Associations of Tumor Necrosis Factor–α G-308A (rs1800629) Gene Polymorphisms with Major Depressive Disorder

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Abstract

Pro-inflammatory cytokine polymorphisms like Tumor Necrosis Factor- (TNF), has been found associated to severe depressive disorder (MDD). The Pro-inflammatory cytokines tumor necrosis factor (TNF-α) G-308A (rs1800629) have been discovered to have an important role in the pathophysiology of depressive disorders and the mechanism of antidepressant treatment. The present study aims to study TNF-α gene polymorphisms in Major depressive disorder using allele specific PCR. The results show that family history are non- significant different between patients and control group (0.6346) and also there was non- significant different in BMI (0.3417), The genotyping data show tow alleles and three genotyping (AG, GG, AA) the statically analysis show non-significant differences between patients and controls groups (0.095, 0.800) for GG, AA and GA respectively with more frequent of GA in patients than control group, the G allele was less frequent in patient than control while A allele was more frequent in patients than control group in significant differences (0.0001). It can be concluded that TNF genotyping didn't impact in the Major depressive disorder but the allele distribution may have potential role in MDD patients.

Key Words: Major Depressive Disorder, TNF-α, Allele Specific PCR.

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Introduction

Depression disorder is associated with a high rate of morbidity, recurrence, disability and mortality (Demyttenaere *et al.*, 2004), although of a prominent cause of global illness burden, MDD pharmaceutical treatments have so far been limited to monoamine neurotransmission modification, just with a partial impact on symptom burden. (Lopez *et al*., 2006). An evidence has proposed that peripheral and central inflammatory response particularly cytokines, perhaps contributed in in the pathophysiology and progression of MDD (Miller *et al*., 2009).

TNF- α is a pleiotropic cytokine that plays a role in a number of physiological and pathological processes, from inflammatory cytokine production to cell survival and apoptosis (Brenner *et al*., 2015), TNF-α is a pro-inflammatory cytokine excessive expressed by macrophages, and is involved in both neurotoxic effects as well as neuroprotective effects, It has been reported that patients with MDD have enhanced (Sriram and O'Callaghan, 2007; Al-Terehi *et. al* 2020; Al-Terehi and Alkaim 2019).

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The level of TNF-α decreased by the treatment with antidepressant drugs (Ma *et al*., 2016). Meanwhile study reported that the anti-TNF-α treatment may be contributed in the depressive symptoms decrement and cognitive impairments repair (Bortolato *et al*., 2015). The location of TNF-gene on chromosome six, with in the class III regions of HLA and numerous single nucleotide polymorphisms have been known in the TNF promote, amongst these mutual promoter sequence polymorphism, a G-to-A replacement at -308 and -238 loci which have been intensively studied. Some revisions have recommended that such allelic differences could have functional significance, but the consequences of these studies have been unpredictable (Allen, 1999).

The TNF gene contains numerous SNPs, the most extensively in the region, 308 A/G SNP (rs1800629) and 238 A/G (rs361525) of promoter sites, It has been created to be complicated in many illnesses outstanding to its capability to change levels of cytokine and medical outcome (Kothari et al.,2013).

Material and Method

Sample collection: A 20 samples of MDD blood were enrolled in present study that collected from (medical clinical, AL-Najaf– Iraq), these samples were diagnosed by specialist physician (prof. Dr. Arafat Al-Dujaile). According to ethical approval of ministry of environment and health of Iraq with written consents from all study subjects, samples of control were collected from healthy people, the age range of study subjects were ranged (20-60) years.

DNA isolation: The DNA extraction kit (Favorgen) was used to extract genomic DNA from white blood cells (WBCs) for both MDD patients and the control groups. The DNA concentration of the samples was assessed using a spectrophotometer (Nanodrop).

Primers and PCR conditions; primers were used in present study mention in table (1) and the PCR conditions were implemented with annealing temperature reached to 65ºC for 50sec (Al-Rayes *et al*., 2011, Al-Terehi et al., 2016).

Table 1. The sequence of TNF-α (G-308A) primers

Descriptive	Sequence	Size product
Sense	5'-TCT, CGG, TTT, CTT, CTC, CAT, CG-3	**184 bp**
Antisense (G allele)	5'-ATA, GGT, TTT, GAG, GGG, CAT, GG-3	
Antisense (A allele)	5'-AAT, AGG, TTT, TGA, GGG, GCA, TGA-3	

Electrophoresis of PCR-product is accomplished according to (Sambrook and Russell, 2001). The data analyzed statically by Qi Square analysis at a level of significance (0.05).

Result and Discussion

The current study's findings included a demographic study and TNF-gene polymorphism; the demographic analysis revealed significant variations in family history between patients and controls, and also, there are significant differences in body mass index (BMI) between patient and control group, there is no significant differences in duration.

Table 2. Mean differences of BMI, duration and family history of study subjects

Variables	Patient	Control	Statics Value	P- Value
History of MDD yes no	 18 2	 17 3	 OR 1.5882 CI% 0.2356 - 10.7048	0.6346
BMI kg/(m2)	24.01±0.86	25.28±1.00	t test 0.9629	0.3417
Duration	7.1525±1.74	0	-	-

The concentration of DNA that was extracted of patients and control, was ranged (50-150 ng/µl) and purity was ranged (1.8 -2.1) (Fig. 1).

