Pak. j. life soc. Sci. (2024), 22(1): 4382-4389 E-ISSN: 2221-7630;P-ISSN: 1727-4915

Pakistan Journal of Life and Social Sciences

www.pjlss.edu.pk

https://doi.org/10.57239/PJLSS-2024-22.1.00321

RESEARCH ARTICLE

Evaluate Catalase Gene Polymorphisms in Beta-Thalassemia Patients in Al- Diwaniyah, Iraq

Hadeer Hayder Inteek[1*], Shaymaa Rabeea Madhkhoor[2]

[1,2]Medical Biotechnology Department, College of Biotechnology, University of AL- Qadisiyah, Al- Diwaniyah, Iraq

ARTICLE INFO

Received: May 21, 2024

Accepted: Jun 21, 2024

Keywords

Catalase gene

Beta-Thalassemia

Patients

Iraq

***Corresponding Author:**

Hadeer.h.I@qu.edu.iq

ABSTRACT

Beta-thalassemia patients require regular blood transfusions, resulti in iron accumulation and irreversible oxidative damage to organs a tissues. This Study aims to investigate the frequency of catala polymorphisms in Iraqi -thalassemia patients. In this case cont study, a total of 105 blood samples were collected during the peri from October 2023 to January 2024. These samples distributed as t groups, study group, n = 60 (patients with β-thalassemia receiving ir therapy) (Age = 2- 36 years) and the control group, n= 45 (healt individuals). By using ELIZA, all samples submitted to measureme the concentration of catalase (CAT). The genotyping of catalase ge was performed in all study participants using allele specific P technique and Tetra-ARMS-PCR. A significant increase was detected the catalase concentration in β-thalassemia patients in comparis with control groups (P<0.005). Tetra-ARMS-PCR revealed that t catalase rs1001179 polymorphism has a likelihood associated with thalassemia in two genetic models (GG: OR = 5.12, P = 0.047 and T OR= 9.01, P= 0.047). The G allele in rs1001179 and the T allele rs7943316 polymorphism are risky alleles for β-thalassemia (O 5.52, P= <0.0001; OR = 1.97, P= 0.041), respectively. There is association between catalase gene polymorphism and risk of thalassemia occurrence in Iraqi population.

INTRODUCTION

Thalassemia is autosomal recessive and affects men and women equally in terms of incidence. It is a class of congenital hemolytic diseases and one of the most prevalent conditions caused by a single gene globally. Chronic hemolytic anemia is caused by aberrant hemoglobin (Hb) production, which is caused by globin gene mutations or deletions. Its core is marrow hematopoiesis that isn't correct, particularly when it comes to in situ hemolysis. However, severe acute infections can also result in acute hemolysis (1).

Beta-thalassemia (β-Thalassemia) is a heterogeneous disorder caused by abnormal hemoglobin synthesis, where the synthesis of normal hemoglobin protein (β-chain) is decreased or completely absent. Patients with β-thalassemia major typically experience iron overload due to frequent transfusions and poor erythropoiesis, which stimulates the generation of free radicals that overwhelm the body's ability to defend against oxidative stress, causing damage to cells and organ (2).

Beta-Thalassemia carriers account for approximately 3 % of the global population (3). β-thalassemia is a major public health concern in Iraq, including the Kurdistan Region with a carrier rate of 4.1% in Sulaymaniyah (northeastern Iraq) (4), and an estimated prevalence rate of 37.1/100,000 in Iraq (5).

Antioxidants are produced by the body under normal circumstances to compensate for the damaging effects of ROS. Oxidative stress, however, can happen when there is an excess of ROS generation or an impairment in the antioxidant defense system (6,7).

Changes in antioxidant activity are linked to many allele variants in antioxidant genes. The endogenous antioxidant enzyme catalase (CT) is essential for regulating the levels of hydrogen peroxide in humans. By breaking down H2O2 into H2O and O2, it.

protects this cell from oxidative stress (8,21)**.**

The CT gene's promoter, 5' and 3' untranslated regions, exons, and introns have all been found to include a variety of polymorphisms. The NCBI database shows that 245 single-nucleotide polymorphisms in various CT gene regions have been documented as of 2012 (9).

MATERIALS AND METHODS

During the period from October 2023 to January 2024, 105 blood samples were collected from patients visited the Thalassemia Center in Al-Diwaniyah, Iraq. The samples were divided to study group (60 thalassemia patients on iron therapy) and control group (45 healthy individuals). A 5ml venous blood were collected from all participants as follow: 3ml in gel tubes to obtain serum samples for measurement the concentration of CAT by used ELISA Kits (BT Lab, China). The Preparation of standard, and 2 ml in EDTA tubes for conducting molecular tests.

