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RESEARCH ARTICLE

Identification and Association Polymorphisms of TGF-β and IFN-γ Genes with Schizophrenia Risk in Iraqi Patients

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Abstract

Genetics study for IFN-γ (Interleukin-γ) T/A +874 and TGF-ß1(Transforming growth factor-beta) +869*C/T genes polymorphisms for schizophrenia patients with mean age 49.95±1.6 was studied by using ARMS-PCR (Amplification refractory mutation system technique). The results revealed the presence of two alleles A and T with three genotypes (TT, TA and AA) for IFN-γ (+874 T/A) gene, and two alleles C and T with three genotypes (CC, TT and CT) for TGF-ß1 (+869*C/T). The result show that high frequency for allele A of IFN-γ +874 T/A as compare with T allele in schizophrenia patients samples (n=38) and associated with etiological fraction (EF) of schizophrenia, dependent on values of odds ratio (OR) and confidence intervals (CI), while T allele associated with preventive fraction (PF) of schizophrenia. The ARMS-PCR analysis results present AA and TT homozygotes genotypes of IFN-γ T/A +874 gene was more than in schizophrenia patients as compared with control and showed that AA and TT genotypes associated with EF in schizophrenia risk, while TA heterozygote genotype present in high frequency in control as compared with schizophrenia patients and associated with (PF). The T allele for TGF-ß1 (+869*C/T) gene exist in high frequency for schizophrenia patients and associated with (EF) schizophrenia, while C allele associated with (PF) schizophrenia. The TT homozygote genotype frequency of TGF-ß1 (+869*C/T) gene was more than in schizophrenia patients and associated with (EF) of schizophrenia, while CC homozygote and CT heterozygotes genotypes present more than in control as compared with schizophrenia patients and associated with (PF).

Keywords: *Polymorphism, Schizophrenia, IFN-γ T/A +874 and TGF-β1 (+869*C/T) genes.*

Introduction

Schizophrenia is a chronic and serious mental disorder that affects in what way a person social behavior. There are several studies showing the correlation between the immune system dysregulation and schizophrenia clinical appearance [1]. Schizophrenia is the most widespread neuropsychiatric illnesses that relatively affecting about 5% of the overall population worldwide [2].

Cytokine variations are ever more recognized as part of schizophrenia pathophysiology, several studies have revealed that there is an inequality between T helper type1 (Th1) T helper type 2 (Th2) cytokines in schizophrenia patients [3], this imbalance was remarked within three remarkable lines; decreased levels in interlukin-2 (IL-2), increased levels in interlukin-6 (IL-6) and interferon-γ production is also diminished due to immune system overstimulation [4].

IL-2 is a protein that controls the activities of lymphocytes especially Th1 and natural kill (NK) cells, while interferon-γ (IFN-γ) is a (20 to 25) kilo Dalton glycoprotein secreted by T-cells and NK cells in response to a different of stimuli [5] that was first described in 1965 by Wheelock [6]. IFN-γ has been shown to be a crucial factor in the immune response for manages a diverse array of cellular programs through transcriptional regulation of immunologically related genes [7].

IFN-γ is secreted by Th1 cells, macrophages, mucosal epithelial cells and NK cells as part of the innate immune response, and by CD4 Th1 and CD8 cytotoxic T lymphocyte (CTL) effector T cells when antigen-specific immunity progresses[8].

It was found a correlation between schizophrenia and IFN-γ expression produced by Th 1 [9].Previous report revealed that the transforming growth factor-beta (TGF-ß) signaling is one of the top classified pathways associated with schizophrenia, this report was showed that increased TGF-ß production correlated with schizophrenia [10].

Dysregulation of cytokine expression occurs with high production of TGF-ß, which has been establish to suppress of Th1 production in schizophrenia patients, as well as the imbalances between Th1 and Th2 cytokines in lymphocytes [11, 12, 13]. TGF-ß is a regulatory cytokine that effects proliferation, differentiation, and survival of lymphocytes, natural killer cells, granulocytes, dendritic cells, mast cells and macrophages [14].

Remarkably, it was found that the differentiation and survival of midbrain dopaminergic neurons was control by TGF-ß [15, 16]. IFN- γ cytokine plays a crucial role in antiviral defense, induces macrophage action and stimulates expression of major histocompatibility complex (MHC) antigens [17, 18].

