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Original article

Evaluation of serum levels of Interleukin -1 and Tumor Necrosis Alpha (TNF-α) and their nucleotide polymorphisms in patients with chronic rhinosinusitis

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ABSTRACT

Background: To evaluate Interleukin-1 and TNF- α serum levels and their single nucleotide gene polymorphisms (SNPs) association in patients with chronic rhinosinusitis. **Methods*:*** A case-control study performed between June 2022 and March 2023 at Benha University Hospitals, Benha, Egypt. 50 patients with chronic rhinosinusitis (CRS) with nasal polyps (Group 1), 50 patients with CRS without nasal polyps (Group 2), and 50 healthy volunteers as the control group (Group 3). TNF-α and IL -1 serum levels were detected by enzyme-linked immunosorbent assay (ELISA). Gene polymorphisms of cytokines IL-1 and TNF-α were delineated through the application of polymerase chain reaction - Restriction fragment length polymorphism (PCR-RFLP) methodology. **Results*:*** Serum concentrations of TNF-α and IL-1 exhibited markedly elevated levels in both groups 1 and 2 when compared with the control group, as evidenced by a highly significant statistical difference [p<0.0001]. Significant genotype distribution of TNF-a 308G>A SNP among (group 1,2) and controls (p < 0.001). The frequency of the GA genotype was found to be more prevalent in group 1 at 48% and group 2 at 50%, compared to a notably lower prevalence of 6% observed in the control group (group 3). Allele-A was significantly higher in groups 1 and 2 than in group 3. No significant differences were found between IL-1A (+4845G/T) genotype distributions in groups 1 and 2 compared to group 3 (p = 0.093). The IL-1B (−511C/T) polymorphism shows a notable correlation in both groups 1 and 2 when compared to the control group (p < 0.001). **Conclusion*:*** Significantly higher TNF- α and IL-1 serum levels and TNF-α and IL-1B (-511C/T) polymorphisms with CRS with or without nasal polyps. Both IL-1B and TNF-α may be involved in the regulation of CRS etiopathogenesis and could represent novel therapeutic targets for its management.
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Introduction

Rhinosinusitis pertains to the inflammatory process involving the nasal cavities as well as the adjacent paranasal sinuses. Chronic rhinosinusitis (CRS) exerts a considerable adverse effect on patient quality of life and incurs substantial

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healthcare expenditures. CRS, a pervasive chronic inflammatory disorder of the sinonasal epithelium.

Epidemiological data indicate the high prevalence of CRS in the general population, up to 12.5% [1]. Another study states that 2.7% to 6.6% of the population are thought to have chronic rhinosinusitis, and the incidence increases in patients with chronic inflammatory lower respiratory disease mainly in association with bronchial asthma and chronic obstructive pulmonary disease (COPD). Chronic sinusitis is present in about 40–75 % of patients with asthma [2].

Chronic rhinosinusitis (CRS) may manifest as one of three major clinical syndromes: CRS without nasal polyps, CRS with nasal polyps, or allergic fungal rhinosinusitis. These classifications possess a great deal of therapeutic significance [3].

Contemporary understanding acknowledges that rhinosinusitis extends beyond a simplistic infectious paradigm, representing a multifaceted and complex disorder. At the core of the pathogenesis of inflammatory conditions, such as rhinosinusitis, lies the synthesis of cytokines, which is mediated not only by immune cells but also by other cellular entities including epithelial cells and fibroblasts. These cytokines are instrumental in mediating the inflammatory response characteristic of this condition. The inflammatory response within the sinonasal mucosa induces mucosal edema, which subsequently obstructs the ostia of the sinus, culminating in mucus retention and consequent infection, thereby facilitating the progression of CRS. Contributory factors associated with this pathology include allergies, atopic conditions, asthma, cigarette smoking, sensitivity to aspirin, and genetic predispositions [4].

Kamath *et al.* [5] found that *Staphylococcus aureus* (*S. aureus)*, MRSA, and *Klebsiella* were the most frequent organisms encountered and anaerobes do not play a prominent role. **Ramakrishnan et al**. [6] found existing evidence lends further support for the role of biofilms (particularly the *S. aureus* phenotype) in more severe, recalcitrant disease and poorer surgical outcomes. Till now the exact bacterial pathogens of CRS remain a matter of debate.

Interleukin 1α and IL-1β, pivotal immunomodulatory proteins, are secreted by a diverse array of activated cells including macrophages, monocytes, epithelial cells, natural killer cells, and fibroblasts. These cytokines initiate the inflammatory cascade through a common receptor pathway. Alongside IL-6 and TNF-α, IL-1α and IL-1β constitute a fundamental triad of inflammatory mediators that orchestrate the body's physiological response to inflammatory stimuli [7].

