Open Access
Baghdad Science Journal **Vol.16(4) 2019**

DOI: http://dx.doi.org/10.21123/bsj.2019.16.4.0871

Thermodynamic Investigation of Partially Purified Paraoxonase in the Sera of Healthy Pregnant Women Compared to Women with Pregnancy Complication

Zaizafoon Nabeel [1] ***Ebaa Zainal*** [2] ***Israa Zainal*** [2*]

Received 1/7/2018, Accepted 23/4/2019, Published 1/12/2019

Abstract

The thermodynamic constanting of "crude and partially purified" Paraxonase(PON) was evaluated in the sera of "healthy and ectopic" pregnant women in order to characterize the reaction of PON with diethyl para-nitro phenyl phosphate as substrate.This study was performed on (17) women with ectopic pregnancy (EP) whose age between (25-55) years and (25) normal pregnant women with a mean age of (25 -55) years as a control group . Samples were collected from the Medical City, AL-Yarmook and Fatema AL-Zahraa hospitals during the period from Sep.2011 to April 2012.The study included the evaluation of "paraxonase activity, specific activity and total protein" in the (crude and partially purified) sera of EP patients & healthy subjects. The results demonstrated that there was significant decline ($p \leq 0.05$) in the activity and specific activity of PON and significant increase ($p \leq 0.05$) in the protein concentration. The study also included the evaluation of the energetic parameters: the Gibb`s free energy (ΔG) enthalpy change (ΔH), heat of activation,(ΔH*), entropy change(ΔS), temperature coefficient (Q10) and activation energy (Ea) with respect to human PON. The results indicated that there was a dramatic increase in each of the thermodynamic parameters (ΔG,Ea,ΔH,ΔS,ΔH*,Pz,Q10) for purified PON compared with enzyme in crude sera. The results indicated also that there was a dramatic increase in each of the thermodynamic parameters (ΔG, Ea, ΔH,ΔS,ΔH*,Pz,Q10) for purified PON compared with enzyme in crude sera which indicated that the purified PON suffered from the lack metals or substances that help enzyme to over come energy barrier, at the same time the purified PON(the freedom enzyme) had the greatest chance to collide and create of the favorable orientation with substrate.

Key words: Ectopic pregnancy, Normal pregnancy, Paraoxonase , thermodynamic.

Introduction:

Paraoxonase (PON)"aryl di alkyl phosphatase" (EC 3.1.8.1) is an ester hydrolase which work on some xenobiotics, such as" organo phosphorates, unsaturated aliphatic esters, aromatic carboxylic esters & possibly, carbamates" (1). Paraxonase has anti-inflammatory and antioxidant properties (2). Paraxonases are a family of three enzymes known as "PON1, PON2 and PON3", they are synthesized in liver, capable of hydrolysis paraoxone, active metabolite of parathion which is an insecticide in organophosphate (3,4,5,6). PON has many roles including "antoxidant, contribution to "innate immunity, detoxification of reactive molecules, bio activation of drugs, and modulation of endoplasmic reticulum stress and regulation of cell proliferation/apoptosis"(7).

[1]Chemistry Department, College of Science, AL-Mustansirya University, Baghdad, Iraq.
[2] Gynecology Department, Azadi hospital.
[3]Chemistry Department, College Of Science, Kirkuk University, Kirkuk, Iraq.
[*]Corresponding author: israaz@yahoo.com

Paraxonase is a Calcium –dependent esterase, is widely distributed among organs such as "liver, kidney and intestine" (8,9,10).

Serum PON activity has been demonstrated to be modified in many diseases including "diabetes mellitus, familial hypercholesterolemia and metabolic syndrome" (11,12). Human serum PON have a molecular weight of 43- 45"kD and bounds with three carbohydrate chains accounting for "15.8"% of its weight (13), and the amino acids sequence was highly conserved among animal species which indicates the important metabolic roles of PON(14).

Emer et al.(15),demonstrated hyperlipdimia and oxidative stress in normal pregnancy, however, did not show any effects of PON1 on lipoprotein metabolism or oxidative –antioxidative system parameters in pregnancy. Harun et.al.(16), showed that women with early pregnancy failure have a decreased PON activity and increased lipid peroxidation level. Normally, metabolic and physiological changes usually occurre during

pregnancy (17). Ectopic (EP) is considered one of the most common complications of early pregnancy, implies that the fertilized egg is embeded outside the uterus, usually in the fallopian tube and rarely, it may be implanted in the women's abdomen,on an ovary or in the cervix (18).This study aimed to evaluate the thermodynamic constants of "crude and partially purified" PON in the sera of "healthy and ectopic" pregnant women.

