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The association between IFN- γ-784 T/A gene with acute myeloid leukemia in Iraqi population

Hassan Nasser Ali , Assit prof Dr. Anwar Abed Nasser Dhabaan
AL-Iraqia University/ College of Education/department of biology, Baghdad, Iraq
Email: dr.anwar.a.nasser@gmail.com and Email: nassirhassan706@gmail.com

Abstract :

Acute myeloid leukemia is a malignant hematological disease characterized by the proliferation of medulloblasts or primary cells that fail to undergo normal differentiation. It can also be defined as leukemia or bone marrow cancer - which occurs in the tissue within the bone in which blood cells are made and in which immature (cancer) cells accumulate (Newell and Cook., 2021). "Acute" in myelogenous leukemia indicates progression It is called myelogenous leukemia because it affects a group of white blood cells that develop normally in different types of mature blood cells such as red blood cells and platelets.This study included 80 acute myeloid leukemia and control samples, comprising 50 samples for Patients and 30 for control healthy (age range from 20 to 70 years), We determined the frequency of IFN - γ -784 T/A gene polymorphism by tetra-amplification refractory mutation system (TARMS-PCR). Also, we determined the association of IFN-γ -784 T/A polymorphism with acute myeloid leukemia in Iraqi patients. The results show high frequency for allele A (88%) for IFN-γ +874 T/A gene as compared with T allele (12%) in acute myeloid leukemia samples and related with etiological fraction (EF) of acute myeloid leukemia, while T allele show high frequency in control (53.33%), compared with T allele (46.67%), associated with preventive fraction (PF) of acute myeloid leukemia.

There was significant difference between genotype frequency of IFN - γ -784 T/A polymorphism for acute myeloid leukemia and control. The result Show AA (82%), TA (12%), and TT (6%) frequency in patients, while in control AA was (36.67%), TA (33.33%), and TT (30%), and show AA genotype related with etiological fraction (EF) in acute myeloid leukemia, while TT and TA genotypes related with preventive fraction (PF). Our findings demonstrate that the IFN gene polymorphism in position-784 T/A may show risk factor for acute myeloid leukemia in Iraqi population and there is association between the IFN - γ -784 T/A polymorphism and acute myeloid leukemia.

Keywords: IFN - γ -784 T/A, Polymorphisms, acute myeloid leukemia

العلاقة بين التعدد الشكلي المظهري لجين الانترفيرون –كاما IFN- γ-784 T/A وسرطان الدم النخاعي الحاد في المجتمع العراقي

حسن ناصر علي ، أ.م.د.انور عبد ناصر ضبعان
الجامعة العراقية / كلية التربية - قسم علوم الحياة

مستخلص:

