

The role of MAOA gene expression in human aggressive behaviors

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ABSTRACT

Aim: To identify whether the association between MAOA gene expression and exposure to antisocial outcomes.

Materials and Methods: A hundred and eighty samples (100 depressed patients going to psychiatric clinics and 80 healthy) of males and females were involved in the present study. Gene expression was performed for patients and controls using the real-time PCR technique to compare the expression of the MAOA gene when exposed to different types and levels of trauma.

Results: The results show a decrease in the expression activity of the MAOA gene in most patients. The data revealed that males have a lower expression of the MAOA gene than females. There were no significant differences down-regulated in the folding expression $2^{-\Delta\Delta Ct}$ of MAOA gene in patients who were prone to trauma exposure (missing someone, domestic violence, exposure to community violence, alcohol abuse, physical abuse, and sexual abuse). In terms of marital status, there was no significant upregulation of fold changes in the expression of the MAOA gene recorded in the patient group ($2^{-\Delta\Delta Ct} = 1.3, 1.3, \text{ and } 1.5$) when compared with the healthy group ($2^{-\Delta\Delta Ct} = 1$).

Conclusions: Our results suggest that the MAOA gene controls aggression and its effect on trauma history and levels.

KEY WORDS: MAOA gene, antisocial behavior, aggression, gene expression, RTPCR

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ABBREVIATIONS

MAOA: *Monoamine oxidase A*

TES total: trauma exposure score

qRT-PCR: Quantitative Real-Time PCR

RNA: Ribonucleic acid

DNA: deoxyribonucleic acid

cDNA: complementary DNA

INTRODUCTION

Monoamine oxidase-A (MAOA) is an enzyme that preferentially breaks down the catecholamine neurotransmitters (norepinephrine, serotonin, and dopamine) and is produced in the mitochondrion [1]. The level of this enzyme was affected directly by the MAOA gene [2]. The MAOA gene is located on the X-chromosome (Xp11.23) and has become known as a significant genetic factor associated with mental disorders [3]. Notably, MAOA gene activity is regulated by the essential region of its promoter, which spans between -71 and -40 bp, and deletion affects the activity of the MAOA gene and reduces it by 64–83% in different human

cell lines [4]. Importantly, when the MAOA gene is normally expressed, it was found that the level of the enzyme is in the normal range [5], but if the gene expression were decreased, the activity of the enzyme would affect human behavior, increasing tension, aggression, and anti-society [6]. Theoretically, the MAOA gene effects human depression and behaviors when the expression is too high or too low, and negative effects will be obtained in both situations [7]. Only a few studies in the past have mentioned and associated aggression with the genetic effect [8]. Studies reported that human behavior is affected by many environmental, external, and genetic factors; these factors interacted with each other to produce human behavior of human [9]. Despite the interaction and overlap of these factors, it is possible to distinguish the role of each factor separately and study their impact. Many evidences refer to the possible link between violent and criminal human behavior and genetics [11]. Although studies indicate that this link is very strongly related to the MAOA gene and especially its protein products, which are shaped and characteristic in a special way to form some hormones [12].

Table 1. Score of trauma history exposure in patients and control

Trauma history Exposure	Male control (40)			Female Control (40)			Male patients (44)			Female Patients (56)		
Scale	0	1	2	0	1	2	0	1	2	0	1	2
Physical Abuse	30	8	2	34	4	2	22	14	8	20	24	12
Sexual Abuse	38	2	0	38	2	0	40	6	0	42	12	2
Domestic Violence	32	8	0	33	6	0	20	18	8	28	22	6
Exposure to Community Violence	20	18	2	26	14	0	22	14	10	26	22	8
Missing some body Bad love experience	30	6	4	30	10	0	32	10	2	36	16	4

Scoring criteria: 0 - means No exposure

Table 2. Age variable in both males and females of the studied groups

Groups	Age (mean± SD)		
	Males	females	p-value
Patients	38.5±10.8	34.2±13.5	0.221
Control	40.7±11.04	39.3±12.2	0.68
p-value	0.43	0.185	

Data was presented as mean± SD according to student t-test

AIM

The aim of this work was to identify whether the association between MAOA gene expression and exposure to antisocial outcomes.