Materials and Methods:

Study included selected pregnant women divided into (healthy & ectopic) pregnancy who were admitted to the units in the medical city"AL-Yarmook & Fatima AL-Zahra hospital" /Baghdad /Iraq during the period from Sep.2011 to April 2012. The samples were divided mainly to 2 groups:
(17) women with ectopic pregnancy (EP) whose age ranged between (25-55) years G1 and (25) normal pregnant women with a mean age of (25 -55) years as acontrol group G2 (crude & partially purified).Samples were selected based on β-human Chronic Gonadotropin > (1000 Units / ml) and ultrasound findings of EP. Specimens collection were done by withdrawing about "5 ml" of venous blood using plastic disposable syringes, left for(20 min) at room temperature ,then centrifuged at "1500" xg for (10 min), then stored at (-20^{o}C) until analysis.

Paraoxonase activity assay

Paraoxonase activity of the enzyme was determined at 37C^{o} with (1.2 mM) paraoxon (diethyl para-nitro phenyl phosphate) as substrate in 0.1M glycine/NaOH, pH=11.2 buffer and 10 mM EDTA solution. PON assay involved estimation of para –nitro phenol formation which has been followed spectrophotometrically at 412 nm. Enzyme activity is calculated by using molar extension coefficient of para – nitro phenol equal to 33300 M^{-} .cm^{-} at pH 11.2) (19).

Purification methods

Paraoxonase was purified partially from serum of human with (normal and ectopic) pregnancy using the following steps:

A mmonium sulfate precipitation

Human crude serum was precipitated with (60-80%) ammonium sulfate as mentioned by Dixon and Webb (20). The precipitate was obtained after centrifugation at 10000 xg for 15 min and re dissolved in 1 ml of 0.1 M glycine- Na glycinate buffer at pH 11.2. Both enzyme activity and protein content were determined for each separate fraction .

Dialysis against buffer

The obtained ammonium sulfate precipitate was dialyzed in the presence of 0.1 M glycine-Na glycinate buffer at pH 11.2 overnight at 4^{o}C. Fractions were checked in terms of both protein amount (280 nm) and enzyme activity.

Gel Filtration chromatography

Enzyme solution, which was dialyzed, was loaded on to the Sephacryl S-200 column after dilution to reach protein concentration (5-10 m gm/ml) then loaded onto the glass column bed (48×1.6) cm pre equilibrated with 0.1M glycine-Na glycinate buffer, pH 11.2

The column was washed with 250 ml buffer. Fractions of. ml/3min were collected and the protein level was monitored by scanning elutes at 280 nm. Enzyme activity and protein concentration of the partially purified samples were checked (21). Tubes having enzyme activity were collected for the kinetic studies.

Estimation of serum total protein

The serum total protein was estimated by Lowry *et al*. method (22), using bovine serum albumin as standard protein.

Studying the thermodynamic parameters

Paraoxonase activity was measured using two temperatures [high (50°C) & moderate (37°C)] for the studied group's samples to determine the following parameters:
Equilibrium constant (K_{eq}), Gibb's energy (ΔG), enthalpy change (ΔH), entropy change (ΔS), activation energy (Ea), stereo-frequency collision factor (PZ), heat of activation (ΔH)* and temperature coefficient (Q_{10}).
Keq value was calculated at [(M=37°C) & (H=50°C)] as follows (23):
"$K_{eq}= 1/K_m$"
Energetic parameters were determined by applying the equation below:
"$\Delta G= -2.303\ RT \log K_{eq}$"
The ΔH was calculated from the integral Van´t Hoff equation between the limits of K_{eq} at (H and M) temperatures:
"$\Delta H= 2.3\ R\ (\log K_{eq}H / \log K_{eq}M)(TH*TM/TH-TM)$"
The Gibb´s Helmholtz equation was used to calculate the ΔS as below:
"$\Delta G = \Delta H - T\ \Delta S$"
The activation energy (E_a) for the reaction was calculated by Arrhenius equation:
"$\text{Log KH /logKM= } E_a /2.303\ R *(TH-TM/TH*TM)$"
The PZ factor was calculated from the following equation:
"$\text{Ln PZ= } (E_a/RT) + \ln K_{eq}$"