Estimation of antioxidant standards: Serum from the collected whole blood was separated by centrifugation at 5000 rpm for 3 minutes. All reagent was let in room temperature (25℃) for 30 minutes before use. 500 mL of wash buffer were prepared by diluting 20 ml of washing buffer with 480 mL of distilled water.

Preparation of catalase standard solutions: Standards of CAT were prepared according to company instructions (BT Lab, China).

Genomic DNA extraction from whole blood according to the instructions of the manufacturer. (**Bosphore® Genomic DNA Extraction Spin Kit**). The concentrations and purity of extracted DNA measured by Nano-drop spectrophotometers.

Single Nucleotide Polymorphism (SNP) Genotyping of Catalase gene: Two SNPs were genotyped in this study. The SNPs of accession numbers *rs*1001179 and *rs*7943316, respectively were genotyped in all study participants using allele specific PCR technique. Next, employing the primer-blast online program, the allele-specific primers were designed manually and computationally verified (National Center for Biotechnology and Information). The allele-specific primer sequences were shown in Table 1.

Table 1. The allele-specific primer sequences of catalase *rs*1001179 SNP.

Primers	Sequence (5'-3')	Product size
Rs1001179- allele C	GCCCTGGGTTCGGCTATC	333bp
Rs1001179- allele G	GCCCTGGGTTCGGCTATG	333bp
Rs1001179- allele T	GCCCTGGGTTCGGCTATT	333bp
Rs1001179-F	CAACACCCATCTGTACGGAGA	686bp
Common Reverse primer	TGTGCAGAACACTGCAGGA	333bp

Amplification Refractory Mutation System (ARMS-PCR) primers for Catalase gene polymorphisms (CT-89 (A > T) _*rs*7943316) were designed in current study utilizing NCBI-SNP data base and Primerl ARMS-PCR primers designed online. These primers were supplied from (Scientific Researcher. Co. Ltd. Iraq) Table 2. According to company instructions (**GoTaq® G2 Green Master**

Mix kit), ARMS-PCR was performed. The amplicons of T-ARMS-PCR were subjected to gel electrophoresis on an agarose gel in TBE buffer (**Bioline CO., USA**).

Table 2. The Tetra-ARMS-PCR Primers for gene CT-89 (A > T) _*rs*7943316 polymorphism.

Primers	Sequence (5'-3')	Product size	Annealing temperature
Forward inner primer (A allele)	GATTGGCTGAGCCTGAAGTCGCCACCGA	205bp	68C^0
Reverse inner primer (T allele)	CAGGCAAATCTGCCTGTTGCCCCGTGA	185bp	68C^0
Forward outer primer	TCTCCGGTCTTCAGGCCTCCTTCGGAGAGC	335bp	68C^0
Reverse outer primer	GCTCGGGGAGCACAGAGTGTACCTGCGC	335bp	68C^0

Statistical analysis

Descriptive statistics were performed using the Statistical Package for Social Sciences (SPSS), version 23. The chi-square test was used to calculate the genotypes and allelic frequencies of the CAT (rs7943316) SNP in β-thalassemia patients and healthy individual controls.

To learn more about the relationship between the CAT (*rs*1001179 and *rs*7943316) SNPs and β-thalassemia, odds ratios (OR) and matching 95% confidence intervals (CI) were computed.

Ethical approval

The study was carried out following the ethical principles that have their origin in the Declaration of Helsinki. Before sampling, the approval of the patient or his companion was taken. The study protocol and the subject data and the approval form were reviewed and approved by Center for Genetic Blood Diseases/ Thalassemia in Al-Diwaniyah according to document number (2476) on (20/12/2023) to get this approval.

RESULTS

There was a highly significant (P<0.0001) in the mean values according to patient's gender. Male and female patients differ statistically significantly, with the former having a higher mean value. There was a statistically significant difference in the mean values according to patient's weight.

The concentration of CAT in serum of healthy control and β-thalassemia patients groups. There is a significant increase in catalase levels in patients in comparison with control groups (*P*<0.005) Figure 1.

