

Study The Relationship Between Smoking And Some Physiological Variables , Genetic Polymorphisms Of Dopamine Beta Hydroxylase In Ramadi Residents Sample .

Omar N. Mohammed ^{1*}, Samir M. Khalaf ¹

¹Department of Biology, College education for pure science, University, of Anbar, Iraq

Abstract

Smoking and the diseases caused by it are one of the largest causes of death in the world at the present time, and it is one of the largest causes of premature death in industrialized countries, The current study was conducted for the purpose of studying the effect of cigarette smoking on a sample of 75 smokers of three age groups, in addition to a control sample consisting of 25 individuals who do not smoke or sit with smokers and of the same age groups, and for the purpose of showing the relationship of dopamine beta-hydroxylase enzyme in smokers and its effect on the genetic future Partially and a case study of the association and effect of some serological factors with smoking for the period from 10/10/2020 to 11/12/2020.

The results of the current study showed a significant increase of $p \leq 0.05$ in the concentration of LDH enzyme in the serum of the study sample compared with its concentration in the serum of the control sample, In addition to the absence of a significant correlation $p \leq 0.05$ between the concentration of dopamine enzyme and the concentration of LDH enzyme in the serum of the study sample members. The results of the current study proved that there was a significant difference, $p \leq 0.05$, in the concentration of vitamin D₃ in the blood of the study sample, compared to its concentration in the blood serum of the control sample, It was also found from the results of the current study that there was a significant inverse correlation $p \leq 0.05$ between the concentration of dopamine enzyme and vitamin D₃ in the blood serum of the study sample,

The results of the current study showed a significant increase, $p \leq 0.05$, in the concentration of Ferritin in the blood of the study sample, as compared to its value in the blood of the control sample members While the results of the current study did not show a correlation between the concentration of the dopamine enzyme and the Ferritin in the blood of the study samples.

The results of the current study also showed the occurrence of a multiplicity of forms or sites of nitrogenous bases (SNPs), as the site rs6351 , rs429699 and rs6880875 showed a polymorphism In the main sites.

Keyword: Smoking, LDH, D₃, dopamine, Ferritin, SNPs

عنوان البحث باللغة العربية

علاقة التدخين ببعض المتغيرات الفسلاجية وتعدد الاشكال الوراثية لأنزيمالدوبامين بيتا هايدروكسيلاز في عينة من سكان مدينةالرمادي

*Email :ome19u1012@uoanbar.edu.iq

عمرناج محمد *, سمير مشرف خلف¹

¹قسم علوم الحياة, كلية التربية للعلوم الصرفة, جامعة الانبار, الانبار, العراق

الخلاصة

يعتبر التدخين والأمراض التي يسببها من أكبر أسباب الوفاة في العالم في الوقت الحاضر ، وهو من أكبر أسباب الوفاة المبكرة في الدول الصناعية ، وقد أجريت الدراسة الحالية لغرض دراسة تأثير تدخين السجائر على عينة من 75 مدخنًا من ثلاث فئات عمرية ، بالإضافة إلى عينة ضابطة مكونة من 25 فردًا لا يدخنون أو يجلسون مع مدخنين ومن نفس الفئات العمرية ، ولغرض توضيح علاقة الدوبامين بيتا - إنزيم هيدروكسيلاز عند المدخنين وتأثيره على المستقبل الجيني جزئيًا ودراسة حالة ارتباط وتأثير بعض العوامل المصلية مع التدخين للفترة من 2020/10/10 إلى 2020/12/11. أظهرت في مصل عينة الدراسة مقارنة بتركيزه في LDH في تركيز إنزيم $p \leq 0.05$ نتائج الدراسة الحالية زيادة معنوية بمقدار LDH بين تركيز إنزيم الدوبامين وتركيز إنزيم $p \leq 0.05$ مصل العينة الضابطة ، بالإضافة إلى عدم وجود ارتباط معنوي ، في تركيز فيتامين د 3 في دم $p \leq 0.05$ في مصل أفراد عينة الدراسة. أثبتت نتائج الدراسة الحالية وجود فرق معنوي ، عينة الدراسة ، مقارنة بتركيزه في مصل الدم للعينة الضابطة ، كما وجد من أظهرت نتائج الدراسة الحالية أن هناك علاقة في مصل الدم لعينة الدراسة ، D3 بين تركيز إنزيم الدوبامين وفيتامين $p \leq 0.05$ عكسية معنوية

في تركيز الفيريتين في دم عينة الدراسة ، مقارنة بقيمته في دم $p \leq 0.05$ أظهرت نتائج الدراسة الحالية زيادة معنوية ، أفراد العينة الضابطة في حين أن نتائج الدراسة الحالية لم تكن كذلك. تظهر علاقة ارتباط بين تركيز إنزيم الدوبامين والفيريتين في دم عينات الدراسة ، كما أظهرت نتائج الدراسة الحالية حدوث تعدد لأشكال أو مواقع القواعد النيوتروجينية وتعدد الأشكال في المواقع الرئيسية rs6880875 و rs429699 ، أظهر rs6351 ، كما في الموقع (SNPs).

