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INTERLEUKIN-1 RECEPTOR ANTAGONIST GENE POLYMORPHISM IN PATIENTS WITH CORONARY ARTERY DISEASES

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ABSTRACT

Cytokine gene variations are contributory factors in inflammatory pathology. Allele frequencies of Interleukin-1 receptor antagonist (IL-1Ra) gene intron 2 VNTR were measured in healthy blood donors (healthy control subjects) and patients with angina, myocardial infarction (MI) and acute coronary syndrome (ACS).

Patients were classified into three groups: thirty one MI patients, twenty two angina patients and thirteen ACS patients. A1/A2 genotype showed significant resistant factor for angina and myocardial infarction and angina (70.97% vs. 29.03%; P=0.0001, 70.97% vs. 31.82%; P= 0.0004, respectively). A1/A1 homozygote was a risk factor in MI and angina (P=0.012; P= 0.0001, respectively), Moreover, A1/A3 and A2/A3 heterozygotes were found in MI only (P= 0.025; P= 0.0047, respectively). All genotypes didn't show any effect on ACS patients.

The data reflect that A1/A1 homozygote can be considered as a significant risk factor associated with angina as well as MI patients, but A1/A2 heterozygote was considered a resistance factor against both diseases.

INTRODUCTION

The genetic basis of coronary artery disease (CAD) may arise from a gene having a direct effect on the initiation of the disease process or a modifying effect on the development of the process or after initiated **[Francis *et al.*, (1999)]**. Interleukin-1 receptor antagonist (IL-1Ra) is a counterinflammatory cytokine encoded by a polymorphic gene in the IL-1 gene family. IL-1Ra is produced locally in various tissues in response to infection or inflammation, and is present in high levels in the circulation secondary to hepatic production as an acute-phase protein **[Gabay *et al.*, (1997)]**. The genes encoding for IL-1Ra are located on the long arm of chromosome 2 in human (2q14-21) **[Steinkasserer *et al.*, (1993)]**. Intron 2 of Il-1Ra gene is 86-bp of tandem repeat polymorphism that leads to the existence of five alleles. The number of times this sequence is repeated in different persons varies from 2 to 6 and the frequency of the individual alleles varies among different ethnic or geographic population **[Bid *et al.*, (2004)]**. Because of its biological plausibility for involvement in atherosclerosis, its association with inflammatory disease and its potential influence on IL-1Ra and other cytokine production ***[Hurme and Santtila, 1998]***, the rationale of the present study was to investigate IL-1Ra in patients with CAD as the first aim.

SUBJECTS AND METHODS

Blood samples were obtained from 66 patients with angiographically-documented coronary artery disease. These patients were admitted to Cardiology Department of Medicine Hospital, Mansoura University between July, 2008 to December, 2009 and the sample were collected under supervision of cardiology physician. An informed consent was obtained from all individuals who participate in the study and they were fully informed of the nature of the disease and the diagnostic procedures involved. These patients were classified into three groups: First group which include 31 patients suffering from myocardial infarction (MI) (22 male, 9 female, mean age 59.71±8.9 y), second group which included 22 patients suffering from angina (14 male, 8 female, mean age 55.5±10.06 y) and third group which included 13 patients suffering from acute coronary syndrome (ACS) (9 male, 4 female, mean age 59.54±10.53 y). All the patients were examined clinically and information pertaining to age, gender, habits and health status was followed by a series of laboratory investigation. 124 healthy adults with no known history of any disease (110 male and 14 female, mean age 25.98±5.7 y) were recruited as a negative control group **Table (1).**

Table (1): Baseline characteristics of MI, angina, ACS and control groups:

