**The Relationship between Tumour Necrosis Factor -alpha, IgE Levels and Oxidative Stress In Iraqi Patients with Allergic Rhinitis**

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**Abstract**

Aim of the studyTo find out the relation between tumour necrosis factor alpha (TNF-α), immunoglobulin-E and antioxidant levels in the patients with mild or moderate/severe cases.

Ninety subject (male , female) were enrolled in this study .Fifty patients with AR were subdivided into two groups i.e. mild (comprising 20 patients) with allergic rhinitis, moderate/ severe (comprising 30 patients) with allergic rhinitis. Forty subjects who are apparently healthy were taken as a control group. Serum TNF-α and IgE were determind by using enzyme- linked immunosorbent assay (ELISA). Malondialdehyde (MDA)levels and catalase activity were determined by using colorimetric method.

Serum levels of TNF-α was significantly higher in mild and moderate/severe groups compared with control group (p<0.01). IgE and MDA were significantly higher in mild group ( p< 0.05) and in moderate/severe group(p< 0.01). Catalase was significantly decreased in mild and moderate/severe groups compared with control group (p<0.05) (p<0.01) respectively .A significant positive correlation between TNF-α , IgE and MDA in AR patients in mild and moderate/severe cases. A significant negative correlation between TNF-α ,IgE and catalase in AR patients in mild and moderate/severe cases. Oxidative stress stimulate the production of TNF-α and IgE in patients of AR.

**الخلاصة**

الهدف من البحثالكشف عن العلاقة بين مستويات عامل نخر الورم الفا والاجسام المضادة IgE ومضادات الاكسدة في المرضى المصابين بالمرض بنوعيه الطفيف المعتدل/ والشديد.

وتم ادراج تسعون شخص في هذه الدراسة خمسون مريض مصابين ب بالتهاب الانف التحسسي، تم تقسيمهم الى مجموعتين، الاولى تتضمن عشرون مريض مع اصابة معتدلة بالمرض ،والثانية ثلاثون مريض مع اصابة معتدلة او شديدة.المجموعه الثالثة تضمنت اربعون شخصا وهم غير مصابين بالمرض وسليمين ظاهريا ليمثلوا مجموعة السيطرة.تم قياس عامل نخر الورم الفا والاجسام المضادة IgE باستخدام تقنية الارتباط المناعي للانزيم وهي ELISA كما تم تقدير فعالية انزيم catalaseو مستوى المالون داي الدهايد بالطريقة اللونية.

ولوحظ حصولزيادة معنوية في مستوى عامل نخر الورم الفا في مصول المصابين بهذا المرض بنوعيه المعتدل/ والشديد مقارنة بمجموعة السيطرة ( P>0.01).ايضا زيادة هامة في الحالات المعتدلة P>0.05)) و في الحالات الشديدة P>0.01)).اظهرت الدراسة انخفاض في فعالية انزيم الكاتليز (P>0.05)(P>0.01) في الحالات المعتدلة والشديدة مقارنة بمجموعة السيطرة .

كانت العلاقة ايجابية ومعنوية بين عامل نخر الورم الفا والاجسام المضادة IgE وMDA لدى المرضى المصابين بالالتهاب الانف المعتدلة /والشديد. فيما كانت العلاقة سلبية ملحوظة بين عامل نخر الورم الفا والاجسام المضادة IgE وانزيم في المرضى المصابين بالالتهاب الانف التحسسي والمعتدله/ والشديدة. وجد ان جهد التاكسد يحفزعلى انتاج عامل نخر الورم الفا والاجسام المضادة IgE لدى مرضى المصابين بالتهاب الانف التحسسي.

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**Introduction**

A

llergic rhinitis (AR) according to the document (Allergic Rhinitis and Its Impact on Asthma) is defined as a symptomatic disorder of the nose, induced after allergen exposure due to an immunoglobulin E (IgE)-mediated inflammation of the membranes lining the nose [1]. AR is a growing problem worldwide, where 10–30% of adults and up to 40% of children suffers from this condition [2].

 The classic symptoms of allergic rhinitis are rhinorrhoea, sneezing, nasal congestion and nasal itching [3]. Symptoms are often severe on waking, improve during the day, and worsen again in the evening. The nasal discharge is usually clear and watery. Sneezing can occur in paroxysms of as many as 10–20 at a time. Nasal congestion may cause the person to mouth-breathe, which dries the mouth, leading to ‘nasal’ Speech and halitosis (bad breath) [4]. ARIA classification of AR according to, Duration of symptoms: persistent and intermittent. Severity of symptoms: mild, moderate/ severe [5]. Mild AR (no impairment of, sleep ,daily activities, leisure or sport, school or work and symptoms not troublesome) . In Moderate/severe AR, One or more of the following criteria are present(sleep disturbance, impairment of daily activities, leisure and sport, impairment of school of work troublesome symptoms)[6].

