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Study the role of interleukin-4 and interferon-γ in patients with penicillin allergy

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Abstract

Penicillin, the most well-known β-lactam antibiotic, is thought to cause allergic responses in 0.7–10% of the human population. Atopic and other allergy illnesses are believed to be developed and regulated in part by excessive secretion of Interleukin-4 (IL-4) and interferon- γ (IFN- γ). In this study, the frequency of penicillin allergy was analyzed by IL-4 and IFN- γ using the enzyme linked immunosorbent assay in 45 patients with an allergy to penicillin and 45 apparently healthy subjects as a control group. Also, we determined the IL-4 receptor α gene (IL-4 Rα) by using tetra primer-amplification refractory mutation system based polymerase chain reaction (T-ARMS-PCR). The findings demonstrated that IFN- γ and IL-4 levels in serum of the patients in the experimental group were significantly higher than in the control group. Although IFN- γ levels were lower than IL-4 in the patients and controls, patients exhibited higher IFN- γ concentrations compared to control subjects. The IL-4 receptor α (Rα) genotype distribution in patients and control groups revealed that genotypes AA, GA, and GG were present in 26 (57.78%), 11 (24.44%), and 8 (17.78%) patient subjects, while in the control group they were present in 17 (37.78%), 21 (46.67%), and 7 (15.56%) subjects. As a result, it seemed that patients had a higher frequency of genotype AA than the control group. In conclusion, penicillin allergy is influenced by IL-4 and IFN- γ, and IL-4 Rα gene polymorphism revealed that AA and GA genotypes may be linked to β-lactam allergy, whereas GG genotypes may offer a strong defense against β-lactam allergy.

Keywords: Penicillin allergy, Interleukin-4, Interferon-γ and IL-4 receptor alpha

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Introduction

Beta-lactam allergies are acknowledged as an important health issue and frequently encountered in routine medical practice.[1] Hypersensitivity reactions are an immune mechanism that primarily mediates the adverse effects of beta-lactams,[2] which account for 6-10% of all adverse drug reactions.[3] Approximately 10% of people report having a penicillin allergy, although after testing, more

than 90% of patients with a documented allergy can tolerate penicillin's.[4,5] Drug challenge and skin testing for penicillin allergy are being applied with growing frequency in clinical practice, supported by recent research confirming their safety and effectiveness.[6-8] The primary immunological effects of a penicillin hypersensitive reaction fall into two categories: immediate and non-immediate reactions.[9] It is believed that the development of an acute allergic reaction to penicillin is greatly influenced by the production of interleukin-4 (IL-4) and interferon-γ (IFN- γ).[10]

IL-4 is essential for humoral and adaptive immunity due to its numerous immunological roles that involve stimulating activated T and B cells.[11] It may also be a significant factor in penicillin allergy with reference to the T helper 1 (Th1)/Th2 system.[12]

In β-lactam hypersensitivity reactions, Th2-derived cytokines are key mediators, largely due to the role of IL-4 in promoting B-cell class switching and subsequent immunoglobulin E (IgE) synthesis. Studies on penicillin allergy have shown that, during the early immune response, the balance between Th1 and Th2 differentiation is shaped more by IL-4 kinetics and concentration than by IFN- γ levels.[13]

IFN is a group of signaling cytokines synthesized and secreted by the host cell in reaction to a number of infections, it takes a role in communication between cells to initiate immune defense reaction.[14] IFN is classified into three types.[15] The immune response is activated and regulated by both IFN-I and IFN-II. In penicillin allergy, IFN- γ influences the generation of IgE, which is crucial for controlling IgE production.[16]

The IL-4 receptor alpha (IL-4 Rα) is a receptor for the cytokine IL-4. It can bind the IL-4 and IL-13 cytokines, which are closely linked and are isogenous due to their chromosomal location and protein molecular. The IL-4 Rα subunit is associated with gamma chain, which will bind specifically to IL-4, and the IL-4 Rα subunit is associated with a different subunit called IL-13 Rα1.[17] The interaction between IL-4 and TNF-α will enhance a structural change in vascular endothelial cells; therefore, it plays a critical role in tissue inflammation. Alternative activation of macrophage result due to binding of IL-4 or IL-13 to IL-4 Rα, this activation will inhibit and down regulate the inflammatory mediators like INF-γ during immune reaction, specifically with regard to helminthic infection.[18] In this study, we aimed to estimate the role of IL-4 and IFN- γ in controlling allergic reactions, as well as to quantify the potential contribution of IL-4 receptor gene polymorphism to the establishment of an immune response against penicillin.

