The Eurasia Proceedings of Science, Technology, Engineering & Mathematics (EPSTEM)
ISSN: 2602-3199

The Eurasia Proceedings of Science, Technology, Engineering & Mathematics (EPSTEM), 2018

Volume 3, Pages 56-71

ICVALS 2018: International Conference on Veterinary, Agriculture and Life Science

Study of Paraoxonase1 Enzyme for Women with Breast Cancer (Biochemical and Molecular Genetics Study)

Luma Abdalmunim BAKER
University of Mosul

Hazha Jamal HIDAYET
University of Salahaddin

Nahida S. AL-CHALABI
University of Mosul

Abstract: The research include studying biochemical and genetic molecular study for paraoxonase1 enzyme and identify some biochemical parameters for serum blood samples 68 women with breast cancer and follow-up after take them three doses of chemotherapy and comparative with 50 normal female as control group.The result showed for women with breast cancer a significant increase in (TC,MDA ,Total protein, U,A , calcium and BMI) when compared with normal women, the results also showed for women who have taken chemical doses and women with breast cancer significant decreased in (PON1, GSH) compared with normal women, the results showed for women who have taken the first dose significant increase in (MDA,TP, Calcium, U.A , BMI), while second dose and the third dose chemotherapy found significant increase in (TP, U.A ,Calcium) and significant decreased for (PON1). The results included sequential analysis for PON1 enzyme ,it has shown results for genetic analyzed selected exon (Exon4), where DNA extraction for normal women and breast cancer women and taken their doses of chemotherapy and measure DNA concentration and purity using Nano-Drop Spectrophotometric Analysis where the result show the DNA concentration ranged between (5.2-68.4) ng/ml where concentration mean for women with breast cancer (26.97) ng/ml and (17.49) ng/ml for normal women while the first dose 24.74 ng/ml , (16.16-9.70)ng/ml for second and third dose respectively . It has also been used PCR technique amplification and test products PCR analysis of sequential technique by using gel electrophoresis where packet appeared clear without deformation and tested PCR product sequencing for exon4 by capillary automated sequencer and comparing the result with the normal sequence of the oxon., the results shows for women have taken first dose deletion mutation called (Remove) frame shift mutation clear nitrogen base one lead to change all codons after deletion site so that all amino acid change in poly protien chain which led to the loss of amino acid (Tyr.) . and mutation type of silent mutation third mutation type of substitution mutation (Replace amino acid (Thr.) by amino acid (Ser.).

Keywords: Paraoxonase1, PON1, Chemotherapy, Molecular genetics

Introduction

Cancer is a group of diseases characterized by being uncontrolled growth and spread of abnormal cells. If the spread is not controlled, it can result in death, , there are several external factors that cause cancer, such as smoking , chemicals, radiation , infectious organisms and internal factors such as , inherited genetic mutations, hormones, immune conditions or changes that occur metabolism, These factors may act together or in sequence to cause cancer. (Blecher *etal*.,2011).

Breast cancer is more prevalent among women, cancers and constitute 21% of cases of cancer that affects females and 14% of deaths of females resulting from cancer in the world, but the survival of life rate is a better than many other cancer cases (Nicholas,2013). It is form a 95% of cancer cases that occur at the age of 40 years of age and older as there are many injuries to women aged between 15 and 35 years old have been diagnosed in

developing countries, rising to about 30% of the incidence rates in Western countries (Coughlin and Cypel, 2013).

In the Middle East Region and Iraq in particular, with an incidence of 24% of the total cancers that infected the women in the Arab world, although some people believe that breast cancer is one of the developed world diseases, but that most of the deaths caused by up to 69% occur in countries developing, where the breast cancer in Iraq, form one-third of the cancer rate of that catch by Iraqi women constitute ratio of 32% (according to a blogger in the Iraqi Centre for cancer) , the last record (2012), which showed that breast cancer ranks first among cancers that catch by Iraqi women , as observed in recent years increasingly evident in the incidence of this disease since most of the cases affecting women Iraq is usually discovered in late stages, making it difficult to control by the treatment and that many of the victims were in the prime of life (Alwan,*etal.* 2012).

The radiation one of the reasons cancer and included UV.irradiation, X -ray, gamma ray through increased generation of free radicals, which directly affect the genetic material DNA updated mutations leading to obtain the growth and division is controlled in the cell which leads eventually to the emergence of tumors Cancer (Coughlin and Cypel, 2013) that the free radicals major role in the activation of cancer genes, along with overlapping free radicals with immune defenses and that work on the spread of cancer to other areas of the body easily (Benhar *etal*., 2002) (Noori, 2012).

Enzyme paraoxonase is a glycoprotein composed of 354 amino acid, as other studies have found that it is composed of 350 amino acids (Gouedard, *etal*.2003), the range of molecular weight of 43 - 49 000 KDa. in humans , 35 - 38 000 KDa in rabbits and 40,000 KDa. in mice (Furiong *etal*.1991) ,the researchers have stated the Isoelectric point IP of the enzyme (7.4 to 8.4) another study indicated that the pH optimum of the enzyme is located the extent of(5.8 to 5.7), while his optimum temperature of 35^{O}C(Askar and Buyukkebletici, 2012).

This enzyme hydrolysis enzymes arylesterase (esterhydrolase) which consists of three enzymes which (Paraoxonase1 (PON1) EC 3.1.1.1) (Arylesterase (PON2) EC.3.1.1.2.) The third enzyme EC3.1.1.3) ((aryldialkyl phophatase PON3).

This enzyme classified by type A which is enzymes of the hydrolysis is working to break the bond ester in aromatic esters, faster than esters aliphatic , It analyzes the phenyl acetate called arylesterase (PON2), it also analyzes the organophosphates in many compounds such as Paraoxonase O, O- diethyl-O- (p-nitrophenyl) phosphate called enzyme paraoxonase1 PON1, as well as the effectiveness of these enzymes measured by depending on the base material PON1 (Paraoxon), PON2 (Phenyl acetate), (PON3 lactones), (Gouederd *etal*.,2003)

The PONs gene cluster contains three gene members, which shares high sequence, namely giving rise to PON1, 2, and 3 and beside their clear protective role against cardiovascular diseases. Paraoxonase plays an explicit role in lipid metabolism.PON1 favorably effects on macrophage cholesterol metabolism PON2 attenuates macrophage triglyceride accumulation and PON3 improvement of bile acid metabolism (Aviram and Rosenblet,2004). The dietary factor which contributes to increase in paraoxonase activity in serum includes consumption of polyphenolrich diets, wine and fruit juice consumption as it contains polyphenols (Han *etal*.2007).

PON1, PON2, and PON3. They all possess antioxidant properties, share 65% similarity at the amino acid level, and the genes are located in tandem on chromosome 7 in humans and on chromosome 6 in mice. the PON1 gene maps to human chromosome 7q21-22. PON1 is synthesized in liver and is found in various tissues and plasma; especially liver, kidneys and intestines. The enzyme takes place in structure of HDL in plasma (Ali et al., 2003). Calcium is required for both activity and stability of enzyme and plays a role in catalytical mechanism. The PON1 arylesterase and PON activities are calcium-dependent and can be totally and irreversibly inhibited by EDTA, while the protection of LDL against oxidation may not require calcium (Bayrak et al., 2005). Serum PON1 activity is inversely associated with oxidative stress not only in serum, but also in arterial macrophages, the hallmark of early atherogenesis, and this phenomenon is associated with enhanced atherosclerotic lesion formation. PON1 has gained currency about its antioxidant properties (Aviram and Rosenblot, 2004).

The PON1 gene has two polymorphisms in the coding region and five in promoter region. PON1 gene substitution of glutamine (Q) by arginine (R) at position 192 and leucine (L) by methionine (M) at position 55 of coding region independently influence the PON1 activity and constitute the molecular basis for inter individual variability (Agachan *etal*.,2004) (Humbert *etal*.1993). In addition to this coding region polymorphism, significant polymorphisms in promoter region have been reported, especially at position 107

which contribute to 22.4 per cent of the variation in paraoxonase gene expression and PON1 serum concentration (James *etal*.2000).