Characteristics	Control (n= 124)	Patient Groups		
		MI (n= 31)	**Angina** (n= 22)	**ACS** (n= 13)
Age (mean±SD)	25.98±5.7	59.71±8.9	55.5±10.06	59.54±10.53
Male	110(88.71%)	22(70.97%)	14(63.64%)	9(69.23%)
Female	14(11.29%)	9(29.03%)	8(36.36%)	4(30.77%)
Diabetes	—	22(70.97%)	15(68.18%)	7(53.85%)
Hypertension	—	21(67.74%)	20(90.91%)	11(84.62%)
Dyslipidemia	—	16(51.61%)	19(86.36%)	9(69.23%)
Smoker	—	20(64.52%)	10(45.45%)	8(61.54%)
Non-smoker	—	11(35.48%)	12(54.55%)	5(38.46%)

n= number of studied cases, (%) = percentage of studied cases, MI= myocardial infarction, ACS= acute coronary syndrome.

After obtaining informed consent, blood samples were drawn following an overnight fasting by venipuncture into tubes, with EDTA.

DNA was extracted from the blood samples of both patients and control groups, then DNA was purified using DNA purification capture column kits (Gentra system, USA). Amplification via PCR technique was carried out for IL-1Ra intron 2 contained the 86 bp VNTR. Each PCR was carried out in 25 µL reaction mixture containing 10 µl master mix (Fermentas, Germany), 8 µl PCR grade water, 2 µl IL-1Ra forward (F) primer, 2 µl IL-1Ra reverse (R) primer (Bio Basic Inc., Canada) and 3 µl extracted DNA according to the method of **[Wilkinson *et al.*,(1999)]**. Primers sequence were

(F): 5'-TCCTGGTCTGCAGGTAA-3'

(R): 5'CTCAGCAACACTCCTAT-3'

PCR conditions: initial denaturation cycle of 95°C for 5 min followed by 35 cycles in the form of 94°C for 30 s (denaturation), 55°C for 30 s (annealing) and 72°C for 1 min (extension) with a final extension cycle of 5 min at 72°C. Amplified segment

was run on agarose gel (3%) electrophoresis, photographed on ultraviolet transilluminator **[Zhang *et al.*, (2004)]**.

STATISTICAL ANALYSIS

Statistical analysis was done using the statistical package of social sciences (SPSS) software version 11.5 **[SPSS, (1999)]**.

RESULTS

The data in the present study revealed three alleles including allele 1 (four repeats, 410 bp), allele 2 (two repeats, 240 bp) and allele 3 (five repeats, 500 bp), and five genotypes of IL-1Ra including A1/A1, A2/A2, A1/A2, A1/A3 and A2/A3 could be recognized among CAD cases and healthy control (Figures 1and 2).

Patients with CAD showing clearly a significant lower frequency of A1/A2 genotype compared to controls (33.33% vs. 70.97%, p= 0.0001, OR= 0.205). This means that A1/A2 genotype might be considered a protective factor against CAD, whereas Allele 2 frequency was responsible for this protective effect (28.79% vs. 43.55%, p= 0.005, OR= 0.524). On the other hand, genotypes A1/A1 (homozygote) and A2/A3 (heterozygote) were demonstrating significant increase in patients compared with controls (45.45% vs. 20.16%, p= 0.0003 and 6.06% vs. 0.00%, p= 0.0137, respectively **Table (2) and Figure (3).**

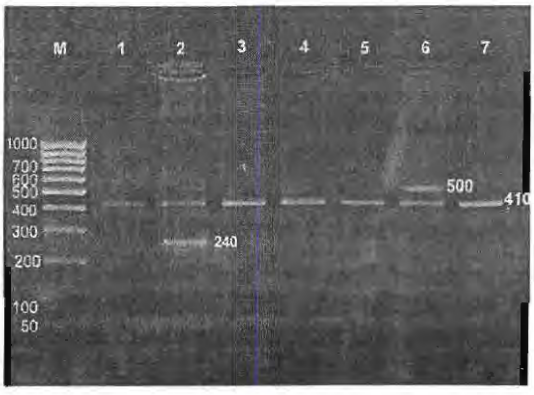

Fig. (1): Agarose gel shows PCR product for IL-1Ra gene intron 2 polymorphism in **CAD patients**.