Materials and Methods

Subjects

Between January and May 2025, a total of 45 patients (21 males and 24 females), aged 10 to 65 years, were identified through positive skin tests for penicillin β-lactam allergy and enrolled in the study. The control group consisted of 45 apparently healthy individuals (15 males and 30 females) aged between 18 and 65 years. All controls had no current or past history of penicillin allergy and tested negative for it. They also had no record of respiratory, dermatological, or autoimmune disorders, including asthma, and were therefore included in the study as controls.

A skin test employing a skin prick test on the inside of the forearm with benzyl penicillin (PG) levels of up to 10,000 IU/ml was conducted.[19] After 15 to 20 minutes, the reaction was considered positive if the wheal diameter was greater than 3 mm and the area around it was erythematous.

Sample collection

Blood samples were collected from all participants by venipuncture. From each participant, whether patient or control, a venous blood sample (5 ml) was collected via vein puncture using sterile, disposable syringes under aseptic conditions. After collection, a blood sample (3 ml) was placed in a sterile plain tube, followed by centrifugation at 2500 rpm for 10 minutes. The resulting serum was portioned into multiple aliquots and promptly stored at –20 °C to preserve integrity and prevent degradation caused by repeated freeze–thaw cycles. The remaining blood sample (2 ml) was

transferred into a tube with ethylenediamine tetra acetic acid (EDTA), for DNA extraction (gene polymorphism of IL-4 Rα)

Immunological Analysis

Human enzyme-linked immunosorbent assay (ELISA) commercial kits (Elabscinece, USA) were employed to determine the IL-4 and IFN-γ levels following the manufacturer's instructions.

Molecular Analysis

Tetra Amplification Refractory Mutation System (T-ARMS)-polymerase chain reaction (PCR) premixed ready-to-use master mix solution kits (GoTaq® G2 Green Master Mix kits) were used. Each sample underwent one reaction using this master mix in accordance with the company's instructions. Following that, these components of the PCR master mix were placed in a PCR thermo cycler (BioRad, USA) after being spun for three minutes at 3000 rpm in a vortex centrifuge (ExiSpin).

DNA was extracted using the quick, simple and accurate T-ARMS-PCR methodology. To assess single-nucleotide polymorphisms (SNPs) in Interleukin-4 receptor α. Thermocycler (Analytic Jena, Germany) was carried out. The T-ARMS-PCR; gene polymorphism primers were created according to the method described by Hammadi, et al., 2023.[20] These primers were commercially obtained (Bioneer Company, Korea), as showed in Table (1).

T-ARMS-PCR was performed using a Thermo cycler, with initial denaturation at 95°C for five minutes. This was followed by 35-cycles, consisted of the following steps, denaturation at temperatures of 95°C for 30 seconds, 57°C for 30seconds for primer connection, 72°C for 40 seconds for extension, and 72°C for five minutes for final extension.

Using agarose gel electrophoresis, on a gel consisting of two percent agarose with 3 µl of ethidium bromide (EB) dye, the PCR products were examined. Next, ultraviolet rays (ATTA, S/Korea) revealed the bands showed in Figure 1.

Statistical Analysis

All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) version 24 and Microsoft Excel 2016. The Chi-square or Fisher's exact tests were used to examine the association between genotype distribution and β-lactam allergy susceptibility, with odds ratios (OR) and 95% confidence intervals (CI) calculated for each genotype. Assessment of IL-4Rα polymorphism was carried out specifically using the chi-squared test. Every statistic was examined using bilateral probability, and every sign was found to be below the standard threshold of p=0.051.

Table 1. Primer sequence of Interleukin-4 receptor α and size of products.

Primer	Sequence (5' - 3')	Product Size (bp)
Fi primer (G allele)	CCTGTGTCTGCAGAGCCCACACGTTTG	172 bp
Ri primer (A allele)	CCCGCGCCTCCGTTGTTCTCAGGTAT	245 bp
Fo primer	TAAGAGGCTGTGGCCAGCAAGAGAGGCAA	364 bp
Ro primer (common)	GCTCACCATGCTCGCTGGGCTTGAAG	