The gene PON have nine exons located on the long arm of chromosome VII in humans and on chromosome sixth for mice , the alloenzyme differences are important, where a recent study found that alloenzyme Q of PON1 has a protective role in heart disease, alloenzyme A PON Includes three amino acids of cysteine connected to a bond (S-S) (disulphide bonds) with the enzyme at Cys 42-353) , the amino acid cysteine third at 284 remain free and there is in the effective site of the enzyme , it has a great job in the excellence and the link with the base material and significant impact on LDL and protect it from oxidation (Askar and Buyukkebletici, 2012).

Moreover, the researchers found that increasing the level of MDA, which is the most important final results of the peroxidation process of fat with injury tumors (Gutowski and Kowalczyk,2013)

Statistical Analysis

The results was Analyzed statistically using (One way analysis of variance) were determining the differences between the groups (Rohlf and Robertl, 2012) and using the test (t-test) between the two groups of patients and control , as well as between women with breast cancer and women who took doses of the chemical , the acceptable level of statistical discrimination was ($p < 0.000$, $p < 0.001$, $p < 0.01$, $p < 0.05$), using SPSS statistical software (version 19)

Aim of the Study

The present study was the impact of chemical doses on some biochemical parameters and molecular genetics for PON1.

Material and Methods

Taking 68 samples from patients who attend Nanakly of hematology hospital , Erbil , between November 2014 – March 2015 , the samples were suffering from advanced breast cancer and the required chemotherapy follow-up take them three doses and comparative with 50 normal females as control group.

Blood samples (5ml) were collected before initiating the treatment in patients . fasting blood samples were divided in two part the first part used to analyze (total cholesterol, HDL-C , T.P, Bilirubin , U.A, S. Creatin, urea, $S.Ca^{+2}$) commercial diagnostic kits from france (BDH) company. serum sample collected for estimation of PON1 were frozen at (-80) °C and were analyzed within one month the kit for estimating PON1 was obtained from MyBiosource company ,U.S.A. depended on ELISA.

The MDA levels in the serums of both groups were examined using Uchiyama and Mihara methods (Uchiyama and Mihara,1978) .The method is based on the production of the pink compound producing maximum absorbance at 535 nm as a result of thiobarbituric acid's reaction with MDA. The GSH level was examined using the Ellman method (Fairbanks and Klee,1986)

The second part of serum blood used to identify mutations in the gene responsible for an enzyme (PON1) , DNA extraction from 63 women with breast cancer patients and take them up after the first dose , the second dose and the third dose of chemotherapy , and 30 women as control group, were measured the concentration and purity of DNA by a spectrophotometric Nano drop (Rinaldi *etal*.2014), selection of the primer was depend on the target region on the sequence of exon4 in the enzyme was designed by using (online primer design program http://workbench.sdsc.edu) the sequence of the foreword and reverse primers were employed as shown in table(1), and test results (PCR) by electrophoresis technique using gel agarose concentration(2%)(W/V) as the size of the package depends on the size of the DNA of the samples used .

Table(1) primer sequence,PCR product and Optimal annealing temperature for the enzyme PON1

Gene	Primer Sequence	Primer length (bp)	Tm °C Optimal Annealing temperature	GC%	Product Length (bp)	Exon Length (bp)
PON1 gene Exon4 Forward	5-GTA TCC TGG AATAAAGAG CTT CAA C-3	25	57.75	40	160	169
PON1 gene Exon4 Reverse	5-TTC ATC TGT GAA TGT GCT AAT CCC -3	24	59.12	41.67	160	169

The results from Table (1)shows primer sequence,PCR product and Optimal annealing temperature for the enzyme PON1.Also the results of the analysis (PCR) of Exon4 for normal women ,women with breast cancer and exposed to chemotherapy by a systematic poetic automatic 96(96-capillary Automated sequence) structured biological Applied 3500 to analyze the genetic chain analyzer) depending on the Sanker method has been done in (the institute of genetic in Tehran which is a scientific center for the analysis of gene sequence).

Results and Discussion

1-Estimate the level of enzyme paraoxonase1 (PON1) in the blood serum of women With breast cancer and who have taken the chemical potions

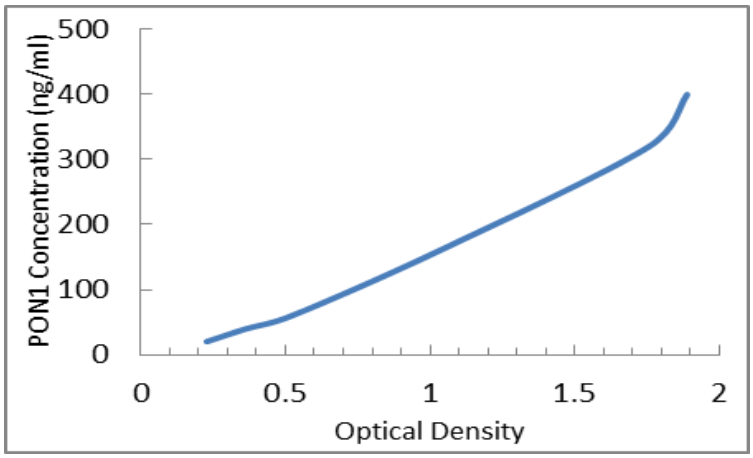


Fig 1. PON1 enzyme standard curve

We found concentrations of (PON1) by standard curve as shown in Figure1.The results showed significant decrease for women with breast cancer at the level of Probability ($p<0.01$) of the enzyme when it reached the level (120.99±10.23 ng/ml) when compared with normal women (195.45± 12.1ng/ml), while the women have taken first dose of chemotherapy shown significant decrease at (119.39± 8.1ng/ml) when compared with normal women, for the same the results shown significant decrease reaching the level of the enzyme (79.28± 5.33ng/ml) (58.34±3.33ng /ml) for women have taken second dose and the third dose at ($p <0.000$, $p <0.001$), respectively, , as shown in Figure(2).

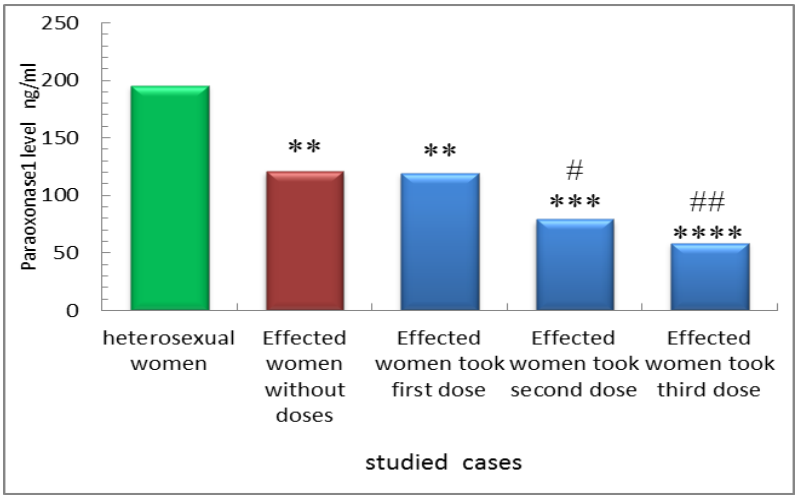


Fig 2. PON1 levels in blood serum for normal and effected women with breast cancer and who have taken chemical doses

(*:p<0.05, **:p<0.01,***: p<0.001,****: p<0.000)at the level of Probability compared to normal
(#:p<0.05, ##:p<0.01, ###:p<0.001, ####;p<0.000) at the level of Probability compared tocancer

Women who have taken the first dose shown not significant decrease when Compared with women with breast cancer, while women with second and third doses shows significant decrease at (p <0.01, p <0.05) The enzyme PON1 one antioxidant enzymatic may be due to decline in cancer patients to attack the free radicals to normal cells, where he found an inverse relationship between the level of an enzyme PON1 and the level of oxidative stress (Das, 2002) , another study found that the reason for the decline in the level of the enzyme when using therapy chemical in mice may be due to the enzyme formed in the liver and through the influence of the toxic treatment on liver cells and attacked a group of proteins, especially those that contain Cystine amino acids and cystain which owns SH group important in building and effectiveness of enzyme PON1 in liver cells (yildirim *etal*., 2012)

The decline of the enzyme PON1 may be due to one side effect of continuing of taking the second and third dose on the acidity of the blood, or due to the so-called(sepsis) and the lack of potassium which affects the enzyme activity and concentration (Inal *etal*., 2015). . One study also pointed to the role of the changes that are occurring in genetic forms that responsible of enzyme encryption, in addition to the low level (HDL) primarily responsible for the transfer of the enzyme.(Elkiran *etal*., 2007).