MATERIALS AND METHODS

SUBJECTS OF THE STUDY

One hundred and eighty males and females (ages 17–50) were involved in this study. One hundred samples comprised aggression and depressed patients who attended a psychiatric clinic, with an age range of males 38.5±10.8 and females 34.2±13.5. In addition, eighty healthy volunteers with an age range of males 40.7±11.04 and females 39.3±12.2 were included in the current study.

ETHICAL APPROVEMENT

The agreement of the Ethics Committees was approved by the Biotechnology Research Center, Al-Nahrain University, Baghdad, Iraq, with reference numbers M.B.8 / 2.1. 23). Informed consent was given by all the participants.

DATA AVAILABILITY STATEMENT

The corresponding author can provide the datasets used in and/or analyzed during the current work upon justifiable request.

DETERMINE THE TOTAL TRAUMA EXPOSURE SCORE (TTES)

Several source and data information were taken to detect trauma exposure history in patients and control. In this study, a form of trauma was evaluated involve (Missing some body, marital status, Domestic Violence, Exposure to Community Violence, Alcohol abuse, Physical abuse and Sexual abuse in different scales from 0 to 2 (Table 1).

Physical abuse rating:

- 1 - excessive physical discipline (e.g., hit with object, no bruising)
- 2 - physical abuse with bruising or more serious injury

Sexual abuse:

- 1 - exposure to pornography or peer-related sexual misconduct
- 2 - sexual abuse with an adult of sufficient severity to warrant criminal or protective services intervention

Domestic violence:

- 1 - frequent intense verbal arguments, throw objects at one another
- 2 - more serious domestic disputes (e.g, hit other, use weapons, injury)

Community violence:

- 1 - gunshots heard in neighborhood, witness serious physical fight
- 2 - witness shooting, death, or serious injury of another.

Missing some body or Bad love experience:

- 1-struggling with upsetting emotion and memories
- 2- effect on all life activities

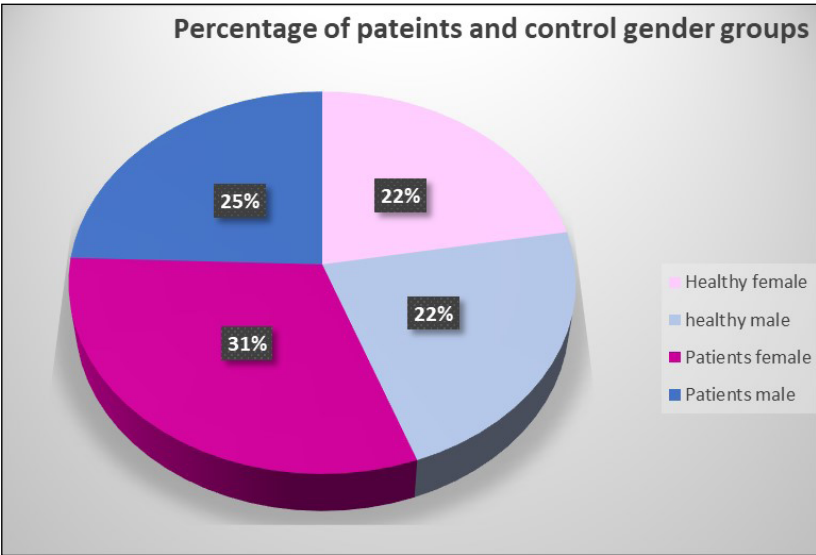


Fig. 1. Percentage of gender for patient and control groups
Source: Own materials

