Genotypes Variation of Interferon-gamma gene with chronic hypertension risk

Variación de genotipos del gen del interferón-gamma con riesgo de hipertensión crónica

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Abstract

The issue of interferon-gamma (IFN-) T/A +874 gene polymorphism has been assessed in various ethnicities with hypertension, and inconsistent outcomes have been provided. Hence, this study is devoted to the genetics study for interferon-gamma gene polymorphism in position T/A +874 for hypertension patients (mean age 56.95±1.9), and the control group (mean age 41.3±2.1). To meet that aim, ARMS-PCR (Amplification refractory mutation system technique) is utilized. The electrophoresis for IFN- +874 T/A gene revealed the presence of two alleles A and T, with three genotypes AA, TA, and TT. The results show a high frequency for allele T (67.11%) for IFN- +874 T/A gene as compared with A allele (32.88%) in hypertension patients' samples and related with an etiological fraction (EF) of hypertension dependent on values of odds ratio (4.52) and confidence intervals 95% (3.09 to 6.61), while A allele associated with a preventive fraction (PF) of hypertension. The ARMS-PCR analysis results present a high frequency of TT (homozygote) and TA (heterozygote) genotypes of IFN- T/A +874 gene in hypertension patients (46.32% and 41.52%, respectively) as compared with control (16.67% and 28.89%, respectively) and associated with Etiological fraction in hypertension risk, and OR was 4.31 with CI 2.32 to 8.02 and the etiological fraction was 35.6% for TT genotype while TA was of OR 1,75 and CI 95% from 1.02 to 3.00. The AA homozygote genotype presents high frequency in control (54.44%) as compared with hypertension patients (12.11%) and is associated with Preventive fraction. the OR was 0.12 with a CI of 95% from 0.06 to 0.21.

Keywords: Polymorphism, hypertension, IFN- T/A +874) gene.

Resumen

El problema del polimorfismo del gen interferón-gamma (IFN-) T/A +874 se evaluó en varias etnias con hipertensión y se proporcionaron resultados inconsistentes. Por lo tanto, este estudio está dedicado al estudio genético del polimorfismo del gen interferón-gamma en la posición T/A +874 para pacientes con hipertensión (edad media 56,95 ± 1,9) y el grupo control (edad media 41,3 ± 2,1). Para lograr ese objetivo, se utiliza ARMS-PCR (técnica de sistema de mutación refractaria de amplificación). La electroforesis para el gen IFN- +874 T/A reveló la presencia de dos alelos A y T, con tres genotipos AA, TA y TT. Los resultados muestran una alta frecuencia del alelo T (67,11%) para IFN- +874 gen T/A en comparación con el alelo A (32,88%) en muestras de pacientes hipertensos y relacionado con una fracción etiológica (FE) de hipertensión dependiente de valores de odds ratio (4,52) e intervalos de confianza del 95% (3,09 a 6,61), mientras que el alelo A se asoció a una fracción preventiva (FP) de hipertensión arterial. Los resultados del análisis ARMS-PCR presentan una alta frecuencia de genotipos TT (homocigoto) y TA (heterocigoto) del gen IFN- T/A +874 en pacientes hipertensos (46,32% y 41,52%, respectivamente) en comparación con el control (16,67%). y 28,89%, respectivamente) y asociado a fracción etiológica en riesgo de hipertensión, y OR fue 4,31 con IC 2,32 a 8,02 y la fracción etiológica fue 35,6% para genotipo TT mientras que TA fue de OR 1,75 e IC 95% de 1,02 a 3,00 . El genotipo homocigoto AA presenta alta frecuencia en el control (54,44%) en comparación con los hipertensos (12,11%) y se asocia a la fracción Preventiva. la OR fue de 0,12 con un IC del 95% de 0,06 a 0,21.

Palabras clave: polimorfismo, hipertensión, gen IFN- T/A +874).