2-Determination of biochemical prameteres level in blood serum of women with breast cancer and who have taken chemotherapy compared with naturalist women

The level of total cholesterol (T C)

The level of tolal cholesterol: Studies have shown when measuring total (TC) cholesterol level that there is a significant increase at the level of probability (p<0.001) for women with breast cancer, which shown (6.0±0.12) mmol/l when compared with normal women's group (4.81± 0.24) mmol/l at the level of propability, (p<0.001) and showed significant increase in total cholesterol level, for women who have taken the second dose (5.87±0.9) mmol/l as well as the results showed an increase significantly in the level of cholesterol for women who have taken third dose (5.94±0.99) mmol/l for the same level of probability, as shown in Figure(3).. and that there is also a not significant decrease for women who have taken chemical doses when compared with women with breast cancer.

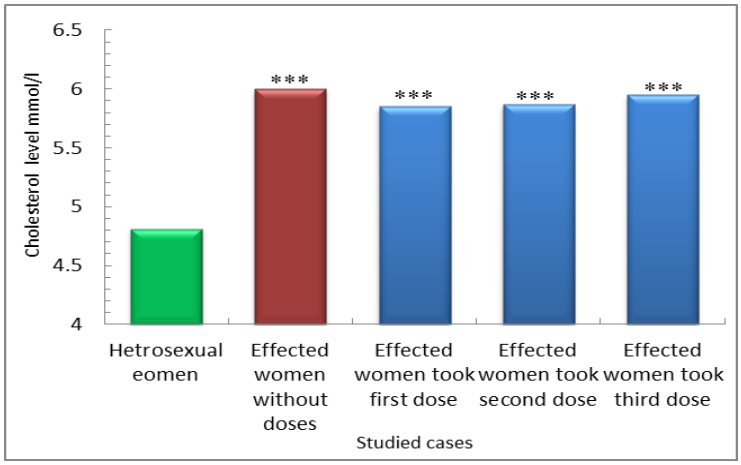


Fig 3. Cholesterol level for normal women ,women with breast cancer and who have taken chemical doses

(*:p<0.05, **:p<0.01,***: p<0.001,****: p<0.000)
(#:p<0.05, ##:p<0.01, ###:p<0.001, ####;p<0.000)

The significant high level of T C for women with breast cancer is predicting breast cancer marker has been attributed to the increase (Etiological) that precedes the disease, there are mechanisms associated with high cholesterol, including its effect on the pathological diagnosis or occurrence disturbances in lipid metabolism may lead to increased production of stimulant hormones, high cholesterol may also associated impact on the immune system of the body, which promotes the growth of tumors, may also be attributed to him the high level of estrogen which leads to increase the likelihood of developing breast cancer, and studies have found that high cholesterol cell cancer lead to increased fluidity of membranes for cancer cells, allowing them to proliferate (Abu Bedair *etal*., 2003) (Yadav *etal*.,2012) (Rzymowska,2011). It may increase the absorption of cholesterol by the tumor cells as it can be liver function have been affected and in turn affect other lipoproteins (Liaveras *etal*., 2011)

The level of malondialdehyde MDA

Found that women with breast cancer and who were not treated with chemotherapy, significantly increase the level of Probability, MDA at (p<0.05) to, which amounted to (2.043±0.061) µm /l compared with normal women, which reached the level to have (1.766±0.081) micromoles / liter as the results showed a significant increase in the level of probability (p<0.01) in women who have taken the firs second doses reaching the levels (2.15±0.061), (2.16 ± 0.031) µm /l , shown a significant rise Level reached , respectively when compared with normal women while who have taken the third dose showed an increase significantly at level of Probability (p<0.001) when compared with normal women's group where amounted (2.24± 0.037) µm /l .

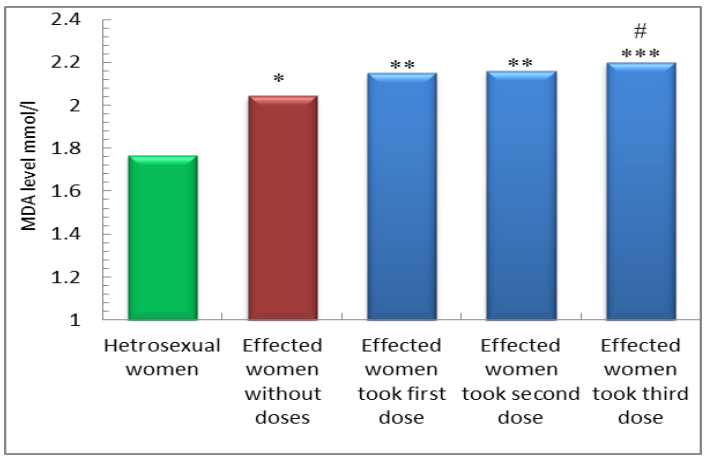


Fig 4. Comparison of malondialdehyde MDA level among patients with breast cancer women and normal women and patient who have taken chemotherapy

(*:p<0.05, **:p<0.01,***: p<0.001,****: p<0.000)
(#:p<0.05, ##:p<0.01, ###:p<0.001, ####;p<0.000)

The results showed when effected women without doses compared to breast cancer with groups who have taken chemotherapy that there is a rise significant when taking the first dose second ,dose as can be seen that there is a rise significantly when the third dose at ($p<0.05$) compared with women with breast cancer.

Based on the results that have been reached it is noted that there is a rise significantly in the level of (MDA) in the case of breast cancer may be attributed the cause to increase the level of free radicals (the primary cause of cancer) that interact with unsaturated fatty acids and converted turn into free radicals (Das U.,2002). These free radicals in turn, works to bring about genetic mutations and cancer genes are active and then formed cancerous tumors by triggering reproduction fibrillation (fibro blast proliferation) leading to cancers including breast cancer (Aghvami *etal*., 2006).

Also found in women that they were taken chemotherapy, which is characterized when taking it does not attack a specific area of the tumor cells but also affect normal cells, which produces a number of reactions in all tissues and this is in agreement with one of the studies which have shown that chemotherapy has a role in increasing the secretion of toxic in all phases of treatment which indicates oxidative stress before, during and after chemotherapy (Junior *etal*., 2015). also attributed the cause of the high level of MDA and who took third dose of chemotherapy to the depletion of most of the antioxidants necessary for Sweeping free radical damage in addition to increasing the level of O_2 and H_2O_2 after taking treatment (Sharma and Rai, 2014)

The level of glutathione

The effect of breast cancer on the level of glutathione GSH found that there is a significant decrease in GSH level in patients with breast cancer at the level of Probability ($p<0.001$) compared with normal women, a group that has been studied and the results found that GSH level in women with breast cancer reached its level (5.083± 0.491) µm /l compared with normal women's group, which reached the level to have (8.699±0.236) µm /l liter while the level for women who have taken the first dose (4.971±0.42) µm /l at ($p<0.001$) while, who have taken the second dose has reached the level (4.43 ± 0.362) µm /l s/liter and who have taken the third dose (4.01±0.56) µm /l at ($p<0.01$). significant decline is observed for the three doses compared with normal women.

As shown results when compared to women who have taken the first dose is decreased not significantly while the results for women who have taken the second and third doses decreased significantly at ($p<0.05$) compared with women with breast cancer. The reason may be due to an increase lipid peroxidation in cancer patients and then increased consumption of (GSH), which is one of the non-enzymatic antioxidant that contribute to the removal of free radicals and their results as well as infected of cancer leads to antioxidant consumption that compound stimulates the enzyme (Thiol transferase) (Nourazarian *etal*., 2014).

