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The Role of TNF-α and Total IgE in Pathogenicity of Iraqi Fuel Stations Workers with Allergic Rhinitis

Mays Qasim Ahmed
Department of Biology, College of Sciences for Women, University of Baghdad, Baghdad, Iraq
mays.qassim2018@gmail.com

Talib Abdullah Hussein
Department of Biology, College of Sciences for Women, University of Baghdad, Baghdad, Iraq
talibah_bio@csw.uobaghdad.edu.iq

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Abstract

Allergic Rhinitis (AR) is a chronic immune system inflammation that occurs when the body overreacts to antigens in the environment (triggers) and produces a variety of symptoms in the nasal mucosa and paranasal sinuses due to the release of many interleukins and cytokines.

We investigated the influence of the allergen on serum levels of TNF-α and Total IgE in patients with AR and their function in initiating allergic rhinitis in Iraqi petrol station workers in Baghdad. One hundred individuals with allergic rhinitis and thirty healthy workers between the ages of 20 and 59 were examined. For immunological testing, sandwich ELISA was used to evaluate serum levels of TNF-α and Total IgE. A complete blood cell count (CBC) was used for hematological testing. Findings showed a positive connection between TNF-α and Total IgE, with a correlation of (r=0.32). The TNF-α binary logistic regression analysis (BLR) was conducted to be highly significant (B = 0.02, OR = 1.02, p =0.065), viewing that an increase of one unit in TNF-α increases the odds of belonging to the AR group. TNF-α had the highest area under the curve (AUC) compared to other markers, with a value of (AUC = 0.995), indicating that it is an excellent predictor and strong marker for AR, with a sensitivity and specificity of 94% and 100%, respectively. After our research, we reached the significance of the study from the clarity of the role of tumor necrosis factor-alpha in the disease and its development and the coincidence of its increase with immunoglobulin E and considering. It is also a diagnostic marker of the disease.

Keywords: TNF-α, Allergic Rhinitis, Eosinophil, gasoline, Risk factors, Total IgE.

1.Introduction

Allergic Rhinitis (AR) is a chronic, inflammatory, immunoglobulin IgE-mediated illness of the nasal mucosa produced by the inhalation of seasonal or perennial allergens. Typical symptoms

include sneezing, rhinorrhea, nasal congestion, nasal irritation, and signs of allergic conjunctivitis [1]. Due to the nature of AR symptoms, the illness is often ignored, misunderstood, and deemed insignificant, even though AR imposes a significant financial burden and negatively impacts sleep duration and quality, productivity, and quality of life [2].

Given the growing frequency of allergic respiratory illness over the last few decades and the rising air pollution associated with urbanization, industrialization, and fast economic expansion, there has been a long-standing interest in the relationship between AR development and air pollution. In addition, environmental variables impacting AR therapy and management techniques have drawn attention; in particular, the management of allergic rhinitis in metropolitan contexts with high levels of air pollution is a rising area of study interest [3]. The genesis of allergies is due to interactions between environmental influences and permissive genetic characteristics [4]. We concentrate on three steps that comprise the allergic rhinitis inflammatory cascade. First (sensitization), the first encounter with an allergen induces the development of IgE antibodies against the allergen in a susceptible individual. These IgE antibodies bind to receptors with high affinity on mast cells and basophils [5]. Second (early-phase reaction), with subsequent allergen exposure, sensitized mast cells are triggered when the allergen cross-links two molecules of bound IgE (antigen) [6].

In the following hours (late-phase reaction), the nasal mucosa is invaded by more inflammatory cells (e.g., eosinophils, neutrophils, basophils, T-cells). This releases more inflammatory mediators, resulting in a prolonged inflammatory response that may last for hours or days. Late-phase symptoms are dominated by nasal congestion [7]. Tumor necrosis factor-alpha (TNF-α) is a pleiotropic cytokine considered to have a significant key role in the pathogenesis of inflammatory disorders, such as allergies. TNF-α is created during the first phase of allergen sensitization and enhances the inflammatory cascade throughout the effector phase of allergic responses. [8].