Heat of activation (ΔH*) was determined using the following equation:

"E_a= ΔH* + RT"

The temperature coefficient Q_{10} was determined using the following equation:

"E_a= (2.3 RTM *TH log Q_{10})/10"

Statistical analysis:

Statistical analysis was done by SPSS statistical software version 10.00. Values were expressed as a(mean±SD). The level of significance was determined by student's t-test when the P value was equal to or less than 0.05 the difference between the two groups is considered statistically significant, less than 0.01 and 0.001 is highly significant.

Results and Discussion:

Table 1 represents the PON["activity & specific activity" and total protein (mean ± SD)] in [ectopic pregnant women compared to healthypregnant subjects:

Table 1. Serum PON activity, specific activity and total protein concentration in ectopic pregnant women compared to healthy subjects.

Groups	No.	PON activity Mean ± SD U/ ml	Total protein Mean ± SD mg/ ml	Specific activity U/mg
Ectopic pregnancy G1	17	102.44±24.50	86	1.2
Healthy subjects G2	25	235.30±85.37	80.6	2.9
P value		p≤0.05	p≤0.05	p≤0.05

*Results with (p≤0.05) considered significant.

The results indicate that there was significant decrease ($p \leq 0.05$) in the [paraxonase activity, specific activity] and significant increase ($p \leq 0.05$) in the total protein concentration in patients compared to healthy subjects. The family of PONs is protecting cells from damage by organophosphate toxins. Paraxonase activity was inhibited during the acute phase response in animals and also in human (24). Pregnancy is a physiological state related with elevated reactive oxygen species and enhances body oxygen requirements (25). Lower PON activity may be associated with early pregnancy failure due to raised lipid peroxidation levels (26). Muge et.al.(27), cleared that patients with complete molar pregnancy are exposed to reactive oxygen species that cause a disease. Many studies investigated the activity of serum PON in "normal and preeclampsia" pregnancy but according to our knowledge present work represents the first study reporting the PON activity in ectopic pregnant women. Roy et.al.(28), concluded that PON1 activity was high during (28 to 32) weeks of normal pregnancy compared with non-pregnant women. Free et.al.(29), reported that PON activity is changing in pregnant women and found the activity of PON in serum was decreased during 32 weeks of pregnancy and at labor. The results of the present study agree with Meera et.al.(30), who showed a reduced in PON activity in preeclampsia and other diseases(31). Aksoy et.al.(32), in the same line, concluded that serum "PON1 and aryl esterase" activity reduced in preeclampsia which may be attributed to elevated lipid peroxidation in these patients. Reduced PON1 activity was reported also in normal pregnancy, pregnancy failure, anemia during pregnancy by Emer et.al.(33) , Harun et.al. (34) and Hakun et.al. (35). In addition, yaghmaei et.al.(36) and Baker et.al. (37), 873anagemented an increase in PON activity in preeclampsia patients and consider PON1 allele 192R is a risk factor for preeclampsia

The activation energy (Ea) of enzyme is a useful parameter for the quantitative measurements of the thermodynamic barrier to be overcome in the cores of catalysis. The present work used Ea to compare PON changes in (ectopic and normal) pregnant women, (Crude & partially purified) sera samples. Although the purified PON has been studied extensively *in vitro* from various sources, there are no known reports on the energetic parameters of PON catalyzed reactions, to the best of researchers' knowldgement. In the present study, equilibrium constant (Keq) and the following energetic parameters of human PON: The Gibb`s free energy (ΔG) enthalpy change(ΔH), heat of activation ,(ΔH*), entropy change(ΔS) , temperature coefficient (Q10) , Ea, and number of collisions Pz with respect to human PON have been studied. Energetic indexes were calculated by using suitable equations above. Basic step of method was to assay the enzyme activity at different temperatures (moderate 37ºC and high 50ºC) and at different substrate concentrations (0.5, 0.75, 1,1.25,1.5,1.75) mM. The values of the equilibrium constant Keq, ΔG, Ea, ΔH, ΔS, Pz, ΔH*, Q10 are presented in Table 2.