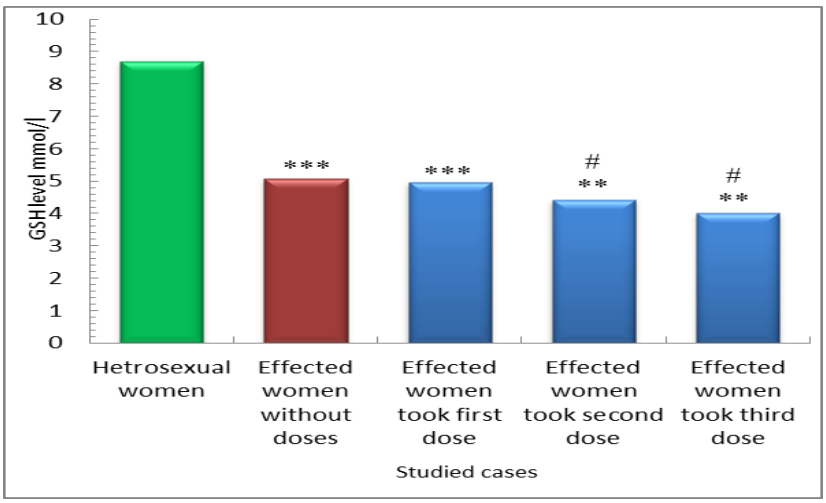


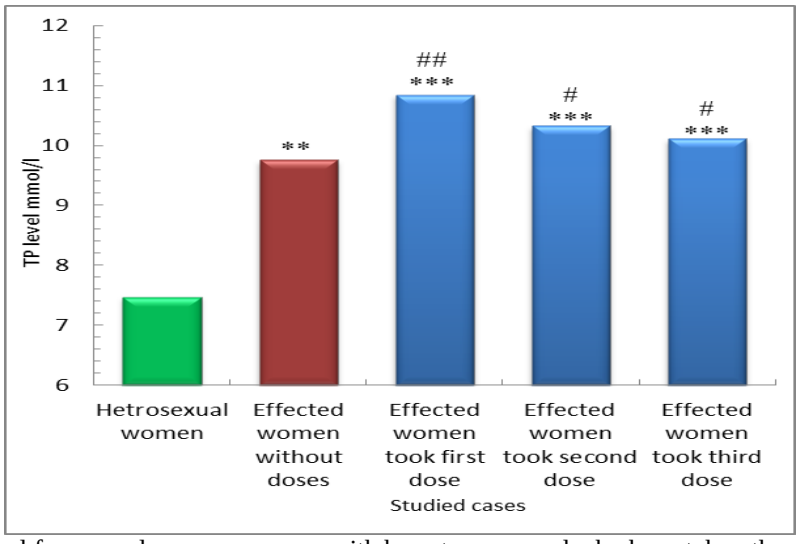
Fig 7. Glutathione level for normal women , women with breast cancer and who took the chemotherapy

(*:p<0.05, **:p<0.01,***: p<0.001,****: p<0.000)
(#:p<0.05, ##:p<0.01, ###:p<0.001, ####;p<0.000)

On the other hand found that the loss of appetite in patients with tumors, especially when subjected to the treatment, which leads to a decrease in the levels of dietary antioxidants, which increases the consumption GSH (Sheeh, 2010) (Nakayamma *etal*., 2011).

The Level of the total protein

The results of comparing the total protein TP in the blood serum of women with breast cancer which not have chemotherapy was significantly higher at the level of Probability ($p \leq 0.01$) its level (9.76±1.251) µm /l when compared with normal women, as shown in Figure.(8) reaching the total protein level (7.472± 1.612) µm /l , the results shown women who have taken doses of chemotherapy first dose increase significantly at the level of probability ($p<0.001$) in the total protein ratio of them (10.84±1.831) µm /l .



Fig(6)Total protein level for normal women, women with breast cancer and who have taken the chemotherapy

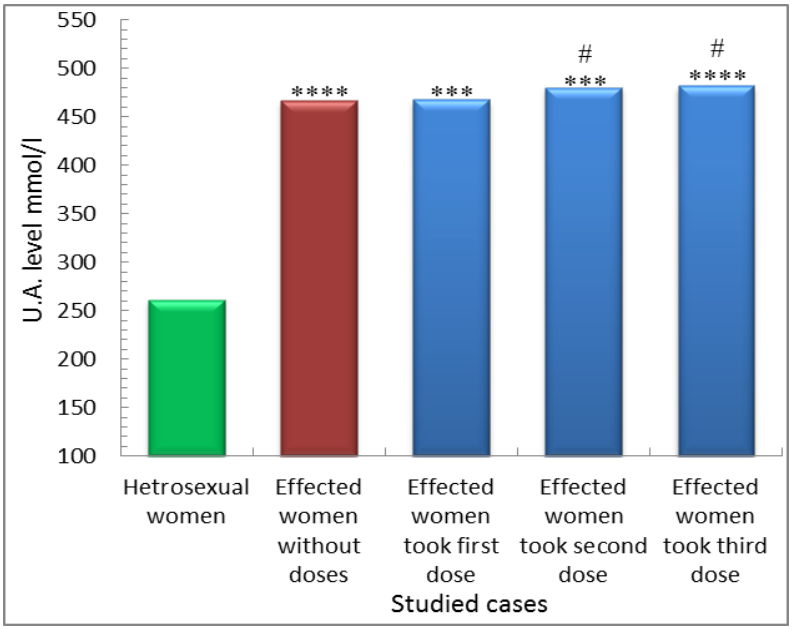
(*:p<0.05, **:p<0.01,***: p<0.001,****: p<0.000)
(#:p<0.05, ##:p<0.01, ###:p<0.001, ####;p<0.000)

The results also showed an increase significantly at the same level of probability for women who took the second dose and the third when compared with countrol group reaching the level (10.33±2.13) µm /l (10.11±1.031) µm /l , respectively Based on the results in our study indicate an elevated level of total protein may be due to the crash of the cells and leakage of cellular contents into the vaccine causing a rise in the level of total protein or as a result of increased globulins increasingly represented in many cancer cases as a result cause of vomiting that accompany malignant diseases of infectious disorders (suga and Tamura, 1972).

The results indicated when women patients taken three doses of chemotherapy show significant increase in the total protein ratio compared with effected women without doses at ($p<0.01$) .But the result for women who took the first dose was indicated while at ($p<0.05$) was indicatedfor women who took the second and third doses . The reason for these increase may have been due to the chemotherapy treatment role lymphocytes in the formation globulins which in turn leads to increased blood serum protein to compensate for the decline in globulins (Fatima, 2013)

The level of Uric acid

The results shown that women with breast cancer display increase significantly at the level of probability ($p<0.000$) reached the level (14. 20± 2.72) µm /l , compared with the control group of women reaching level (10. 21±1.32) µm /l and also the results showed an increase significantly when taking all doses. where women who have taken the first dose reached the level (467.61± 8.24) µm /l , (480.33±8.31) µm /l for women that they were taken away the second dose and at a level of probability ($p <0.001$) and also women who took their third dose showed a significant rise in the level of probability ($p <0.000$), reaching (482.11±9.933) µm /l .



Fig(7) Uric acid levels in normal women, women with breast cancer and who took the chemotherapy

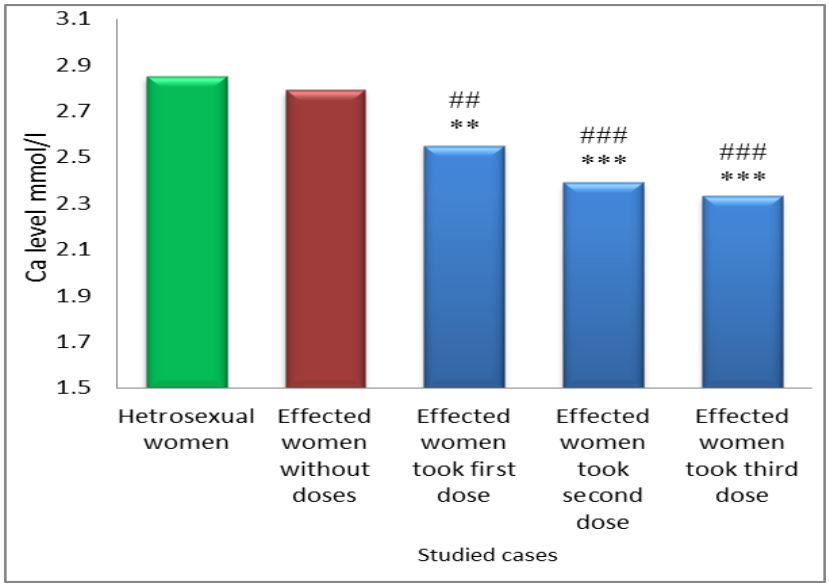
(*:p<0.05, **:p<0.01,***: p<0.001,****: p<0.000)
(#:p<0.05, ##:p<0.01, ###:p<0.001, ####;p<0.000)

The results showed women treated with chemotherapy who take the first dose was not significant increase when compared with women with breast cancer while women who took the second dose, and the third, there are significantly higher at (p<0.05).

