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STUDY ASSOCIATION OF THE *ANKK1* GENE POLYMORPHISMS AND D2 DOPAMINE RECEPTOR GENE EXPRESSION WITH INFERTILITY AMONG SOME OF IRAQI FEMALES

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Abstract

Infertility is the most common endocrine disease occurring in women of reproductive age. This study aimed to clarify the effect of *ANKK1* polymorphism in *DRD2* gene expression and altered dopamine concentrations with infertility in women and to study their correlation with some biochemical parameters. The study was conducted at the Institute of Genetic Engineering and Biotechnology for Postgraduate Studies - University of Baghdad from January 2022 to October 2023. The patients were taken from Baghdad's Kamal Al-Samarrai Infertility Treatment Hospital. The study included (50) patients and (50) controls. Methods included biochemical examinations, including serum concentrations of Dopamine (DA), Luteinizing hormone (LH), follicle-stimulating hormone (FSH), and prolactin were measured. The RNA and DNA were extracted from whole fresh blood. The concentration and Purity of DNA and RNA Based on a nanodrop device were performed, then converted to cDNA to measure the level of gene expression for *DRD2*, a single-nucleotide polymorphism *ANKK1* rs1800497 (SNP) was genotyped. In this study, the results showed a significant decrease in Dopamine levels in infertile Women in comparison to the control group ($P< 0.01$). In addition, the analysis of hormone levels of LH, FSH, and Prolactin (PRL) in infertile females showed a highly significant increase in PRL and LH compared to the control group ($P\leq0.01$) and a non-significant difference in the level of FSH. The fold expression of *DRD2* was down-regulated in patients, whereas in the control group, it was up-regulated. Genotype Results of *ANKK1* rs1800497 were observed to have no significantly different frequencies in the homozygous GG and mutant AA genotypes. In contrast, it has significantly different frequencies in the heterozygous GA in patients compared to the control group. Consequently, Assessing the impact and correlation between *ANKK1* rs1800497 variation and the expression of *the DRD2* gene as a risk of infertility showed a significant negative correlation between polymorphism in *ANKK1*gene with folding expression for *DRD2.*

Key words: D2 dopamine receptor, *ANKK1* polymorphism ,infertility, Dopamine ,*DRD2* gene expression

Introduction

Infertility is defined as the failure to conceive a pregnancy after engaging in regular unprotected sexual intercourse for 12 months. Approximately 85% of couples experiencing infertility can attribute their condition to a specific reason. Ovulatory dysfunction, male factor infertility, and tubal illness are the primary causes of infertility. The remaining 15% of couples who are unable to conceive are classified as having "unexplained infertility." Lifestyle and environmental variables, such as smoking and obesity, can negatively impact fertility. Ovulatory

problems contribute to over 25% of infertility diagnoses, with 70% of women experiencing anovulation being diagnosed with polycystic ovary syndrome[1,2]. Infertility can serve as an indicator of an underlying chronic disease that is linked to infertility. In addition to hereditary reasons and genetic developmental problems, these factors significantly contribute to female infertility [3]. Dopamine is involved in several essential brain functions, including locomotion, behavior, cognition, motivation, and neuroendocrine secretion. These effects are achieved through the activation of dopamine receptors. Specifically, the dopamine D2 receptors have been linked to the brain's reward systems. A malfunction of the D2 dopamine receptors results in abnormal behavior characterized by the pursuit of substances [4].

Dopamine governs a range of physiological and behavioral functions, including the regulation of reproduction. In vertebrates, this regulation occurs through the hypothalamic-pituitary-gonadal (HPG) axis .

