patience, and support to make this thesis a reality.

And to my family may mother , my brothers and sisters for their patience, encouragement, endless support, understanding, kindness, and love.

Special thanks go to my friends who assisted me in some form and to everyone who helped me light my pathway.

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Summary

Allergic rhinitis is the inflammation of nasal mucosal membranes accompanied with many sign such as the swelling of nasal mucosa, nasal secretion ,itching and other symptoms. Allergic rhinitis is caused by allergens, which are usually harmless substances but trigger an allergic reaction in some people it is a chronic disease. .An essential part of the inflammatory process is played by cytokine .Tumor necrosis factor alfa (TNF-α) produced mainly by macrophages and monocytes. inhibition of TNF-α delayed the development of allergic rhinitis TNF-α may be involved in AR. TNF-α is regulated by TNF-α gene, a 233 amino acid .single nucleotide polymorphism of TNF-α gene is located on band q21.33 of chromosome 6 consist of 4 exons.

This study was designed to investigate the possible relation of TNF-α, IgE, Eosinophilis and electrolytes (Na^+ ,K^+ ,Cl^- , Ca^{+2}) in patients with AR in Babylon province. and to evaluate the role of TNF-α genepolymorphism by T-ARMS PCR and its associated with the risk of AR.

To achieve this aim, the present study included 100 subjects divided into 2 group The first group include 50 patients with AR with a mean age 37.5 ± 7.1 years and an age range of 30-45 years and the second group included 50 apparently healthy individuals with a mean age of 36 ± 7.6 years and an age range of 29-44 Besides, The TNF-α protein level and Ig-E were determined in serum by enzyme linked immunosorbent assay(ELISA). An Abbott device was used to measure electrolytes (Na^+ ,K^+ ,Ca^{+2}, Cl^-) concentrations. Eosinophil were determined by analyzing the blood sample count by hematology analyzer. DNA was extracted from blood and genotyped for the SNP rs769178 G > T by amplified refractory mutation system(

T-ARMS PCR).Genotyping was achieved with specific primers to amplify fragment The product was analyzed by agarose gel electrophoresis.

Various statistical analysis were applied to analyze the data our result found there was a significant increase in TNF-α ,IgE and Eosinophils in patients with AR compared to the control group ($P< 0.05$), While no significant difference in levels of Sodium ,potassium, Calcium and Chloride in patients with AR compared to the control group ($P> 0.05$). polymorphism of TNF alfa gene rs769178 G >T was not associate with allergic rhinitis .TNF alpha and Ig-E were positively correlated with age ,gender, smoker and BMI , While , Sodium ,potassium, Calcium and Chloride No correlated with age ,gender ,smoker and BMI in patients with AR.

TNF-α polymorphisms of the genetic mutation rs769178G < T in patients with allergic rhinitis did not show a significant association with the disease (AR).

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Abbreviation

	Details
AR	Allergic Rhinitis
ARMS	Amplification Refractory Mutation System
BMI	Body mass index
Ca	Calcium
Cl	Chloride
CI 95%	Cofidence Interval
CLDN	Claudin
DC	Dendritic cell
DNA	Deoxyribonucleic acid
EDTA	Ethylene diamine tetra acetic acid
ELISA	Enzyme- linked immunosorbent assay
Fcr	Fc recepter
GM-CSF	Granulocyte Macrophage Colony Stimulating Factor
HDM	House dust mite
HRP	Hours radish peroxidase
Ig-E	Ig-E Immunoglobulin E
IL	Interlukin
ILC2	Iinnate lymphoid Cell group 2
INF	Interferon

(LT) C4	leukotriene C4
LTD4	Leukotriene D4
LTE4	Leukotriene E4
M	Mean
MCTc	connective tissue mast cells
MHC-II	Major Histocompatibility complex Class II
mRNA	Messenger Ribonucleic
MR	Mixed Rhinitis
NARES	Non –allergic rhinitis with eosinophilia
Na	Sodium
OR	Odd ratio
K	Potassium
OCLN	Occludin
PCR	Polymerase chain reaction

PGD2	prostaglandin D2
PGE2	Prostaglandin E2
RFLP	Restriction Fragment Length Polymorphism
SD	Standard deviation
SP-A	Surface protein A
SP-D	Surface protein D
SNP	Single nucleotide polymorphisms
TBE	Tris-borate EDTA
TCR	T cell receptor
TGF	transforming growth factor
TH2 cells	type2T helper cell
TNF	Tumor Necrosis Factors
TSLP	Thymic stromal lyphopoietin
Th1	T helper type 1
Treg,	Regulatory T cell
VCAM-1	vascular cell adhesion molecule 1

1. Introdution and literature Review

1.1 Allergic Rhinitis

Allergic rhinitis (of which the seasonal type is called hay fever) is the inflammation of nasal mucosal membranes accompanied with many sign such as the swelling of nasal mucosa, nasal secretion ,itching and other symptoms. AR is caused by allergens, which are usually harmless substances but trigger an allergic reaction in some people **(1).**

Rhinitis (also known as coryza) is a reaction that happens in the eyes, nose, and throat when allergens in the air trigger histamine to be released in the body ,is irritation and inflammation of the mucous membrane inside the nose. Rhinitis is very common. Allergic rhinitis is more common in some countries than others; in the United States, about 10–30% of adults are affected annually. Mixed rhinitis (MR) refers to patients with non allergic rhinitis and allergic rhinitis **(2)**. MR is a specific rhinitis subtype. It may represent between 50 and 70% of all AR patients. However, true prevalence of MR has not been confirmed yet. Rhinitis is categorized into three types ,infectious rhinitis includes acute and chronic bacterial infections, non allergic rhinitis and allergic rhinitis, allergic rhinitis (AR) is the most common form of rhinitis **(3)**.

inflammation in the nose that occurs when the immune system over react to allergens in the air symptom onset is often within minutes following allergen exposure, and can affect sleep and the ability to work or study. Some people may develop symptoms only during specific times of the year, often as a result of pollen exposure. Many people with allergic rhinitis also have asthma, allergic conjunctivitis, or atopic dermatitis **(4)**.

An allergen is a type of antigen that produces an abnormally vigorous immune response. In technical terms, an allergen is an antigen

that is capable of stimulating a type-I hypersensitivity reaction in atopic individuals through immunoglobulin E (IgE) responses. Most humans mount significant Immunoglobulin E responses**(5)** . However, some individuals may respond to many common environmental antigens. This hereditary predisposition is called atopy. In atopic individuals, non-parasitic antigens stimulate inappropriate IgE production, leading to type I hypersensitivity. Sensitivities vary widely from one person (or from one animal) to another. A very broad range of substances can be allergens to sensitive individuals **(6).**

Allergens can be found in a variety of sources, such as dust mite excretion, pollen, pet dander, or even royal jelly. Food allergies are not as common as food sensitivity, but some foods such as peanuts , nuts, seafood and shellfish are the cause of serious allergies in many people**(7).** metals and other substances can also contribute to issues . Food, insect stings and medications are common causes of serious reactions. Their development is dependent on genetic and environmental factors**(8)**.

Seasonal allergy symptoms are commonly experienced during specific parts of the year, usually during spring, summer or fall when certain trees or grasses pollinate. This depends on the kind of tree or grass. Fungal allergens described as being possible airborne allergens allergies are associated with seasonal asthma **(9).**

1.2. Symptoms:

Hay fever symptoms can appear throughout the year. Outdoor allergies are worse in the spring, summer and early fall. In warm weather, weeds and flowers bloom, and pollen counts are higher. Indoor allergies,

such as those that result from pet dander and dust mites, can get worse in winter because people spend more time indoors **(10).**

Symptoms of hay fever include:

- Nasal stuffiness (congestion), sneezing and runny nose.
- Itchy nose, throat and eyes.
- Headaches, sinus pain and dark circles under the eyes.
- Increased mucus in the nose and throat.
- Fatigue and malaise (general feeling of discomfort).
- Sore throat from mucus dripping down the throat (postnasal drip).
- Wheezing, coughing and trouble breathing **(11).**

1.3. Epidemiology

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**(12)**. In Europe, the prevalence of AR has steadily increased among Danish adults, over the last three decades, from 19% to 32%.**(13).**

The prevalence in countries around the world of allergic rhinitis varies because of geographical differences which lead to the differences in the allergens' types and potential in these areas, Reports from various sources suggest that the variations in allergic rhinitis prevalence especially in developing countries may be related to the environmental and physical changes and the adoption of an urbanized lifestyle**(14**) .

The prevalence of AR has increased over the years along with increased urbanization and environmental pollutants thought to be some of the leading causes of the disease.

In Asia, this disease affects a large population, ranging from 27% in South Korea to 32% in the United Arab Emirates . It is a prevalent yet underappreciated atopic disorder which is commonly characterized by the presence of at least one of the following clinical symptoms: persistent nasal obstruction and mucous discharge, sneezing, and itching**(15)** .

1.4 Classification of Rhinitis

Table 1-1: Classification of Rhinitis (16)

A-Systematic atopy of allergic rhinitis	**B-Allergic rhinitis without systemic atopy**	**C-Non allergic rhinitis including the following**
1- Classically graded 2-Aeroallergen exposure time: permanent, seasonal, and jobs a-persistent and intermittent. b-mild, Systematic atopy allergic rhinitis moderate, sever	a-Classical classify-cation according to Time of exposure to aeroallergens :perennial, seasonal and occupational. b-ARIA classification according to duration and severity.	❖ A-infectious ❖ B-drug indued ❖ C-Hormonal ❖ D-Food ❖ E-Emotional ❖ F-Atrophic ❖ G-non –allergic rhinitis with eosinophilia NARES ❖ H-rhinitis of the elderly .

functional classification of allergic rhinitis according on severity of symptoms, Mild (No weakening of beeding days activities and not troublesome) Moderate to severe(A fallowing one or more is present

Sturge of sleep, Every day movement disability and troublesome symptoms) **(17).**

1.5 Risk Factors Of Rhinitis

Risk factors affecting the presentation of allergic rhinitis Apart from the demographic factors, smoking and drinking habits, pet adoption, education attainment, and family history were the risk factors of AR, commonly studied in Asian countries . Conversely, Western countries focus more on the effects of pollens, drugs, pets, and family history on the presentation of AR**(18).**

Increased risk of occupational rhinitis has been found in the following professions: Furriers, Bakers ,Livestock breeders ,Food-processing workers, Veterinarians ,Farmers ,Electronic product assemblers and Boat builders**(19)**.

Environmental ,Many possible factors have been suggested, such as lifestyle changes, increased exposure to allergens, pollution and irritants. Dietary modifications responsible for diminution of protective nutrients. Pollution certainly increases symptomatic rhinitis and diesel exhaust particles may induce a Th2-like inflammation**(20)**.

Other risk factors for allergic rhinitis include ,The presence of other allergic diseases such as asthma, eczema, Parental rhinitis ,Allergic sensitization to common household aeroallergens, Obesity/being over weight, Elevated exhaled Nitric oxide, High total serum IgE, Exposure to parental smoking, Exposure to pets, Exposure to fossil fuel and traffic related pollutants. Increased dampness and poor ventilation from tightly insulated modern homes (increases sensitization and allergy to molds) and Early introduction of infants to formula/food(**21**).

The best established risk factor for allergic rhinitis is a family history of allergy especially of allergic rhinitis . If a child has one parent with atopy (allergies), he or she has a 30% risk of developing allergic rhinitis. If both parents are atopic, then risk increases to 50–70**% .**Having other allergic conditions also predispose a person to allergic rhinitis, and someone with a history of infantile eczema has a 70% chance of developing allergic rhinitis, asthma, or both**(22) .**The genetics of rhinitis has not been studied as much as that of asthma and atopy , because there is a difficulty in the precision of the allergic rhinitis phenotype. However, there are several genes which appear to be involved in atopy. Those include an area on the 5q chromosome where genes exist for interleukin 1L-4 and IL-13 with markers associated with the presence of a high level of serum IgE **(23)**.

1.6. Pathophysiology:

Allergens are usually proteins with molecular weight ranging from 10 to 40 kD that induce type I hypersensitivity by reacting with particular Ig E antibodies in response to allergens that lead to the histologic features of allergic rhinitis are covered below**(24)**.

1.6.1. Sensitization:

During the sensitization phase, allergens are taken up by dendritic cells in the nasal mucosa and induce a series of events leading to the generation of plasma cells that produce allergen-specific immunoglobulin E (IgE) that binds to mast cells and basophils and to a pool of memory allergen-specific type2 T helper cells (TH2 cells) and IgE, B cells. In individuals who are sensitized to the allergen, subsequent allergen exposure activates basophils and mast cells in the nasal mucosa,

triggering the release of allergic mediators (including histamine and sulfidopeptide leukotrienes)**(25)**, leading to the acute symptoms of allergic rhinitis. Subsequent cytokine production by memory allergen-specific TH2 cells induces an inflammatory infiltrate (eosinophil recruitment) within a few hours, leading to more symptoms and changes in the functional aspects of the nasal mucosa that mimic chronic rhinitis. **(26)**.The allergic response in AR can be divided into two phases i.e., the early phase and the late phase.

1.6.2 . Early phase Response:

starts within 20 min after exposure to harmful allergens. Antigen presenting cells such as dendritic cells in the mucosal surface uptake, process and present peptides from allergens on the major histocompatibility complex (MHC) class II molecule **(27)**. The antigen complex and the MHC class II molecule serve as a ligand for T cell receptors on naïve CD4+ T cells, resulting in differentiation of naïve CD4+ T cells into allergen-specific Th2 cell. Cytokines such as IL-4 and IL-13 released from the activated Th2 cells interact with B-cells to produce allergen-specific Ig-E. This allergen-specific Ig-E binds to high-affinity Fc receptor for IgE (FcεR) present on mast cells, leading to mast cell activation (**28)**.

Cross-linking of the FcR on mast cells causes release of allergic mediators consisting of histamine, proteases and lipid mediators such as leukotriene (LT) C4, and prostaglandin D2 (PGD2) that cause vascular leak, bronchoconstriction, inflammation, and intestinal hypermotility **(29)**. These mediators induce mucosal edema and watery rhinorrhea characteristic of AR by causing the blood vessels to leak. Histamine is the major mediator in AR where it activates H1 receptors on sensory nerve

endings and causes sneezing, pruritus, and reflex secretory responses, and it also interacts with H1 and H2 receptors on mucosal blood vessels, leading to vascular engorgement(nasal congestion and plasma leakage**(30)**.

1.6.3. Late phase Response:

After 4–6 h of allergens exposure, the late phase of allergic response is initiated, nasal mucosal inflammation occurs with the influx and activation of a variety of inflammatory cells such as T cells, eosinophils, basophils, neutrophils, and monocytes into the nasal mucosa **(31)**. Recruitment of these inflammatory cells is triggered by cytokines such as IL-4 and IL-5. These cytokines upregulate the expression of adhesion molecules such as vascular cell adhesion molecule 1 (VCAM-1) on endothelial cells which facilitate inflammatory cellular influx **(32)**. The activation of structural cells in the nasal mucosa, such as epithelial cells and fibroblasts, can promote the release of additional chemokines (e.g., eotaxin and RANTES) that facilitate cellular influx from the peripheral blood The mechanisms underlying the infiltration of inflammatory cells are an exciting new chapter in the annals of allergic rhinitis. Circulating leukocytes bind to endothelial cells of postcapillary venules. The shear forces of blood flow force these leukocytes to roll along the surface of the endothelium**(33)** The schematic representation of pathophysiology of AR is illustrated in Figure 1-1

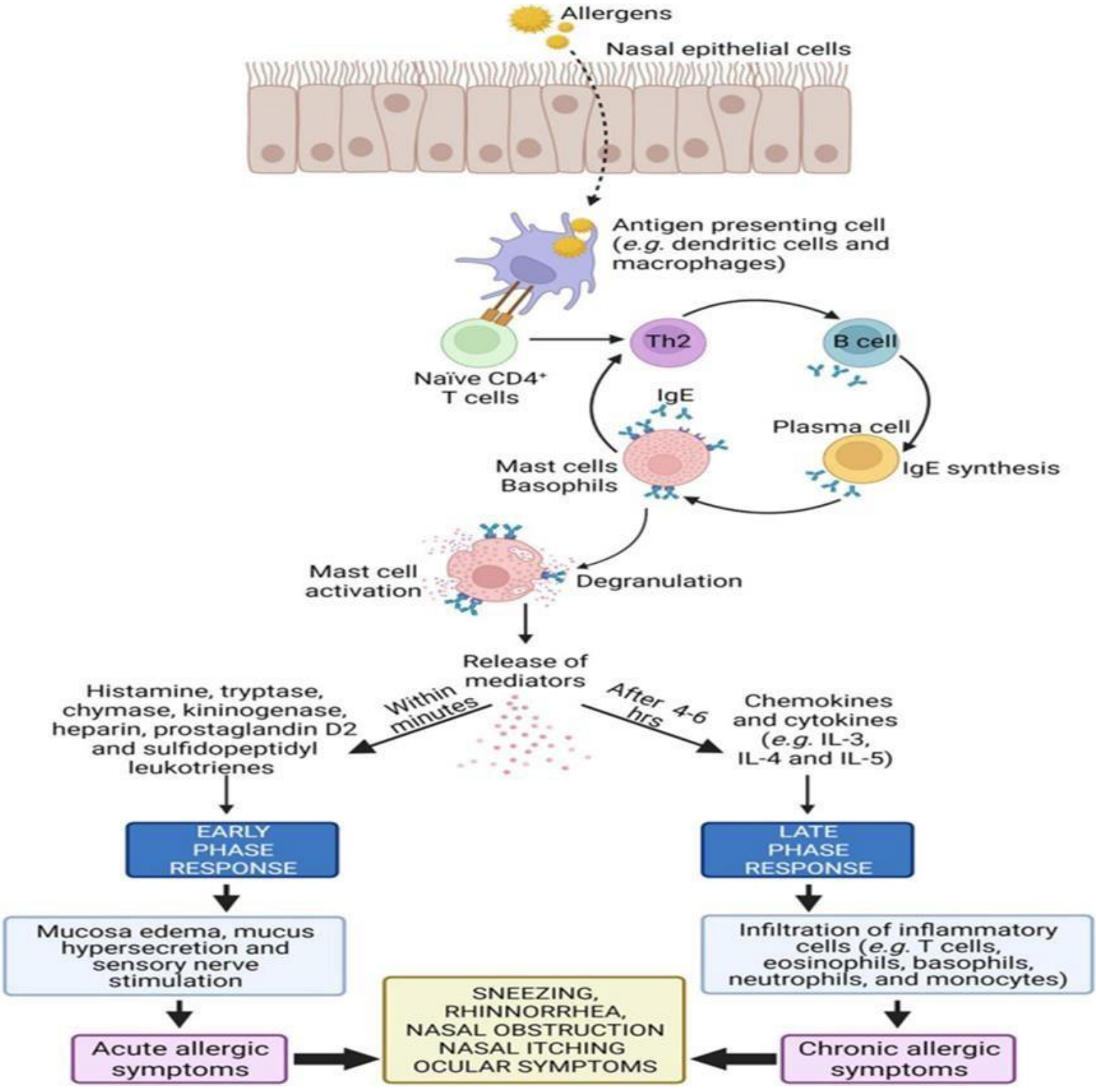

Figure1-1: This figure explains the step-by –step process that starts from the allergen and release of mediators in the early or late response phase that leads to acute or chronic allergic rhinitis and hence the onset of symptoms(**34)**. TH2 cells type2T helper cell; IL Interlukin.