Furthermore, during allergen exposure, sensitized epithelial barriers and immune cells generate TNF-α (such as macrophages, mast cells, and dendritic cells (. It can stimulate Th2 reactions. [9], causing high levels of IL- 4, IL-5, and IL-13, which promote trigger eosinophil's, mast cells, and basophils [10]. TNF-α is important in AR for the recruitment of eosinophils to the site of allergic inflammation by inducing adhesion molecules [11]. Eosinophils are a critical element of the pulmonary inflammation associated with allergic airway disorders. In addition to other measures, eosinophil counts were used to diagnosing AR [12].

The objective of this research is to shed light on (TNF-α and Total IgE) and their functions in the onset and progression of allergic rhinitis in Iraqi gasoline station workers by measuring their blood levels to find the correlation between them.

2.Materials and Methods

The research included (100) petrol station personnel with Allergic Rhinitis between the ages of 20 to 59 and a healthy control group (30) of station employees. The samples for the patients were obtained at Al-Rusafa and Karkh gas stations in Baghdad.

The sample was obtained between (October 2021 to April 2022). Diagnostic criteria for respiratory diseases and rhinitis are specialized in these conditions, clinically diagnosed following worldwide recommendations by meeting patients and healthy controls face-to-face. We provided all patients and healthy controls with a questionnaire including their information (name, age, stress, employment, accommodation location, the Period of treatment, infection with bacteria or Fungi, and date of the beginning of all symptoms).

Under sterile protocols, eight milliliters of blood were extracted from each patient using 10 milliliters of disposable plastic syringes. Blood was collected in a sterile Ethylene diamine tetra acetic acid (EDTA) tube for the hematological test (total WBC Count, Eosinophil). The tubes were passed through a roller mixer before hematological analysis using a Mindaray BC-5000 analyzer counter.

Blood was collected in a six-ml gel tube and allowed to clot at room temperature (20-25°C). After blood coagulation, the serum was centrifuged for 10 minutes at 3000 rpm before being divided into three equal quantities and kept at -20°C for immunological testing of Total IgE and TNF-α. Immediately after blood collection on EDTA, eosinophil and total WBC levels are determined. Utilizing the counting technique of the Mindaray Analyzer, their proportions were computed. Human Total IgE and TNF-α levels were determined using an ELISA kit manufactured by the Chinese company Cusabio. Whereas statistical analysis was done with conventional statistical methods, SPSS 26 and Excel 2016 were used to study and interpret the results, including the following:

I-Descriptive statistics (percentages, Median, observed frequencies, T-test, probability (P-value) on <0.05, mean, standard deviation, standard error (SE), and cumulative percentage and graphical display using bar charts)

II- Inferential Statistics: (These were used to determine if statistical hypotheses were accepted or rejected. They included: the receiver operating characteristics (ROC) analysis, Binary Logistic Regression, Chi-Square test ($\chi 2$), and Pearson Correlation by Relation coefficient).

3.Results and discussion

The research comprised (130) gasoline station employee samples. The distribution of study groups based on several factors is as follows:

3.1 The Distribution according to Age

This study demonstrated the correlation between the control and patient group's age. It was found that the individuals with the greatest AR prevalence are the age group (40-49) years (n = 31, 31.00 %). There were no significant differences in the incidence of age groups between the categories (P = 0.594), (P<0.05) (**Table 1**), (**Figure 1**)

Table 1. Frequency table for age group Chi-square tests of the relationships between each age category and the group.