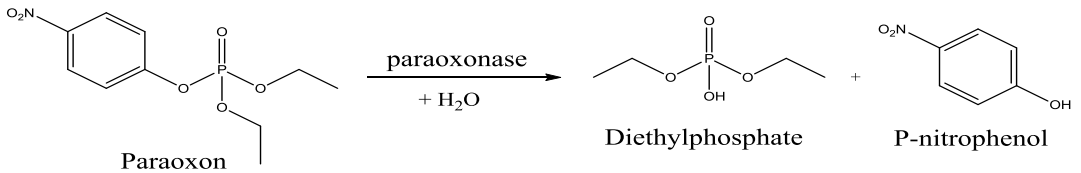
Table 2. Energetic parameters of PON reaction in the "purified & crude sera" samples of ectopic pregnancy women compared to healthy subjects.

Samples	K_{eq} mM	ΔG (Kcal/mM)	Ea (Kcal/ mM)	ΔH (Kcal/ mM)	ΔS (Kcal/ mM)	Pz mM	ΔH* (Kcal/ mM)	Q_{10}
Crude sera of NP	0.62	2.88	136	-164.85	-0.541	$2.6X10^{4}$	192.922	0.298
Purified sera of NP	0.62	2.88	193.707	-164.85	-0.541	$3.17X10^{13}$	187.569	0.428
Crude sera of EP	0.62	2.88	110.98	-78.601	-0.262	$4.4X10^{7}$	104.842	0.243
Purified sera of EP	0.4	5.625	258.398	-266.29	-0.877	$7.67X10^{17}$	252.26	0.566

Table (2) shows that there was a increase in the E_a of PON in the "partially purified compared to crude" sera samples in "normal and ectopic" pregnancy, and the ectopic purified sample had Ea higher than normal purified sample and this indicated the presence of change that increased the thermodynamic barrier in patients samples leading to delay in the breakdown [ES] complex and the formation of the products. The data also reflect that there were increase in the energy barrier in purified sample compared to crude sera due to the absence of cofactor or substances essential for PON activity. Table (1) also shows that the Keq values were the same in healthy subjects samples (0.62), while in ectopic the purified sample was decreased (0.4). The results point out that the direction of enzymatic reaction from right to left is more favored in this group than others, which refers that very little [ES] is present at equilibrium(38):

$$E + S \underset{K_{-1}}{\overset{K_1}{\rightleftharpoons}} ES \xrightarrow{K_2} E + P$$

ΔG was determined based on the Keq determined, ΔG has positive values in both EC and healthy subjects which indicates the formation of activated complex [ES] is a nonspontaneous process. ΔG value of this reaction is independent from the molecular pathway of mechanism of transformation (39). ΔH values of PON in both EC and healthy subject were negative and this indicates that the enzymatic reaction is exothermic and these state may be attributed to the breakdown of phosphoester linkage and produce the high energy compound (phosphate ion) as clear in the equation below (39):



ΔS values of PON in EC and healthy subjects were negative. This indicates that the activated complex (ES) involved in the binding process had a more arranged structure than the starting reactant (substrate and enzyme). Negative value of entropy indicates the randomness in the structure of PON. Because ΔS is the measure of inherent probability of the occurrence of the transition state, apart from energetic considerations, the negative value of ΔS indicates the formation of a transition state of PON molecules with the substrate molecules. Moreover the differences in the value of ΔS among the studied groups are an indication of variation in their conformational stability, molecular flexibility, complexity and structure rigidity (38).

However, the number of collisions Pz factor for PON indicated that the number of energetically and sterically favorable collision was done between paraoxon and PON enzyme in partially purified sample more than in crude sera for both ectopic and healthy pregnancy, which means that the main factor controlling the collision potency of the partially purified enzyme was the creation of favorable orientation and a distribution of potential energy of colliding molecules.The values of Pz agree favorably with the Q10 value, it is found to be (0.428, 0.566) in purified enzyme when compared to that in crude sera (0.298, 0.248) for ectopic and healthy pregnancy respectively. The rate constant of purified enzyme was increased by raising the temperature 10ºC compared with that found in crude sera enzyme.