1. Introduction

Smoking cigarettes is one of the most serious problems that threaten human health and society together, and it is also one of the causes of death in the world [1]. One of the studies mentioned that about three million people die per year in the world due to smoking or due to its pathological complications, half of them before reaching the age of seventy years[2]The vast majority of smokers start this habit before they reach 20 years of age [3].

Diseases caused by smoking are one of the largest causes of death in the world at present, and it is one of the largest causes of premature death in industrialized countries. In the United States of America, about 500,000 deaths annually are due to smoking-related diseases, and the World Health Organization has predicted that smoking deaths in India may exceed 1.5 million by 2022[4]

Young smokers want to appear mature, self-confident, independent, and achieve high[5], On the other hand, it is considered sensory issues such as bad taste or smell, and religion and the consequences of health negative and poor physical performance and response physiological negative and problems related to the family of some of the reasons for not smoking, as predicting attitudes and religious beliefs about smoking intentions and start smoking. Whereas, religious beliefs towards smoking are considered as a deterrent to starting smoking and as a motivator to quit smoking [6].

Therefore, we started our directions in this study for

- 1- Study the effect of smoking on the enzyme LDH and vitamin D3 And Ferritin
- 2- Estimation of the dopamine enzyme in the blood of smokers and the study of its relationship to the molecular effect on the genetic receptor.

2. methods

2.1 Study sample collection:

The study samples were collected for the period from 2020/10/10 to 2020/11/12 This study included the collection of 100 blood samples 75 for smokers and 25 Garmdechnon males were divided into four groups depending on the age of smokers as follows :

- The first group included smokers aged from 20 to 30 years old by 25 samples.
- The second group included smokers aged from 31 to 40 years by 25 samples.
- The third group included smokers aged 41 years and over, with 25 samples.
- The fourth group included the control group, which included 25 samples that were distributed according to the age groups of the study samples .

A pre-designed questionnaire was made for this purpose ,including name, age, sample number, phone number, housing, and academic achievement .Smoking period, type of cigarettes, do you smoke hookah, do you suffer from chronic diseases, what kind of treatment .According to the rules of ethics for scientific research and after obtaining the consent of all individuals included in the study.

2.2 Collect and store blood samples:

5ml of venous blood was drawn from the study subjects with a single-use Disposable syringe After sterilizing the place of withdrawal with ethanol at a concentration of 70% Then 2.5 ml of the drawn blood was placed in a plastic tube containing EDTA-K2 Anticoagulant and then shaken quietly for 5 minutes. Then placed the remainder of sample 2.5ml into Gel Tube. Then the gel tube tubes were placed in the centrifuge speed 3000 rpm for 10 minutes .Serum was removed from other blood components and transferred to Eppendorf 2ml capacity tubes. The information of each sample was recorded on it and kept in the freezing position at a temperature of 20C° until tests were conducted on it.

2.3 Determination of serum Lactate Dehydrogenase (LDH) concentration

Rated Lactate dehydrogenase in the blood serum using the ready-made analysis kit from the company linear (CHEMICALS, SLU SPAIN)[7] .

2.4 Dopamine concentration estimation

The concentration of dopamine hormone was estimated for all study samples by following the attached steps with its ready-made analysis kit and according to the manufacturer's instructions (CALBIOTECH) Relying on an immunological method, you know Enzyme-linked immunosorbent assay (ELISA) using a device ELISA Reader.

2.5 Estimation of the level of vitamin D3 in the blood serum

The serum vitamin D3 level was estimated by using the Ichroma kettate (ICHROMA Korean) Korea Origin.

2.6 Estimation of Ferritin in the blood

The serum ferritin level was estimated by using the Ichroma kettate (ICHROMA Korean) Korea Origin.

2.7 Molecular study.

2.7.1 DNA extraction

Genomic DNA was extracted from the blood of smokers 75 samples as well as 25 control samples according to the extraction method approved by the supplying company (Geneaid American).