ابيضاض الدم النخاعي الحاد (AML) Acute myeloid leukemia هو مرض دموي خبيث يتميز بتكاثر الأورمات النخاعية أو الخلايا الأولية التي تفشل في الخضوع للتمايز الطبيعي. يمكن تعريفه ايضا بأنه سرطان الدم اونخاع العظم – الذي يحدث في النسيج الموجود داخل العظم الذي تُصنع فيه خلايا الدم والذي تتراكم فيه الخلايا (السرطانية) غير الناضجة (Newell and Cook.,2021). تشير كلمة «حاد» في ابيضاض الدم النقوي إلى التقدم السريع للمرض، ويطلق عليه ابيضاض الدم النقوي نظرًا لأنه يؤثر على مجموعة من خلايا الدم البيضاء والتي تتطور بشكل طبيعي ضمن الأنواع المختلفة من خلايا الدم الناضجة كخلايا الدم الحمراء والصفائح الدموية. شملت الدراسة 80 عينة دم، كان منها 50 عينة لأشخاص مصابين بسرطان الدم النخاعي الحاد Acute Myeloid leukemia، و30 عينة لأشخاص أصحاء (عينات سيطرة)، تراوحت أعمارهم ما بين (70–20) سنة. درس التعدد الشكلي المظهري لجين الإنترفيرون – كاما (IFN – γ) المتضخم باستعمال تقانة تفاعل البلمرة لنظام الممانعة للتضاعف Amplification refractory mutation system (ARMS-PCR)، تم تحديد العلاقة جين الإنترفيرون وسرطان الدم النخاعي الحاد في المرضى المصابين ومقارنتها بالعينة القياسية. أظهرت النتائج وجود فروق معنوية مع نسبة تكرارا عاليا للأليل A في عينة المصابين بالمقارنة مع الأليل T (٪88 و 12 بالتتابع)، وظهر الأليل A كأليل مسبب مرتبط مع خطر الإصابة بسرطان الدم النخاعي الحاد، بينما سجل الأليل T نسبة اعلى من الأليل A في العينة القياسية (٪53 و ٪46 بالتتابع)، وظهر الأليل T كأليل مرتبط مع الجزء وقائي للمرض. ظهر النمط الوراثي AA كنمط مرتبط وبصورة معنوية مع الجزء المسبب للإصابة بالمرض، وكانت نسبته لدى المصابين ٪82 بينما لدى الاصحاء ٪36.67، بينما ظهر النمطان الوراثيين TT و TA كنمطين مرتبطين وبصورة معنوية مع الجانب ألوقائي من خطر الإصابة بسرطان الدم ألنخاعي الحاد، وكانت نسبتهما لدى المرضى ٪12 و ٪6 بالتتابع، بينما لدى الاصحاء كانت ٪33.33 و ٪30 بالتتابع، كما أظهرت نتائج التحليل الإحصائي وجود علاقة معنوية ما بين جين IFN - γ وسرطان الدم النخاعي الحاد، وأظهرت هذه العلاقة ارتباط معنوي لبعض الانماط الوراثية لجين الإنترفيرون – كاما في الموقع T/A 784 مع تطور خطر الإصابة بسرطان الدم ألنخاعي الحاد.

الكلمات المفتاحية: جين الإنترفيرون – كاما، ألتعدد الشكلي المظهري، سرطان الدم النخاعي الحاد.

Introduction

Interferon- gamma (IFN - γ) It is a glycoprotein with a low molecular weight between 17-25 kDa has been implicated in the pathogenesis of a large number of human diseases (Elahi at al.,2009). which produced by immune cells having the ability to suppress tumor cell proliferation and induce tumor regression (Feng et al., 2014). It is also known as Type II interferon or macrophage-activating factor (MAF). The IFN - γ gene is located on chromosome 12 in region 24 of the long arm of chromosome 12, and it consists of 4 exons and three introns (Calvo at al., 2002). Since It was first distinguished in 1965 AD in Murine Kupffer cells and macrophage. The production of interferon-gamma is stimulated by several cells, including active Th1 helper T cells and CD8+ cells, and it can also be produced by cytosolic killer cells. IFN-y directly inhibits viral proliferation and antigen scaling (Saha et al., 2010). Cytokines and IFNγ production is expressed by the IFN-y gene (Saha et al., 2010). IFN -γ expression is associated with a number of autoinflammatory and autoimmune diseases, and the importance of IFN- γ in the immune system lies in its ability to directly inhibit viral replication, as IFN- γ is mostly produced by natural killer (NK) cells as part of The innate immune response, and by CD8 and CD4 lymphocytes (CTLs) during the development of antigen-specific immunity as part of the acquired immune response (Novikov et al., 2011). Cancer of acute myelogenous leukemia (AML) is not clear, but the infection may appear in patients who have problems with blood tissue, or it may occur in people exposed to harsh chemicals or radioactive agents, or it may occur in people with a genetic history of the disease or event They have genetic changes in the genes responsible for the normal proliferation, differentiation and maturation of myeloid cells (Shah et al., 2013). **The aim of study**:

1- Determination of the polymorphisms of the interferon- gamma gene IFN-γ at the +874 T/A site using ARMS-PCR technology in samples of the infected and the standard sample..

