

## Research Article

### Mitochondrial DNA A10398G Mutation is not Associated with Breast Cancer Risk in a Sample of Iraqi Women

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**Abstract:** The aim of this study was to investigate if there is a relationship between mtDNA polymorphism (A10398G) and breast cancer in a sample of 59 Iraqi women. Breast cancer is the second most common diagnosed cause of cancer death in the developed countries and accounts for 23% of the total cancers. Different studies reported that breast cancer accounts for 14% of all cancer deaths in females. It is well documented that the different factors such as genetics and environment factors are involved in tumorigenesis. Mutations in the mitochondrial DNA D-loop region and somatic mutations are emerging as early genetic markers of cancer. Identification of such markers for breast cancer would prevent late detection and increase the chance of recovery and survival rate. In breast cancer different mtDNA alterations were reported. The A10398G mutation in NADH Dehydrogenase (ND3) a subunit of complex I of the Oxidative Phosphorylation process (OXPHOS) is perhaps one of the most studied mutations with conflicting reports of its association with breast cancer. Genomic DNA was extracted from 21 unrelated women with malignant tumors, 22 women with benign tumors and 16 healthy women blood donors. Subsequently, PCR amplification was performed using specific primers, PCR products were subjected to a suitable restriction enzyme. No genetic variants were identified in mtDNA among malignant tumoral group and controls while 9% of benign tumor cases exhibited the variant. Our finding indicated that A10398G polymorphism cannot be used as a biomarker for breast cancer detection in Iraqi women.

**Keywords:** Benign, malignant, mtDNA, PCR, polymorphism

## INTRODUCTION

According to the most recent Iraqi Cancer Registry in 2010, breast cancer has a propensity in young aged women; this disease is classified as the first amongst malignancies in the Iraqi population (Iraqi Cancer Board and Iraqi Cancer Registry, 2008). In order to establish breast cancer control programs, the World Health Organization (WHO) recommends early detection and screening to reduce associated mortality (WHO). In a cross-sectional, questionnaire-based study comprising 387 Iraqi females and males, 75% of participants believe that early detection is the best way to control the disease occurrence (Alwan *et al.*, 2012).

For this we need to emphasize on the importance of genetic screening approaches and to test potential genetic markers, one approach is to test mitochondrial DNA possible markers for early detection of this disease.

Mitochondria are responsible for the production of the energy needed by our cells in the form of ATP; they play a central role in cellular metabolism (Chan, 2006; Wallace, 2007). The human cells contain hundreds of mitochondria; each harbors several copies of the mitochondrial genome with its own translation

machinery independent of the nuclear genome. Mitochondria DNA (mtDNA) is a double stranded DNA molecule of 16569nt, compactly organized encoding for 37 genes 13 of which are involved in cellular energy production. mtDNA represents <0.1% of total cellular DNA, contains no introns or histones and maternally inherited. The mtDNA mutation frequency is 20 times greater than that of nuclear DNA (Anderson *et al.*, 1981; Ishikawa *et al.*, 2008).

These organelles generate much of the endogenous Reactive Oxygen Species (ROS) and regulate programmed cell death (apoptosis) via the mitochondrial permeability transition pore (Wallace, 2005). Because apoptosis plays a critical role in cancer development and in cellular response to anticancer agents, the significance of mtDNA mutations in cancer is obviously an important area of some investigations (Wang, 2001; Higuchi, 2007; Asari *et al.*, 2007; Lee and Wei, 2009).

mtDNA changes were reported in colorectal, bladder, head and neck, lung, pancreatic, gastric, hepatic, renal, ovarian and breast cancers and haematological diseases among others (Brandon *et al.*, 2006). Several studies have identified mutations in the

non-coding and coding regions of mtDNA and have investigated their potential use as somatic markers for early tumor detection (Carew and Huang, 2002; Miyazono *et al.*, 2002; Parrella *et al.*, 2003; Zhou *et al.*, 2007). The role of mtDNA mutation in tumor formation still needs to be elucidated, mtDNA alterations such as germ line and/or somatic point mutations, mtDNA depletion and Microsatellite Instability (MSI) were reported in most cases of breast cancer (Salgado *et al.*, 2008). The A10398G mtDNA polymorphism has received the most attention (Salgado *et al.*, 2008). The mutation causes a non-conservative amino acid substitution from theormine to alanine NADH Dehydrogenase (ND3) subunit of complex I (Anderson *et al.*, 1981). This polymorphism is believed to contribute to increased levels of ROS which was associated to mitochondrial disease such as Parkinson's disease (Kosel *et al.*, 1998; Shoffner *et al.*, 1993). Conflicting reports of A10398G mutation involvement as a risk factor in breast cancer was reported by many researches.