2.8.2 Electrophoresis on agarose gel Agarose gel electrophoresis

DNA Extract and technology product PCR were detected using electrophoresis on agarose gel, the molecular weights of DNA Extracted were estimated based on the distance traveled in the gel compared to the marker of DNA known molecular weight (Genomic marker 1Kbp) as for the molecular weights of the resulting pieces PCR It was estimated by comparison with a volume guide for the output PCR (DNA Ladder 100bp).

2.9.3 reaction of PCR

The reaction of PCR was done using several-Premix Processed by the Korean company Intron with a final size of 25µL. Two pairs of specialized primers were used (Table 1) for each SNP. Primers designed by the researcher according to the system tetra-primer amplification refractory mutation system—a polymerase chain reaction. The prefixes are prepared lyophilized from company Bioneer and dissolved in distilled water, and a final concentration of 10 picols/µl. The reaction solution consists of Taq PCR PreMix 5µl And 0.5µl Each of the four prefixes used has a specialized prefix, and 1.5µl from template DNA and 16.5 µl of distilled water to the final volume 25µL The materials were mixed and transferred to Thermocycler device For amplification, it included a protocol PCR The first stage Initial Denaturation 93°C And for 3 minutes and then a stage Denaturation-2 93°C for 35 seconds and then a stage annealing 59 °C for 35 seconds and then a stage Extension-1 72 °C for 35 seconds and finally a stage 72 °C Extension-2 for 7 minutes the first step was one round, then the 3 steps (35 round) and the last step was also one round.

Table(1) Prefixes Primers used in this study with their sequences and expected size.

Primer	Sequence	Tm (°C)	GC (%)	Prod. size base pair
Inners6351	F 5'-CGATCCTGGGCCTCCACG - 3'	62.2	72.2	219 bp
	R 5'-GGAGCAGAACGGAGTGCT - 3'	57.6	64.7	
Outers6351	F 5'- AAAGTCGATCTTCTTGCCCC - 3'	57.8	50.0	
	R 5'- CAAGGAGCAGAACGGAGTGC - 3'	61.2	60.0	
Inner rs429699	F 5'- TGCCGGCTTGGCTGCCTT - 3'	65.3	66.6	242bp
	R 5'- TATGGTGAGTCCGGGGG - 3'	57.0	64.7	
Outer rs429699	F 5'- GGGGAGTGGCACAGCCA - 3'	61.0	70.5	
	R 5'- TTTGGAGTGCTCATCGA - 3'	52.6	47.0	
Inners6880875	F 5'- CTGCCCGGTCATCTGC - 3'	57.0	68.5	238bp
	R 5'- CAGCGACGACATCCA - 3'	52.0	60.0	
Outers6880875	F 5'- AGCACAGCGCCAGT - 3'	58.2	66.7	
	R 5'- GGGGGAATGCGCTGC - 3'	57.8	73.3	

3. Results and discussion

3.1 The effect of smoking on the concentration of basic dehydrogenase enzyme (LDH)

The results of the current study showed a significant increase $p \leq 0.05$ In enzyme concentration LDH In the serum of the study sample (279.8 ± 110.2) U/L compared with its concentration in the serum of the control sample, where its concentration reached (136.2 ± 42.39) U/L. Figure (1)

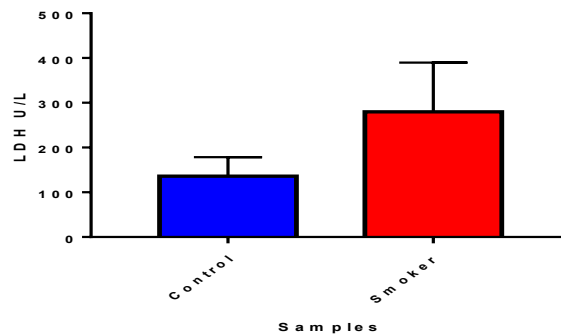


Figure (1) The concentration of LDH U/L

One of the studies mentioned that the reason for the high LDH is due to tissue damage or Necrosis in organs and in the case of renal infarction, where this enzyme is found in almost every cell of the body, including the blood, muscles, heart, brain, and kidneys, and it also rises when metabolic processes are accelerated, as this enzyme converts sugar into energy [8], while the study indicated that there was no significant association $p \leq 0.05$ between the concentration of the enzyme dopamine and the concentration of the enzyme LDH In the serum of the study sample The value of the correlation coefficient was (0.08269), Figure (2).