Variable		AR	Control	Chi-squared	DF	Significance level
Age categories	(20- 29)	28 (28.00%)	11 (36.66%)	1.899	3	P = 0.594
	(30-39)	23 (23.00%)	5 (16.67%)			
	(40- 49)	31 (31.00%)	8 (26.67%)			
	(50-59)	18 (18.00%)	6 (20.00%)			
	Total	100 (100%)	30 (100%)			

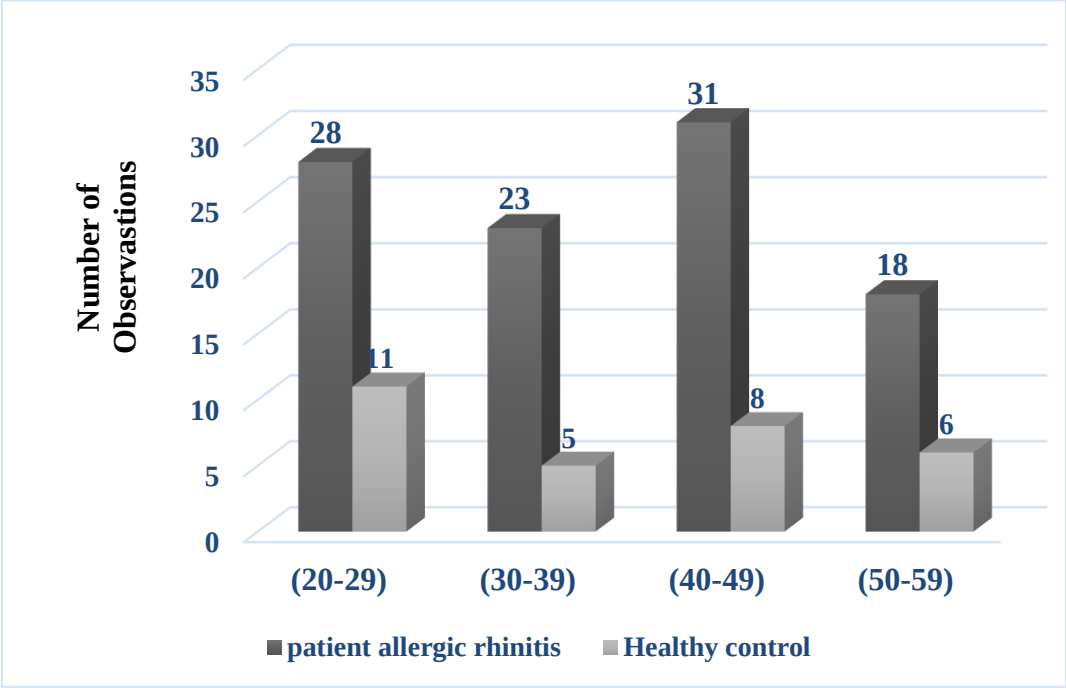

Figure 1. Demonstrates the distribution of age groups among the patient and control groups with the greatest degree of allergies to rhinitis (40-49 years).

3.2-Immunological and hematological results

There was a significant increase in AR patients compared to healthy controls. Compared to the control group, blood total WBC was significantly higher in AR patients (8.20 ±1.67 10^9/L) than in the control group (7.10± 1.00 10^9/L) with p-value (P<0.001) on (P<0.05). In addition, the eosinophil count means for the patient was (3.63 ±1.66%), whereas the mean for the control was (1.66± 0.77%). There was a highly significant increase in AR relative to the control group (P=0.001) on (P<0.05). In contrast, TNF-α had a mean of (78.67±44.63 Pg/ml) for AR and (48.03±6.63 Pg/ml) for health workers. It increased significantly in patients compared to the control group (P<0.001). Also, there was a significant rise in the total IgE level in patients (P=0.02), which was (314.38± 112.01 IU/ml) in the patient group compared to (278.37± 54.87IU/ml) in the healthy group (**Table 2**).

