

**Polymorphism of Catalase Gene and Serum Antioxidant Level in Iraqi Women Suffering from Spontaneous Abortion**

Auras A. Hatem* and Hamdiyah A. Ali

Department of Gynecology, Collage of Medicine, University of Kufa, Kufa, Iraq

ARTICLE INFO*Article history:*

Received 06 November 2020

Revised 28 April 2021

Accepted 07 July 2021

Published online 02 August 2021

Copyright: © 2021 Hatem and Ali. This is an open-access article distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

ABSTRACT

Cupressus sempervirens (*Cupressaceae*) “cypress” is a specie native to the Mediterranean. Spontaneous abortion is a pregnancy failure that happens spontaneously before 20 weeks of gestation. Catalase (CAT) has been implicated to play a vital role in the protection of early pregnancy. The current study was aimed at investigating the polymorphism of catalase gene in relation to serum antioxidant level in Iraqi women suffering from spontaneous abortion. Fifty women with spontaneous abortion (study group) and fifty stable pregnant women (control group) were recruited for this study. Blood sample was collected from each subject for analyses. Vitamin D was measured by AFIAS-6 Compact Desktop Immuno-analyzer, while catalase activity was evaluated from serum by Colorimetric analysis. Also, the amount of Malondialdehyde (MDA) was determined. DNA was isolated from the blood samples and used for detecting polymorphism by RT-PCR. The results revealed that vitamin D and catalase activity were highly significant ($p < 0.0001$) in the control than patient group. Conversely, the level of MDA was significantly ($p < 0.0001$) higher in the patient group than control. The polymorphism assay indicated that CC genotype showed a significantly ($p = 0.003$) higher frequency in the patients (38%) than control (10%), while the TT genotype was higher in the control than in the patients group. Also, the CT genotype showed higher frequency in the control than patients group, but the difference was insignificant.

Keywords: Spontaneous abortion, Malondialdehyde, Catalase activity, Vitamin D, RT-PCR

Introduction

Spontaneous abortion is the termination of pregnancy prior to 20 weeks from conception or if the weight of the fetus was less than 500 g.¹ There are several causes of abortion, so it is difficult to ascertain the exact process. In spite of the several causes, there is now a compelling evidence that abortion is linked with placental oxidative stress. Abnormal placentation can contribute to placental oxidative stress, which can cause severe bleeding resulting in disastrous impacts on syncytiotrophoblast.² The human body has several antioxidant systems to stop the development of excessive reactive oxygen species (ROS). When the body has an extreme amount of ROS, it results in oxidative stress which is a cause of early pregnancy loss.³ Catalase (CAT) is an enzymatic antioxidant capable of eliminating hydrogen peroxide (H_2O_2), thereby preventing peroxidation of lipids in cell membrane and acting as free radical scavengers.⁴ The role of CAT is quite necessary during pregnancy. CAT plays a role in implantation in early pregnancy by shielding blastocysts from superoxide radicals in the endometrium.⁵ Vitamin D is essential for natural reproductive health, and vitamin D receptor (VDR) has been found in the ovaries, uterus, placenta, hypothalamus, and pituitary gland.⁶ Low level of vitamin D during pregnancy has been connected with preeclampsia gestational diabetes, bacterial vaginosis, and impaired intrauterine growth.^{7,8}

In humans, the CAT gene is encoded by the nuclear chromosome

11p13. The polymorphism (C-262T) of this gene is located on the promoter region and influences transcription factors-binding, altering the basal transcription and consequent expression of this enzyme.⁹ The aim of the current study was to investigate the polymorphism of catalase gene (cat c-262t) in relation to serum antioxidant level in Iraqi women suffering from spontaneous abortion.

Materials and Methods*Study population, sample collection and analyses*

This study was conducted in Baghdad Teaching Hospital, Baghdad, Iraq from September 2019 to March 2020. Ethical approval was obtained from the Baghdad Teaching Hospital ethics committee. One hundred women were involved in this study, of which 50 of them with spontaneous abortion were regarded as the study group, while 50 stable pregnant women were the control group. An aliquot of 10 mL of blood sample was collected from each subject under aseptic condition and used for the measurement of IgM and IgG antibodies by Enzyme linked Immunosorbent Assay (ELISA) technique (MyBioSource, cat# MBS2509204, MBS2511279). Vitamin D was measured by AFIAS-6 Compact Desktop Immuno-analyzer. Also, catalase activity was determined from serum samples by Colorimetric method using kit (Abcam, USA, Cat No. ab83464), while MDA was evaluated using the method reported by Nsaif.¹⁰