In the context of rhinosinusitis, IL-1α, liberated from compromised epithelial cells, potentiates the activation of monocytes and T-cells. This cytokine significantly upregulates the expression of adhesion molecules, notably ICAM-1 and VCAM-1, on monocytes, which are critical for the eosinophil recruitment process. Concurrently, IL-1β amplifies the release of Chemokine (C-C motif) ligand 5 (CCL5) from fibroblasts within nasal polyps, fostering the attraction and trans-endothelial migration of eosinophils, monocytes, and memory T-cells to the inflammatory locus. Beyond its pro-inflammatory roles, IL-1β also induces glucocorticoid resistance in nasal polyp tissues, thereby complicating the efficacy of localized treatments [8].

TNF-α amplifies inflammatory responses by (1) promoting the congregation and accumulation of eosinophils via the enhancement of VCAM-1 and the chemokine eotaxin within nasal polyps, and (2) augmenting the expression of CCL2, a critical chemoattractant for monocytes, within fibroblast cultures originating from nasal polyps. Of particular significance, heightened serum concentrations of TNF-α are consistently observed across the diverse array of chronic rhinosinusitis endotypes [9].

Ultimately, the advent of novel pharmacological formulations has markedly expanded the therapeutic arsenal available for the pharmacologic management of CRS. Continued exploration into the cytokine expression profiles within inflammatory sinus disorders promises to enhance our comprehension of their pathophysiological underpinnings and facilitate the innovation of new therapeutic strategies [10].

The current investigation aims to assess the serum levels of interleukin 1 and TNF-α, and to explore the correlation between their single nucleotide gene polymorphisms among patients suffering from chronic rhinosinusitis (both without and with nasal polyps) compared to a control group.

Patients and methods

Patients

This case-control investigation was conducted on a cohort of 150 participants from June

2022 and March 2023. The participants were categorized into:

- **Group 1**: 50 patients of chronic rhinosinusitis with nasal polyps (CRSwNP).
- **Group 2**: 50 patients of chronic rhinosinusitis without nasal polyps (CRSsNP).

Patients were non-randomly selected and after they were diagnosed with rhinosinusitis were evaluated at the outpatient clinic of the department of Otorhinolaryngology at Benha University Hospitals they were put into both study groups either chronic rhinosinusitis with nasal polyps (CRSwNP) or without nasal polyps (CRSsNP). The diagnostic confirmation of CRS was meticulously achieved through comprehensive clinical anamnesis and was further substantiated by direct observation via nasal endoscopy, adhering rigorously to the diagnostic criteria stipulated by the European Position Paper on Rhinosinusitis and Nasal Polyps (EPOS) 2020 [11] Additionally, patients with a history of polypectomy, where the diagnosis was confirmed by pathological reports, were also included.

Nasal symptoms were assessed in accordance with the European Position Paper on Rhinosinusitis and Nasal Polyps (EPOS 2020) [11] Additionally, the survey gathered information on the history of previous sinus surgeries, duration of symptoms, and predisposing factors for CRS, including family history of nasal polyps, presence of atopy, allergies, asthma, and aspirin intolerance. Detailed findings from nasoendoscopy were recorded, noting the presence and grading of nasal polyps as per Lund's criteria [12], obstructions in the middle meatus, mucopurulent discharge, and mucosal edema.

Subjects afflicted with concurrent significant medical pathologies—such as diabetes mellitus, renal, hepatic, or cardiovascular conditions—were precluded from participation in this study. Furthermore, exclusion criteria extended to those suffering from either acute or chronic systemic or dermatological infections, existing autoimmune or systemic allergic disorders such as atopic disease, malignancies, or any form of immunosuppression. Specific pathologies warranting exclusion also included diagnosed cases of inverted papilloma, young syndrome, Kartagener syndrome, or antrochoanal polyp.

- **Group 3**: Control group

This cohort non randomely comprised 50 healthy volunteers, meticulously matched with the patient group in terms of age and sex.

This investigation received ethical clearance from the Ethical Committee of the Faculty of Medicine at Benha University (REC-FOMBU), Egypt, under the approval code RC-12-5-2023. Conducted in strict adherence to the Helsinki Declaration of 1975, as amended, the study ensured compliance with international ethical standards. Written informed consent was duly obtained from all participants.

The study was undertaken at the Department of Medical Microbiology and Immunology, located within the Faculty of Medicine at Benha University in Benha.

Methods

A standardized questionnaire was employed to collect comprehensive data from all participants. This encompassed demographic parameters like gender, ethnic background, age at diagnosis, and historical tobacco use.

Blood samples: A 5 ml specimen of whole venous blood was meticulously drawn under conditions of stringent asepsis. One-half of the sample was placed into an Ethylenediaminetetraacetic acid (EDTA) tube. The remaining portion was transferred into serum separator tubes and subjected to centrifugation at 1000 x g for 15 minutes. Subsequently, all samples were preserved at -80°C until further analysis.

