## Catalase Gene Polymorphism (CAT C-262T) and Serum Level in Iraqi Women Suffering from Spontaneous Abortion‏.

**Abstract**

Spontaneous abortion is a pregnancy failure that happens spontaneously before 20 weeks of gestation. Early pregnancy loss applies only to spontaneous abortion in the first quarter. A total of fifty patients with Spontaneous abortion and fifty control group were enrolled in this study, was conducted between September 2019 to march 2020 samples were collected from Baghdad Teaching hospital. The Vitamin D was measured by AFIAS-6 Compact Desktop Immuno-analyzer, as well the catalase activity was measured from serum by ELISA. The results of this study revealed that vitamin D and catalase activity were significantly higher (p<0.0001) in control group than in patients group, while were significantly higher in MDA (µmol/I) levels in patients group than those of control group(p<0.0001). The results by RT- PCR The CC genotype showed a significant (p= 0.003) higher frequency in patients (38%) than the control which showed only (10%)frequency , while the TT genotype were higher in control than in patients, finally the CT genotype showed also higher frequency in control than in patients but the relation was insignificant.

**Key Words:** Spontaneous abortion, Malondialdehyde ,Catalase Activity, Vitamin D ,Real-Time.

**Introduction**

Spontaneous abortion is characterized as the termination of pregnancy prior 20 weeks from pregnancy or if the weight from the fetal is less than 500 g (Dongol *etal.,*2011 ).There are several variables involved with abortion, so it is difficult to ascertain the exact process. Despite several possibilities, there is now compelling proof that abortion is linked with placental oxidative stress. Abnormal placentation can contribute to placental oxidative stress , resulting in disastrous impacts on syncytiotrophoblast (Speroff, *etal.,*2005 ). The body has several antioxidant systems to stop the development of excessive Reactive Oxygen Species ( ROS), and antioxidants stay in balance in a healthy body. When the body has an extreme amount from ROS, oxidative stress (OS) is a cause fromearly pregnancy loss (Agarwal *etal.,*2005). Catalase (CAT) is an enzymatic antioxidant capable of eliminating hydrogen peroxide ( H2O2), preventing lipid peroxidation in the cell membrane and acting as free radical binding(Kohen *etal.,*2002).The role of CAT quite necessary during pregnancy, CAT plays a role in implantation in early pregnancy by shielding blastocysts from superoxide radicals in the endometrium(Yusrawati *etal.,*2017). Vitamin D is essential for normal reproductive health; VDR have been found in the ovaries, uterus, placenta , hypothalamus, and pituitary gland(Sejourne *etal.,*2010). Low vitamin D status through pregnancy has been connected with preeclampsia (Lerchbaum *etal.,*2012) gestational diabetes,( Cho *etal.,*2013)bacterial vaginosis, and impaired intrauterine growth(Bodnar *etal.,*2009).

**Subjects, Materials, and Methods**

The study was conducted in Baghdad Teaching hospital from September 2019 to march 2020. 100 women were involved in this study, 50 women with spontaneous abortion were included as a study group compared to 50 control group . 10 ml of blood was taken under aseptic technique and measurment IgM and IgG antibodies. The Vitamin D was measured by AFIAS-6 Compact Desktop Immuno-analyzer, also the Catalase activity was measured from serum samples by enzyme-linked immunosorbent assay (ELISA) using kit (Abcam, USA, Cat No. ab83464), while were investigated MDA was evaluated by Nsaif (13), 2ml of blood were transferred into anticoagulant EDTA container for estimating . P-value of <0.05 was considered indicative from a statistically significant difference. All the statistical analyses were done by utilizing the SPSS program (version-20) and the Excel application program.

**Genotyping**

The DNA were extracted by using (zymo Quick-DNA Microprep Kit, CAT; D3020). And then the genotype were revealed by SNP genotyping assay kit by thermo fisher (CAT# C\_\_11468118\_10) by adding 0.5 µl of the SNP assay kit and 10 µl of the probe master mix, then 3 µl of the eluted DNA and then finally the volume were completed to 20 µl by nucleases free water. And then the samples were placed in the thermo cycler instrument.

**Statistical analysis**

All the statistical tests were done by using SPSS (version 23).The differences in genetic distributions between patients and controls were estimated by chi-square (χ2) test. Odds ratios (OR) and 95% confidence intervals (95% CI) and those tests were calculated for each genotype. A value of *p* < 0.05 was considered statistically significant.

**Results**

As presented in figure (1), the Mean ± SE serum levels of MDA in spontaneous abortion group was 9.60±.018 whereas in control group was 4.17±0.22 there were higher significantly differences (p<0.0001).

**Figure (1): Levels of MDA(μmol/L) of spontaneous abortion and control groups.**

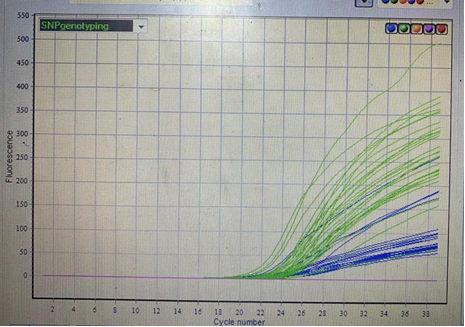
Figure (2) show the Mean ± SE of CAT (nmol/ml), 0.1290± 0.0043 is highly significant (P ≤ 0.0001) in control group comparing with patients with spontaneous abortion 0.0055 ± 0.0022.