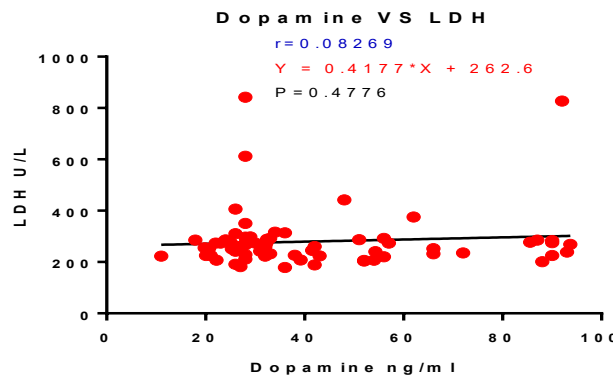


Figure (2) Dopamine correlation coefficient with LDH concentration In the blood serum

Cigarette smoking increases levels of lactate dehydrogenase LDH In serum as well as saliva as an indicator of tissue damage in the oral cavity, the researcher's study [9] indicated that saliva is a better test media than serum in determining lactate dehydrogenase levels, but the study did not indicate a significant association between LDH and dopamine secretion in smokers compared to controls, lactate dehydrogenase (LDH) is an enzyme required during the process of converting sugar into energy for the function of the cells, there is LDH in many types of organs and tissues within the body including liver, heart, pancreas, kidneys, skeletal muscle, lymph tissue, and blood cells. When oxidative stress or oxidative damage occurs in the body, it may release LDH and raises the level in the blood, the high-level LDH in the blood leads to acute or chronic cell damage, and an elevated level of LDH To several conditions such as stroke, cancer, heart attacks, lack of blood flow, hemolytic anemia, hepatitis, muscle

injury, and tissue death, and high dopamine may help release high amounts of platelets that will block the course of the veins and help in the clotting process and the occurrence of heart attacks and stroke, so the researcher's study indicates an association, but it is not significant, and the study also evaluated the effect of smoking on antioxidants in the blood (LDH And CAT And SOD And GXP) between smokers and control, and the results showed a significant decrease and increase in serum antioxidant enzymes concentrations when compared with the control group of non-smokers, indicating a gradual depletion and sequential accumulation of antioxidants. Conclusively, smoking depletes many of the antioxidants in the blood needed to curb excess free radicals, thus increasing the rate of lipid peroxidation, and the positive relationship between dopamine and antioxidants, including LDH[10].

3.2 The effect of smoking on vitamins D3

The results of the current study showed a significant difference $p \leq 0.05$ in vitamin D3 concentration of the study sample (30.29 ± 8.518) ng/ml compared with its concentration in study sample, where its average concentration was (18 ± 6.564) ng/ml.

The significant difference in the concentration of vitamin D3 can be explained through previous studies, where the study [11], which included 295 samples of smoking men aged between 35-64 years, which confirmed that smoking is not associated with a deficiency levels D3. This is in agreement with the results of other studies such as [12], [13], which indicates that there is no association between smoking and vitamin deficiency D3. This is in agreement with the results of this current study. While other studies have indicated that smoking is linked to vitamin deficiency D3, where he indicated [14] In his studies that levels (OH)D 25 In smokers 58%, increased the risk of vitamin D deficiency in smokers compared to non-smokers between the ages of 20 and 29 years, and the reason for this deficiency was explained that smoking is usually accompanied by a less healthy lifestyle (less physical activity, alcohol consumption). and bad eating habits), which leads to reduced exposure to the sun, and therefore smoking has a causative role in vitamin deficiency D3 It affects the synthesis of the vitamin and, therefore the effect of smoking cannot be excluded. and also a study [15] in which he indicated that (47% of male smokers) aged 30-79 years had a lower concentration of (OH)D25 in serum. Figure (3).

The inconsistency between different studies in terms of the association of smoking with low levels of vitamin D3 It can be explained by the different ways in which smoking is done, heterogeneity (homogeneity in smoking intensity), and also due to the different methodology used to measure the level of smoking. D3 In the blood serum, or it may be due to the difference in the intensity of solar radiation between different countries, where the sun's rays in Iraq are intense compared to other countries, especially European ones.