Detection of TNF-α, and IL -1concentrations by the ELISA test

The quantitative assessment of IL-1 and TNF-α concentrations was conducted employing ELISA kits from BioLegend's LEGEND MAX, based in San Diego, CA, USA. The assays were conducted in strict accordance with the protocols stipulated by the manufacturer.

Detection of TNF-α, and IL -1 single nucleotide polymorphisms (SNPs) by PCR-RFLP test

DNA extraction: DNA extraction using QIAamp® DNA Mini Genomic DNA Purification kit (QIAGEN) and the procedure was done according to the manufacturer's instructions.

For TNF-a 308G>A SNP -:- The primers used for amplifications are shown in **table (1)** [13]. Amplification was carried out using a Biometra thermal cycler based in Germany. Each 50µl reaction tube was provisioned with the following reagents: 25µl of PCR Master Mix, utilizing the 2X

TOPsimple™ DyeMIX-nTaq kit (Enzynomics, Korea), 2.5 µl of the forward primer, 2.5 µl of the reverse primer, 5 µl of template DNA, and 15 µl of nuclease-free water. The thermal cycling regimen was initiated with a preliminary denaturation phase of genomic DNA at 95°C, extending over a period of seven minutes. Subsequently, the protocol comprised 35 cycles, with each cycle incorporating a denaturation phase at 95°C for 30 seconds, a primer annealing phase at 60°C for 30 seconds, and an elongation phase at 72°C for 45 seconds. The sequence of amplification culminated in a final elongation phase maintained at 72°C for an extended duration of 10 minutes.

The PCR amplicons were subjected to enzymatic digestion with 10 units of NcoI enzyme at 37°C overnight, in strict adherence to the protocol provided by BioLabs, New England. The digestion products were subsequently separated on a 4% agarose gel. The wild-type G-allele PCR products, in their homozygous state, underwent cleavage into two distinct fragments measuring 192 and 20 base pairs. Conversely, the homozygous variant A-allele PCR products remained unfragmented at 212 base pairs. Meanwhile, the heterozygous GA genotypes resulted in three disparate fragments of 212, 192, and 20 base pairs **(Figure 1)**.

Figure 1: -Restriction fragment length polymorphism (RFLP) analysis of -308G>A of TNF-a polymorphism using Nco1 restriction enzyme

Lane 1 contains the molecular weight ladder. Lane 2 represents the homozygous reference GG genotype, exhibiting 192- and 20-bp fragments (the 20-bp fragment is not visible due to its small size). Lane 3 displays the heterozygous GA genotype, showing 212-, 192-, and 20-bp fragments. Lanes 4 and 10 demonstrate the homozygous AA genotypes, indicated by the undigested 212-bp fragment.

For IL-A and IL-B SNP: - The primers utilized for IL1A and IL1B are delineated in Table 1. For the amplification procedure targeting both genes, the reaction milieu was meticulously composed, featuring 1 µL of both forward and reverse primers, 3 µL of genomic DNA, 5 µL of DNase-free water, and 10 µL of an enriched PCR Master Mix containing HF Buffer (2X TOPsimple™ DyeMIX) as provided by Enzynomics. The protocols governing the PCR cycling conditions were delineated as follows:

(a) The amplification of IL-1A (+4845G/T) began with a first denaturation stage at 94°C, which lasted for three minutes. Later on, the amplification process was enhanced to consist of 30 cycles. Each cycle consisted of a denaturation phase at 98°C for one minute, followed by an annealing phase at 54°C for one minute, and culminated with an extension phase at 72°C for two minutes. This sequence concluded with a terminal extension maintained at 72°C for an extended duration of 5 minutes.

(b) The protocol for IL-1B (-511C/T) initiated with an initial denaturation phase at 94°C, which lasted for 10 minutes. This phase was succeeded by a sequence of 30 cycles, each of which consisted of a denaturation step at 94°C for 45 seconds, an annealing phase at 55°C for 45 seconds, and an extension phase at 72°C for 60 seconds in order. The amplification process was concluded with a terminal extension phase at 72°C, extending for an additional 10 minutes.

Post-amplification, 4.0 µL of the PCR product was subjected to enzymatic digestion. This was achieved by incorporating 0.5 µL of the specific restriction enzyme, 18.0 µL of nuclease-free water, and 2.5 µL of CutSmart® buffer into the mixture. The resultant solution was then incubated at 37°C for 20 minutes to facilitate the enzymatic activity. For the detection of IL-1A (+4845G/T), the PCR product was processed with the Sat I restriction enzyme (New England Biolabs®, UK). This enzymatic activity produced fragment sizes of 124 and 76 base pairs in the presence of the G allele, and a singular 200 base pair band indicative of the T allele **(Figure 2)**

Figure 2: The Sat I restriction profiles of (+4845G/T) polymorphic sites of IL-1 A gene Lane 1 is the ladder, lanes 2 represents homozygous GG genotype (124- and 76-bp fragment). Lane 3 represents the homozygous genotype (undigested 200-bp fragment), while lane 4 represents the heterozygous genotype GT (124-, 76-, and 200-bp fragments).

