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Research Article

ASSOCIATION OF INTERLEUKIN-4 CYTOKINE AND IL-4Rα GENE POLYMORPHISM IN β-LACTAM ALLERGIC PATIENTS

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ABSTRACT

Objective: The present study was carried out to estimate the possible role of Interleukin-4 (*IL-4)RαQ576R* genes polymorphism in the development of immune reaction against penicillin, as well as to study the effect of IL-4 cytokine in regulating allergic reactions.

Materials and Methods: Measurement of serum IL-4 concentration was done using enzyme-linked immunosorbent assay technique; *IL-4RαQ576R* gene polymorphisms were genotyped using polymerase chain reaction-restriction fragment lengths polymorphisms. Comparisons for statistical significance were performed using Mann–Whitney U-test.

Results: Comparing with control subjects, there was a significantly increased level of IL-4 (348.53 pg/ml) in penicillin allergic patients versus (284.72 pg/ml) in sera of control subjects. The *IL-4RαQ576R* alleles were significantly higher in the penicillin allergic individual compared with apparently healthy control subjects.

Conclusions: Data study suggested that IL-4 cytokine have some important roles in penicillin hypersensitivity reaction, additionally the *IL-4RαQ576R*gene polymorphisms might involve in modulating of penicillin hypersensitivity.

Keywords: β-lactam, Allergy, Genotype, Interleukin-4.

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INTRODUCTION

Beta-lactams(BL) antibiotics are the most widely used in clinical practice worldwide and constitute the most common inducer of adverse drug reactions (ADRs) with an incidence rate of 0.7–10% of all population [1,2]. Adverse effects of BLs are mainly mediated by an immunological mechanism that is known as hypersensitivity reactions (HRs) [3], that account for 6–10% of all ADRs [4], which considered a problem of great concern for regulatory agencies, healthcare system and industry [5]. Undiagnosed beta-lactam allergy is significant and mounting public health issue due to its limitation in drug selection of alternatives, which can be either expensive or more side effects, so misdiagnosis of BLs allergy will increase the risk of frequent hospitalization, the emergence of antibiotic-resistant infection and increase of medical coast [6]. About 10–20% of total population has been marked as allergic to penicillin [7]. However, it has been reported that over 90% of BLs allergic patients were proven to be able to tolerate penicillin antibiotic when administered [8,9]. Penicillin hypersensitivity is mainly related to specific immunological reactions, that being classified as immediately and noon immediate reaction [10]. The immediate reaction usually occurs during 1 h after drug administration and initiated by certain IgE antibody which responsible for immediate anaphylactic reaction sign and symptoms [11]. Non-immediate reaction usually appears within 24 h after BLs administration [12]. Production of IgE is greatly affected by certain cytokines that released by stimulated T-lymphocyte such as Interleukin-4 (IL-4) and IFN-γ [13]. Therefore, excessive release of IL-4 and interferon-γ is believed to be very important in modulating of immediate HR to penicillin [14].

METHODS

Subject

The current study was carried out during period from second of January 2017 to the end of June 2017, 50 patients with penicillin allergy (24 males and 26 females), 28 of them are allergic/atopic (have an atopic disorder) and 22 only allergic to penicillin, they were recruited from dermatology Outpatients Department of AL-Diwaniya Teaching Hospital. Informed consent obtained from the patients also an ethical approval obtained. Other 50 apparently healthy subjects (13 males and 37 female) they were included as a control group; blood samples were collected from both groups. Skin testing was done with Benzylpenicillin (PG) using SPT on inner of forearms with a concentration of reagent up to 10000 IU/ml (15, 9, 16, 17). The reaction read after 15–20 min it considered +ve when a wheel diameter was ≥3 mm with surrounding areas of erythema. A blood sample was drawn from patients at the tof +ve skin test. Sera were separated from clotting blood at room temperature for 1 h by centrifugation then stored data −30°C until *in vitro* tests were performed. Another 3 mL of blood was collected in Ethylenediaminetetraacetic acid tubes then stored at 4°Cnfor DNA extractions for detection of IL-4Rα polymorphisms by polymerase chain reaction-restriction fragment lengths polymorphisms (RFLP-PCR) technique.

Immunological study

Serum level of IL-4 was detected using enzymes linked-immunosorbent assay (ELISA) which purchased from CALBIOTECH (USA), according to instructions of the company.