The hypothalamus releases gonadotropin-releasing hormone (GnRH1), formerly known as luteinizing hormone-releasing hormone, which stimulates the pituitary gland to release luteinizing hormone (LH) and follicle-stimulating hormone (FSH) into the bloodstream. Gonadotropic hormones directly modify the reproductive capacity by stimulating the production of gonadal steroid hormones such as testosterone, estrogen, and progestin [5]. In addition, Dopamine attaches to *DRD2* receptors in the pituitary lactotrophs and reduces the amount of cyclic adenosine monophosphate inside the cells. This, in turn, hinders the production of prolactin [6, 7]. The *ANKK1* rs1800497 single nucleotide polymorphism (SNP), also known as Taq1a polymorphism (rs1800497, Glu713Lys), is located approximately 10 kb downstream from the drd2 gene in the ankyrin repeat and kinase domain containing 1 (*ANKK*1) gene. This SNP encodes a signaling protein that indirectly modulates the expression of *DRD2*. The genes Neural Cell Adhesion Molecule 1 (*NCAM1*) and Tetra tri co peptide Repeat Domain 12 (*TTC12)* are part of the gene cluster (NTAD) located on Chr11q22–23. Furthermore, evidence indicates that genetic variation in a specific gene within the *NTAD* cluster can indirectly influence the expression of another gene within the same cluster[8]. So, this study was conducted to clarify the effect of *ANKK1* polymorphism in *DRD2* gene expression and altered concentrations of Dopamine with infertility in Iraqi women.

Materials and Methods

Study Design

The current study was conducted at the Institute of Genetic Engineering and Biotechnology for Postgraduate Studies - University of Baghdad from January 2022 to October 2023. Patients were selected from Kamal Al-Samarrai Hospital in Baghdad for infertility treatment. The study included 50 patients and 50 controls.

Sample collection

This observational study consisted of 50 infertile females and 50 as controls. Blood samples (5ml) were collected using EDTA tubes, (250) µl of blood from each EDTA tube was added to 750µl of Triazol in an Eppendorf tube kept in a deep freezer (-20 ºC) used for molecular

analysis**,** and the rest of the samples were centrifuged. The serum was collected and refrigerated at (4 ºC) for a biochemical test.

The Ethical approval

The study was conducted in conformity with the ethical principles of the Declaration of Helsinki. The study commenced after obtaining the patients' verbal and written consent before their recruitment for study. The study protocol, subject information, and permission form underwent a thorough evaluation and received approval from the institutional ethics committee (Reference: 3306, dated 12-12-2022).

Methods

Endocrine assessments were incorporated. Serum concentrations of Dopamine, Prolactin, LH, and FSH by Enzyme-linked Immunosorbent assay (ELISA) and enzyme-linked fluorescent assay(ELFA) technique to estimate DNA concentration and purity through absorbance measures of samples' microvolumes using the NanoDrop spectrophotometer[9] .RNA, DNA purity and concentration were determined Then, synthesis of the cDNA form mRNA. The expression levels of the *DRD2* gene were assessed using the reverse transcription-quantitative polymerase chain reaction (qRT-PCR) method. A quantitative real-time qRT-PCR SYBR Green test was employed to validate the expression of the target gene. The endogenous control gene *GAPDH's* mRNA levels were amplified and utilized to normalize the *DRD2* gene's mRNA levels. DNA was extracted from the peripheral blood of all participants, *and ANKK1* rs1800497 was genotyped by HRM –PCR (High-Resolution Mult), a sensitive method for identifying variations in the target region [10]. A Rotor gene Q Real-time PCR System (QIAGEN) was used to perform qPCR-HRM, followed by an HRM analysis with 0.2 °C scaling from 55 to 95 °C. 2xTransStart® Tip Green qPCR Super Mix Synthetic SNP sequences were evaluated using duplicates. To identify allelic differences, qPCR-HRM was used on triplicate synthetic controls, and normalized melting curves (NMC) and differential curves (DC) were constructed using the HRM Tool included in the integrated program (Rotor gene 4.4). Components of Real-time PCR were performed in a 20 µl reaction volume including 10 µl 2xTransStart® Tip Green qPCR Super Mix, 1 µl Forward Primer (10 µM), 1 µl Reverse Primer (10µM), 5 µl Nuclease free water, 3 µl Template DNA. According to the thermal profile, the cycling protocol was programmed for the following optimized cycles: one cycle enzyme activation step of 30 sec at 94ºC hold followed by 40 cycles of denaturation at 94ºC for 5 sec, annealing 60ºC for 15 sec and extension 72ºC for 20 sec, followed by 40 cycles of HRM at 55-95ºC for 0.2sec for 1 degree The primers were designed using the Primer 3plus, V4, and double checked by the University Code of Student Conduct (UCSC) programs, and with their reference sequences in the National Center for Biotechnology Information (NCBI) database. They were synthesized and lyophilized by Alpha DNA Ltd. (Canada). Table (1,2) displays all primer sequences in this study's assays.