ΔH* in the present investigation was calculated (187.569, 252.26) Kcal/mM for partially purified enzyme and (129.922, 104.842) Kcal/mM for crude sera enzyme in both ectopic and healthy pregnancy respectively. This demonstrated that partially purified enzyme increased the value of

ΔH* from crude sera enzyme. These large values of ΔH* indicated that a large amount of stretching , squeezing or even breaking of chemical bonds occurred during the formation of the transition state(40). The biological significance of the data shown in Table (1) can be explained as follows:

There was a dramatic increase in each of the thermodynamic parameters (ΔG, Ea, ΔH, ΔS, ΔH*,Pz,Q10) for purified PON compared with enzyme in crude sera, which indicated that the purified PON suffered from the lack metals or substances that help enzyme to overcome energy barrier, in the same time partially purified PON(the free form of enzyme) had the greatest chance for the collision and creation of favorable orientation with substrate. Additionally , when comparing the most obtained results (for partially purified enzyme) of ectopic and healthy pregnancy , noticed that in patients samples the thermodynamic barrier of PON reaction, increased and these large changes in ΔG, Ea, ΔH and ΔS confirm the effect of pathological case on enzyme mechanism and activity. On the other hand, in healthy pregnancy E and S interact is more favorable for facilitating conversion of substrate to product via weakly interacts not covalently bond to the active site through interaction like (Hydrogen bonding, Vander Waal`s interactions, ect…) All these interactions may produce stabilization to the whole structure of enzyme-substrate complex (41).

Conclusions:

The results of this study indicate that there is a dramatic increase in each of the above thermodynamic parameters for purified PON compared with enzyme of crude sera which indicates that the purified PON sufferes from the lack metals or substances that help enzyme to over come energy barrier, in same time purified PON(the freedom enzyme) has the greatest chance to collision and creation of favorable orientation with substrate.

Conflicts of Interest: None.

References:

1. Hatzihidiroglou A, Makedou K, Savopoulos C. Prevalence of paraoxonase polymorphisms in diabetes mellitus type 2 Greek patients. Hipp .2016;20(2): 176.
2. Kunutsor S, Bakker S, James R, Dullaart R. Serum paraoxonase-1 activity and risk of incident cardiovascular disease: the prevend study and meta-analysis of prospective population studies. Ather. 2016;245:143–54.
3. Blatter M, James R, Messmer S, Barja F, Pometta D.Identification of a distinct human high-density lipoprotein subspecies defined by a lipoprotein-associated protein, K-85: identity of K-85 with paraoxonase. Eur J Biochem. 2016; 211:871-879.
4. Katarzyna K, Ewelina S, HalinaM.The Role of Paraoxonase in Cardiovascular Diseases.Ann. Clin. & Lab. Sci. 2015;45(2):226-233.
5. Emel T, Ahmet H, Erkan C, Tarik U, Ozlem G, Ulkan K .Serum Paraoxonase 1 Activity and Oxidative Stress in Pediatric Patients with Pulmonary Tuberculosis .Med Princ Pract.2014; Sep23(5): 426–431.
6. Jose J C, Fernando T, Asta T. Serum paraoxonase 1 (PON1) measurement: an update. BMC Vete Res. 2014;**10:**74.
7. Martinelli N, Consoli L, Girelli D, Grison E, Corrocher R, Olivieri O.Paraoxonases: ancient substrate hunters and their evolving role in ischemic heart disease. Adv Clin Chem. 2013;59:65–100.
8. Hussein M, Nagwa S, Saadeldin A, Abdelzaher M, Mohammad M. Paraoxonase activity and gene polymorphism in colorectal cancer Egyptian patients. New York Sci J. 2017;10(4):27.
9. Andre de S, Alissana E, Felipe A, Monica M, Rebecca J, Clement E ,et al. Paraoxonase 1 (PON1) Q192Rgenotypes and their interactionwith smoking strongly increase atherogenicity and the Framingham risk score. Arch End Met. 2016;60(5):426-35.
10. Jose J, Fernando T, Asta T.Serum paraoxonase 1 (PON1) measurement: an update BMC Vet Res. 2014;10: 74.
11. Kathryn L, Lunetta A, Cupples R , Robert C. Green M, Clinton T. et al .Serum paraoxonase activity is associated with variants in the PON gene cluster and risk of Alzheimer disease. Neur Agi . 2012;33(5): 1015.
12. Moushira Z, Walaa A, Walaa Y , Eman Y, Tamer H. Serum paraoxonase-1 activity in Egyptian premenopausal women with metabolic syndrome and its relation with recurrent pre-eclampsia risk. J of App Pha Sci . 2018; 8 (02): 054-059.
13. Souade I, Hicham B, Olivier K, Echarki Z, Abdelouahed K. Human paraoxonase 1 overexpression in mice stimulates HDL cholesterol efflux and reverse cholesterol transport. Plose one.2017;12(3): 1- 18.
14. Gerard A, Anabel G, Marta G, Anna R, Raúl, Judit M, et al.Serum paraoxonase-3 concentration in HIV-infected patients. Evidence for a protective role against oxidation. J Lip Res. 2012; 53: 168-174.
15. Xue-Lian L, Dan-Feng Du, Shang-Jie C, Sai-Hua Z, Arier C, Qi C. Trends in ectopic pregnancy, hydatidiform mole and miscarriage in the largest obstetrics and gynaecology hospital in China from 2003 to 2013. Rep Heal. 2016; 13:58.
16. Yuk J, Kim Y, Hur J, Shin J.Association between socioeconomic status and ectopic pregnancy rate in the Republic of Korea. Int J Gynaecol Obstet. 2013; 122(2):104-7.
17. Esra D, Muhammet F. *In Vitro* Inhibition of Three Different Drugs Used in Rheumatoid Arthritis Treatment on Human Serum Paraoxanase 1 Enzyme Activity. Pro Pep Let. 2016;23, 3-8.
18. Emily H, Bernard N. Using subcutaneous methotrexate to prolong durationof methotrexate

