How to Cite:
Abed, D. S., & Salloom, D. F. (2022). The effect of PD-1 concentration level and its polymorphism in male infertility. *International Journal of Health Sciences*, *6*(S7), 3347–3354.
https://doi.org/10.53730/ijhs.v6nS7.12472

The effect of PD-1 concentration level and its polymorphism in male infertility

Dhuha S. Abed
Department of biology, College of science, University of Baghdad, Baghdad-Iraq

Dunya F. Salloom
Department of biology, College of science, University of Baghdad, Baghdad-Iraq

***Abstract*---**Infertility has become a major public health issue, impacting a significant number of couples. This study aimed to examine the association of serum concentration level of PD-1 and its polymorphism with male infertility. The study consisted of 63 infertile male as a patients group their ages 33.37±0.86 years as well as 80 healthy fertile men each of them has at least one child, their ages 34.39±0.61) years also enrolled in this study were examined at Kamal Al-Samarrai Hospital for infertility and In vitro Fertilization from April 2021 until January 2022, excluded criteria included patients suffering from Varicocele (according to the clinical examination and ultrasonic waves), chronic diseases e.g. cardiovascular, diabetes mellitus, and hypertension, men with a history of specific childhood illnesses or conditions such as bilateral cryptorchidism or unilateral, testicular trauma the result showed there was significant differences between studied groups p≤0.05 the results of gene polymorphism after sequences showed that one of the SNPs is rs 741861 and when studied in in patients compared to controls showed that the genotype AG was risk factor infertility patients

***Keywords*---**Programmed death-1(*PD-1*), polymorphism, autoimmunity, male infertility.

Introduction

Infertility is an increasing public health issue confronting developed countries today; it affects over 186 million people worldwide (Uddin *et al.*, 2018). Infertility is defined as "a disease of the reproductive system defined by the failure to achieve a clinical pregnancy after a period of 12 months or more of regular unprotected sexual intercourse with no other cause" (Barbu, 2021). A reduction in the ability of male fertility may be caused by congenital or acquired factors such as urogenital abnormalities, varicocele, infections of the genital tract,

genetic abnormalities, hormonal abnormalities, testicular failure, immunologic problems, cancer, systemic diseases, altered lifestyle, and exposure to gonadotoxic factors (Dohle *et al.*, 2005), (Jungwirth *et al.*, 2012). In spite of the advances in diagnostic tests, the cause of fertility disability cannot be determined in many cases. (Jungwirth *et al.*, 2012; and Rowe *et al.*, 1993). Programmed cell death-1 (*PD-1*, *Pdcd1*), an immunoreceptor from the CD28-CTLA-4 family, negatively regulates antigen receptor signaling by attracting protein tyrosine phosphatase, SHP-2 upon interacting with either of two ligands, PD-L1 or PD-L2. Several hundreds of genes are required for normal sexual development. Its biological significance influences a variety of immune responses, including autoimmune, tumor immunity, infectious immunity, allergies, and immunological privilege. This is due to the wide spectrum of ligand distribution in the body (Okazaki and Honjo, 2007). On the other hand, studies on *PD-1*-deficient mice with the C57BL/6 background, which display hyperactivation of the immune system including splenomegaly and in vitro increased proliferation of B cells, first identified the relationship between PD-1 deficiency and autoimmunity (Nishimura *et al.*, 1998). The aims of this study were to determine the effect *PD-1* gene polymorphism with male infertility and assessment of PD-1 concentration level related infertility.

Materials and method Subjects

One hundred and forty-three males participated in this study (all of them were Iraqis), which was divided into two fundamental groups: infertile and fertile. Infertile group, which included 63 patients, their ages, was 33.37±0.86 years as well as 80 healthy fertile male with age 34.39±0.61 years each of them has at least one child the sample were collected examined at Kamal Al-Samarrai Hospital for infertility and In vitro Fertilization from April 2021 until January 2022. At first the aim of study was explained for all participants and after obtaining their oral and signed consent they have been studied. From each participating subject (patient and control), about 5 ml of venous blood were collected by using 5ml disposable syringe. The blood was distributed into two aliquots; the first was dispensed in a plain tube to collect serum (after clotting, blood was centrifuged at 2000 rpm for 15 minutes at room temperature, and then serum was separated and stored at -20°C until assayed), while the second aliquot was drawn in EDTA tube and stored at -20°C until DNA extraction. The serum was used for sero-diagnosis of PD-1 concentration level, while EDTA blood was used to extract DNA for the determination of *PD-1* gene polymorphisms, the number of patients for sequencing gene polymorphism was32, while the number of control was 18