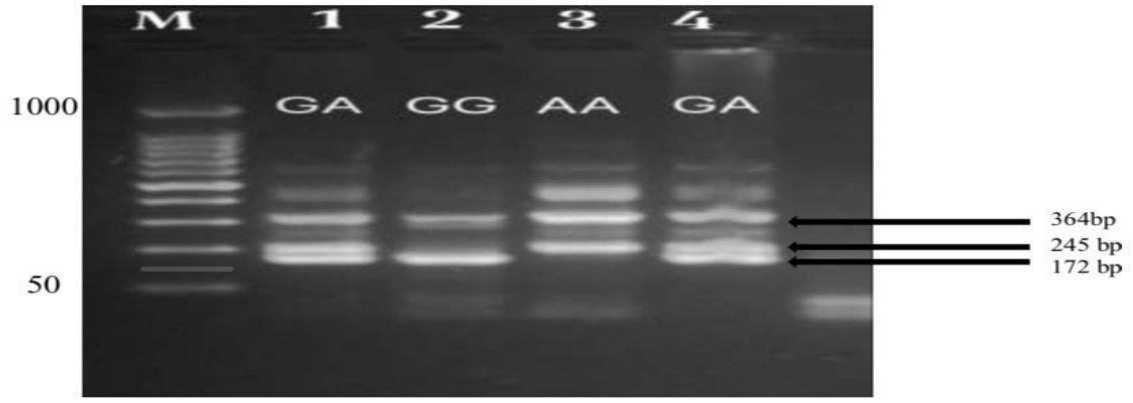

Figure 1. Agarose gel (2%) electrophoresis image, showing the Tetra Amplification Refractory Mutation System (T-ARMS) - polymerase chain reaction (PCR) result for evaluation of the interleukin-4 (IL-4) gene in the a patient sample. Lane GG show wild type homozygote, observed at two bands (364 bp control band and 172 bp G allele), lane AA mutant type homozygote, observed at two bands (364 bp control band and 245 bp A allele), and lane GA heterozygote, observed at three bands digested at (364 bp control band, 172 bp G allele, and 245 bp A allele) where M: marker (50 bp to 1000 bp).

Results

In this case-control study, 45 participants with a known allergy to β-lactam medications were included. They were between the ages of 10 and 65, with an average age of 35.36 ±12.54 years. Furthermore, the trial included 45 control subjects, ranging in age from 18 to 65 years, with a mean age of 33.36 ±13.00 years and no history of β-lactam antibiotic resistance. The study and control groups' mean ages were not statistically significantly different (p=0.074).

The gender distribution of the patients group was 21 (47%) male and 24 (53%) female, whereas the control group included 15 (33%) male and 30 (67%) female subjects (Table 2). Gender did not significantly influence the distribution of control or the patients groups (p=0.420).

Table 2. Gender distribution of patients with penicillin allergies and controls.

Gender	Patients group No = 45	Control group No = 45	χ^2 p-value
Male	21 (47%)	15 (33%)	NS
Female	24 (53%)	30 (67%)	

χ^2: Chi-square test $p > 0.05$ is not significant (NS).

According to the study data, the serum levels of the patient group and the median interquartile range (IQR) of IL-4 and IFN-γ were considerably greater than those of the control group (Table 3). The IL-4 median concentration revealed a significant relationship with penicillin allergy (p=0.001), excessive production of IL-4 explains the important role of this interleukin in immunopathology of penicillin HR and atopy. The IFN-γ median concentration showed a significant correlation between IFN-γ serum level and penicillin allergy. The result showed higher level of IFN-γ in patient's sera (184.32 pg/ml) compared with control subjects (145.72 pg/ml), but the level of IFN-γ was lower than IL-4 in both patients and controls.

Table 3. Median serum of interferon-γ (IFN-gamma) and interleukin (IL)-4 levels in both patient and control groups.

Characteristic	Patients group No = 45		Control group No = 45		*p* -value †
IFN-γ (pg/ml), median (IQR)	184.32	134.33	145.72	47.78	0.001
IL-4 (pg/ml), median (IQR)	357.28	245.57	274.76	125.55	0.001

IQR: Interquartile range; †: Mann–Whitney U test. *p* <0.05 is significant.

The genotype distribution in patients and control groups is presented in Table 4. Among the patients, the AA genotype was observed in 26 cases (57.78%), GA in 11 cases (24.44%), and GG in 8 cases (17.78%). In contrast, the control group had these genotypes in 17, 21, and 7 subjects, respectively, and they accounted for 37.78%, 46.67%, and 15.56%. Therefore, it seemed that patients had a higher frequency of genotype AA than the control group. (*p*=0.071). Additionally, there was no significant difference in the rates of genotype GG (17.78% compared to 15.56%) between the patient and control groups (*p*=0.799), while the patients' genotype GA rate was considerably lower than the control group (24.44% against 46.67%, separately; *p*=0.035).

AA seemed to be more closely linked to β-lactam medication allergies. Additionally, it was observed that genotype GA was moderately linked to β -lactam allergy, but genotype GG revealed to have a strong protective impact on β-lactam medication allergy.

Table 4. Distribution of interleukin-4 receptor α gene (IL-4Rα) genotype in the control and patient's groups.

