International Journal of PharmTech Research
CODEN (USA): IJPRIF, ISSN: 0974-4304, ISSN(Online): 2455-9563
Vol.9, No.11, pp 324-333, 2016

# FASL Gene Polymorphism with Oxidative Stress of Iraqi Females with Breast Cancer

**Ferdous A. Jabir[1], WatheqJaber[2] and Mohammed AbdulwahabAti Al-Askeri[3]***

**[1]Biochemistry Dept., College of Medicine, University of Al-Qadisiyah, Iraqz**
**[2]Chemistry Dept., College of Science, University of Al-Qadisiyah, Iraq**
**[3]Mdical Biotechnology Dep., College of Biotechnology, University of Al-Qadisiyah,Iraq.**

**Abstract :** The aim of current study is to explore the probable role of FASL gene polymorphism and oxidative stress in breast cancer occurring in the Iraqi female patients. Blood samples from patients and healthy volunteers were collected and used for the succeeding experiments. The results were obtained by (T-ARMS PCR) technique for the FASL gene were shown there was a significant difference in 2124 A/G polymorphism of FASL between treated group and control group (P less than 0.05).
**Keyword :** Brest Cancer, FASL gene, MDA, Oxidative stress,T-ARMS PCR and Polymorphism.

## Introduction

Cancer disease begins when normal cells set up to grow out of ordinary body control. Cells in almost any part of the body can become cancer under certain inside and outside factors, and may spread to other parts of the body. Breast cancer is "a malignant tumor that begins in the breast cells"[1].

The key risk factors interrelated to breast cancer susceptibility are related to clinical history, elongated periods of hormonal, age, family history, lifestyle factors and delayed first pregnancy2.The other important risk factor for beginning and development of breast cancer is oxidative stress[3,4], breast tumors are normally livinginan unbelievably pro-oxidative surroundings, while the mammary gland is abundance in close adipose tissue. Therefore, override ROS speedily acts on the surrounding area of lipid yielding many of energetic metabolites that can regulate a broad variety of cellular processes. Well- known examples are derived of lipid peroxidation like low-molecular-weight aldehydes that have reported as new supposed indicators of the oxidative status in breast cancer patients are Malondialdehyde, 8-F2-isoprostanes and 4- hydroxynonenal[5-7].These prooxidant surroundingsappear to be essential through the early stages of disease for probably cancer developing to an advanced stage, besides it may influence adaptability tumor cells against the reactive species (RS) derived from antineoplastic drugs[8].Oxygen radicals are connected with diverse steps of breast carcinogenesis during formation of adducts, dealing with oncogenes and tumor suppressor genes by DNA damage which affects immunologicalmechanisms[9]. The indirectcreation of "DNA adducts" through launching autocatalytic peroxidation of lipid which cause a great mixture of possible genotoxic subside products including Aldehydes, Malondialdehyde (MDA), Peroxyl radicals (ROO) and Alkoxyl (RO). Thus, DNA is a permanently became damaged and oxidatively amended. Mutations can cause by an oxidative error that is not repaired, increasing the risk of carcinogenesis[10].

8-hydroxy-2'-deoxyguanosine (8-OHdG) is a very important marker for determining the impact of exogenous or endogenous oxidative affects to DNA. It was studied as a potential factor in initiation and development of carcinogenesis also[11].In last few years, 8-OHdG was targeted mostly in several studies as a biomarker for the estimation of endogenous oxidative DNA damage amount and as a risk factor for various diseases involving cancer. The estimation of 8-OHdG based on a simple technique for the estimation of oxidative DNA damage. Many studies showed levels of urinary 8-OHdG were considerably higher in breast cancer patients[12].

Increase expression of a number of genes by reason of certain polymorphisms or mutations increases the breast cancer occurrence risk. In view of the fact that mutations that are familiar to increase breast tumors in families are very rare, recognition of these SNPs is so essential. The most important positions which contain mutations are "STK11, RAD50, BRCA1, BRCA2, CDH1,ATM, PTEN, CHEK2, PPM1D, MLH1, MSH2, MRE11, BARD1, MSH6, MUTYH, NBN, PMS1, PMS2, BRIP1, RAD51C, and TP53"the existence of SNPs in those genes increases the breast cancer risk and correlated diagnostic indicators are between the most credible for estimating prognosis in breast cancer[13].

FAS or "Apo-1 or CD95" is an active element of the death receptor group, which performs an important role in apoptotic signaling mechanisms[14].FAS protein reacts with its "FAS-ligand (FASL)" to motivate the cell death signal and hence stimulate apoptotic cells death[42].