Table (1): The study's designed primers of *DRD2* and *GAPDH*

Primer	**Sequence (5′→3′ direction)**	**primer size bp**	**Product size bp**	**Ta** °C
DRD2 (Gene Expression)				
Forward	CTGCAGACCACCACCAACTA	20	154	58
Reverse	TGACGTCCAGAGTGACGAAG	20		
***GAPDH*- Glyceraldehyde 3-phosphate dehydrogenase**				
Forward	GAAATCCCATCACCATCTTCCAGG	24	160	58
Reverse	GAGCCCCAGCCTTCTCCATG	20		

Table 2: The study's designed primers of *ANKK1*rs1800497

Primer	**Sequence (5′→3′ direction)**	**primer size bp**	**Product size bp**	**Ta** °C
Forward	CAACACAGCCATCCTCAAAG	20	71	58
Reverse	CAGCTCACTCCATCCTGGAC	20		

Statistical analysis

The statistical analysis was conducted using the SPSS version. The categorical variables were displayed as frequencies and percentages. The study used the chi-square and Fisher exact tests to compare the percentages (frequencies). The study utilized odds ratios (ORs) and 95% confidence intervals to assess the potential correlations between the expression of dopaminergic genes and the likelihood of experiencing infertility. A P value was considered significant for all tests if it was less than or equal to 0.05.

Results and Discussion

Hormones analysis

Table 3 compares Serum Dopamine in Infertile females with healthy women, and The results indicate a substantial decrease in Dopamine levels among infertile females (13.4966±2.53960 vs 22.3652±15.62011.; P≤0.01). These results agree with another study by Wasilewski *et al*. [11], which found that a decrease in dopamine metabolism can result in menstrual irregularities and increase the likelihood of miscarriage. This reduction in dopamine levels can lead to elevated prolactin concentration, subsequently causing hyperprolactinemia and disturbances in ovulation, which are considered the main cause of infertility in females.

Table 3: Comparison between patients and control groups in Dopamine

Groups	**Mean(pg/ml)**	**Std. Deviation**	**Std. Error of Mean**	**p-value**
Patients	13.4966	2.53960	.35561	0.001**
Control	22.3652	15.62011	3.59669	

Total	16.3345	10.87201	1.25539	

Table No. (4) and Figure (1**)** show the Receiver Operating Characteristic (ROC) of DA to determine the accuracy of hormone level in predicting female infertility. The ROC was used as a binary classifier system. Diagnostic testing to determine a disease's presence or absence is essential in clinical practice. which indicated that the level of Dopamine hormone in blood samples is a very good marker for predicting female infertility with a predictive cut off value of 14.3398 U/ml, AUC(area under the curve) 0.81,which means the test can correctly discriminate positive and negative results with a probability of(0.81), specificity 76%, sensitivity (83)[12].

Table 4: Receiver Operating Characteristic curve data of the studied gene

Parameters	**AUC**	**Explanation**	**P value**	**The best Cut off**	**Sensitivity %**	**Specificity%**
Dopamine	0.81	Very good	0.001	14.3398	83	67