Increases of TGF-ß production occur with dysregulation of cytokine expression, which has been found to suppress of Th1 and IFN-γ production [19]. This deregulation in an imbalance in cytokines expression was reported frequently in schizophrenia patients [21, 20].

The main factor of schizophrenia still unknown, but the strong hereditary background people of schizophrenia and genetic factors may also trigger immune system deregulation and abnormal cytokine production observed in schizophrenia [22]. Hence, some authors have concentrated on functional polymorphisms located in genes encoding cytokines such as TGF-ß and IFN-γ that concerned in schizophrenia [23].

The cytokine encoding gene location associated with the risk of schizophrenia, IFN-γ gene located on the long arm of chromosome 12 in locus 14 (12q14) [24], while TGF-ß gene located on the long arm of chromosome 9 in locus 13.1-13.3 (9q 13.1-13.3) [2]. Previous report showed that TGF-ß gene is the candidate gene for schizophrenia [25]. However, data in respect to polymorphism for particular genes related disease, especially in schizophrenia are limited in Iraq, so amplification refractory mutation system-PCR (ARMS-PCR) was used to investigate for the first time whether polymorphisms in the TGF-ß (+869C/T) and IFN-γ (+874T/A) genes are associated with clinical manifestation in schizophrenia in the Iraqi population.

Material and Methods

Study Population

The study population consisted of 38 patients with schizophrenia (20 female and 18 male) and 27 individuals (16 female and 11 male) as control of range age 20-70 years. All the patients were recruited from Ibn-Rushed Psychiatric Hospital in Baghdad.

They had an established diagnosis of schizophrenia 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 questionnaire, which provides a diagnosis of schizophrenia that involve the following criteria: the patient's history of neurological illnesses, material addiction, acute physical health injuries by a full medical examination.

The control group involved of healthy volunteers free from present, past and family history (first-degree relatives) of psychiatric disease, no present infections, allergies, nor current and past history of autoimmune disorders. The study was approved by the Ethical Committee of the College of Medicine, Al-Iraqia University, and Baghdad.

Genotyping Procedures

Genomic DNA was extracted by take five ml of blood from each patient and healthy control by venipuncture, later, 2.5 ml was added in to EDTA tubes then DNA was extracted by DNA isolation kit (Geneaid, Taiwan) and according to manufacture instructions manual. DNA purity was qualified by nanpdrop and it was about 1.5 ±1.8. All samples were kept at 15 ° for further study.

Polymorphism of IFN-γ (+874A/T) and TGF-β1 (+869C/T) was 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, Canada) that designed according to [13,26, 27] table (1), 20 µl was the total volume of reaction mix (Bioneer, Korea), and the molecular marker size (Bioneer, Korea)100-500 base pair. Amplification was accomplished using thermal cycler (Gene Technologies, UK); PCR cycling conditions were summarized in Table (2). The genotypes were established by analyzing electrophoresed 2% agarose gel stained with ethidium bromide.

Statistics

Differences in the frequencies of IFN-γ and TGF-β1alleles for schizophrenia patient in this study with control groups were analyzed with a value 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.

Results

The genetic polymorphism of IFN-γ (+874T/A) and TGF-β (+869C/T) alleles in thirty-eight schizophrenia patients with mean age 49.95±1.6 years, and twenty-seven of healthy individuals as a control sample with mean age 39.7 ±2.4years. Notably, the two alleles A/T are more present for IFN-γ (+874T/A) with TT, TA and AA genotype in schizophrenia group (figure 1).

Allele frequency and genotype distribution for each tested polymorphism for healthy control and schizophrenia patient are presented in table (3). With respect to the IFN-γ +874T/A polymorphism, there was a significance in schizophrenia patient in compare with control group (P>0.05), and the A and T alleles were different in frequency, so allele A frequency was 54% for schizophrenia patient while allele T frequency was 46%, as compared with A and T alleles in control group that it's frequency was 51.9% and 48.1% figure (3).