DNA extraction and genotyping analysis

An aliquot of 2 mL of blood were transferred into anticoagulant EDTA container. DNA was extracted from the blood samples (that have been collected in the EDTA tubes) by using Zymo Quick-DNA Microprep Kit (CAT; D3020). Genotypes of the catalase gene were determined by Real Time-PCR for the SNP genotyping assay by employing the kit from Thermo Fisher (CAT# C_11468118_10). The reaction was set up by adding 0.5 μ L of the SNP assay kit, 10 μ L of the probe master mix, 3 μ L of the eluted DNA and finally the reaction volume was made up to 20 μ L by nuclease free water. The samples

*Corresponding author. E mail: aurasadihatem@gmail.com

Tel: +964 7830974696

Citation: Hatem AA and Ali HA. Polymorphism of Catalase Gene and Serum Antioxidant Level in Iraqi Women Suffering from Spontaneous Abortion. Trop J Nat Prod Res. 2021; 5(7):1192-1195. doi.org/10.26538/tjnpr/v5i7.4

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

were placed in a thermo cycler machine. And the amplification program was set as follow; 95°C for 5 min, then 40 cycles of 5°C for 20 secs and 60°C for 20 min.

Statistical Analysis

Data were expressed as mean \pm standard error (SE). Statistical analyses were done by utilizing the SPSS program (version 23) and Excel application program. $P < 0.05$ was considered to have statistically significant difference. The variations in genetic distributions among the study and control groups were calculated by chi-square (χ^2) test. More so, odd ratio (OR) and 95% confidence interval (95% CI) were determined for each genotype.

Results and Discussion

As presented in Figure 1, there was a high significant difference ($p < 0.0001$) in the serum level of MDA in the spontaneous abortion group with a value of $9.60 \pm 0.018 \mu\text{mol/L}$, compared to the control with a value of $4.17 \pm 0.22 \mu\text{mol/L}$. There was a high significant difference ($P \leq 0.0001$) in the amount of CAT in the control ($0.1290 \pm 0.0043 \text{ nmol/mL}$) compared with the patients with spontaneous abortion ($0.0055 \pm 0.0022 \text{ nmol/mL}$) as shown in Figure 2. Meanwhile, there was a high significant difference ($P \leq 0.0001$) in the level of value of vitamin D ($25.36 \pm 1.38 \text{ ng/mL}$) in the control group in relation to the group of patients with spontaneous abortion, which had vitamin D concentration of $11.15 \pm 0.66 \text{ ng/mL}$ (Figure 3). The curve obtained for the Real Time-PCR is presented in Figure 4 and each curve of the amplification of the SNP region represented an allele type. The results in Table 1 summarize the allele and genotype frequencies of the study and control groups. The CC genotype showed a significant ($p = 0.003$) higher frequency in the patients with spontaneous abortion (38%) compared to the control which showed only 10% frequency and 3.6 odd ratio. Meanwhile, the TT genotype was higher in the control than in patients group. More so, the CT genotype showed a higher frequency in the control compared with the patients with spontaneous abortion, but the relationship was insignificant. In addition, the allele frequency showed a significantly higher frequency of C allele in the patients in relation to the control with high odd ratio (2.597), while the T allele showed lower frequency in the patients compared with the control and low protective ratio (0.698).

In this study, the mean MDA level in patients with spontaneous abortion was observed to be higher than the control. This observation is in agreement with the results obtained in studies performed by Abdul-Barry,¹¹ as well as Ozkaya,¹² where the serum MDA levels of patients with a history of repeated spontaneous abortion were significantly higher than in women with a stable pregnancy. Since MDA is considered as a by-product of lipid peroxidation, its excess may indicate an overproduction of lipid peroxide and may overwhelm the antioxidant defense mechanism. Jauniaux and his workers proposed a hypothesis that free radicals in abortion was higher than in natural pregnancy,¹³ in their research, it was discovered that during abortion, the intervillary O_2 flow is generated much earlier which led to an excessive development of free radicals that could induce abortion.¹³ Sugino's team found that total CAT activity is decreased while there was an increased prostaglandin synthesis in cases of spontaneous abortion.¹⁴ They showed that pregnancy termination could be caused by decreased CAT activity, which promotes the production of prostaglandins. This finding was consistent with the study by Yigenoglu *et al.*,¹⁵ which found that overall antioxidant potential was lower in pregnant female with a spontaneous abortion history than in stable pregnant women. Meanwhile, the research conducted by previous study¹⁶ indicated that CAT activity was higher in patients than in the control group, an observation which is not in agreement with the current study. CAT is important for fetal growth and pregnant women protection. In pregnancy, CAT levels were found to increase and low plasma or placenta CAT activity was observed in cases of spontaneous abortion. Also, other study suggested that low levels of antioxidants increased the risk of miscarriages,¹⁷ a finding consistent with the observation in the current study. The results obtained in the current study revealed that most patients with spontaneous abortion had vitamin deficiency.