2- Studying the relationship between the studied gene in patient samples and comparing it with healthy people and determining the extent of the relationship between these genes and the risk of developing acute myelogenous leukemia.

Materials and methods

for hematological analysis and DNA extraction. These blood sample were obtained from 50 patients whose ages ranged between 40-70 years with Acute myeloid leukemia attending the hematology hospital of Medical City-Baghdad, Iraq during the period from September to November 2021, another 30 sample are apparently healthy as controls. Blood samples were collected according to the ethical consideration and the hospital mangers approvals.

Genomic DNA extraction

Genomic DNA was extracted from 200 µl of whole blood using the ReliaPrep^{TM}Blood gDNA Miniprep System kit (promega). Concertation and purity ware detected using nanodrope.

Cytokine genotyping

The **IFN-** γ gene T and A alleles at positions -784 were genotyped using the amplification refractory mutation system (ARMS-PCR) reaction. The PCR Primers sequences were used in present study are shown in table (1). I used the ARMS-PCR program as shown in the table 2. (Depending on [9] and [10] sources). Electrophoresis was dose by using 2 % agarose gel concentration for 1 hour, 75 Vol, stained with ethidium bromide.

Table 1: The primers sequence IFN- γ-784 T/A of genotypes.

No	Primers **names**	Sequence of Genetic primer
1	Generic	5` -TCAACAAAGCTGATACTCCA-3`
2	A allele	5`-TTCTTACAACACAAAATCAAATCA-3`
3	T allele	5`-TTCTTACAACACAAAATCAAATCT-3`

Table 2: The ARMS-PCR Technique using for IFN- γ-784 (T/A) genotype.

Target gene	steps	Temperature (c°)	Cycles	seconds
IFN-γ + 874 A/T	Pre-denaturation	96		180
	Initial denaturation	95	15	15
	Annealing	65		50
	Extension	72		40
	Initial denaturation	95	20	50
	Annealing	55		50
	Extension	72		50
	Final Extension	7		420

3. Statistical analysis

The differences in allele/genotype (IFN- γ-784 T/A) frequencies and significant between patients and controls were analyzed bythe Fisher's exact test,Pvalues less than 0.05, were considered significant. The strength of the association of disease with respect to a particular allele/genotypeis expressed by odd ratio (OR), and Confidence Intervals (CI) interpreted as relative risk (RR) following the Woolf's method as out lined by Schallreuter and his colleagues [11].

4. Results

The genetic frequency of IFN- γ-784 T /A gene in acute myeloid leukemia patients and control by using Nasted -PCR (amplification refractory mutation system-polymerase chain reaction). Note, three genotypes TT, TA and AA are present in patients and control (figure 1 and 2). Table 1 showed the alleles frequencies of IFN- γ-784 T /A in acute myeloid leukemia and control individuals. The frequency of A allele ratio was 88% in patients and in control 53.33%, and present associated with acute myeloid leukemia risk, the odds ratio (OR) was 7.87 and confidence intervals (CI 95%) was ratio ,(21.85-2.83), while T allele was 12% in patients and 46.67% in control, and OR was 0.15 and CI 59% was (0.60 -0.04) (Table 3 and Figure 3). The A have etiological fraction (EF) with acute myeloid leukemia risk, while T allele have preventive fraction (PF).

