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Invastigate The Serum Level of Interleukin-4 In Patients with Chronic Rhinosinositis with Nasal Polyps and Healthy Individuals

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ABSTRACT

Objective: Interleukin-4 plays several biological roles, including stimulating the proliferation of activated B and T cells and differentiating B cells into plasma cells. This study aimed to determine whether the etiology of chronic rhinosinusitis with nasal polyps (CRSwNPs) is associated with serum levels of interleukin-4. Patients and Methods: a blood samples from 35 Iraqi patients—21 men and 14 women—who had nasal polyps as well as a control group of 35 healthy volunteers were collected. The serum was then separated in a plain tube, and an enzyme-linked immunosorbent assay method was used to determine the optical density of Interleukin-4 in the serum (ELISA) Results: Serum levels of interleukin-4 were significantly higher in the patient group compared to the control group ($P < 0.001$). There was no significant correlation between serum interleukin-4 levels and age or gender in either group. Conclusions: Individuals who have NPs had elevated degrees of IL-4.

Keywords: Serum, ELISA, Interleukin-4, Nasal Polyps.

1 Introduction

A subset of chronic rhinosinusitis is known as chronic rhinosinusitis with nasal polyps, which is a chronic upper respiratory tract condition that develops from significant irritation of the nose and paranasal sinuses [1]. Depending on how much eosinophil infiltration there is in the nasal tissue, CRSwNP may be further divided into eosinophilic and noneosinophilic groups [2]. The clinical illness and concomitant asthma are more severe in eosinophilic CRSwNP [3].

Furthermore, Numerous research has shown a significant rise in the percentage of Among Asians with eosinophilic CRSwNP nations, with at least 50% of NP now exhibiting tissue eosinophilia and a T helper type 2 (Th2) cytokine profile [4]. People that suffer from CRS have a markedly reduced quality of life due to reduced health value, mental anguish, reduced physical and social engagement. The yearly cost of disease-related expenses is estimated to be $6 billion [5,6].

Referred to in historical records as far back as 2000 BC, this condition is one of the earliest ear, nose, and throat ailments known to science. Hippocrates, the father of medicine, gave these nasal nodules the name "polypus". Although it often poses little danger to life or physiological function, CRSwNP significantly lowers quality of life [7]. The normal diagnostic age range is 40-60 years, while the average age of onset for chronic rhino sinusitis with nasal polyp is 42 years. Face discomfort, purulent nasal discharge, nasal blockage, decreased sense of smell during persistent inflammation, and endoscopy-confirmed nasal polyp presence are among the symptoms. These signs persist for longer than 12 [8]. Numerous gene groups have an impact on how chronic rhinosinusitis develops. The CFTR locus gene, HLA genes, genes relating to innate immunity, TH2-inflammatory response development, tissue remodeling in the paranasal sinus, metabolism of arachidonic acid, cells that genes xenobiotic transformation, and other pro-inflammatory genes are among them [9]. Inheritance is a classic indicator of the genetic component in the creation of CRS. Although studies verifying CRS heredity are lacking, a long-standing theory proposed a

genetic foundation for CRS development [10]. A study revealed that relatives were of the first degree of CRSwNP Patients have a 4.1-fold increased likelihood of have nasal polyps [11]. It is not quite clear what the underlying processes are that lead to the persistent Inflammation of the sinuses seen in persistent rhinosinusitis with nasal polyps. Numerous research teams have concentrated on investigating the potential roles that infections, the host immune system, and sinusoidal epithelial cells may have in the pathophysiology of CRSwNP [12]. It is believed that a number of flaws the natural role of the airway epithelial barrier, such as reduced antimicrobial product expression and barrier integrity loss, along with bacterial and fungal colonization, probably play a significant part in the development of chronic inflammation in CRSwNP [13].