- Lane (M) : shows DNA Ladder (100 bp).
- Lane (1, 3 , 4, 5 and 7): shwed A1/A1 homozygote polymorphism of IL- IRa at 410 bp.
- Lane (2) : shows heterozygote polymorphism of IL-1Ra A1/A2 genotype A1 at 410 bp and A2 at 240 bp.
- Lane (6): shows heterozygote polymorphism of IL-1Ra A1/A3 genotype where A1 at 410 bp and A3 at 500 bp

B and A3 at 5

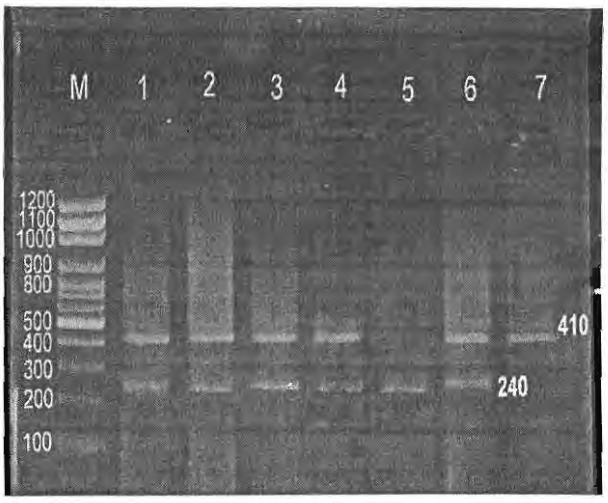

Fig. (2): Agarose gel shows PCR product for IL-1Ra gene intron 2 polymorphism in **control**.

- Lane (M): shows DNA ladder (100 bp).
- Lane (1, 2, 3, 4, and 6): shows A1/A2 heterozygote polymorphism of IL-1Ra at 410 bp and A2 at 240 bp.
- Lane (5): shows homozygote polymorphism of IL-1Ra A2/A2 genotype where A2 at 240 bp.
- Lane (7): shows homozygote polymorphism of IL-1Ra A1/A1 genotype where A1 at 410 bp.

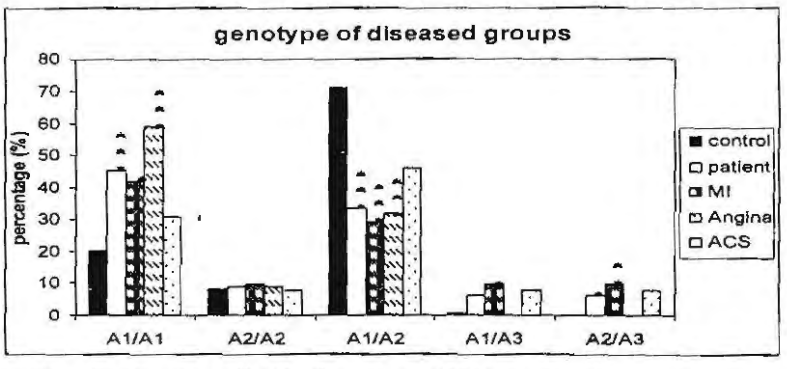

Fig. (3): Genotype distribution among CAD cases and controls, where * significant, ** very significant and *** highly

Table (2): Comparison between allele frequencies of IL-1Ra polymorphism in different genotypes in different studied groups.

