Gebelikte Enzimler: Sitozolik Karbonik Anhidraz, Katalaz, Paraoksonaz 1 ve Ksantin Oksidaz Düzeyleri

Ender ŞİMŞEK[1], Ayşe Filiz YAVUZ[2,3], Emine TERZİ[1], Tuğba Kevser UYSAL[1], Beyza Ecem ÖZ BEDİR[1], Emin HAQVERDİYEV[2], Özen ÖZENSOY GÜLER[1]

Öz

Gebelik, birçok vücut fonksiyonu için yüksek enerji ve dolayısıyla daha fazla oksijen gerektiren fizyolojik bir durumdur. Oksijen ve enerji gereksinimlerindeki bu artış, oksidatif streste artışa neden olmaktadır. Gebeliğin 1., 2. ve 3. trimesterlarında yüksek enerji ve artmış oksijen ihtiyacı duyulur. Bu biyokimyasal değişiklikler ile bazı enzim aktiviteleri arasında anlamlı ilişki olabilir. Yapılan bu çalışmada gebelik sırasında sitozolik CA, CAT, PON 1 ve XO enzim aktiviteleri araştırılmıştır. Antioksidan enzimlerin aktiviteleri Biotek cihazı ile spektrofotometrik olarak ölçülmüştür. Sitozolik CA aktivitesi, Wilbur ve Anderson yöntemine göre CO2'nin hidratasyonu ile ölçülmüştür. Bulgulara göre, gebelikte CAT ($p=0.048$) ve sitozolik CA ($p<0.001$) enzim aktiviteleri azalırken, PON 1 ($p<0.001$) ve XO ($p=0.016$) enzim aktiviteleri artmıştır. Bununla birlikte, sitozolik CA, CAT, PON 1 veya XO enzim düzeyleri açısından 1., 2. ve 3. trimesterlar arasında istatistiksel olarak anlamlı bir fark saptanamamıştır ($p>0.05$). Farklı antioksidan enzim aktiviteleri hamilelikte artabilir veya azalabilir. Ayrıca, gebe grubunda sitozolik CA enzim düzeyleri azalmasının fetal ve maternal sağlığın biyokimyasal ve fizyolojik yönleri üzerinde önemli etkisi olabilir.

Anahtar Kelimeler: Gebelik, katalaz, paraoksonaz, ksantin oksidaz, sitozolik karbonik anhidraz

Yayın Bilgisi

Gönderi Tarihi:12.07.2018

Kabul Tarihi:18.09.2018

Online Yayın Tarihi:31.03.2019

DOI: 10.26453/otjhs.409112

Sorumlu Yazar

Özen ÖZENSOY GÜLER

Enzymes During Pregnancy: Cytosolic Carbonic Anhydrase, Catalase, Paraoxonase 1 and Xanthine Oxidase Levels

Ender ŞİMŞEK[1], Ayşe Filiz YAVUZ[2,3], Emine TERZİ[1], Tuğba Kevser UYSAL[1], Beyza Ecem ÖZ BEDİR[1], Emin HAQVERDİYEV[2], Özen ÖZENSOY GÜLER[1]

Abstract

Pregnancy is a physiological condition which requires high energy and therefore more oxygen for many body functions. This increase in oxygen and energy requirements leads to an increase in oxidative stress. High energy and increased oxygen are needed in the duration of pregnancy - 1st, 2nd and 3rd trimesters. There can be significant correlations between biochemical changes and some enzyme activities. This study investigates the activities of cytosolic CA, CAT, PON 1 and XO enzymes during pregnancy. The antioxidant enzymes' activities were measured spectrophotometrically using the UV assay method on a Biotek. Cytosolic CA ctivity was measured by the hydration of CO2 in accordance with Wilbur and Anderson's method. According to the findings of this study, CAT ($p=0.048$) and cytosolic CA ($p<0.001$) activities decreased during pregnancy whereas both PON 1 ($p<0.001$) and XO ($p=0.016$) activity levels were higher in pregnancy. However, there were no statistically significant difference between 1st, 2nd and 3rd trimesters of pregnancy in terms of cytosolic CA, CAT, PON 1 or XO enzyme levels ($p>0.05$).Different antioxidant enzymes' activities may increase or decrease during pregnancy. The decrease of CA enzyme levels in the group consisting pregnants may have significant impact on biochemical and physiological aspect of fetal and maternal health.