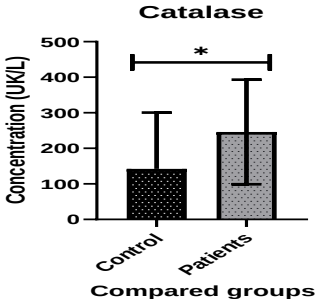

Figure 1. The concentration of CAT in serum of healthy control and thalassemia patient's groups. This shows a significant increase of CAT in Patient in comparison with control (*P*<0.005)

The distribution of CAT (*rs*1001179) and (*rs*7943316) polymorphism was detected by Tetra ARMS-PCR technique.

The CAT (*rs*1001179) genotyping including three alleles (C, G, and T) that were detected at 686bp and 333bp PCR product, respectively (Figure 2 A, B, and C).

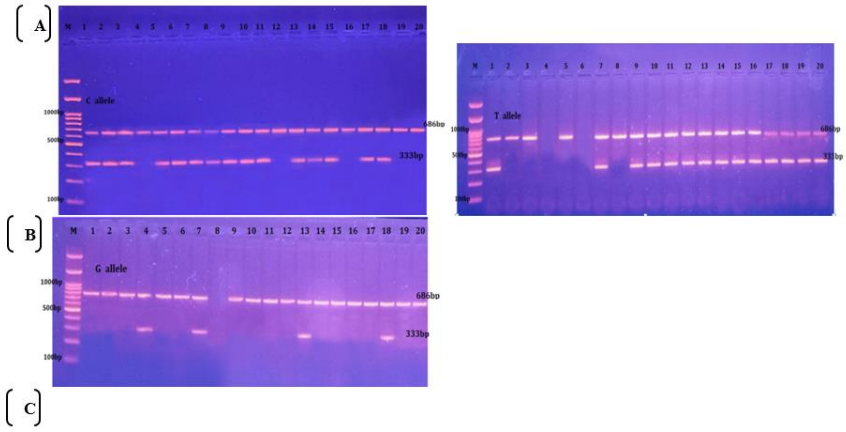

Figure 2. Agarose gel electrophoresis image that showed the Tetra-ARMS-PCR product analysis of CAT (*rs*1001179) polymorphism in β-thalassemia patients. M: DNA marker; A: Lane (1,2,3,5,6,7,8,9,10,11,13,14,15,17, and 18) C allele (686bp, 333bp); B: Lane (4,7,13, and 18) G allele (686bp, 333bp); C: Lane (1,7,9,10,11,12,13,14,15,16,17,18,19, and 20) T allele (686bp, 333bp).

The 205 bp PCR products for the CAT (*rs*7943316) genotyping included lane (AA) homozygote and solely displayed the A allele. While the (AT) heterozygote displayed both the A and T allele at 205bp and 185bp T-ARMS-PCR product as well as the outside internal control at 335bp PCR product, the lane (TT) homozygote only displayed the T allele at 185bp PCR product Figure 3 and 4.

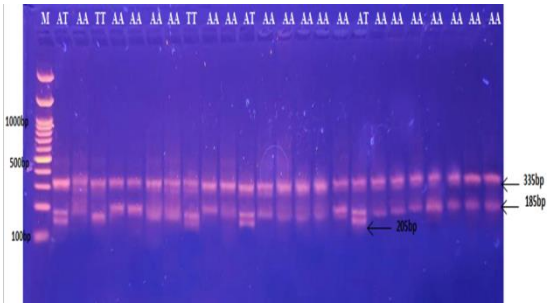

Figure 3. Agarose gel electrophoresis image that showed the Tetra-ARMS-PCR product analysis of CAT (*rs*7943316) gene polymorphism in β-thalassemia patients. M: DNA marker; Lane 3 and 8: homozygous TT genotype (335bp, 185bp) ; Lane 1,11, and 17: heterozygous AT genotype (335bp, 205bp, 185bp); Lane 2,4,5,6,7,9, 10 ,12,13,14,15,16,18,19,20,21,22, 23, and 24 : homozygous AA genotype (335bp, 205bp).

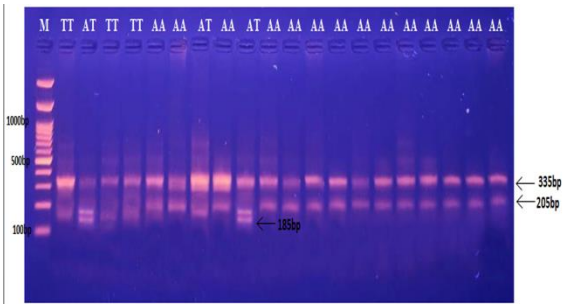

Figure 4. Agarose gel electrophoresis image that showed the Tetra-ARMS-PCR product analysis of CAT (*rs*7943316) A/G gene polymorphism in healthy control. M: DNA marker; Lane 1,3, and 4: homozygous TT genotype (335bp, 185bp); Lane 2,7, and 9: heterozygous AT genotype (335bp, 205bp, 185bp); Lane 5,6,8, 10,11,12,13,14,15,16,17,18,19, and 20: homozygous AA genotype (335bp, 205bp).