To determine the presence of IL-1B (-511C/T), the PCR products underwent enzymatic digestion using the restriction enzyme Ava I, supplied by New England Biolabs®, UK. This enzymatic cleavage yielded a 305-bp band indicative of the C allele, whereas the presence of the T allele was confirmed by the generation of 190-bp and 115-bp bands **(Figure 3).**

Figure 3: The Ava I restriction profiles of (-511C/T) polymorphic sites of IL-1 A gene Lane 1 is the ladder, Lanes 2 represents homozygous CC genotype (305-bp fragment). Lane 3 represents

heterozygous genotype CT (190-, 115-, and 305 -bp fragments), whereas lane 4 represents the homozygous TT genotype, indicated by bands at 190 bp and 115 bp.

Statistical analysis

The sample size was calculated using G* power software version 3.1.9.2 , with test family ((X^2 - tests), statistical test (Goodness-of-fit tests: Contingency tables), type of power analysis (A priori: Compute required sample size - given α , power and effect size), input parameters, effect size w = 0.47, α error= 0.05, power(1- β)= 0.95, resulting output parameter was total sample size of 90 (30 patients in each group).

The data were recorded on an "Investigation report form". These data were tabulated, coded then analyzed using the computer program SPSS (Statistical package for social science) version 26. Descriptive statistics were calculated for the data in the form of mean and standard deviation (±SD), Median and interquartile range (IQR) and Number and percent. In the statistical comparison between the different groups, the significance of difference was tested using student's *t*-test to compare between mean of two groups of numerical data, for continuous non-parametric data, Mann-Whitney *U*- test was used for inter-group analysis, Anova to compare between mean of more than two groups of numerical data, for continuous non- parametric data, Kruskal Wallis test was used for inter-group analysis Inter-group comparison of categorical data was performed by using chi square test (X^2-value). *P* value <0.05 was considered statistically significant.

Table 1. Sequences of the primers used in PCR-RFLP for detection of SNP of TNF-α and IL-1.

Name	Sequences (5' > 3')
TNF-α Forward primer Reverse primer	5' AGGCAATAGGTTTTGAGGGCCAT 3'; 5' TCCTCCCTGCTCCGATTCCG3';
IL- 1 A Forward primer *Reverse primer*	5'-ATG GTT TTA GAA ATC ATC AAG CCT AGG GCA-3' 5'-AAT GAA AGG AGG GGA GGA TGA CAG AAA TGT 3'
IL-1B Forward primer Reverse primer	5'- TGGCATTGATCTGGTTCATC-3' 5'- GTTTAGGAATCTTCCCACTT-3'

Figure 1. Restriction fragment length polymorphism (RFLP) analysis of -308G>A of TNF-a polymorphism using Nco1 restriction enzyme.

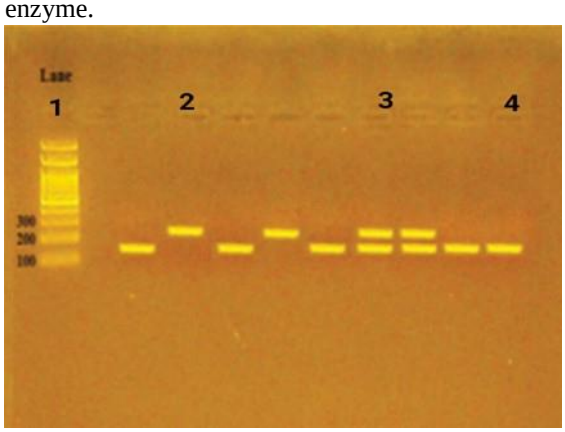


Figure 2. The Sat I restriction profiles of (+4845G/T) polymorphic sites of IL-1 A gene.

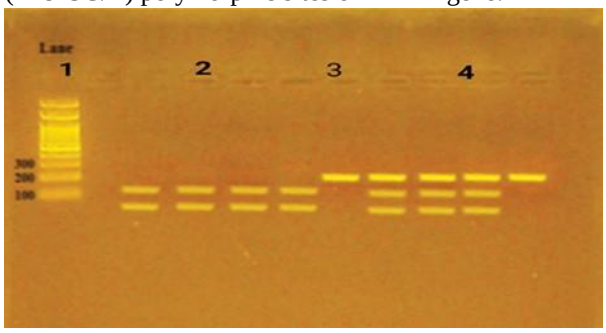
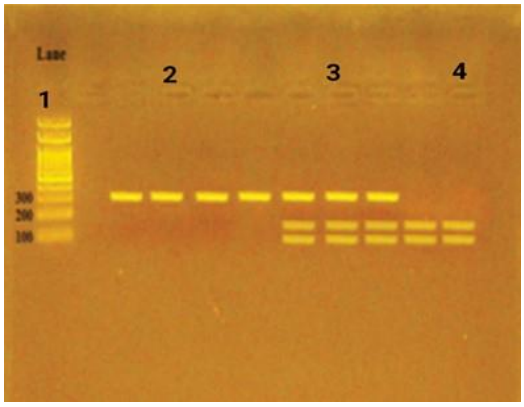


Figure 3. The Ava I restriction profiles of (-511C/T) polymorphic sites of IL-1 A gene.