Keywords: Pregnancy, catalase, paraoxanase, xanthine oxidase, cytosolic carbonic anhydrase

Article Info

Received: 12.07.2018

Accepted: 18.09.2018

Online Published:31.03.2019

DOI: 10.26453/otjhs.409112

Corresponding Author

Özen ÖZENSOY GÜLER

[1] Ankara Yıldirim Beyazit University, Deparment of Medical Biology, Bilkent Campus, Ankara, Turkey.
[2]Ankara Yıldirim Beyazit University, Department of Obstetrics and Gynecology, Bilkent Campus, Ankara, Turkey.
[3]Ankara Atatürk Research and Training Hospital, Deparment of Obstetrics and Gynecology, Bilkent, Ankara, Turkey.

INTRODUCTION

Pregnancy is a physiological process that affects the balance between oxidant and antioxidant levels.1 This process increases oxygen demand and requires high energy for many body functions.2 Antioxidants play crucial roles in the development of the fetus and contribute to the maintenance of homeostasis.3 Oxidative stress occurs due to the harmful effects of free radicals. Antioxidants are one of the most important defense mechanisms for the inactivation of the free radicals.4 In normal physological conditions, there is a balance between the occurence of the free radicals and the antioxidant defence system. Many cells and tissues might get damaged by actions that shift this balance towards the free radicals' side.5

The catalase (CAT) enzyme, which contains a heme group, is found in the cytoplasm and peroxisomes6 of every type of cells at different concentrations. CAT regulates hydrogen peroxide (H2O2) levels and removes it by converting it to water. High levels of H2O2 can damage cells and might be a risk factor for various diseases.6-8 Changes in CAT activity levels are associated with oxidative stress and few specific diseases.9 Paraoxonase (PON), one of the important enzymes in the antioxidant defence system, is a calcium-dependent ester hydrolase. The PON enzyme family consists of PON1, PON 2 and PON 3. PON1 is associated with HDL and metabolizes oxidized lipids.10 It also inhibits low density lipoprotein (LDL) oxidation by hydrolysing H2O2.11 PON1 has a high antioxidant capacity aganist lipid peroxidation resulting from the free radicals' activities.12 In addition, low levels of PON 1 are a risk factor for atherosclerosis.1

Xanthine oxidase (XO), is an iron and molybdenum-containing enzyme which catalyzes the oxidation of hypoxanthine to xanthine, and xanthine to uric acid.[13-15] XO is commonly found in the liver, lung, kidney, heart, brain and in the plasma.[16,17] XO produces reactive oxygen species by producing superoxide anion radicals.[18] Increased XO activity is related to oxidative stress in diabetes, cardiovascular diseases and cancer.[19-21]

Carbonic anhydrase (CA) enzymes are metalloenzymes that are commonly found in almost all living organisms that catalyze the hydration/dehydration of CO_2/HCO_3^-. [22] It is of great interest as an enzyme because it is one of the fastest known enzymes.[23] There are 16 isoenzymes of carbonic anhydrase. Some of the CA enzymes are cytosolic (CA-I, CA-II, CA-III, CA-VII, CA-XIII) and

some are membrane-dependent. (CA-IV, CA-IX, CA-XII, CA-XIV and CA-XV), CA-VA and CA-VB are mitochondrial and CA- VI is secreted from the salivary gland.[24] CA-I and CA-II are the two major isoforms found in mammalian red blood cells.[25] CA-I, hemoglobin-free protein, is the most abundant isoenzyme in red blood cells.[26] CA-II, the most studied isoenzyme with the highest turnover rate among CAs, is highly excreted in most organs and contributes to many important physiological processes.[27,28] A number of studies have suggested that there is a relationship between antioxidant enzymes, cytosolic carbonic anhydrase enzymes and pregnancy. Based on this literature, we hypothesize that these enzyme levels may vary in relation to the trimesters of pregnancy. In this sense, this study aimed to experimentally investigate the differences between enzyme activity levels during different stages of pregnancy. We hypothesize that these enzyme levels may vary in relation to the trimesters of pregnancy. These enzymes' activities may increase or decrease. These decreases or increases may lead to some complications during pregnancy.