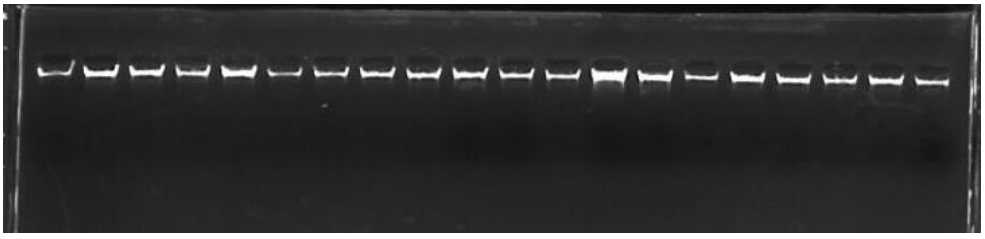

Fig. 1. Electrophoreses pattern of DNA extracted from whole blood of MDD patient and control, 1% Agarose, 75 v, 20 mA for 60 Second (10µl in each well).

The result of the TNF genotyping product has Tow band about (184bp for G allele and others for A allele) for both patient and the control group as shown in **Fig. 2.**

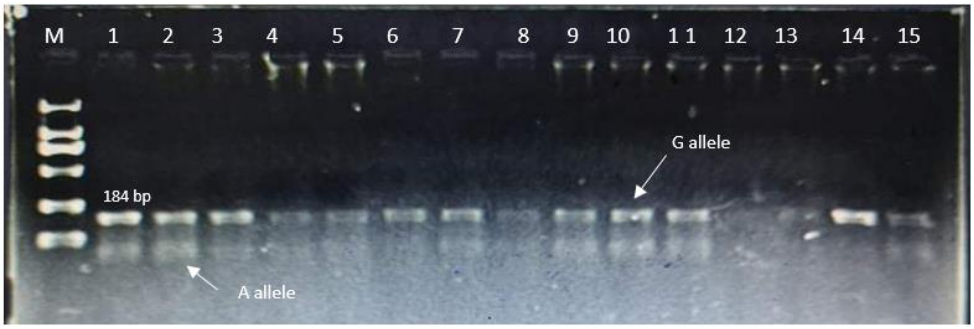

Fig. 2. Electrophoresis pattern of TNF genotyping, lane M (100 bp) DNA marker, lane 1-10 TNF genotype for patients, lane11-15 TNF genotype for control.

Table 3. The genotype distribution of TNF-α (G-308A) gene polymorphism for patients and control

TNF-α Genotype	Patients MDD (17)	Control (19)	sig-value	Odd ratio
GG	**5**	**16**	**0.095**	15.0000
AA	**2**	**0**		0.6200 - 362.9028
GA	**11**	**3**	0.8009	0.6571 0.0251 - 17.1811
G	**0.61**	**0.92**	0.0001	7.3525
A	**0.39**	**0.078**		3.2166 - 16.8060

The result of polymerase chain reaction of TNF gene polymorphisms showed two band (G allele and A allele), three genotyping of TNF appeared in this study AA GG and GA (table 3 and figure 2), AA was low frequent in patient while disappeared in control subjects, GA was high frequent in patient than control while GG low frequent in patient than control, these differences were non-significant differences (sig value 0.095, 0.8009) for GG, AG and AA respectively. The allele frequency according to hardy-weinberg show that A allele low frequent in patients and G was more frequent in control in significant differences (0.0001), in a study implemented by Jun *et al*., (2003) that they found potential role of TNF polymorphism–G308A for susceptibility to MDD in the Korean population.

The TNF genotyping have been found to be linked with excessive expression in autoimmune/inflammatory diseases; although of non-significant association between present genotyping with the MDD but it can be noticed that allele frequency was strong association with MDD, the present study agrees with a study conducted by Cerri *et al*., (2009). That show the polymorphism of TNF- gene's G308A may have a role in determining depression susceptibility, in addition the TNF-system enhancement may have role in the development of MD in the elderly.

Other evidence proposed that There was no linkage between AA genotype and MDD, While others show to be in opposition to the findings of a research conducted on Asian population. (Jun *et al*., 2003) which has discovered a linkage between the A allele, increased TNF-a production, However, it should be noted that various ethnic groups may have distinct susceptibility genes and/or risk factors on a nearby locus that interact with TNF-α in different ways (Padyukov *et al*., 2001). On the other hand Cerri et al. (2010) found that the GG genotype increased the likelihood of acquiring MDD, Furthermore, Kim et al. concluded that the GG genotype enhanced the probability of suicide attempts in MDD patients. (Kim *et al*., 2012).

The A allele of this polymorphism can contribute to higher nuclear factor binding affinity to the TNF promoter, resulting in high transcription activity and TNF secretion levels. As a result, the TNF-G-308A polymorphism was proposed as a possible risk factor in the depression episode (Wang et al., 2020).

In present study there was deletion mutation was observe in (1) control and (3) patients in PCR amplification products.

Conclusion

In conclusion, there was week association between TNF-α genotypes and MDD but significant association with allele frequency; Furthermore, this association had little to do with the type of depression Given that depression is polygenic, meaning it is affected by a variety of factors such as ethnicity, environment, gender, and age.

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