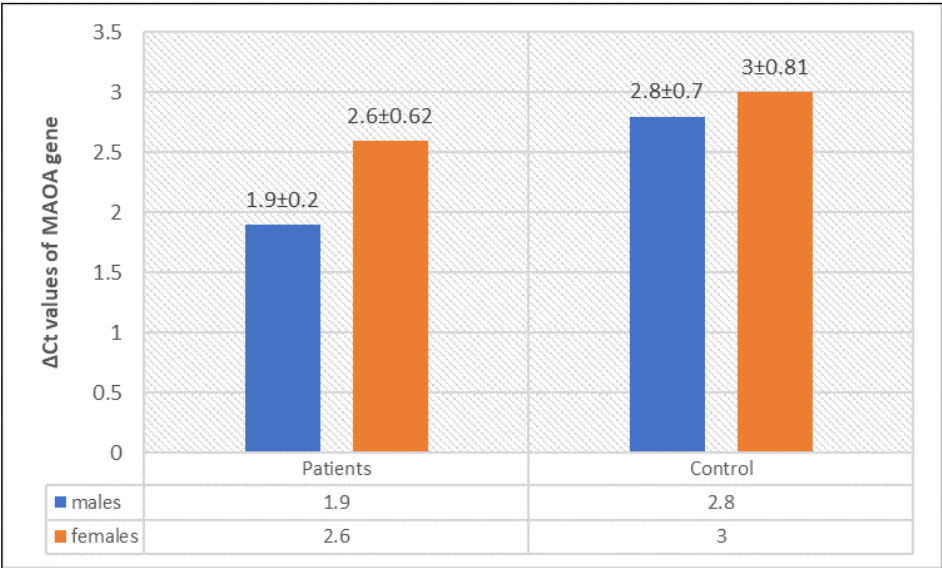


Fig. 2. Δ Ct value of MAOA gene in both males and females of patients and control group
Data was presented as mean \pm SD according to student t-test
Source: Own materials