Human PD-1/ PDCD1 (Programed cell death protein 1) using ELISA kit (Al-shkairate establishment for medical supply/ Jordan). The serum sample was used to determine the concentration level of PD-1 protein in the infertile and control serum sample. Human PD-1/ PDCD1

(Programed cell death protein 1) using Enzyme-Linked Immunosorbent Assay kit is based on sandwich enzyme-linked immune-sorbent assay technology.

DNA extraction

Peripheral venous blood samples were collected from the subjects in

EDTA coated tubes. The genomic DNA was extracted by using **Transgene biotech Company (Transgene biotech china).** According to the manufactures' protocol. DNA purity and concentrations were confirmed by spectrophotometric measurement of absorbance at 260/280 nm.

Determination of *PD-1* genotype

Polymerase chain reaction (PCR) was utilized to determine the *PD-1* polymorphism. The PCR primers were designed by using the National Center for Biotechnology Information (NCBI), the primer sequences were 5-TTGCTGGAAAATGTGGAGGC-3 (forward) and 5- CGAAGCTCTCCGATGTGTTG -3 (reverse). Amplification was performed under the following conditions: An initial denaturation for 5minat 94oC , followed by denaturation at 94oC, annealing at 57 oC, and extension at 72oC for40 s, followed by a final extension at 72oC for 7 min. A final volume of 25 µL of extract was used to detect the PD-1 polymorphism. Subsequently the amplified PCR products subjected to gel electrophoresis and monitored by ethidium bromide. Then DNA sequencing Tanique was used for determine PD-1gene polymorphism

Statistical analysis

The IBM SPSS version 28.0 used to calculate the mean and standard error for the parametric data, ANOVA table, independent T-test used to calculate the probability. While in non-parametric data, Pearson chisquare used to calculate the probability. The probability was significant when it less than 0.05. Online Hardy-Weinberg equilibrium calculator used to calculate the odd ratio, 95% CI and Fisher's exact probability.

Results and discussion

The presented result showed significant value according to concentration level of PD-1 gene ($p>0.05$) when the mean value of infertile patient 0.77 ± 0.12 and mean value of control group 1.86 ± 0.14 (Table1-1).

Table 1-1: The differences between mean of PD-1 concentration for studied groups.

Studied groups	PD-1 level ± SE (pg/ml)	Probability
Infertile patients	**0.77 ± 0.12**	**7.6×10^{-8}**
Control	**1.86 ± 0.14**	

The reason for the decrease in PD-1 in infertile group compared to control group in presented study could be due to several factors such as mild osteoporosis, Multiple sclerosis, Rheumatoid arthritis, Inflammatory bowel disease and other

diseases (Nagahama., *et al* 2004; and Francisco., *et al* 2010). The results of this study are showed that the low concentration level of (PD-1) have been associated with advanced male infertility this concept may be due to its effect on the immune tolerance in the patient affected with male infertility due to its critical role in the regulation of immune environment.