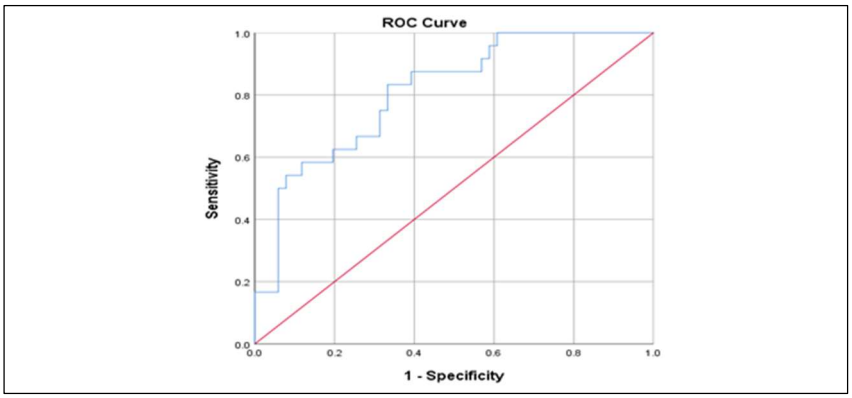

Figure 1: Receiver Operating Characteristic curve of the Dopamine

Tables (5 and 6) compare patients and healthy controls in LH and prolactin levels Serum. There was a significant increase in LH (6.2466±3.819400 vs. 3.9680 ±.92327; P≤0.01) and a significantly elevated increase in PRL (22.226 ±8.7353 vs. 12.446 ±2.26290; P≤0.01). These findings are close to the study conducted by Al-Juaifari and Al-Jumaili [13], which concluded that infertile females showed a significant increase in LH levels compared to healthy control females, Which causes alteration in gonadotropin-releasing hormone secretion and disturbances in ovulation, In addition, High levels of LH not only affect oocyte maturity and human reproduction but also causing lower fertility and higher miscarriage prevalence [14]. Furthermore, Hamed et al.[15] in their study founded a significant increase in serum prolactin concentrations in infertile females compared to healthy women in PRL level lead to hyperprolactinemia which contribute gonadal dysfunction in infertile females. Also, hyperprolactinemia might be linked to an increased risk of metabolic syndrome and probably become a metabolic risk. In women, it frequently leads to ovulatory disorder, menstrual galactorrhoea, and infertility[16].

Table 5: Comparison between patients and control groups in LH

Groups	Mean	Std. Deviation	Std. Error of Mean	p-value
Patients	6.2466	3.81940	.54015	0.0001**
Control	3.9680	.92327	.13057	

Table 6: Comparison between patients and control groups in PRL

Groups	Mean(ng/ml)	Std. Deviation	Std. Error of Mean	p-value
Patients	22.226	8.7353	1.2354	0.0001**
Control	12.446	2.2629	.3200	

Table (7) showed a non-significant decrease in Serum FSH levels in the infertile females compared to healthy control females (5.1442 ±3.28634 *vs* in control 5.3180±.99297). The low level of FSH and significantly higher LH hormone levels lead to increased LH/FSH ratio in infertile females**. These results agreed with studies done by Al-Faisal and Al-Deresawi** [17], which reported high LH and low FSH levels in these individuals prevented them from producing a mature egg, which caused infertility. Abnormality of LH In infertile females is associated with less response for progesterone and defect in the frequency of FSH. This leads to an elevated baseline LH/FSH ratio in women, associated with poor ovulatory response [18].

Table 7: Comparison between patients and control groups in FSH (mμ/ml)

Groups	Mean	Std. Deviation	Std. Error of Mean	p-value
Patients	5.1442	3.28634	.4647600	0.7
Control	5.3180	0.99297	0.14043	
Total	5.2311	2.41684	.24168	

2.Fold expression of *DRD2* gene

Table (8) displays the Ct value of *DRD2*, a housekeeping gene used in this work. A quantitative RT-PCR test was used in the ongoing study to evaluate *DRD2* mRNA expression and compare it between fertile and control groups. The relative quantification equation was used to determine gene expression fold change In the present study [19]. The calculated ratios for *DRD2* gene fold expression in infertility and healthy groups were (0.6791) and control (1.00), respectively. The results showed significantly lower in infertile women than apparently healthy control in gene expression of *DRD2*. These results agreed with Chaudhari *et al.* [20] , who described that a significant decrease in the expression of *DRD2* led to hypersecretion of prolactin and increased release of LH in infertile females, particularly in females with Polycystic ovary

syndrome (PCOS)which cause metabolic disorder[21]. Furthermore, a study conducted by Gómez *et al*.[22], reported a decrease in *DRD2* expression caused an increase in the secretion of Vascular endothelial growth factor(VEGF) and increased risk of ovarian hyperstimulation syndrome. Also, *DRD2* gene expression decreased in endometriosis areas, and there was a negative correlation of DRD2 with PRL level and dopaminergic regulation decrease, leading to local PRL level and elevation and VEGF in infertile females[23].

Table 8: Comparison between patients and control groups in *Fold of* Gene expression**.**

Groups	***Means Ct of DRD2***	***Means Ct of GAPDH***	***ΔCt (Means Ct of DRD2)***	***$2^{-\Delta Ct}$***	***experimental group/ Control group***	***Fold of gene expression***
Patients	27.8373	14.7944	13.0433	0.000118	0.000118/0.000191	0.6791
Control	27.1066	14.7593	12.3473	0.000191	0.000191/0.000191	1.00

3.Genotype and allele frequencies detected by HWE law of *ANKK1* gene polymorphism (rs1800497) between patient group and control group.