The OR for allele A was 1.1 with CI 0.54-2.12 at 95 % table (3), and it was 0.44 as an etiological fraction (EF), while for T allele there is no significance and OR was 0.92 with CI 0.46-1.84 at 95% and the value of T allele as preventive fraction (PF) was 0.39. Previous report on polymorphism of IFN-γ (+874T/A) show that may A allele be an etiological fraction and also, it's describe that the T allele may be a preventive fraction that correlated with the risk of schizophrenia. According to genotyping for polymorphisms for IFN-γ (+874T/A) by ARMS-PC , there is a significance in genotype frequency in healthy control and schizophrenia patients, so, AA and TT genotype showed the high frequency in schizophrenia patient as compared with health control group, and it was 29 % and 22% respectively for AA genotype also, OR was 1.42 with CI 0.46-4.40 and the etiological fraction was 0.86, while for TT allele the frequency was 21% and 19% for patient and health group respectively, also the OR was 1.17 and CI was 0.34-3.34 and the value for TT as etiological fraction was 0.31.

For TA genotype, the frequency for schizophrenia patients was higher than the healthy control; it was 59% and 50% respectively. OR was 0.69 with CI 0.26-1.83 and the value of TA genotype as a protective fraction was 0.18 as illustrated in figure (5) and table (4). Briefly, the result showed that AA and TT genotype were correlated with the risk of schizophrenia, whilst TA genotype was correlated with the protective fraction of schizophrenia.

The genotyping study found the difference in genotype distribution and allele frequency of the TGF-β 1 polymorphism that was significant with a diagnosis of schizophrenia and healthy controls, however, this result presented that C and T alleles were significantly higher in patients with schizophrenia and healthy control subjects within codon 10 +869 for TGF-β 1 gene with CC, CT and TT genotype as illustrated in figure (2). It was a difference in frequency distribution for C allele; it was 40% for schizophrenia patient while for healthy group it was 48% figure (3).

The OR and CI at 95% for allele C were 0.74 and 0.37-1.49 respectively as summarizes in table (3). However, the allele C was correlated with the preventive fraction that was 0.12. Remarkably, the T allele had a high frequency as compared with C allele; it was 59.2% for patient while for control it was 52%.

For the allele T, the OR was 1.35 with CI at 95% was 0.67-2.71, and it was represented as etiological fraction for the schizophrenia that was 0.15. It was a high significance in genotype frequency for CC, CT and TT polymorphism for healthy group and schizophrenia patient table (4), The TT genotype had a higher frequency for schizophrenia patient as compared with the healthy group (control) and it was 42.1% and 29.1 % respectively. The OR and CI values were 1.73 and 0.62-4.84 respectively. Thus, the TT genotype represents the etiological fraction that was 0.17.

Additionally, it was observed that the CT genotype was higher in frequency for healthy group as compared with the schizophrenia patient and it was %44.5 and %34.2 respectively. The OR was 0.65 while the CI was 0.24-1.76.

The CC genotype value as preventive fraction was 0.15. It was observed that the CC genotype frequency was higher for healthy control subject as compared with the patient who suffering from schizophrenia and it was 25.9 % and 23.7 % respectively and there is no significance was noted, also the OR and CI were 0.89 and 0.29-2.72 respectively. Also, the CC genotype represents the preventive fraction with value 0.29 Table (4).

Discussion

The role of the immune system in the pathogenesis of schizophrenia was showed by numerous authors, but to the best of our knowledge, it is the first study in Iraq showing the association between IFN-γ (+874 T/A) and TGF-β1 (+869C/T) polymorphism and schizophrenia. Cytokines are thoroughly linked with the works of the central nervous system and are known to be concerned in the pathophysiology of diverse types of schizophrenia. Cytokines might work together with neurons, influencing neurotransmission and neurodevelopment [28].

The polymorphism at single nucleotide level for two alleles was revealed a correlation with susceptibility of schizophrenia pathogenesis, and any change in genotype distribution and allele frequency for IFN-γ (+874 T/A) and TGF-β1(+869 C/T) has effect on the progress of schizophrenia risk [29].

Another report was showed that the schizophrenia had an effect on the immune system [29, 30]. Dorata and *et al*., was reported that any change in the IFN-γ gene expression may increase the risk of the schizophrenia [22]. The AA genotype correlated with the decreasing of IFN-γ expression and TT genotype was correlated with the increasing of IFN-γ expression while the AT genotype was in between [31]. The macrophage cytokine IL-1β in combination with IFN- γ and TNF-α, plays an important stimulation role for auto-immune cells [32, 33]. Also, the IFN- γ expression was organized by MHC cells that have an important role for cell death through $CD8^+$ during autoimmune pathogenesis [34, 35].