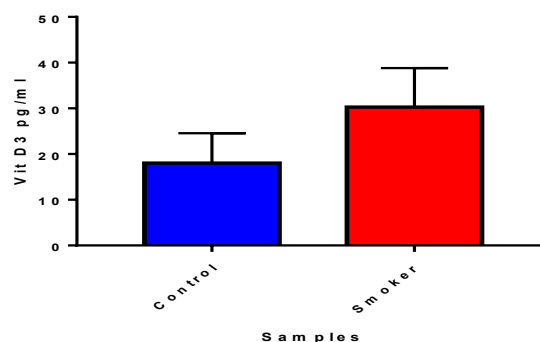


Figure (3) Vitamin D3 concentration in The blood serum pg/ml

The results of the current study also showed a significant correlation $p \leq 0.05$ The inverse of the concentration of the enzyme dopamine and vitamin D 3 In the blood serum of the study sample, the value of the correlation coefficient was . (0.3555-) Figure.(4)

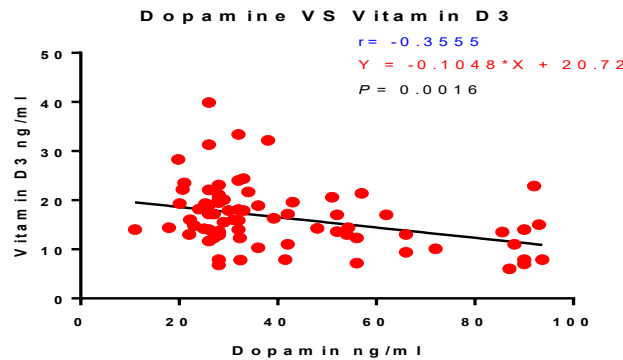


Figure (4) Dopamine correlation coefficient with vitamin concentration D3 In the blood serum

The researchers instructed[16] that cigarette smoking is associated with lower vitamin D3 levels and symptoms of depression in patients with acute stroke, the cells of our bodies consist of calcitriol(1,25)-OH and vitamin D2, which is the active form of vitamin D3. It is an integrated biological substance that influences a large number of biological processes. While a high prevalence of vitamin D3 deficiency has been revealed in populations worldwide, vitamin D3 deficiency is observed not only due to low levels of vitamin D3 in the diet, low exposure to sunlight, and low vitamin D synthesis. cutaneous, but also due to consumption of certain medications, excessive alcohol intake, and tobacco smoking, vitamin D3 is known to affect levels of estradiol, dopamine, and the pro-inflammatory cytokine and is closely related to these factors, along with being involved in the regulation of hormone-related mechanisms such as glucocorticoids, when Vitamin D binds to its receptors located in the central nervous system. It is noted that it is responsible for regulating the functions of neurons in the brain. Vitamin D receptors are found in tissues and cells of the nervous system, especially dopamine nerves. In the instantaneous phase of brain development, vitamin D may act as A neurohormone in the fields of neurotransmission, neuroprotection, and neuroimmune modulation, the vitamin D receptor belongs to the hybrid class of the nuclear receptor superfamily, which is activated by vitamin D, a neurohormone that Playing its main role in the nervous system by following the mechanisms of regulating ions Ca^{2+} , balance, neurotrophic modulation, activation of key brain hormones and enzymes for neurotransmitter metabolism. VDR It is a large molecular weight protein molecule weighing 50-60 kDa, which consists of several functional binding domains, specifically and typically for all steroid hormones responsible for binding, DNA binding, asymmetric transformation, and ligand activation of transcription factors in prostates. The a close link between dopamine and vitamin D3[17]

3.3 The effect of smoking on the concentration of iron stores in the blood

The results of the study showed a significant increase $p \leq 0.05$ In the concentration of iron stores in the blood of the study sample, its value reached $(313.1 \pm 215.6 \text{ ng/ml})$ Compared with its value in the blood of the control sample $(195 \pm 130.1 \text{ ng/ml})$. Figure (5).

Ferritin is the primary iron storage protein in tissues and is also an acute phase reactant .An elevated level is found in many chronic conditions, infections, and liver disease.Determination of serum ferritin provides a direct measurement of iron stores in the body and thus helps distinguish iron deficiency anemia from anemia due to chronic infection or inflammation.[18], Where the study indicated a significant increase in the average level of ferritin in the blood of smokers than non-smokers[18] ,While another study indicated a link to smoking and drinking also increase the level of ferritin in the blood. [19]Increased hemoglobin concentration observed in smokers due to the inhalation of carbon monoxide, leading to the formation of hemoglobin Alkrboxa which reduces the level of oxyhemoglobin in smokers. [18]High blood ferritin is not only a sign of iron stores but also an indicator of inflammation and oxidative stress. [18]Thus, the results showed that smoking has a great effect on the concentration of hemoglobin.