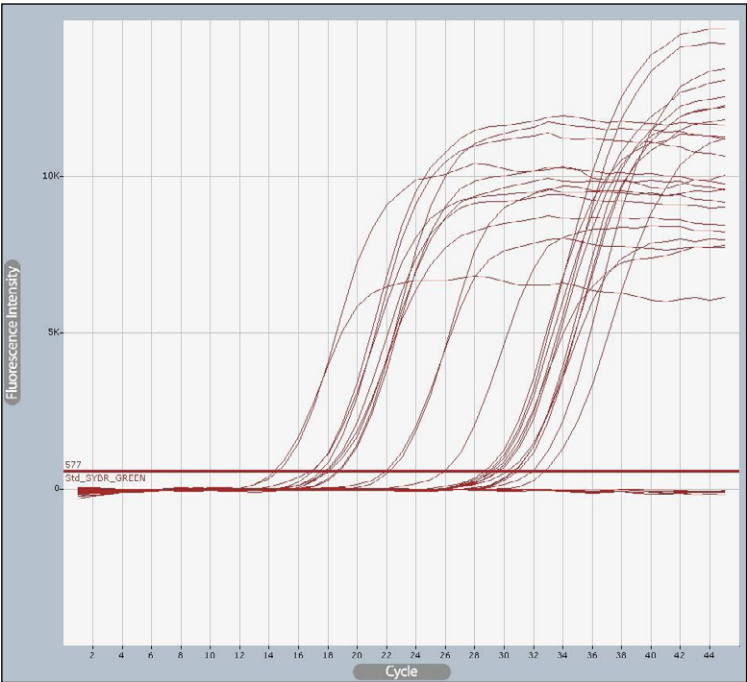


Fig 3. Amplification plots of MAOA gene done by qPCR
Source: Own materials

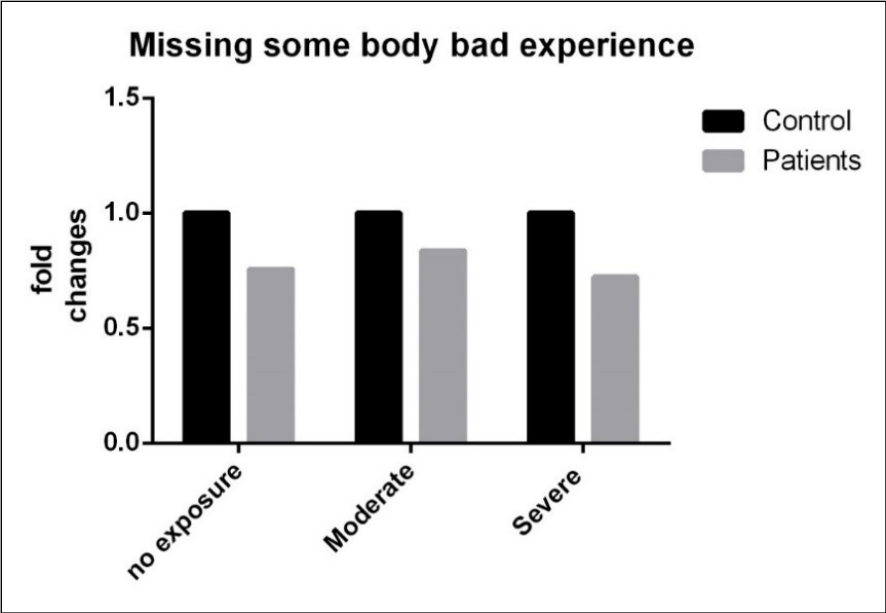


Fig 4. Comparison the effect of the loss of a person or bad love experience in relative fold changes of studied groups
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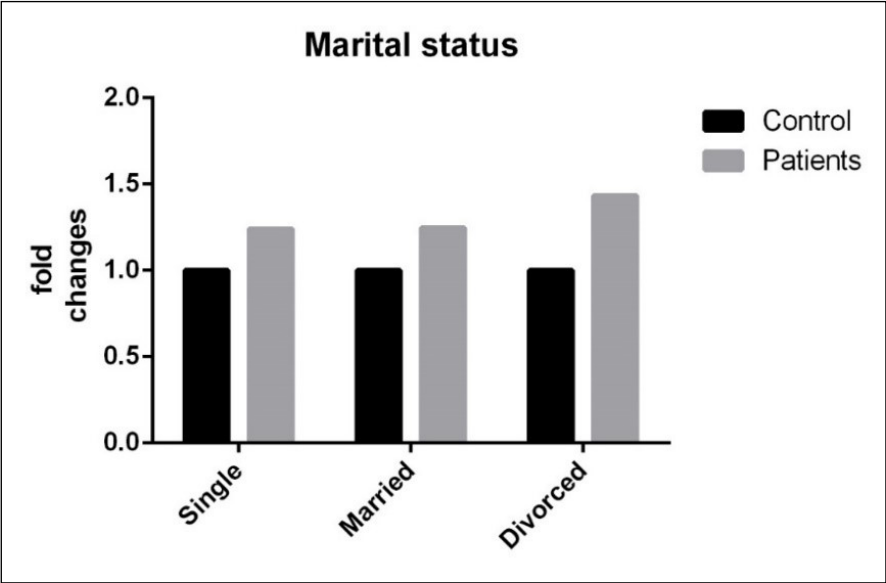


Fig 5. Comparison the effect of Marital status in fold changes for healthy and patients' group
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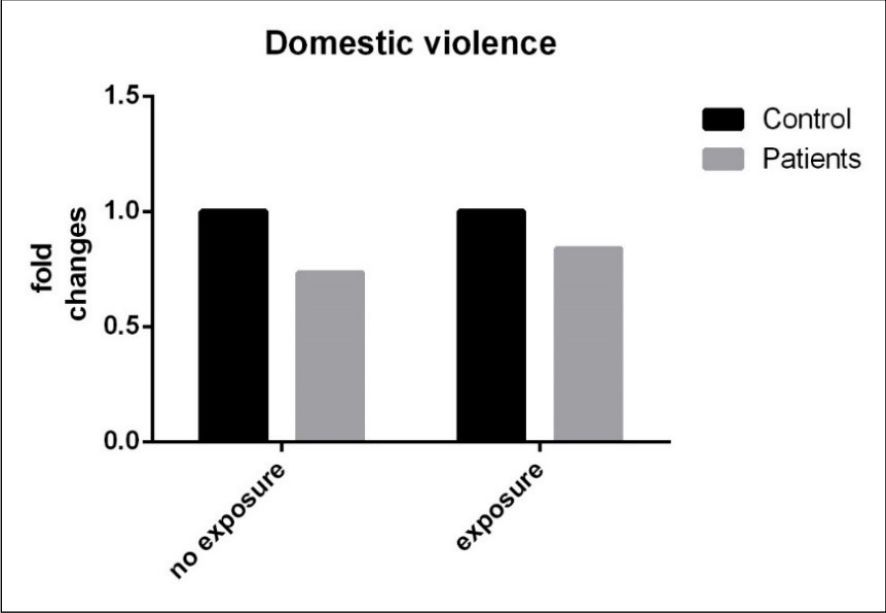