1.6.4. Cellular Recruitment Phase:

The mediators of the immediate-phase response generate the acute symptoms of itch, rhinorrhea, congestion, and sneezing of allergic rhinitis. As the mediators are metabolized and cleared from the mucosa, the symptoms wane. However, the release of cytokines and activation of endothelial cells leads to a latent recruitment phase that ushers in the inflammatory late-phase response**(35).**

1.6.5. Epithelial Cell Changes In Allergic Rhinitis

The influx of inflammatory cells and upregulation of endothelial cell function are not the only changes in allergic rhinitis. Epithelial cells also appear to be activated, because they express increased amounts of GMCSF, IL-l, IL-8, IL-1 receptors, TNF receptors, and class II HLA **(36)**. Cytokines and ECP also upregulate ICAM-1 expression. Endothelin-1, which is known to be expressed by epithelial and submucosal gland cells in human mucosa and the enzyme that generates endothelin-1 are upregulated in chronic rhinitis. NO is present in nasal air, and its levels in exhaled nasal air are increased during allergic rhinitis**(37)**. It may include endothelial cells that express constitutive Type III NO synthetize, parasympathetic neurons that express Type I NO synthetize and colocalized vasoactive intestinal peptide, or macrophages, neutrophils, mast cells, arterial smooth muscle cells, and fibroblasts that may be induced to express Type II NO synthetize. NO may promote vasodilatation, glandular secretion, and immunomodulation, and because it is a free radical, it may have an antibacterial function**(38**). Submucosal gland area is increased in perennial allergic rhinitis compared with the normal state and hypertrophic rhinitis. Submucosal glands represent approximately 15% of the lamina propria in people without allergies. However, in perennial allergic rhinitis and chronic sinusitis, gland area

increases to approximately 25%. This is consistent with the mucus hypersecretion that has been suggested to be present in allergic airway disease(**39)**.

In normal physiological state (left panel) figure 1-2, intact epithelial barrier prevents allergens infiltration and hence homeostasis of immune components and functions are maintained. In AR such as HDM-sensitized AR (right panel), proteases released by HDMs disrupt tight junctions leading to disrupted epithelial barrier that allows infiltration of allergens. This triggers a cascade of IgE overproduction by B cells, cleaved CD40 on the surface of DCs disrupts the production of thiols by DCs causing decreased Th1 proliferation and collectively with increased IL-6 secretion leads to biased Th2 proliferation. Th2 cells produce the hallmark AR cytokines IL-4 and IL-13**(40).** HDM proteases also cleave the pulmonary surfactants SP-A and SP-D, causing decreased lung clearance of allergens. CLDN, Claudin; DC, Dendritic cell; HDM, House dust mite; IL-4, Interleukin 4; IL-12, Interleukin 12; IL-13, Interleukin 13; IL-25, Interleukin 25; IL-33; Interleukin 33; IFNγ, Interferon gamma; OCLN, Occluding; SP-A, Surface protein A; SP-D, Surface protein D; Th1, T helper type 1; Th2, T helper type 2; Trig, Regulatory T cell; TSLP, Thymic stromal lyphopoietin **)(41).**

A summary of the effects resulting from proteolytic activity of HDM is presented in Figure 1-2.

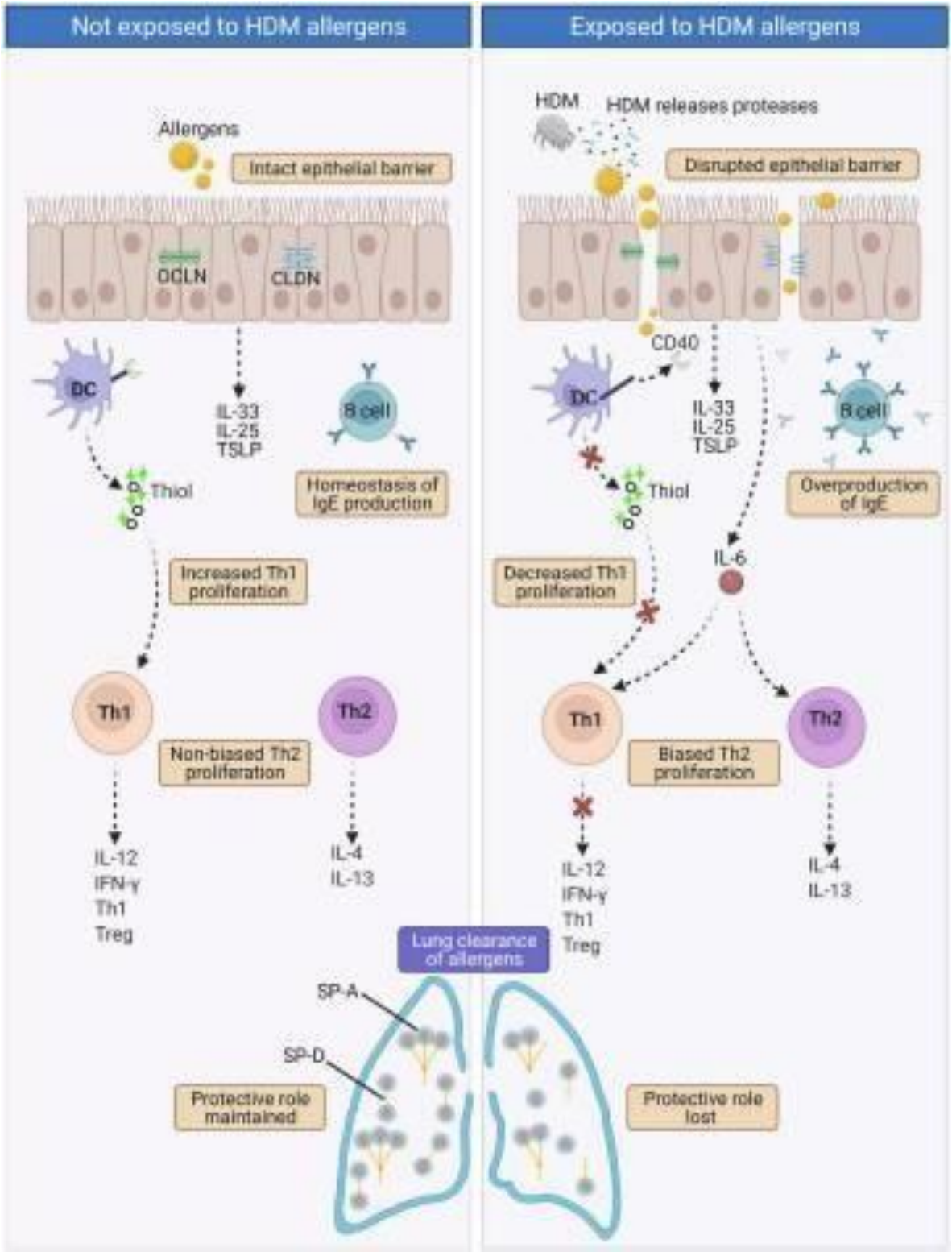

Figure 1-2 :A summary of the effects resulting from proteolytic activity of HDM**(42) .** TSLP:Thymic stromal lyphopoietin;TH2 cells:type2T helper cell; ILInterlukin; HDM House dust mite; CLDN, Claudin, DC, Dendritic cell; IFNγ, Interferon gamma.

1.6.6 Mast Cells

Circulating Ig-E binds to high-affinity Fce receptors (Fce-RI) on the surfaces of mast cells and basophils. It is unclear if mast cells circulate through regions of Ig-E production to pick up their Ig-E or if they passively adsorb the Ig-E from plasma or interstitial fluid. Mast cells exit in post capillary venules in the mucosa and reside in the submucosal regions. These mast cells are thought to express chymase, tryptase, and tumor necrosis factor (TNF-alpha) and are called connective tissue mast cells (MCTc) **(43)**. This cell population represents 85% of the IL-4-positive mast cells in the nasal lamina propria. Mast cells in the lamina propria also express IL-13, although it is unclear which subset or another is involved .During allergen assault, there is an increase in the proportion of epithelial mast cells. These cells produce predominantly layers of the lamina propria. Epithelial mast cells have a higher rate of cell division in allergic rhinitis compared with nasal mucosa not affected by allergy. Epithelial mast cell density is decreased by topical nasal application of glucocorticoids. Mast-cell degranulation is the critical initiating event of acute allergic symptoms**(44).**The consequences of mast cell degranulation are illustrated with an examination of results with nasal allergen provocation models . Application of allergen to the nasal mucosa of a person leads to a rapid, statistically significant increase in sneezing, nasal discharge, and resistance of nasal airways**(45).**Histamine, tryptase, prostaglandin (PG)D2, PGF2, and bradykinin are released rapidly during this immediate allergic response. Mast cell kininogenase releases bradykinin from plasma kininogens. Cytokines, including TNF-alpha , IL-4, IL-5, IL-6, transforming growth factor (TGF), and IL-13, also may be released. Mast cells can degranulation in response to many histamine-releasing factors; their roles in allergic inflammation **(46)**.Cross-linking

of IgE on the surface of the mast cell activates tyrosine kinases. The activation leads to activation of phospholipase A2, which releases arachidonic acid from the A2 position of membrane phospholipids. Arachidonic acid can be metabolized by cyclooxygenase, are under investigation**(47)**. which is present as a constitutive enzyme or an inducible form that can be upregulated in some forms of inflammation. In mast cells, PGD2 is the predominant product. Epithelial cells generate predominantly PGE2, endothelial cells PGI2, and platelets thromboxane A 2. The phospholipid backbone may become a substrate for the formation of platelet activating factor**(48)**. Myeloid series cells also possess 5-1ipoxygenase, which combines with 5-1ipoxygenase activating protein to generate leukotriene LTA4 .In neutrophils, LTA4 is metabolized to LTB4, a potent neutrophil chemoattractant. Other cells, including mast cells and eosinophils, preferentially generate the peptidyl leukotrienes LTC4, LTD4, and LTE 4 (slow-reacting substance of anaphylaxis)**(49).** These interact with LTD 4 receptors to induce glandular exocytosis and vascular permeability and may contribute to tissue eosinophilia and airway mucosal hyper responsiveness **(50**). The explosive degranulation of mast cells induced by allergen leads to release of a complex cascade of mediators, which may have synergistic effects on resident cells in tissues . Leukotrienes and chymase stimulate glandular exocytosis and mucous secretion. Histamine, bradykinin, leukotrienes, and platelet-activating factor activate the endothelial cells of post capillary venues to induce vasodilatation, vascular permeability, and cellular adhesion. Contraction of post capillary venue endothelial cells opens gaps, which allows hydrostatic intravascular pressure to force plasma into the interstitial space(**51**).

Histamine is an important mediator of mast-cell degranulation

it accounts for approximately half the symptoms of allergic rhinitis . Histamine is released in the immediate phase by mast cells and by basophils in the late-phase response. Histamine Hi-receptor mRNA is upregulated in allergic rhinitis. H 1 receptors are present on endothelial cells, where they induce vascular permeability and the watery rhinorrhea of allergic rhinitis (**52**).

1.6.7 Eosinophils

The influx of eosinophils produces profound changes in the airway mucosa. Eosinophils are activated by interactions of CD40 with CD40 ligand on other inflammatory cells, mediators such as platelet-activating factor (PAF), C5a, IL-16, and MCP-3, and allergen-antibody complexes involving IgA, IgG, and possibly Ig-E (low affinity Fce-RII)(**53**). The presence of IL-3, IL-5, and GM-CSF promotes eosinophil survival in tissue. Because these cytokines can be produced by eosinophils, autocrine positive feedback loops may lead to autonomous eosinophilic inflammation . Eosinophil granules release very basic, highly charged polypeptides that include major basic protein (MBP), ECP, eosinophil-derived neurotoxin, and eosinophil peroxidase(**54**).

These highly charged cations may bind to basement membrane proteoglycans and hyaluronic to cause cellular disaggregation and epithelial desquamation. These proteins can also act on cell membranes, which lead to cell death. Eosinophil-derived neurotoxin may inactivate mucosal nerves. Eosinophil peroxidase may play a role in the generation of free radical damage of cells(**55**). Eosinophils express 5-1ipoxygenase and are an important source of LTC 4. LTC 4 and ECP are potent glandular secretagogues. Eosinophils also appear to be an important source of cytokines. In addition to IL-3, IL-5, and GM-CSF, which

promote eosinophil survival, eosinophil chemoat tractant polypeptides, including eutaxon, IL-5, and RANTES, are released. These may act in an autocrine manner; IL-3, IL-4, IL-5, and IL-8 receptors have been identified on their surfaces(**56**). Eutaxon is also expressed by nasal polyp epithelial cells; its release might promote migration through the epithelium and into the airway lumen. IL-3, IL-4, and GM-CSF promote mast cell and Ig production. IL-8 may promote neutrophil and basophil chemo attraction. IL-10 may reduce production of Thl cytokines such as interferon(**57**). TNF-a and IL-le~ upregulate endothelial cell adhesion marker expression and may circulate systemically to promote proinflammatory responses in the hypothalamus and liver that generate low-grade fever, malaise, fatigue, and acute-phaseprotein responses. Transforming growth factor and platelet-derived growth factor can induce fibroblast collagen production that may contribute to thickening of collagen deposits beneath the epithelial basement membrane and fibrosis with "stiffening" of nasal polyp, nasal mucosa, and bronchial airways(**58**). Although eosinophils are an important component of the anti parasite function of Th2-IgE-mast cell mucosal immune response, their misguided toxic assault on innocent allergens contributes to airway morbidity. The percentage of lymphocyte subsets in the nasal mucosa of persons with allergic rhinitis has been compared with that of persons with chronic infectious rhinitis both during and out of allergen season. CD4 cells are increased(**59**). There is an increase in CD4+CD45 memory T cells, suggesting that these proliferate in the mucosa of patients with allergic rhinitis. The percentage of CD8-positive T-cytotoxic cells also appears to be increased. CD3+CD4-CD8 - cells with a predominant T-cell receptor type also are increased in allergic rhinitis. Numbers of B lymphocytes (CD20) are higher in allergic rhinitis than in chronic infectious rhinitis, whereas numbers of natural killer cells are lower.

Numbers of lymphocytes in normal nasal mucosa are too low to allow accurate comparison with a fluorescence-activated cell sorter for each of these lymphocyte subsets(**60**)

1.7 Complications of allergic rhinitis:

Unfortunately, allergic rhinitis itself can‟t be prevented. Treatment and management are keys to achieving a good quality of life with allergies. Some complications that can arise from hay fever include :inability to sleep from symptoms keeping you up at night, development or worsening of asthma symptoms, frequent ear infections, sinusitis or frequent sinus infections anxiety and insomnia absences from school or work because of reduced productivity and frequent headaches .Complications can also arise from antihistamine side effects. In rare cases, antihistamines can cause gastrointestinal, urinary, and circulatory effects(**61**).

1.8 Diagnosis

The first steps in the diagnosis are the clinical history and physical examination of the patient, recognize the type of rhinitis (allergic or non-allergic). interrogatory includes age of onset, duration, frequency, severity, timing during the year, suspected triggers, pattern of presentation, and progression of the symptoms. Also, the past therapeutic and effectiveness, the personal and family history of atopy conditions, especially asthma, AR coexists with asthma in 75 to 100% of the patients. Also asking about potential triggers at home and work like pollen, animals, tobacco smoke, humidity(**62**). the physical examination includes, especially in children, a growth assessment, since airways problems are associated with growth reduction, the inhaled corticosteroids reduce height at high doses. the presence of conjunctivitis, allergic nasal crease, allergic salute or double creases beneath the eyes or

Dennie-Morgan lines, allergic pimples, allergic salute, rubbing of the nose can guide the physician about a case of AR(**63**).Patients with moderate to severe AR and with uncontrolled symptoms needs a nasal examination, with rhinoscopy where the typical appearance in nasal cavity is swollen pale bluish inferior turbinate edema with copious clear secretions it is also performed an ear inspection, the otitis media is a comorbidity in children with AR. When the clinical history and physical examination suggest AR, the confirmatory diagnosis is by skin prick test or blood tests to identify specific antigens and vitro of specific Ig-E antibodies(**64**).

1.9 Immunoglobulin E (IgE):

Immunoglobulin E (Ig-E) is a type of antibody "isotype" that has been found only in mammals. Ig-E is synthesised by plasma cells. Monomers of Ig-E consist of two heavy chains (ε chain) and two light chains .

Immunoglobulins are a group of plasma proteins that function as antibodies, recognizing and binding foreign antigens. This facilitates the destruction of these antigens by elements of the cellular immune system.