therapy in rheumatoid arthritis. Eur J Rhe .2018; 17113.
19. Mueller R, Hormung S ,Furlong C. Plasma PON Polynorphism:A newenzyme assay, population, family ,Biochemical and linkage studies. Am J Hum Gene. 1983;35:393-408.
20. Dixon M, Webb E. Enzymes, 2nd ed. New York. 1964;Academic press.Unc.
21. Ammar M S .Studies on the rmophilic bacteria Ph.D,Thesis, Botany department, Facutty of Science, Al-Azhar university,Cairo, Egypt, 1975.
22. Mohamed A, Abudallah S, ALI A, Abudalazzea A. Thermodynamic Analysis of Human Retinal Acetylcholinesterase Inhibition Using an Anti-Alzheimer's Drug, Tacrine, through the Development of a Dual Substrate and Temperature Model. Proc Natl Sci Counc ROC(B). 2000 ; 24(3):108-114 .
23. Lowry O,Rose N,Farr L , Ronall J. protein measurement . J Biol Chem. 1951; 193:265-275.
24. 24- Vlaicu S , Tatomir A, Mekala A , Mircea P , Niculescu F. The role of complement activation in atherogenesis: the first 40 years. Imm Res. 2016;64(1):1-13.
25. Mark B, Yeon S, Hye-Jin S, Jung M, Young-Jin C, Yong-In K, et.al. Paraoxonase-1 (PON1) induces metastatic potential and apoptosis escape via its antioxidative function in lung cancer cells. Onco. 2017; Jun 27:8(26): 42817–42835.
26. Lenarcik A, Bidizinska B.The role of inflammatory markers, homostasis factors, and oxidative stress in pathogenesis of polycystic ovary. Adv Clin Exp Med. 2007;16(2):317-22.
27. Rong W, Jiafu F, Yuwei Y, Chunmei D, Anyang L, Jie L. Significance of Serum Total Oxidant Antioxidant Status in Patients with Colorectal Cancer. Plose one. 2017;12(1).
28. Katarzyna K, Ewelina S, Halina M .The Role of Paraoxonase in Cardiovascular Diseases. Pol Ann Clin & Lab Sci. 2015;45(2):226-232.
29. Ayla K, Onur E, Hamit Y, Fatma D, Esin E, Necat Y . Effects of serum paraoxonase and paraoxonase phenotypic distribution on the parameters of first trimester screening. Bio Res. 2017; 28 (7): 3286-3292.
30. Shikha S, Thimmaraju K, Prem C , Srivastav K, Mallick B, Nishita S, et.al.Role of dyslipidaemia and lipid peroxidation in pregnancy induced hypertension. J Clin Sci Res.2015;4:205-12.
31. Quemeneur T,Martin F ,Harton P . PON,a new biomarker of cardio –vascular disease. Sem Arth Rh . 2007;37(3) :149.
32. Şerefden A, Ülkü Ö, Murat C, Berrak G, Görkem M, Suat D, et.al. Levels of Oxidized LDL, Estrogens, and Progesterone in Placenta Tissues and Serum Paraoxonase Activity in Preeclampsia. Med Infl. 2013; Article ID 862982: 6 pages.
33. Yahya Y, Basima S, Husham K. Assessment of the Relationship between Myeloperoxidase and Paraoxonase Levels in Prediction of Preeclampsia. Nt J Pharm Sci Rev Res. 2015 ;31(2): 186-191.
34. Harun T,Hakan C. Assessment of Serum Paraoxonase and Arylestrase Activities in Early Pregnancy Failure. Swiss Med Weekly. 2009 ;39(5-6):76-81.
35. Hakan C ,Harun T ,Mehmet V ,Alysun C, Abdullah T ,Hakin C.Serum PON and aryl esterase activities in iron deficiency anemia during pregn –ancy.Turk J Med Sci .2011;41(2):185-191.
36. Yaghmaei M ,Hashemi M , Azarian A ,Mozzeni A ,Mokhtari M .Association of L55M and Q192 R polymorphisms of PON. Arch Med Res. 2011; 42(4):324-8.
37. Ozlem B, Enver S, A Savas K, aglar Y, Ismail A , Murat A, et al. Evaluation of Maternal Serum 25-Hydroxyvitamin D, Paraoxonase 1 Levels, and Neutrophil-to-Lymphocyte Ratio in Spontaneous Preterm Birth. Med Sci Monit. 2016; 22: 1238–1243.
38. Zaizafoon N N.Extent of metabolic changes in normal and abnormal pregnancies in Iraq.Ph.D. thesis. University of Al-Mustansiriya College of Science, Chemistry department; 2013.
39. Richard A,Mcpherson R. Clinical diagnosis and 876anagement by laboratory method.21st ed. Philadephia:Saunders; 2006.
40. Donald T. Haynie. Biological Thermodynamics (2ed.). Cambridge University Press. 2008; P. 26.
41. Iram U, Muhammed M, Fazel A. Kinetics and thermodynamic studies of alpha –amylasemis mutant.Pak J Bot. 2010;42(5): 3507-3516.