Additionally, the sense mutations in "FAS and FASL" genes that weaken apoptotic signal transduction were revealed to be linked with a bigger risk of a range of cancers[15]. Consequently the "FAS/FASL system" is possibly important during beginning, growth, and progression of cancer. SNPs, which may have the possibility to change the expression levels of FAS and/or FASL, were suggested to be considerable in the genetic predisposition to cancer[15].This gene locates on site1q23 and composed of 4 exons across 8 kb and encoding 281 amino acids[16]. Many studies scrutinized the role of FAS and FASL gene polymorphisms in the causes of various cancers involving "breast, cervix, bladder, prostate, lung, head and neck and esophagus"[17].

Therefore, FASL is sometimes over-expressed in various human tumors, including "breast cancer"[18]. So, we assumed that the polymorphisms in the encoding sequence, "FASL 2124 A/G" may raise the breast cancer probability in anIraqi population.

## Materials and methods

Blood samples have been collected from twenty-one healthy persons and thirty-one patients, the healthy individuals divided into two groups those with no previous family background of cancer (15 persons) considered as a "control group" and those with a family history of breast cancer (sisters of some patients) as related group (6 persons). Blood samples were collected from patients who confirmed by pathohistological examination having breast cancer ;those also divided into two groups those were taken therapy (31 persons) as treated group and those without therapy as an untreated group (15 persons) .The patients and healthy groups are matched in age as shown in the table (1). Five milliliters of venous blood samples were withdraw and collected in tubes, two milliliters were mixed in Na-EDTA tubes, and three milliliters were collected in gel tubes, the gel helps in good separating for serum. The serum and whole blood kept frozen at (-20°C) until used for different assays and DNA extraction.

Lipid peroxidation, estimated by MDA formation was determined by the method of Guidet, B. and Shah. (1989)[19].The nitrite/nitrate concentrations were determined by Griess reagent according to the method described by Chaea, S.Y. *et al.* (2004)[20].Nitrite/nitrate concentration in serum was determined using the reference standard curve that made.

The levels of 8-OHdG in sera were estimated using ELISA assay by using the 8-OHdG kit, (Elabscincecompany, USA ).

FASL polymorphisms were detected by using "tetra-amplification refractory mutation system–polymerase chain reaction (T-ARMS PCR)"as described previously by Mohammad Hashemi et al.(2013)[21].

PCR product sizes were: "300 bp for the A allele, 197 bp for the G allele and 438 bp for control band" using specific PCR primers as it shown in the table (2).

The statistic analysis between the frequencies of alleles and genotype distributions of the four groups were set by the binary logistic regression at *p*- value<0.05 using SPSS18 software through calculating the odds ratio (OR) and their confidence intervals values (95% CI) and $\chi^2$values.

Comparison among groups for biochemical tests conducted by paired sample T-test at (*p*-value< 0.05) and as a significant variation.

**Table(1):The PCR oligonucleotides used in current study**

| Primer name | Sequence (5'–>3') | Tm (ºC) |
|---|---|---|
| FasLgene polymorphism A/G (rs5030772) | Sense outer primer<br>5'GGTCTTCTTGGATTAGTCACCCAACTT 3'<br>Antisense outer primer<br>5' CACTTTCCTCAGCTCCTTTTTTTCAG 3'<br>Sense inner primer<br>5' CTGCAGTTCAGACCTACATGATTAGTCTG 3'<br>Antisense inner primer<br>5' TTAAAACCGTAAATGGCAACAGTCTAAAAT 3' | 57 |

**Table (2): Samples and groups used in the experiment**

| No. | Group | Age (mean ±SE) | Range of Age | No. of Cases |
|---|---|---|---|---|
| G1 | Control | 42.33 ± 3.54 | (23-65) | 15 |
| G2 | Related | 46.67 ± 4.13 | (26-53) | 6 |
| G3 | Treated | 48.55 ± 1.27 | (33-62) | 31 |
| G4 | Untreated | 46.73 ± 3.06 | (23-65) | 15 |

SE= Standard Error

## Results:

### A-Biochemical tests:

Malondialdehyde (MDA),Nitric Oxide(NO) and 8-Hydroxydeoxyguanosine**(**8-OHdG) levels in the sera of patients who have breast cancer treated and untreated groups (G3 and G4) were indicated to be significantly higher compared to healthy group (G1) at (p-valueless than 0.001) as illustrated in table (3). Also, when compared the levels in sera of treated group (G3) to the untreated group (G4) were found to be different, less than G4, this difference is statistically significant (P less than 0.05), While when compared the levels in sera of related group (G2) to control group (G1) were found to be different, but this difference is not statistically important (P greater than 0.05), as shown in the table (3).