(Garutti *et al.,* 2021) also concluded that immune checkpoint inhibitors ICIs (i.e., anti-PD1, anti-PDL1, and anti-CTLA4) have revolutionized cancer treatments because of their extraordinary efficacy. Therefore, it is anticipated that their use is going to increase further in the near future. At the same time, they concluded that these compounds could cause primary hypogonadism (referring to the direct damage of gonads, that is, ovaries or testicles). From a clinical perspective, this translates into a reduced or impaired production of viable oocytes or spermatozoa and a fertility compromise. From a biochemical perspective, primary hypogonadism can be suspected of a reduced level of sexual hormones. Also, causing secondary hypogonadism, which refers to damage in the hypothalamus or in the pituitary gland, causes a reduced activation of the hypothalamicpituitary-gonadal axis. Clinically, this translates into a reduced or impaired production of viable oocytes or spermatozoa and compromised fertility. Biochemically, secondary hypogonadism can be suspected when there are reduced levels of sexual hormones. The results of presented study are in agreement with (Wang, *et al.*, 2005) they explained that the PD-1 deficiency results in the spontaneous development of some features of a late onset lupus-like disease characterized by autoantibodies and mild glomerulonephritis in C57BL/6 mice. Autoimmunity is accelerated by PD-1 deficiency on autoimmune-prone backgrounds providing further evidence for a role for PD-1 in the induction and/or maintenance of tolerance. (Fife and Pauken 2011) they also found similar results about the role of PD-1 in regulating T cell tolerance and autoimmunity was first suggested by the autoimmune phenotype of Pdcd1−/− mice. Our results are in accordance with the recently published results by (Scovell *et al.*, 2020), who found impaired spermatogenesis in most immune checkpoint inhibitors ICIs patients post mortem in testicular biopsies.

The result showed that one of the SNPs after sequencinf of PD-1 gene polymphism was A/G rs7421861 as shown in fig (1-1)

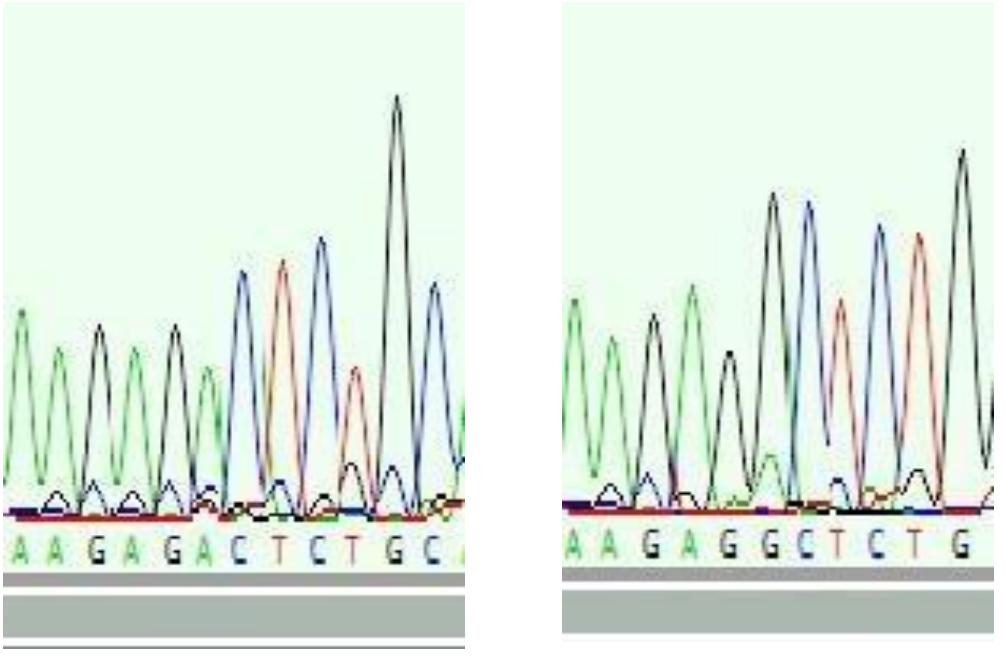

Figure: **rs741861 AG** AAGAG**R**CTCTGC

The SNP of *PD-1* gene (A/G rs7421861; located on Chromosome 2; 241853198 bp) was presented with three genotypes (AA, AG, GG) and two alleles (A and G). It was observed that genotypes frequencies in both groups of subjects were in a good agreement with Hardy-Weinberg (HW) equilibrium, and there were no significant differences between the observed and expected frequencies (Table 1-2).

Table 1-2: Number and percentage frequencies of *Pdcd1* gene genotypes rs7421861 and their Hardy-Weinberg equilibrium (HWE) in patient group and control group in blood samples.