The genotype frequencies and allele distribution of the CAT (*rs*1001179) SNPs C/T polymorphism in the control and β-thalassemia patients groups are illustrated by the results shown in Table 3.

Table 3. Distribution of genotypes between CAT (*rs*1001179 control and β- thalassemia patient groups and their association with β-thalassemia.

Genotype	Groups		X2	P value	OR	CI
	Control N= 45	Patients N= 60				
CC	9(20%)	8(13.33%)	0.842	0.359(NS)	0.61	0.2169 1.7461
CG	2(4.44%)	4(6.66%)	0.236	0.627(NS)	1.53	0.2686 8.7785
GG	4 (8.88%)	20(33.33%)	8.71	0.003**	5.12	1.6990 16.3246
CT	28(62.22%)	16(26.66%)	13.35	<0.0001**	0.22	0.0962 0.5068
TT	2(4.44%)	7(11.66%)	1.71	0.191(NS)	2.83	0.5607 14.3800
TG	0(0)	5(8.33%)	3.93	0.047*	9.01	0.4856 167.46
Allele Frequency						
G	10(11.11%)	49(40.83%)	22.48	<0.0001**	5.52	2.6042 11.7053
C	48(53.33%)	36(30%)	11.66	0.001**	0.37	0.2123 0.6625
T	32(35.35%)	35(29.16%)	0.966	0.326(NS)	0.74	0.4161 1.3385

* (P≤0.05), ** (P≤0.01), NS: Non-Significant.

The genotype frequencies and allele distribution of the CAT (*rs*7943316) SNPs A/T polymorphism in the control and thalassemia patient groups are illustrated by the results shown in Table 4.

DISCUSSION

Various studies have connected oxidative stress to a variety of medical conditions. Furthermore, oxidative stress has been linked to the emergence of tissue damage and inflammation, highlighting its widespread effects on human health (10).

Table 4. Distribution of CT-89 (A > T) _rs7943316 genotypes and allele frequency between control and thalassemia patient groups and their association with thalassemia.

Genotype	Observed		Expected		χ^2	P-value	OR	C.I.
	Control N=45 (%)	Patients N=60 (%)	Control N=45 (%	Patients N=60 (%)				
AA	34(75.55)	38(63.33)	31 (68.89	31.10 (51.84)	1.78(NS)	0.182	0.55	0.236 1.319
AT	7(15.55)	10(16.66)	12.70 (28.22)	24.19 (40.32)	0.023(NS)	0.878	1.08	0.378 3.115
TT	4(8.88)	12(20)	1.30 (2.89	4.70 (7.84	2.45(NS)	0.117	2.56	0.767 8.558
Total	45	60						
Allele Frequency								
A	75(83.33)	86(71.66)	--		3.91*	0.041	0.50	0.225 1.000
T	15(16.66)	34(28.33)	--		3.91*	0.041	1.97	0.999 3.909

* (P≤0.05), NS: Non-Significant.

A key antioxidant enzyme in maintaining cellular homeostasis, catalase aids in the breakdown of hydrogen peroxide, a reactive oxygen species that, in excess, can result in oxidative stress. This study

observes a significant increase in catalase levels in patients in comparison with control groups (P<0.005) Figure 1. Despite oxidative stress is not the main cause of thalassemia, it is a mediator of its pathologies. The primary factors contributing to oxidative stress in thalassemia are iron overload and the breakdown of unstable hemoglobin, both of which increase the generation of excess free radicals (11). The body's defense mechanisms cause an increase in catalase production in response to elevated hydrogen peroxide levels. Catalase then breaks down hydrogen peroxide into water and oxygen, thereby decreasing oxidative stress and minimizing cellular damage (12,13). Catalase activity can be affected by a number of factors, such as genetic mutations that change the enzyme's structure or function or environmental variables that affect the enzyme's expression or activity. Toxins, medications, and radiation exposure, for example, can reduce catalase activity and exacerbate oxidative stress (14).