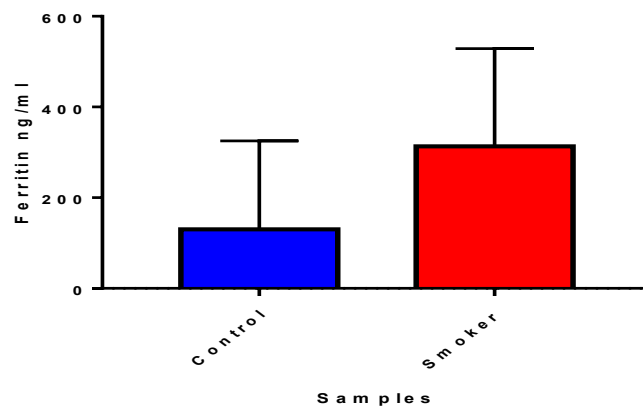


Figure (5)Concentration of iron in the blood (ng/ml)

While the results of the current study did not show a correlation between the concentration of dopamine enzyme and iron stores in the blood of the study samples, the value of the correlation coefficient was . (0.0563) Figure .(6)

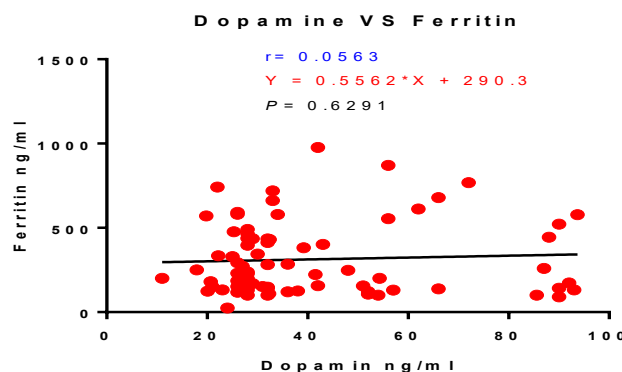


Figure (6)The correlation coefficient of dopamine with the value of iron in the blood

A study indicates[20]indicated that iron deficiency may alter the dopamine pathways involved in the treatment of smoking addiction, as the study showed the effect of iron status on the ability to quit smoking, which depends on the integrity of dopamine pathways, and this indicates the link between dopamine and iron stores in the blood, iron is one of The most abundant and indispensable transitional element for almost all living things .While iron's

ability to participate in redox chemistry is a prerequisite for participating in a range of vital enzymatic reactions, this same feature of iron also makes it dangerous for the generation of hydroxyl radicals and superoxide anions. Given the high local oxygen tensions in the lung, regulation of iron acquisition, utilization, and storage becomes critically important, perhaps more so than any other biological system. Iron plays an important role in the biology of basically every cell type in the lung and the rest of the body, in particular, changes in iron levels have important implications for immune function and the body's microenvironment, and cigarette smoke causes an imbalance in iron regulation, which means that iron The link between smoking and smoking-related lung disease[21], considered Ferroptosis A type of controlled cell death characterized by iron-dependent deposition of lipid hydroperoxide to lethal concentrations. Ferroptosis Pathological conditions lead to neurodegenerative diseases, cancer, heart attacks, hemorrhagic stroke, traumatic brain injury, ischemia and reperfusion injury, and renal dysfunction. Dopamine action and consequently loss of control of the nervous system and general weakness[22].

3.4 molecular study molecular study

3.4.1 extraction of DNA

The current study included DNA extraction DNA From (75) smokers' blood samples, as well as from (25) control samples, depending on the method of extracting the DNA From the total blood described in the separation of materials and methods of work, the purity and concentration of the samples were also measured by a device) (Nano Drop If the purity and concentration were within the ideal limits and appropriate for the reaction of the (tetra-primer ARMS-PCR All samples are transported into the relay) Gel Electrophoresis (To ensure the success of the extraction process by detecting the presence of DNA by following its movement on agarose gel at a concentration of .(%1) Picture(1)



Figure(7) DNA Extract on agarose gel at a concentration of 1%5 vol. /cm for 1:15 hour

2.4.2 Results of PCR products of single allelic polymorphisms rs6351

The results of the current study show that table (2 and 3) and figure (8) the occurrence of a multiplicity in the forms or locations of nitrogenous bases (SNPs), showing the site rs6351 Multiple occurrences in the primary site G(125/94) ,(in which the number of bands appearing in it was 16 (21.3%) in the study sample members compared to 23 (92%) in the control sample, while the study showed the occurrence of polymorphism in the form of nucleotide above, as it showed two additional forms :G(125) And G(94) The number of occurrences and frequency reached 25 (33.3%) and 34 (45.4%), respectively, compared to the number of times they appeared in the control sample, which amounted to 2 (8%) and (0%).