Genotype study

Extraction of genomic DNA from blood samples of patients and control done using Genomic DNA mini kit extraction kit (Frozen Blood) Geneaid (USA) and the procedure was done according to company instructions. The extracted blood genomic DNA was checked by using Nanodrop spectrophotometer (THERMO. USA), which measures the concentration of DNA (ng/μL) and checked the purity of DNA through reading absorbance (260/280 nm). RFLP-PCR technique was performed for genotyping and detection of IL-4Rα *(IL-4Rα-Q576R*) gene polymorphisms in patients with beta-lactam allergy and in healthy control blood samples. This method was carried out according to a

method that has described by Qiao *et al*., (2005) [19]. The IL-4RαQ576R polymorphism was amplified with the following primer, GCC CCC ACC AGT GGC TACC and GAG GTC TTG GAA AGG CTT ATAC. Preparation of PCR master mix occurred by the use of AccuPower PCR PreMix Kit, and preparations of the kit were occurred according to instructions of the manufacturer (DNA template 5 µl, IL-4Rα forward primer 1.5 µl, IL-4Rα reverse primer 1.5 µl, and PCR water 12 µl). Next, these PCR master mix component putted in standard AccuPower PCR PreMix Kit which contains all the other components that required for PCR reactions such as Taq DNA polymerase, dNTPs, Tris-HCl pH: 9.0, KCl, $MgCl_2$, stabilizer, and loading dye, after that all PCRD tubes transferred into Exispins vortex centrifuge for 3 min at 3000 rpm. Moreover, then put in PCR Thermocyclers(MygenegKorea). The program completed with 38 cycles for 30 s at 95°C, for 30 s at 58°C, 50 s at 72°C, and last 5 min extension at 72°C. IL-4RαQ576R polymorphism PCR products were 204 bp in length and were digested by 5 U of *NapII* restrictions enzymes. Separation of the digested fragment was carried out with 1% agarose gels, all results of genotypes seen in Figs. 1 and 2.

Statistical analysis

Analysis of data was performed using Statistical Package for the Social Sciences (SPSS) software version 20 in association with Microsoft Excel 2010. Significant associations among beta-lactam allergic susceptibility and the genotype distribution were assessed using Fisher's exact test or Chi-squared tests to estimate the odds ratio (OR) and 95% of the confidence interval (CI) for genotypes. Hardy–Weinberg equilibrium for IL-4Rα polymorphism was tested by the Chi-squared test. All statistics were inspected by performing bilateral probability, and all signs were determined as being below the conventional levels of p=0.051.

RESULTS

Presented results were based on the analysis of a random sample of 50 cases who were known to have beta-lactam allergy. Their mean age was 37.36±13.55 years and their age range was from 8 to 62 years. In addition, the study included 50 control subjects who had no history of β-lactam drug allergy; the mean age was 32.38±14 years and their age ranged from 18 to 65 years. Statistically speaking, no significant differences in mean age among control group and study groups (p=0.074). Distribution of patients according to 10 years' age intervals are shown in Fig. 3 which demonstrated that majority of patients aged 30–49 years. In addition, the patient's group included 24 (48%) male subjects and 26 (52%) female subjects, while control group included 13 (26%) male subjects and 37 (74%) female subjects. There was no significant difference in the distribution of patients and control group regarding gender (p=0.420). This mandatory to assure matching regarding gender between control and patient's groups, as shown in Table 1. The female percentage, 52% indicates that the female appeared to be more likely to develop BLs allergy than male (48%).

Association between cytokine and penicillin allergy

Significantly there were higher levels of IL-4 cytokine in patient serum comparing with the control group, it was 348.53 (245.57) versus 284.72 (125.55), 194.33, respectively, with (p=0.001), as shown in Table 2. This data found that an increased level of IL-4 might be correlated with HRs in penicillin allergic patients compared with healthy control subjects.

Genotypes of IL-4Rα and allele frequencies in control and study groups

To be sure about the random selection of control subjects enrolled in the present study, Hardy–Weinberg equilibrium was carried out and show no significant difference between observed and expected genotype frequencies (p=0.556), as shown in Table 3.