MATERIALS AND METHODS

Patients

The voluntary participants had no risk factors for potential problems related to pregnancy. The mean age of the subjects was 28. The study was conducted with 33 non-pregnant and 96 pregnant women for cytosolic CA enzyme analyses, 33 non-pregnant and 94 pregnant women for CAT enzyme analyses, 33 non-pregnant and 96 pregnant women for PON 1 enzyme analyses, and 34 non-pregnant and 98 pregnant women for XO enzyme analyses. Pregnant women were separated according to their trimesters. Exclusion criteria were any women with the presence of any disease or anemia.

Ethical Approval

Approval for the study was granted by the AYBU Atatürk Training and Research Hospital Ethics Committee (26379996/45), and informed consent forms were signed by all participants.

Blood Samples

Blood samples were taken at the AYBU Atatürk Training and Research Hospital, Gynecology and Obstetrics Clinic in Ankara, Turkey. Peripheral venous blood samples (5 mL) were collected into BD

Vacutainer sodium citrate tubes and placed on ice.

Materials

All chemicals were of analytical grade and obtained from Sigma - Aldrich (Taufkirchen, Germany) or Merck (Darmstadt, Germany).

Sample Preparation

The blood samples were centrifuged at 2208 g for 20min, and then the separated plasma and buffy coat were collected. These plasma samples were used to determine the activities of XO and PON 1. The packed red cells were washed twice with NaCl (0.9%), and then erythrocyte lysates were prepared by putting the cells through three freeze - thaw cycles in dry ice with the addition of five volumes of ice- cold distilled water. The hemolysates were used to determine the cytosolic CA (hCA-I, hCA-II) and CAT activity levels.[9]

Assay of Cytosolic Carbonic Anhydrases Activities

Carbonic anhydrase activity was measured by the hydration of CO_2 according to the method of Wilbur and Anderson[29], which is based on the determination of the time required for the pH of solution decreasing from 10.0 to 7.4 due to the hydration of CO_2. Assays were performed at least twice on each lysate, and the mean value was determined to the formula: EU=(to-tc)/tc.[8]

Assay of Catalase Activity

CAT activity was measured spectrophotometrically using the UV assay method on a Biotek (Winooski, VT, USA). CAT - catalyzed decomposition of H2O2 was monitored for 5 min by measuring the decrease in absorbance at 240 nm, at 25ºC. The results were expressed as units per liter (U/L).9 The enzyme units were calculated according to the following formula:

$$EU = \frac{Total\ volume\ x\ enzyme\ volume\ x\ dA/dT}{240\ x\ (1\ cm\ cuvette)}$$

Assay of Paraoxonase Activity

PON 1 activity towards paraoxon as a substrate was quantified spectrophotometrically by the method described by Gan et al.[30] The reaction was observed for 2 min at 37°C by monitoring the appearance of p - nitrophenol at 412 nm on a Biotek (Winooski, VT, USA) automated recording spectrophotometer. The initial substrate concentration during enzyme assay was 2 mM, and all rates were determined in duplicate and corrected for the non - enzymatic hydrolysis. A molar extinction coefficient (ϵ) of 17,100 $M^{-1}cm^{-1}$ for p - nitrophenol at pH 8.0 in 100 mM Tris buffer was used for the calculation. One unit PON 1 activity is accepted as p-nitrophenol which

occurred in one minute, µmol.[31] Enzyme units were calculated from the absorbance change according to the following formula.[11]

$$EU = \frac{Reaction\ volume\ (mL) x \frac{dA}{dT} x\ 1000}{\varepsilon 412\ x\ enzyme\ volume\ (mL) x\ d\ (cm)}$$

Assay of Xanthine Oxidase Activity

XO activity was analyzed essentially according to the method described by Roussos.[32] The change in absorbance was recorded at 290 nm at 15 s interval for 1 min on a Biotek (Winooski, VT, USA). The appropriate control was run simultaneously. Roussos[24] defined 1U of activity as change in absorbance at 290 nm in 1 min by 1 mL enzyme preparation.[9]

Statistical Analysis

The distribution of enzyme levels were examined visually with using the Shapiro Wilk test. The enzyme levels were presented as mean ± standard deviation (mean ± s) and median (min-max: minimum-maximum). Mann-Whitney U tests were used to compare the enzyme levels between non-pregnant and pregnant groups. In addition, Kruskal Wallis tests were used for comparing pregnancy terms (1st trimester, 2nd trimester, and 3rd trimester). The relationship between enzyme levels were examined using Spearman's rank correlation coefficients. Test statistics and p values are given for test results. A $p < 0.05$ value was accepted as statistically significant. All statistical analyses and calculations were performed through IBM SPSS Statistics 21.0 (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp.).