The over expression of IFN-γ and TNF cause the destruction of human and mice tissues [36].Kim and *et al*., study concluded that there was a correlation between the polymorphism of IFN- γ and the risk of schizophrenia in Korean population [23].

Furthermore, IFN-γ induces MHC class I antigen expression on both neuronal and glial cells and also induces MHC class II expression on microglia, some population of astrocytes, and endothelial cells [24], otherwise, TGF-β1 expression was organized the differentiation and also, the TGF-β1 has biallelic single nucleotide polymorphisms both triggering amino acid substitutions and both known to influence modifications in TGF-β synthesis [37, 38].

Schizophrenia is associated with the imbalance in Th1 and Th2 when immune system was dysfunctional by changed expression of numerous cytokines in the brain and peripheral blood [39], thus, TGF-β1 expression was depressed the immune induction for Th1 and cause the imbalance in lymphocyte [40]. Remarkably, it has been found that TGF- β1 promotes estrogen and estradiol production via enhancing the basal secretion of follicle-stimulating hormone (FSH) for schizophrenia patients [43, 445].

It has been shown that the level of TGF- β1 is significantly increased in schizophrenia patient [41].The risk of schizophrenia higher in carriers of T allele with CT and TT genotypes than individuals with CC genotype [42].

Conclusion

The present study demonstrated that the polymorphism IFN-γ T/A +874 and *TGF-β1* (+869*C/T) genes are associated with schizophrenia risk in Iraqi patients.

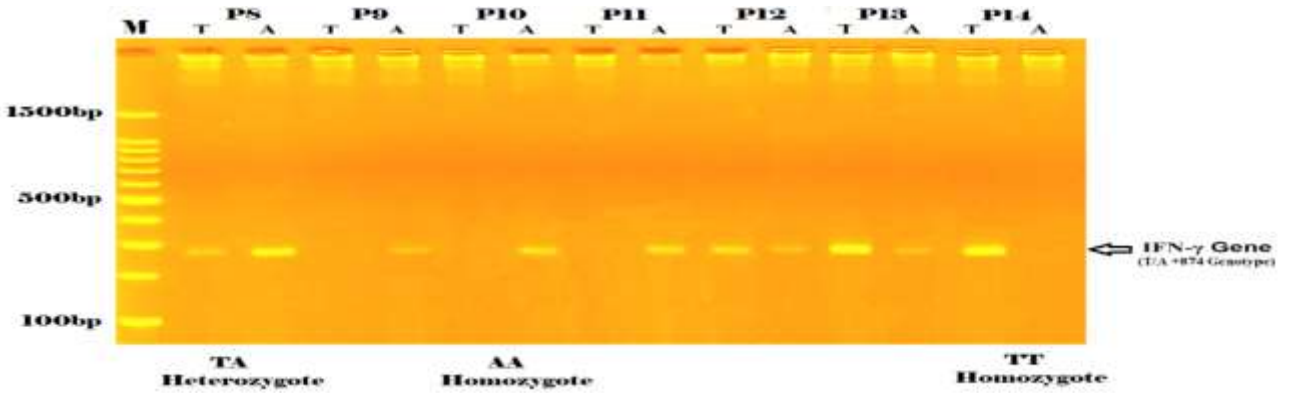

Figure 1: Electrophoresis for *IFN-γ* T/A +874 genotype in Schizophrenia patients, lane M DNA marker (100 to 1500 bp), lane 1,12 and 13 TA genotype, lane 9,10 and 11 AA genotype, lane 14 TT genotype

