



Study Genotype of TNF- α in Breast Cancer Tissue in Iraqi Women

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Abstract : The present study aims to detection TNF-308 genotype in breast cancer tissue using ARMS technique, he results show that highly significant association between gene polymorphisms and cancer, it were (0, 94.44, 5.55) for GG,AA and AG respectively, while it was (0, 4.98, 95.12)% in control with highly significant, the present study concluded that TNF-308 gene polymorphism was association with breast cancer in Iraqi women.

Keywords: breast cancer, TNF-308, gene polymorphisms, ARMS.

Introduction:

Breast cancer (BC) is the most frequent of cancer world. Siegel et al., (1) Its resulted from dis-regulation of cells proliferation which cusses by DNA damage, there are two types of Breast cancers, in situ carcinomas and invasive (or infiltrating) carcinomas. The in situ carcinomas may arise in either ductal or lobular epithelium, but remain confined there, with no invasion of the underlying basement membrane that would constitute extension beyond epithelial boundaries there is extension of the ductal or lobular malignancy beyond the basement membrane that constitutes the epithelial border, then the malignancy is considered invasive (or infiltrating) ductal or lobular carcinoma.

The potential for metastases and ultimately death occurs in invasive disease (2)

Cancer is heterogeneous disease in regards to its clinical, histological and molecular profile. It diagnosis by different methods but only estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (Her2) are typically used for BC diagnosis in routine clinical practice. BC intrinsic subtypes, including triple-negative breast cancer (TNBC) and Her2⁺, luminal A, and luminal B BCs, are characterized by immunohistochemistry (IHC) and have important differences in phenotype and prognosis (3,4).

Genetics plays a limited but important role as a risk factor for breast cancer. Only 5% to 6% of breast cancers are considered hereditary (5) BRCA-1 and BRCA-2 account for an estimated 80% of hereditary breast cancer, but again, this only represents 5% to 6% of all breast cancers. BRCA-1 and/or BRCA-2 positive women have a 50% to 85% lifetime risk of developing breast cancer and a 15% to 65% risk of developing ovarian cancer, beginning at age 25 (6).

Tumor necrosis factor- α (TNF- α), a multi-functional cytokine, it is used in inflammatory responses and have major role in the pathogenesis of inflammatory, autoimmune and malignant diseases (7). It used in stimulation angiogenesis during endothelial cell proliferation and the enhancement of the expression of other pro-angiogenic factors (8) addition of these it induces expression of adhesion molecules involved in the invasion (9,10). increment of plasma levels of TNF- α have been detected in many malignancies and are often associated with poor prognoses (11-14).

Knockdown of the TNF- α gene is associated with cell proliferation inhibition and apoptosis in TNBC(1).

Because of the TNF- α is a tumorigenic *in vitro* and *in vivo*, it can be hypothesized that the TNF- α -308G>A polymorphism may have an important function in different BC subtypes and be related to the characteristics of different BC subtypes, especially in highly aggressive BC (15).

Materials and methods

About 30 breast cancer embedded tissue was collect from histopathology unit and 30 blood sample of control. DNA was extracted from embedded tissue according to the leaflet of Genaid manufacture with modification, in briefly 40 mg of tissue was put in 1.5ml tube contain 300 μ l of xylene, then it grinded used micropestel for 2 min, then it fallow guides in the kit leaflet, in the last step DNA was eluted using d H₂O. the consternation and purity estimated using nanodrpe (optezen). The primer used in present study are Forward 5'-TCT CGG TTT CTT CTC CAT CG-3, and two reverse 5'-ATA GGT TTT GAG GGG CAT GG-3 for G allele, 5'-AAT AGG TTT TGA GGG GCA TGA-3 for A allele (16), the PCR condition are summarized in table (1)

Table (1) PCR conditions for ARMS Technique

Subjects	Cycles	Heat	Time
Pre-denaturation	1	94	5 min
Denaturation	10	94	15 sec
Annealing		65	50 sec
Extension		72	40 sec
Denaturation	25	94	20sec
Annealing		59	50 sec
Extension		72	50 sec
Final extension	1	72	7 min

Results and discussion

The results of present study show significant variation between alleles in patient and control gouge, GG allele was no present in patient and control while AA was more present in patients than control (94.4%) (5%) respectively, GA was less in patients it was (5.55)% while it was (95%) in control group table(2) figure (1).