In the present study we have chosen to screen for the A10398G mutation in a sample of Iraqi women, 21 and 22 from malignant and benign tumors respectively in addition to the control group of 16 women to test the association of this mtDNA mutation and breast cancer in Iraqi women.

## MATERIALS AND METHODS

**Sample collection:** Fifty nine blood samples were collected from unrelated females aged between 18-70 years, consisting of 21 females with breast malignant tumors, 22 females with Breast Benin tumors (mean age 45.3) and 16 healthy females (mean age 46.6) considered as a control group, using EDTA tubes. Blood samples and Fine Needle Aspirates (FNA) were collected from donors admitted to the National Center for Tumors Prediagnosis/Medical City/Iraq. Breast cancer was diagnosed by cytological test of FNA; suspected samples were further subjected to histological testing to for confirmation. Blood samples were immediately transferred to the laboratory for genomic DNA extraction.

**DNA isolation:** Genomic DNA was extracted from whole blood by using DNA extraction kit provided by (Bioneer, Korea). Extracted DNA was tested using 0.8% agarose gel electrophoresis. Yields and purity of DNA samples were estimated by using spectrophotometer.

**DNA amplification (specific PCR):** The following primers were used for PCR amplifications, forward primer 5'-ACA TAG AAA AAT CCA CCC CT-3' with nucleotide positions 10147-10166 and reverse primer 5'-CTA GGC ATA GTA GGG AGG AT-3' with

nucleotide positions 10569-10550 to amplify a region of mitochondrial DNA spanning 10147-10569 encoding for ND3 subunit, primers were provided by (Alpha DNA, Canada) upon request. PCR reactions were performed in a total reaction volume of 25  $\mu$ L containing 9.5  $\mu$ L Distilled water, 12.5  $\mu$ L (1X) GoTaq® Green Master Mix containing (Reaction buffer (pH 8.5), Taq polymerase, 200 mM each of dATP, dCTP, dGTP, dTTP and 1.5 mM of MgCl<sub>2</sub>) provided by (Promega U.S.A.), 0.5  $\mu$ L (10 pmol/ $\mu$ L) of each primer (Forward and Reverse) and 2  $\mu$ L of 50 ng template DNA. The mixture was incubated in (Multigene™ Gradient Thermal Cycler, Labnet International, Korea) with the following cycling conditions: An initial denaturation at 95°C for 10 min, followed by 35 cycles of denaturation at 95°C for 60 sec, annealing of primers at 50°C for 60 sec, extension at 72°C for 70 sec and a final extension cycle 72°C for 10 min., a negative control reaction was prepared will all amplifications to ensure reliability of results. PCR products were electrophoresed in 2% agarose along with 100 bp DNA ladder supplied by (Promega, U.S.A.).

**RFLP screening:** PCR amplification products of 422 bp were subjected to digestion reaction with (+*AluI*<sub>10397</sub>) was performed on ice, in a total volume of 30  $\mu$ L. Ten  $\mu$ L of digestion reaction consists of: {(6.4  $\mu$ L of sterile distilled water, 3  $\mu$ L of restriction buffer (10X), 0.3  $\mu$ L of BSA (100X), 0.2  $\mu$ L of restriction enzyme (10 U/ $\mu$ L)} supplied from (Promega, U.S.A.) and 20  $\mu$ L of amplified DNA. Then the digestion reaction was incubated at 37°C for 3 h. Digestion products were run on 2.5% agarose gel electrophoresis and stained with 0.5  $\mu$ g/mL ethidium bromide. The resulting fragments were visualized under the U.V fluorescence. Gel images were captured using a gel documentation system.