Since every immunoglobulin molecule is specific for one antigenic determinant, or epitope, there are vast numbers of different immunoglobulins .All share a similar basic structure **(65).** This is described in figure 1-3:

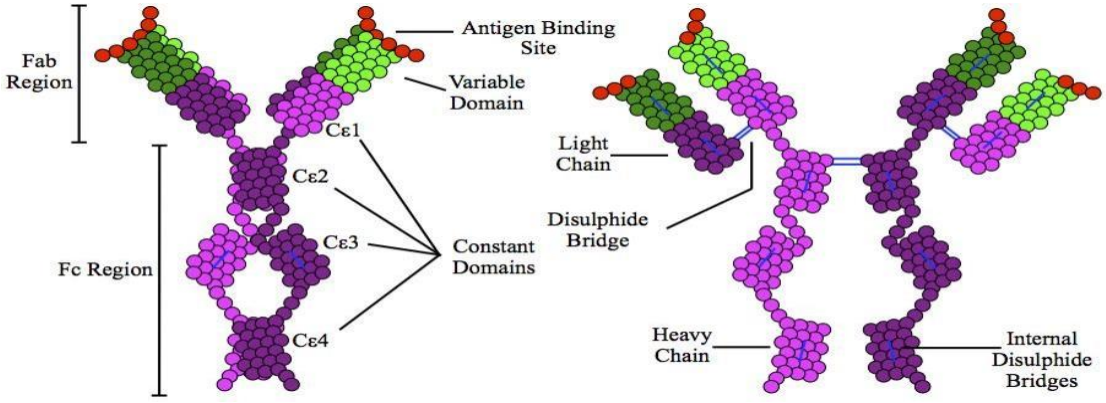

Figure1-3:immunoglobulin structure **(66).**

The five primary classes of immunoglobulins are IgG, IgM, IgA, IgD, and IgE. These are distinguished by the type of heavy chain found in the molecule. IgG molecules have heavy chains known as gamma-chains; IgMs have mu-chains; IgAs have alpha-chains; IgEs have epsilon-chains; and IgDs have delta-chains. The N-terminal amino acid sequences of both the heavy and light chains show considerable variation between individual immunoglobulin molecules; those form the part of the immunoglobulin molecule responsible for recognition of the antigen (the antigen binding site**)(67).**

The amino acid sequence of the rest of the chains varies little within one immunoglobulin class; this constant part of the molecule is concerned with complement activation and interaction with the cellular elements of the immune system increases and decreases of plasma immunoglobulin concentrations can be either physiological or pathological in origin **(68).**

The essential features of IgE function involve interactions between members of the protein network and the role of the different ells outlined as following **.**

Immunoglobulin E (IgE) plays a critical role in the allergic inflammatory process in diseases such as allergic rhinitis. Cross-linking IgE bound to its receptor on cells by multivalent allergens initiates a chain of events resulting in allergic immune responses. Mast cells and basophils are involved in the early, immediate response, which is marked by cellular degranulation and the release of proinflammatory mediators, including histamine(**69**). Antigen-presenting cells are also activated by allergen-loaded Ig-E, resulting in immunomodulation of T-cell responses.

In allergy and asthma, the populations of B cells and plasma cells in the respiratory tract mucosa are heavily biased towards the production of Ig-E **(70).**Approximately (4%) of the B cells and (12–19%)of the plasma cells express IgE in the nasal mucosa of patients with rhinitis, compared

with 1% and <1% respectively in healthy subjects**.** The concentration of Ig-E in the serum of healthy individuals is 104 times less than that of IgG .IgE-expressing plasma cells in the bone marrow are comparably sparse, suggesting that, in individuals with rhinitis, the nasal mucosa is the major source of IgE .Local production of Ig-E may account for the generally higher allergen-specific fraction up to a half in the tissue than in the blood ,where allergen-specific IgE may stem wholly or partly from other sources **(71).** The diagnosis of AR is based on the demonstration of the production of allergen specific IgE and on the concordance between allergy testing and history, such as the symptom occurrence after inhalation of the sensitizing allergen. The blood usually has small amounts of IgE antibodies. Higher amounts can be a sign that the body overreacts to allergens, which can lead to an allergic reaction (**72**).

The activity of the antibodies presumably depends on the avidity with which the antibodies bind allergens and the probability that the antibodies will be found in high density on effecter cells.Areliable assay for measuring specific IgE (sIgE) can help clinicians diagnose and manage allergy-like symptoms appropriately**,** detecting serum IgE is thought to be an indicator of the degree of Ig-E-mediated sensitivity to a specific allergen**(73).**

Both IgE and mast cells are concentrated in the mucosal tissues, and so IgE antibodies are among the first defense molecules that an invading pathogen will encounter. The hallmark of an allergic response, which is mediated by the Ig-E-FceRI complex on mast cells, is immediate hypersensitivity(**74).**This reveals itself in characteristic signs and symptoms in the different target organs of allergy, the skin (atopic dermatitis or eczema), the nose (rhinitis), the lungs (asthma) and the gut (food allergic reactions**).** Cross linking of IgE– FceRI complexes on the mast-cell surface by allergens leads, within minutes, to the so-called

„early phase" of the allergic reaction, which involves mast-cell degranulation and the synthesis of lipid mediators (**75**). Cytokines and chemokines liberated in this early phase initiate the „late phase", which peaks some hours later and involves the recruitment and activation of inflammatory cells at sites sensitive to allergen**.** Similarly, but without overt symptoms, allergens activate the IgE-sensitized APCs, which in turn promote IgE production by B cells to replenish the Ig-E consumed in the allergic reaction, thereby maintaining mast-cell and APC sensitization (**76**).The processes of mast-cell and APC recruitment and IE production in the mucosal tissues are central to the functions of Ig-E, and no less so in guarding against systemic anaphylaxis. Mast-cell precursors are generated in the bone marrow and migrate to mucosal tissue before expressing FceRI **.** The receptor is highly expressed (~500,000 copies per cell) on tissue mast cells, probably as a result of IgE mediated upregulation of FceRI expression **(77).**The concentrations of IgE required for the upregulation of FceRI in the mucosal tissues, which are higher than those normally present in the circulation, may be derived from IgE synthesis by local B cells. The rate of this process is more than sufficient to maintain saturation of the FceRI molecules on the mast cells, and any excess IgE is preferentially directed into secretions, rather than entering the circulation **(78).**

1.10 Tumor Necrosis Factors (TNF):

Cytokines are a broad and loose category of small proteins (~5–25 kDa) important in cell signaling. Cytokines are peptides and cannot cross the lipid bilayer of cells to enter the cytoplasm. Cytokines have been shown to be involved in autocrine, paracrine and endocrine signaling as immunomodulating agents **(79).**

Cytokines include chemokines, interferons, interleukins, lymphokines, and tumor necrosis factors, but generally not hormones or growth factors . Cytokines are produced by a broad range of cells, including immune cells like macrophages, B lymphocytes, T lymphocytes and mast cells, as well as endothelial cells, fibroblasts, and various stromal cells; a given cytokine may be produced by more than one type of cell **(80).** They act through cell surface receptors and are especially important in the immune system; cytokines modulate the balance between humoral and cell-based immune responses, and they regulate the maturation, growth, and responsiveness of particular cell populations. Some cytokines enhance or inhibit the action of other cytokines in complex ways. They are different from hormones, which are also important cell signaling molecules **(81).**

TNF is lymphokines that exist in either an α or β form; they are capable of causing in vivo hemorrhagic necrosis of certain tumor cells ,but not affecting normall cells .They also have been used as experimental anticancer agents but can also induce shock when bacterial endotoxins cause their release .TNF-α contains 157 amino acids .TNF-β is a lymphotoxin and contains 171 amino acids **(82).**

TNF-α was formerly known as cytotoxic ,cytotoxic factor , differentiation–inducing factor, hemorrhagic factor ,macrophage cytotoxic factor, macrophage cytotoxic ,and necrosis .TNF-β was formerly known as cytotoxin, differentiation-inducing factor, and lymphotoxin (LT) (**83).**

TNF-α is synthesized by an enzyme called ADAM-17 a disintegrin and metalloprotease domain 17 ; also referred to as TNF-α converting enzyme and TNF-α converting activity .It is non glycosylated protein with molecular weight 25KDa .The amino terminus is intracellular, but it's carboxy terminus is extracellular .This enzyme catalyzes the cleavage of pro-TNF-α (a 26 KDa protein) to TNF-α which is the active form with M.wt 17KDa **(84,85).**

TNF-α is produced primarily by activated monocytes or macrophages ,activated T cells ,B cells, NK cells ,mast cells, endothelial cells ,fibroblasts, astrocytes ,Kupffer cells, and smooth muscle cells. The primary sources of TNF-α are CD14 monocytes or macrophages. Cellular sources for TNF-β are few, mostly activated T and B cells, NK cells and astrocytes **(86)** .

The up regulation of TNF-α production is stimulated by Nitric oxida, reactive oxygen intermediates, nonsteroidal anti-inflammatory drugs and hypoxia. The increased production of TNF-α by hypoxia may be due to decreased PGE2 .Also TNF-α is inhibited by IL-10, PGE2,dexamethasone**(87)** .

There are two different TNF receptors of 55 and 75 KDa, each of which is encoded by a separate gene and is bound both TNF-α and TNF-β**.** The type 1(TNFR-1,type B) receptor is expressed on many different types of cells, and type Π (TNFR-2,type A)receptor is expressed predominantly on hematopoietic cells. Each of these receptors has a large cytoplasmic domain and can transmit signals through it **(88)**.

The inflammatory response is a highly regulated process, initiated by tissue damage, infiltrating pathogens or both. They are tightly regulated through soluble mediators cytokines and monokines that orchestrate inflammation. Pro-inflammatory cytokines such as TNF-α, IFN-γ, and IL-1 enhance antimicrobial functions of immune cells and facilitate the

pathogen clearance TNF-α is a central inflammatory mediator, initially identifed as a serum component capable of eliciting "hemorrhagic of necrosis" certain tumors (89).Since discovery, TNF-α has been found to be produced by many cell-types and confer an increadible range of immune processess . In the context of inflammatory responses, TNF-α promotes the chemotaxis of neutrophils and monocytes/macrophages.

TNF-α is considered as a growth factor for fibroblasts and a major stimulator of acute phase response .TNF-α together with IL-1 influence the behavior of many types of cells (90).

Allergic rhinitis represent an inflammatory reaction that is characterized by the chemotaxis and activation of various cell populations. A high degree of cell-to-cell communication is needed to orchestrate this inflammatory immune response. variety of cytokines and adhesion receptors seem to play an important role in the allergic late reaction (91) . Some cytokines ,such as TNF-α and IFN are thought to be essential in orchestrating airway inflammation in both upper and lower airways and seem to be implicated in the development of the late allergic reaction (92).

Genetic factors play an equally important role in the risk factors related to the incidence of AR as same as and environmental factors. The emergence of gene polymorphism changes the coding sequence or changes the process of transcription and translation, which are involved in the occurrence and development of the disease by regulating the character, activity and dose of protein expressed by gene. Therefore it is one of the important ways for researchers to discover the genetic mechanism of human complex diseases and to prevent and treat complex diseases by searching for the gene loci closely related to human complex diseases **(93).** Numerous single nucleotide genetic polymorphisms have been foundto be associated with allergic rhinitis.. It has been suggested that TNF-α may be involved in the up regulation of expression of

endothelial cell adhesion molecules and activation of various cell populations in human nasal mucosa during allergic inflammation **(94).**

1.11 Electrolytes

Electrolytes perform several functions which are absolutely essential for the very existence of the organism. Principal elements and trace elements are the different categories for the mineral The majority (60–80%) of the inorganic material in the body is made up of the seven major elements (macro minerals). Calcium, phosphorus, magnesium, sodium, potassium, chloride, and sulfur are some of them over 100 mg per day is needed for the essential components. electrolytes . like sodium, potassium, magnesium, and calcium have both direct and indirect roles in regulating your immune system. So whether you‟re sick, healthy, or in-between you want to be getting enough electrolytes. In fact, preventing electrolyte deficiencies is essential for good immune health (**95).** Sodium is the chief cation of the extracellular fluid , about 50% of body sodium is present in the bones, 40% in the extracellular fluid and the remaining (10%) in the soft Biochemical functions: In association with chloride and bicarbonate, sodium regulates the body‟s acid base balance and Sodium is required for the maintenance of osmotic pressure and fluid balance. It is necessary for the normal muscle irritability and cell permeability (**96**) . Sodium is involved in the intestinal absorption of glucose, galactose and amino acids . It is necessary for initiating and maintaining heart beat tissues also Sodium regulates a myriad of functions in the human body, including: conducting nerve impulses and protecting against certain infections decreased levels of sodium may be cause by excessive use of diuretic prolonged vomiting a decrease in the

intake of sodium in the diet and metabolic acidosis. Increased levels ofsodium may be found in severe dehydration, or in high levels of saltintake without an adequate supply of water(**97**).

Chloride is the major extracellular anion. The majority of ingested chloride is absorbed, and the excess is excreted along with other ions into the urine. Low levels of chloride are observed in the case of prolonged vomiting accompanied by the loss of hydrochloric acid (HCl), in some cases of metabolic alkalosis in which there is an increased accumulation of organic anions, , and in kidney disease resulting in loss of salt**(98**). Elevated levels of chloride are observed in metabolic acidosis associated with prolonged diarrhea and with loss of sodium bicarbonate ($NaHCO_3$), and in the case of renal tubular diseases in which there is a decreased excretion of hydrogen ion (H^+), which causes in turn a decrease in the reabsorption of bicarbonate ion (HCO_3^-). Elevated levels of serum chloride are also implicated in certain cases of hyperparathyroidism(**99**).

Potassium is the principal intracellular cation . It is equally important in the extracellular fluid for specific functions. The concentration of potassium in the erythrocytes is approximately 23 times concentration in plasma. For this reason ,must be used only unhemolyzed samples must be used (**100**) . Decreased levels of extracellular potassium characterized by weakness in the muscles, irritability, paralysis accelerated heartbeat, and eventually cardiac arrest, and may be caused by a poor intake of potassium in the diet, by a redistribution of extracellular potassium, and by an increased loss of body fluids rich in potassium. Abnormally elevated levels of extracellular potassium produce mental confusion, general weakness, numbness flaccid paralysis in the extremities, a slowed heart rate, and eventually collapse of the peripheral vascular system and cardiac arrest. Causes of increased potassium levels may be linked to

inappropriate intravenous therapy, dehydration, shock, diabetic ketoacidosis, and severe burns (**101**) .Biochemical functions Potassium maintains intracellular osmotic pressure ,It is required for the regulation of acid base balance and water balance in the cells ,The enzyme pyruvate kinase (of glycolysis) is dependent on K^+ for optimal activity ,Potassium is required for the transmission of nerve impulse , Adequate intracellular concentration K^+ is necessary for proper biosynthesis of proteins by ribosomes ,Extracellular K^+ influences cardiac muscle activity **(102).**Sodium and potassium (K^+) provide more than 90 per cent of plasma cation concentration in the healthy subject; other includes low concentrations of calcium (Ca^{2+})The anion gap represented as A– in the following equations, is the difference between the total concentration of measured cations (Na^+ and K^+) and measured anions (Cl^- and HCO_3^-);it is normally about(15–20) mEq/L. Therefore (**103**):

$[Na^+]+ [K^+] =[HCO^-3] + [Cl^-]+ [A^-]$ 140 + 4 = 25 + 100+ 19mEq/L

$[A^-] = [Na^+] + [K^+] - [HCO^-_3] + [Cl^-]$

Potassium ions are not only the most abundant cation in the body but are also capable of regulating T cell function. Under normal physiological conditions, sodium and potassium ions have opposite actions. Increased sodium chloride concentrations enhance the induction of TH17 cells by activating the p38/MAPK pathway and serum/ glucocorticoid- regulated kinase 1 (SGK1)(**104**) . These TH17 cells secrete large amounts of proinflammatory cytokines GM-CSF, TNF-α and IL-2 that coincided with severe forms of autoimmune disease .whereas elevated intracellular K^+ concentration suppresses T cell function by inhibiting Akt and mTOR protein kinases . Hence, maintaining the balance between Na^+ and K^+ across the cell membrane is critical not only for T cell function but also for other cells such as endothelial cells to prevent inappropriate

production of pro-inflammatory cytokines (endothelial cells also produce cytokines)(**105**).

Calcium is the most abundant among the minerals in the body biochemical functions development of bones and teeth, muscle contraction, blood coagulation, nerve transmission, membrane integrity and permeability, activation of enzyme, second messenger for such hormonal action on heart Ca^{2+} acts on myocardium and prolongs systole**(106).**

Hypercalcemia can result from hyperparathyroidism, hypervitaminosis D, multiple myeloma, and some neoplastic diseases of bone. Long- term lithium therapy has been reported to cause hyperparathyroidism in some individuals, with resulting hypercalcemia. Hypocalcemia can result from hypoparathyroidism, hypoalbuminemia, renal insufficiency, and pancreatitis(**107**). Calcium is of central importance in allergic reactions. The transmembrane influx of calcium is an important step in the activation and degranulation of basophils and mast cells after cross-linking of Ig-E molecules on the cell membrane. In a number of in vitro studies on human basophils and rat mast cells it was possible to reduce the release of histamine by means of calcium channel blocking drugs(**108**). studies suggest that altered calcium mobilization in respiratory tract muscle is directly implicated in the induction of respiratory tract hyperactivity, possibly caused by toxic oxygen products. Finally, capillary permeability ("vascular leakage") is a function of the partially calcium-dependent adhesion and contraction of endothelial cells(**109**).

It may be speculated that calcium activity in allergic rhinitis most probably relies on the reduction in the permeability of blood vessel walls an effect resulting from the inhibition of histamine release from mast cells. studies can be found , the efficacy of oral or intravenous calcium

preparations in allergic diseases such as allergic rhinitis, urticaria, and allergic bronchial asthma(**110**).

1.12 Single Nucleotide Polymorphism:

Single nucleotide polymorphisms or SNPs is a substitution of a single nucleotide at a specific position in the genome, that is present in a sufficiently large fraction of the population **(111)**. DNA sequence variations that occur when a single nucleotide (A,T,C, or G) in the genome sequence is altered. For example a SNP might change the DNA sequence AAGGCTAA to ATGGCTAA. For a variation to be considered a SNP, it must occur in at least 1% of the population. SNPs, which make up about 90% of all human genetic variation, occur every 100 to 300 bases along the 3-billion-base human genome. About 12 million of SNPs have been known **(112).** Mutations defined as a sequence variations that occur in less than 1% of the population while the more widespread variation are known as polymorphisms. The main common genetic variations are single nucleotide polymorphisms (SNPs) **(113).**

The common form of SNPs are transition (pyramidine -pyramidine C↔T or purine-purine A↔G) or transversion (pyramidine-purine or purine-pyramidine) replacements. About 2/3 of SNPs are transition replacements, while 1/3 of SNPs are transversion replacements(**114).**

The SNPs classification is based on their genomic site. The coding SNPs (cSNP) are situated in translated sequences (exons) of DNA and non-coding SNPs are located in untranslated sequences (introns) of DNA. SNPs may be either synonymous or non-synonymous**(115) .**

The synonymous cSNPs are classically silent and change the sequence of DNA, but do not change the sequence of amino acid in protein while the non-synonymous cSNPs change the sequence of DNA in a coding region and it change the sequence of amino acid in protein. Because of a changes in the amino acid function and structure, these cSNPs are considered as genetic markers for several diseases **(116).**

The bulk of SNPs are situated in the non-coding region of genome. On the other hand, some of these intronic SNPs have no recognized role but may participate in a regulation of the gene expression of coding regions, these SNPs are known as regulatory SNPs (rSNPs). Regulatory SNPs situated in the promoter area may influence on the sites of transcription factor **.**In general the non-synonymous SNPs in a coding sequence are more likely to affect the function or availability of a protein than other SNP classes **(117).**

1.13 TNF-α Gene:

The gene encoding TNF-α located in chromosome 6, at the short arm as shown in Figure 1- 4 **(118).**

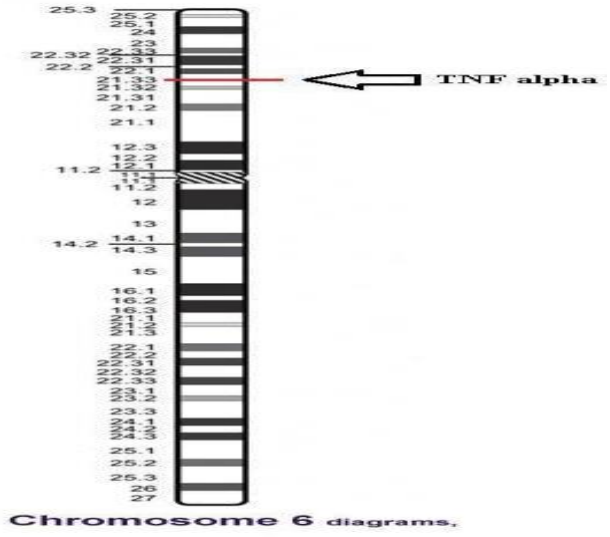

Figure 1-4 : Location of TNF-α gene in chromosome 6.