Genotyping of rs741861	Patients group frequency (%)		Control group frequency (%)	
	Observed	Expected	Observed	Expected
AA	15 (46.88)	13.78	9 (50.0)	7.35 (40.82)
		(43.07)		
AG	12 (37.50)	14.44 (45.12)	5 (27.78)	8.31 (46.14)
GG	5 (15.63)	3.78 (11.82)	4 (22.22)	2.35 (13.04)
Total	32 (100.0)	32 (100.0)	18 (100.0)	18 (100.0)
P-HWE	0.3396		0.0913	

P- HWE: Probability of Hardy- Weinberg equilibrium. I degree of freedom (d.f.) for Chi-squared distribution.

Inspecting PD-1 gene genotypes and allele frequencies in patients group and control group showed that there were non-significant differences in genotype frequency (Table 1-3).

Genotype or alleles of PD-1(rs741861) polymorphisms were not associated with either male infertility susceptibility or protection; therefore these polymorphisms might have no role in the genetic etiology of male infertility, the limited sample size may be an important reason of the results. (Dong *et a*l., 2016) also reported no associations between cancer risk and PD-1.9 (rs2227982) or PD-1 rs7421861 in any genetic model or allele. On the other hand (Zamani *et al.*, 2015) investigated for PD1 gene polymorphism in patients with antisperm antibody related infertility in Iranian men. They performed genotyping by PCR and restriction enzyme digestion (PCR-restriction fragment length polymorphisms (RFLP) for PD 1.3 polymorphism in 145 cases (61 of ASA related infertility and 84 controls). They found a lower frequency of GG and GA and higher frequency of AA genotype in patients vs. controls (29.6%, 32.7%, and 37% in patients vs. 41.6%, 38.2%, and 20.2% in controls (p=0.042, p=0.035, p=0.0001) respectively. Also a significantly higher frequency of an allele in patients (55.8%, p=0.0012) as compared to controls (39.1%) was observed. Hence they concluded that a correlation exists between PD-1 gene polymorphism and susceptibility to ASA related infertility in their group investigated. However, our results firstly revealed that genotype AG weas risk factor in infertility patients

Table 1-3: Genotype and allele frequencies of *Pdcd1* gene rs7421861 of patients group and control group in blood samples.

Genotyping of rs741861	Patients group frequency (%)	Control group frequency (%)	OR (95% CI)	EF or PF %	Fisher's exact probability
AA	15 (46.88)	9 (50.0)	0.88 (0.29 – 2.73)	6	1.0
AG	12 (37.50)	5 (27.78)	1.56 (0.46 –	14	0.548
			5.31)		
GG	5 (15.63)	4 (22.22)	0.65 (0.15 – 2.71)	8	0.705
Total	32 (100.0)	18 (100.0)			
Alleles frequencies					
A	42 (66.0)	23 (64.0)	1.08 (0.53 – 2.19)	5	1.0
G	22 (34.0)	13 (36.0)	0.93 (0.40 – 2.15)	3	

OR: odd ratio; CI: confidence interval; EF: Effective fraction; PF: protective fraction.

The impact *PD-1* gene polymorphisms on serum level of PD-1. The three genotypes of patients group (AA, AG and GG) for PD-1 showed a nonsignificant difference. Generally, the GG genotype in patients was observed with the highest mean (1.33 ± 0.73 pg/ml) compared to other genotype, while in control group the highest mean was for AA genotype (2.61 ± 0.65 pg/ml). There was a significant differences between the AA genotype in patients and control groups (0.90 ± 0.20 pg/ml vs. 2.61 ± 0.65 pg/ml; p= 0.003) (Table 1-4).

Table 1-4: level distribution of PD-1 on patients group and control group according to the genotypes.

Genotyping of rs741861	PD-1 level mean ± SE (pg/ml)		Probability
	Patients	Control	
AA	0.90 ± 0.20^{A}	2.61 ± 0.65^{A}	0.003
AG	1.06 ± 0.26^{A}	0.63 ± 0.24^{A}	0.340 NS
GG	1.33 ± 0.73^{A}	1.10 ± 0.56^{A}	0.812 NS
Duncan test: the similar letters referred to a non significant difference among the genotyping of the same group.			