**Table (3): The mean and comparison of concentrations of biochemical tests**

| variables | G1, n=15 (Control) | G2, n=6 (Related) | G3, n=31 (Treated) | G4, n=15 (Untreated) | Comparisons | P value |
|---|---|---|---|---|---|---|
| NO Cons. (mean ±SE) | 33.52 ± 1.04 | 40.26 ±7.47 | 54.56 ± 3.27 | 113.56 ±19.24 | G2 with G1<br>G3 with G1<br>G4 with G1<br>G4 with G3 | 0.506<br>0.001 *<br>0.001*<br>0.006 * |
| MDA Cons. (mean ±SE) | 2.38 ± 0.05 | 2.78 ± 0. 15 | 3.02 ± 0.10 | 3.4 ± 0.16 | G2 with G1<br>G3 with G1<br>G4 with G1<br>G4 with G3 | 0.068<br>0.001 *<br>0.000 *<br>0.001 * |
| OHdG Cons. (mean ±SE) | 121.67±16.30 | 188.6 ±59.75 | 243 ± 19.19 | 377.80±161.24 | G2 with G1<br>G3 with G1<br>G4 with G1<br>G4 with G3 | 0.485<br>0.000 *<br>0.000 *<br>0.036 * |

(*) Represent the value is statically significant,(SE) Represent Standard Error

**B- Association between various polymorphisms with possible risk of breast cancer**

The current study examined the probable association between rs5030772polymorphisms with the risk of breast cancer .The study was conducted by means of "Tetra-Amplification Refractory Mutation System–Polymerase Chain Reaction (T-ARMS-PCR)"[21].Figure (1) shows the PCR product of FASL gene polymorphism for the four groups (G1,G2,G3and G4), and the PCR product size is, G allele (197 bp),an allele (300 bp) and control band (438 bp) .

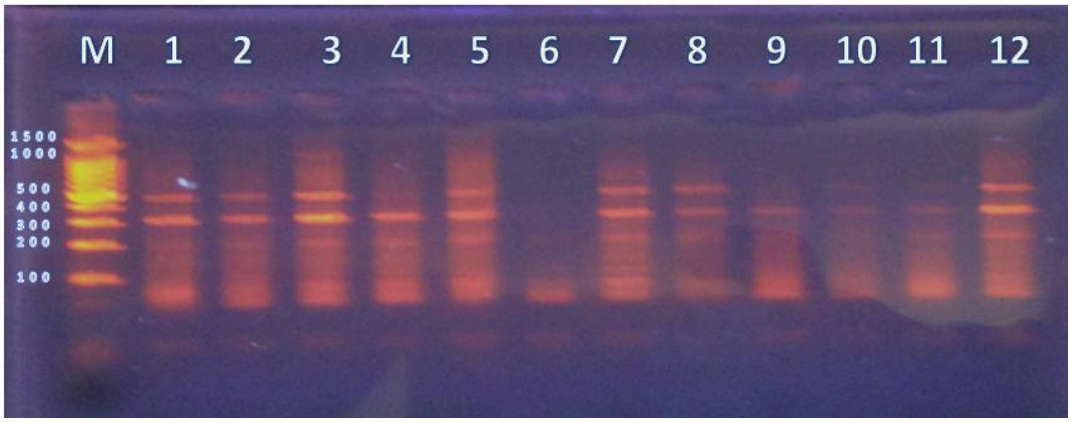


**Figure (1):Electrophoretic pattern of T-ARMS-PCR for detection of polymorphisms for FASL gene [2124 A/G, rs5030772]; lane No. of (1, 2) refer to G4 (Untreated group);lane(3,12) refer to G1(Control);lane (4,5,6,7,8,9,10) refer to G3 (Treated) ;lane (11) refer to G2Related(sister of the patient No. 10); M: Refer to (100bp DNA Ladder).**

**1. Related(G2)with Control(G1)**

The genotype frequencies and distributions of allele towards 2124 A/G polymorphism of FASL in related (G2) and control (G1)groups are revealed in the table (4). The results indicated that the frequency of "GG" is 0%, "AG" is 100% and "AA" is 0% in G1. The frequency of 2124 A/G polymorphism in G2 was 0% for "GG", 100 % for "AG" ,and 0% for AA. There was no significant difference in 2124 A/G polymorphism of FASL between G2 and G1 (p=0.233).As shown in the table (4),for G1 the "A" allele frequency was 53.3% and the "G" allele frequency was 46.7%, and in the G2 the frequencies were 50% for "A" allele and 50% for "G" allele. The frequency of allele was not significantly different among G1and G2 (p=1.000).