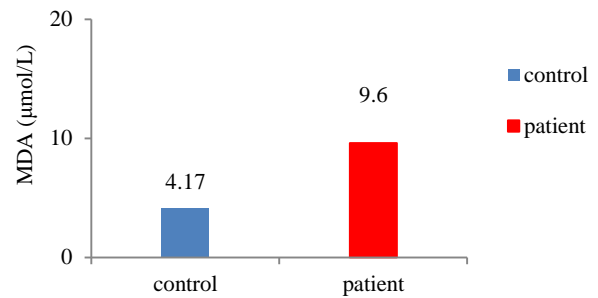


Figure 1: Levels of MDA of spontaneous abortion and control groups

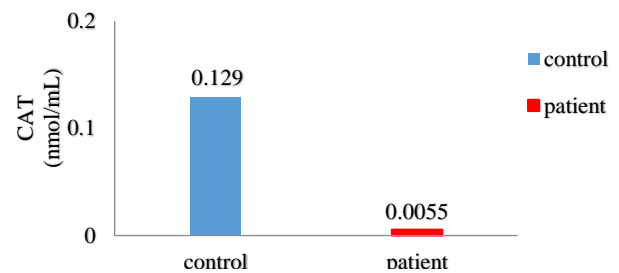


Figure 2: Levels of CAT activity of spontaneous abortion and control groups

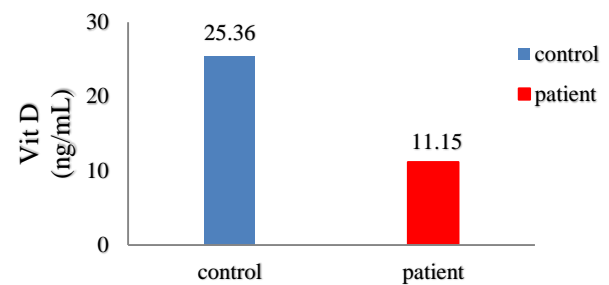


Figure 3: Levels of vitamin D of spontaneous abortion and control groups

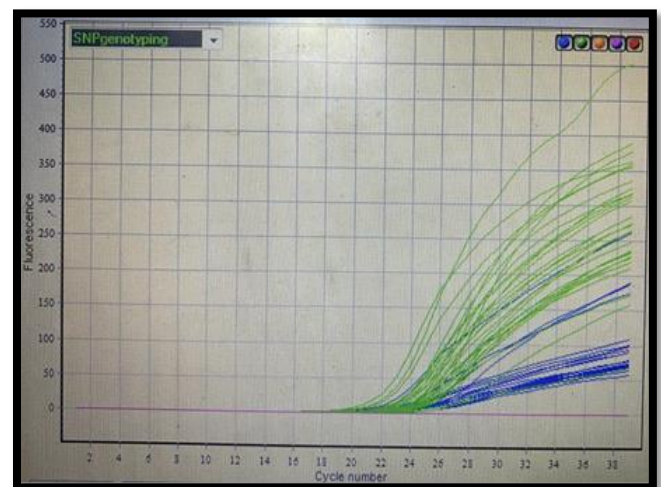


Figure 4: RT-PCR curves for the determination of catalase gene polymorphism

Table 1: Genotype and allele frequencies among patients and control groups

CI95%	P-value	O.R.	Control		Patients		Genotype
0.441-1.0441	0.108	0.67	56%	28	38%	19	TT
0.288- 1.613	0.513	0.764	34%	17	26%	13	CT
1.702- 15.05	0.003	3.6	10%	5	36%	18	CC
0.622- 1.889	0.002	0.698	73%	73	51%	51	T
1.439- 4.668	0.002	2.597	27%	27	49%	49	C