From Table (9), the *ANKK1* rs1800497 SNP was observed to have three genotypes (G.G., GA, and A.A.) that were correspondent to two alleles, which were G and A.the distribution of genotype and allele frequencies analyses for *ANKK1* rs1800497 among patient groups comparison to the control group Out of 50 infertile Iraqi women, as co Dominant gene genotypes 14 were homozygous, giving a total frequency of (28%) (14/50)., 20 females with a frequency of (40%) (20/50) were heterozygous and A homozygous mutant was identified in 16 females, with a frequency of (32%) (16/50) The frequencies of these genotypes did not differ significantly from the control group26 (52%),12 (24%),12 (24%) respective. In Co-dominant, the odd ratio (95 % CI)for GA. and AA. genotypes were 3.02 (0.9471 to 10.1151) and 2.4 (0.7426 to 8.2564) respectively No statistically significant differences were found (p=0.06 and p=0.1) Implies that these genotypes did not have a higher risk of infertility than the wild type in Dominant genotypes allele GA+ AA frequencies in patient group and Control)was 36 (72%)for the patient and 12 (48%) for control the O.R(95% CI) was 2.7 (1.0265 to 7.5601);(P=0.04) with significant difference from the control group revealed that there was significant variation between these frequencies and there was a positive correlation (etiological factor) in Dominant genotypes with infertility, this means that females who caring Dominant GA+ AA genotype have a higher risk for infertility than other genotypes The frequencies of Recessive genotypes **A.A.** did not differ significantly from the control group 16 (32%)for patient and12(24%)for control O.R. and 95% CI was1.4 (0.4993 to 4.4474);(P=0.4)with no statistically significant differences Implying that these genotypes did not have a higher risk of infertility than the wild type The OR (95% CI)for A allele were1.9 (0.9582

to 3.8711); p = 0.060 When the comparison was made at the allele level, allele frequency was increased in patients compared to the control (0.52 vs. 0.36) In contrast, the wild allele (G) significantly decreased frequency in patients (0.48 vs. 0.64) These findings suggested that an A allele-related risk factor for infertility in Iraqi women, while the wild allele (G) might have a protective effect.

ANKK1 rs1800497 polymorphism G to A substitution is located at 11q22-q23. The G allele is considered wild type, and the A allele is polymorphic [24], these Results agreed with Eken *et al,* [25]showing no significant association was found in co dominant and recessive genotypes state, which suggested showing no significant association was found in the co-dominant and recessive genotypes state, which suggested that it may not be associated with the susceptibility of infertility in females with these genotypes. In contrast, these polymorphisms in a heterozygous genotype state had significant variation (P=.0.04), which suggested (the GA) genotype may be associated with the susceptibility of infertility in females with dominant genotypes. the rs1800497 polymorphism affects dopaminergic nerve functions, and it was reported that the AA genotype of *ANKK1* rs1800497 polymorphism was lower when compared to AG and GG genotypes[26, 27]. Furthermore, the functional polymorphism rs1800497 in the *ANKK*1 gene is associated with induced Hyperprolactinemia (HPRL). Since dopamine suppresses the release of prolactin by attaching to D2 receptors found on the outer surface of lactotrophs in the anterior pituitary gland, the variations in the *ANKK1* gene could influence the expression of DRD2 and potentially contribute to the development of HPRL, this condition can lead to infertility in women by disrupting the functioning of the gonadotropic axis at various levels. Elevated levels of PRL inhibit the release of LH and FSH directly from the pituitary gland [28,29]. Taq IA polymorphism (rs1800497) in the *ANKK1* gene (specifically GA heterozygot genotypes) has been linked to a decrease in the density of DRD2 in important brain regions associated with reward, particularly in the striatum [30]. on the other hand, Studies done by Aslan et al [27] concluded that the genotype distributions, the AG and GG, were found, and No AA genotype was found for (rs 1800497). When allelic distributions were examined in the athlete group, it was found that the A allele was lower than the G allele. Also, they believe that the A allele is associated with addiction and athletic performance.