**Table (4): Genotypes and allele distributions for "FASL gene" polymorphism in G2 and G1**

| Polymorphisms FAS/FASL (-124 A/G) | G1 Control)( N=15(%) | G2 Related)( N=6(%) | $\chi^{2a}$ | P-value [a] | OR (95%CI) [b] | P value [b] |
|---|---|---|---|---|---|---|
| AA | | | 0.000 | 1.000 | $1.0^{ref}$ ($1.0^{ref}$) | |
| AG | 15(100) | 6(100) | | | c | c |
| GG | | | | | c | |
| A allele | 15(50) | 6(50) | 0.000 | 1.000 | $1.0^{ref}$ ($1.0^{ref}$) | |
| G allele | 15(50) | 6(50) | | | 1.000 (0.262-3.815) | 1.000 |

[V]alues about genotype distribution, [b]Are calculated relative to subjects with the A/A genotypes and Anallele c the variables is constant

**2. Treated G3 with Control(G1)**

The genotype frequencies and allele distributions of 2124 A/G polymorphism of FASL in treated (G3) and control (G1) groups are shown in the table (5). The results showed that the frequency of "GG" is 0% , "AG" is 100% and "AA" is 0% in G1. The frequency of 2124 A/G polymorphism in G3 was 0% for "GG", 82.4% for "AG" and 9.7% for "AA". There was a significant difference in 2124 A/G polymorphism of FASL between G3 and G1.As shown in Table (5) in G1, "A" allele frequency was 50% and the "G" allele frequency was 50%, and in the G3 the frequencies were 58.8 % for "A" allele and 41.2% for G allele. Logistic regression analyses proved that the 2124 A/G"AG" and "GG" genotypes were linked to breast cancer risk compared with the AA genotype ($P$ = 0.044). There was a significant difference when comparing the "G" allele with the reference "A" allele ($P$ = 0.480).

**Table (5) :Genotypes and allele distributions of "FASL polymorphism gene" in G3 and G1**

| Polymorphisms FAS/FASL (-124 A/G) | G1 Control)( N=15(%) | G3 Treated)( N=17(%) | $\chi^{2a}$ | P-value [a] | OR (95%CI) [b] | P value [b] |
|---|---|---|---|---|---|---|
| AA | | 3(9.7) | 4.068 * | 0.044 * | $1.0^{ref}$ ($1.0^{ref}$) | |
| AG | 15(100) | 14(82.4) | | | 1.731 (0.000) | 0.999 |
| GG | | | | | | |
| A allele | 15(50) | 14(41.2) | 0.501 | 0.479 | $1.0^{ref}$ ($1.0^{ref}$) | |
| G allele | 15(50 | 20(58.8) | | | 0.700 (0.260-1.882) | 0.480 |

[V]alues about genotype distribution, [b]Are calculated relative to subjects with the A/A genotypes and Anallele,*the value is statically significant

**3. Untreated(G4) with Control(G1)**

Genotype frequencies and allelic distributions of 2124 A/G polymorphism of FASL in treated (G4) and control (G1) groups are illustrated in Table (6). The results showed that the frequency of "GG" is 0%, "AG" is 100% and "AA" is 0% in G1. The frequency of 2124 A/G polymorphism in G4 was 0% for "GG", 100% for "AG",and 0% for "AA". There was no significant variation in 2124 A/G polymorphism of FASL in between G4 and G1($P$ = 1.000).As shown in Table (6) in G1, "A" allele frequency was 50% and the "G" allele frequency was 50%, and in the G4 the frequencies were 50 % for "A" allele and 50% for "G" allele. There was a significant difference when comparing the "G" allele with the reference "A" allele ($P$ = 1.000).

**Table (6):Genotypes and allele distributions of "FASL polymorphism gene" in G4 and G1**

| Polymorphisms FAS/FASL (-124 A/G) | G1 Control)( N=15(%) | G4 Untreated)( N=11(%) | $\chi^{2a}$ | P-value [a] | OR (95%CI) [b] | P value [b] |
|---|---|---|---|---|---|---|
| AA | | | 0.000 | 1.000 | $1.0^{ref}$ ($1.0^{ref}$) | |
| AG | 15(100) | 11(100) | | | c | c |
| GG | | | | | c | |
| A allele | 15(50) | 11(50) | 0.000 | 1.000 | $1.0^{ref}$ ($1.0^{ref}$) | |
| G allele | 15(50) | 11(50) | | | 1.000 (0.333-3.005) | 1.000 |

[V]alues about genotype distribution, [b]Are calculated relative to subjects with the A/A genotypes and An allele, cthe variables is constant

**4. Treated(G3) with Untreated(G4)**

Genotype frequencies and allelic distributions of 2124 A/G polymorphism of FASL in treated (G3) and control (G4) groups are indicated in the table (7). The results indicated that the frequency of "GG" is 0% , "AG" is 100% and "AA" is 0% in G4. The frequency of 2124 A/G polymorphism in G3 was 0% for "GG", 82.4% for "AG" ,and 9.7% for "AA". There was the difference in 2124 A/G polymorphism of FASL in between G3 and G4 but this difference is not significant (p = 0.07).As shown in the table (7) in G4, "A" allele frequency was 50% and the "G" allele frequency was 50%, and in the G3 the frequencies were 58.8 % for "A" allele and 41.2% for "G" allele .There was a "significant difference" when comparing the "G allele" with the reference "A" allele (*P* = 0.517).