Fig 6. Comparison the effect of Domestic violence in fold changes for healthy and the patients group
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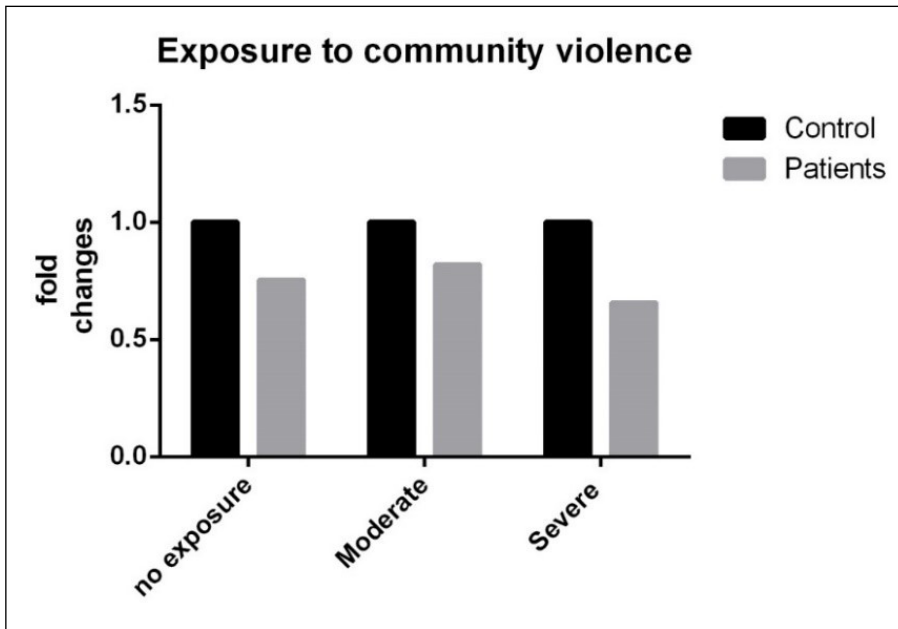


Fig. 7. Comparison the effect of exposure to community violence in fold changes for healthy and the patients group
Source: Own materials

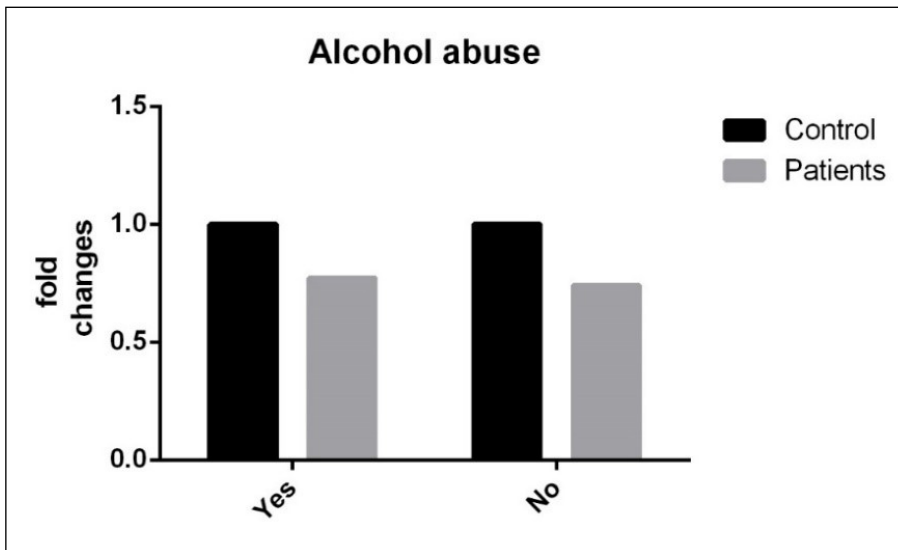


Fig. 8. Comparison the effect of alcohol abuse in fold changes for healthy and the patients group
Source: Own materials