At least in the Europe and the US, CRSwNP, among the CRS subtypes with the most study, is generally considered to have a type 2 inflammatory environment. Type 2 inflammatory response is a response that is mostly brought on by the type 2 cytokines IL-4, IL-5, and IL-13. Mast cells in large quantities, basophils, and eosinophils infiltrate the area during such inflammations. In individuals with CRSwNP, nasal polyps exhibit higher amounts and more active Thymic stromal lymphopoietin (TSL), a crucial cytokine generated from epithelial cells in activating type 2 responses, in comparison to healthy sinonasal tissue [14]. This highlights the role that type 2 inflammation plays in development of eosinophilic CRSwNP. Several immune cells, such as Th2 cells, mast cells, and group 2 innate lymphoid cells (ILC2s), may generate IL-4. In these cell types, both innate and adaptive signals may generate type 2 cytokines [15, 16]. Saline rinses, biologics, macrolide antibiotics, and oral corticosteroids are some of the other options for treating nasal polyposis. The long-term usage of intranasal corticosteroids is also important [17]. Although surgical therapy of nasal polyps offers a valuable therapeutic option after conservative measures have been exhausted, it is not a curative measure since over 40% of patients have polyposis recurrence six months following surgery. Since there haven't been many notable advances in the surgical and medicinal therapy of CRSwNP in the last ten years, the high risk of recurrence calls for more study into the pathophysiology and management of the condition [18]. This study aimed to determine whether the etiology of chronic rhinosinusitis with nasal polyps (CRSwNPs) is associated with serum levels of interleukin-4.

2 Material and Methods

This research was carried out at the AL-Diwaniyah Teaching Hospital's Otorhinolaryngology Department. Under the direction of of ENT experts and blood were taken from 35 healthy persons and 35 patients with nasal polyps and chronic rhinosinusitis, including 21 male and 14 female patients with CRSwNP. When the study's controls were enrolled, they had no systemic illness, allergy symptoms, CRS, atopy, or any kind of rhinitis. A 2.5 ml sample of aseptic venous blood was extracted using a disposable syringe, placed in a gel tube, and allowed to clot. The serum was then separated by centrifugation at 1500 rpm for five minutes. The serum was drawn into a plane tube and kept at 20 degrees Celsius so that the ELISA test could measure the amount of IL-4 in the serum.

2.1 Assessment of cytokine levels by ELISA

Using an ELISA kit (Sandwich enzyme immunoassay) and IL-4 levels were determined by following the manufacturer's instructions. In a nutshell, the kit's plate came pre-coated with an IL-4-specific antibody. After adding the samples, blank, and standard to the ELISA plate, it was incubated for 90 minutes at 37°C. After draining each well's contents, add the Biotinylated Detection Ab working solution and incubate at 37°C for one hour. After aspirating the solution, repeat three plate washes, add the HRP conjugate, and continue incubating for an additional thirty minutes. After aspirating the solution, repeat five plate washes, add the TMB substrate solution, and let it sit for fifteen minutes. Put a stop solution in every well. After then, the color instantly becomes yellow, and the microplate reader measures it at 450 nm.

2.2 Calculation of results

The duplicate reading average for every standard and sample's optical density was used to generate the ELISA findings. Plotting the y-axis concentration versus the x-axis mean optical density (OD) value for each standard and creating a best curve between graph's points is next step in creating a standard curve.

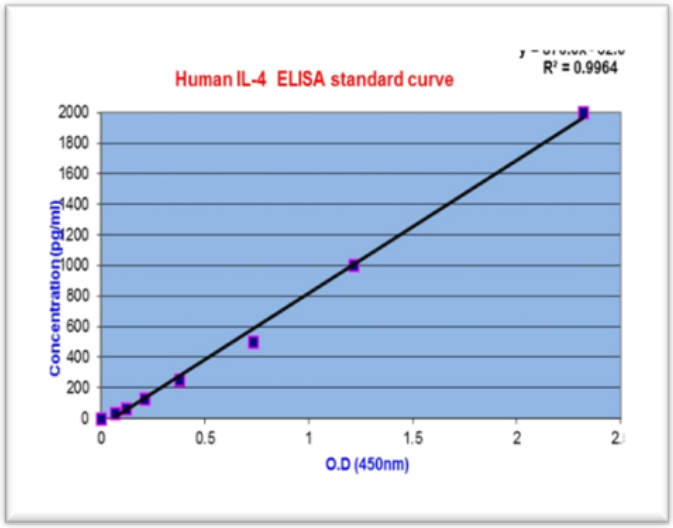

Fig. 1. Standard curve of Interleukin-4.

3 Results

3.1 *Patients' profiles*

The study included 70 individuals, including 35 cases and 35 controls, ages ranged from 20 to 60 years, with a mean age of 39.91 ± 10.19. P = 0.905 and P = 1.000, respectively, indicated no significant differences in age or gender between these two groups.