Odd ratio (95% CI)	**P value**	**Frequencies (%)**		***SNP rs1800497***
		Control (n= 50)	**Patients (n= 50)**	
Co-dominant				
1.00 (Reference)	---	26 (52%)	14 (28%)	**GG**
3.02 (0.9471 to 10.1151)	0.06	12 (24%)	20 (40%)	**GA**
2.4 (0.7426 to 8.2564)	0.1	12 (24%)	16 (32%)	**AA**
Dominant				
1.00 (Reference)	---	26 (52%)	14 (28%)	**GG**

2.7 (1.0265 to 7.5601)	0.04*	24 (48%)	36 (72%)	**GA+ AA**
Recessive				
1.00 (Reference)	---	38 (76%)	34 (68%)	**GG +GA**
1.4 (0.4993 to 4.4474)	0.4	12 (24%)	16 (32%)	**AA**
Allele				
1.00 (Reference)	---	0.64 (32)	0.48 (48)	**G**
1.9 (0.9582 to 3.8711)	0.06	0.36 (18)	0.52 (52)	**A**

Table 9: Genotype and allele frequencies detected by HWE law of *ANKK1/DRD2* gene polymorphism ((rs1800497) between patient group and control group**.**

4.The impact of (*ANKK1*rs1800497) SNP on *DRD2* expression in control and patient group

When comparing the impact of *ANKK*1rs1800497 SNP on *DRD2* expression in control and patient group there is statistically significant different(P=0.02) which was explained There was significant impact of the*ANKK1*(rs1800497) SNP genotype on the expression fold of *DRD2* (Table 10). The functional implications of the genetic variant (rs1800497) at the molecular level modified substrate binding selectivity and lower *DRD2*densities[31]. Additionally, there is a connection between the expression of the *DRD2* gene and *ANKK1* rs (1800494) in The *NTAD* gene cluster on Chr11q22–23 consists of these two genes, as well as *NCAM*1 and *TTC12*.An intriguing characteristic of this cluster arises from the data indicating its functionality as a cohesive entity. the NTAD cluster, which consists of genes associated with neurogenesis and neurotransmission, includes evidence indicates that genetic variation in one gene of the NTAD cluster might indirectly influence the expression level of another gene within the same cluster[32]. Furthermore, *ANKK1* rs1800497 (G/A transition) The G allele is considered wild type and related to the high number of the *DRD2* receptor molecules on the cell membrane, the A allele is considered a polymorphic allele, and linked with lower *DRD2* receptor numbers and dopamine levels [8,25]. This result was compatible with Polat et al [26] who reported the *ANKK1* (rs1800497) polymorphisms have an effect on dopaminergic nerve functions and reported that a Low dopamine level due to (rs1800497) polymorphism, which causes Low dopamine levels, also reported the AA genotype of *ANKK1/DRD2* rs1800497 polymorphism was lower when compared to AG and GG genotypes. It also shows that the expression of *DRD2* decreased [33]. So, the present study showed potential influences of variations of *the ANKK1* gene on the expression of *DRD2* in a group of Iraq infertile women, causing lower gene expression of *DRD2*gene.

Table 10: Compared the impact of (*ANKK1*rs1800497) SNP in *DRD2* expression in control and patient groups**.**

Groups	rs1800497	Mean 2-ΔΔCt	Std.Deviation	p-value	p-value
Patients	**Wild GG**	0.7587	0.32961	0.5	0.02*
	Hetero GA	0.6765	0.38993		
	Mutant AA	0.4500	0.15766		
Control	**Wild GG**	1.3070	0.91266	0.7	
	Hetero GA	1.0713	0.39988		
	Mutant AA	.8890	0.26267		

Conclusions

According to the findings, this study concluded:

Infertility can be considered as a complex, heterogeneous condition triggered by the interactive effect of genetic and environmental factors. A low expression value of *DRD2*gene and Dopamine serum level in infertility women was found. One of the major findings of the present study was the effectiveness of the SNP genotype *ANKK*1 rs1800497 on the relative expression of *DRD2* in the target genes, so the new finding was that *ANKK1* rs 1800497 polymorphism and *DRD2* gene expression level is an intrinsic characteristic of infertility among some of Iraqiin females and can be utilized as biomarkers for early diagnosis of female infertility in Iraqi women.