There are 2772 single nucleotide variants for TNF-α Gene . In spite of the presence of many variants for this gene, but there are few studies deal with them. SNP were suggested to be engaged in changes of the effectiveness of function of TNF-α gene rs769178 G>T, as shown in figure 1-5(**119**).

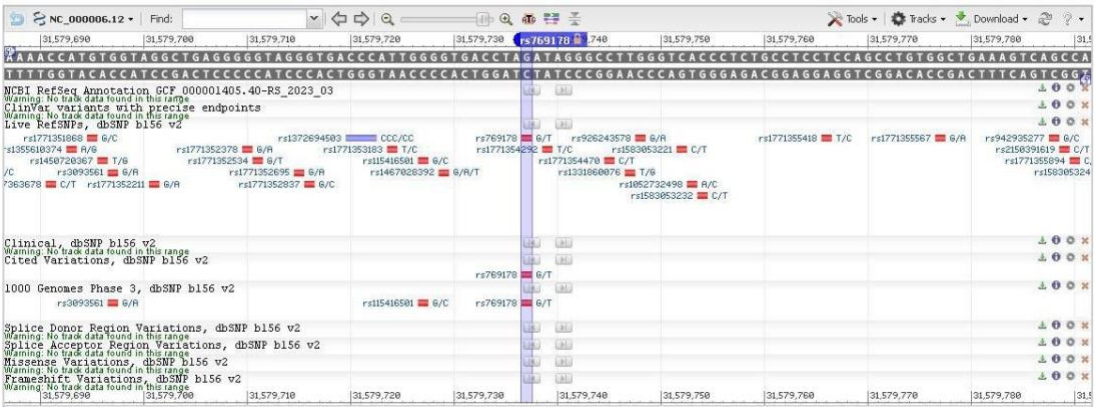

Figure 1-5:Positions of the SNP (rs769178 G>T) in the TNF-α **gene of Homo sapiens chromosome 6.**

1.14 Aims of study:

1- Investigate TNF alpha ,Ig-E, Eosinophil and electrolytes (Na^+ ,K^+ ,Cl^- , Ca^{+2}) inpatients with AR in Babylon province.

2- To find the possibility of correlation between TNF –alpha ,Ig-E and electrolyte parameters with age ,gender ,smoker and BMI in AR patients.

3-To evaluate the role of TNF–alpha gene polymorphism by T-ARMS PCR and its associated with the risk of AR.

2.1 Materials and methods:

2.1.1 Chemicals and Kit: Chemicals and Kits that were used in the present study listed in table below:

Table 2-1: Chemical substances used in the study

NO	Chemical substance	Origin
1.	Agarose	Bio Basic (China)
2.	Calcium kit	USA) Medical, Italy
3.	DNA extraction kit (blood)	Addbio/Korea
4.	DNA ladder 50 bp	Bioneer (Korea)
5.	Electrolyte kit(Na^+ ,K^+ ,$Cl^{-)}$	USA) Medical, Italy
6.	Ethanol 70%	BDH, UK
7.	Gel loading dye	Biolabs (New England)
8.	Ig-E Kit	Inter Medical, Italy
9.	PCR Master Premix kit	Promega(USA)
10.	Primers	Macrogen
11.	TBE buffer	Intron (korea)
12.	TNF-α Kit	Kama Biotech, Korea

2.1.2 Instruments and tools:

Instruments and tools were used in this study showed in table 2-2.

Table 2-2: Instruments and equipment's were used in this study

NO	Instruments and Tools	Origin
1.	Absorbance ELISA microplate reader	Biotech (USA
2.	Balance	Sartorius, Germany
3.	Centrifuge	Taiwan
4.	Disposable pipette tips	Kartell (dispolab)
5.	Disposable syringes (5 mL)	China

6.	EDTA-K3 tube (5ml)	China
7.	ELISA microplate washer	Bio Tek (Germany)
8.	Eppendorf tube (1.5ml)	China
9.	Freezer	Liebhe, Austria
10.	Gel tube	China
11.	hematology analyzer	Sysmex Japan
12.	Incubator	Fisher Scientific, German
13.	Medical cotton	China
14.	Micropipettes	Slamed (Germany)
15.	PCR Thermo cycler	Bioneer(Korea)
16.	Vortex (Electronic)	Kunkel (Germany)
17.	Water bath	GFL (Germany)

2.1.1 In the study design:

In this investigation Patients and healthy control from the Imam Sadiq Hospital, Hilla Teaching Hospital and Allergy center in the province of Babylon All samples were taken between November, 2022, and March, 2023.

The practical side of the study was performed at the laboratory of chemistry and Biochemistry Department/ College of Medicine University of Babylon.

2.1.2 Study design:

This is case control study

2.1.3 Study population:

There were 100 participants in this study, ranging in age from 29 to 45. The subjects were split into two groups, the first of which included 50 patients with allergic rhinitis and the second of which included 50 individuals who appeared to be in good health. The body mass index had been used by everyone to determine the prevalence of obesity (BMI).

2.1.4 Data collections:

Following are the criteria for inclusion and exclusion:

a-Inclusion criteria:

Participants in this study were separated into two groups: the patient group (Rhinitis women and men) and control group (apparently healthy women and men). with age between ranged in age from (29 to 45) and normal weigh ,over weigh, smoker and nonsmoker .

b- Criteria for exclusion :

Individual with Asthma , Patients with rheumatoid arthritis, any other autoimmune diseases.

2.1.5 Ethical approval and concent

The following depends on:

a) The Scientific Committee's acceptance by the Babylon Medical College(Babylon University, Iraq) and the Department of Biochemistry at the same institution.

b) Scientific Committee approval of Hilla Teaching Hospital and Imam Sadiq Hospital in Babylon province , Hilla city.

c) All participants in the present study were clarified in order to obtain verbal recognition by the goals and methods of that study.

2.1.6 Study instruments

The characteristics socio- demographic that composed of age ,gender, height (m), weight(kg) , marital status, medical history , family history ,over weight, smoking,. body Mass Index (BMI) had calculated by weight (in kilograms) divided by the square of height (in meters); weight and height are measured by the same scale for the all sample subjects.

BMI = weight in Kg /(height in m)^2.

2.1.7 Blood collection :

Five mililiters of venous blood was obtained from healthy control subjects and patients by 5ml disposable syringe, and slowly pushed into two tube 2 mL blood in EDTA tube for genetic study and 3 mL blood in gel tube) . Gel tube of blood was centrifuged for 10-15 minut at 1500 xg.,then the serum had divided into three parts and stored at -20˚C which would be used in biochemical test. for this 2 ml of the blood was drained into EDTA tube and mixed gently , the blood in EDTA (ethylene diamine tetra acetic acid) tube stored at -20 ˚C and used for DNA extractio

2.2 Methods (Biochemical Part) :

2.2.1 Determination of Serum TNFα

2.2.1.1 Principle:

The TNF-α Elisa Kit is a solid phase enzyme –linked immune sorbent assay (ELISA), based on the sandwich principle .The micro titer wells are coated with a monoclonal antibody directed towards a unique antigenic site on TNF-α molecule.

As aliquot of sample, containing endogenous TNF-α is incubated in the coated well with a specific biotinylated monoclonal anti-TNF-α antibody .A sandwich complex is formed. After incubation, the unbound material is washed off and strepto-avidin peroxidase enzyme complex is added for the detection of the bound TNF-α . Having added the substrate solution, the intensity of color developed is proportional to the conce ntration of TNF-α in the sample (**120**) .

2.2.1.2 Reagent:

1- 96 wells ; Microtiter wells , 12x8 strips , wells coated with anti-human TNF-alpha .

2- Human TNF-alpha Standard : (Lyophilized) 55 ng (2 vial) of recombinant Human TNF-alpha was reconstituted in 55 µ l of sterile water for a concentration of 1 µL /ml.

3- Detection Antibody : (Lyophilized) 2.75 ng (2 vial) of biotinylated antigen-affinity purified anti-Human TNF-alpha should be reconstituted in 275 µL sterile water for a concentration of 10 µL g/ml.

4- Color Development Enzyme Streptavidin – HRP (horseradish peroxidase) conjugate (600 ul).

5- Assay Diluent: 0.1% (Bovien serum albumin) BSA in (phosphate buffer solution) PBS (50 ml)

6-Color development Reagent A: (tetra methyl benzidin) TMB solution (10 ml).

7-Color development Reagent B: Substrate (H_2O_2) Solution (20 ml).

8-Stop Solution 2M H_2SO_4 (10 ml).

9-PBS powder Pouch for 1 L.

10-Tween-20 (50%) 1 ml.

11-Plate Sealer.

2.2.1.3 Procedure:

1- Avolume of 100 µl of standard and serum were added to each well.

2-The wells were incubated for 2 hours at room temperature (with covering the plate).

3-Then wells were aspirated to remove the liquid and the plates were washed 4 times by 300 µl of washing solution for each well.

4-Avolume of 100 µl of the diluted detection antibody (0.5 µg/ml) was added to each well.

5-The wells were incubated for 2 hours at room temperature (with covering the plate).

6-The wells were aspirated and washed 4 times by adding 300 µl of washing solution for each well.

7-Avolume of 100 µl of diluted color development enzyme (1:20 dilute) per well was added.

8-The plate was incubated 30 minutes at room temperature (with covering the plate).

9-The wells were aspirated and washed 4times as in previous step.

10-Avolume of 100 µl of color development solution-B was added to each wells.

11-The well were incubated at room temperature (4-14 minutes)

12-Then avolume of 100 µl of stop solution was added for each well.

13-The absorbance of each well was read at 450 nm after adding the stop solution.

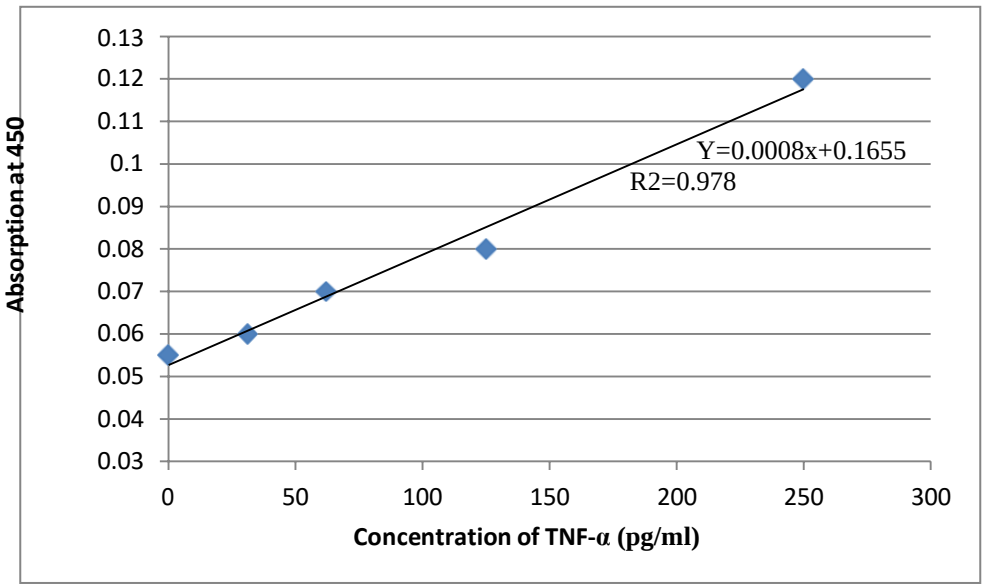

Figure 2-1 :The standard curve of TNF-α (R^2=0.978) .

2.2.2 Determination of Serum Ig-E:

2.2.2.1 Principle:

The (Ig-E total) is a one –step immunoassay ,based on principle of (sandwich) method. The assay utilize two high affinity and specificity monoclonal antibodies (enzyme conjugated and immobilized)that can bind to two different epitopes on the intact IgE molecule. The sample is allowed to react simultaneously with these two antibodies ,resulting in the Ig-E molecules being sandwiched between the solid phase and enzyme –linked antibodies .After incubation ,The wells were washed with washing-solution to remove unbound labeled antibodies .A solution of TMB- substrate is added and incubated ,resulting in the development of a blue color .The color development is stopped with the addition of Stopping Reagent ,changing the color to yellow. The concentration of IgE is directly proportional to the color intensity of the test sample. The bsorbance is measured spectrophotometrically at 450 nm(**121**).

2.2.2.2 Reagent:

1. Microtiter wells, 12x8 strips, 96 wells; wells coated with monoclonal anti Ig-E antibody.

2-Standard (standard 0-6) ,2ml; 6 vails concentrations (0,62.5,125,250,500,1000 IU/ml .contain 0.05% proclin 300,0.004% Gentomycin sulfate 0.1% phenol as a preservative.

3-Control (1vial) ,0.5 ml, contain 0.05% proclin 300 ,0.004% gentomycin sulfate, 0.1% phenol as preservative.

4-Conjugate solution ,(1 vial), 15.0 ml ,monoclonal anti-IgE antibodies conjugated to horse raddish peroxidase .contain 0.05% proclin 300, 0.004% gentamycin sulfate ,0.1% phenol as apreseiverva.

5-Washing solution ,1vail,50 ml.

6-Substrate solution ,(1 vial), 14 ml ,is ready to use ,tetramethyl benzidine in citric acid buffer.

7-Stop solution ,1 vial, 25ml ,is ready to use, contains of 0.2M sulphuric acid .

2.2.2.3 Procedure:

1-Avolume of 20 µl of each calibrators, control serum and samples was added into appropriate wells.

2-Avolume of 150 µl of conjugate was added ineto each well.

3-The plate was incubated for 45 minutes at (37.0±0.5) c^o (with covering the plate).

4-The wells were washed 5 times with 300 µl of diluents.

5-Avolume of 100 µl TMB –substrat added into each well.

6-The plate was incubated for 20 minutes at room temperature in dark place .

7-Avolume of 150 µl of stopping reagent added into each well.

8-The plate was read at 450 nm within 20 minutes after addition of stopping reagent.

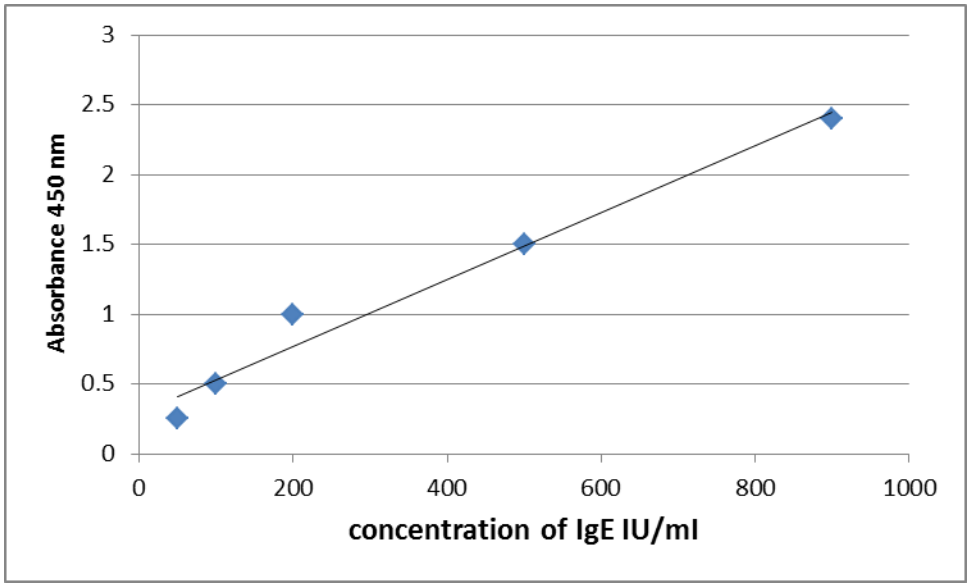

Figure 2-2: The standard curve of Ig-E(R^2=0.956)

2.2.3 Determination of Serum electrolytes: (Na^+ ,K^+ ,Cl^-)

An Abbott device was used to measure electrolyte concentrations using ICT (Integrated Chip Technology) is used for the quantitation of sodium, potassium, and chloride in human serum,

2.2.3.1 principles of the procedure:

Ion-selective electrodes for sodium, potassium, and chloride utilize membranes selective to each of these ions. An electrical potential (voltage) is developed across the membranes between the reference and measuring electrodes in accordance with the Nernst equation. The voltage is compared to previously determined calibrator voltages and converted into ion concentration. Methodology: Ionselective electrode dilute (Indirect)(122)

2.2.3.2 Preparation for Analysis:

Serum complete clotting was confirmed before centrifugation. Centrifuge according to tube manufacturer‟s specifications to ensure proper separation of serum from blood cells. The sample volume for the Integrated Chip Technology (ICT) assays 15 µL. This volume is mixed with 69 µL of ICT Sample Diluent and 276 µL of system water.

Reagent kit contents ICT (Na+, K+, Cl-) Sample Diluent 2P32. Supplied as a liquid, ready-to-use single reagent kit.

Table2-3 The normal range for electrolytes (**123**).

Serum/ Plasma	Range (mmol/L)
Sodium	136 to 145
potassium	3.5 to 5.1
Chloride	98 to 107

Reagents (Kit contents):

Table 2-4 kit(Na^+, K^+,Cl^-) contents:

Supplied as a liquid, ready-to-use single reagent kit.

REF	2P32-11	2P32-50
Σ	12,000*	7,000*
R1	10 x 93 mL	10 x 54 mL

2.2.4 Determination of Serum calcium:

Principles of the procedure Arsenazo-III dye reacts with calcium in an acid solution to form a blue-purple complex. The color developed is measured at 660 nm and is proportional to the calcium concentration in the sample. Methodology: Arsenazo III

REAGENTS Kit Contents Calcium 3L79. Supplied as a liquid, ready to use single-reagent kit(**124**)

Reagents :

Kit contents:

Table 2-5 :Kit Calcium contents:

Supplied as a liquid, ready-to use single-reagent kit.