Table (2) Number of registered genotypes

NS	SNP	G(125/94)	G(125)	G(94)	
1	rs6351	GG=16	GC=25	GT=34	Patients Control
		GG=23	GC=2	GT=0	

2	rs429699	G(104/138)	G(138/190)	G(138)	Patients
		CC=22	CA=14	CT=39	
3	rs6880875	CC=21	CA=4	CT=0	Control
		G(136/102)	G(136)	G(102)	
		TT=52	TA=12	TC=11	Patients
		TT=18	TA=7	TC=0	

Table (3) genotypes of rs6351SNP

SNP:1 rs6351	Patients No. (%)	Control No. (%)	P-value	Square is -chi (X 2 - - - - -)
GG	16 (21.34%)	23 (92.00%)	0.0001	5.80 **
GC	25 (33.33%)	2 (08.00%)	0.896	1.22 N
GT	34(45.33%)	0 (0.00%)	0.0001	2.47 *
Allele	Frequency			
G	0.19	0.90	--	--
C	0.81	0.10	--	--

** ($P \leq 0.05$).



Figure (8) Electrophoresis product rs6351 on agarose gel at a concentration of 1.5%5 vol. /cm for 1:15 hour.

2.4.3 Results of PCR products of single allelic polymorphisms rs429699

The results of the current study also showed Table (4) and figure (9) the occurrence of a multiplicity of forms or locations of nitrogenous bases) SNPs ,(showing the site rs429699 Multiple occurrences in the primary siteG(104/138) (in which the number of packets appearing 22 (%29.34) in the members of the study sample compared to 21 (84%) in the control sample, while the study showed the occurrence of polymorphism in the form of nucleotides above, as it showed two additional forms :G(138/190) And G(138) And the number of appearances and frequency reached 14 (18.66%) and 39 (52%) respectively compared to the number of times they appeared in the control sample, which amounted to 4 (16%)and 0 (0%).

Table (4)in situ single-nucleotide electrophoresis product rs429699On agarose gel at a concentration of 1.5%5 vol. /cm for 1:15 hour.

SNP:2 rs429699	Patients No. (%)	Control No. (%)	P-value	Square is -chi (X 2 - - - - -)
Genotype				
CC	22 (29.34%)	21(84.00%)	0.0001	2.87 **

CA	14 (18.66%)	4 (16.00%)	0.173	0.02 N
CT	39 (52.00%)	0 (0.00%)	0.0001 **	2.70 **
Allele	Frequency			
C	0.19	0.93	--	--
T	0.81	0.07	--	--

** ($P \leq 0.05$).

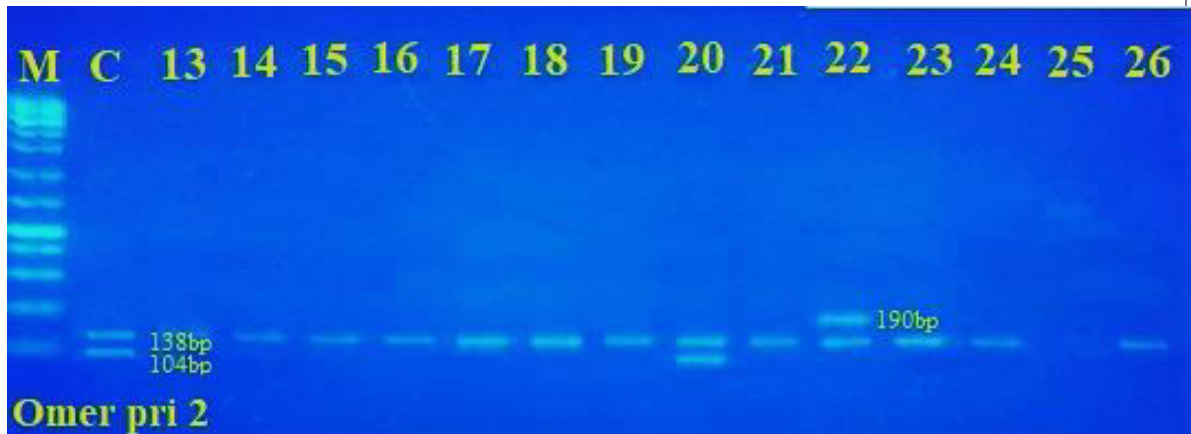


Figure (9) In situ single-nucleotide electrophoresis product rs429699 on agarose gel at a concentration of 1.5% 5 vol. /cm for 1:15 hour.

the study indicated [23] and a relationship between genotype C/T and Attention Deficit Hyperactivity Disorder in Taiwan, and the results of the current study indicated in Table (5) the occurrence of a multiplicity in the forms or locations of nitrogenous bases) SNPs, (showing the site rs6880875 Multiple occurrences in the primary site G(136/102) ,(in which the number of bands appearing was 52 (69.33%) in the study sample members compared to 18 (72%) in the control sample, while the study showed the occurrence of polymorphism in the form of nucleotides above, as it showed two additional forms :G(136) And G(102) And the number of appearances and frequency reached 12 (16%) and 11 (14.67%) respectively compared to the number of times they appeared in the control sample, which amounted to 7)28 (% and 0(0% . (Table (3) and Picture.(4)

Table (5) in situ single-nucleotide electrophoresis product rs6880875.