This observation supports the analysis carried out by Hantoosh which was in accordance with previous studies conducted among female of reproductive age groups (76%),¹⁸ and Al-Hilali (65%),¹⁹ and the finding was supported by studies in Iran (26.1%),²⁰ and (33%).²¹ Those with deficiency in 25-hydroxyvitamin D were mainly miscarriage 96 (52.7%) and was lower than normal pregnancy 86 (47.3%); 25-hydroxyvitamin D was lower in those with miscarriage (11.3 ± 5.3 ng/mL) than in normal pregnancy (15.9 ± 11.2 ng/mL). These observations are in agreement with a study in Iraq which found that 60% from those with recurrent pregnancy loss had vitamin D deficiencies, with an average of 21.5 ± 11.88 ng/mL.²² It was found in Iran that 33% of people with recurrent pregnancy loss had vitamin D deficiency.²¹ In a prospective cohort study by Mumford *et al.*, adequate preconception vitamin D serum levels was found to be associated with lower rates of pregnancy loss.²³

There are two well-known antioxidant enzymes; glutathione peroxidase 1 (GPX1) and catalase (CAT). Both of these enzymes can protect the cells from the ROS by controlling hydrogen peroxide concentrations through its conversion to water and oxygen.²⁴ CAT encodes H₂O₂, detoxifying antioxidant enzymes and protects cells from oxidative damage. Various studies have shown a functional polymorphic site at position-262 (C-262 T) in the CAT promoter area that could influence blood expression and enzyme levels, thereby contributing to certain pathological conditions.²⁵

An allele substitution in a gene that encodes antioxidant enzyme may lead to functional changes, so in this study, one of CAT most relevant polymorphism for determining its function in spontaneous abortion was evaluated. The results showed a strong relationship between the presence of C allele and CC genotype and the occurrence of abortion. A previous study done by Forsberg *et al.*, in which they found that the transcriptional activity of the T allele was substantially greater than that of the C variant, and due to this reason higher catalase levels showed in the genotype T/T.²⁶ As there is a greater defense against H₂O₂ accumulation in high catalase expression cells, the variability related to this polymorphism can play a role in responding to oxidative stress.²⁷

Conclusion

This study showed that vitamin D and catalase activity were significantly higher in the control than in patients with spontaneous abortion. Conversely, MDA was significantly ($p < 0.0001$) higher in the patients than control. The results from the RT-PCR revealed that the genotype of CC showed a significantly ($p = 0.003$) higher frequency in the patients (38%) compared to the control with only 10%, while the genotype of TT was higher in the control than in patients. Conclusively, the genotype CT also showed a higher frequency in the control than in patients, but the relationship was negligible.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgements

The authors express their profound gratitude to all members of Baghdad Teaching Hospital and the Department of Gynecology, College of Medicine, University of Kufaa who contributed to the success of this study.

References

- Dongol A, Mool S, Tiwari P. Outcome of pregnancy complicated by threatened abortion. Kathmandu Univ Med J (KUMJ). 2011; 9(33):41-44.
- Zhou J, Huang Z, Pan X, Leung WT, Li C, Chen L, Zhang Y, Wang L, Sima Y, Zhang N, Qiu X, Li L, Wang L. New thoughts in exploring the pathogenesis, diagnosis, and treatment of threatened abortion. Biosci Trends. 2019; 13(3):284-285.
- Saalu LC. The incriminating role of reactive oxygen species in idiopathic male infertility: an evidence based evaluation. Pak J Biol Sci. 2010; 13(9):413-422.
- Kohen R and Nyska A. Oxidation of biological systems: oxidative stress phenomena, antioxidants, redox reactions, and methods for their quantification. Toxicol Pathol. 2002; 30(6):620-650.
- Yusrawati A, Pebrina M, Herman RB. Differences in malondialdehyde and catalase activity levels between abortion and normal pregnancy. J Med Sci. 2017; 17:22-25.
- Natalene S, Stacey C, Henri C. Support following miscarriage: What women want. J. Reprod Infant Psychol. 2010; 28(4): 403-411.
- Lerchbaum E and Obermayer-Pietsch B. Vitamin D and fertility: a systematic review. Eur J Endocrinol. 2012; 166(5):765-778.
- Cho GJ, Hong SC, Oh MJ, Kim HJ. Vitamin D deficiency in gestational diabetes mellitus and the role of the placenta. Am J Obstet Gynecol. 2013; 209(6):560.e1-8.
- Shen Y, Li D, Tian P, Shen K, Zhu J, Feng M, Wan C, Yang T, Chen L, Wen F. The catalase C-262T gene polymorphism and cancer risk: a systematic review and meta-analysis. Med. 2015; 94(13):e679.
- Ghufran SN, Amer HA, Najwa SA, Wafaa RA. Evaluation of estradiol and some antioxidant in breast cancer Iraqi women. Al-Nahrain J Sci. 2018; 21(1):35-40.
- Jamal A, Al-Rubai SA, Qasim QA. Study of oxidant-antioxidant status in recurrent spontaneous abortion. Thi-Qar Med J. 2011; 5(1):35-46.
- Okan OM, Mekin S, Hakan K. Serum malondialdehyde, erythrocyte glutathione peroxidase, and erythrocyte superoxide dismutase levels in women with early spontaneous abortions accompanied by vaginal bleeding. Med Sci Monitor. 2008; 14(1):CR47-CR51.