دراسة ثرموديناميكية لانزيم الباروكسنيز المنقى جزئيا في امصال الحوامل الاصحاء و الحمل خارج الرحم

زيزفون نبيل [1] **اباء زنيل [2]** **اسراء زينل[3]**

[1] قسم الكيمياء، كلية العلوم، الجامعة المستنصرية، بغداد، العراق.
[2] مستشفى ازادي ،قسم النسائية
[3] قسم الكيمياء، كلية العلوم، جامعة كركوك ، كركوك، العراق.

الخلاصة

اجريت هذه الدراسةِ على 17 إمرأةِ حبلى (حمل خارج الرحم) ، متوسط اعمارهن (26.14±6.99) سنة و 25 امرأة (حمل طبيعي) بمتوسط اعمار(25.16±5.99) كمجموعة سيطرة، جمعت عينات الامصال من مستشفيات (مدينة الطب، اليرموك و فاطمة الزهراء للفنرة من ايلول 2011 الى نيسان 2012. تضمنت الدراسة تقييم (الفعالية و الفعالية النوعية)لانزيم الباروكسنيز وتقدير تركيز البروتين في امصال(الخام و المنقاة جزئيا في دراسة سابقة) للحوامل (خارج الرحم) ومقارنتها بمجموعة السيطرة ولوحظ وجود نقصان معنوي في تركيز البروتين في امصال المرضى مقارنة بالاصحاء($P\geq0.05$) في الفعالية والفعالية النوعية للانزيم وزيادة معنوية. ($P\leq0.05$)

شملت الدراسة ايضا قياس الدوال الخاصة بالطاقة لتفاعل انزيم الباروكسنيز والتي تضمنت ($\Delta G, Ea, \Delta H, \Delta S, \Delta H^*, Pz, Q10$) للمجاميع اعلاه واظهرت النتائج وجود زياده ملحوظه في المتغيرات الحرارية الديناميكية للانزيم المنقى جزئيا مقارنة بالانزيم الخام للمجاميع المدروسة.

الكلمات المفتاحية: الحمل خارج الرحم ، الحمل الطبيعي، الباراكسونيز و الثرموديناميك.