Genotype	Patients group No = 45	Control group No = 45	*p*-value	OR	95%CI		EF	PF
					Lower	Upper		
AA	26 (57.78 %)	17 (37.78 %)	NS	2.08	0.93	4.62	0.31	---
GA	11 (24.44 %)	21 (46.67 %)	0.035	1.14	0.17	0.95	0.06	---
GG	8 (17.78 %)	7 (15.56 %)	NS	0.40	0.42	3.10	---	0.34

OR: Odds ratio; CI: Confidence interval; EF: Etiologic fraction; PF: Preventive fraction. *p* > 0.05 is not significant (NS).

Table 5, shows correlations between IL-4 Levels and the genotypes GG, GA, and AA. IL-4 levels in patients with these genotypes were not substantially different (*p*=0.595).

Table 5. Correlations between serum of interleukin-4 (IL-4) levels and genotypes.

IL-4 (pg/ml)	Genotype		
	AA	GA	GG
Median	357.36	330.76	270.62
p value		NS	

p > 0.05 is not significant (NS).

Discussion

Penicillin is the most prevalent medication class for allergy and beta-lactam antibiotic allergy.[21] The anti-inflammatory Th2 cytokine IL-4 can connect to IL-4 Rα to control the immune system, IgE synthesis, and embryonic implantation. [22, 23] According to a number of research studies, allergy disorders are linked to IL-4 and/or IL-4R gene variants. [24] The findings of the current study demonstrated that the sick group had considerably greater serum of IFN-γ and IL-4 levels than the control group. There was a significant relationship between penicillin allergy and the median IL-4 levels (p=0.001). These results indicated a significant correlation between penicillin allergy and the median IFN-γ concentration. The patients' serum levels of IFN-γ were higher (184.32 pg/ml) than those of the control subjects (145.72 pg/ml); however, both patients' and controls' levels of IFN-γ were lower than IL-4. Measuring IL-4 levels serves as a precise marker for detecting hypersensitivity reactions triggered by β-lactam antibiotics. Furthermore, a more sensitive method for identifying these drug-induced reactions may be to measure IL-4 in conjunction with INF-γ.[25] The findings of a previous study,[25] which examined the relationship between IL-4, IL-6, IL-10, IL-12 and IFN-γ and penicillin hypersensitivity reactions in 80 pediatric patients in Saudi Arabia, showed that the level of serum IL-4 in the patients was higher than in the control group (p<0.0001). They came to the conclusion that IL-4 is a specific indicator for the identifying allergies caused by β-lactams.[25] The information on IL-4 and IFN-γ is completely consistent with the Th1/Th2 balancing theory, which postulates that the cytokines are responsible for cutaneous reactions because they can alter the antibody response. [26] Among these are Th2 cytokines, such as IL-4, which play a role in stimulating B-cell differentiation and proliferation, as well as promoting isotype class switching toward IgE antibody production. The delayed type hypersensitive reaction, on the other hand, is caused by IFN-γ, the most important Th1 cytokine, which negatively control antibody-mediated reactions by inhibiting the generation of IgE that is generated by IL-4.[26]

The current study determined the distribution of IL-4 receptor α genotype in controls and penicillin allergy patients. The genotype GA was significantly higher in the control group than in the patient group, although the genotype AA seemed to be more common in the patients compared to the control group. It seemed that genotype AA was more linked to β-lactam medication allergies. IL-4 receptor α is found on chromosomes 16p11–16p12. Because it stimulates the production of IgE, this component is important in allergic diseases.[27] In a previous study of the IL-4Rα Q576R polymorphism, individuals with penicillin allergy had a greater incidence of the AA genotype (76% vs. 64%, p=0.005). Compared to controls, the penicillin allergy individuals had the A allele more frequently (87 vs. 80%, p=0.003). The group with urticaria was more probable than the control group to have the AA genotype for IL-4Rα I75V (32 vs. 19%, p=0.049). The Q576/I75 polymorphism was more common in the penicillin allergy patients than in the controls, according to haplotype analysis (42% vs. 35%, p=0.037).[28]

In conclusion, penicillin allergy is influenced by IL-4 and IFN-γ, and IL-4 receptor α gene polymorphism revealed that AA and GA genotypes may be linked to β-lactam allergy, whereas GG genotypes may offer a strong defense against β-lactam allergy.

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Author Contributions

RSMA, wrote the manuscript and created the outlines. HNH and RSMA, conducted the experiments and analyzed the data. The first draft of the work was written by SMK and HNH. The scientific information included in the publication was examined by RSMA, SMK, and HNH. Every author has read and approved the manuscript's final submission.

Declaration of Conflicting Interests

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Ethical approval

The protocol of the study was reviewed and approved by the Medical Ethics Committee at the Faculty of Polytechnic College/Al-Qadisiyah. (Approval no: 14, dated October 2025).

Informed consent

All patients provided informed consents and confidentiality was respected.

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