The genotype distribution had not deviation from Hardy–Weinberg equilibrium in all study groups. Genotypes AA, GA, and GG were seen in 19, 22, and 9 control subjects, respectively, and in terms of percentages they accounted for 38%, 44%, and 18%, respectively, whereas in patients group they were seen in 28, 12, and 10 patients, respectively, and accounted for 56%, 24%, and 20%, respectively. Hence, it

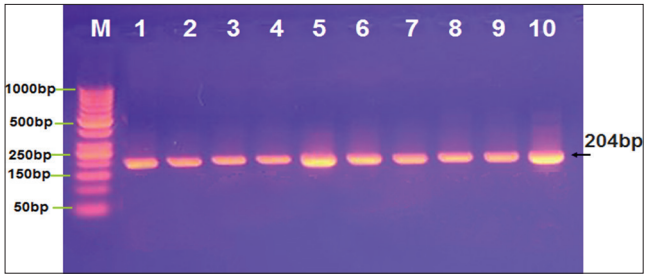

Fig. 1: Agarose gel electrophoresis image of PCR product analysis for IL-4RαQ576

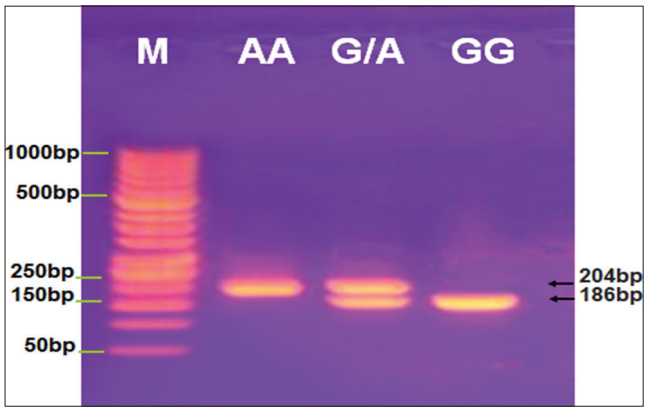

Fig. 2: Agarose gel electrophoresis image of RFLP-PCR products analysis of IL-4RαQ576R gene polymorphism

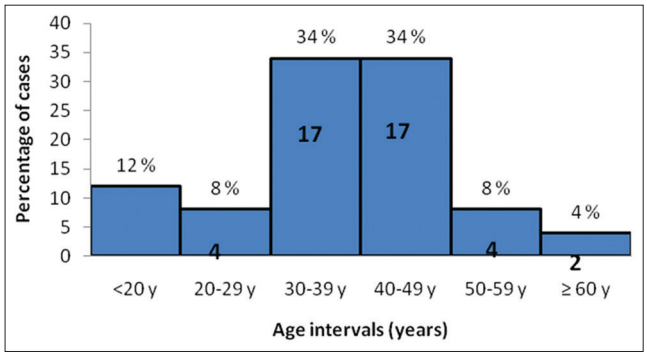

Fig. 3: Histogram showing the distribution of patients group according to 10 years' age intervals

Table 1: Distribution of control and penicillin allergic patients according to gender

Gender	Control group n=50 (%)	Patients group n=50 (%)	χ^2	p value
Male	13 (26)	24 (48)	0.649	0.420*
Female	37 (74)	26 (52)		

*Not significant at P≤0.05

appeared that genotype AA was more frequent in patients than in control groups; however, the difference did reach statistical (p=0.071). By another, genotype GA was largely lower in patients compared with the control group, 24% versus 44%, respectively (p=0.035). No significant difference was seen in the rate of genotype GG between patients and control groups, 20% versus 18%, respectively (p=0.799). Genotype AA appeared to be more associated with allergy to β-lactam drugs with OR of 2.081 (95% CI: 0.93-4.62), and the etiologic fraction (EF) was 0.31, also it was found that genotype GG carried a significant

Table 2: Serum median of IL-4 in control and study groups

Characteristic	Control group n=50		Patients group n=50		p value †
IL-4 (pg/ml), median (IQR)	284.72	125.55	348.53	245.57	0.001*

n: number of cases, IQR: Interquartile range, †: Mann–Whitney U-test, *significant at P<0.05

Table 3: Hardy–Weinberg equilibrium in control group

Genotype	Observed	Expected	p
AA	19	18.00	0.556*
GA	22	24.00	
GG	9	8.00	

*Not significant at P≤0.05

Table 4: Genotype and allele frequency distribution of IL-4Rα in control and patients group

Genotype	Control group n=50 (%)	Patients group n=50 (%)	P	OR	95% CI		EF	PF
					Lower	Upper		
AA	19 (38)	28 (56)	0.071	2.08	0.93	4.62	0.31	-
GA	22 (44)	12 (24)	0.035*	1.14	0.17	0.95	0.06	-
GG	9 (18)	10 (20)	0.799	0.4	0.42	3.10	-	0.34
Allele	**Control group n=100 (%)**	**Patients group n=100 (%)**	**P**	**OR**	**95% CI**		**EF**	**PF**
					Lower	Upper		
A	60 (60	68 (68)	0.239	1.42	0.79	2.53	0.16	-
G	40 (40)	32 (32)	0.239	0.71	0.40	1.26	-	0.16