REF	3L79-21	3L79-31	3L79-41
Σ	1,500*	11,440*	24,370*
R1	5 x 13 mL	10 x 41 mL	10 x 84 mL

*Calculation is based on the minimum reagent fill volume per kit.

Reactive Ingredients	Concentration
R1	
Arsenazo-III dye	0.94 mmol/L
Sodium acetate	271 mmol/L

2.2.4.1 Specimen Dilution Procedures:

Serum and plasma specimens with calcium values exceeding the 24.0 mg/dL (6.00 mmol/L) are labeled and may be diluted by following the Manual Dilution Procedure, or the Automatic Dilution Protocol . Manual Dilution Factor = (Volume of Specimen + Volume of Dilution Reagent) / Volume of Specimen.

After the calibration was done, and after marking the samples, they were entered into a special rack of the device, been chosed the type of analysis required on the device screen, and been gave the order to measure, where the samples were drawn, and after a few minutes, the result appeared directly, the concentrations of electrolytes in the samples, and they were recorded.

2.2.5 Determination of Eosinophils :

All subjects had their antecubital fossas venepunctured, and 2 ml of blood was taken in EDTA anticoagulant under stringent aseptic guidelines. Eosinophil levels were determined by analyzing the blood sample. count by hematology analyzer, Sysmex Japan eosinophil as a percent **(125,126)**

2.3 Methods (Genetic part) :

2.3.1 DNA Extraction:

DNA extraction from whole Blood by (AddPrep Genmic DNA Extraction Kit)(**127**) .

2.3.1.1 Principle (Silica Based Technology):

Silica matrices have special properties for DNA binding. They have positive charge and consequently they have large affinity for the negative charge of the DNA.high salt conditions and pH are performed to lyse cells and degrade protein, allowing DNA to bind to the glass fiber matrix of the spin column, by using sodium cations that bind strongly to the negatively charged oxygen of the DNA phosphate.

Contaminants were removed with multiple washing steps by using a wash solution (containing ethanol) and the purified genomic DNA was eluted by low ionic strength (pH ≥7) through TBE buffer. These methods was faster and simpler procedure than other methods like organic extraction method .

2.3.1.2 DNA Extraction Procedure:

The protocol of DNA extraction from blood involved several steps:

1-Two hundred µl of blood was curry over to a 1.5 ml micro centrifuge tube, then 200 µl of lysis was added.

2-Twenty µl of proteinase K (20mg/ml) was added and briefly mixed. Then it was incubated for 15 minutes at 56℃.

3-Two hundred µl of binding solution was added to the samples then mixed by shaking vigorously.

4-The sample was incubated in a 56℃ water bath for 10minutes, during incubation the sample was inverted every 3 minutes.

5-Two hundred µl of absolute ethanol (DNA binding) was added to the test and vortex for 15 second.

6-The lysate was transfer into the upper reservoir of the spin with 2ml collection tube.

7- The sample were centrifuged for 1 minutes at full speed (13,000 rpm).

8-The 2ml collection tubes containing the flow-through were discared, after that the spin column was placed in a new 2ml collection tube.

9-The spin column was washed with 500 µl washing 1 solution (wash) and centrifuged for 1 minutes at (13,000 rpm) then the flow-through was discarded.

10-The spin column was putted back in the 2ml collection tube and washed with 500 µl of washing 2 solution then centrifuged for 1 minute at (13,000 rpm) and discarded the flow-through.

11-The spin column was placed back in the 2ml collection tube and centrifuged for additional 3 min to dry the column.

12- The spin column was placed to a new 1.5 ml microcentrifuge tube.

13- One hundred µl of Elution solution was added to the spin column and the spin column stayed standing at least 1 min.

14- Centrifuged for 1 minutes at (13,000 rpm) in order to elute the purified DNA.

15- The DNA fragment was stored at 4°Cor -20°C.

2.4 Determination of Concentration and Purity DNA

Agarose gel electrophoresis and spectrophotometric methods were used to measure the concentration and purity of extracted DNA.

2.4.1 Spectrophotometric Method:

The purity and concentration of DNA were measured by nanodrop instrumnt (absorbance method)(**128**).Absorbance readings were acomplished at 260 nm and at 280 nm. At 260 nm the DNA strongly absorbs light while at 280 nm, the protein absorbs light most strongly. DNA purity can be estimated by A260/A280 ratio. The A260/A280 ratio between 1.7 and 2.0 is generaly acepted and it represent a high quality DNA sample(**129**).

2.4.1.1 Procedures:

•One microliter of distiled water was applied on the highly senstive micro detector of nanodrop as blank.

•The micro detctor was cleaned up from blank.

•Then 1 µL of sample was aplied on the micro detector of nanodrop.

•The concentration and A260/A280 ratio of DNA were recorded from the instrument(**130**).

2.4.2 Agarose Gel Electrophoresis Method:

The quality of the isolated DNA was assessed by running 8 µL of each DNA sample on 1% and 2% agarose gel stained with RedSafe nucleic acid staining solution, then DNA sample was visualized by U.V. transilluminator **(131)**.

2.4.2.1 principle:

When charged molecules are placed in an electric field, they migrate towards either the positive or negative pole according to their charge**(132)**

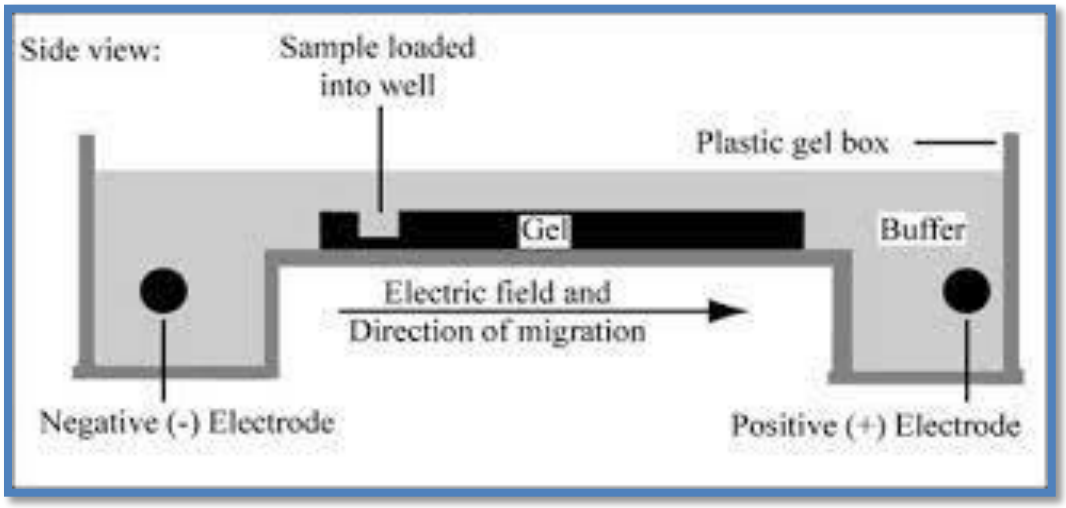

Figure 2-3 Principle of Electrophoresis.

Requirements :

1. Electrophoresis unit
2. Casting tray
3. Combs
4. Boat
5. Micropipette
6. Microwave oven

2.5 Primers Used in This Study Primers of Nested T-ARMS PCR

Table 2-6 The forward and reverse primer for the SNP rs769178 G > T of TNF alfa gene .

Target gene		SNP rs769178G<T of TNF-α gene Sequence (5‟-3‟)	M.T	GC %	Length Ta	Product sizes
outer	F	CTGGGTGACAGAACGAGACCCTG TCTCA	73	50	28 30	370
	R	TCCCATTTAGGGTGAGACGGAAA ATCCTCA	73	52		
Inner T allell	F	GTAGGGTGACCCATTGGGGTGAC CCAT	74	63	27	201
Inner G allell	R	GCAGAGGGTGACCCAAGGCCCTA CAC	73	60	26	222

2.5.1 Primers:

A primer is a short segment of nucleotide, which is complimentary to a section of the DNA, which is to be amplified in the PCR reaction. Primers are annealed to the denatured DNA template to provide an initiation site for the elongation of the new DNA molecule.

According to instruction of the primer synthesiser company, the primers (originally lyophilized), were dissolved in the free ddH2O to obtain a final concentration of 100 µM/µl which served as a stock solution that stored at -20 °C. A concentration of 10 µM/µl was prepared from the stock primers to be used as a work primer.

Table 2-7 : Components of materials of T-ARMS PCR

Forward Primers 5 Pico moles	4µl
Reverse Primer 5 Pico moles	4µl
Master mix	25µl
DNA sample dd H_2O	4µl 13 µl

These ingredients were needed to amplify one DNA sample ,total volume 50µl was taken in each microfuge tube. These tubes were kept in the DNA thermo cycler the first end into the holder. Start the machine and observe the capillary tube extruding from the holder carefully, Close the hot top lid slightly, but not so tight to crush the tubes.

2.5.1.1 Principle of T-ARMS- PCR:

The principle of the present technique relies on the modification of primers to amplify a specific allele. The 3‟ end of the primer is modified in such a way that one primer can amplify a mutant allele while the other can amplify the normal allele. To do this, researchers modify a few bases from the primer‟s 3‟ OH end.(**133**)

PCR is used to amplify a specific region of a DNA strand (the DNA target). Most PCR methods typically amplify DNA fragments of up to ~10 kilo base pairs (kb), although some techniques allow for amplification of fragments up to 40 kb in size. A basic PCR set up requires several components and reagents. These components include(**134**):

a) DNA template that contains the DNA region (target) to be amplified.

b) Two primers that are complementary to the 3' (three prime) ends of each of the sense an anti-sense strand of the DNA target.

c) Taq polymerase or another DNA polymerase with a temperature optimum at around 70 °C (should be thermo stable).

d) Deoxynucleoside triphosphates (dNTPs; also very commonly and erroneously called deoxynucleotide triphosphates), the building blocks from which the DNA polymerases synthesizes a new DNA strand.

e) Buffer solution, providing a suitable chemical environment for optimum activity and stability of the DNA polymerase.

f) Divalent cations, magnesium or manganese ions; generally Mg^{+2} is used, but can be Mg^{+2} utilized for PCR-mediated DNA mutagenesis, as higher concentration Mg^{+2} increases the error rate during DNA synthesis (Pavlov AR)

g) Monovalent cation potassium.

The method relies on thermal cycling which involves cycling of different temperatures for DNA melting and enzymatic reactions.

2.5.1.2 procedure:

To carry out a PCR experiments the targeted DNA is mixed with a pair of gene specific oligonucleotides primers deoxynucleotide and Taq DNA polymeras

Start the programme:

Step 1: initialization: Heat the mixture at 95^0C for 5 minutes to ensure that the DNA strands as well as the primers have melted.

The DNA Polymerase can be present at initialization, or it can be added after this step.

Step 2: denaturation: Heat at 94 ^{0}C for 1 minute.

Step 3: annealing: Heat at 56 ^{0}C for 30 sec.

Step 4: extension: Heat at 72 ^{0}C for 1 minute.

Step 5:step 2-4 are repeated to 35 cycles until the target DNA is amplified to target level.

Step 6: final extension: Heat at 72 C for 7 minutes to make sure all extensions is completed.

Step 7: 4 (or) 120C for 30 minutes.

Step 8**:** end The machine will hold the tubes at 40 C until the reaction tube are removed(**135**)

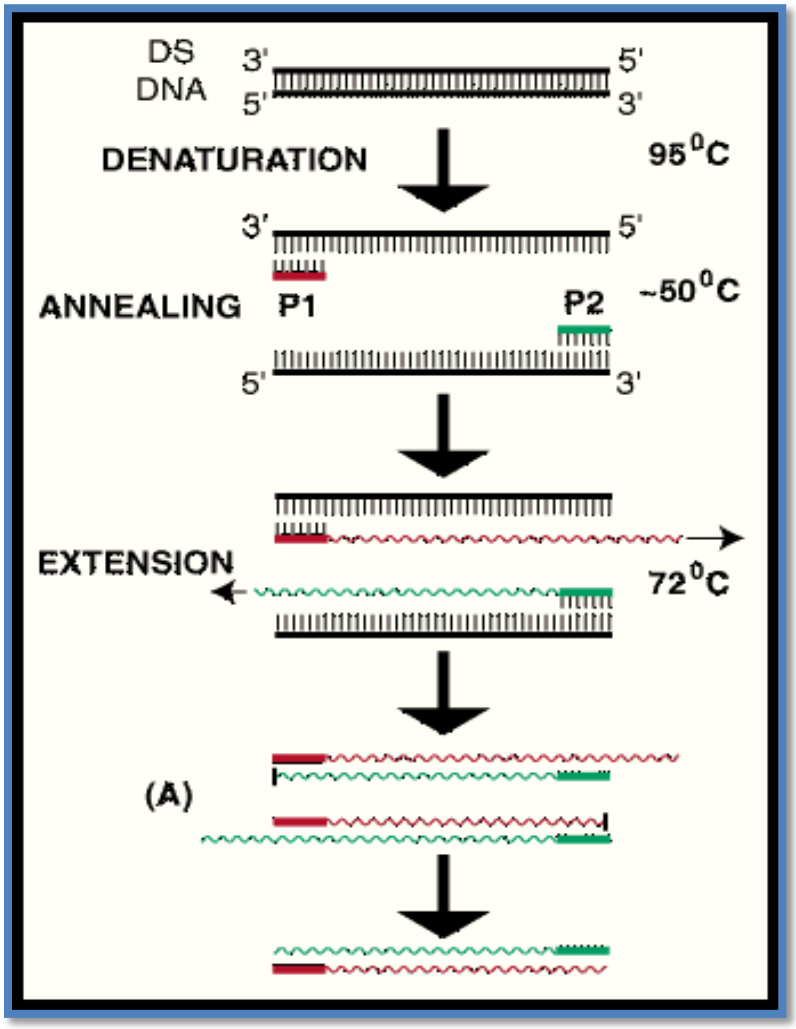

Figure2-4: Steps of PCR(136)

2.5.2 Optimization of T-ARMS PCR Conditions:

A slight modification of T-ARMS PCR technique was implemented in which an outer PCR product was amplified prior to the T-ARMS PCR. This outer-PCR would serve as a DNA template for the T-ARMS PCR. In brief, it is consisted of Taq polymerase and four primers with two outer primers. The ratio of the outer primer to the allele-specific primer is 1:10 PCR products on a 2% agarose gel electrophoresis at 3–5 volts/cm for 40 min. The genotypes are differentiated by checking the amplicon sizes in reference to molecular size markers.

Table 2-8 : Amplification conditions of TNF alfa gene rs769178 G > T.

Gene	Initial	Denaturation	Annealing	Extension	Final Extension
Omentin-1	94°C 5min	94°C 30sec	56°C 30sec	72°C 1min	72°C 7min
	1 cycle	35 cycle			1 cycle

2.5.3 Detection of PCR product by agarose gel electrophoresis:

The PCR products should be fragments of DNA of defined length, the simplest way to check for the presence of the fragments is to load a sample taken from the reaction product, along with the appropriate molecular weight markers, on to an 2% agarose gel which contains 0.8-4.0%Red safe DNA bands on the gel can then be visualized under UV gel doc.

2.6 Statistical Analysis:

In the current study a t-test was performed to determine whether group variance was significant or not, the difference were considered significant when the P value less than 0.05. Data were expressed as mean ±standard deviation in expression of results. Statistical analyses were performed with SPSS (version 20).

3.Results and discussion

3.1 General Characteristic of the Study group.

3.1.1 Age

The present case control study included 50 patients with Allergic rhinitis with a mean age 37.5 ± 7.1 years and an age range of 30-45 years. Besides, the study included 50 apparently healthy individuals with a mean age of 36 ± 7.6 years and an age range of 29-44 years , The age of subjects is stratified into 2 groups to elucidate the effect of age on study parameters; the results show no significant differences ($p > 0.05$) in age between control group and Allergic rhinitis group; as demonstrated in, table (3-1) .

Table 3-1: Means Age ± SD of Allergic rhinitis and control groups.

Subjects	**Number**	**Age (Years) Means ±SD**	**Range**	**P value**
Allergic rhinitis group	50	37.5 ± 7.1	30-45	P<0.05
Control group	50	36.5 ± 7.6	29-44	

SD: standard deviation; non-significant at $p > 0.05$

The frequency distribution of patients with AR according to age was as following: 35 (70 %) cases from 30-37 years, 15 (30%) cases and between 38- 45 year, as demonstrated in figure 3-1.

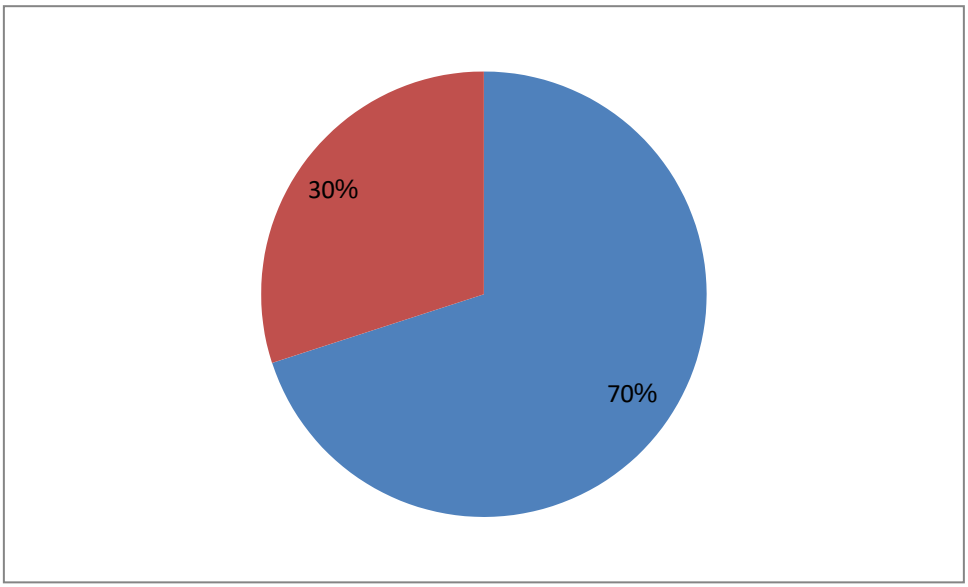

Figure 3-1: Frequency distribution of AR patients according to age

Age distribution of our study showed that most people were in the age range of 30-37 years, and this is relabel with another reviews like Yang Liu,*et al* (2022) (**137**) who said that the peak of prevalence of allergic rhinitis with regard to age gradually.