Conclusion

According to these results, there is a reverse effect for PD-1 concentration level on the male infertility and there is no correlation between gene polymorphism and susceptibility to infertility in our study group.

References

Uddin , S., Ibne Wahed, I., Uddin S., , Anwarul Haque , I., Nejum, R.(2018). Current Consequence and Research of Human Infertility in Bangladesh. *Journal of Reproductive Endocrinology & Infertility*, 3 (4): 1-8.

Barbu, M. G., Thompson, D. C., Suciu, N., Voinea, S. C., Cretoiu, D., & Predescu, D. V. (2021). The roles of micrornas in male infertility. *International Journal of Molecular Sciences*, *22*(6), 2910.

Dohle, G. R., Colpi, G. M., Hargreave, T. B., Papp, G. K., Jungwirth, A., Weidner, W., & Infertility, E. A. U. W. G. on M. (2005). EAU guidelines on male infertility. European Urology, 48(5), 703–711.

Jungwirth, A., Giwercman, A., Tournaye, H., Diemer, T., Kopa, Z., Dohle, G., … Infertility, E. A. U. W. G. on M. (2012). European Association of Urology guidelines on Male Infertility: the 2012 update. European Urology, 62(2), 324– 332.

Rowe, P. J., Comhaire, F. H., Hargreave, T. B., Mellows, H. J., & Organization, W. H. (1993). WHO manual for the standardized investigation and diagnosis of the infertile couple.

Okazaki, T., & Honjo, T. (2007). PD-1 and PD-1 ligands: from discovery to clinical application. *International immunology*, *19*(7), 813-824.

Nishimura, H., Minato, N., Nakano, T., & Honjo, T. (1998). Immunological studies on PD-1 deficient mice: implication of PD-1 as a negative regulator for B cell responses. *International immunology*, *10*(10), 1563-1572.

Nagahama, K., Aoki, K., Nonaka, K., Saito, H., Takahashi, M., Varghese, B. J., ... & Ohyama, K. (2004). The deficiency of immunoregulatory receptor PD-1 causes mild osteopetrosis. *Bone*, *35*(5), 1059-1068.

Francisco, L. M., Sage, P. T., & Sharpe, A. H. (2010). The PD-1 pathway in tolerance and autoimmunity. *Immunological reviews*, *236*(1), 219-242.

Garutti, M., Lambertini, M., & Puglisi, F. (2021). Checkpoint inhibitors, fertility, pregnancy, and sexual life: a systematic review. *ESMO open*, *6*(5), 100276.

Wang, J., Yoshida, T., Nakaki, F., Hiai, H., Okazaki, T., & Honjo, T. (2005). Establishment of NOD-Pdcd1-/-mice as an efficient animal model of type I diabetes. Proceedings of the National Academy of Sciences, 102(33), 11823-11828.

Fife, B. T., & Pauken, K. E. (2011). The role of the PD-1 pathway in autoimmunity and peripheral tolerance. Annals of the New York Academy of Sciences, 1217(1), 45-59.

Suryasa, I. W., Rodríguez-Gámez, M., & Koldoris, T. (2021). Get vaccinated when it is your turn and follow the local guidelines. International Journal of Health Sciences, 5(3), x-xv. https://doi.org/10.53730/ijhs.v5n3.2938

Scovell, J.M., Benz, K., Samarska, I., Kohn, T.P., Hooper, J.E., Matoso, A. and Herati, A.S., 2020. Association of impaired spermatogenesis with the use of immune checkpoint inhibitors in patients with metastatic melanoma. *JAMA oncology*, *6*(8), pp.1297-1299.

Zamani, M. R., Asbagh, F. A., Massoud, A. H., Salmaninejad, A., Massoud, A., & Rezaei, N. (2015). Association between a PD-1 gene polymorphism and antisperm antibody-related infertility in Iranian men. *Journal of Assisted Reproduction and Genetics*, *32*(1), 103-106.

Dong, W., Gong, M., Shi, Z., Xiao, J., Zhang, J. and Peng, J., 2016. Programmed cell death-1 polymorphisms decrease the cancer risk: a meta-analysis involving twelve case-control studies. *PloS one*, *11*(3), p.e0152448.