Table (3) allelic frequencies of IFN- γ-784 T /A gene polymorphisms acute myeloid leukemia patients and controls

Alleles	acute myeloid leukemia N.50 (%)	Control N.30 (%)	P-value (Two-tailed)	OR	CI 95%
A	88%	53.33%	0.000**	7.87	(21.85 - 2.83)
T	12%	46.67%	0.000**	0.15	(0.60 - 0.04)

The results showed that AA genotype had higher frequency in acute myeloid leukemia compared with control, and with the ratio of AA 82% vs 36.67% sequentially, and TT was 6% vs 30% sequentially. The odds ratio (OR) and confidence intervals (CI 95%) of AA and TT genotypes was 7.87 (2.83 to 21.85) and 0.15 (0.04 to 0.60) sequentially, while TA genotype show higher frequency in control compared to patients, and ratio was 33.33% vs 12% sequentially, and OR was 0.27 and CI 95% was (0.09 to 0.84) (Table 4 and Figure 4). The presence of AA genotype associated with etiological fraction (EF) for acute myeloid leukemia patients' risk, while TT and TA genotypes show a preventive IFN- γ-784 T /A fraction with acute myeloid leukemia patients' risk (table 4).

Table (4) Genotypes frequencies of IFN- γ-784 T /A gene polymorphisms in acute myeloid leukemia patients and controls

Genotype	acute myeloid leukemia N. 50 (%)	Control N. 30 (%)	P-value (Two-tailed)	OR	CI 95%
AA	82%	36.67%	0.000**	7.87	(2.83 - 21.85) 7.05)
TA	12%	33.33%	0.041*	0.27	(0.09 - 0.84)
TT	6%	30%	0.007	0.15	(0.04-0.60)

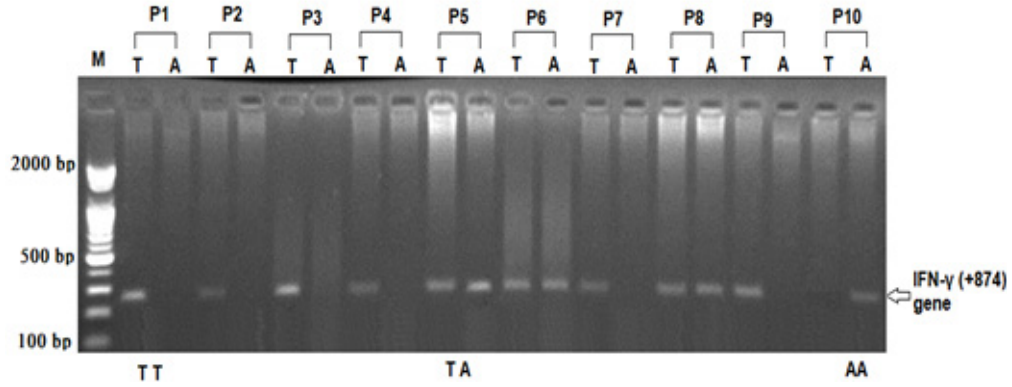

Figure (1) Electrophoresis for IFN- γ-784 T /A genotype in same in acute myeloid leukemia patients.

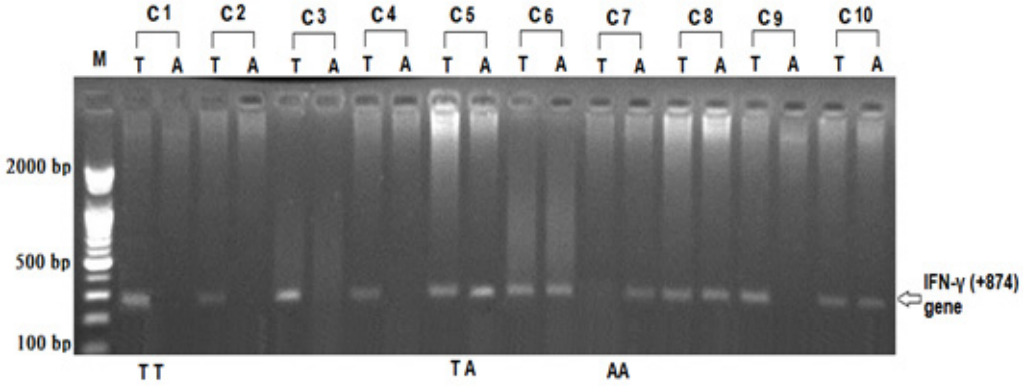

Figure (2) Electrophoresis for IFN- γ-784 T /A genotype in same in control.