The high level of uric acid when compared with normal women may be because of injury of cancer leads to increased metabolism of purines (rules nitrogen component consisting of nucleic acid crashes DNA), and the high level of uric is evidence of the destruction of the cancer cells, where studies have shown that uric acid is a ring important link between cancer disorders and inhibition of the effectiveness of the enzyme xanthine oxidoreductase (XOR) inside the tumor cell (Fini *etal*., 2012). These results agreed with (Ames *etal*., 1981) that an increase in uric acid, a sign of increased oxidative stress , also may be due to rise among patients who were treated with chemotherapy treatment is that these substances have a significant impact on the overall causing inflammation renal glomeruli, one of the side effects of these treatments causing a buildup of uric acid in the blood serum (Vidhya *etal*.,2010). As one of the studies pointed to the effect of taking doses of chemotherapy for breast cancer patients who have taken the chemotherapy, which leads to increased production of free radicals as a result of the consumption of antioxidant treatment in serum (Abdel-Salam *etal*.,2011). As noted one study that giving the mice a drug Doxorubicin, one of the types of chemotherapy for cancer patients with multiple doses cause deterioration in kidney function and increased permeability of the capillaries in the glomerulus renal (Soliman *etal*.,2014)

The level of calcium

The study of the impact of cancer on the level of calcium showed the level of calcium decline significantly for women with breast cancer, reaching the level (2.79±0.01) mmol/l when compared with normal women level attained (2.85±0.125) mmol/l while the level (2.55±0.067) mmol/ l for women who have taken the first dose as the results showed a significant decrease in the level of probability (p<0.01). women who have taken the second dose third dose have shown a significant decrease in the level of probability (p<0.001) reaching the level (2.39±0.043) mmol/l and (2.33±0.011) mmol/l, respectively, as shown in Figure (8). But when you make a comparison between the women who have taken the chemotherapy dose for the first shows a significant decrease at (p<0.01) when compared with infected women also effected women without doses effected who took second and third doses showed a significant decrease at (p <0.001) when compared with women with breast cancer without doses.



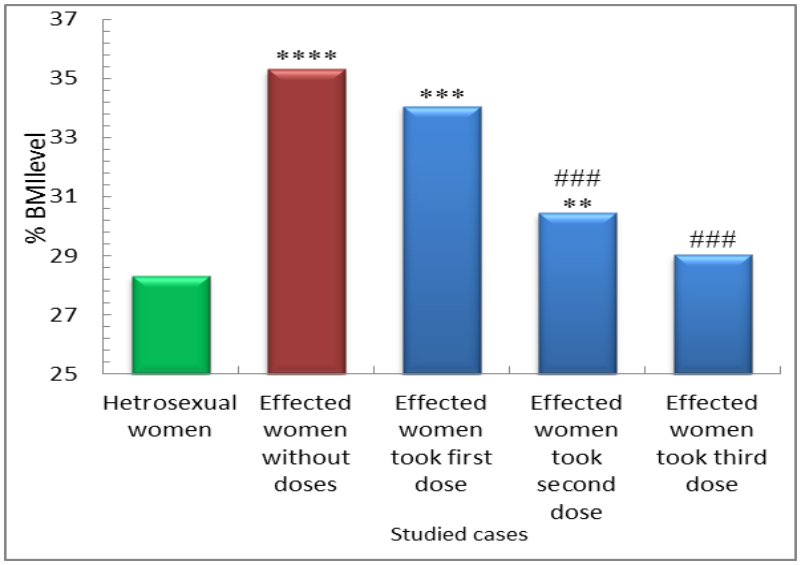
Fig(8) level of calcium for normal women , women with breast cancer and who took the chemotherapy

(*:p<0.05, **:p<0.01,***: p<0.001,****: p<0.000)
(#:p<0.05, ##:p<0.01, ###:p<0.001, ####;p<0.000)

The reason may be due to that there is a direct correlation between the incidence of cancer and the level of calcium in the blood serum, where a recent study found that the level of calcium is linked to the likelihood of developing breast cancer as the calcium is the second messenger for transfer of signals between cells through its participation in regulating of Reproduction and apoptosis of the cell and influence calcium receptors in the thyroid , kidneys and other tissues as colon and breast then influence the secretion of thyroid hormone, kidney, calcium receptor has an important role in maintaining the calcium concentration inside and outside the cells and regulation of many cellular processes including proliferation of cancer cells (Yadav etal., 2012) , it may also attributed the cause to be (Toxal) one kind of chemical treatments that work mechanism programmed death of cancer cells affects the distribution of calcium inside and outside the cells, therapy causing flow of calcium into the cell , in addition to the breast cancer patients suffer from bone destruction and lack of calcium absorption intestine (Pan *etal*.,2014; Coombes *etal*,1977), this has relation of the spread of cancer cells. (Almquist *etal*., 2009) , generally chemotherapy types have effect on calcium channels effort in cell membranes and makes them more permeable, allowing the entry of calcium into cells, causing reducing its level in the blood serum (Florea and Busselberg, 2009)

The level of BMI

Also the results shown that there were high significant at the level of probability (p<0.000) for women with breast cancer, which reached the level of BMI to have (35.313±0.051)% compared with normal women, reaching the level (28.31±1.08)%, as well as women who have taken the first dose (34.06±1.07)% at a level of probability (p<0.001) as well as who take them the second dose was (30.44±2.077)% at a level of probability (p<0.01), but women who took the third dose showed not significant increase which reached (29.03±2.31)% when compared with normal women



Fig(9) BMI level for normal women , women with breast cancer and who took the chemotherapy

(*:p<0.05, **:p<0.01,***: p<0.001,****: p<0.000)
(#:p<0.05, ##:p<0.01, ###:p<0.001, ####;p<0.000)

Also the results shown these women who took the first dose have not significant decrease when compared with women with breast cancer without doses while women who have taken the second dose and third dose have shown a significant decrease at (p<0.001) when compared with infected women without doses, the reason may be due the level of BMI has relation with human breast cancer through several mechanisms, including its increased may affect the immune system and the factors that regulate cell growth, such as growth IGF-1, and sex hormones like Estrogen where the height increases the activity in adipose tissue surrounding (Singh and Jangra,2013) (American cancer Society, 2015). Other studies showed that BMI height has the role in the incidence of breast cancer which is linked to 25% with an increased likelihood of developing breast cancer, although the mechanism of BMI link and the risk of injury is still not clear, but some studies have pointed to an increase fatty tissue may lead to increased cell division and promote tumor also found that higher BMI has an effect on the metabolism of chemotherapy like Tamoxifen , low BMI after taking the treatment may be due to the negative effects that accompany chemotherapy Vomiting , nausea and loss of appetite after taking the third dose affecting body weight. (Berclaz *etal*.,2004) also showed another study on the effect of multiple doses on the hormone leptin (satiety hormone), which has a role precisely body mass (Escribano *etal*.,2014)

The Study Molecular of Genetics Gene for Enzyme Paraoxonase 1 Gene(Exon4)

The results of the study of genes responsible on the types of enzyme paraoxonase1 of exon PON1 (Exon 4) for women, normal and female patients with breast cancer and then take them chemotherapy first dose second dose third dose which included this study also identified the mutations in the genetic analysis of mutations of genes and the study included the following steps.