Single nucleotide polymorphism (SNP) combinations within or across different genes may contribute to the development of illness. SNPs influence the phenotypic expression of a gene that has been found, hence raising a person's risk of contracting a certain disease. Consequently, research on genetic associations has started investigating at how SNPs affect the outcome of disease (15). SNPs in the genes encoding antioxidative enzymes, like catalase, may be in charge of changing the activity, substrate selectivity, or structure of the enzymes, which in turn may change the enzyme's ability to fend off oxidative stress.

According to the NCBI database, 245 catalase single-nucleotide polymorphisms were found and characterized in various CAT gene regions (9,22). Many studies were documented significant correlations of the catalase polymorphisms and various diseases including C-262T -*rs*1001179 and A-89T -*rs*7943316 (16).

Regarding the CAT (*rs*7943316) SNP, the current study found that the T allele (OR =1.97, P= 0.041) was significantly increase (OR= 2.56, P= 0.041) in β-thalassemia and not significant increase of TT genotype (OR = 2.56, P= 0.117) Table 2. Tripathi *et al* (8) found that T allele (OR= 2.78) have higher frequency in CT-89 (A > T) polymorphisms in thalassemia major patients than healthy controls and the AT genotype was the higher frequency than TT genotype in β-thalassemia. The rs794316 polymorphism (A-15 T) has been detected in the promoter region close to the CAT starting site, and the host's response to oxidative stress is probably influenced by the endogenous variability of this promoter. (17,24).

According to an Iraqi study's findings, the T-ARMS-PCR assay demonstrated a strong correlation between breast cancer and the A/T allele of the CAT gene (rs7943316). To be more precise, people with the TT genotype were more likely to get breast cancer than people with the AA genotype (18).

Regarding the CAT (*rs*1001179) SNP, this study indicated a correlation of the *rs*1001179 polymorphism with β-thalassemia represented by TG (OR=9.01, P= 0.047) and GG (OR= 5.12, P= 0.003) Table 1. Galasso *et al*. (19) discovered that since the CT/TT genotypes were associated with higher levels of CAT and decreased levels of methylation, it is possible that the rs1001179 T allele and CpG methylation interact to regulate CAT expression in chronic lymphocytic leukemia.

A different study found that people with the TT genotype had a higher chance of developing pathospermia (20,23). In the promoter region of CAT, the rs1001179 polymorphism (C-262T) affects transcription factor binding, modifies basal transcription, and ultimately changes the expression of the encoded enzyme (17).

CONCLUSION

There is an association between catalase gene (*rs*1001179 and *rs*7943316) polymorphism and risk of β-thalassemia occurrence in Iraqi population. This research is the first study at the CAT C-262T (*rs*1001179) SNP in thalassemia patients with triallelic SNP at the position-262 in the catalase gene. Tetra-ARMS-PCR was a quick, cost-efficient, and high-throughput approach to SNPs detection.

References

1- Chen, D., Shen, X., Wu, C., Xu, Y., Ding, C., Zhang, G., ... & Zhou, C. (2020). Eleven healthy live births: a result of simultaneous preimplantation genetic testing of α-and β-double thalassemia and aneuploidy screening. *Journal of assisted reproduction and genetics*, *37*, 549-557.

2- Bazvand, F., Shams, S., Esfahani, M. B., Koochakzadeh, L., Monajemzadeh, M., Ashtiani, M. T. H., & Rezaei, N. (2011). Total antioxidant status in patients with major β-thalassemia. *Iranian journal of pediatrics*, 21(2), 159.

3- Jaing, T. H., Chang, T. Y., Chen, S. H., Lin, C. W., Wen, Y. C., & Chiu, C. C. (2021). Molecular genetics of β-thalassemia: *A narrative review*. Medicine, 100(45), e27522.

4- Al-Allawi, N. A., Faraj, A. H., & Ahmed, N. H. (2008). Prevalence of haemoglobinopathies in Sulaimani-Iraq. *Duhok Med J,* 2, 71-9.

5- Kadhim, K. A., Baldawi, K. H., & Lami, F. H. (2017). Prevalence, incidence, trend, and complications of thalassemia in Iraq.

6- Eddaikra, A., & Eddaikra, N. (2021). Endogenous enzymatic antioxidant defense and pathologies. In Antioxidants-benefits, sources, mechanisms of action. *IntechOpen*.

7- Habtemariam, S. (2019). Antioxidant and anti-inflammatory mechanisms of neuroprotection by ursolic acid: Addressing brain injury, cerebral ischemia, cognition deficit, anxiety, and depression. *Oxidative medicine and cellular longevity*, *2019*(1), 8512048.