IL-1Ra Genotype		Groups				
		Control (n= 124)	Patient (n= 66)	MI (n= 31)	Angina (n= 22)	ACS (n= 13)
A1/A1	n (%)	25 (20.16%)	30 (45.5%)***	13 (41.93%)*	13 (59.1%)***	4 (30.77%)
	P	—	0.0003	0.012	0.0001	0.4732
	OR (95% CI)	—	3.3 (1.72-6.34)	2.86 (1.24-6.61)	5.72 (2.2-14.89)	1.76 (0.5-6.19)
A1/A2	n (%)	88 (70.97%)	22 (33.3%)***	9 (29.03%)***	7 (31.8%)***	6 (46.16%)
	P	—	0.0001	0.00۰۱	0.0004	0.067
	OR (95% CI)	—	0.205 (0.11-0.39)	0.167 (0.07-0.4)	0.191 (0.07-0.51)	0.351 (0.11-1.12)
A2/A2	n (%)	10 (8.06%)	6 (9.09%)	3 (9.68%)	2 (9.09%)	1 (7.69%)
	P	—	0.808	0.724	1	1
	OR (95% CI)	—	1.14 (0.4-3.29)	1.221 (0.32-4.73)	1.14 (0.23-5.6)	0.95 (0.11-8.07)
A1/A3	n (%)	1 (0.81%)	4 (6.06%)	3 (9.68%)*	—	1 (7.69%)
	P	—	0.0502	0.025	1	0.1814
	OR (95% CI)	—	7.935 (0.87-72.52)	13.18 (1.32-131.5)	—	10.25 (0.6-174.5)
A2/A3	n (%)	—	4 (6.06%)*	3 (9.68%)**	—	1 (7.69%)
	P	—	0.0137	0.0047	—	0.0949
	OR (95% CI)	—	—	—	—	—

MI= myocardial infarction, ACS= acute coronary syndrome, n= number of cases, (%)= percentage, OR= odds ratio, CI= confidence interval, P= probability and it is obtained from chi-square test for cells which contain expected values >5 and Fisher's exact test for cells contain expected value <5.
Genotypes is expressed as number of cases (proportion in % within brackets). * significant P≤0.05, ** very significant P≤0.01 and *** highly significant P≤0.001.

Both MI and angina groups showed a significant lower frequency of A1/A2 genotype when compared with controls (29.03%, P=0.0001, OR=0.167 and 31.82%, P=0.0004, OR=0.0191, respectively), whereas a significant higher frequency of A1/A1 genotype, when compared with controls (41.93%, P=0.012, OR=2.86 and 59.09%, P=0.0001, OR=5.72, respectively). Also, homozygote A2/A2 showed non-significant in MI and angina groups (9.68%, P=0.724, OR=1.221 and 9.09%, P=1, OR=1.014,

respectively). In MI group, A1/A3 and A2/A3 were significant higher when compared with controls (9.68% vs. 0.806%, P=0.025, OR=13.18 and 9.68% vs. 0.00%, respectively **Table (2) and Figure (3).**

On the other hand, acute coronary syndrome group (ACS) showed statistically non-significant in all IL-1Ra genotypes **Table (2) and Figure (3).**

DISCUSSION

This study divided patients into three groups: myocardial infarction group (MI), angina and acute coronary syndrome group (ACS). In MI and angina groups, we found that genotype A1/A1 was significantly increase (41.93%; P<0.05 for MI and 50.09%; P<0.001 for angina) when compared with control (20.16%). Seripa et al. **[2003]** were agreed with ours. They published that the frequency of A1/A1 genotype was significantly higher in stroke survivors when compared with controls (77.2% and 45.5%, respectively; P< 0.001).

Moreover, our results showed that A1/A3 and A2/A3 heterozygotes were act as another risk genotypes beside A1/A1 for MI patients only (P< 0.05 and P< 0.001, respectively) and this was due to the haplotype 3 (allele 3) of the gene encoding for IL-1Ra was associated with reduced mRNA levels of this gene which is the principal antagonist of IL-1 and this increased the incidence of MI [**van Minkelen *et al.*, (2009)**].