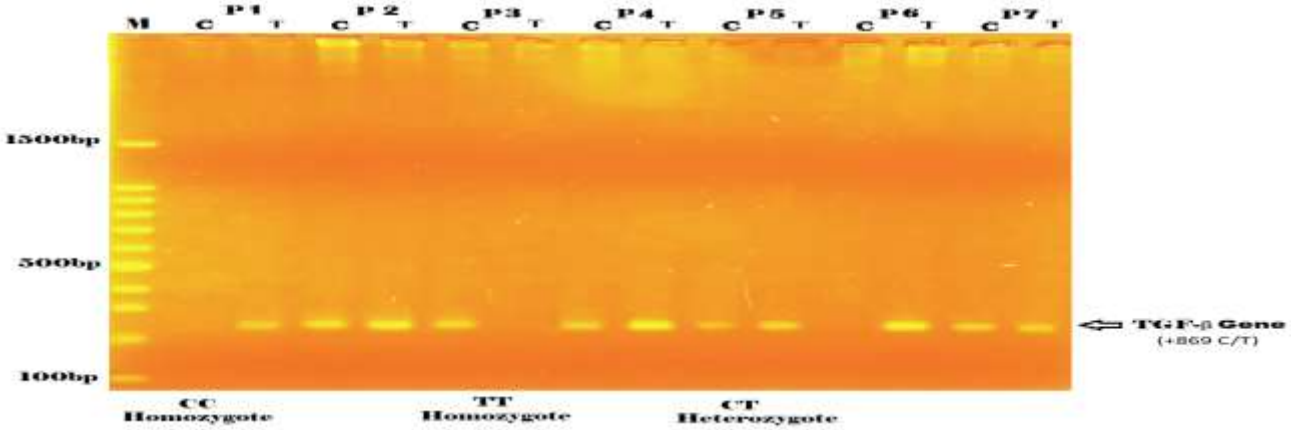

Figure 2: Electrophoresis for *TGF-β1*(+869*C/T) genotype in Schizophrenia patients, lane M DNA marker (100 to 1500 bp), lane 1 and 6 CC genotype, lane 2,4,5 and 7 CT genotype, lane 3 TT genotype

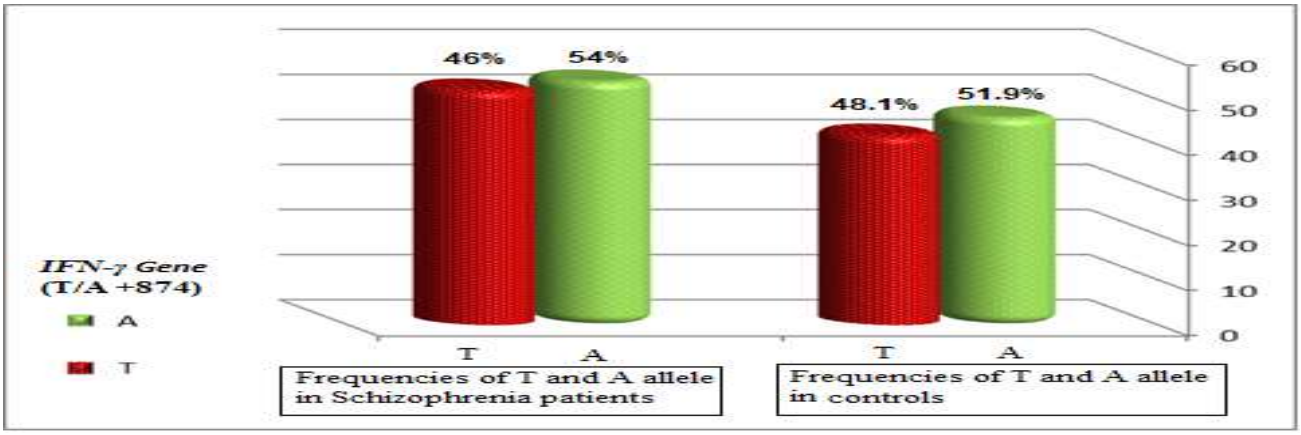

Figure 3: Allelic frequencies of *IFN-γ* T/A +874 polymorphism Schizophrenia patients and controls