RESULT AND DISCUSSION

The median values of CAT enzyme activity for the non-pregnant women were calculated to be 11.95 EU / mL (min - max: 2.11 - 29.53), while those for the pregnant women were

9.14 EU / mL (min - max: 0.70 - 35.86) (Table 1). Thus the CAT levels of the pregnant group were lower than those of the non-pregnant group (p= 0.048).

Similarly cytosolic CA enzyme levels of pregnant group were low compared to non-pregnant group (p<0,001). The median value of cytosolic CA enzyme for the non-pregnant and pregnant groups was calculated respectively 2,34 EU/mL (min-max: 1,37- 3,06) and 1,50 EU/mL (min-max: 0,38-3,23) (Table 1).

The median values of PON 1 enzyme activity for the non-pregnant and pregnant groups were calculated to be 30.7 EU / mL (min - max: 1.23 - 87.19) and 58.64 EU / mL (min - max: 4.91 - 209.39), respectively

(Table 1). The PON 1 enzyme levels of the pregnant group were thus higher than those of the non-pregnant group ($p < 0.001$).

The median values of XO enzyme activity were calculated to be 25.06 EU / mL (min - max: 0.00 - 106.69) and 39.61 EU / mL (min - max: 1.62 - 177.82) for the non-pregnant and pregnant women, respectively (Table 1). As with PON 1, the XO enzyme levels of the pregnant group were higher than those of the non-pregnant group ($p =0.016$).

Overall, there were no statistically significant differences between the 1st trimester, 2nd trimester and 3rd trimester pregnant women for either cytosolic CA, CAT, PON 1 or XO enzyme levels ($p > 0.05$) (Table 2).

There is no relationship between antioxidant enzyme levels in the 1st and 2nd trimesters. There is negative and a weak correlation between PON with cytosolic CA enzymes activity levels in 3rd trimester pregnant. However, there is a negative correlation between XO and both PON and CAT enzyme levels in the 3rd trimester ($p < 0.05$; Table 3).

Pregnancy is a process that affects the balance between oxidant and antioxidant levels.[1] This process increases the sensitivity against oxidative stress. There is a balance between reactive oxygen species (ROS) and antioxidant status. Increased ROS levels in pregnancy can cause oxidative stress and can also affect maternal and fetal health.[33]

In this study, we analyzed CAT, PON 1 and XO levels in pregnancy. Additionally, we examined the relationships between these enzyme activities across the trimesters of pregnancy. According to our results, the antioxidant enzymes may play an active role in pregnancy, as their levels change during pregnancy, though not necessarily across the broad time course of pregnancy.

One of the important components of the antioxidant defense system is CAT that catalyses the conversion of H_2O_2 into H_2O and O_2.[34] There are different reports of decreases, increases and no changes in CAT activity in pregnant women. Although Ademuyiwa et al.[35] reported that CAT activity did not change, Djordjevic et al.[36] showed increased CAT activity during the course of pregnancy. Yuksel et al. suggested that CAT activity in maternal blood decreases during pregnancy, consistent with the studies carried out in pregnant ewes.[37] Similarly, the blood CAT activity of pregnant women was decreased compared with non-pregnant women in the study of Góth et al.[38] In our study we also found that

CAT activity levels were lower in pregnant women than non-pregnant women.

Lekharu et al. stressed a gradual decrease in the activities of CAT throughout the three trimesters of pregnancy.[39] In another study, CAT activity was the lowest in the 3rd trimester of pregnancy.[40] However, in our study, there were no significant differences between the 1st, 2nd and 3rd trimesters of pregnancy with respect to CAT enzyme levels.