Results

The current study included 100 chronic rhinosinusitis (CRS) cases; their mean age was 41.2 ± 11.6 years. They were 35(35%) males and 65 (65%) females. 50 patients (50%) were diagnosed with CRSwNP and 50 (50%) with CRSsNP. The control group was selected to be matched in age and sex. Their mean age was 41.1 ± 12.5 years; they were 20 males (40%) and 30 females (60%). Cigarette smoking was significantly associated with CRS patients in comparison with control (*p*-value < 0.001***).

Concentrations of TNF-α in serum were markedly elevated in group 1, registering 157.28±6.64, and in group 2, measuring 139.67±4.24, in contrast to the control group (group 3), which demonstrated a level of 93.43±3.24 [p<0.0001***], as detailed in **table (2).**

Furthermore, the serum concentrations of IL-1 exhibited a notable elevation in both group 1 and group 2 when compared to those observed in group 3 [p<0.0001***] as shown in **table (2).**

A marked disparity was noted in the genotype distribution of the TNF-a 308G>A single nucleotide polymorphism (SNP) between groups 1 and 2, and the control group (group 3), with a statistical significance (p < 0.001***). Specifically, the frequency of the GA genotype was substantially elevated in cases of CRSwNP at 48%, and CRSsNP at 50%, compared to a mere 6% observed within the controls. Moreover, the presence of allele-A was also significantly more prevalent in groups 1 and 2 relatives to the healthy controls, where it was found at 8% **(Table 3**).

In our investigation, the genotype distributions of IL-1A (+4845G/T) did not exhibit statistically significant differences across the three groups (p = 0.093). Conversely, a pronounced association was discerned for the IL-1B (−511C/T) polymorphism in the comparative analysis between groups 1 and 2 against group 3 (p < 0.001***). Of particular note, the prevalence of the IL-1B CC genotype was markedly higher in groups 2 and 3 (p < 0.001), whereas the CT genotype was predominantly observed in group 1. Additionally, the T allele demonstrated a robust association with group 1, distinctly contrasting with its occurrence in groups 2 and 3 (p < 0.001***), as documented in **table (3).**

Table 2. Comparison of the TNF-α levels and IL-1 levels of the three groups (pg/ml).

	Group 1 CRSwNP (50)	**Group 2 CRSsNP (50)**	**Group 3 Control group (50)**	**t-test**	***P*-value**
TNF-α levels	157.28±6.64	139.67±4.24	93.43±3.24	12.866	<0.001***
IL-1 levels	29.83±4.78	22.48±2.21	12±2.21	9.818	<0.001***

Table 3. Genotype distribution and allele frequencies of TNF-a 308G>A SNP, IL-1A (+4845G/T), and IL-1B (−511C/ T) genes in different groups.

	Group 1 CRSwNP (50)	**Group 2 CRSsNP (50)**	**Group 3 Control group (50)**	***P*-value**
TNF-a 308G>A SNP Genotype				<0.001***
GG	23 (46%)	20 (40%)	43 (86%)	(Highly
GA	24 (48%)	25 (50%)	6 (12%)	significant)
AA	3 (6%)	5 (10%)	1 (2%)	
TNF-a 308G>A SNP Allele				
G	N=100	N=100	N=100	
A	70 (70%)	65(65.0%)	92 (92%)	
	30 (30%)	35 (35.0%)	8 (8%)	
***IL-1A* (+4845G/ T)**				0.093
Genotype				(non- significant)
GG	19(38%)	13(26%)	25(50%)	
GT	22(44%)	24(48%)	18(36%)	
TT	9(18%)	13(26)	7(14%)	
Allele				
G	60 (60%)	50(50%)	68 (68%)	
T	40 (40%)	50(50%)	32(32%)	
***IL-1B* (−511C, −511T)**				<0.001***
Genotype				(Highly
CC	10(20%)	26(52%)	29(58%)	significant)
CT	27(54%)	17(34%)	20 (40%)	
TT	13(26%)	7(14%)	1(2%)	
Allele	47(47%)	69 (69%)	78 (78%)	
C	53 (53%)	31 (31%)	22 (22%)	
T				

Discussion

Chronic rhinosinusitis (CRS), a multifaceted inflammatory disorder, is typified by localized inflammation of the sinonasal mucosa. The pathogenesis of CRS is intricately linked to adaptive immune responses, which involve localized increases in T cell subsets and the cytokines they secrete [14].