3.1.2. Gender

The gender of Allergic rhinitis patients and control group in the present study was include both males and females. the results show no significant differences ($p > 0.05$) in gender between control group and Allergic rhinitis group.

The frequency distribution of patients with AR and control groups according to gender was as following: Table 3-2 and figure 3-2 . The percentage of males was 66 % and percentage of females was 34% for patients , this finding was similar with the study conducted by ,Safa Z. Jaleel *et al* (2022) (**138**) demonstrated that there was a significant increase in the risk of Allergic rhinitis in men compared to women as shown in figure3-2.

Table 3-2: Distribution of Study Groups According to gender.

Gender	AR		control	
	Count	%	Count	%
Male	33	66%	34	68%
Female	17	34%	16	32%

AR= Allergic rhinitis

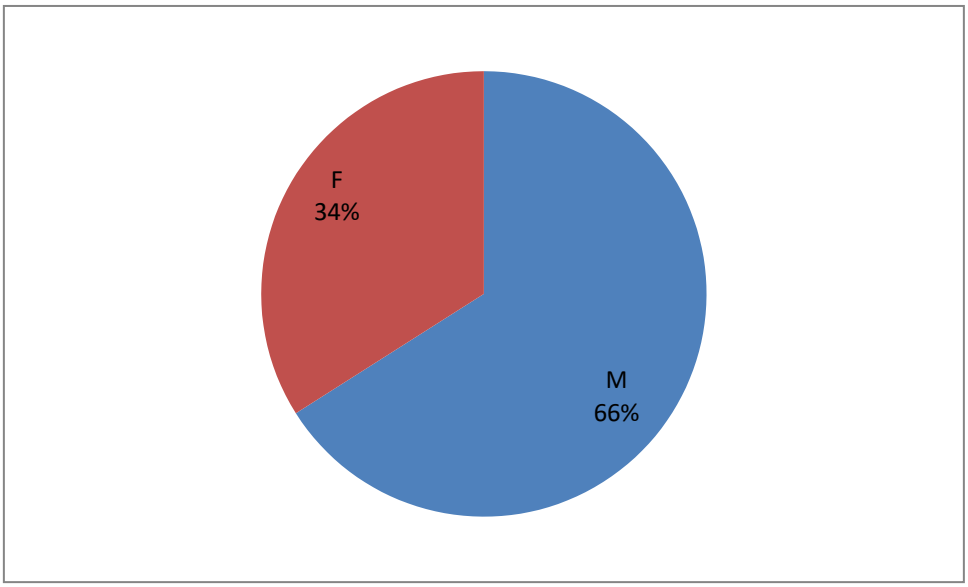

Figure 3-2: Percentage of Gender in AR group.

3.1.3 Body Mass Index (BMI)

In this study ,The BMI (mean ±SD) for normal-weight AR patients (23.2 ±0.31), normal-weight controls (22.9 ±1.3), and the (mean ±SD) for over weight (27.14 ± 0.55) AR patients and (26.3 ± 1.1) control patients.

Table 3-3 shows no significant differences in BMI between patients with AR and control group.

The frequency distribution of patients with AR according to BMI was as following: Figure 3-3 showed that the percentage of over

weight patients were 64 % and percentage of normal weight was 36% for patients group. An increased body-mass index (BMI) has been demonstrated to worsen the disease outcome of Allergic Rhinitis. this finding was similar with the study conducted by ,Almaraz *R G et al* (2020) **(139)**.

Table 3-3: Means BMI ± SD of patients and control groups.

Subjects	Number Of normal weight	BMI Means ±SD	P-value
Normal weight AR	18	23.2 ±0.31	P > 0.05
Normal weight Control group	17	22.9 ±1.3	
Subjects	Number Of over weight	BMI Means ±SD	P-value
Over weight AR	32	27.14 ± 0.55	P >0.05
Over weight Control group	33	26.3 ± 2.1	

BMI: Body Mass Index ;SD: standard deviation; non-significant at p > 0.05

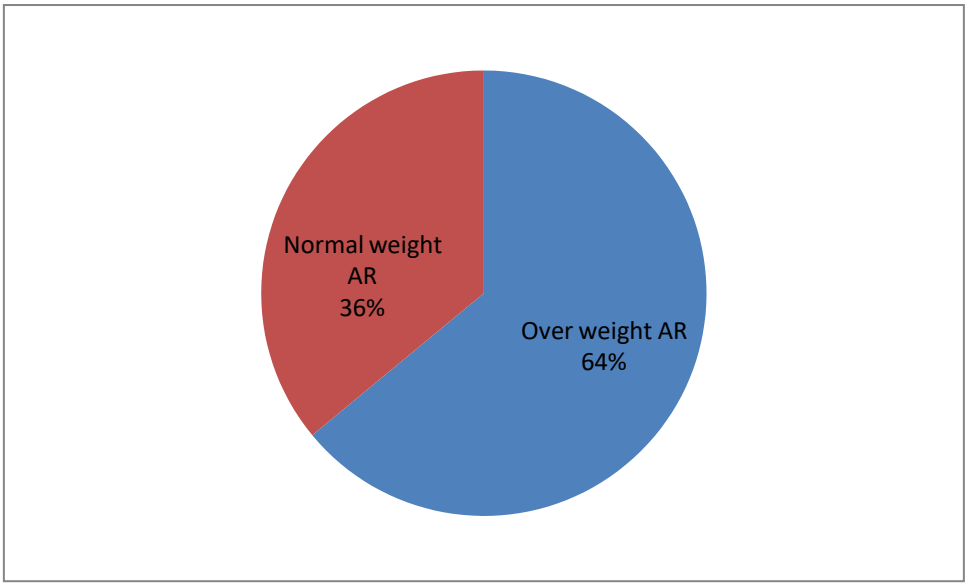

Figure3-3: The frequency distribution of AR paints according to BMI

3.1.4. smoker and non – smoker

In This study there was no significant difference between smoker and non – smoker between AR and control group . Also, showed the percentage of smoker AR were 30 (60%) and percentage of Non- smoker AR were 20 (40 %)smoker=30,non smoker=20 for patients as demonstrated in, figure(3-4).. this finding was similar with the study conducted by , Hisinge-lknen ,H .*et al* (2018) (4) . demonstrated that there was a significant increase in the risk of Allergic rhinitis in smoker compared with non -smoker .

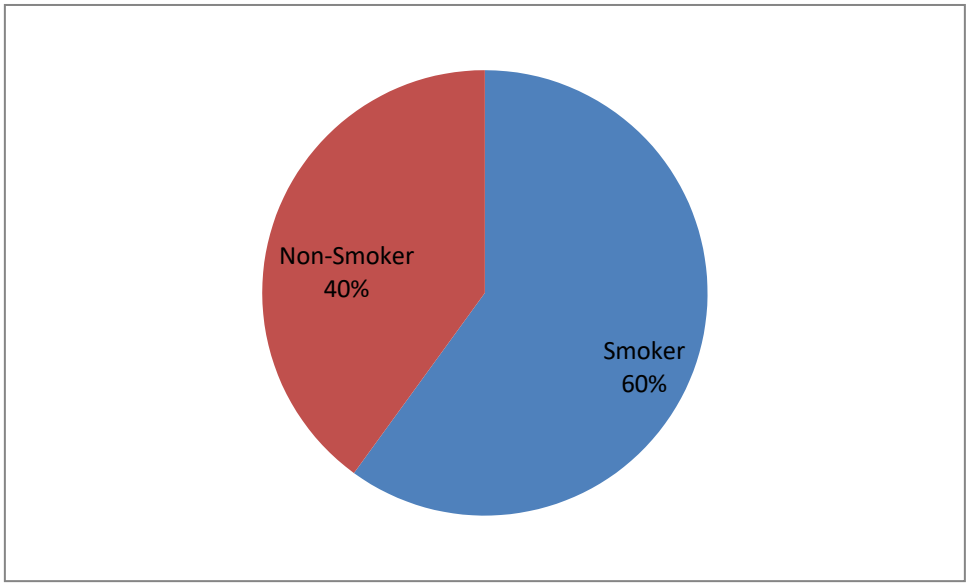

Figure 3-4: Percentage of smoker and non-smoker in AR group

3.2 Biochemical results:

The present case control study Table (3-4) ,demonstrates the base line characteristics of the study, which includes the data of the patients and the control groups. It contains the arithmetic results for the measurements of the levels of each of:

Tumor necrosis factor-alpha (TNF–α) ,ImmunoglobulinE (IgE) Eosinophils, Sodium, Potassium, Calcium and Chloride in patients with allergic rhinitis and control.

The levels of Tumor necrosis factor-alpha (TNF–α) and Immunoglobulin-E (Ig-E) , were discovered to be significantly higher in AR patients than in the control group, where ($p<0.05$). Also eosinophils found to be significantly ($p\leq0.05$) increase in patients compared to control, as mean 4.50 and SD 3.98 for patient compare to control as mean 2.40 and SD 1.19 , $p < 0.05$ While no significantly difference in levels of Sodium, Potassium, Calcium and Chloride in patients with allergic rhinitis and control ($p >0.05$).

Table 3-4: Criteria Clinical Measurements of the Samples Population

Variables	Group	No	Mean ± SD	95% confidence interval for Mean		Sig. value
				Min	Max	
TNF- α Pg/ml	patients	50	78.84±5.1	73.74	83.94	p < 0.05
	control	50	11.88±7.2	11.68	26.08	
IgE IU/ml	patients	50	297.1±15.2	281.9	312.3	p < 0.05
	control	50	93.2±12.3	80.9	105.5	
Eos. (%)	patients	50	4.50±3.98	3.691	5.180	P < 0.05
	control	50	2.40±1.19	1.980	2.740	

Sodium mEq/L	**patients**	**50**	**139.2±2.1**	**127.1**	**141.3**	**P > 0.05**
	control	**50**	**140.5±1.5**	**139**	**142**	
Potassiu mEq/L	**patients**	**50**	**4.5 ± 0.2**	**4.3**	**4.7**	**p > 0.05**
	control	**50**	**4.4 ± 0.15**	**4.25**	**4.55**	
Calcium mEq/L	**patients**	**50**	**2.1±0.05**	**2.05**	**2.15**	**p > 0.05**
	control	**50**	**2.2±0.15**	**2.04**	**2.35**	
Chloride mEq/L	**patients**	**50**	**103 .05±1.2**	**101.85**	**104.25**	**p > 0.05**
	control	**50**	**105.2±1.11**	**104.09**	**106.31**	

SD: standard deviation; significant at P>0.05

Allergic rhinitis is a type of inflammation in the nose that occurs when the immune system overreacts to allergens in the air .AR a significant cause of widespread morbidity, high medical treatment costs, reduced work productivity, low quality of life and can be associated with conditions such as fatigue, headache, cognitive impairment, and sleep disturbance. Avariety of cytokines and adhesion receptors seem to play an important role in the allergic rhinitis (**140)**.

The results of increased serum levels of TNF-α and IgE were in agreement with the Minhas *et al*.(**141**) and Widegren *et al*. (**142**) Jung *et al*.(**143**). In the current investigation, patients with allergic rhinitis had higher serum eosinophil levels was found (R=0.78), indicating the importance of these cells in allergic rhinitis. Eosinophilia can be viewed as a result of the entire process because Ig-E is involved early in the inflammatory cascade and can be regarded a cause of allergic rhinitis

The biological background that is "driving" the inflammation should be taken into account when choosing a course of treatment because it will likely indicate how well the patient will respo(**144**) The increase in those parameters and its interfering effects can be explained by the following . Allergen is mediated by sensitizedmast cells whose high-affinity Ig-E receptors (FceRI receptors) are occupied by IgE directed against specific allergens.(**145**).

Activation of Th cells leads to the production of various cytokines, such as interleukin IL-3, IL-4, IL-5, IL-6, IL-13, interferon (IFN), and TNF-α . During the late phase ,all these cytokine recruitment; with transendothelial migration and infiltration of activated T-cells,

eosinophils, basophils, neutrophils and macrophages into the nasal mucosa(**146**). Eosinophils are the predominant cell, they generate vasoactive mediators and cytotoxic proteins. Activated basophils are responsible for histamine release. All these events amplify the allergic inflammatory response, leading to a real cascade of reactions(**147**). Cytokines (produced due to activation of Th-cells and mast cells) lead to increased production of histamines, leukotrienes and prostaglandin that have multiple activities .They cause the characteristic watery rhinorrhoea by stimulating gland and goblet cell secretion, vasodilation and blood vessel leakage(**148**).Vessel dilation and the pooling of blood in the cavernous sinusoids already produce a certain degree of nasal congestion during this early phase. Sensory nerve stimulation leads to itching sensations and sneezing reflexes (**149**).

TNF-α causes changes in the ionized calcium flux within smooth muscle. which lead consequently to the promotion of IgE production. IgE triggers the release of mediators that are responsible for arteriolar dilation, increased vascular permeability ,itching ,rhinorrhea and mucous secretions**(150).**

Table 3-5:Association of Biochemical results in AR patients with age groups

Parameters	**Age 30-37 (years) N=35 (Mean ± SD)**	**Age 38-45 (years) N=15 (Mean ± SD)**	**P value**
TNF- α pg/ml	**81.44±2.5**	**71.22±2.49**	**< 0.05**
IgE IU/ml	**307.01 ±5.29**	**290.3 ±8.4**	**< 0.05**
Sodium	**134.75 ±6.55**	**134.1 ±7.01**	**> 0.05**
Potassium	**4.51±0.19**	**4.48 ±0.19**	**> 0.05**

mEq/L			
Calcium mEq/l	**2.11 ±0.04**	**2.09±0.04**	**> 0.05**
Chloride mEq/l	**103.08±1.17**	**103.03±1.18**	**> 0.05**

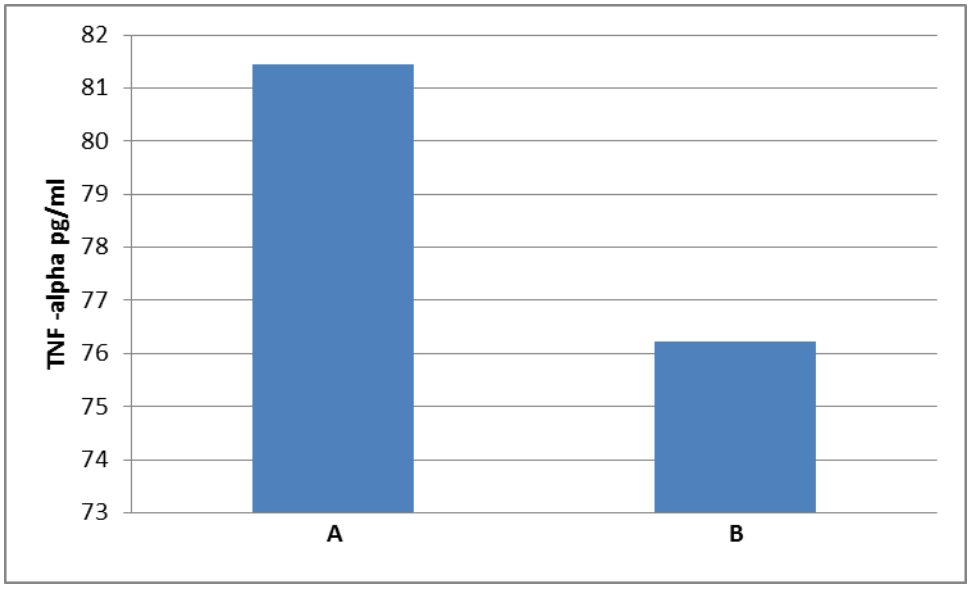

Figure 3-5 Mean of TNF-alpha in two groups, A(30-37)and B(38-45)

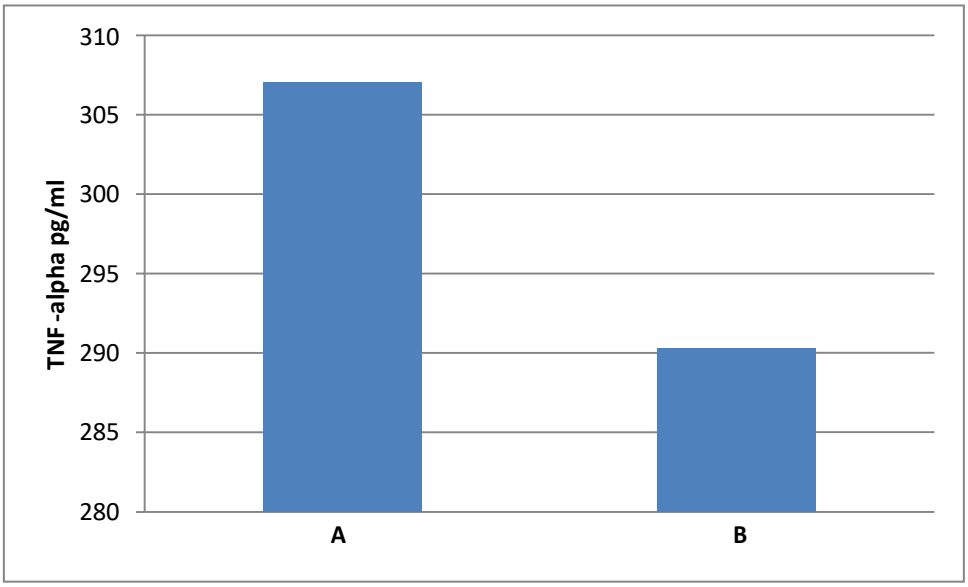

Figure 3-6 Mean of Ig-E in two groups, A(30-37) and B(38-45)

The Age was similarly distributed in healthy controls and AR patients , as demonstrated in, table (3-5) and figures(3-5) (3-6).the current study findings indicate that the majority of AR patients were in their age between (30-37) years of life with percent (70 %) and this is in accordance with other studies like Li *et al.*, (**151**) and Yonekura *et al.*, (**152**)

Although Allergic Rhinitis may occurs at any age, but most occurs mainly in the age <40 years of old that this agreed with British study (**153**),another study (**154**).

The current study indicates that there was significant difference in mean of TNF –alpha and IgE in patients with age between (30-37) compared with other group , most affecting AR where pervious mainly in the age <40 years of old that this agreed with American study(**155**).While ,No significant difference in mean of Sodium ,potassium, Calcium and Chloride in patients with age between (30-37) compared with other group.