PON 1 is an effective antioxidant enzyme[1] and protects lipoproteins from oxidative modification.[41] There is a strong relationship between PON 1 and HDL. PON 1 prevents lipid peroxdiation by playing a crucial role in the oxidation of LDL.[12] Increased HDL and LDL levels during pregnancy have been reported.[42-44] Thus, it may be that PON 1 activity increases in this process. According to the results of our study, PON1 activity levels were higher in pregnant women compared to non-pregnant women. Thus, PON 1 may have a role in combatting oxidative stress in pregnant women. This data contradicts with that of Vlachos et al. who reported that PON 1 activity decreased in pregnancy.[45] Furthermore, Stefanović et al. indicated that PON 1 activity significantly decreased especially during gestational week 32.[1] However, increased serum PON 1 levels were measured at the weak 28 and 32 of normal pregnancy by Roy et al.[46] In our study, there were no differences in PON enzyme levels between the 1st, 2nd and 3rd trimesters of pregnancy. This indicates that the PON 1 levels effective against the oxidative stress, remain stable during all trimesters of pregnancy.

One of the cellular redox enzyme in our metabolism is XO which is a metabolic pathway for uric acid production.[47] Tsutsumi et al. found that XO activity levels during pregnancy and after delivery were not statistically different.[48] According to the results of our study, the XO enzyme levels of the pregnant group were higher than those of the non-pregnant group. It is suggested that XO may contribute the generation of ROS in pregnancy. In addition, our results did not indicate any significant changes in XO activities across trimesters, therefore we consider that XO does not play a crucial role in the time course of pregnancy.

The balance between oxidant and antioxidant levels have a significant role during pregnancy. In this current study, we determined the relations between oxidative stress and pregnancy. Interestingly, CAT enzyme levels are comparatively lower for

pregnant women, whereas PON 1 and XO enzyme levels are significantly higher. Further studies are needed to explain the roles of different antioxidant enzymes in the physiological process of pregnancy.

In additional to these, cytosolic CA enzyme are crucial for exchanging gaseous in pregnancy.[49] But, there are limited studies about cytosolic CA levels and its importance in pregnancy. Uesato demonstrated that cytosolic carbonic anhydrases enzyme levels were higher than the control group in 2nd trimester of pregnancy. These levels reached maximum levels in 3rd trimester of pregnancy. It is told that increased CA levels is related with gaseous exchange in placenta.[49] In another study, Shepherd and Spencer indicated that there are no statistically significant difference for RB-HCA1 concentrations between pregnant women and healthy volunteers during menstrual cycle.[50] In contrast to these studies, cytosolic CA enzyme levels decreased in pregnant group in our study. One of the most important functions of cytosolic CA-I and CA-II isoenzymes is to provide CO_2/HCO_3^- transport and pH/CO_2 homeostasis. Moreover, maternal ventilation and blood gas changes during pregnancy and the CO_2 pressure (pCO_2) are decreased. Our group suggested that decreasing CO_2 levels in pregnancy cause lower CA enzyme levels.

REFERENCES

1. Stefanović A, Ardalic D, Kotur-Stevuljević J, et al. Longitudinal changes in PON1 activities, PON1 phenotype distribution and oxidative status throughout normal pregnancy. Reprod Toxicol. 2012; 33(1): 20-26.
2. Gitto E, Reiter RJ, Karbownik M, et al. Causes of oxidative stress in the pre-and perinatal period. Bion Neonate. 2002; 81(3): 146-157.
3. Al-Gubory KH, Fowler PA, Garrel C. The roles of cellular reactive oxygen species, oxidative stress and antioxidants in pregnancy outcomes. Int J Biochem Cell Biol. 2010; 42(10): 1634-1650.
4. Little RE, Gladen BC. Levels of lipid peroxides in uncomplicated pregnancy: a review of the literature. Reprod Toxicol. 1999; 13(5): 347-352.
5. Verit FF, Erel O, Sav M, Celik N, Cadirci D. Oxidative stress is associated with clinical severity of nausea and vomiting of pregnancy. Am J Perinatol. 2007; 24(9): 545-548.