Interleukin -1β and TNF-α, which are robust pro-inflammatory cytokines, are produced across a varied spectrum of cell types, including macrophages, eosinophils, and epithelial cells. These cytokines exhibit synergistic interactions within chronic inflammatory processes, mediated through the presence of Th1 and Th2 cytokines. Recently, there has been a notable increase in the approval of therapeutic modalities specifically designed to target the adaptive immune system [8].

This study revealed that levels of TNF-alpha and IL-1 were significantly elevated in CRS patients (groups 1,2) when compared with the control group (group 3). This agrees with **Lennard *et al***. [15] and **WEI *et al.*** [16]. These findings substantiate the hypothesis that the inflammatory response observed in chronic rhinosinusitis is intricately associated with augmented levels of pro-inflammatory cytokines.

These findings are also consistent with **Plewka *et al.*** [17] who prove an enhanced expression of these cytokines in the nasal mucosa of chronic rhinosinusitis patients especially in polyp tissues Prior investigations have indicated increased expression levels of this cytokine in nasal polyps, as seen by many researchers [18-20].

TNFA gene is situated within the extensively polymorphic MHC class III region on chromosome 6, precisely at the 6p21.3 locus. Variations in the form of SNPs within the -308-promoter region of the TNF-α gene give rise to the development of two discrete allelic forms. The predominant allele, denoted as TNFA-1, features a guanine (G) nucleotide at this site. In contrast, the substitution of guanine with adenine (A) produces the less common TNFA-2 allele [21].

This study observed a significant difference in the genotype distribution of TNF-a 308G>A SNP among groups 1 and 2 when compared to controls (group 3) ($p < 0.001$), where the GA genotype frequency was higher in CRSwNP (group 1) (48%) and CRSsNP (group 2) (50%) than controls (6%). Furthermore, the frequency of allele-A was observed to be significantly elevated in both group 1 and group 2 compared to healthy controls, where it was present at a rate of 8%.

Bernstein *et al.* [22] determined that the A allele of TNF-α at the -308 position is correlated with a two-fold increase in the incidence of nasal polyps (NP) when compared to control groups. **Szabó *et al.*** [23] further elucidated that the A allele is implicated in the susceptibility to NP development, particularly in the context of acetylsalicylic acid sensitivity. In contrast, **Erbek *et al.*** [24], **Endam *et al***. [25], and **Fajardo-Dolci *et al.*** [26] found no statistically significant associations between the genotypic or allelic distributions of TNF-α and the development of nasal polyps.

The discrepancies observed across these studies can be attributed to genetic heterogeneity and the diverse ethnic backgrounds of the study populations. Moreover, the impact of allelic variations in the promoter region of the TNF-α gene on TNF-α transcription remains ambiguous. While some research has not identified a correlation between genotype and transcriptional activity, in-vitro studies have demonstrated elevated TNF-α production in individuals with the GA genotype compared to those with the GG genotype [27].

Another crucial aspect is that the regulation of TNF-α expression is not limited to the TNFA gene locus; it also involves the TNFB gene. Additionally, the extensive linkage disequilibrium in the vicinity of the TNFA locus may play a role in the genetic predisposition to developing nasal polyps and chronic rhinosinusitis [25].

Our study found no statistically significant differences in the genotype distributions of IL-1A (+4845G/T) among the three groups ($p = 0.093$). Nevertheless, the involvement of the IL-1A gene in CRS has been corroborated by other research findings (20, 28), which identified a significant correlation between IL-1A (+4845G/T) polymorphism and CRS. This discrepancy may be attributed to ethnic variations affecting the frequency of genetic alleles. Furthermore, the absence of an association between the IL-1A (+4845G/T) gene polymorphism and chronic rhinosinusitis observed in our study may be attributed to epigenetic factors, such as environmental interactions with the genome [29].

The IL-1B (-511C/T) polymorphism was found to be statistically significant in groups 1 and 2 compared to group 3, as evidenced by a *p*-value of less than 0.001. This observation aligns with the

findings of **Erbek *et al.*** [24] in a Turkish cohort, suggesting the potential involvement of the SNP at the IL-1B locus -511 on chromosome 2 in the intricate inflammatory pathways of CRS. However, the precise mechanistic relationship between this genetic variant and the clinical progression of CRS remains unclear. It is possible that the IL-1B (-511C/T) polymorphism influences or modulates the transcriptional activity of other cytokine genes implicated in the pathogenesis of CRS [30].

Limitations of the study

Small sample size and limited facilities.

Conclusion

Significantly higher TNF- α and IL-1 serum levels and TNF-α and IL-1B (-511C/T) polymorphisms with CRS with or without nasal polyps. Both IL-1B and TNF-α may be involved in the regulation of CRS etiopathogenesis and could represent novel therapeutic targets for its management.

Conflict of interest

None to declare.