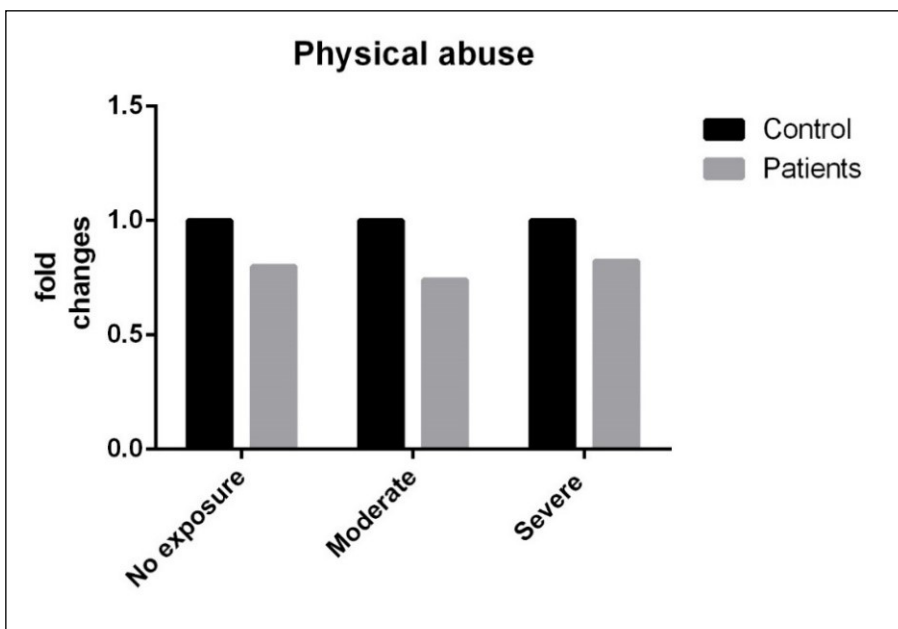


Fig. 9. Comparison the effect of physical abuse in fold changes for healthy and the patients group
Source: Own materials

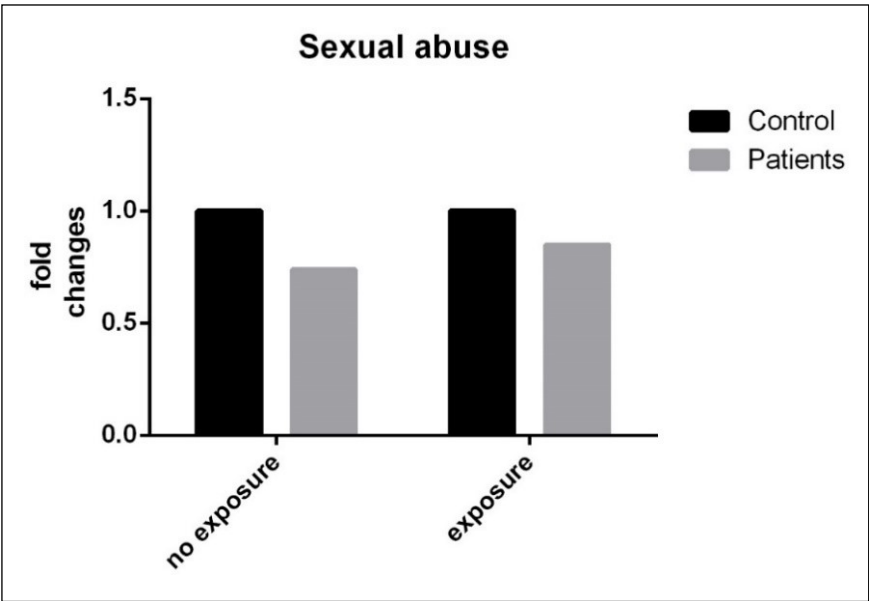


Fig 10. Comparison the effect of sexual abuse in fold changes for healthy and the patient groups
Source: Own materials

RNA EXTRACTION

The quantification of gene expression of the MAOA gene was identified by qRT-PCR. Five ml of blood were collected from the patients and healthy subjects, and then the total RNA was isolated from the peripheral blood samples using TRIzol® LS Reagent according to manufacturer protocol (Invitrogen™). In brief, TRIzol™ Reagent was added, followed by 1 ml of chloroform for lysis, then thoroughly mixed by shaking, and incubated for 2–3 minutes. The mixture was centrifuged for 15 minutes at 12,000 × g at 4°C, then the mixture separated into a lower phenol-chloroform, an interphase, and a colorless upper aqueous phase and transferred the aqueous phase containing the RNA to a new tube. 0.5 ml of isopropanol was added to the aqueous phase, incubated for 10 minutes at 4°C, and centrifuged for 10 minutes at 12,000 × g at 4°C. The supernatant was discarded. Washed the RNA using ethanol, then solubilized the RNA using RNase-free water, then stored at –20°C until further processing.