 An etiology of allergic rhinitis develop as a result of an (IgE) mediated immune response to an inhaled allergen (allergens are antigens that induce and react with specific IgE antibodies). The allergic inflammatory cascade has three phases [7]. First (sensitization) in a susceptible person , initial contact with an allergen leads to the production of IgE antibodies against the allergen. These IgE antibodies bind to high-affinity receptors on mast cells and basophile [8]. Second (early-phase response) on further exposure to the allergen, sensitized mast cells are activated when two molecules of bound IgE are cross-linked by the allergen(antigen) [9].

 Third (late-phase response) over the next few hours the nasal mucosa is infiltrated by other inflammatory cells (e.g. eosinophils, neutrophils, basophils, T-cells). These release further inflammatory mediators, producing a sustained inflammatory reaction which may persist for hours or days. The predominant late-phase symptom is nasal congestion[10] Allergic rhinitis can be caused by (Common causes) :House dust mite, Pollens, Animals (Less common) Moulds [11].

 Previous studie indicated the changes in TNF-α and IgE levels in AR patient. [12] found tumor necrosis factor-alpha, interferon gamma, soluble inter cellular -1 and soluble vascular adhesion molecular-1 in bronchia asthma and allergic rhinitis: relation with disease severity .Other study reported the role of antioxidant in AR patient. [13]. Increased oxidative stress and altered antioxidants status in patients with chronic allergic rhinitis**.**In this study, the relationship between TNF-alpha, IgE and antioxidant was investigated in mild and moderate/severe cases of the disease.

**Patients and Methods**

This study was conducted in Babylon Maternity and Pediatric Teaching Hospital and in the laboratory of Biochemistry Department, College of Medicine , University of Babylon in the period starting from December 2012 to May 2013.Fifty patients with AR .These selected patients were divided into two groups according to severity of disease.

 The first: Mild group included 20 patients with mild AR, their age ranged between (20 - 25) years. Second moderate/severe group included 30 patients with moderate/severe AR, their age ranged between (20 - 25) years. Full history was taken from all patient which include: age, sex, residence, smoking, family history of allergic rhinitis , medical history drug history and surgical history, No drugs were prescribed to those patients that may interfere with the measured parameters.

 Control group is comrrised of forty with age rang (20-25) which approximately similar mean to age of patients, they were chosen as healthy people.they don't have any history of chronic disease, as diabetes mellitus, hypertension inflammatory disease such as rheumatoid arthritis and not smoking.

 Five milliliters of blood were obtained from patients and healthy person, then collected in tubes without anticoagulants and were left for 15 minutes at room temperature to clot. After that, the blood samples were centrifuged at 1500 xg for approximately 10 minutes. The serum was separated and divided into six eppendrof tubes ( 1ml) and stored at

(-20°C) until time of use. Serum TNF-α, IgE , were determined using ELISA kit provided by Kama Biotech, Korea, Inter Medical, Italy respectively. **Statistical analysis**

All statistical analysis was performed by using SPSS version 18 for windows. Data were expressed as Mean ± SD. The normality of the distribution of all variables was assessed by the Student′s F-test and Pearson correlation analysis that have been used to determine the significant difference between the two groups. P values less than 0.05 is considered significant.

**Results and Discussion**

The results in( table- 1) reveals a highly significant increase in the sera level of TNF-α in patients with allergic rhinitis in both mild and moderate/severe cases compared with those of control group .(P1<0.01)and (P2<0.01). as well as a significant increase(P3<0.01) between the mild and moderate/severe cases (P3<0.01) between the mild and moderate /severe cases.

 IgE levels were observed in the sera of moderate/severe patients in comparison with control group while a significant increment in IgE was seen in mild cases of AR patients compared with those of control group (p2<0.01) (p1<0.05) respectively . The same level of significancy was recorded between the mild and moderate/severe cases (p3<0.05) .

 The same trend was observed in MDA levels in the sera of patients and healthy control group.

 Serum catalase activity showed ahighly significant decrease in moderate/severe when compared with those of healthy subjects (P2<0.01) while significant decrease was observed between mild cases and control group (P1<0.05).

 The same level of significancy was recorded between mild and moderate/severe cases.

 The results in table (1) indicates the role of oxidative stress in the induction of TNF-α .Oxidative stress results in an imbalance between the oxidants and antioxidants defense mechanism which is in favor of injury that has been implicated in the pathogenesis of asthma and allergic rhinitis .This was confirmed by the concomitant decrease in catalase activity and increase in MDA levels in the patients with AR. A similar results were reported by Tass. et al [14] .Such changes were observed to cope with the severity of disease in the corresponding cases .