Introduction

Hypertension is the most common cardiovascular disease that increases the risk of heart, kidney, brain, and other diseases[1]. Several studies show the correlation between genetic system dysregulation and hypertension clinical appearance[2]. The chronic inflammatory state in atherosclerosis is an important risk factor for cardiovascular outcomes, hypertension is a chronic disease and affects about 33% between the years 2010 and 2030 of the world's total population, and an estimated 1.28 billion adults aged 30-79 years worldwide have hypertension[1]. Cytokine variations are ever more recognized as part of hypertension pathophysiology, several studies have revealed that there is an inequality between T helper type1 and T helper type 2 cytokines in hypertension patients[3], this imbalance increases the levels of some cytokines such as Interferon-gamma (IFNγ) production[4]. Serum levels of several inflammatory cytokines such as Interferon-gamma are elevated in individuals with other diseases[5]. In most of the population, IFN-gamma is highly expressed in atherosclerosis and has emerged as an important factor in cardiovascular disease development[6]. It is known that the genetic characteristic of cytokine production is affected by polymorphism. Single nucleotide polymorphism in situ +874 T → A of the IFN-gamma gene may influence its increase or decrease secretion of interferon- gamma[7]. IFN-γ is secreted by T helper type1 cells, macrophages, natural killer cells, and mucosal epithelial cells as part of the innate immune response, and by CD4+ cells and CD8+ cytotoxic effector T helper cells when antigen-specific immunity progresses[8]. The cytokine encoding gene location associated with hypertension risk, IFN-ɣ gene located on the long arm of chromosome 12 in locus 14[9]. However, data with respect to polymorphism for particular genes related diseases, especially hypertension are limited in Iraq, so amplification refractory mutation system- Polymerase chain reaction (ARMS-PCR) was used to investigate for the first time whether polymorphisms in the IFN-γ (+874T/A) gene are associated with clinical manifestation in hypertension in the Iraqi population.

Materials and methods

Study population

The study population consisted of 190 patients with hypertension (range age 43-75 years) and 90 individuals as control of range age 30-68 years. All the patients were recruited from some hospitals in the Baghdad governorate. They had an established diagnosis of hypertension by the senior board-certified investigators based on specific interviews, clinical examination, medical records (hospital and patient clinic case observations), and family data. All patients were assessed for lifetime psychotic symptomatology using a questionnaire, which provides a diagnosis of hypertension that involve the following criteria: the patient's history, material addiction, and acute physical health injuries by a full medical examination. The control group involved healthy volunteers free from present, past, and family history (first-degree relatives) of psychiatric disease, no present infections, allergies, or current and past history of autoimmune disorders. The study was approved by the Ethical Committee of the College of Medicine, Al-Iraqia University, Baghdad.

Genotyping procedures

Genomic DNA was extracted by taking five ml of blood from each patient and healthy control by venipuncture, later, 1.5 ml was added to EDTA tubes then DNA was extracted by DNA isolation kit (Geneaid) and according to the manufacturer instructions manual. DNA purity was qualified by nanodrop, and it was about 1.6 ±1.8. All samples were kept at 20 ° for further study. Polymorphisms of the IFN-γ gene in local +874A/T were examined using ARMS-PCR method. For each allele, PCR reaction was carried out on a DNA template with a pair of specific primers (Alpha DNA for Canada) by source[10], (Table 1), 20 µl was the total volume of reaction mix (Bioneer, Korea), and the molecular marker size100-2000 base pair (Bioneer, Korea). Amplification was accomplished using a thermal cycler by ARMS PCR cycling conditions were summarized in table 2 by source[10]. The genotypes were established by analyzing electrophoresed 2% agarose gel stained with ethidium bromide, and a power voltage of 75V.

Table 1. Primer sequences of IFN-γ +874 A/ T gene for hypertension patients and control groups samples.