Table 2: Descriptive statistics for WBC, Eosinophil, TNF-α, and T-IgE Using t-Tests to compare mean differences between AR patients and control workers.

n=number, SEM= Standard Error for Mean , t= T-test , P=P-value , M=Mean , SD= Standard deviation

Parameters	group	n	Median	Mean± SD	SEM	*t*	P
WBC	AR	100	7.90	8.20± 1.67	0.17	4.39	<0.001*
	Control	30	7.19	7.10± 1.00	0.19		
Eosinophil	AR	100	3.20	3.63± 1.67	0.17	9.009	< 0.001**
	Control	30	1.45	1.66± 0.77	0.14		
T-IgE	AR	100	347.98	314.38± 112.01	11.26	2.371	0.02*
	Control	30	289.80	278.37± 54.87	10.19		
TNFα	AR	100	69.06	78.67±44.63	4.46	6.63	< 0.001*
	Control	30	48.23	48.03±6.63	1.21		
(P<0.01)**= Highly significant							

3.3 Pearson Correlation Analysis

A Pearson correlation analysis determined the relationship between WBC, eosinophil, total IgE, and TNF-α. The correlations between T-IgE and TNF-α showed a significant positive correlation (r=0.32) and effect size (p = 0.007, 95% CI = [0.13, 0.49]). This indicates that T-IgE levels increase simultaneously with TNF-α. No more associations of significance were discovered. The findings of the correlations are shown in (**Table 3**).

3.4 Binary Logistic Regression (BLR)

A binary logistic regression analysis was performed to determine if WBC, eosinophil, total IgE, and TNF-α had a statistically significant impact on the likelihood of belonging to the AR group. The control group acted as a point of reference for the group. Variance inflation factors (VIFs) were used to assess the absence of multicollinearity between predictors. All predictors in the regression model have VIFs less than 10, which are considered acceptable [13].

Statistically demonstrating WBC, eosinophil, total IgE, and TNF-α significantly impacted the likelihood of belonging to the AR group. The binary logistic regression model was statistically significant, with $\chi 2$ (4) = 60.21, p< 0.001, and McFadden R2 = 0.43. The McFadden R2-squared value for this model was (0.43), suggesting that the model is well-fitting. The WBC had a statistically significant effect on the likelihood of being in the AR group (B = 0.38, OR = 1.46, p =0.105). Eosinophil showed a very considerable influence (B = 1.45, OR = 4.27, p<0.001), indicating that an increase of one unit in eosinophil increased the likelihood of belonging to the AR group. The effect of TNF-α was significant (B = 0.02, OR = 1.02, p = 0.065), indicating that a one-unit increase in TNF-α increases the likelihood of belonging to the AR group. The effect of total IgE was significant (B = 0.0003, OR = 1.00, p = 0.920), indicating that an increase in total IgE is associated with an increased risk of developing rhinitis. The findings of the regression model are given in (**Table 4**).

Table 3. The results illustrate the Pearson correlation (correlation coefficient) between T-WBC, Eosinophil, TNF-α, and T-IgE.

Spearman Correlation Results Among WBC, Eosinophil ,T-IgE, and TNFα				
Combination	***r***	**95.00% CI**	***n***	***p***
T-WBC -Eosinophil	0.21	[0.01, 0.39]	100	0.168
T-WBC -T-IgE	0.07	[-0.13,0.26]	100	0.964
T-WBC -TNFα	0.03	[-0.17, 0.22]	100	0.964
Eosinophil -T-IgE	0.15	[-0.05, 0.34]	100	0.388
Eosinophil -TNFα	0.21	[0.02, 0.39]	100	0.168
T-IgE -TNF-α	0.32	[0.13, 0.49]	100	0.007*
The relationships were analyzed with an alpha value of 0.05. r: Pearson Correlation P: probability				

Table 4. Logistic regression findings with WBC, Eosinophil, TNF-α, and Total IgE predicting collection, showing that the eosinophils was highly significant p<0.001.