 Catalase is one of the major intarcellular antioxidant enzyme which is responsible for detoxifying the hydrogen peroxide produced under physiological conditions to O2 and H2O . Excessive H2O2 is harmful to almost all cell components [15]. Consumption of catalase in the protection of epithelial cells of respiratory tract was reported by investigator Marple B.F [16] . this enzyme protect the cells from H2O2 induced apoptosis. Thus, decreased enzyme activity of catalase is attributed to antioxidant action of enzyme leading consequently to enzyme depletion .

 The generation of reactive oxygen species (ROS) through normal cellular metabolism and by means of exogenous insults is a constant problem for which cells have developed multiple protective mechanisms to survive. Elevated levels of ROS such as hydroxyl radicals, superoxides and peroxides may induce a variety of pathological changes that are highly relevant in nasal and airway mucosa **[17].** These include lipid peroxidation, increased airway reactivity, and nasal mucosal sensitively and secretions , production of chemoattractant molecules and increased vascular permability . Such association between chronic inflammation and oxidative stress is well documented by many investigators [18,19].

 Lipid peroxidations leads to oxidative stress (represented by MDA levels) which stimulate the production of TNF-α. This is confirmed by the highly significant correlation between MDA and TNF-α (Table-2).TNF-α in turn ,stimulate promotion of IgE production by T-helper cells (Th)2 . infiltrate the nasal lining upon exposure to allergen and lead to a release of cytokines that promote IgE production. This is confirmed by the significant correlation between TNF-α and IgE (Table-3). Parris etal **[ 20]** reported that TNF-α causes changes in the ionized Ca influx within smooth muscle which lead consequently to the promotion of IgE production.IgE triggers the release of mediators such as histamine and leukotriene that are responsible for arteriolar dilation, increased vascular permeability ,itching ,rhinorrhea and mucous secretions .

 The unique idea in our work is the relationship among MDA, catlase, TNF-α and IgE since previous studies lack the correlations among those parameters and the results in table 2 and 3 confirmed such relationships among different parameters and the induction of TNF-α production since the latter was used as a therapeutic that is administered infranasally to patients with AR.

**Table 1** Biochemical parameters of allergic rhinitis and control groups.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **P values** | **Control****n=40**  | **Moderate/ Severe****n= 30** | **Mild****n=20**  | **Parameter** |
| **0.01\*\* P1 <****0.01\*\* P2<****0.01\*\* P3<** | 17.88**±**3.9713.70-25.2)) | 100.84**±**12.5590.2-124.1)) | 37.70**±**4.15 (34.7-50.1) | **TNF –α pg/ml** **Mean± SD****Range** |
| **0.05\*  P1<****0.01\*\* P2<****0.05\*  P3<** | 94.57**±**15.3045.30-110.5)) | 316.55**±**67.74200.6-430.5)) | 173.8**±**61.35101.40-270.2)) | **IgE IU/ml****Mean± SD****Range** |
| **0.05\*  P1 <****0.01\*\* P2<****0.05\*  P3<** | 2.31±0.172.1-2.71)) | 5.27±0.593.70-5.96)) | 4.142±0.473.40-4.95)) | **MDA μM****Mean± SD****Range** |
| **0.05\*  P1<****0.01\*\* P2<****0.05\*  P3<** | 28.3±0.56727.5-29)) | 23.92**±**1.2022.1-27.4)) | 25.4**±**1.1724.02-27.20)) | **Catalase kU/L****Mean± SD****Range** |

**P1= Mild Vs Control P2 = moderate/severe Vs Control P3 = Mild Vs Moderate / Highly significant \* Significant \*\* severe**

**Table 2** Pearson's correlation between the levels of catalase, MDA and TNF in different groups (n= 90)

|  |  |  |  |
| --- | --- | --- | --- |
|  | **TNF-α**  | **Groups** | **parameters** |
| **p** | **r** |
| **0.05** | **-0.533** | **mild** | **catalase** |
| **0.05** | **-0.54** | **Moderate/severe** |
| **0.05** | **-0.59** | **control** |
| **0.05** | **0.45** | **mild** | **MDA** |
| **0.01** | **0.601** | **Moderate/severe** |
| **0.05** | **0.66** | **control** |

**Significant = P< 0.05 high significant = P< 0.01**

**Table 3** Pearson's correlation between the levels of TNF-α and IgE each of mild , moderate/severe and control groups (n=90).

|  |  |  |  |
| --- | --- | --- | --- |
| **parameters** | **Groups** | **Number**  | **IgE** |
| **r** | **p** |
| **TNF-α** | **mild** | **20** | **0.63** | **0.01** |
| **TNF-α** | **Moderate/severe** | **30** | **0.78** | **0.01** |
| **TNF-α** | **control** | **40** | **-0.32** | **0.05** |

**Significant = P< 0.05 high significant = P< 0.01**

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