CDNA SYNTHESIS AND GENE EXPRESSION USING QUANTITATIVE REAL-TIME PCR (QRT-PCR)

The gene expression process includes two steps. In the first step, RNA was converted to cDNA to be utilized in the real-time PCR reaction using a cDNA synthesis kit (Geneaid kit, Korea). Secondly, the gene expression of the MAOA gene was assessed by qRT-PCR and (SYBR)™ qPCR Kits using two primers forward and reverse (F-CTGCCATCATGGGCTT and R-TTGCTGATCCACA) at 154 bp [13]. Another set of primers was used to amplify the housekeeping gene GAPDH F-GAAATCCCATCACATCTTCCAGG-3 and R-GAGCCCCAGCCTTCTCCATG-3

as a reference gene for normalizing the level of gene expression.

The program of thermal reaction was: pre-denaturation or enzyme activation at 95°C for 10 minutes of 1 cycle, followed by denaturation at 95°C for 10 seconds of 35 cycles, annealing at 53°C for 30 seconds, and extension for 30 seconds at 60°C. The level of gene expression was determined by the folding expression (2-ΔΔCt) method [14].

STATISTICAL ANALYSIS

GraphPad Prism software was used for comparison of the folding expression (2-ΔΔCt). A student t-test was performed to compare the mean values of age and ΔCt. The scoring criteria for trauma exposure were presented as a Z-score (0 = no exposure, 1 = moderate exposure, 2 = severe exposure)

RESULTS

In our study, we measure and determine the mean level of expression of MAOA gene in the males and females of the studied groups (Fig. 1). The number of patients include males 44 (25%), females 56 (31%) and 40 (22%) of both males and females as control group. In the Table 2, no significant association was noted in the age between the study groups in both genders (p>0.05).

In the design used in the current study and according to the range of ΔCt values of the MAOA gene (Fig. 2) in healthy people and patients, we found that the level of gene expression is lower in patients than in healthy people, and the patients with lower expression have more antisocial behavior and aggression than the others (Fig. 3).

The results indicate that the level of MAOA gene expression is higher in females than males. It was demonstrated that male patients are more frequently attending psychiatric clinics than female patients. A significant interaction between exposure to trauma levels and the activity of MAOA gene expression shows a relationship with the risk of aggression.

Some influencing factors were studied and their effects on patients and healthy participants analyzed. In respect to the factors of our study, they include Missing someone, bad love experience, marital status, domestic violence, exposure to community violence, alcohol abuse, physical abuse, and sexual abuse. As the results show in the figures below, the relative fold changes alter with the level of trauma history or factor levels. Specifically, the findings of missing some body or bad love experience showed low activity or downregulation of MAOA gene expression $2^{-\Delta\Delta Ct} < 1$ in the patient group (no exposure, moderate, and severe) when compared with the control group with no significant differences (Fig. 4).

In the marital status (single, married, and divorce), as shown in Figure 5, there was no significant upregulation of fold changes in the expression of the MAOA gene recorded in the patients' group ($2^{-\Delta\Delta Ct} = 1.3, 1.3$, and 1.5 , respectively) compared with the healthy group ($2^{-\Delta\Delta Ct} = 1$).

Thus, the results of the expression level of the MAOA gene showed lower activity in people exposed to domestic violence ($2^{-\Delta\Delta Ct}$ of no exposure = 0.71 and $2^{-\Delta\Delta Ct}$ of exposure = 0.86 , respectively) than the control group ($2^{-\Delta\Delta Ct} = 1$), and the findings had no significant differences (Fig. 6). In addition, the exposure to community violence observed a non-significant downregulation of the expression level of the MAOA gene in the patient group (no exposure, moderate, and severe) with a ($2^{-\Delta\Delta Ct} < 1$) compared to the control group (Fig. 7).

Hence, alcohol abuse is another important factor affecting the gene expression of the MAOA gene. According to the data analysis, the results revealed a reduction in the activity of the relative expression $2^{-\Delta\Delta Ct} < 1$ of the selected gene in the patients' group when compared to the control subjects. However, the reduction is not statistically significant (Fig. 8).

Regarding the physical abuse, the statistical comparison between the studied groups recorded a downregulation of MAOA gene expression ($2^{-\Delta\Delta Ct} < 1$) in the patient group (no exposure, moderate, and severe) with the corresponding value of the control group ($2^{-\Delta\Delta Ct} = 1$), and no significant differences were observed (Fig. 9).

Further data analysis of the MAOA gene expression with respect to sexual abuse revealed no significant decrease in the folding changes in the patient group

(no exposure, exposure) ($2^{-\Delta\Delta Ct} < 1$) compared with control subjects (Fig. 10).