**Table (7):Genotypes and allele distributions of "FASL polymorphism gene" in G4 and G3**

| **Polymorphisms FAS/FASL (-124 A/G)** | **G4 Untreated)( N=11(%)** | **G3 Treated)( N=17(%)** | $\chi^{2a}$ | **P-value [a]** | **OR (95%CI) [b]** | **P value [b]** |
|---|---|---|---|---|---|---|
| AA | | 3(9.7) | 3.224 | 0.070 | $1.0^{ref}$ ($1.0^{ref}$) | |
| AG | 11(100) | 14(82.4) | | | 1.269 (0.000) | 0.999 |
| GG | | | | | | |
| A allele | 11(50) | 14(41.2) | 0.420 | 0.517 | $1.0^{ref}$ ($1.0^{ref}$) | |
| G allele | 11(50) | 20(58.8) | | | 0.700 (0.238-2.060) | 0.517 |

[V]alues about genotype distribution, [b]Are calculated relative to subjects with the A/A genotypes and An allele

## Discussion

Reactive oxygen species "ROS" have been identified to take part in a significant function in the initiation and development of carcinogenesis and have been involved in carcinogenesis in animal models as well as humans[22]. Former studies showed that perpetual alteration of genetic material due to oxidative damage causes carcinogenesis [23]. It has been demonstrated that ROS are correlated with the various steps of carcinogenesis either in the course of structural DNA damage or effecting tumor suppressor genes, oncogenes and immunological mechanisms [22].

Our study has proved higher levels of MDA in breast cancer patients, Malondialdehyde (MDA) is the final product of lipid peroxidation, as a result of its high cytotoxicity, it has been proposed to act as a

carcinogenic agent and a tumor promoter. Increase in MDA levels in carcinoma could be used as an important parameter to the risk of this disease fundamentally due to its double roles as a tumor promoter and a mutagen [22]. Elevated amounts of the final products of lipid peroxidation have shown in tumor tissue itself obviously pointing to the source of elevated "MDA" levels in patients who have with cancer[24]. Similarly, Mohini Aiyengar Tupurani et al (2013)[22]and Aghvani et al, (2006)[25] demonstrated average MDA levels were in patients who have breast cancer.

Our study has also revealed higher levels of NO in breast cancer patients.NO is a free "radical gas, water-soluble" which perform an important role in a variety of pathological and physiological mechanisms. It is made by a multifarious family of nitric oxide synthase (NOS) enzymes. The role of "NOS" in tumor biology was specified clearly. It was suggested to have both tumor-promoting impacts in addition to tumoricidal which rely on its real-time reaction, concentration, and site [26]. These high NO levels may cause by the elevated NOS II activity which was catalyzed by a host protection system versus tumor development. NO-"derived reactive nitrogen species" and NO stimulate nitrosative and oxidative stress which caused DNA damage and the inhibition of DNA repair enzymes during indirect or direct mechanisms[27].It was suggested that the biological impacts of NO could be mediated by the productions of diverse NO metabolites. "NO" can cause damage to DNA through the production of $N_2O_3$ and peroxynitrite ($ONOO^-$). Peroxynitrite can cause oxidation and nitration for DNA and may because of single-strand DNA breaks during the attack on the sugar-phosphate backbone. Different Studies have explained that levels of NO can be implicated in stimulating or preventing the cancer etiology[28].

Increased NO-generation in a cell may choose mutant p53 cells and participate to tumor angiogenesis by up-regulating VEGF (vascular endothelial growth factor). Also, NO may modulate mechanisms of tumor DNA repair by up-regulating p53, poly (ADP-ribose) polymerase (PARP). A comprehension at the molecular level of the role of NO in cancer will have deep therapeutic implications for the diagnosis and treatment of cancer[28]. Our study has also authenticated higher levels of NO in breast cancer patients compared to controls,these results are similar to those obtained previously studies [22,29].

Our study showed that an association between risk of breast cancer and elevated 8-OHdG levels.

8-OHdG caused by ROS-induced a wide range of oxidative damage of DNA, is widely utilized as anindicator of oxidative damage to DNA, which is essential in carcinogenesis and mutagenesis processes [30]. Urinary levels of 8-OHdG are therefore considered as an oxidative stress general biomarker. Because elevated levels were noticed among patients with prostate cancer[31], lung cancer [32], urine and serum and 8-OHdG levels in patients with breast cancer[31,33,34] and colon cancer[35,36], when compared to healthy controls.8- OHdG may be beneficial as an evidence of cancer risk. 8-OHdG can be determined by enzyme-linked immunosorbent assay (ELISA), immunohistochemistry and high-pressure liquid chromatography (HPLC)[30,46].