Logistic Regression Results with WBC, Eosinophil, IgE, and TNFα Predicting group						
Variable	**B**	**SE**	**χ^2**	**p**	**OR**	95.00% CI
(Intercept)	-6.63	2.18	9.29	0.002	-	-
WBC	0.38	0.23	2.62	0.105	1.46	[0.92, 2.30]
Eosinophil	1.45	0.35	17.69	< 0.001*	4.27	[2.17, 8.40]
IgE	0.0003	0.003	0.01	0.920	1.00	[0.99, 1.01]
TNFα	0.02	0.01	3.40	0.065	1.02	[1.00, 1.05]
Note. $\chi^2(4) = 60.21$, $p < 0.001$, McFadden $R^2 = 0.43$.						

Note : OR=Odd Ratio, CI=Confidence interval

3.5 The Receiver Operating Characteristics (ROC)

The receiver operating characteristics (ROC) curve was constructed to assess each AR parameter's predictive value and decide the cut-off value. The areas under the curve (AUC) for each predictor were determined. To compare the diagnostic abilities of two or more tests or to estimate the capacity of two or more markers for the same illness, this statistical model (ROC curve) is helpful. It can distinguish between two or more tests when the area under the ROC curve is considered. There is a variety of 0 to 1 from areas under the curve in this simulation. To put it another way, statistically, the values of 0, 0.5-0.6, 0.6-0.7, 0.7-0.8, 0.8-0.9, more than 0.9, and 1 in the ROC curve are perfect. These numbers mean (an inaccurate test, a failed test, a poor test, a fair test, a good test, an excellent test, or a perfectly accurate test) respectively [14]. The findings of the ROC curve indicated that TNF-α had the highest AUC compared to other parameters with (AUC = 0.995), making it an excellent predictor and strong marker for diagnosing AR with a sensitivity and specificity of 94% and 100%, respectively when the cut-off value was (>61,397). The second-largest AUC was for Total IgE (0.924), with a sensitivity and specificity of 83% and 96.67%, followed by Eosinophil (0.892), with a sensitivity and specificity of 76.00% and 96.67%. All are regarded as excellent indicators. These are outlined in (**Table 5**) and, (**Figure 2**).

Table 5. ROC analysis for the research parameters (WBC, TNF-α, Total IgE, and Eosinophil), the assessment of the areas under the curve (AUC), (the closer the value is to1, the better).

Variable	AUC	SE	95% CI	Cut off	Sensitivity	Specificity
WBC	0.679	0.0483	0.592 to 0.758	>8.2	44.00	93.33
Eosinophil	0.892	0.0309	0.825 to 0.939	>2.6	76.00	96.67
T-IgE	0.924	0.0230	0.864 to 0.963	>348.933	83	96.67
TNF-α	0.995	0.00329	0.963 to 1.000	>61.397	94	100

Standard Error (SE), Confidence interval (CI) and best Cut-off value.