DNA extraction

It was extracted the DNA of 93 blood samples, including 63 women patients with breast cancer and follow-up after she took them the first dose second dose third dose of chemotherapy, and 30 blood samples of women naturalist group control , it was measured the concentration and purity of DNA by using spectrophotometric analysis Nano – drop device . (Rinaldi *etal*.,2014)

PCR and gel electrophoresis

The product of Polymerase chain reaction technique PCR are tested by using electrophoresis technique using Agarose gel at concentration of 2% and using DNA safe stain dye to show the outputs clearly where packets

DNA appeared (bands DNA) clearly as in Figure(15) PON1(Exon4) where there is no deformation smear did not include any of the shows on the gel packs prepared layer.

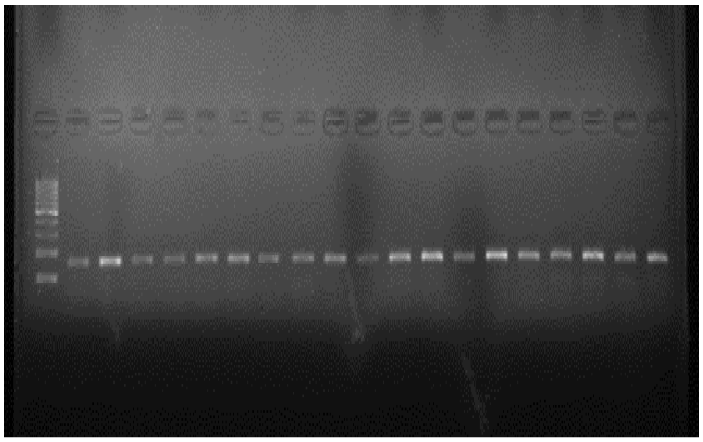


Fig 10. Products analysis shows the polymerase chain reaction by using electrical immigration agarose2% and dab using (Safe stain) of the PON1Exon4

Sequencing

It was found the sequence analysis for 4model of the enzyme PON1, which is used sequence sequential technique for same samples selected within Exon No.(4) in a gene PON1, also sequential sequence analysis technique are made and compare the results studied categories in website (NCBI) National center for biotechnology in formation.

The Study Molecular of Genetics Enzyme Paraoxonase1 Gene (Exon4)

The Sequence technique shows of exon4 for women naturalist and women with breast cancer and women showed after she took them the first dose (Query sequence) and compared with the original source of the sequence of DNA of Exon (reference sequence) figure (11) for model natural for women of Exon4PON1 gene Sequencing where It notes the lack of a mutation when compared with the original source of exon
CGCGAGCAACTTAAATACTTCTGATGGACCTGAATGAAGAAGATCCAACAGTGTTGGAATTGGGG
ATCACTGGAAGTAAATTTGATGTATCTTCATTTAACCCTCATGGGATTAGCACATTCACAGATGAA
AACT

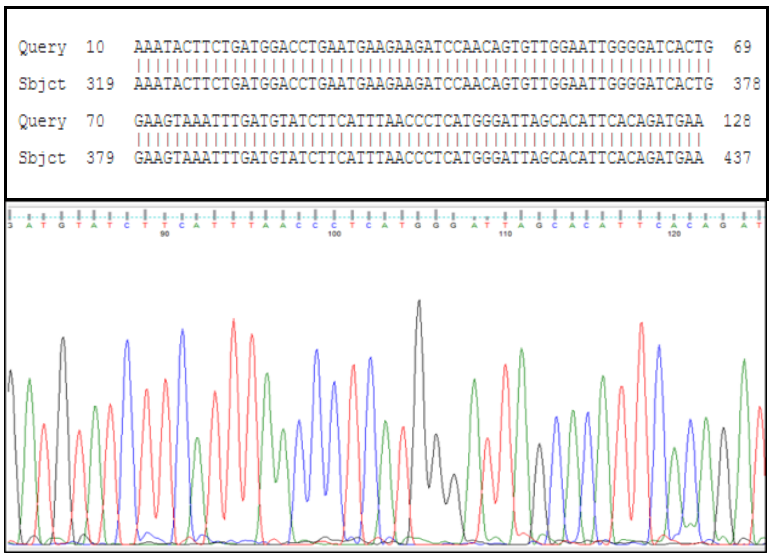
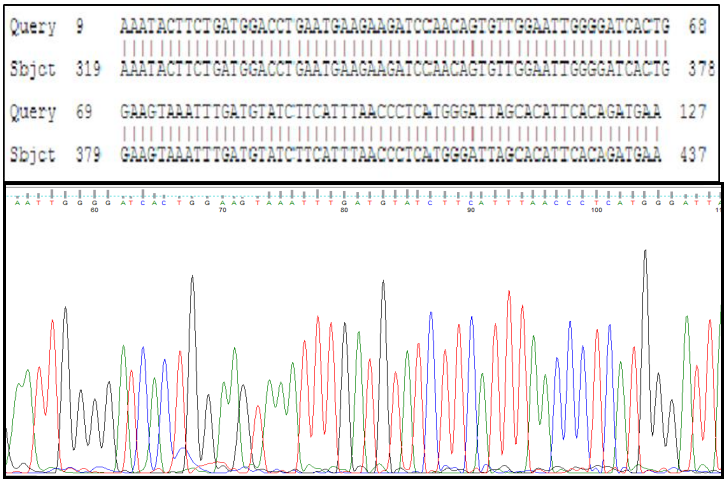


Fig 11. Sequence sequential enzyme PON1 (Exon 4) for the control group (normal women)

CAGAGAACGCAAATACTTCTGATGGACCTGAATGAAGAAGATCCAACAGTGTTGGAATTGGGGAT
CACTGGAAGTAAATTTGATGTATCTTCATTTAACCCTCATGGGATTAGCACATTCACAGATGAAAA



Fig(12)Sequence sequential of enzyme PON1 (Exon4) for a group of patients (women with breast cancer) who did not take their chemical potions

The results showed in Figure(13) three different types of mutations within exon No. (4) of the gene PON1 for women with breast cancer (after she take the first dose of chemotherapy) which represent the first mutation (a mutation type deletion mutation) and the so-called Frame shift mutation (shift) deleted one nitrogen base and lead to change all codons after the deletion site therefore change all amino acids in the multi-acid chain amino , delete the nitrogenous base at position 610 (this mutation lead to the loss of amino acid (TAC) Tyr) the second mutation for the type of (point mutation) of the substitution mutation which is two mutations at site 647 (silent mutation where codon variable is giving same amino acid (glycine Gly) GGC to GGA) and location 648 (Thr. acid turns into amino acid Ser. TCT (ACA)). also found the third mutation type of deletion mutation at position 659 (loss of amino acid Asp (GAC)).

CAAACTAGAGTAGATATTCTGCCACCAGAGATGCACTATTTTACTGACTCCCTCCTGTTGATTTTTT
GAGATGATCTTGGCACATCGCTGGATTATGTTCTTTTCTACAGCCCAAGGGAGGTTAAAGTGGTGG
CCAAAGGATTTTGTAGTGCCAATGGGATCACACTTCTCACTCAGACCAGAA

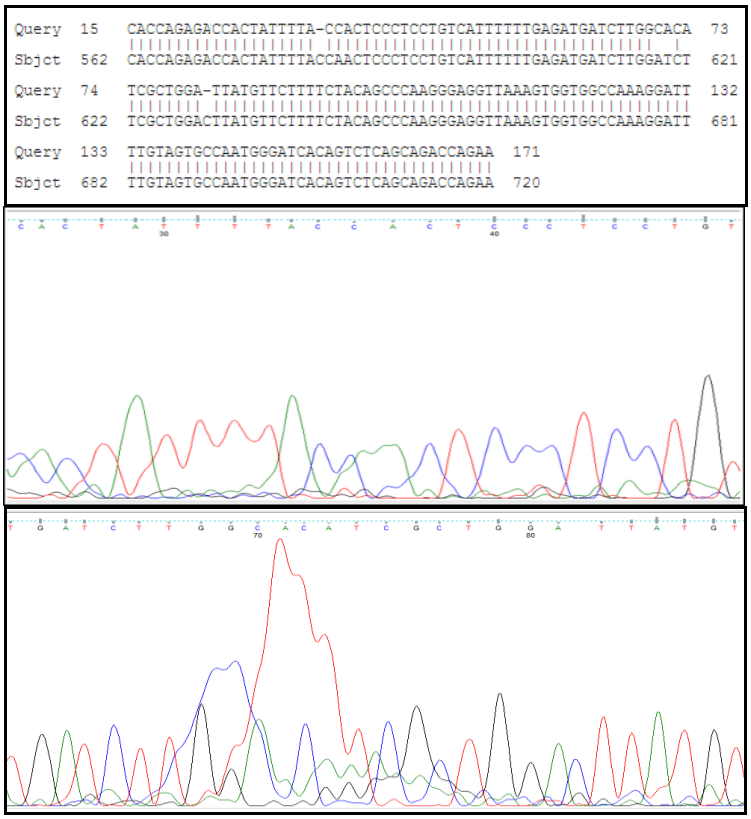


Fig 13. Sequence sequential for enzyme PON1 (Exon 4) for a group of women with breast cancer who took the first dose of the chemical

References

Abdel-Salam O., Youness E., Hafez H.,(2011)" The antioxidant status of the plasma in patients with breast cancer undergoing chemotherapy"Open Journal of Molecular and Integrative Physiology,1:pp:29-35.