Genotype A1/A2 was acting as a resistant factor against MI and angina as shown in our study. This resistant heterozygote genotype (A1/A2) showed another difference between risk and protective haplotype because of the presence of allele 1 in the risk haplotype and allele 2 in the protective haplotype. In this case the role of this polymorphism as a susceptibility marker for cardiovascular disease is controversial, with positive and negative results reported in the international literature. For instance, [**Francis *et al.*, (1999)**] reported an association of allele 2 in single vessel coronary disease, but not in multi-vessel coronary disease. These findings were not confirmed in a subsequent study suggesting that this marker is not related with the risk of developing cardiovascular disease **[Iacoviello *et al.*, (2000)]**. **[Tolusso *et al.*, (2006)]** reported that healthy blood donors homozygous for allele 2 had decreased plasma levels of IL-1Ra as compared with non-allele 2 carriers. In contrast, **[Hurme and Santtile, (1998)]** found that individuals with allele 2 have 1.2-fold increased IL-1Ra plasma levels. Besides, in vitro studies suggested a high production of IL-1Ra in individuals with allele 2 **[Vamvakopoulos *et al.*, (2002)]**. These findings are agreeing with our results, since the individuals with these haplotypes could produce more IL-1Ra, diminishing the pro-inflammatory effects of IL-1.

Acute coronary syndrome (ACS), in which the inflammatory stimulus is much stronger and pathogenetic mechanism is complicated by many additional factors, the effect of allele 2 might no longer be evident.

Our study didn't show any effect to A2/A2 homozygote genotype, this is because in every population studied to date, most persons are either A1/A1 homozygous (which is named IL-1RN*1) or A1/A2 heterozygote (IL-1RN*1/ IL-

1RN*2). The prelevance of A2/A2 homozygote (IL-1RN*2) is typically <10%. In black Africans and African American persons, the frequency of IL-1RN*2 homozygote is considerably lower than in white population **[Rider *et al.*, (2000); Mwantembe *et al.*, (2001)]**.

In conclusion, we speculated that subjects having the IL-1Ra A1/A1 genotype were in higher risk for CAD. MI subjects also had A1/A3 and A2/A3 genotypes. Whereas those with A1A2 genotype seemed to be more protected against such infection.

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الملخص العربى

دراسة التعدد الشكلي لجين مضاد المستقبل انترليوكين-١ فى مرضي الشريان التاجى.

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يعتبر جين مضاد المستقبل انترليكين -١ من الجينات المضاده للالتهاب، يحتوى انترون ٢ من هذا الجين على ٨٦ زوج من القواعد، و هذا العدد يتكرر من مرتين الى ست مرات ،وهذا التكرار يختلف من شخص لآخر بإختلاف العرق. وبسبب مساهمات هذا الجين فى إنتاج البروتين الخاص به فى مناطق تجمع الدهون حيث تتكون الجلطات، لذا تتم الإستعانة به فى هذه الدراسة لرؤية مدى تأثيره على مرض الشريان التاجى حيث إن هذا المرض هو السبب الرئيسى الثانى للوفاة فى العالم.

وكان الهدف من هذه الدراسة هو إيجاد العلاقة بين التعدد الشكلى للجين الخاص بمضاد المستقبل انترليوكين-١ ومرض الشريان التاجى، ومدى تأثيره أيضاً على الجهد التأكسدى وبعض القياسات البيوكيميائية الأخرى لهؤلاء المرضى.

تم عمل تكبير لجين مضاد المستقبل انترليوكين-١ بإستخدام تفاعل البولمريز المتسلسل للمرضى الذين تم تقسيمهم الى ثلاث مجموعات (مرضى الذبحة الصدرية وعددهم ٢٢ مريض، مرضى المصابين بموت فى جزء عضلة القلب وعددهم ٣١ مريض، ومرضى الشريان التاجى الحاد وعددهم ١٣ مريض). وقد تم تجميع ١٢٤ عينة من أشخاص أصحاء من الذين لا يعانون من وجود أى أمراض وهم من المتبرعين لبنك الدم بمستشفى الأطفال الجامعى بجامعة المنصوره.

ومن خلال هذه الدراسة وجد أن للجين طرز عديده بين المرضى المصابين وكذلك الأصحاء، منها الطراز الجينى A1/A2 وهو مميز للأصحاء عن المصابين. أما طراز A1/A1 فإن نسبة ظهوره فى المرضى أعلى من الأصحاء. بينما وجد الطرازان A1/A3 و A2/A3 فى المرضى المصابين بموت جزئى فى عضلة القلب.