Target Gene	primer	Primer sequences (5`→ 3`)	Size (bp)
IFN-γ +874 A/T	Specific T	TTCTTACAACACAAAATCAAATCT	262
	Specific A	TTCTTACAACACAAAATCAAATCA	
	Antisense	TCAACAAAGCTGATACTCCA	

Statistics

Differences in the frequencies of IFN-γ 1alleles for hypertension patients in this study with control groups were analyzed with a value of P<0.05 by Fisher's exact test. Odds ratios (OR) and confidence intervals (CI) were calculated using Compare 2 Ver.3.04 software (J. H. Abramson (2003-2013). Preventive Fraction (PF) and Etiologic Fraction (EF) results were compared with Hardy-Weinberg equilibrium and according to the software within the following website: www.had2know.com.

Table 2. The cycling condition for ARMS-PCPR program for detection of IFN-γ +874 A/T in hypertension patients and control group samples.

Target gene	steps	Temperature (c°)	Cycles	seconds
IFN-γ +874 A/T	Pre-denaturation	96		180
	Initial denaturation	95	15	15
	Annealing	64		50
	Extension	72		40
	Initial denaturation	95	20	50
	Annealing	55		50
	Extension	72		50
	Final Extension	7		420

The genetic polymorphism of IFN-γ in position +874T/A was studied in one hundred and ninety hypertension patients with a mean age of 56.95±1.9 years, and ninety control individuals as a healthy group with a mean age of 41.3 ±2.1 years. Notably, two

Figure 1. Electrophoresis for IFN-γ T/A +874 genotype in hypertension patients, lane M DNA marker (100 to 2000 bp), lane 1,4 and 7 TA genotype, lane 6 AA genotype, lane 2, 3, 5, and 8 TT genotype.

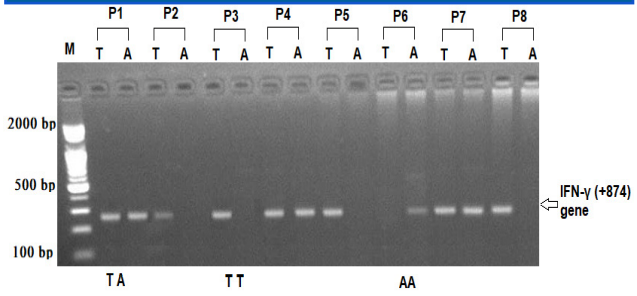
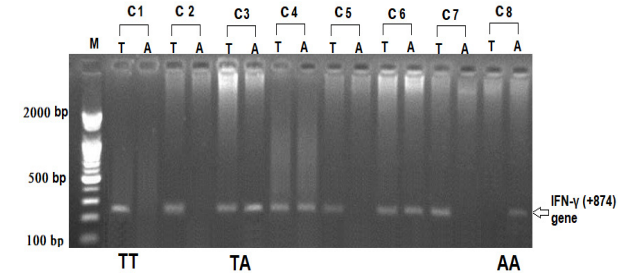


Figure 2. Electrophoresis for IFN-γ +874 A/ T genotype in control lane M DNA marker (100 to 2000 bp), lane 1, 2, 5, and 7 TT genotype, lane 3, 4, and 6 TA genotype, lane 8 AA genotype.



Allele frequency and genotype distribution for each tested polymorphism for healthy control and hypertension patient are presented in Table 3. With respect to the IFN-γ +874T/A polymorphism, hypertension patient was significantly higher compare with the control group (P>0.05), and the A and T alleles were different in frequency, so allele T frequency was 67.11% for hypertension patient and 31.11% for control group, while allele A frequency was 32.89% in the control group and 68.89% in hypertensive ones (~~Figure~~ Table 3). The OR for allele T was 4.52 with CI 3.09 to 6.61 at 95 % (Table 3), and it was 52.2% as an etiological fraction, compared OR for A allele was 0.22 with CI 0.0.15 to 0.32 at 95% and the value of A allele as preventive fraction was 53.6%. The previous report on polymorphism of IFN-γ (+874T/A) shows that T allele may be an etiological fraction (EF) and it's described that the A allele may be a preventive fraction (PF) that correlated with the risk of hypertension in Iraqi population.