Abu-Bedair F.A.,El-Gamal B. A. ,Ibrahim N.A., El-Aaser A.A.,(2003) " Serum lipids and tissue DNA content in egyptian female breast cancer patients " Jpn. J. Clin. Oncol. ,33(6) ,278-282.

Agachan B, Yilmaz H, Karaali Z, Isbir T.(2004)" Paraoxonase 55 and 192 polymorphism and its relationship to serum paraoxonase activity and serum lipids in Turkish patients with non-insulin dependent diabetes mellitus" *Cell Biochem Funct* ,22 : 163-8.

Aghvami T., Djalali M.,Keshavarz A., Sadeghi M.R.,Zeruati H.,Yeganeh H.H., Negahdar M., (2006) " Plasma level of antioxidant vitamins and lipid peroxidation in breast cancer patients " Iranian J. Pubi. Health , 35(1),pp.42-47.

Aksoy-Sagirli P, Cakmakoglu B, Isbir T, Kaytan SE, Kizir A, Topuz E, et al.(2011)" Paraoxonase-1 192/55 polymorphisms and the risk of lung cancer in a Turkish population" *Anti cancer Res,* 31(6): 2225–9.

Ali, A.B., Zhang, Q., Lim, Y.K., Fang, D., Retnam, L., (2003)"Expression of major HDL associated antioxidant PON-1 is gender and regulated during inflammation" *Free Rad Bio & Med*, 34, 824-829.

Almquist M., Anagnostaki l.,Bondeson L. , Berrgguist S., Landberg G., Malina J., Manjer J., (2009) " Serum calcium and tumour aggressiveness in breast cancer: a prospective study of women" Eur. J. Cancer Prev.;18(5) :354-360.

Alwan N., Al Attar W, Eliessa R, Al-Madfaie Z and Nedal,F.,(2012)" Knowledge and practices of women in Iraqi universities on breast self examination" *Eastern Mediterranean Health Journal*, Vol.18, Issue.7,pp:- 742-748

American cancer Society (the History of cancer)1-800-227-2345 (2014) www.bordet ,be/en/presentation /history / cancer / cancer1 , htm.

American Cancer Society 2015(Body weight and cancer risk) ,31-800-227-2345.

American cancer Society(2014)"Kideny cancer (Adult)-Renal cell carcinoma" www.bordet ,be/en/presentation// Renal cell carcinoma

Ames B.N., Cathcart R., Sehwiers E., Hochstein P., (1981) " Uric acid provides an antioxidant defense in humans against oxidant –and radical –caused aging and cancer : a hypothesis " Proc. Natl. Acad. Sci . USA;78(11) :6858-6862.

Arpaci A, Gormus U, Dalan B, Berkman S, Isbir T. (2009)"Investigation of PON1 192 and PON1 55 polymorphisms in ovarian cancer patients in Turkish population" *In Vivo,* 23(3):421–4.

Askar T.K., Buyukkebletici O., (2012) "Paraoxonase : Anew biochemical marker of oxidant –Antioxidant status in atherosclerosis" ,Oxidative stress,145-154

Aviram, M., Rosenblat, M.,(2004)"Paraoxonases1,2 and 3,oxidative stress, and macrophage foam cell formation during atherosclerosis development" *Free Radical Biology &Medicine*,37,304–1316

Aynacioglu AS, Cascorbi I, Mrozikiewicz PM, Nacak M, Tapanyigit EE, Roots I.(1999)" Paraoxonase 1 mutations in a Turkish population"*Toxicol Appl Pharmacol,*157(3): 174–7.

Bayrak, T., Bayrak, A., Demirpençe, E., Kılınç, K., (2005)"Yeni bir kardiyovasküler belirteç adayı: Paraoxonaz," *Hacettepe Medical Journal*, 36,147-151.

Benhar M.,Engelberg D., Levitzki A., (2002) " Roes, Stress-activated kinases and Stress Sig naling in cancer "EMBO reports

Berclaz G., Li S., Price K.N.,Coates A.S., Castiglione-Gertsch M., Rudenstam C. -M.,Holmberg S.B.,Lindtner J., Erzen D., Collins J.,*etal*.,(2004)" Body mass index as a prognostic feature in operable breast cancer: the international breast cancer study group experience "Annals of Oncology , 15:875-884.

Blecher E., Chaney-Graves K. , Desantis C., Edward B. . Ferlay J. , Forman D., Grey N., Harford J., Kramer J.,Mc Mikel A., etc., (2011) "Global cancer Facts and food"2nd ed,American cancer society, pp:1-55

Coombes R.C., Powles T.J., and Joptin G.F.,(1977)"Calcium metabolism in breast cancer " Proc. Roy. Soc. Med.,Vol.70,pp195-199.

Costa, LG., Vitalone, A., Cole, TB., Furlong, CE.(2005)"Modulation of paraoxonase (PON1) activity"*Biochemical Pharmacology,* 69: 541-550.

Coughlin S., and Cypel Y., (2013) " Epidemiology of breast cancer in women " Chapter 2 Springer Science Business Media New York , pp.13-34.

Dai-Hua F, Cong-Hai F, Qiang J, Bo-Xiang Q, Juan L, Lu W. (2012)"Differential effects of paraoxonase 1 (PON1) polymorphisms on cancer risk: evidence from 25 published studies" *Molecular Biology Reports,* 39:6801–9.

Das U.,(2002) "A radical approach to cancer " Med. Sci. Monit., 8(4) : RA79-RA92

Davies HG, Richter RJ, Keifer M, Broomfield CA, Sowalla J, Furlong CE.(1996)"The effect of the human serum paraoxonase polymorphism is reversed with diazoxon, soman and sarin" *Nat Genet ,14* : 334-6.

Durrington P.O., Mackness B., Mackness M.L.(2001) " Paraoxonase and atheresclerosis" arteriosclerosis , *thrombosis and vascular biology*;21:473-480

Elkiran E., Mar N. , Aygen B., Gursu F.,Karuoglu A., and Koca S., (2007) " Serum paraoxonase and arylesterase activities in patients with lung cancer in turkish population " Bio. Med. Cancer, pp1-8.

Escribano B., Moreno A., Tasset I., Tunez I.,(2014)" Impact of light /dark cycle patterns on oxidative stress in an Adriamycin-induced nephropathy model in rats " Plos one,Vol.9,Issue.5,pp.1-10.

Fairbanks V, Klee GG, (1986)."Biochemical aspects of hematology. In: N.W. Tietz Editor" Textbook of clinical chemistry W.B. Saunders, Philadelphia. 1532–1534.

Fatima T., (2013) " Circulatory proteins in women with breast cancer and their chemotherapeutic responses" Pakistan J. Zool., Vol.45, pp: 1207 -1213.

Fini M.A., Elias A., Johnson R.,and Wright R.,(2012) " Contribution of uric acid to cancer risk , recurrence , and mortality " Clinical and Translational Medicine J. ; 1 : 16 (http://www.clintransmed.Com/ content / 1 /1/16.

Florea A., Busselberg D., (2009) " Anti-cancer drugs interfere with intracellular calcium signaling " Neurotoxicology .,30,803-810.

