Genetic Polymorphisms of Mitochondrial Genes (ND1, ATP6c) in Depression Patients

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Abstract

Depression become the most health problem especially after COVID-19 pandemic in Iraq by increased the psychiatric disorder, the present study focused on the Mitochondrial Genes (ND1, ATP6c) in Depression patients using PCR-SSCP technique, samples collection with patients data were collected from psychiatric clinic, then DNA isolation and genes polymorphisms were detected, the results show that patients recorded in age more than 40 years and in married than single individuals in significant differences, non- significant differences were observed in occupational status, the genotyping found tow haplotypes in ATP6c gene (A and B) in significant differences between for B haplotype frequent in patients, the ND1 polymorphisms show three haplotypes (A, B and C) that varied in non-significant differences between patients and control, the present results concluded association between ATP6c and depression disease but didn't found linked with ND1 polymorphism.

Key Words: Depression, ND1, ATP6c, Haplotypes, PCR-SSCP.

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Introduction

Mitochondria play critical roles in energy production, lipid metabolism, steroid metabolism, and protein metabolism, as well as in cellular stability management, such as modulating Ca2+ levels, maintaining ROS levels, and regulating apoptosis (Scheffler, 2011). Thus, mitochondrial malfunction not only makes it difficult for cells to meet their energy needs, but it may also play a role in neural communication and cellular resilience, both of which contribute to mood and psychotic disorders. (Rezin et al., 2009).

The concept of neuroplasticity is at the center of a new mood disorder hypothesis, which focuses on depression and bipolar disorder. The term "neuroplasticity" refers to the brain's malleability, which includes both synaptic and non-synaptic plasticity, Synaptic plasticity is the process of neurons adjusting to changes in their internal or external environment by producing changes in brain pathways and synapses. It includes synaptic-genesis, axon and dendritic growth, and the elimination of superfluous connections between neurons, Mitochondria play a key role in neuroplasticity, and it has been well established that stress causes structural and functional impairment in numerous brain regions of depressed patients, resulting in reduced neuroplasticity (Sheline et al., 2003; Al-Terehi *et. al* 2020; Al-Terehi and Alkaim 2019).

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The Major depressive disorder is a major public health in a prevalence with a 12-month in about 7% of adults individuals, the WHO found that about 350 million people worldwide have been affected (Kessler *et al*., 2005; World Health Organization 2012). The changing in lifestyle habit like eating and sleeping patterns, apathy, restlessness, the slowed down feeling, weariness, worthlessness feeling, excessive guilt, impaired capacity to focus or concentrate, indecisiveness, or repeated thoughts of death are all signs of major depressive episode (Shao et al., 2008).

These symptoms must last for at least two weeks and create significant impairment in functioning; they do not include cases where bipolar disorder, substances, or a general medical condition play a role in the etiology. (American Psychiatric Association 2013). The present study was suggested to clarify the mitochondrial gene polymorphisms relation with depression which included ND1 and ATP6c.

Material and Method

Sample collection: present study included 49 depression cases and 20 control individuals, patients were diagnosed by specialist physician prof Dr. kareem Naser that attended to psychiatric clinic in Marjan hospital in Babil province, control group included 20 blood samples collected from healthy people, there age range from (23-70) years for both groups, all data and samples collected according to ethical approval of Ministry of environment and health in Iraq with approval from study subjects.

DNA isolation: Genomic DNA was isolated from white blood cells (WBCs) both patients and the control group using a DNA extraction kit (Favorgen), The DNA concentration of samples was estimated by using a spectrophotometer (Nanodrop).

Primers and PCR programs: The primer of **ATP6 gene** were F: 5-TCATCAGCCTACTCATTCAACCAATAGC-3, R: 5- TAGGTGGCCTGCAGTAATGTTAGC-3The PCR amplicon size (123bp) (Jiang *et al*., 2007). The PCR experiments completed with annealing temperature reached to 60ºC for 20sec. Electrophoresis of PCR-product is accomplished according to (Al-Hussainee, 2016). The primers of **ND1 gene** were used: Forward 5- GGATCAGGACATCCCGAT -3 Reverse: -5- GGTTTTAGGGGCTCTTTGG- 3 PCR amplicon size 420bp. The PCR experiments completed with annealing temperature reached to 59ºC for 60sec.

Haplotypes polymorphisms: The SSCP for Haplotypes polymorphisms was implemented according to Al-Terehi et al., (2018).

All data were analyzed statically by X^2 analysis at a level of significance (0.05) (using the number of samples), and data represented by percentages (100%) in all tables.

Result and Discussion

The result of present research found that The ages of the study subjects were ranged (23–70) years, the result showed there was significant differences (P 0.019) in age categories groups between patient and control and more frequent of disease papered in the age with more than 40 years (79.1%), the result show that disease accrued in female (66%) than male (33.3%) in significant differences also (P 0.000), also there are significant differences (P 0.019) for the married (0.95.8%) and non-married status, finally there are non-significant differences in Occupational status (table 1), from this results, it have been proved that the social status were strongly linked with depression infected and this was proved by some evidences (Steger and Kashdan,2009; Slavich and Irwin, 2014).