References

1) . Al-Juaifari , B. A. J. and Al-Jumaili, E.F.A. (2019). Allelic estrogen receptor beta (ESR2) gene association with Polycystic Ovary Syndrome in unmarried Iraqi female of Baghdad Governorate. Iraqi Journal of Biotechnolgy .2019, vol.18,no.3, 26-32.

2) . Nader, Mohammed & Mezaal, Mohammad. (2019). Correlation between Insulin Resistance and Polycystic Ovary Syndrome in Iraqi Women. African Journal of Medicine and Medical Sciences. 47. 2051-5731

3) Beke A. Genetic causes of female infertility. Genetics of Endocrine Diseases and Syndromes. 2019:367-83.

4) AL-Awadi SJ. Association of the A1 allele of D2 dopamine Receptor gene Polymorphisms with Alcohol and Drug abuse among some of Iraqi Population. Iraqi journal of biotechnology. 2017;16(1).

. 5) Dufour S, Sebert ME, Weltzien FA, Rousseau K, Pasqualini C. Neuroendocrine control by dopamine of teleost reproduction. Journal of fish biology. 2010;76(1):129-60.
6. Abbas OA, Ibrahim IG, Ismail A-GE. Therapeutic effects of nano-HAp in a rat model of AlCl3 induced neurotoxicity. Iranian Journal of Pharmaceutical Research: IJPR. 2019;18(3):1309.
7. Koeneke A, Ponce G, Troya-Balseca J, Palomo T, Hoenicka J. Ankyrin repeat and kinase domain containing 1 gene, and addiction vulnerability. International journal of molecular sciences. 2020;21(7):2516.
8. Niu Y-M, Zhang J, Tang H, Cao L-H, Jiang T-Y, Hu Y-Y. Association between DRD2/ANKK1 rs1800497 C> T polymorphism and post-traumatic stress disorder susceptibility: a multivariate meta-analysis. Frontiers in Neuroscience. 2023;17:1102573.
9. García-Alegría AM, Anduro-Corona I, Pérez-Martínez CJ, Guadalupe Corella-Madueño MA, Rascón-Durán ML, Astiazaran-Garcia H. Quantification of DNA through the NanoDrop spectrophotometer: methodological validation using standard reference material and Sprague Dawley rat and human DNA. International journal of analytical chemistry. 2020;2020.
10. Sharma L, Watts E, Singh P. High resolution real-time PCR melting curve assay for identification of top five Penaeidae shrimp species. LWT. 2020;133:109983.
11. Wasilewski T, Łukaszewicz-Zając M, Wasilewska J, Mroczko B. Biochemistry of infertility. Clinica Chimica Acta. 2020;508:185-90.
12. Nahm FS. Receiver operating characteristic curve: overview and practical use for clinicians. Korean journal of anesthesiology. 2022;75(1):25-36.
13. Al-Juaifari BJ, Al-Jumaili EF. Correlation of Body Mass Index and Some Hormones (Estradiol, Luteinizing, Follicle Stimulating Hormones) with Polycystic Ovary Syndrome among Young Females [20 to 35 Years]. Biomedical and Pharmacology Journal. 2020;13(1):193-8.
14.Kadhim SJ, Abdul-hassan IA. Correlation between vitamin D receptor gene polymorphisms and levels of some hormones in Iraqi infertile women with polycystic ovary syndrome. Iraqi journal of biotechnology. 2017;16(4).
15.Hamed EA, Sayyed HG, Abbas AM, Gaber MMA, Abd El Aleem HM. Nesfatin-1, dopamine, and NADPH levels in infertile women with polycystic ovary syndrome: is there a relationship between their levels and metabolic and hormonal variables. Journal of Reproduction & Infertility. 2022;23(3):160.
16. Andersen M, Glintborg D. Metabolic syndrome in hyperprolactinemia. Metabolic Syndrome Consequent to Endocrine Disorders. 2018;49:29-47.
17. AlFaisal AHM, Al-Deresawi MSG. The correlation between thyroid hormones, reproductive hormones, body mass index (BMI) and hirsute in Iraqi women with polycystic ovary syndrome (PCOS). J Univ Anbar Pure Sci. 2013;7:1-619.