Furiong C., Richter R., Chapline C., and Crabb J., (1991) " Purification of rabbit and human serum paraoxonase " Biochemistry ,30,10133-10140

Gallicchio L, McSorley MA, Newschaffer CJ, Huang HY, Thuita LW, Hoffman SC, et al.(2007) "Body mass, polymorphisms in obesity-related genes, and the risk of developing breast cancer among women with benign breast disease" *Cancer Detect Prev* 2007; 31(2): 95–101.

Gouedard C.,Koum-Besson N.,Barouki R., Moral Y.,(2003)"Opposite regulation of the human paraoxonase-1 gene PON-1 by fenofibrate and statins" Molecular Pharmacology ,Vol.63, pp.945-956.

Gutowski M., and Kowalczyk S., (2013) "Astudy of free radical chemistry :their role and pathophysiological significance "ACTA Biochemical polonical ,Vol.60,No.1, pp1-16.

Guyton A.C., and Hall j.E.,(2001)" Texbook of medical physiology " 11th ed.,Elsvier.,Sauuders com .,philndelphia, pp.46-47.

Han, X., Shen, T., Lou, H. (2007)"Dietary Polyphenols and Their Biological Significance" *Int J Mol Sci*. ,8: 950-988.

Hejmadi M. (2010) "introduction to cancer biology "2nd ed.,Momna Hejmadi & bookboon.com, pp.7,22,11,36,66.

Humbert R, Adler DA, Disteche CM, Hassett C, Omiecinski CJ, Furlong CE.(1993)"The molecular basis of the human serum paraoxonase activity polymorphism"*Nat Genet* ,3 : 73-6.

Inal V., Yamanel L., Taskin G., Tanpan S., Comert B., (2015) "Paraoxonase1 activity and survival in sepsis patients " Balkan Med J.,32:PP183-188.

James RW, Leviev I, Ruiz J, Passa P, Froguel P, Garin MC.(2000)" Promoter polymorphism T(-107)C of the paraoxonase PON1 gene is a risk factor for coronary heart disease in type 2 diabetic patients" *Diabetes* ,49 : 1390-3.

Junior A.L.G.,Paz M., De silva L., Carvalho S., Sobral A., Machado K., Ferreira P., Satyal P., (2015) " Serum oxidative stree markers and genotoxic profile induced by chemotherapy in patients with breast cancer : A Pilotstudy " Hindavi Pubishing Corporation ,1-18.

Liaveras G.,Danilo C.,Mercier I.,Daumer K.,Capozza F.,Williams T.M.,Sotgia F.,Lisanti M.P.,and Frank P.G.,(2011)"Role of cholesterol in the development and progression of breast cancer "American Journal of Patbology ,Vol.178,No.1 ,pp402-421

Lurie G, Wilkens LR, Thompson PJ, McDuffie KE, Carney ME, Terada KY, Goodman MT, (2008)"Genetic polymorphisms in the Paraoxonase 1 gene and risk of ovarian epithelial carcinoma" *Cancer Epidemiol Biomarkers Prev*,17(8): 2070–7.

Moren X, Deakin S, Liu ML, Taskinen MR, James RW.(2008)"HDL subfraction distribution of paraoxonase-1 and its relevance to enzyme activity and resistance to oxidative stress" *J Lipid Res.*49 :1246-53.

Nakayamma A., Alladin K., Lgbokw C., White J., (2011) " Systematic review : generating evidence based guide lines on the concurrent use of dietary antioxidant and chemotherapy or radiotherapy " Cancer Invest.; 29(10) : 655-667.

Nicholas,T.,(2013)"Cancer "Hindwi foundation for education and culture pp.17,17,18,47

Noori S., (2012) " On overview of Oxidative stress and antioxidant defense system "Citation Open Access,Nooti;1-8 ,1:413-,109

Nourazarian A., Kangari P., Salmanineejad A., (2014) " Roles of oxidative stress in the development and progression of breast cancer " Asian Pacific Journal of Cancer , Vol.15,4745-4751.

Pan Z.,Avila A., Gollahon L.,(2014)" Paclitaxel induced apoptosis in breast cancer cells through different calcium –regulating mechanisms depending on external calcium conditions"Int. J. Mol. Sci.Vol.15,2672-2694.

Rinaldi C., Anglo R., Ruggeri A., Calabro M., Scimone C., and Sidoti A.,(2014) " PON1 gene polymorphisms Cancer :A case –control study in apopulation from sothern italy " J. Mol. Biomarker and Diagnosis ,5: ISSNE.2 ;2-6.

Rzymowska J., (2011) " Effect of cytotoxic chemotherapy on serum lipid levels in breast cancer patients " Pathobiology ;67,: 129-132.

Searles Nielsen S, Mueller BA, De Roos AJ, Viernes HM, Farin FM, Checkoway H. (2005)" Risk of brain tumors in children and susceptibility to organophosphorus insecticides: the potential role of paraoxonase (PON1)" *Environ Health Perspect*,113(7): 909–13.

Sharma N., Rai M.,(2014)" Lipid peroxidation status in different stages of breast cancer women treated by chemotherapy"European Academic Research,Vol.11,Issue.4,pp.5661-5668.

Sheeh A.,(2010) "Comparative study of renoprotective effects of captopril and aminophylline against cisplatine induced nephrotoxicity in rats " Al-Kindy College. Med. J. 6 , (1),pp:1-12.

Singh M., and Jangra, B.,(2013) "Association between body mas index and Risk of Breast cancer among femaler of north India "South Asia J. Cancer ,2(3):121-125

Sokal R.R. and Rohlf F.J.,(2012)"Biometry : the principles and practice of statistics in biological research " 4th ed. W.H.Freeman and Co.newyork.937pp.

Soliman H., Ahmed R., Gomaa H., Ali A.,(2014)"Assessment of the chemo-preventive effects if variouse plant constituents against doxorubicin-induced toxicity in rats"J. of American Science ;10(9):153-164.

Sorenson RC, Bisgaier CL, Aviram M, Hsu C, Billecke S, La Du BN.(1999)" Human serum paraoxonase/arylesterase's retained hydrophobic N-terminal leader sequence associates with HDLs by binding phospholipids: apolipoprotein A-I stabilizes activity" *Arterioscler Thromb Vasc Biol,*19 : 2214-25.

Suga S., and Tamura Z., (1972) " Analysis of serum protein changes in patients with special reference to x-globulin fractions " Cancer Research ,32,426-429.

Tang WH, Hartiala J, Fan Y, Wu Y, Stewart AF, Erdmann J. (2012)" Clinical and genetic association of serum paraoxonase and arylesterase activities with cardiovascular risk" *Arterio scler Thromb Vasc Biol* , 32(11):2803–12.

Uchiyama M, Mihara M, (1978)"Determination of malonaldehyde precursor in tissues by thiobarbituric acid test" *Anal. Biochem*.,34:271-278.

Vekic J, Kotur-Stevuljevic J, Jelic-Ivanovic Z, Spasic S, Spasojevic-Kalimanovska V, Topic A, *et al.* (2007)"Association of oxidative stress and PON1 with LDL and HDL particle size in middle-aged subjects" *Eur J Clin Invest,*37 :715-23.

Vidhye R., Jayanthi M., Kanagavalli U.,(2010) " Protective effect of captoxrll against cisplatine in auced nephrotoxicity in rats " Pharmacology online.,1:12-15.

Yadav N.K.,Poudel B.,Thanpari C., Koner B., (2012) " Assessment of biochemical profiles in premenopausal and postmenopausal women with breast cancer " Asian Pacific J. Cancer Prev., Vol.13, pp.3385-3388.

Yildirim S., Kisa F., KLarddeniz A.,Yildirim A., Karakor A., Can I., Kara A., Simsek N.M.,(2012)"effects of pomegranate seed extract on liver paraoxonase and bcI-XLactivites in rats treated with cisplatin" Journal of Medicinal Plants Research ,Vol.6(12),pp.2317-2323.

Yılmaz N.(2012)" Relationship between paraoxonase and homocysteine: crossroads of oxidative disease" *Arch Med Sci* ,8(1): 138–53.

Author Information

Luma Abdalmunim Baker
University of Mosul
Mosul / Iraq
Contact e mail: Nahida_alchalabi@yahoo.com

Hazha Jamal Hidayet
University of Mosul
Mosul / Iraq

Nahida S. Al-Chalabi
University of Mosul
Mosul / Iraq