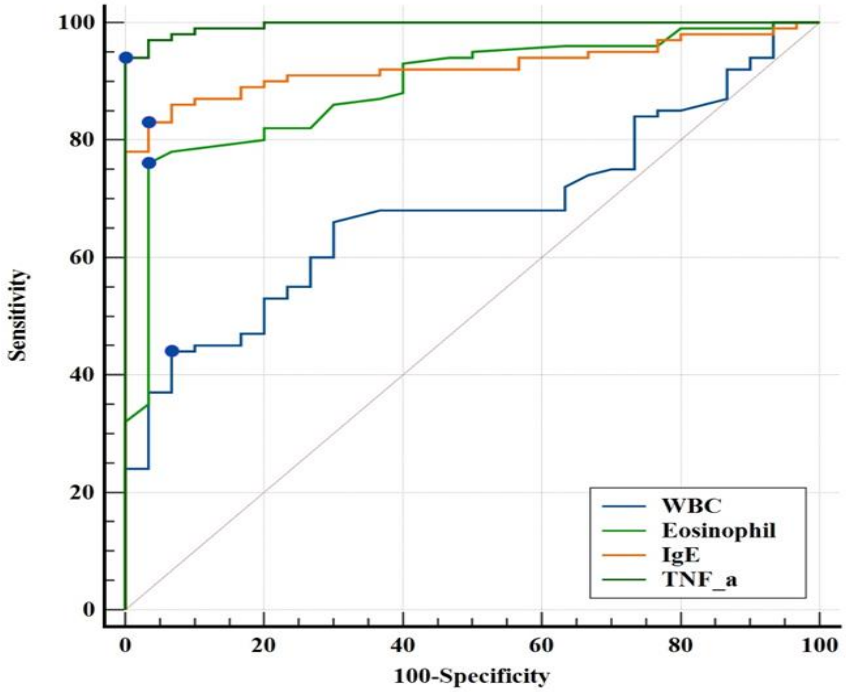

Figure 2. compares the ROC curves of the research parameters (WBC, TNF-α, Total IgE, and Eosinophil) as they differentiate between **the** patient and the control group.

4.Discussion

This study investigated the connection between patient and control groups' age. The age group with the highest prevalence of rhinitis allergies is between the ages of 40 to 49 years. These results support Ava and Wasim's claims [15].

Consistent with earlier results, the absolute number of total WBCs in AR patients was considerably higher than in the control group [17]. In contrast, Hasoon and Hussein [18] discovered WBC within normal limits without significance in AR. Allergic rhinitis patients may have abnormalities in hematological markers because of systemic inflammation [16]. The allergic process causes an increase in the number of eosinophil cells and B cells, which aid in the generation of plasma cells that generate IgE. This development may lead to a rise in WBC cells [17].

In this study, we significantly increased the percentage of eosinophils in patients than in the control, which was accepted [18]. And according to studies [19]. In AR and after allergen exposure, the inflammatory late-phase response may increase circulating blood eosinophils [20].

After DC presents antigen to T helper2 cells (Th2), the latter produces Th2 cytokines (IL-4, IL-5, and IL-13), which have an essential role in the pathogenesis of allergic inflammation and the development of allergy sensitization [21]. Where TNF-α levels were significantly increased as predicted, there were comparable findings in our study with the study of Al-khyat, T.H.A et al., [22] and with Alnahas et al., [23]. Moreover, sensitized epithelial barriers and immune cells such as; macrophages, mast cells, and DCs generate TNF-α in response to allergen exposure. It can stimulate Th2 responses [9]. TNF-α has been shown to increase IgE production by altering the ionized Ca influx into smooth muscle. Arterial dilatation, increased vascular permeability, itching, rhinorrhea, and mucus discharges are caused by mediators like histamine and leukotriene that are released in response to IgE [24]

Allergen-specific IgE is produced when cytokines interact with B lymphocytes to cause the release of IgE, which then binds to a high-affinity IgE receptor (FcεRl) on mast cells to release histamine and other chemokines, inflammatory cytokines, and other mediators of AR. one of important these cytokines was (IL-4) because it promotes the production of IgE. Therefore, in addition to clinical criteria, measuring total and specific IgE levels is recommended for diagnosing AR and asthma [25, 26]. Our results showed a highly significant total IgE rise in patients' AR compared to healthy controls. This finding agrees with Al-khyat, T.H.A, *et al.* [22] and Hussein *et al.* [27].

5.Conclusions

We established the importance of tumor necrosis factor alpha. It was a significant and close relationship with the disease development since it was increased in the patient, accompanied by an increase in total immunoglobulin E. We considered it an excellent diagnostic factor for the disease, and the results of our research confirmed this. In the future, we hope to find treatments to reduce the levels in the blood and, thus, reduce the rate of the disease.

Because allergic rhinitis was type I, hypersensitivity was linked essentially with immunoglobulin E. Finally, the recovery of AR patients depends on several factors, including environmental, psychological, and physical, which we hope to consider in the future in dealing with the disease.

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