3.2 *Cytokine profile of patients with CRSwNP versus controls*

The median IL-4 levels in the patients' serum were 132.27 pg/ml compared to 38.44 pg/ml in the control group (P = 0.001), indicating a significantly higher level in patients

4 Discussion

In chronic rhinosinusitis , the pathophysiology of nasal polyps is yet unknown [19], According to this research, compared to healthy controls, patients with CRSwNP exhibited greater levels of IL-4 in their sera.

The explanation behind this is that IL-4 have an important part in halting death of activated T cells by promoting the transformation of naïve Th0 lymphocytes into Th2 cells. The primary cytokine that causes B-cell immunoglobulin class switch to the IgE phenotype is IL-4 [20]. IL-4 also stimulates eosinophil migration by increasing the expression of VCAM-1 on human endothelial cells.

Table 1. Serum *IL-4* in control and patient's groups.

Serum IL-4	Patients N=35	Control N=35	P
Median	132.27 (66.00)	38.44 (38.00)	<0.001 £
Range	31.00 -235.00	3.67 -93.00	HS

IQR: inter-quartile range; £: Mann Whitney U test; HS: highly significant difference at P $\leq$ 0.01

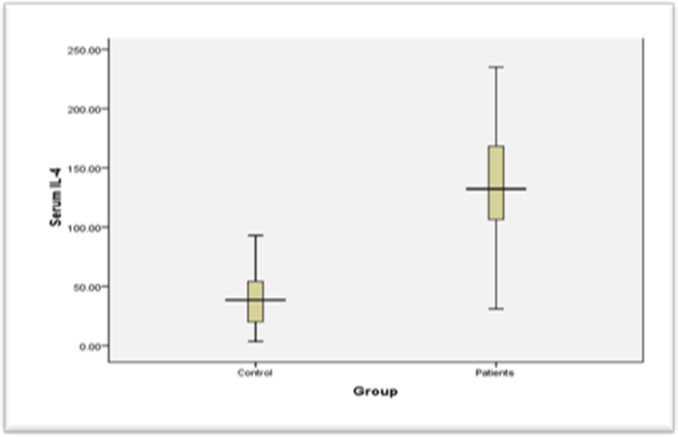

Fig. 2. Serum IL-4 in control and patient's groups

It has been shown that nasal polyps and sample taken from individuals with disease contain mRNA transcription of IL-4 and receptor (IL-4R) [21]. Several comparative studies have shown that nasal polyps (NPs) exhibit higher IL-4 concentrations compared to controls. Ozkara et al., [22], Serum IL-4 levels in nasal polyps and controls were compared. They discovered that NPs and allergic rhinitis patients had statistically greater levels of IL-4 than in the control group.

Steinke et al., [23], shown that greater levels IL-4 in CRSwNP with allergy and aspirin-exacerbated respiratory illness Therefore, it seems that IL-4 is exclusively harmful in NPs subgroups with the AERD phenotype or in cases where allergies.

Du et al., [24], also discovered that following endoscopic sinus surgery, the levels of IL-2, IL-4, IL-5, and IL-17 in NPs patients remained elevated. Furthermore, a correlation has been than the control seen between the severity of NPs and the scores on (SNOT-22) and higher levels of IL-2, IL-4, and IL-22 [25]. Another research showed that compared to controls, A. flavus-positive NPs patients had considerably lower serum levels of the cytokine IL-4 [26]. a study done by Ekinci and Ozcan, who discovered no statistically significant variations in the level of IL-4 (3.25 pg/ml vs. 3.38 pg/ml) in the control and the NPs (p=0.799) [27]. Nagarkar et al., [28], found that there was no discernible difference in the two groups' blood levels of IL-4 in their investigation of NPs.

5 Conclusions

The current findings demonstrated that the patients' blood levels of IL-4 were greater than those of the control group, which may help identify NPs patients and forecast the degree of the illness's intensity.

The mean age of patients and the controls did not vary statistically. Furthermore, in comparison to the control, adult patients specifically, those in the 30-39 and 40-49 year , age ranges—had the greatest prevalence of nasal polyps.

Conflict of Interest: The authors declare no conflict of interest.
Financing: The study was performed without external funding.
Ethical consideration: The study was approved by University of Al-Qadisiyah, Al-Qadisiyah, Iraq.

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