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Ethical approval statement

This study received approval from the Research Ethics Committee of Benha Faculty of Medicine (REC-FOMBU), Egypt, under protocol approval number RC-12-5-2023. The study adhered to the principles outlined in the Helsinki Declaration of 1975 and its subsequent amendments. Written informed consent was obtained from all participants involved in the study.

Authors' contributions

The authors' contributions to the paper are as follows: AFG and RAE contributed to the study idea and design. MGE, MGS, and RAE were involved in data collection. EFA conducted the analysis and interpretation of results, as well as prepared the draft manuscript. All authors critically reviewed the results and approved the final version of the manuscript. The corresponding author affirms that all named authors have read and approved the manuscript for submission. The authors confirm that they have met the criteria for authorship as defined, and each believes the manuscript reflects honest scholarly work.

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List of abbreviations

- TNF-α Tumour Necrosis Alpha
- CRS Chronic rhinosinusitis
- SNPs Single Nucleotide Gene Polymorphisms
- IL-1 Interleukin-1
- ICAM-1 Intercellular Adhesion Molecule-1
- VCAM-1 Vascular Cell Adhesion Protein 1
- CCL5 Chemokine (C-C Motif) Ligand 5
- EDTA Ethylenediaminetetraacetic acid
- ELISA enzyme-linked immunosorbent assay
- EPOS European Position Paper on Rhinosinusitis and Nasal Polyp
- PCR-RFLP Polymerase chain reaction -Restriction Fragment Length Polymorphism
- CRSsNP Chronic rhinosinusitis without nasal polyps
- CRSwNP Chronic rhinosinusitis with nasal polyps
- NP Nasal polyps

References

1- **Reda A, Lashin BI, Alaaraj MM, Abouelkheir M, Ahmed MI, Shah J, et al.** The impact of chronic rhinosinusitis on COVID-19 risk and outcomes: A systematic review and meta-analysis. Frontiers in Immunology 2023;14:1139031.

2- **Hosny MHM, Khaled AM, Mohammed RSE, Fahim RS, Abdel Tawab MA.** Study of The Effect of Functional Endoscopic Sinus Surgery on Spirometry in Patients with Chronic

Rhinosinusitis and Chronic Obstructive Lung Disease. Egyptian Journal of Medical Research 2022;3(1):106-19.

3- **El-Beltagy YF, Ghita AF, Mady OM, Ibrahim AM.** The relation between chronic rhinosinusitis and sleep-disordered breathing. The Egyptian Journal of Otolaryngology 2019;35:155-61.

4- **Bartier S, Coste A, Béquignon E.** Biotherapy and treatment of adult primary chronic rhinosinusitis with nasal polyps: Cellular and molecular bases. European Annals of Otorhinolaryngology, Head and Neck Diseases 2021;138(5):355-62.

5- **Kamath MP, Shenoy V, Mittal N, Sharma N.** Microbiological analysis of paranasal sinuses in chronic sinusitis–A south Indian coastal study. Egyptian Journal of Ear, Nose, Throat and Allied Sciences 2013;14(3):185-9.

6- **Ramakrishnan Y, Shields R, Elbadawey M, Wilson J.** Biofilms in chronic rhinosinusitis: what is new and where next? The Journal of Laryngology & Otology 2015;129(8):744-51.

7- **Kao SS-T, Bassiouni A, Ramezanpour M, Finnie J, Chegeni N, Colella AD, et al.** Proteomic analysis of nasal mucus samples of healthy patients and patients with chronic rhinosinusitis. Journal of Allergy and Clinical Immunology 2021;147(1):168-78.

8- **Carsuzaa F, Béquignon É, Dufour X, de Bonnecaze G, Lecron J-C, Favot L.** Cytokine signature and involvement in chronic rhinosinusitis with nasal polyps. International Journal of Molecular Sciences 2021;23(1):417.

9- **Hanlon MM, Rakovich T, Cunningham CC, Ansboro S, Veale DJ, Fearon U, et al.** STAT3 mediates the differential effects of oncostatin M and TNFα on RA synovial fibroblast and endothelial cell function. Frontiers in immunology 2019;10:2056.

10- **Gubernatorova EO, Namakanova OA, Gorshkova E, Medvedovskaya AD, Nedospasov SA, Drutskaya MS.** Novel anti-cytokine strategies for prevention and treatment of respiratory allergic diseases. Frontiers in Immunology 2021;12:601842.

11- **Fokkens WJ, Lund VJ, Hopkins C, Hellings PW, Kern R, Reitsma S, et al.** European position paper on rhinosinusitis and nasal polyps 2020. Rhinology: official organ of the International rhinologic society 2020.

12- **Lund VJ.** Fortnightly review: diagnosis and treatment of nasal polyps. Bmj 1995;311(7017):1411-4.

13- **Ohori J, Ushikai M, Sun D, Nishimoto K, Sagara Y, Fukuiwa T, et al.** TNF-α upregulates VCAM-1 and NF-κB in fibroblasts from nasal polyps. Auris Nasus Larynx 2007;34(2):177-83.