The results show that ARMS technique which used in present study was easy, low cost and high specify for detection gene polymorphisms than other technique like PCR-RFLP, PCR-SSCP and PAMSA which used in our DNA laboratory because it depending on touchdown PCR and no post PCR manipulation, the review of literature for used different technique to detection TNF polymorphisms show different types of techniques like PCR-RFLP polymerase chainreaction-restriction fragment length polymorphism which used by Park, (17)Kohaar, (18) and Gomez Flores-Ramos (19), also Kamali-Sarvestani, (20)and Karakus, (21) used ASO-PCRpolymerase chain reaction sequence-specificprimers method, all these study found variety of genotype that depending on population and the loci of the gene which may be effected by other factors.

Inflammatory responses play critical roles in tumor development, including pathogenesis, invasion, and metastasis, thus, inflammatory cytokines are important components of tumor progression. TNF- α and its receptors belong to the TNF-TNFR superfamily, these genes interaction regulate inflammation and the invasive activity increment and metastatic potential of tumor cells (22) , thus TNF -308 was choose in present study to determinate polymorphism in Iraqi women.

The genotype of TNF-308 in breast cancer tissue in present study don't deal with other studies such asSarvestaniet al., (23) found no differences in the TNF- α and TNF- β alleles and genotypes frequencies between breast cancer patients and control subjects and The correlations between TNFA or TNFB alleles or genotypes and clinic pathological indices were also insignificant in Iranian females. In ChineseXu et al ., (22) suggest that rs1061622 and rs1061624 in TNFRSF1B may affect breast cancer risk, and SNPs in TNFRSF1A are associated with the clinical features of breast cancer.

In meta-analysis there are two studies reported that the TNF- α -308GA and AA genotypes were significantly associated with decreased BC risk in Caucasians(24,25).

Table (1) distribution of allele frequency and genotype of TNF in case-control study

GG	0	0	30.401*	0.0001	0.0031 0.0002 to 0.0534
AA	94.44	4.98			
GA	5.55	95.12			
A	0.972	0.525			
G	0.028	0.475			

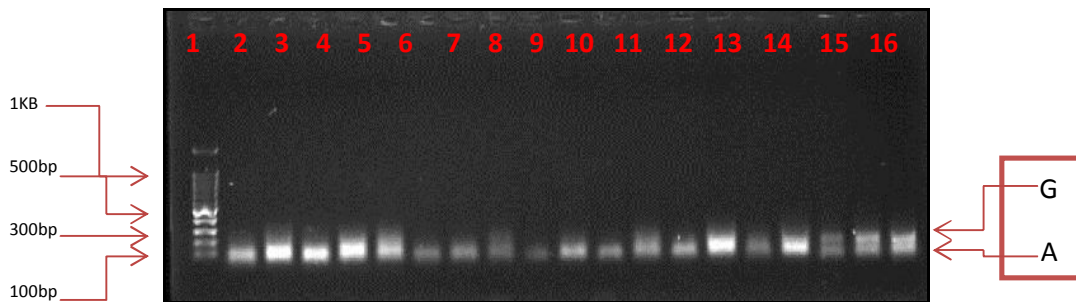


Figure (1) electrophoresis pattern of TNF - α genotype in breast cancer tissur, lane 1 DNA marker (100bp-1kb), lane 2-13 AA genotype, lane 14-16 AG genotype.

Karakuset *al.*, (21) found in Turkish population that *TNF- α* -308 genotype had no effect in breast cancer susceptibility by Genomic DNA extraction from EDTA-preserved peripheral venous blood for patients and controls using a salting-out method and genotype was analyzed by polymerase chain reaction, allele-specific oligonucleotide polymerase chain reaction, and restriction fragment length polymorphism. From all mention above the differences between present study and previous studies may be some reasons, in the first of all Iraqi environments which contaminated by weapons and Remnants of arms and radioactive isotopes because of the war in last decades, this causes damage in DNA and disruption of repair system genes like BRAKA1 and BRAKA2 which increment in last decades (6) , breast cancer have been in the first rank of cancer types in Iraq it accounted for 16% of all Iraqi patients (26) in the other hand life style have major role in diseases incidence and development, in Iraq the nutrition was unstable and unhealthy because of decline the stander of living and low educational level.

The variation of association TNF -308 with cancer resulted from genetic of population diversity according to environments, educational, nutrition and mating low. In Iraq the closed mating in the family lead to enhanced genetic predisposition of some disease like cancer, it may found that large family members have been infected in Iraqi population, this can be clarify by that this persons have genetic predisposition to infected cancer which enhanced by environment factors, this study need more investigators about factors that related with cancer incidence in Iraqi population.

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