**Figure (2): Levels of CAT activity (nmol/ml)of spontaneous abortion and control groups.**

Whilst, the Mean ± SE of Vit D3 , 25.36± 1.38 is highly significant (P ≤ 0.0001) in control group comparing with patients with spontaneous abortion 11.15± 0.66, as seen in figure (3).

**Figure (3): Levels of Vit D (ng/ml) of spontaneous abortion and control groups.**

The results in figure (4) showing the resulted curves after the Real time- PCR run were completed and the every curve represented a sample result the results of the C allele showed in the Hex while the results of the T allele.



**Figure (4): RT-PCR curves representing the amplification of the SNP region each curve represents an allele type.**

The results in table (1) summarize the allele and genotype frequencies of subjected cases and compared them to control. The CC genotype showed a significant (p= 0.003) higher frequency in patients (38%) than the control which showed only 10% frequency and the odd ratio of this relation were 3.6. while the TT genotype were higher in control than in patients. Then finally the CT genotype showed also higher frequency in control than in patients but the relation was insignificant. In addition, the allele frequency showed a strong significant higher frequency of C allele in patients than in control with high odd ration (2.597) while the T allele showed lower frequency in patients than in control and low protective ratio (0.698).

**Table (1): Genotype and allele frequencies between patients and control groups.**

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

**Discussion**

In this study , the mean MDA level in patients was found to be higher than the control group. In a study performed by (Abdul-Barry *et al.,*2011), serum MDA levels were significantly higher in patients with a history of repeated spontaneous abortion than in women with a stable pregnancy, as well Ozkaya *et al.,*( 2008), this agreement with our research. Since MDA is a side-product from lipid peroxidation, Excess in the MDA level may reflect an overproduction of lipid peroxides and may alter the antioxidant defense mechanism. Jauniaux *et al.,*(2006) proposed a hypothesis from how free radicals are higher in abortion than in normal pregnancy. They found that the intervillary O2 flow had emerged much earlier in the abortion community so that it could lead to an excessive development of free radicals that could induce abortion (Jauniaux *et al.,* 2006). Sugino *et al.,* (2000) found that total CAT activity decreased and that increased prostaglandin synthesis in cases of spontaneous abortion with vaginal bleeding. They indicated that termination of pregnancy could be triggered by decreased CAT activity, which promotes the synthesis of prostaglandins6. This study was consistent with the study by Yigenoglu *etal.,*(2011), which found that overall antioxidant potential in pregnant women with a history of spontaneous abortion was lower than in healthy pregnant women. It is also consistent with a study conducted by Biri *et al.,*(2006) that found that CAT behavior was higher in the abortion group than in the control group, this inagreement with our study. CAT is as well important for embryonic growth and the preservation of early pregnancy.CAT levels were found to increase during pregnancy and low plasma or placenta CAT activity was found in cases of spontaneous abortion .Another research also indicates that low antioxidant levels raise the risk of spontaneous abortion(Bilici, *etal.,* 2014), this agreement with our study. This study showed that most patients had vitamin deficiency. This goes with the analysis of Hantoosh , this is in line with previous studies conducted among female reproductive age groups (76 %)( Hantoosh *etal.,*2019), and Al-Hilali (65 %)(2016) ,and is backed by studies in Iran (26.1%) (Salek *etal.,* 2008 ), (33 %) (Ghaedi *etal.,*2016). Those with deficiency of 25-hydroxyvitamin D were mainly miscarriage 96 (52.7 %) compared to those with normal pregnancy 86 (47.3 %); 25-hydroxyvitamin D was lower among those with miscarriage 11.3±5.3 than normal pregnancy 15.9±11.2.This is consistent with a study in Iraq that found that 60% of those with recurrent pregnancy loss had vitamin D deficiency, with a mean of 21.5±11.8 (Sharef *et al.,* 2020)In Iran, it was found that (33%) of those with recurrent pregnancy loss had vitamin D deficiency (Ghaedi *etal.,*2016) .In a prospective cohort study, Mumford *et al.,* (2018) found that adequate preconception of Vitamin D serum levels was correlated with lower pregnancy loss rates.

There are two well-known anti-oxidant enzymes, the first is glutathione peroxidase 1 (GPX1) and the other one is catalase (CAT), both of those enzymes can protect the cells from the ROs by conversion of the hydrogen peroxide to water and oxygen (Rohrdanz *etal.,* 1998). Catalase (CAT) encode an antioxidant enzymes that detoxify H2O2 and protect the cells from oxidative damage. Various studies proved a functional polymorphic site at the CAT promoter region at position −262 (C-262T) which might effect on the expression and enzyme blood levels, leading to some pathological clinical conditions (Eskafi *et al.*, 2014).

 As the allele substitutions in a gene encode antioxidant enzyme may lead to functional changes, so in this study, we evaluated one of the most important polymorphism of CAT to determine their role in spontaneous abortion. The results showed a stron relation between the presence of C allele and CC genotype and the occurrence of the abortion. A previous study done by Forsberg *et al.*, (2001) in which they demonstrated that the T allele has a significantly higher transcriptional activity than the C variant, and due to this reason higher catalase levels showed in the genotype T/T. Since there is more protection against H2O2 accumulation in cells with high catalase expression, it is possible that the variability associated with this polymorphism plays a role in response to oxidative stress (Chang *etal.,*1999).

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