Table3-6:Association of Biochemical results in AR patients with gender

Parameters	**Male (N=36) (Mean ± SD)**	**Female (N=14) (Mean ± SD)**	**P value**
TNF-α pg/ml	**80.27±3.67**	**74.52±0.78**	**< 0.05**
Ig-E IU/ml	**301.4±10.9**	**285.05±3.16**	**< 0.05**
Sodium mEq/l	**134.6±6.7**	**133.65 ±6.35**	**> 0.05**

Potassium mEq/l	**4.55±4.15**	**4.43±0.14**	**> 0.05**
Calcium mEq/l	**2.10±0.05**	**2.08±0.03**	**> 0.05**
Chloride mEq/l	**103.15 ±1.1**	**102.525±0.675**	**> 0.05**

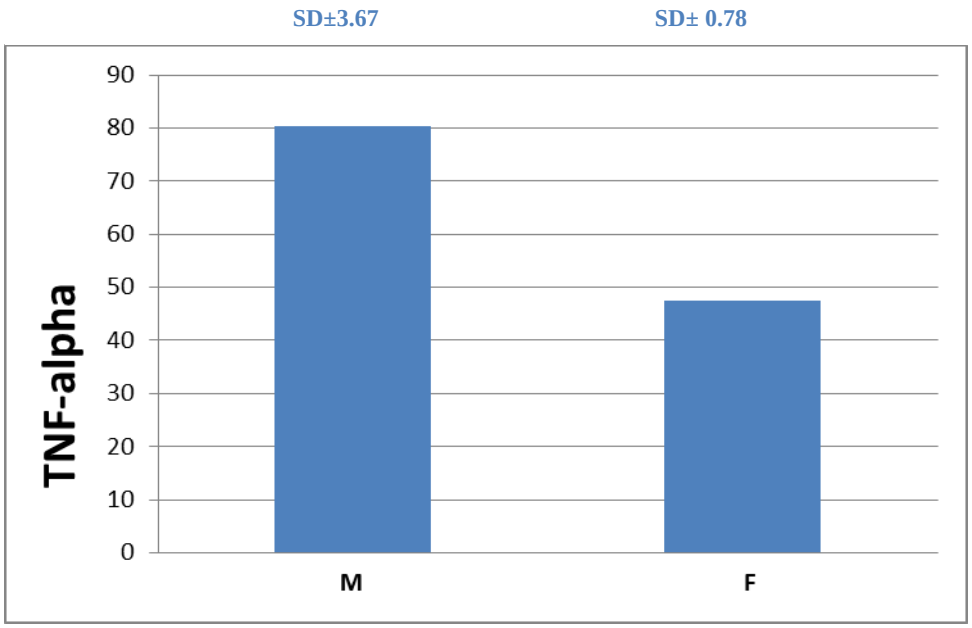

Figure 3-7:Mean of TNF-alpha in two groups(M and F)

SD±10.9 SD±3.16

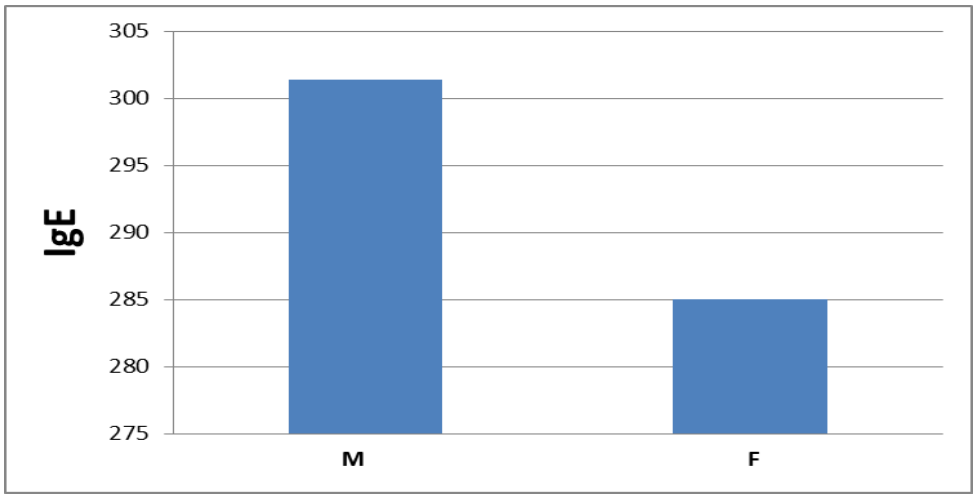

Figure 3-8:Mean of Ig-E in two groups(M and F)

Our results are consistent with previous research on the differences in prevalence according to sex. AR prevalence were higher in males in comparison to females .Differences in condition prevalence is explained by higher levels of endogenous sex steroids hormones with increased Th2 response in women, whereas in men, testosterone works by suppressing the Th2 response**(156).**

Hong Lv et al in china studies suggested non significant difference in age, sex was observed between patients and healthy control in patient with Allergic Rhinitis(**157**). The AR and comparison cohorts showed no significant differences based on distributions of age, sex and comorbidities(**158**).

The current study indicates that there was significant difference in mean of TNF –alpha and Ig-E in Male compared with Female patients ,table (3-6) and figures(3-7) (3-8) While ,No significant difference in mean of Sodium ,potassium, Calcium and Chloride in patients with gender between groups.

Table 3-7:Association of Biochemical results in AR patients with normal and over weight BMI

Parameters	**Overweight (N=32) (Mean ± SD)**	**Normal weight (N=18 (Mean ± SD)**	**P value**
TNF-α pg/ml	**81.07±2.87**	**77.57±2.83**	**< 0.05**
IgE IU/ml	**302.6±9.7**	**286.20±4.31**	**< 0.05**
Sodium mEq/l	**134.34±6.96**	**133.5 ±6.4**	**> 0.05**
Potassium mEq/l	**4.53±0.17**	**4.46±0.17**	**> 0.05**

Calcium mEq/l	**2.125 ±0.025**	**2.095±0.045**	**> 0.05**
Chloride mEq/l	**103.18±1.07**	**102.98 ±1.13**	**> 0.05**

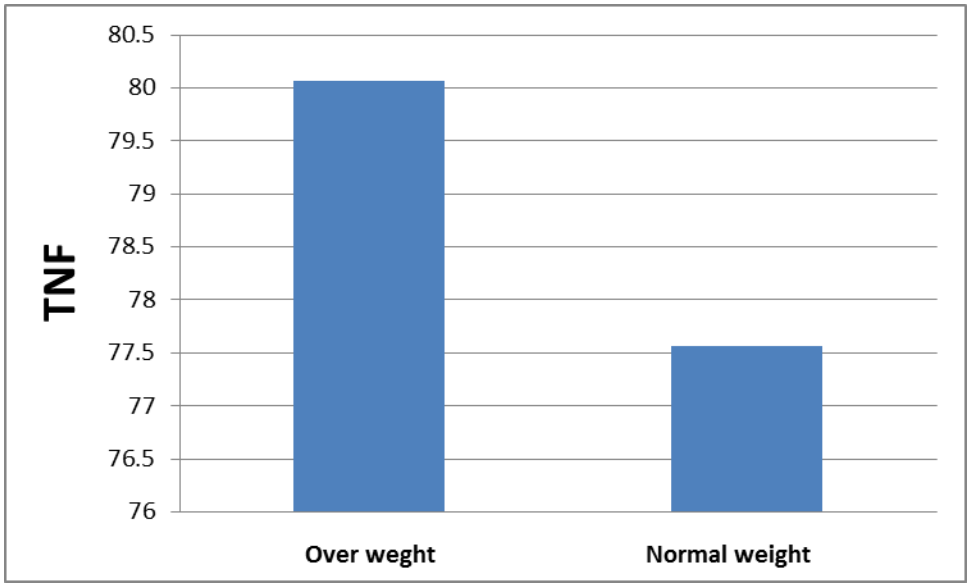

Figure 3-9: Mean of TNF-alpha in two groups(over weight and normal weight)

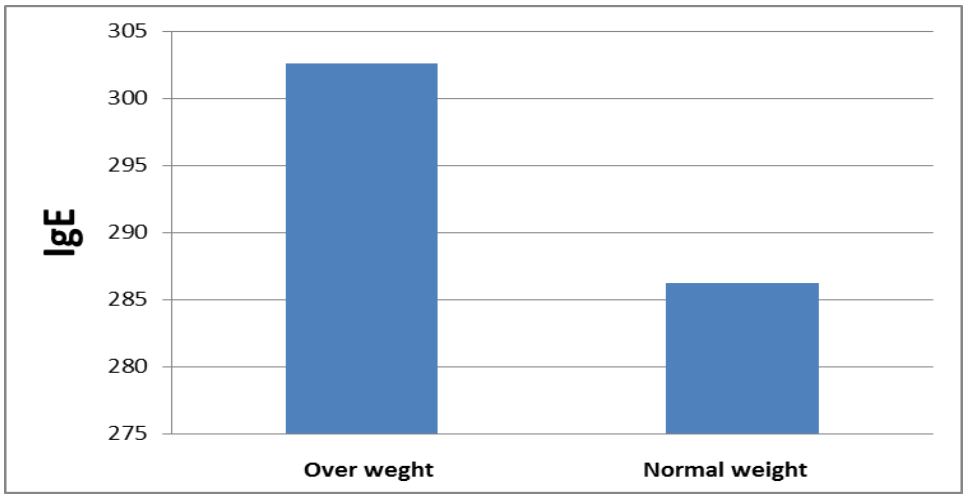

Figure3-10:Mean of Ig-E in two groups over weight and normal weight.

Overweight/obesity has emerged as one of the most serious public health challenges during the last decades and is a strong risk factor for several adverse health effects and chronic diseases. According to the World Health Organization (WHO), overweight and obesity are defined as "abnormal or excessive fat accumulation that may impair health (**159**).

Obesity may be associated with allergic diseases (including atopy, asthma, and allergic rhinitis) body mass index (BMI) may be associated with bronchial hyper-responsiveness. Nevertheless, the relationship between histamine skin reactivity and BMI is poorly understood. Some studies showed a significant association between obesity and allergic responses. Bibi et al(**160**).

As the prevalence of being overweight has increased worldwide, investigators are paying closer attention to the relationship between weight status and allergic disease(**161**).

A positive association has been shown between central obesity and allergic rhinitis, and waist circumference is an isolated risk factor for asthma (**162**)

The current study indicates that there was significant difference in mean of TNF –alpha and Ig-E in overweight compared with norml weight patients **,** table (3-7) and figures(3-10) (3-11). While ,No significant difference in mean of Sodium ,potassium, Calcium and Chloride in patients compared with other group.

Table3-8:Association of Biochemical results in smoker and non smoker AR patients

Parameters	smoker (N=30) (Mean ± SD)	Non -smoker (N=20) (Mean ± SD)	P value
TNF-α pg/ml	82.015±1.93	75.025±1.28	< 0.05
Ig-E IU/ml	305.5±6.8	290.55±8.65	< 0.05
Sodium mEq/l	134.61 ±6.69	134.15±7.05	> 0.05
Potassium mEq/l	4.63 ±0.07	4.49±0.2	> 0.05
Calcium mEq/l	2.14 ±0.01	2.08±0.03	> 0.05
Chloride mEq/l	103.25 ±1.01	103.01±1.16	> 0.05

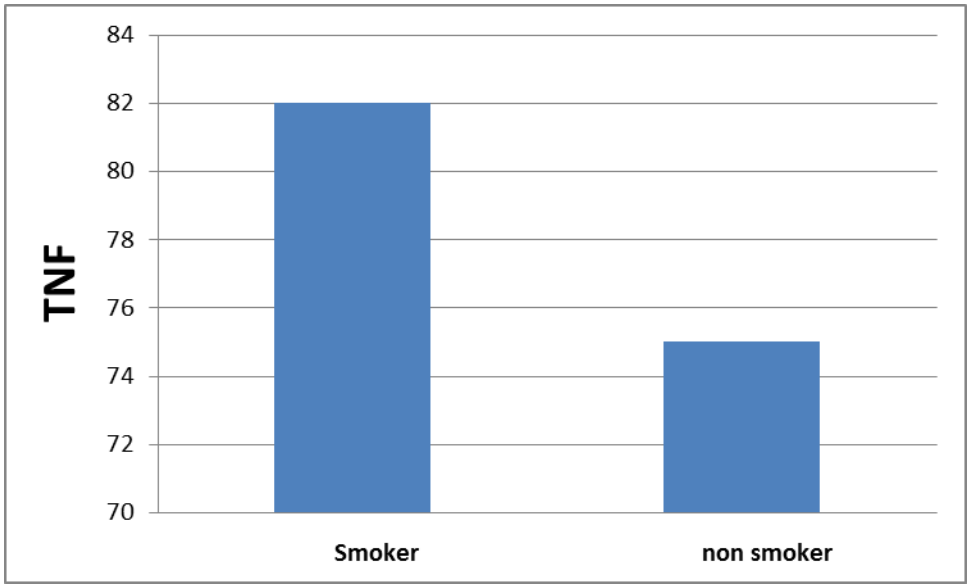

Figure 3-11: Mean of TNF--α in two groups(smoker and non smoker

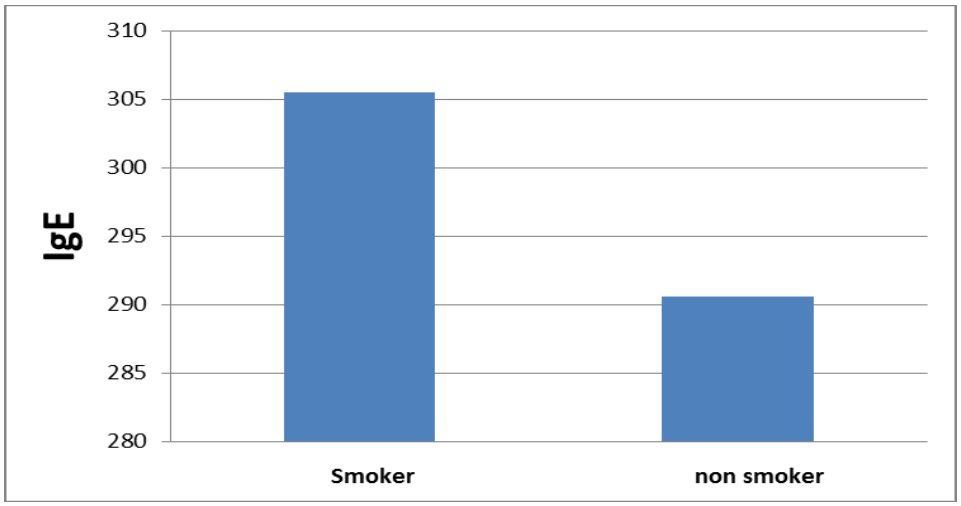

Figure3-12:Mean of Ig-E in two groups(smoker and nonsmoker)

Rhinitis symptoms is significantly more common among smoking individuals than among non-smokers. Smoking is also associated with the development of nasal polyposis. The combined effects of smoking, environmental tobacco smoke and occupational irritants on nasal symptoms have been less well estimated. Current smoking in combination with occupational exposure to gases, dusts or fumes resulted in increased occurrence of chronic nasal symptoms suggesting an additive harmful effect. Smoking alone was associated with chronic rhinitis and nasal congestion. Occupational irritants were associated with runny . The risk increase is modest but obviously becomes significant in large populations. The prevalence of longstanding nasal symptoms was high: 36.9% reported chronic rhinitis, 29.8% nasal congestion, and 27.9% runny nose. These symptoms were even more common than previously reported in Sweden (**163**).

smoking was associated with chronic nasal symptoms and increased the risk of nasal congestion and suggest that the association between nasal symptoms and tobacco exposure might be independent of allergy.

also evidence that tobacco smoke exposure would prevent from allergic sensitization**(164)** We observed that environmental tobacco smoke both at home and at work slightly increased the occurrence of chronic nasal symptoms, which is in line with previous observations(**165**).

The current study indicates that there was significant difference in mean of TNF –alpha and Ig-E in smoker compared with non-smoker patients, table (3-8) and figures(3-12) (3-13). While ,No significant difference in mean of Sodium ,potassium, Calcium and Chloride in patients compared with other group.

3.3. Genotyping

3.3.1. DNA Samples extraction:

The isolated DNA was running in agarose gel stained with Red Safe nucleic acid staining solution .

3.3.2. Estimation of DNA concentration and purity:

A ratio of absorbance at 260 nm and 280 nm was measured for the estimation of DNA concentration and purity of studied samples. Data was demonstrated in Table 3-9 and figures 3-14 ,3-15 respectively.

Table 3-9 : The Purity and concentration of the extracted DNA

DNA Purity and Concentration	Mean ± SD
DNA Concentration (ng /µl)	33.07 ±6.43
DNA Purity (260/280)	1.84 ± 0.09

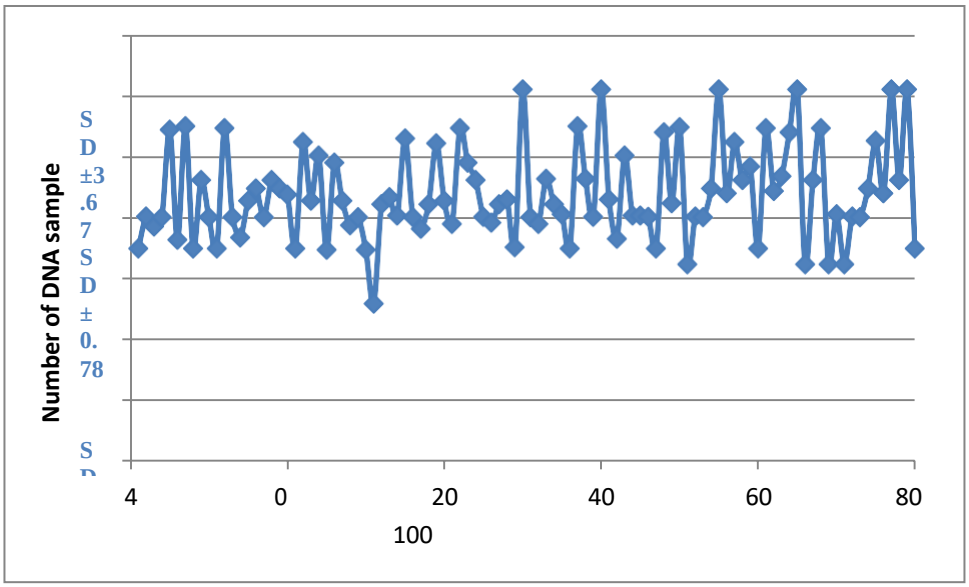

Figure 3-13: Concentration of Extracted DNA Samples

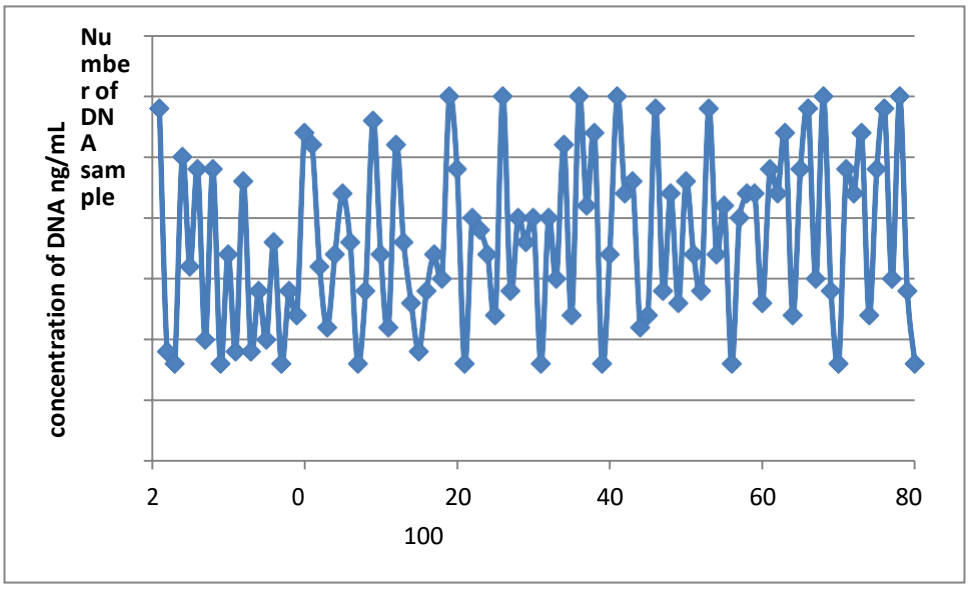

Figure 3-14 :Purity of Extracted DNA Samples.