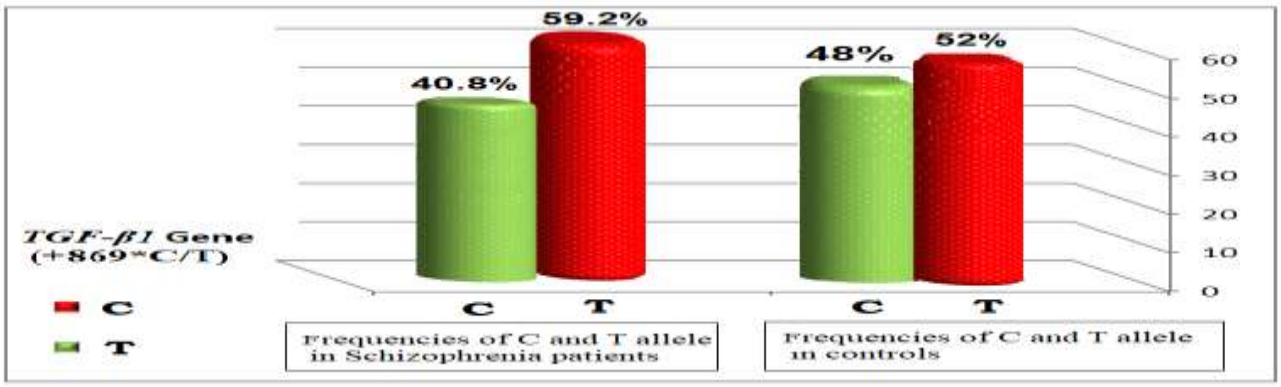

Figure 4: allelic frequencies of *TGF-β1* (+869*C/T) polymorphism Schizophrenia patients and controls

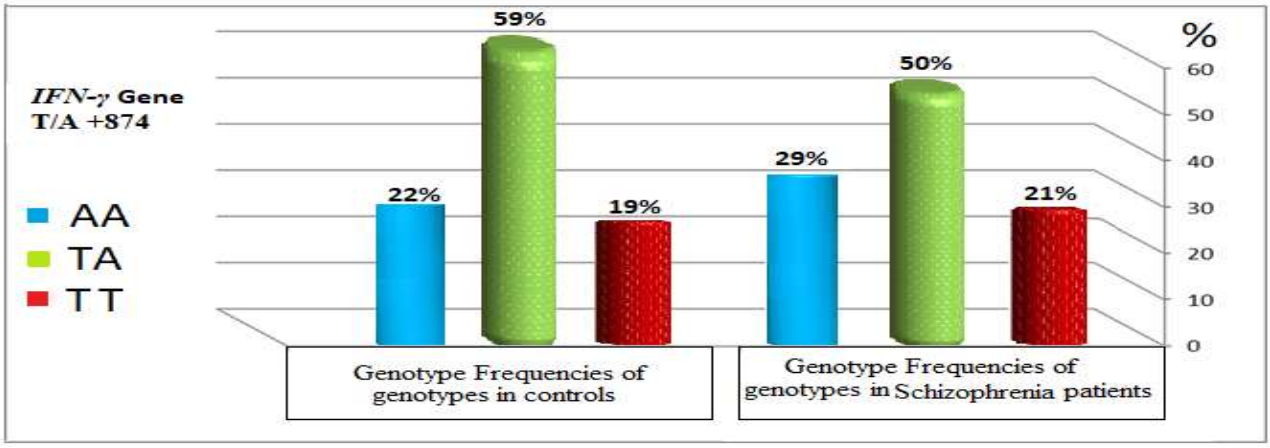

Figure 5 Genotype frequencies of *IFN-γ* T/A +874 polymorphism in Schizophrenia patients and controls