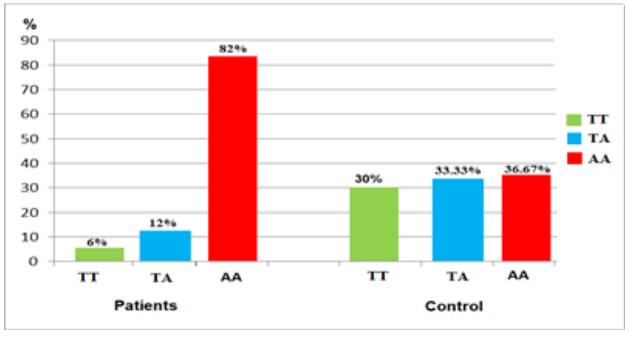

Figure (3) Genotypes frequencies of IFN- γ-784 T /A gene polymorphisms in acute myeloid leukemia patients and controls

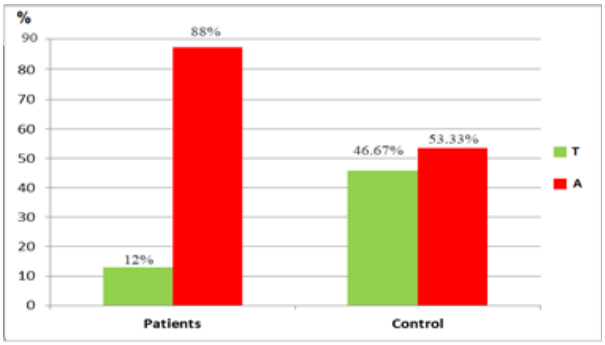

Figure (4) Alleles frequencies of IFN- γ-784 T /A gene polymorphisms in acute myeloid leukemia patients and controls

DISCUSSION

The Interferone -gamma gene (IFN gene) many studies showed that the increase in the gene expression activity of IFN-γ has a significant role in the development of some diseases (Cope et al., 1997). If the IFN-γ gene is disrupted, its expression is inhibited, or its receptors are disrupted, it increases the risk of developing diseases (Akalin, E. and Murphy, B. 2001). The results also show that people with homozygous AA genotype have a higher risk of developing the disease, and these results indicate the importance of the role of the TA and TT genotypes in the prevention of disease.

The present study showed association between polymorphisms of IFN- γ gene with Acute myeloid leukemia patients for Iraqi population. This study was similar for the results reached by Wu and colleagues in 2016 which state that people who carry the TT and TA genotypes are less likely to develop leukemia, while people who carry the AA genotype have a risk of developing leukemia (Wu et al., 2016). Interferon may be associated with other cancers, not only acute myeloid leukemia. In a study in Turkish women for the same gene, the AA and TA genotypes posed a risk of breast cancer (Karakus at al., 2011). IFN-γ is also involved in the activity of tumor-killing macrophages, and this cytokinesis is central to the immune response of CD4+ T cells and macrophages as it acts as an immune control machine for tumor cells. Tumor (Corthay et al., 2005). The production of interferon gamma gives cooperation between it and CD4 + T cells and macrophages and this is necessary for the success of immune surveillance in cancerous infections, such as myeloma, B-cell lymphoma, breast cancer, in addition to many other cancers, and this production is considered to be a positive effect, although Although a high level of IFN-γ can protect some immune cells such as natural killer NK cells from collateral damage through certain mechanisms, these same mechanisms may also allow cancer cells to resist immune system attack and this may represent a negative effect of interferon - gama in the injury area (Merlino & Zaidi ;2011) .

Conclusion: That the allele A and genotype AA for IFN- γ gene They had a significant association with the causative fraction of acute myeloid leukemia While the T allele and the TT and TA genotypes showed an association with the protective portion of the disease risk.

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