SNP:3	Patients	Control	P-value	Square is -chi (X 2 - - - -)
rs6880875	No. (%)	No. (%)		
Genotype				
TT	52 (69.33%)	18 (72.00%)	0.0001	0.01 **
TA	12 (16.00%)	7 (28.00%)	0.894	0.17 N
TC	11 (14.67%)	0 (00.00%)	14.61**	0.44 **
Allele	Frequency			
T	0.83	0.12	--	--
A	0.17	0.88	--	--

** ($P \leq 0.05$).

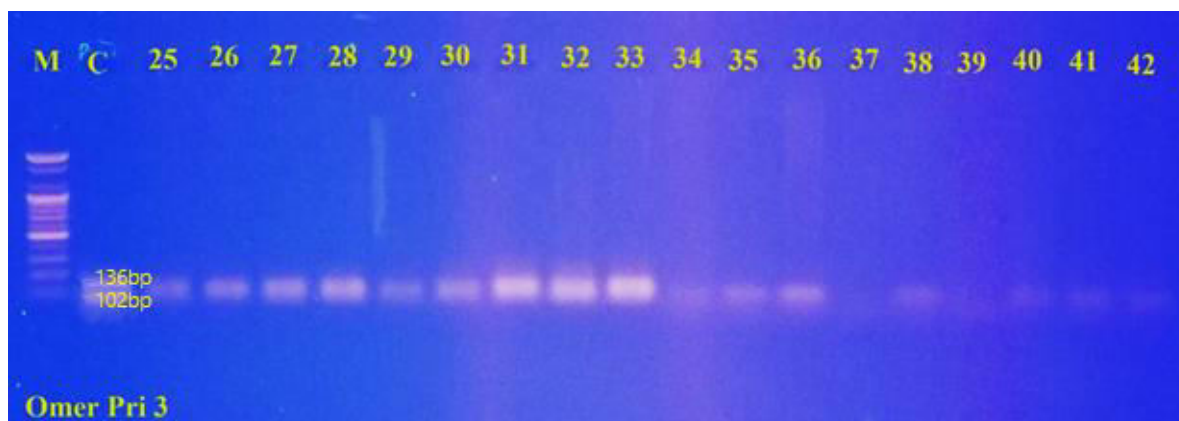


Figure (10) in situ single-nucleotide electrophoresis product rs6880875 on agarose gel at a concentration of 1.5% vol. /cm for 1:15 hour.

Cigarette smoke is known to contain many carcinogens, Polycyclic aromatic hydrocarbons (PAHs). In addition to aromatic amines, and Alaldehyadat which are harmful substances that cause dangerous and harmful to the genetic content of the organism due to be free radicals, which in turn caused damage DNA ,studies have indicated that the hormone estrogen turned by smoking to catechol estrogensWhich causes the production of many free radicals, which are the cause of significant damage to the DNA. [24]Studies also indicate that cigarette smoking may lead in one way or another to the development of DNA methylation Which would activate many cases of modulation in the gene expression of many genes, as cigarette smoke is considered one of the strongest environmental modifiers of DNA methylation, which shows the causes of substances in the composition of cigarettes such as (arsenic, chromium, formaldehyde and polycyclic aromatic). Hydrocarbons and nitrosamines double-strand breaks in DNA.

Cigarette smoke may also modulate DNA methylation through the effects of nicotine on gene expression. Nicotine binds to and activates nicotinic acetylcholine receptors (which are abundant in the central and peripheral nervous systems), thereby increasing intracellular calcium and leading to activation of a response element-binding protein .cAMP Cigarette smoke alters DNA methylation indirectly by modulating the expression and activity of DNA-binding factors .As cigarette smoke increases the expression ofSp1and it's binding to DNA in lung epithelial cells. (Sp1is a common transcription factor that binds to regions rich inGCIn gene inducers) or cigarette smoke may alter DNA methylation via hypoxia, smoke contains carbon monoxide that binds to hemoglobin (competitively with oxygen) and thus reduces tissue oxygenation which in turn leads to response factor dependence.hypoxia response HIF-1 α Put methionine SAM transferase ,which is a major donor to the operations of DNA methylation[25]

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