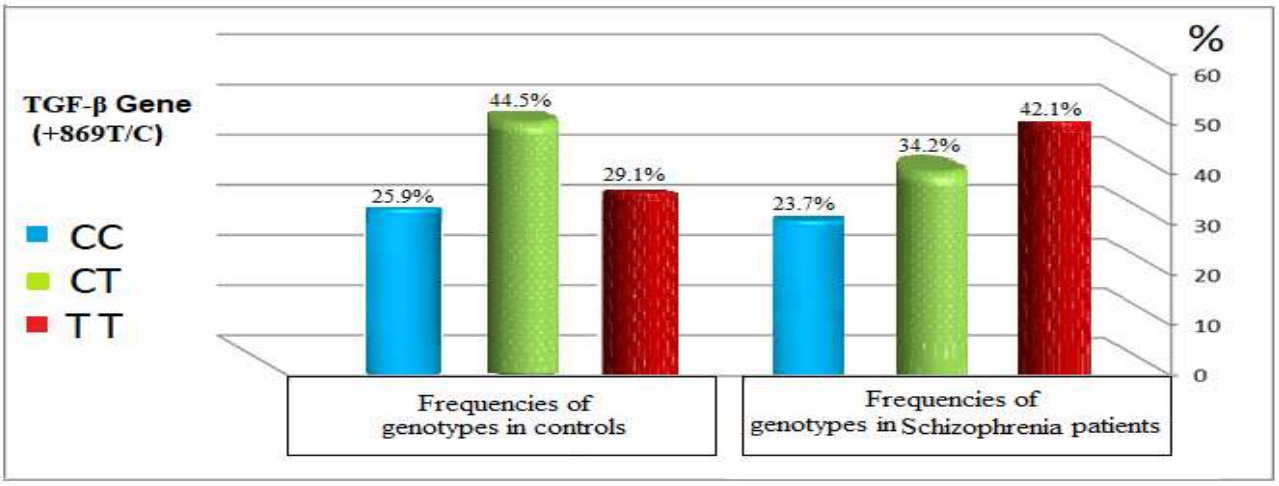

Figure 6: Genotype frequencies of *TGF-β1* (+869*C/T) polymorphism in Schizophrenia patients and controls

Table 1: primer sequences of IFN-γ +874 A/ T and *TGF-β1* +869C/T genes

Target Gene	primer	Primer sequences (5`→ 3`)	Size (bp)
IFN-γ +874 A/T	Specific T	TTCTTACAACACAAAATCAAATCT	262
	Specific A	TTCTTACAACACAAAATCAAATCA	
	Antisense	TCAACAAAGCTGATACTCCA	
TGF-β1 +869C/T	Allele C	GCAGCGGTAGCAGCAGCG	233
	Allele T	AGCAGCGGTAGCAGCAGCA	
	(Generic)	TCCGTGGGATACTGAGACAC	

Table 2: The cycling condition for ARMS-PCPR program for detection of IFN-γ +874 A/T and *TGF-β1*(+869*C/T) in schizophrenia patient and healthy control groups samples

Target gene	steps	Temperature (c°)	Number of cycles	Time (seconds)
IFN-γ +874 A/T	Pre-denaturation	95		180
	Initial denaturation	95	10	15
	Annealing	65		50
	Extension	72		40
	Initial denaturation	95	20	50
	Annealing	55		50
	Extension	72		50
	Final Extension	72		420
TGFβ1(+869*C/T)	Pre-denaturation	95		60
	Initial denaturation	95	10	15
	Annealing	65		15
	Extension	72		40
	Initial denaturation	95	20	20
	Annealing	56		20
	Extension	72		20
	Final Extension	72		7

Table 3: Genotype distribution and allele frequencies of polymorphisms in *IFN-γ* T/A +874 and *TGF-β1*(+869*C/T) genes in healthy and schizophrenia patient samples

Target Gene	Allele	Schizophrenia Patient (%) Number	Control (%) Number	(95%CI) Or	P-Value
IFN-γ +874 T/A	A	(%54)41	(%51.9) 28	(2.12-0.54)1.1	0.447
	E.F	0.44			
	T	(%46)35	(%48.1)26	(1.84-0.46)0.92	
	P.F	0.39			
TGF-β1(+869*C/T)	C	(%40.8)31	(%48)26	(1.49-0.37)0.74	0.256
	P.E	0.12			
	T	(%59.2)45	(%52)28	(2.71-0.67)1.35	
	E.F	0.15			

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 and *TGF-β1*(+869*C/T) genes for healthy and schizophrenia patient samples

Gene	Genotype	Schizophrenia Patient Number (%)	Healthy (%) Number	(CI 95%) OR	P-value
IFN-γ T/A +874	AA	(%29)11	(%22) 6	(4.40-0.46)1.42	0.37
	E.F	0.86			
	TA	(%50)19	(%59)16	(1.83-0.26)0.69	0.31
	P.F	0.018			
	TT	(%21)8	(%19)5	(3.99-0.34)1.17	0.52
	E.F	0.31			
TGFβ1 +869C/T	CC	(%23.7)9	(%25.9)7	(2.72-0.29)0.89	0.53
	P.E	0.29			
	CT	(%34.2)13	(%44.5)12	(1.76-0.24)0.65	0.28
	P.E	0.15			
	TT	(%42.1)16	(%29.1)8	(4.84-0.62)1.73	0.22
	E.P	0.17			

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

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