3.3.3.Results Of Amplification Reaction:

In current study the gene polymorphism of TNF- α gene was studied in AR and Control groups. The genotyping were detected by T-ARMS-PCR . SNP **rs769178 G>T**, within the TNF- α gene included in present study.

The amplification of SNP of TNF- α gene rs769178 G>T, was showed in 370 bp as in (Fig 3-15).

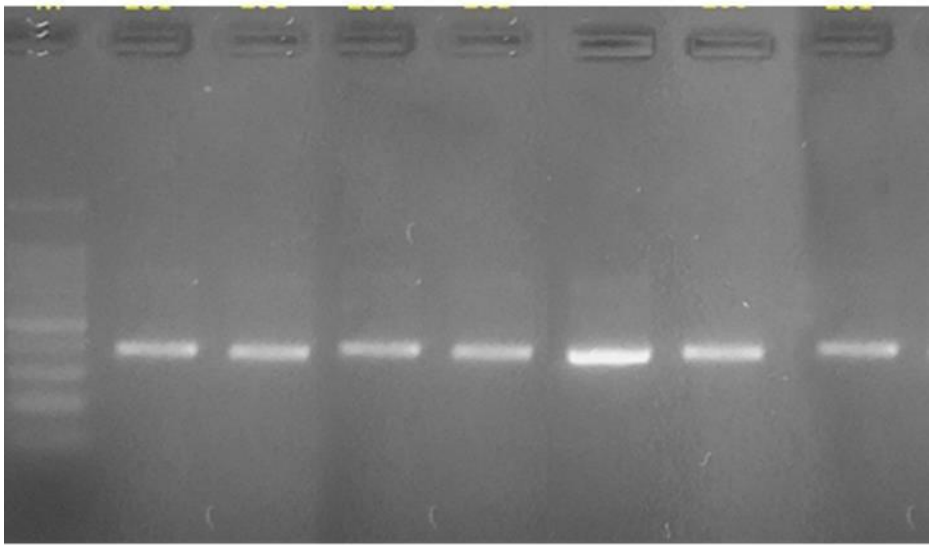

Figure 3-15 : The Outer PCR for SNP is: rs769178 G>T= 370 bp ,The agarose gel was 1% and the DNA dye is RedSafe (Intron, Korea). V: 95, Time: 45 minutes. M: DNA stairway. as in (Fig 3-15)

3.3.4 Tumor necrosis factor-alpha Gene (rs769178 G>T) SNP Polymorphism.

The subjects enrolled in present study were reported into three genotypes, These homozygous for the G allele (GG) wild type, heterozygous (GT) and the last one was homozygous for the allele (TT).

* Two band (222 bp) and(370 bp) is the wild genotype (GG).

* Three bands (222 bp), (201 bp) and (370 bp) are heterozygous genotype (GT).

*Two bands (370 bp) and (201 bp) are the homozygous genotype (TT).as in fig (3-16).

The genetic power was calculated. It represents the power to detect a significant difference at level of 0.05 for TNF-alpha gene rs769178 G>T It is found to be (40%). It seemed to be less than the optimal level (80%). Mostly, these findings are due to relatively small sample size (100)**(166)**.

3.3.5 The Hardy–Weinberg Equilibrium (WHE) model for (rs769178 G>T)

The result from Hardy-Weinberg equilibrium (WHE) exact test revealed the AR group the genotype frequency not follow the Hardy-Weinberg equilibrium , but follow with the Hardy-Weinberg equilibrium in control group indicating that the investigated allele frequencies are constant between generations as in table (3-10).

Table: 3-10 Results of Hardy Weinberg Equilibrium for TNF- α Gene rs769178 G>T SNP genotypes in AR and control groups.

Group	X2	P-value
AR	5.80	**0.05**
Control	0.94	**0.62**

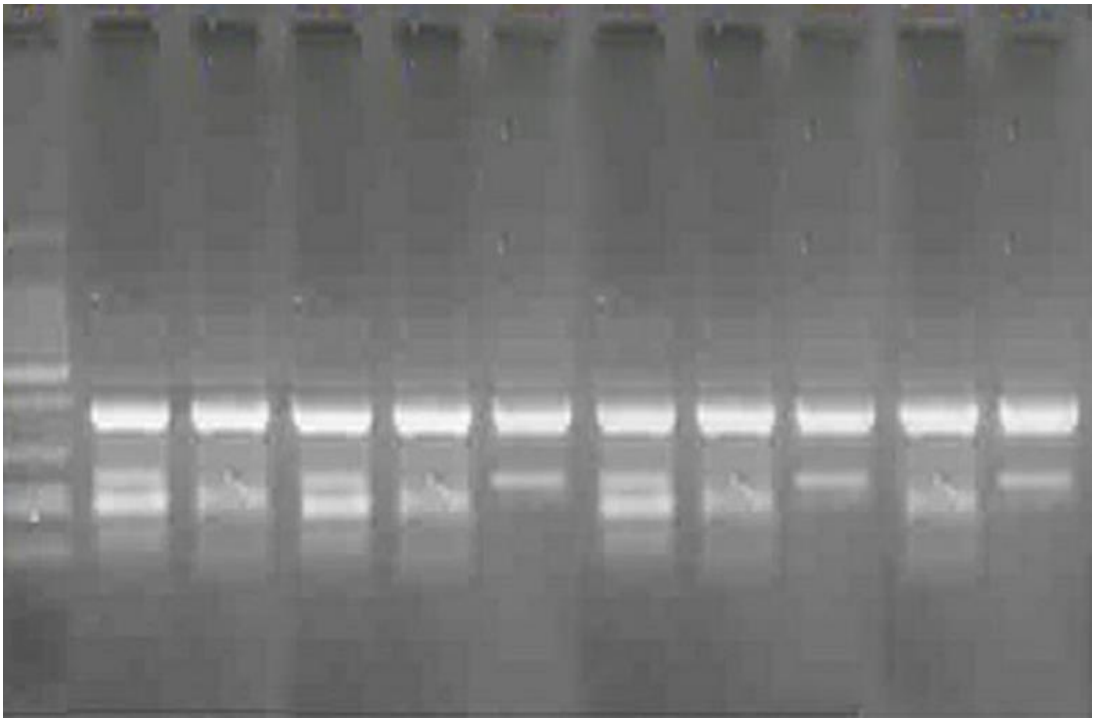

Figure 3-16 : T-ARMS-PCR bands of human gene TNF- α rs769178 G>T SNP, DNA marker (100-1500 bp).

Lane (1,3 ,and 6) heterozygosity (rs769178 G>T) (370 bp-222 bp-201 bp).

Lane (2,4,7,and 9) homozygote mutant (rs769178 G>T) (370 bp- 222bp).

Lane (5,8 and 10) homozygote wild type (rs769178 G>T) (370 bp-201bp).

3.3.6. Relevance of TNF- α Gene (rs769178 G>T) Polymorphism

The genotypes distribution and frequency of TNF- α gene (rs769178 G>T) SNP as shown in ,Table 3-11 . The analysis of results indicated that the TNF- α gene (rs769178 G>T) SNP genotype frequencies of wild genotype (GG), heterozygous (GT) and homo-

zygous genotype (TT) were, (50 %, 30% and 20 %) in AR group and (56%, 34 % and 10 %) in control group respectively.

The heterozygous genotype (GT) of TNF- α gene (rs769178 G>T) was found to be non significantly difference (OR = 1.01 , CI 95% , (0.42-2.43) ,P =0.97) the risk of AR with respect to those of the wild genotype (GG) of TNF- α gene.

The homozygous genotype (TT) of TNF- α gene (rs769178 G>T) SNP was found to be non- significantly difference (OR = 0.44 , CI 95% , (0.134-1.48) ,P =0.188). the risk of AR by with respect to those of the wild genotype (GG) of TNF- α gene .

Table 3- 11: Genotypes Distribution and Frequency of TNF- α Gene (rs769178 G>T)SNP in AR, and control Groups. GG: Wild genotype, GT: Heterozygous and TT : Homozygous genotype

Geno-type	AR N=50	Control N=50	OR	(95% CI)	P-value
GG	25 50%	28 56%	Refe	Refe	Refe
GT	15 30%	17 34 %	1.01	(0.42-2.43)	0.97
TT	10 20%	5 10 %	0.44	(0.134-1.48)	0.188

The allele distribution and frequency of G and T of TNF- α *gene***(**rs769178 G>T) SNP were found to 65 % and 35 % in the AR group respectively and 73% and 27 % in the control group respectively. The minor allele frequencies (T) of TNF- α *gene* **(**rs769178 G>T) SNP in AR and control groups were found to be 35% and 27% respectively. It was non-significantly difference (P >0.05) in AR compared with that of the control group as shown in Table 3-12 **.**

Table 3-12:Alleles Distribution and Frequency of TNF- α **gene (**rs769178 G>T **SNP) in AR and control Groups.**

Allele		**AR**	**Control**	**OR**	**95% CI**	**P-value**
G	**No.**	**65**	**73**	**Refe**	**Refe**	**Refe**
	%	**65**	**73**			
T	**No.**	**35**	**27**	**0.68**	**037-1.25**	**0.22**
	%	**35**	**27**			
Total	**NO**	**100**	**100**			

Table 3-13: Mean Serum TNF- α Concentrations for TNF- α *gene* (rs769178 G>T) in Patients with AR.

	Genotype	Mean ± SD
TNF-alpha pg/ml	**GG**	**74.17 ± 1.18**
	GT	**77.5 ± 1.4**
	TT	**81.57 ± 2.31**

GG: Wild genotype, GT: Heterozygous and TT: Homozygous genotype

The results of present study revealed that significant increase ($P < 0.05$) of serum TNF- α concentration in the homozygous genotype (TT) of TNF- α gene (rs769178 G>T SNP) when compared with wildgenotype (GG) of TNF-alpha gene (rs769178 G>T SNP) in patients with AR . However, other genotypes show non-significant variations as shown in table 3-13.

Genetic factors and ethnicity can play significant role in the development of Allergic rhinitis. single nucleotide polymorphism (SNPs) has a significant role in the fluctuation of symptoms in allergic disease (**167**).

Various genetic single-nucleotide polymorphisms have been investigated in AR including single-nucleotide polymorphisms in the TNF-alpha gene (**168**).

In the present study, we identified the rs769178 locus polymorphism of TNF-α gene was associated with the increased risk of AR . The rs769178 locus polymorphism was also associated with Ig-E expression level(**169**). Brozek et al showed that the interaction between the genetic

susceptibility and environmental factors are the fundamental cause of AR (**170**) .

Genetic factors play an equally important role in the risk factors related to the incidence of AR as same as and environmental factors .The emergence of gene polymorphism changes the coding sequence or changes the process of transcription and translation, which are involved in the occurrence and development of the disease by regulating the character, activity and dose of protein expressed by gene(**171**).Therefore, it is one of the important ways for researchers to discover the genetic mechanism of human complex diseases and to prevent and treat complex diseases the gene loci closely related to human complex diseases(**172**).

. Previous studies shown that expression of TNF-α could be associated with some pulmonary diseases. The highly expressed TNF-α was observed in nasal mucosal mast and epithelial cells. Similarly, the highly expressed cytokine of TNF receptors is also found in patients with AR(**173**).The vivo suggested that inhibition of TNF-α delayed the development of AR. TNF-α is regulated by TNF-α gene. Several single nucleotide polymorphisms (SNP) of TNF-α gene have been reported, and studies have identified several SNP of TNF-α. In the present study, we screened target SNP of TNF-α gene and analyzed the associations between this SNP polymorphism and AR but no signifecant(**174**).

3-4 Conclusion:

1. TNF alpha and Ig-E were positively correlated with age ,gender ,smoker and BMI , While , Sodium ,potassium, Calcium and Chloride No correlated with age ,gender ,smoker and BMI in patients with AR.
2. Allergic rhinitis patients in Babylon province have higher serum level of TNF-α and Ig-E and eosinophils comparing to normal subjects .
3. The TNF-α gene SNP (rs769178 G>T) do not associate with AR patients in Babylon province.

3-5 Recommendations: ::

1-The measurements of Ig-E and TN F-α and eosinophils levels are essential to specify the severity of the disease and monitoring of treatments in patients with allergic rhinitis.

2-Analysis of more SNPs of TNF-α gene and observing the relation with progression of AR in Iraqi population.

3-Determine the genotyping of TNF -α gene in other provinces in Iraq to give a complete picture about genotype distribution in Iraqi population.

4-Conduct whole TNF-α gene sequencing to detect the possible SNPs related to AR.

5-Studying other elements, such as Magnesium and trace elements whose concentration may have an effector or influence on my disease AR

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Study Of Same Electrolyte Imbalance And Tumour Necrosis Factor Alpha (TNF-α) Gene Polymorphism In Patients With Allergic Rhinitis In BabylonProvince In Iraq

Patient in information

Name:		**Age:**
Height:	Weight:	BMI:
Gender: Male: female: Smoker		Family history of disease:
Criteria for exclusion: Individual with Asthma, Patients with rheumatoid arthritis, diabetes mellitus and any other autoimmune diseases.		Duration of disease:

Other disease:

Biochemical testes:

Electrolyte:	Na^+	K^+	Cl^-	Ca^{++}
EILSA	TNF alfa level		Ig-E level	
Eosinophils				

الخلاصة

التهاب الانف التحسسي (AR) هو التهاب الاغشية المخاطية للأنف مصحوباً بعلامات كثيرة مثل تورم الغشاء المخاطي للأنف , وافرازات الانف والحكة واعراض اخرى , ينتج التهاب الانف التحسسي عن مسببات الحساسية وهي مواد غير ضارة عادة ولكنها تؤدي الى رد فعل تحسسي لدى بعض الاشخاص وهو مرض مزمن , يلعب السايتوكين جزءاً مهماً من العملية الالتهابية , وينتج عامل نخر الورم الفا بشكل رئيسي عن طريق الضامة والخلايا الاحادية ,ان تثبيط عامل نخر الورم الفا يؤخر تطور (AR).

قد يكون TNF−α مشاركاً في حساسية الانف الموسمية , يتم تنظيم TNF−α بواسطة TNF−α جين وهو233 حامض اميني , تقع تعدد اشكال النيوكليوتيدات الفردي لجين TNF−α على النطاق 21.33 q من الكروموسوم 6 المكون من 4 exons تم تصميم هذه الدراسة لتحقيق في العلاقة المحتملة بين IgE, TNF−α, الازينوفيل والالكتروليتات (Na^{+} , K^{+} , Ca^{+2} , Cl^{-1}) لدى مرضى AR في محافظة بابل ولتقييم دور تعدد الاشكال الجينية لل TNF−α بواسطة نظام T−ARMS−PCR وعلاقته كعامل خطورة لحساسية الانف الموسمية.

لتحقيق هذا الهدف اشتملت الدراسة الحالية على 100 شخص مقسمه الى مجموعتين , المجموعة الاولى تضم 50 مريضاً يعانون من AR بمتوسط عمر (37.5) , والمجموعة الثانية 50 شخصاً يتمتعون بصحة جيدة على ما يبدو وبمتوسط عمر (36) , تم تحليل مستوى TNF−α و IgE في السيرم بواسطة الممتز المناعي المرتبط بالانزيم, تم تحليل مستوى الالكتروليتات (صوديوم, بوتاسيوم ,كالسيوم ,كلوريد) بواسطة جهاز الابوت, وتم تحديد الازينوفيل بواسطة جهاز محلل امراض الدم, تم استخراج الحامض النووي من الدم والتنميط الجيني $r_s769178G< T(SNP)$ بواسطة نظام طفره حرارية متضخمة, ثم تحقيق التنميط الجيني بأستخدام بادئات محددة لتضخيم القطع ثم تحليل الناتج عن طريق الترحيل الكهربائي في الهلام الاكاروز.

تم تطبيق التحليل الاحصائي المختلف لتحليل البيانات التي توصلت اليها نتائجنا, كان هناك زيادة معنوية في TNF-α و IgE و الازينوفيل في المرضى الذين يعانون من حساسية الانف الموسمية $P< 0.05$ مقارنة بمجموعة التحكم, بينما لا يوجد فرق معنوي في مستويات الصوديوم والبوتاسيوم والكالسيوم والكلورايد في المرضى الذين يعانون من حساسية الانف الموسمية مقارنة بمجموعة التحكم $P> 0.05$ ويوجد ارتباط ايجابي لل TNF-α و IgE مع العمر والجنس والتدخين ومؤشر كتله الجسم في مرضى (AR).

ان تعدد الاشكال الجينية لل TNF-α للطفرة الوراثية $r_s769178G < T$ لدى مرضى حساسية الانف الموسمية لم تبين ارتباطاً مهماً للمرض.

جمهورية العراق

وزارة التعليم العالي والبحث العلمي

جامعة بابل كلية الطب

قسم الكيمياء والكيمياء السريرية

دراسة بعض الالكتروليتات وتعدد الاشكال الجينية لعامل نخر الورم الفا (TNF-α) في المرضى المصابين بحساسية الانف الموسمية في محافظة بابل /العراق

رسالة

مقدمة الى مجلس كلية الطب / جامعة بابل استيفاء جزئي لمتطلبات درجة الماجستير في الكيمياء الحياتية السريرية

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