*Significant at P<0.05, OR: Odds ratio, CI: Confidence interval, EF: Etiologic fraction, PF: Preventive fraction

Table 5: Level of serum markers according to genotype in control and study groups

Serum marker	Genotype	Median	IQR	p†
IL-4 (pg/ml)	AA	357.36	119.97	0.595*
	GA	332.42	433.24	
	GG	268.77	625.12	

protection and might be considered as a preventive effect against β-lactam drugs allergy with preventive fraction (PF) (0.34), as shown in Table 4, while genotype GA showed to be slightly associated with beta-lactam allergy with an effective fraction (EF=0.06). Regarding allele distribution, the results showed that the A allele was associated with beta-lactam allergy with an effective fraction (EF=0.16), and G allele carries A significant preventive action against beta-lactam allergy with a PF=0.16.

Association between IL-4RαQ576R genotypes and IL-4 serum level

The correlations between genotypes AA, GA, and GG and serum concentrations of IL-4 were shown in Table 5. There was no significant difference in the IL-4 level among patients with genotypes AA, GA, and GG (p=0.595).

DISCUSSION

Regarding the ELISA results of this study that revealed a significant association between IL-4 concentration and penicillin hypersensitivity (p=0.001), this result agrees with Qiao *et al.,* (2005) [19] study, in which they showed significantly higher level of IL-4 (100 ng/L) in penicillin allergic patients having +ve specific IgE (p<0.010) and lower IL-4 level (7 ng/L) in allergic patient with -ve specific IgE (p<0.05). These findings also consistence with Khaled *et a*l., (2016) [20], they study the relation of IL-4, IFN-γ, IL-10, IL-6, and IL-12 with penicillin HRs in 80 pediatric patients in KSA, the results declared that serum IL-4 level in sera of patients was significantly higher as comparing with apparently healthy control (p<0.0001), with average 178.0±110.2 and 48.1±35.1 pg/ml, respectively, and they conducted that IL-4 is specific marker for diagnosis of BLs induced HRs and may play an important role in BLs allergy.

This results in genotype frequency are consistent with Qiao *et al*., (2005) [19] they showed that the AA, GA, and GG level is higher in allergic patients with positives IgE, they were 77 (79%), 19 (19%), and 2 (2%), respectively, compared with healthy control 52 (60%), 32 (37%), and 3 (3%), respectively, which indicated that AA genotype was associated with allergic reaction and considered etiological genotype for hypersensitivity and more frequent in allergic patient than in control 77 (79%) versus 52 (60%). While GA genotype was slightly lower in patients 19% versus 37% in control, which might provide a slightly effective action against beta-lactam dug allergy. GG genotype was carried significant protection or preventive effect against beta-lactam allergy 2 (2%) in patients versus 3 (3%) in control. The results inconsistent with Gugliel *et al*., [13] that showed the genotype frequency of IL-4RαQ576R were: AA was slightly lower in patients 65.9% versus 78.65 in control, while AG was slightly higher in patients 34% versus 16.6 in control, and GG genotype has not been found in patients 0% and in control was 4.8%. This disagreement may be related to reasons belong to population sample, like ethnicity since Gugliel *et al.* made their study in France, but in general they found that the IL-4RαQ576R polymorphism associated with BLs HR.

Regarding allele distribution comparing between the result of this study that showed that the A allele percentage inpatient was higher, 68% versus 60% in control, while G allele was 32% in patient versus 40% in control subjects. G allele carries a significant protection or preventive effect against drug allergy, and in Qiao *et al*. (2005) [19], A allele percentage was 88% in patients versus 78% in control, and G allele was 12% in patients versus 22% in control, also the results disagree with Gugliel *et a*l., [13] they showed that allele frequency was**: A** 0.83 in patients compared with 0.875 in control, were as G was 0.17 in allergic patients versus 0.13 in healthy control**.**

CONCLUSION

Results of this study suggested that IL-4 cytokine play a crucial role in modulating and development of β-lactam allergy and can be

considered as a specific marker for the diagnosis of penicillin allergy. The IL-4RαQ576R polymorphism may have a role in penicillin allergy, and AA genotype approved to be associated with BLs allergy and, while GG genotype have a significant protection effect against penicillin allergy, moreover it was found that GA genotype might be also slightly associated with beta-lactam allergy, and regarding allele frequency it was found that A allele associated with beta-lactam allergy, while G allele provide significant protection against beta-lactam hypersensitivity.

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