The increase of 8-OHdG levels in patients considers a marker of elevated oxidative stress, lacked antioxidant defense or not insufficient repair of oxidative DNA damage. 8-OHdG is one of the high-oxidized bases was characterized. 8-OHdG in DNA could direct to misincorporation of adenines adverse the 8-OHdG lesion thus inducing "G: C to T: A" mutations in genomic DNA. Elevated 8-OHdG levels in breast cancer patients may be attributable to defects in 8-oxoguanine DNA glycosylase-1 (OGG1) repair gene causing aggregation of mutations, cancer appearance and progression[37,47].

Karki et al.(2014)reported that high sensitivity, specificity, and accuracy of bio-indicators of oxidative stress comprising serum 8-OHdG can be utilized as distinguishable markers for reliable diagnosis of breast cancer[38].These results are similar to those obtained previously studies[12,31,37]. The chemotherapy has reduced the oxidative stress and levels of 8-OHdG in patients who have breast cancer. This is consistent with the previous study[39].

Our study also investigates the association between −607C/A (rs1946519) and 2124 A/G (rs5030772) polymorphisms with risk of breast cancer. In our study, we found no relationship between FAS expression in the untreated group (G4) or primary stages of breast cancer and the risk of breast cancer, while we found an association between FAS expression in the treated group (G3) or advanced stages and the risk of breast cancer. It was confirmed that the FAS need in the primary tumor was correlated with perilymphatic fat permeation and metastasis process either to the bones or to the surrounding lymph nodes [40,41,49].

FAS protein reacts with "FAS-ligand (FASL)" to initiate the death signal molecules and hence stimulate apoptotic cells death [42]. The interactions between "FAS and FASL" were shown to be included in the creating of an immune response status against tumor by stimulating "FAS-mediated apoptosis in tumor-specific lymphocytes"[43,50]. Additionally, the sense mutations in "FAS and FASL genes" that weaken programmed cells death signal transduction were proved to be related with an increased risk of a variety of cancers[44]. Many studies investigated the role of "FAS and FASL gene" polymorphisms in the causes of various cancers involving breast, bladder, prostate, lung, head, cervix, neck and esophagus[45,48].

## Conclusions

The "FAS/FASL" system possibly important in the beginning, growth, and progression of cancer, and "single nucleotide polymorphisms (SNPs)" which have the possibility to change the expression levels of "FAS and/or FASL" is possible to be considerable in the genetic susceptibility to breast cancer [17].

## Acknowledgement

The authors thank Mr.Mudher Al-Khozay for his technical assistance.

### References

1. American cancer society. What is breast cancer? 2015. Sep 18, [18 Mar 2015].
2. Garcia M et al. Global Cancer Facts & Figures. Atlanta, GA: American Cancer Society, 2007
3. Halliwell B and Gutteridge JMC: Free radicals in biology and medicine. New York, Oxford University, 4th edition, 2007.
4. Pani G;Giannoni E;Galeotti T and Chiarugi P: Redox-based escape mechanism from death: the cancer lesson. Antiox Redox Signal. 2009; 11(11): 2791-2802.
5. Manello F; Tonti GA;Pagliarini S; Benedetti S;Canestrari F; Zhu W; Qin W and Sauter ER: The 8-epimer of prostaglandin F (2 alpha), a marker of lipid peroxidation and oxidative stress,is decreased in the nipple aspirate fluid of women with breast cancer. Int J Cancer. 2007;120(9): 1971-1976.
6. ManelloF; Tonti GA and Medda V: Protein oxidation in breast microenvironment: nipple aspirate fluid collected from breast cancer women contain increased protein carbonyl concentration.CellOncol. 2009;31: 383-392.
7. Gago-Dominguez M; Jiang X and Castelao JE: Lipid peroxidation and the protective effect of physical exercise on breast cancer. Med Hyp.2007;*68*: 1138-1143.
8. Farias J W; Furtado FS;Guimaraes SB;Silva Filho AR and Vasconcelos PR: Oxidative stress parameters in women withbreast cancer undergoing neoadjuvant chemotherapy and treated with nutraceutical doses of oral glutamine. Acta Cir Bras.2011;*26(1-2)*: 82-87.
9. Kang DH: Oxidative stress, DNA damage, and breast cancer. AACN Clin Issues. 2002;13(4): 540-549.
10. Arsova- Sarafinovska Z;Eken A;Matevska N;Erdem O;Sayal A;Savaser A;Banev S, Petrovski D;Dzikova S;Georgiev V;Sikole A;Ozgök Y;Suturkova L;Dimovski AJ;Aydin A: Increased oxidative/nitrosative stress and decreased antioxidant enzyme activities in prostate cancer. ClinBiochem, 2009;42(12): 1228-1235.
11. *Halliwell, Barry*:Oxidative stress and cancer: have we moved forward?*Biochem. J. 2007*; *401 (1): 1–11*.
12. Kuo HW; Chou SY; Hu TW; Wu FY; Chen DJ.: Urinary 8-hydroxy-2-deoxy guanosine (8-OHdG) and genetic polymorphisms in breast cancer patients. Mutat Res .2007; 631:62–8.
13. Kooshyar Mohammad Mahdi; Mohammad Reza Nassiri; KhadijehNasiri :Hereditary Genes Associated with Breast Cancer.2013;14.6.3403.
14. Andera L: Signaling activated by the death receptors of the TNFR family. Biomed Pap Med FacUnivPalacky Olomouc Czech Repub. 2009; 153:173–180..
15. Wajant H: The Fas signaling pathway: more than a paradigm. Science. 2002;296:1635–1636.
16. Takahashi T; Tanaka M;Inazawa J; Abe T;Suda T; et al.: Human Fas ligand: gene structure, chromosomal location and species specificity. International immunology.1994;6: 1567–1574.
17. Yang M; Sun T; Wang L; Yu D; Zhang X; et al. : Functional variants in cell death pathway genes and risk of pancreatic cancer. Clin Cancer Res 2008;14: 3230–3236.
18. Müllauer L;Mosberger I;Grusch M, Rudas M and Chott A: Fas ligand is expressed in normal breast epithelial cells and is frequently up-regulated in breast cancer. J Pathol.2000;190:20–30.