13. Eric J, Lucilla P, Burton GJ. Placental-related diseases of pregnancy: involvement of oxidative stress and implications in human evolution. *Hum Reprod Update*. 2006; 12(6):747-755.
14. Sugino N, Nakata M, Kashida S, Karube A, Takiguchi S, Kato H. Decreased superoxide dismutase expression and increased concentrations of lipid peroxide and prostaglandin F₂ α in the decidua of failed pregnancy. *Mol Hum Reprod*. 2000; 6(7):642-647.
15. Yusrawati A, Pebrina M, Herman RB. Differences in malondialdehyde and catalase activity levels between abortion and normal pregnancy. *J Med Sci*. 2017; 17:22-25.
16. Biri A, Kavutcu M, Bozkurt N, Devrim E, Nurlu N, Durak I. Investigation of free radical scavenging enzyme activities and lipid peroxidation in human placental tissues with miscarriage. *J Soc Gynecol Invest*. 2006; 13(5):384-388.
17. Bilici M. The importance of oxidative stress in early week pregnancy losses. *Crescent J Med Biol Sci*. 2014; 1(4):151-153.
18. Hantoosh HA, Mahdi MH, Imran BW, Yahya AA. Prevalence of vitamin D deficiency in Iraqi female at reproductive age. *Med J Babylon*. 2019; 16(2):119-122.
19. Al-Hilali KA. Prevalence of hypovitaminosis D in adult Iraqi people including postmenopausal women. *Sci Res J*. 2016; 4:53-62.
20. Salek M, Hashemipour M, Aminorroaya A, Gheiratmand A, Kelishadi R, Ardestani PM, Nejadnik H, Amini M, Zolfaghari B. Vitamin D deficiency among pregnant women and their newborns in Isfahan, Iran. *Exp Clin Endocr Diab*. 2008; 116(6):352-356.
21. Neda G, Sedighe F, Jaleh Z, Mehrab S, Azar N, Khadijeh K. Vitamin D deficiency and recurrent pregnancy loss in Iranian women. *Glob Adv Res J Medic Med Sc*. 2016; (5)6:194-198.
22. Alya ARS, Samira SH, Fatehiya MN. Vitamin D3 deficiency and early pregnancy loss. *Middle East J Fam Med*. 2020; 18(1):76-80.
23. Mumford SL, Garbose RA, Kim K, Kissell K, Kuhr DL, Omosigbo UR, Perkins NJ, Galai N, Silver RM, Sjaarda LA, Plowden TC, Schisterman EF. Association of preconception serum 25-hydroxyvitamin D concentrations with livebirth and pregnancy loss: A prospective cohort study. *Lancet Diab Endocrinol*. 2018; 6(9):725-732.
24. Röhrdanz E and Kahl R. Alterations of antioxidant enzyme expression in response to hydrogen peroxide. *Free Rad Bio Med*. 1998; 24(1):27-38.
25. Sabet EE, Salehi Z, Khodayari S, Zarafshan SS, Zahiri Z. Polymorphisms of glutathione peroxidase 1 (GPX1 Pro198Leu) and catalase (CAT C-262T) in women with spontaneous abortion. *Syst Bio Reprod Med*. 2014; 60(5):304-307.
26. Forsberg L, Lyrenäs L, de Faire U, Morgenstern R. A common functional CT substitution polymorphism in the promoter region of the human catalase gene influences transcription factor binding, reporter gene transcription and is correlated to blood catalase levels. *Free Rad Bio Med*. 2001; 30(5):500-505.
27. Seog CM, Geun LS, Mo RH. Transcriptional activation of Cu/Zn superoxide dismutase and catalase genes by panaxadiol ginsenosides extracted from *Panax ginseng*. *Phytother Res*. 1999; 13(8):641-644.