6. Natarajan V. Oxidants and signal transduction in vascular endothelium. J Lab Clin Med. 1995; 125(1): 26-37.
7. Armstrong D. Free Radical and Antioxidant Protocols. New Jersey, NJ: Humana Press; 1998.
8. Ozensoy O, Kockar F, Arslan O, Isik S, Supuran CT, Lyon M. An evaluation of cytosolic erythrocyte carbonic anhydrase and catalase in carcinoma patients: An elevation of carbonic anhydrase activity. Clin Biochem. 2006; 39(8): 804-809.
9. Cengiz FP, Beyaztas S, Gokce B, Arslan O, Guler OO. Catalase, carbonic anhydrase and xanthine oxidase activities in patients with mycosis fungoides. J Enzyme Inhib Med Chem. 2015; 30(2): 212-215.
10. Kaya MO, Sinan S, Güler ÖÖ, Arslan O. Is there a relation between genetic susceptibility with cancer? A study about paraoxanase (PON1) enzyme activity in breast cancer cases. J Enzyme Inhib Med Chem. 2016; 31(6): 1349-1355.
11. Bilen Ç, Beyaztaş S, Arslan O, Güler ÖÖ. Investigation of heavy metal effects on immobilized paraoxanase by glutaraldehyde. . J Enzyme Inhib Med Chem. 2013; 28(3): 440-446.
12. Abuhandan M, Cakmak A, Taskın A, Karakaya E, Kocyigit A, Kılıc H. The effect of age on the paraoxonase and arylesterase activity of pregnant mothers and their infants. J Clin Lab Anal. 2012; 26(4): 302-306.
13. Kaya MO, Kaya Y, Çelik G, Kurtuluş F, Arslan O, Güler ÖÖ. Differential in vitro inhibition studies of some cerium vanadate derivatives on xanthine oxidase. J Enzyme Inhib Med Chem. 2015; 30(2): 286-289.
14. Brondino CD, Romão MJ, Moura I, Moura JJ. Molybdenum and tungsten enzymes: the xanthine oxidase family. Curr Opin Chem Biol. 2006; 10(2): 109-114.
15. Beyaztaş S, Arslan O. Purification of xanthine oxidase from bovine milk by affinity chromatography with a novel gel. J Enzyme Inhib Med Chem. 2015; 30(3): 442-447.
16. Sathisha KR, Khanum SA, Chandra JN, et al. Synthesis and xanthine oxidase inhibitory activity of 7-methyl-2-(phenoxymethyl)-5H-[1, 3, 4] thiadiazolo [3, 2-a] pyrimidin-5-one derivatives. Bioorg Med Chem. 2011; 19(1): 211-220.
17. Bytyqi-Damoni A, Genç H, Zengin M, Beyaztas S, Gençer N, Arslan O. In vitro effect of novel β-lactam compounds on xanthine oxidase enzyme activity. Artif Cells Blood Substit Immobil Biotechnol. 2012; 40(6): 369-377.

18. Isık H, Aynıoglu O, Tımur H, et al. Is Xanthine oxidase activity in polycystic ovary syndrome associated with inflammatory and cardiovascular risk factors?. J Reprod Immunol. 2016; 116: 98-103.
19. Miric DJ, Kisic BM, Filipovic-Danic S, et. al. Xanthine oxidase activity in type 2 diabetes mellitus patients with and without diabetic peripheral neuropathy. J Diabetes Res. 2016; 2016: 4370490.
20. Feoli AM, Macagnan FE, Piovesan CH, Bodanese LC, Siqueira IR. Xanthine oxidase activity is associated with risk factors for cardiovascular disease and inflammatory and oxidative status markers in metabolic syndrome: effects of a single exercise session. Oxid Med Cell Longev 2014; 2014: 587083.
21. Güleç M, Akın H, Yüce HH, et al. Adenosine deaminase and xanthine oxidase activities in bladder washing fluid from patients with bladder cancer: a preliminary study. Clin Biochem. 2003; 36(3): 193-196.
22. Alterio V, Di Fiore A, D'Ambrosio K, Supuran CT, De Simone G. Multiple binding modes of inhibitors to carbonic anhydrases: how to design specific drugs targeting 15 different isoforms?. Chem Rev. 2012; 112(8):4421-4468.
23. Chegwidden WR, Carter, ND. Introduction to the carbonic anhydrases. EXS 2000; 90:13-28.
24. Thiry A, Dogné JM, Masereel B, Supuran CT. Targeting tumor-associated carbonic anhydrase IX in cancer therapy. Trends Pharmacol Sci. 2006; 27(11):566-573.
25. Tashian RE, Hewett-Emmett D, Goodman N. On the evolution and genetics of carbonic anhydrases I, II, and III, Isozyme. Isozymes Curr Top Biol Med Res. 1983; 7:79-100.
26. Imtaiyaz Hassan M, Shajee B, Waheed A, Ahmad F, Sly WS. Structure, function and applications of carbonic anhydrase isozymes. Bioorg Med Chem. 2013; 21(6):1570-1582.
27. Silverman DN, Mckenna R. Solvent-mediated proton transfer in catalysis by carbonic anhydrase. Acc Chem Res. 2007; 40(8):669-675.
28. Tashian RE. Genetics of the mammalian carbonic anhydrases. Adv Genet. 1992; 30:321-356.
29. Wilbur KM, Anderson NG. Electrometric and colorimetric determination of carbonic anhydrase. J Biol Chem 1948; 176:147-154.
30. Gan KN, Smolen AN, Eckerson HW, La Du BN. Purification of human serum paraoxonase/arylesterase. Evidence for one