19. Guidet B, Shah SV. Enhanced in vivo H2O2 generation by rat kidney in glycerol-induced renal failure. American Journalof Physiology. 1989 Sep;**257**(3 Pt 2):F440–F445.
20. Chaea Y, LeeM , Kim S and Bae Y: Protection of Insulin Secreting Cells from Nitric Oxide Induced Cellular Damage by Crosslinked Hemoglobin. Biomaterials, Vol. 25, No. 5, 2004, pp. 843-850
21. Hashemi M;Fazaeli A;Ghavami S;Eskandari-Nasab E;Arbabi F; et al. : Functional Polymorphisms of FAS and FASL Gene and Risk of Breast Cancer – Pilot Study of 134 Cases. PLoS ONE 2013;8(1): e53075.
22. Mohini Aiyengar Tupurani; Chiranjeevi Padala; Rajesh G Kumar; Kaushik Puranam; Sanjeeva Kumari; Surekha H Rani: Oxidative stress/Nitrosative stress in breast cancer .Hyderabad, Ab , 2013; India- 500 007
23. Farias JW; Furtado FS;Guimar菊s SB;Silva Filho AR;Vasconcelos PR: Oxidative stress parameters in women with breast cancer undergoing neoadjuvant chemotherapy and treated with nutraceutical doses of oral glutamine. Acta Cir Bras.2011; (26)1: 82-87.
24. Gönenç A, Özkan Y, Torun M, Şimşek B. Plasma malondialdehyde (MDA) levels in breast and lung cancer patients. Journal of clinical pharmacy and therapeutics. 2001 Apr 1;26(2):141-144.
25. Aghvami T;Djalali M;Keshavarz A;Sadeghi MR;ZeraatiH;SadrzadehYeganeh H;Negahdar M: Plasma level of antioxidant vitamins and lipid peroxidation in breast cancer patients. Iranian J Publ Health. 2006;35(1): 42- 47,.
26. Choudhari SK;Chaudhary M;Bagde S;Gadbail AR; Joshi V. Nitric oxide and cancer: a review. World J SurgOncol. 2013;11:118.
27. . Muntané J; la Mata MD: Nitric oxide and cancer. World J Hepatol. 2010;2:337–344.
28. Xu W; Liu LZ;Loizidou M, Ahmed M; Charles IG: The role of nitric oxide in cancer. Cell Res.2002;12(5-6): 311- 320,.
29. YaraRashed;Tawfik R. Elkhodary;Amal M. El-Gayar;Liala A. Eissa.:Evaluation of Serum Levels of HER2, MMP-9, Nitric Oxide, and Total Antioxidant Capacity in Egyptian Breast Cancer Patients: Correlation with Clinico-Pathological Parameters.2013; doi: 10.3797/scipharm.1306-18 .
30. Wu LL;Chiou CC; Chang PY; Wu JT: Urinary 8-OHdGa marker of oxidative stress to DNA and a risk factor for cancer, atherosclerosis and diabetic. *ClinChimActa.*2004; 339: 1-9.
31. Calişkan-Can E;Firat H;Ardiç S;Simşek B; Torun M;Yardim-Akaydin S: Increased levels of 8-hydroxydeoxyguanosine and its relationship with lipid peroxidation and antioxidant vitamins in lung cancer. *ClinChem Lab Med.*2008; 46 (1): 107-112.
32. Gackowski D;Speina E;Zielinska M;Kowalewski J;Rozalski R;Siomek A;PaciorekT;Tudek B;Olinski R : Products of oxidative DNAdamage and repair as possible biomarkers of susceptibility to lung cancer. *Cancer Res.*2003; 63: 4899-4902.
33. Atukeren P;Yavuz B;Soydinc HO;Purisa S;Camlica H;Gumustas MK;Balcioglu I: Variations in systemic biomarkers of oxidative/ nitrosative stress and DNA damage before and during the consequent two cycles of chemotherapy in breast cancer patients. *ClinChem Lab Med* 2010; 48(10): 1487-1495.
34. Himmetoglu S;Dincer Y;Ersoy YE;Bayraktar B;Celik V;Akcay T: DNA oxidation and antioxidant status in breast cancer. *J Invest Med*2009;57(6): 720-723.
35. Obtulowicz T;Swoboda M;Speina E;Gackowski D;Rozalski R;Siomek A;Janik J;Janowska B;CieslaJM;Jawien A;Banaszkiewicz Z;Guz J;Dziaman T;Szpila A;Olinski R;Tudek B: Oxidative stress and 8-oxoguanine repair are enhanced in colon adenoma and carcinoma patients. *Mutagenesis* 2010; 25 (5): 463-471.
36. Sato T; Takeda H;Otake S;Yokozawa J;Nishise S;Fujishima S;Orii T; Fukui T; Takano J; Sasaki Y;Nagino K;Iwano D;Yaoita T;Kawata S: Increased plasma levels of 8 hydroxydeoxyguanosine are associated with development of colorectal tumors. *J ClinBiochemNutr,*2010;47 (1): 59-63,.
37. Sanaa A. El-Benhawy and Heba G. El-Sheredy : Evaluation of the role of oxidative DNA damage marker 8-hydroxy-2 deoxyguanosine in patients with breast cancer . 2014;2320-5407.
38. Karki K;Pande D;Negi R;Khanna S;Khanna RS;Khanna HD : Expression of Serum Toll-Like Receptor 9 and Oxidative Damage Markers in Benign and Malignant Breast Diseases. DNA Cell Biol 2014; ahead of print doi:10. 1089/dna. 2405.
39. O. M. E. Abdel-Salam *et al*. :The antioxidant status of the plasma in patients with breast cancer undergoing chemotherapy.2011 ; 29-35.
40. Bebenek M;Dus D;KozlakJ : Fas expression in primary breast cancer is related to neoplastic infiltration of perilymphatic fat. Adv Med Sci 2008; 53: 49–53.