14- **Czerwaty K, Piszczatowska K, Brzost J, Ludwig N, Szczepański MJ, Dżaman K.** Immunological Aspects of Chronic Rhinosinusitis. Diagnostics 2022;12(10):2361.

15- **Lennard CM, Mann EA, Sun LL, Chang AS, Bolger WE.** Interleukin-1β, interleukin-5, interleukin-6, interleukin-8, and tumor necrosis factor-α in chronic sinusitis: Response to systemic corticosteroids. American journal of rhinology 2000;14(6):367-74.

16- **Wei L, Zhang Y, Tan H.** Changes of TNF-α, PDGF and HA in nasal secretions and olfactory function of patients with chronic sinusitis before and after endoscopic sinus surgery. Experimental and Therapeutic Medicine 2018;16(4):3413-8.

17- **Plewka D, Grzanka A, Drzewiecka E, Plewka A, Misiołek M, Lisowska G, et al.** Differential expression of tumor necrosis factor α, interleukin 1β, nuclear factor κB in nasal mucosa among chronic rhinosinusitis

patients with and without polyps. Advances in Dermatology and Allergology/Postępy Dermatologii i Alergologii 2017;34(3):199-206.

18- **Zhang N, Van Zele T, Perez-Novo C, Van Bruaene N, Holtappels G, DeRuyck N, et al.** Different types of T-effector cells orchestrate mucosal inflammation in chronic sinus disease. Journal of Allergy and Clinical Immunology 2008;122(5):961-8.

19- **Chen F-H, Deng J, Hong H-Y, Xu R, Guo J-B, Hou W-J, et al.** Extensive versus functional endoscopic sinus surgery for chronic rhinosinusitis with nasal polyps and asthma: a 1-year study. American Journal of Rhinology & Allergy 2016;30(2):143-8.

20- **Oyer SL, Mulligan JK, Psaltis AJ, Henriquez OA, Schlosser RJ.** Cytokine correlation between sinus tissue and nasal secretions among chronic rhinosinusitis and controls. The Laryngoscope 2013;123(12):E72-E8.

21- **Sethi JK, Hotamisligil GS.** Metabolic messengers: tumour necrosis factor. Nature metabolism 2021;3(10):1302-12.

22- **Bernstein JM, Anon JB, Rontal M, Conroy J, Wang C, Sucheston L.** Genetic polymorphisms in chronic hyperplastic sinusitis with nasal polyposis. The Laryngoscope 2009;119(7):1258-64.

23- **Szabó K, Kiricsi Á, Révész M, Vóna I, Szabó Z, Bella Z, et al.** The–308 G> A SNP of TNFA is a factor predisposing to chronic rhinosinusitis associated with nasal polyposis in aspirin-sensitive Hungarian individuals: conclusions of a genetic study with multiple stratifications. International immunology 2013;25(6):383-8.

24- **Erbek SS, Yurtcu E, Erbek S, Atac FB, Sahin FI, Cakmak O.** Proinflammatory cytokine single nucleotide polymorphisms in nasal polyposis. Archives of otolaryngology–head & neck surgery 2007;133(7):705-9.

25- **Endam LM, Cormier C, Bossé Y, Filali-Mouhim A, Desrosiers M.** Association of IL1A, IL1B, and TNF gene polymorphisms with chronic rhinosinusitis with and without nasal polyposis: a replication study. Archives of Otolaryngology–Head & Neck Surgery 2010;136(2):187-92.

26- **Fajardo-Dolci G, Solorio-Abreu J, Romero-Álvarez JC, Zavaleta-Villa B, Cerezo-Camacho O, Jiménez-Lucio R, et al.** DQA1 and DQB1 association and nasal polyposis. Otolaryngology—Head and Neck Surgery 2006;135(2):243-7.

27- **Batikhan H, Gokcan MK, Beder E, Akar N, Ozturk A, Gerceker M.** Association of the tumor necrosis factor-alpha− 308 G/A polymorphism with nasal polyposis. European archives of oto-rhino-laryngology 2010;267:903-8.

28- **Elahi MM, Asotra K, Matata BM, Mastana SS.** Tumor necrosis factor alpha− 308 gene locus promoter polymorphism: an analysis of association with health and disease. Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease 2009;1792(3):163-72.

29- **Shi JB, Fu Q, Zhang H, Cheng L, Wang Y, Zhu D, et al.** Epidemiology of chronic rhinosinusitis: results from a cross-sectional survey in seven C hinese cities. Allergy 2015;70(5):533-9.

30- **İsmi O, Özcan C, Polat G, Kul S, Görür K, Pütürgeli T.** TNF-α and IL-1 β cytokine gene polymorphism in patients with nasal polyposis. Turkish archives of otorhinolaryngology 2017;55(2):51.