18. Ibraheem, Q. A., Al Obaidy, L. H. A., Nasir, G. A., & Al Obaidi, M. T. (2020). Fat Mass and Obesity Association gene Polymorphism in PCOS Iraqi Women. Baghdad Science Journal, 17(3 (Suppl.)), 1103-1112
.

19. Schmittgen TD, Livak KJ. Analyzing real-time PCR data by the comparative CT method. Nature protocols. 2008;3(6):1101-8.

20. Chaudhari N, Dawalbhakta M, Nampoothiri L. GnRH dysregulation in polycystic ovarian syndrome (PCOS) is a manifestation of an altered neurotransmitter profile. Reproductive Biology and Endocrinology. 2018;16:1-13.

21. Majid Raheem, N., & Hassan Marouf, B. . (2022). Impact of Vitamin D Deficiency on the Pathogenesis of Polycystic Ovary Syndrome. Al-Rafidain Journal of Medical Sciences (ISSN 2789-3219), 2, 1–7. https://doi.org/10.54133/ajms.v2i.53

22. Gomez R, Ferrero H, Delgado-Rosas F, Gaytan M, Morales C, Zimmermann RC, et al. Evidences for the existence of a low dopaminergic tone in polycystic ovarian syndrome: implications for OHSS development and treatment. The Journal of Clinical Endocrinology & Metabolism. 2011;96(8):2484-92.

23. Yarmolinskaya M, Suslova E, Tkachenko N, Molotkov A, Kogan I. Dopamine agonists as genital endometriosis target therapy. Gynecological Endocrinology. 2020;36(sup1):7-11

24. Silar Ç, Karagöz E, Polat T, Yilmaz ÖÖ, Aslan BT, Doğan CS, et al. Distribution of Dopamine Receptor 2 (DRD2) rs1800497 Polymorphisms In Cyclist. The Journal of Neurobehavioral Sciences. 2020;7(3):170-3.

25.EKEN BF, GEZMIŞ H, Yasemin Ü, ERDOĞDU A, DOĞAN CS, KIRAÇ D, et al. Türk Kökenli Futbolcularda Apolipoprotein-E Genotiplerinin Belirlenmesi. Eurasian Research in Sport Science. 2019;4(2):53-8.

26. Polat T, Dogan CS, Dogan M, Akcay T, Ulucan K. Distribution of α-actinin-3 rs1815739 and angiotensin-1 converting enzyme InDel polymorphisms in Turkish bodybuilders. Biomedical Reports. 2020;13(6):1-.

27. ASLAN BT, YILMAZ ÖÖ, POLAT T, ŞILAR Ç, EKEN BF, DOĞAN CS, et al. Distribution of Dopamine Receptor 2 DRD2 rs1800497 Polymorphisms in Professional Football Players. Turkish Journal of Sport and Exercise. 2021;23(2):185-9.

28. Fedorenko OY, Paderina DZ, Loonen AJ, Pozhidaev IV, Boiko AS, Kornetova EG, et al. Association of ANKK1 polymorphism with antipsychotic-induced hyperprolactinemia. Human Psychopharmacology: Clinical and Experimental. 2020;35(4):e2737.

29. Maiter D, editor Mild hyperprolactinemia in a couple: What impact on fertility? Annales d'endocrinologie; 2022: Elsevier.

30. White MJ, Morris CP, Lawford BR, Young RM. Behavioral phenotypes of impulsivity related to the ANKK1 gene are independent of an acute stressor. Behavioral and Brain Functions. 2008;4(1):1-9.

31. Świtała K, Bojarczuk A, Hajto J, Piechota M, Buryta M, Leońska-Duniec A. Impact of the DRD2 Polymorphisms on the Effectiveness of the Training Program. International Journal of Environmental Research and Public Health. 2022;19(9):4942.

32. Mota NR, Rovaris DL, Kappel DB, Picon FA, Vitola ES, Salgado CA, et al. NCAM1-TTC12-ANKK1-DRD2 gene cluster and the clinical and genetic heterogeneity of adults with ADHD. American Journal of Medical Genetics Part B: Neuropsychiatric Genetics. 2015;168(6):433-44.

33. Robert F, Pelletier J. Exploring the impact of single-nucleotide polymorphisms on translation. Frontiers in genetics. 2018;9:507.