Table 1. The socio-demographic distribution of study subjects

Variable	Study group		X2	p- value
	Patient	control		
Age group				
20-30	**(8.3%)**	**(40%)**	7.9262	0.019004.
30-40	**(12.5%)**	**(20%)**		
more than 40	**(79.1%)**	**(40%)**		
Sex				
Male	**(33.3%)**	**(100%)**	17.4977	0.000029
Female	**(66%)**	**0**		
Occupational status				
Employed	**(54.1%)**	**(30%)**	0.7513.	0.386064
Non- Employed	**(45.8%)**	**(70%)**		
Social status				
Married	**(95.8%)**	**(70%)**	5.442.	0.019659
Non-married	**(4.1%)**	**(30%)**		

DNA was extracted from the whole blood of patients and control, its concentration was ranged (50-150 ng) and purity was ranged (1.6 -2.4). The result of the Polymerase chain reaction of the ATP6c gene showed the products have one band with size about (123bp) for both patient and the control (Fig. 2).

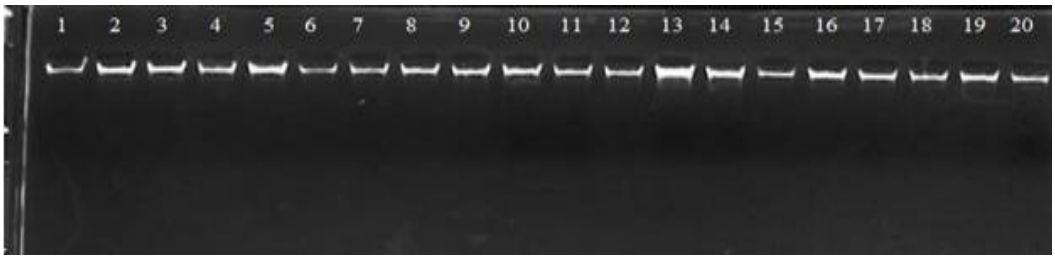

Fig. 1. Electrophoreses pattern of DNA extracted from whole blood of Depression patient and control,1% Agarose, 75 v, 20 mA for 1 hour (10µl in each well). Lane 1-10 DNA from patients, lane 11-20 DNA from control.

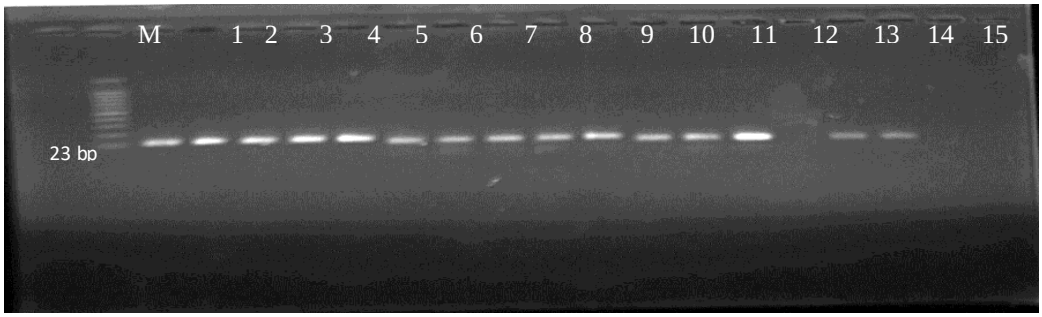

Fig. 2. Electrophoreses pattern of PCR of ATP6c gene in patients and control ,1% Agarose, 75 v, 20 mA for 1 hour (10µl in each well). Lane 1-8 control products, lane 9-18patients products.

Table 2. The Genotype of ATP gene polymorphism using PCR-SSCP technique of patients and the control groups

Pattern	Patient group %	Control group %	X^2	P value
A	40	99	10.7724	0.00103
B	60	1		

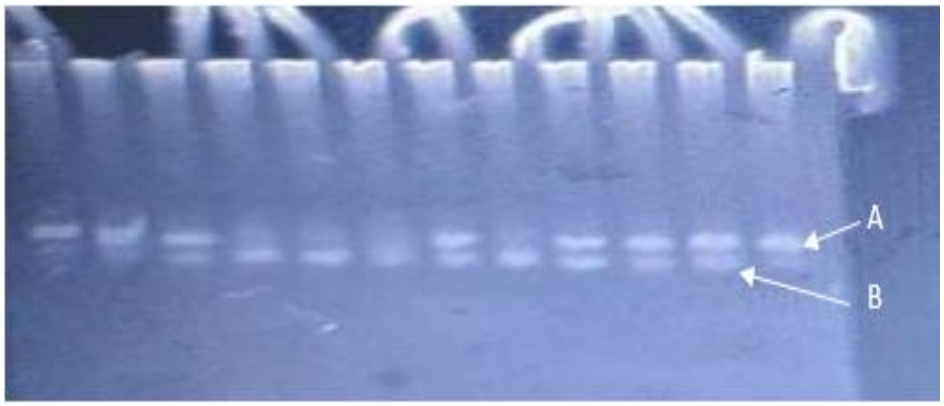

Figure 3. Electrophoresis pattern of PCR-SSCP for PCR product (123bp) of ATP represented by two haplotypes (A and B).