41. Sun T; Miao X; Zhang X; Tan W;Xiong P; et al. : Polymorphisms of death pathway genes FAS and FASL in esophageal squamous-cell carcinoma. Journal of the National Cancer Institute. 2004; 96: 1030–1036.
42. Kim R; Emi M; Tanabe K; Uchida Y and Toge T: The role of Fas ligand and transforming growth factor β in tumor progression: molecular mechanisms of immune privilege via Fas-mediated apoptosis and potential targets for cancer therapy. Cancer. 2004;100:2281–2291..
43. Reichmann E: The biological role of the Fas/FasL system during tumor formation and progression. Semin Cancer Biol. 2002;12:309–315.
44. Wajant H: The Fas signaling pathway: more than a paradigm. Science. 2002;296:1635–1636.
45. YangM; SunT; WangL; YuD; ZhangX; et al.: Functional variants in cell death pathway genes and risk of pancreatic cancer. Clin Cancer Res 2008;14: 3230–3236.
46. Al-Terehi1, M. al-kilabi2, L.H., AL –Mamoori1, A., Al-Jboori, M.J., Al-Saadi1, A H. Zaidan H.K. Some Heavy Metals Concentrations in Tumor Tissue, International Journal of ChemTech Research CODEN (USA): IJCRGG 2016,9, ,03 ,407-411.
47. Mona Al-Terehi1, Haider K. Zaidan2, Ayad M.J. AL –Mamoori2; Ali Hmood Al-Saadi2, IsraaHarjan Effective of different factors on trace elements concentrations in Iraqi lactating mother'smilknternational Journal of Pharm Tech Research,Vol.8, No.10, pp 151-157, 2015.
48. HAYTHEM ALI ALSAYIGH, ENDOTHELIAL NITRICOXIDE GENE POLYMORPHISMS IN IMPEDED BREAST CANCER TISSUE, Int J Pharm Bio Sci 2016 July ; 7(3): (B) 557 – 561.
49. Mona Al-Terehi1, Rana Ghaleb2, Shaimaa A. Al-Oubaidy2, Ali H. Al-Saadi1, Haider K. Zaidan,(2015) Study TNF-α gene polymorphism in Type 1 Diabetic Patients Using Amplification Refectory Mutation System (ARMS) technique , JCPS Volume 9 Issue 3. 1107-1111.
50. AizharHamzihHasan (2015)Study Genotype of TNF-αin Breast Cancer Tissue in Iraqi Women, International Journal of PharmTech ResearchVol.9, No.5, pp 387-391, 2016.

*****