DISCUSSION

Exposure to violence was linked to increased signs and symptoms of behavior disorder in young adulthood, which led to antisocial personality traits. This study was an assessment to explore the correlation between the expression level of the MAOA gene and the social disorder of Iraqi patients with aggressive behavior. The gene codes for the catabolic enzyme monoamine oxidase A (MAOA), which metabolizes several kinds of neurotransmitters in the brain. MAOA has been identified in catecholaminergic neurons in the human brain and metabolizes serotonin and norepinephrine more efficiently [15].

These neurotransmitters are affected by some hormonal disorders and external environmental factors. The MAOA gene influences aggressive behavior in the broader population [16].

Evidence of the current results indicates that the expression level of the MAOA gene was higher in females than males in both groups. The study by [17] found that antisocial behavior was more likely in males with the genotype for low MAOA activity who had been mistreated. Other studies found that the number of men who attend psychiatric clinics is greater than that of females, although it seems that the matter is due to the difference in culture and nature of societies. Furthermore, the previous work by [18] documented and showed the influence of MAOA on extraversion in males than in females, demonstrating a sex-specific link between the MAOA gene and personality traits. In contrast, [19] found in their studies the opposite effects of MAOA in women and mentioned that the high activity of MAOA gene expression is associated with an increased risk of aggression and depression. Other important findings by [20] suggested that the testosterone hormone can modify and alter the effect of the MAOA gene on the behavior of these women.

Our results observed a low expression form of MAOA gene $2^{-\Delta\Delta Ct} < 1$ in all the study factors or trauma exposure, but only marital status has upregulated MAOA gene expression $2^{-\Delta\Delta Ct} > 1$ when compared with the corresponding value of control $2^{-\Delta\Delta Ct} = 1$.

A team led by clinical psychologists in different colleges and countries found that exposure to physical or sexual abuse affects hormone status and some genetic products depending on the levels of abuse [21]. The deficiencies in MAOA gene expression have been associated with higher levels of aggression and violence [22].

A single nucleotide polymorphism SNP (missense mutation) of the MAOA gene was discovered in the Dutch family. Males who repeatedly engaged in violent criminal behavior over several generations exhibited an anomaly in MAOA expression, and this mutation prevented the synthesis of MAOA enzyme while the females were still unaffected [23]. Further investigation by [24] documented that the low-level expression of the MAOA-L gene estimates greater expressed happiness in females.

As noted earlier by [25, 26], the level of aggression is linked with the history of violence exposure in most cases. Moreover, studies by [27] suggested that divorce was significantly linked with aggression and made people more prone to antisocial behaviors. On the other hand, alcohol abuse causes damage to the brain by affecting the endings of nerve receptors, making it difficult for those nerve receptors to send important nerve signals. It also has an effect on hormone receptors and neurotransmitters [28]. An important study of 174 alcoholic violent offenders found that the MAOA-H genotype and childhood physical abuse before the age of 13 were linked to a higher risk of severe, impulsive, and aggressive recidivism [29].

Human experiments demonstrated for the MAOA-VNTR genotype that a low level of transcription activity was more prevalent in young men who had poor family relationships, suffered from sexual abuse or mistreatment in childhood, and reported a higher intake of alcohol [30]. Furthermore, a study published by Caspi and colleagues

in 2002 examined the effect of childhood mistreatment on the antisocial personality. An allele providing low activity of the monoamine oxidase A (MAOA) gene causes behavioral problems and violent crimes [16].

A previous study by [31] reported that the MAOA gene's tandem repeat (VNTR) variant affects the efficiency of gene expression. Carriers with alleles with 2 or 3 repeats have lower gene expression (MAOA-L) compared to those with 3.5 or 4 repeats (MAOA-H).

Another important result is that McDermott, et al. [32] discovered that individuals who carry the allele of low production (MAOA-L) had a more aggressive response when prone to provoke action compared to individuals who carry the allele of high production (MAOA-H) of the MAOA gene.























In general, managing the level of gene expression of the MAOA gene is a complex process that involves the interaction between genetic, epigenetic, and hormonal influences.

CONCLUSIONS

In our study, the decreased expression level of the MAOA gene is relatively associated with trauma exposure based on the small sample size. The lower expression of the MAOA gene increases the risk of aggression. The males have slightly lower expression of the MAOA gene than the females. Therefore, it is essential for this study to be closely replicated in the future with a large sample size.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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