The result of genotyping of ATP6c gene shows two haplotypes (A, B), and there is significant differences between patient and control group, The ATP6c gene is a mitochondrial gene that is involved in the oxidative phosphorylation process, which is required for ATP synthesis. The mutant in the ATP6 gene and adjacent genes can impair mitochondrial oxidative phosphorylation, which has been linked to a variety of disorders, The results of the current study agree with other studies conducted Allen *et al.*, (2018) that showed the mitochondrial dysfunction and oxidative stress declined may be associated with abnormal brain function and mood disorders, such as depression, Neurotransmitters

like serotonin and norepinephrine, which are linked to depression, require an adequate amount of ATP for their synthesis, release, and reabsorption. As a result, a link between mitochondrial function and depression can be assumed (Kim *et al*., 2011).

Mitochondria are intracellular organelles that produce ATP by oxidative phosphorylation and provide over 95 percent of a cell's energy demand. Body organs that use a lot of energy, such as the brain, have a lot of mitochondria and are more susceptible to mitochondrial malfunction. As a result, disruption to mitochondria's electron transport system has been proposed as a key role in the etiology of psychiatric disorders such as bipolar disorder, depression, and schizophrenia (Rezin *et al*., 2009). Several studies the activity of mitochondrial electron transport complex I, III, and IV in the cerebrum and cerebellum of depression rats was found to be lower than in controls. (Rezin *et al.,* 2008).

The product PCR of the second gene (ND1gene) use in this study was 420bp in both patients and control groups as showed in Fig. 4.

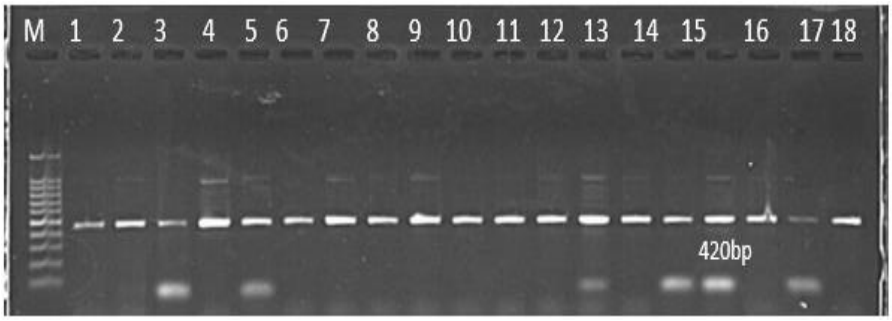

Fig. 4. Electrophoreses pattern of PCR of ND1 gene in patient and control ,1% Agarose, 75 v, 20 mA for 1 hour (10µl in each well). Lane 1-10 DNA from control, lane 11-18 DNA from control.

The result of genotyping of ND1 genes shows three haplotypes (A, B and C), C allele was more frequent in patient than control group, and A allele also more frequent in patient than control while B allele is less than A and C, and there were no significant differences between patient and control group (P 0.778).

Figure 5. Electrophoresis pattern of PCR-SSCP for PCR product (420bp) of ND1, which shows three haplotypes (A, B and C).

Table 3. The Genotype of ND1 gene polymorphism using PCR-SSCP technique of patients and the control groups.

Pattern	Patient group %	Control group %	X^2	p
A	30	37.93	0.5078	0.778
B	20	24.13		
C	50	41.37		

In this study we found no associated between ND1gene and incident od depression, and this disagreement with study conducted by Beech *et al*., (2012) that showed increased expression of multiple components of the mitochondrial ETC may be a primary deficit in bipolar depression, rather than an effect of medication. Reactive oxygen species induce oxidative damage, which leads to poorer physiological function and a shorter lifespan. In the elderly brain, oxidative DNA damage, particularly that affecting mtDNA, increases (Milaneschi *et al.,* 2013). The mtDNA 5178 A>C variant of the complex I subunit 1 (MT-ND1) gene was studied by Rollins et al., 2009, and the 5178C genotype was considerably more common in severe depression disorder subjects

with a maternal family history compared to controls. Multiple studies have suggested that alterations in energy metabolism may play a role in the pathogenesis of depression, these include studies showing decreased levels of phospho-creatine in the frontal lobes of patients with bipolar depression, increased levels of lactate in cerebrospinal fluid of patients with depression, and decreased levels of messenger RNA (mRNA) for creatinine kinas (Mac Donald *et al*., 2006).

Conclusion

In conclusion there was an association between ATP6c polymorphisms represented by haplotypes and depression but the ND1 didn't association with diseases at least in present study.

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