8- Tripathi, P., Agarwal, S., Tewari, S., & Mandal, K. (2022). Status of catalase, glutathione peroxidase, glutathione S-transferase, and myeloperoxidase gene polymorphisms in beta-thalassemia major patients to assess oxidative injury and its association with enzyme activities. Journal of Pediatric Genetics, 11(03), 198-212.

9- Crawford, A., Fassett, R. G., Geraghty, D. P., Kunde, D. A., Ball, M. J., Robertson, I. K., & Coombes, J. S. (2012). Relationships between single nucleotide polymorphisms of antioxidant enzymes and disease. *Gene*, *501*(2), 89-103.

10- Chatterjee, S. (2016). Oxidative stress, inflammation, and disease. In Oxidative stress and biomaterials (pp. 35-58). *Academic Press*.

11- Fibach, E., & Dana, M. (2019). Oxidative stress in β-thalassemia. Molecular diagnosis & therapy, 23, 245-261.

12- Alfonso-Prieto, M., Biarnés, X., Vidossich, P., & Rovira, C. (2009). The molecular mechanism of the catalase reaction. *Journal of the American Chemical Society,* 131(33), 11751-11761.

13- Zucca, P., Rescigno, A., Rinaldi, A. C., & Sanjust, E. (2014). Biomimetic metalloporphines and metalloporphyrins as potential tools for delignification: Molecular mechanisms and application perspectives. *Journal of Molecular Catalysis A: Chemical*, *388*, 2-34.

14- Xiao, X., Luo, H., Vanek, K. N., LaRue, A. C., Schulte, B. A., & Wang, G. Y. (2015). Catalase inhibits ionizing radiation-induced apoptosis in hematopoietic stem and progenitor cells. *Stem Cells and Development*, *24*(11), 1342-1351.

15- Chanock, S. (2003, October). Genetic variation and hematology: single-nucleotide polymorphisms, haplotypes, and complex disease. In *Seminars in hematology* (Vol. 40, No. 4, pp. 321-328). WB Saunders.

16- Kodydková, J., Vávrová, L., Kocík, M., & Zak, A. (2014). Human catalase, its polymorphisms, regulation and changes of its activity in different diseases. *Folia biologica*, *60*(4), 153.

17- Liu, K., Liu, X., Wang, M., Wang, X., Kang, H., Lin, S., ... & Dai, Z. (2016). Two common functional catalase gene polymorphisms (rs1001179 and rs794316) and cancer susceptibility: evidence from 14,942 cancer cases and 43,285 controls. *Oncotarget*, *7*(39), 62954.

18- Hoidy, W. H., Nubgan, A., & Al-Saadi, M. H. (2023). Development of a T-ARMS-PCR Assay for Detecting Genetic Polymorphism in the Catalase (rs7943316) Gene in the Iraqi Population with Breast Cancer. *Asian Pacific Journal of Cancer Prevention: APJCP*, *24*(9), 3283.

19- Galasso, M., Dalla Pozza, E., Chignola, R., Gambino, S., Cavallini, C., Quaglia, F. M., ... & Scupoli, M. T. (2022). The rs1001179 SNP and CpG methylation regulate catalase expression in chronic lymphocytic leukemia. *Cellular and Molecular Life Sciences*, *79*(10), 521.

20- Savikina, K. G., Abd Ali, A. H., Shkurat, T. P., Lomteva, S. V., & Karantysh, G. V. (2021). Association of CAT C262T (rs1001179) polymorphism with male infertility: Meta-analysis. *Meta Gene*, *30*, 100974

21- Kanval, N., Ihsan, H., Irum, S., & Ambreen, I. (2024). Human Capital Formation, Foreign Direct Investment Inflows, and Economic Growth: A Way Forward to Achieve Sustainable Development. Journal of Management Practices, Humanities and Social Sciences, 8(3), 48-61.

22- Rashid, A., Jehan, Z., & Kanval, N. (2023). External Shocks, Stock Market Volatility, and Macroeconomic Performance: An Empirical Evidence from Pakistan. Journal of Economic Cooperation & Development, 44(2), 1-26.

23- Jam, F.A., Khan, T.I., Zaidi, B., & Muzaffar, S.M. (2011). Political Skills Moderates the Relationship between Perception of Organizational Politics and Job Outcomes.

24- Jam, F., Donia, M., Raja, U., & Ling, C. (2017). A time-lagged study on the moderating role of overall satisfaction in perceived politics: Job outcomes relationships. Journal of Management & Organization, 23(3), 321-336. doi:10.1017/jmo.2016.13