Based on genotyping of IFN-γ polymorphisms in local +874T/A by ARMS technology, genotypes frequency is significantly higher in hypertension patients and the control group (healthy), so, TT homozygote and TA heterozygote genotypes showed a high frequency in hypertension patients as compared with a low control group, it was 46.32% for TT in hypertension patients and 16.67% in control. Also OR was 4.31 with CI 2.32 to 8.02 and the etiological fraction was 35.6%, while TA the frequency was 41.58% for patients and 28.89% for health group, also the OR was 1.75 and CI was 1.02 to 3.00 and the value for TA as etiological fraction was 17.8%. Showing the AA homozygote genotype, the recurrence rate of the control group was higher than the hypertensive patients. They were 54.44% in control and 12.11%, in hypertensive patients. The OR was 0.12 with a CI of 0.06 to 0.21 at 95 %, and the AA genotype value as a protective fraction was 48.2% as shown in (Table 4). Briefly, the result showed that TT homozygote and TA heterozygote genotypes were correlated with the risk of hypertension, while the ~~TA~~ AA genotype was correlated with the protective fraction of hypertension.

Table 3. Genotype distribution and allele frequencies of polymorphisms in IFN-γ T/A +874 gene in healthy and hypertension patients' samples

Target Gene	Allele	hypertension patients Number (%)	Control Number(%)	OR (95%CI)	P-Value
IFN-γ +874 T/A	A	32.89%)125)	68.89% (124)	0.22(0.15 to 0.32)	**0.0001
	P.F	%53.6			
	T	67.11%(255)	31.11% (56)	4.52(3.09 to 6.61)	
	E.F	%52.2			

Notes: OR= Odds ratio, CI= Confidence Interval, P.F= Preventive fraction E.F= Etiological fraction, P<0.05 by Fisher's test.

Table 4. Genotypes of IFN-γ T/A +874 gene for healthy and hypertension patients' samples

Gene	Genotype	hypertension patients Number	Healthy Number	OR(CI 95%)	P-value
IFN-γ T/A +874	AA	12.11%)23(	54.44%(49)	0.12(0.06 to 0.21)	**0.0001
	P.F	%48.2			
	TA	41.58%)79)	28.89%)26)	1,75(1.02 to 3.00)	*0.041
	P.F	%17.8			
	TT	46.32%(88)	16.67%(15)	4.31(2.32 to 8.02)	**0.0001
	E.F	%35.6			

Notes: OR= Odds ratio, CI= Confidence Interval, P.F= Preventive fraction E.F= Etiological fraction, P<0.05 by Fisher's test.

Discussion

Interferon-gamma (IFN-γ) is a pleiotropic cytokine stimulated in Pro-inflammatory and anti-inflammatory immune responses by the balance of other cytokines[11]. Furthermore, the IFN-γ expression was organized by MHC (Major Histocompatibility Complex) types that have an essential role in cell killing through T- cytotoxic (CD8+) during pathogenesis. An increase in the expression of other cytokines, such as TNF-a, IL-1, and IL-6 destroys tissues[12]. Cytokines are thoroughly linked with diseases of the circulatory system and heart; cytokines might work together with cardiovascular, influencing blood pressure[13].

Studies have shown a relationship between the genotypes of the interferon gene and people with high blood pressure[14]. In hypertensive patients' groups, we demonstrated that patients with the TT homozygote genotype in the IFN-y polymorphisms had a higher etiological risk and cardiovascular disease. It was shown an increase of T allele related to patients with unstable angina and myocardial infarction[15-17]. Similarly, Saurus et al.[14] showed in hypertensive individuals that the presence of the T allele and TT genotype of IFN-gamma at the T/A + 874 local polymorphisms resulted in a higher incidence of hypertension. These results are consistent with the results of our study in terms of an association of T allele and TT genotype with hypertensive patients. The results of the study are also in line with the study of Manginas et al.[15] which examined the inflammatory cytokine gene variants in coronary artery disease patients in Greece.

Conclusions

The present study demonstrated that the polymorphism of the IFN-γ gene in local T/A +874 is associated with hypertension risk in Iraqi patients.

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