esterase catalyzing both activities. Drug Metab Dispos. 1991; 19(1): 100-106.

31. Arslan M, Gencer N, Arslan O, Guler OO. In vitro efficacy of some cattle drugs on bovine serum paraoxonase 1 (PON1) activity. J Enzyme Inhib Med Chem. 2012; 27(5): 722-729.
32. Roussos GG. Xanthine oxidase from bovine small intestine. Methods Enzymol. 1967; 12: 5-16.
33. Saker M, Mokhtari NS, Merzouk SA, Merzouk H, Belarbi B, Narce M. Oxidant and antioxidant status in mothers and their newborns according to birthweight. Eur J Obstet Gynecol Reprod Biol. 2008; 141(2): 95-99.
34. Negara I, Surya IG, Sanjaya H, Anantasika AA, Mahardika I. Lower Serum Catalase Level is Associated with Preterm Labor among Pregnant Women at Sanglah Hospital Denpasar, Bali-Indonesia. Bali Medical Journal. 2016; 5(3):47-50.
35. Ademuyiwa O, Odusoga OL, Adebawo OO, Ugbaja RN. Endogenous antioxidant defences in plasma and erythrocytes of pregnant women during different trimesters of pregnancy. Acta Obstet Gynecol Scand. 2007; 86(10): 1175-1180.
36. Djordjevic A, Spasic S, Jovanovic-Galovic A, Djordjevic R, Grubor-Lajsic G. Oxidative stress in diabetic pregnancy: SOD, CAT and GSH-Px activity and lipid peroxidation products. J Matern Fetal Neonatal Med. 2004; 16(6): 367-372.
37. Yüksel S, Yiğit AA. Malondialdehyde and nitric oxide levels and catalase, superoxide dismutase, and glutathione peroxidase levels in maternal blood during different trimesters of pregnancy and in the cord blood of newborns. Turk J Med Sci. 2015; 45(2): 454-459.
38. Góth L, Tóth Z, Tarnai I, Bérces M, Török P, Bigler WN. Blood catalase activity in gestational diabetes is decreased but not associated with pregnancy complications. Clin Chem. 2005; 51(12): 2401-2404.
39. Lekharu R, Pradhan R, Sharma R, Sharma D. A Study of Lipid Peroxidation and Antioxidant Enzymes in Normal Pregnancy. GCSMC J Med Sci 2014; 3: 55-56.
40. Patil SB, Kodliwadmath MV, Kodliwadmath SM. Study of oxidative stress and enzymatic antioxidants in normal pregnancy. Indian J Clin Biochem. 2007; 22(1): 135-137.
41. Patra SK, Singh K, Singh R. Paraoxonase 1: a better atherosclerotic risk predictor than HDL in type 2 diabetes mellitus.

Diabetes Metab Syndr. 2013; 7(2): 108-111.

42. FÅhraeus L, Larsson-Cohn UL, Wallentin L. Plasma lipoproteins including high density lipoprotein subfractions during normal pregnancy. Obstet Gynecol. 1985; 66(4): 468-472.
43. Stone NJ. Secondary causes of hyperlipidemia. Med Clin North Am. 1994; 78(1): 117-141.
44. Kourtis A, Gkiomisi A, Mouzaki M, et. al. Apelin levels in normal pregnancy. Clin Endocrinol (Oxf). 2011; 75(3): 367-371.
45. Vlachos GD, Bartzeliotou A, Schulpis KH, et. al. Maternal–neonatal serum paraoxonase 1 activity in relation to the mode of delivery. Clin Biochem. 2006; 39(9): 923-928.
46. Roy AC, Loke DF, Saha N, Viegas O, Tay JS, Ratnam SS. Interrelationships of serum paraoxonase, serum lipids and apolipoproteins in normal pregnancy. Gynecol Obstet Invest. 1994; 38(1): 10-13.
47. Stevens CR, Millar TM, Clinch JG, Kanczler JM, Bodamyali T, Blake DR. Antibacterial properties of xanthine oxidase in human milk. The Lancet. 2000; 356(9232): 829-830.
48. Tsutsumi K, Kotegawa T, Matsuki S, et al. The effect of pregnancy on cytochrome P4501A2, xanthine oxidase, and N-acetyltransferase activities in humans. Clin Pharmacol Ther. 2001; 70(2): 121-125.
49. Uesato T. Carbonic Anhydrase Levels of Erythrocytes in Normal Pregnancy. Tohoku J. Exp. Med 1980;130(2):153-158.
50. Shepherd JN, Spencer N. Human carbonic anhydrase I concentration in erythrocytes during pregnancy and the menstrual cycle. Clin Biochem 1985;18(6):369-372.

Table 1. Distribution of enzyme levels in the non-pregnant and pregnant groups

EU / mL	**Non-pregnant group** **Mean ± S** **Median (min - max)**	**Pregnant group** **Mean ± S** **Median (min - max)**	**Z**	**p**
CAT enzyme activity levels	n = 33 13.61 ± 8.05 11.95 (2.11 - 29.53)	n = 94 10.43 ± 6.78 9.14 (0.70 - 35.86)	1.975	**0.048**
PON enzyme activity levels	n = 33 33.44 ± 26.53 30.7 (1.23 - 87.19)	n = 96 67.26 ± 46.57 58.64 (4.91 - 209.39)	4.019	**< 0.001**
XO enzyme activity levels	n = 34 34.38 ± 30.48 25.06 (0.00 - 106.69)	n = 98 55.89 ± 46.43 39.61 (1.62 - 177.82)	2.420	**0.016**
CA enzyme activity levels	n = 33 2,31±0,53 2,34 (1,37-3,06)	n = 96 1,57±0,64 1,50 (0,38-3,23)	5.341	**<0.001**

Table 2. Comparing enzyme levels in pregnancy terms.

EU / mL	1st **Trimester** **Mean ± S** **Median (min - max)**	2nd **Trimester** **Mean ± S** **Median (min - max)**	3rd **Trimester** **Mean ± S** **Median (min - max)**	χ^2	**P**
CAT enzyme activity levels	n = 30	n = 33	n = 31	0.774	**0.679**
	9.66 ± 4.92	12.02 ± 8.73	9.5 ± 5.79		
	8.79 (0.70 - 19.69)	10.55 (1.41 - 35.86)	8.44 (1.41 - 24.61)		
PON enzyme activity levels	n = 30	n = 32	n = 34	0.786	**0.675**
	59.19 ± 38.19	68.45 ± 45.65	73.25 ± 53.92		
	52.5 (8.60 - 168.86)	53.11 (7.37 - 182.98)	66.32 (4.91 - 09.39)		
XO enzyme activity levels	n = 30	n = 33	n = 35	0.423	**0.809**
	56.47 ± 50.65	57.46 ± 42.73	53.9 ± 47.3		
	39.61 (1.62 - 164.89)	51.73 (4.85 - 147.11)	35.56 (8.08 - 177.82)		
CA enzyme activity levels	n=28	n=32	n=36	1.800	**0.406**
	1,45±0,46	1,53±0,59	1,71±0,78		
	1,49 (0,64-2,26)	1,4 (0,60-2,99)	1,62 (0,38-3,23)		

Table 3. The correlations between enzyme levels in trimesters.

Spearman rho coefficient		PON	CAT	CA
1st Trimester				
	XO	0.065	-0.300	-0.001
	PON	1.000	0.033	0.012
	CAT	0.033	1.000	-0.340
2nd Trimester				
	XO	0.033	0.004	0.038
	PON	1.000	0.035	-0.185
	CAT	0.035	1.000	-0.107
3rd Trimester				
	XO	-0.376*	-0.360*	0.135
	PON	1.000	0.183	-0.363*
	CAT	0.183	1.000	-0.096

*p<0.05