## RESULTS

Extracted genomic DNA yields ranged between (285-1020) ng/ $\mu$ L with purity of (1.1-1.3). A region of mtDNA was amplified using PCR technique. All three groups of malignant, benign and controls a total of 59 sample were subjected sequentially to PCR amplification to amplify the fragment of 422 bp which harbor nucleotide position 10398 (Fig. 1).

The resulting 422 bp PCR fragment encompassing the potential *AluI*<sub>10397</sub> site and another *AluI*<sub>10232</sub> which served as an internal control for digestion efficacy. The *AluI* digestion resulted in two different patterns of restriction: an acquisition of *AluI*<sub>10397</sub> site (+10397) produces three fragments sized 85, 165 and 172 bp, respectively. Wild type digestion results in two bands with molecular weights of 85 and 337 bp, respectively.

After amplification, restriction reactions were performed with the PCR products (Fig. 2). The results

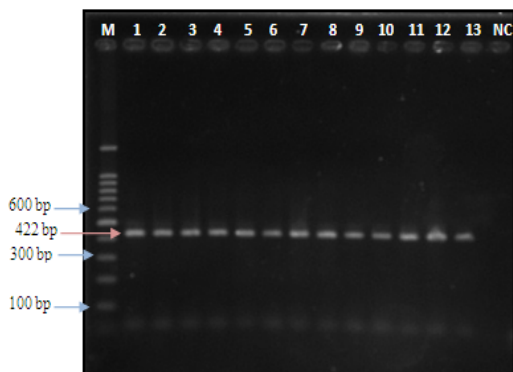


Fig. 1: PCR amplification of 422 bp region of mtDNA  
1-13: Ethidium bromide stained agarose gel (2%) in benign tumor DNA; M: Molecular weight marker (100 bp); NC: PCR negative control

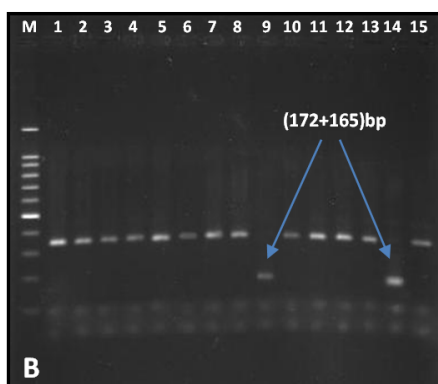
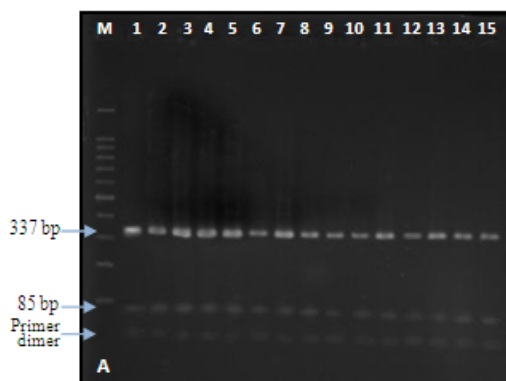


Fig. 2: Restriction reaction with (*AluI*) product of 422 bp. Ethidium bromide stained agarose gel (2.5%) (A): showing a band of (337 bp) and (85 bp), M: Molecular weight marker (100 bp), Lane (1-15): Malignant tumor DNA samples, (B): Showing a band of (165 + 172 bp) and (85 bp), M: Molecular weight marker (100 bp), Lane (1-15): Benign tumor DNA samples

shows that no one in control groups and malignant have the mutation while just two individuals with benign tumor show this mutation.

## DISCUSSION AND CONCLUSION

Mitochondrial DNA is a rich template for genetic variation that exhibits exclusively maternal transmission (Chan, 2006; Ishikawa *et al.*, 2008). We focused our attention on the mtDNA A10398G polymorphism because recently, many studies provide evidence that this mtDNA variant modify a woman's risk of developing breast cancer (Sultana *et al.*, 2011; Nadiyah *et al.*, 2012). While other studies showed there is no association between this polymorphism and breast cancer (Setiawan *et al.*, 2008). The mtDNA A10398G polymorphism has been reported to alter the ND3 subunit of the electron transport chain Complex I and to cause oxidative stress (Asari *et al.*, 2007; Lee and Wei, 2009; Carew and Huang, 2002; Miyazono *et al.*, 2002). A strong association of this polymorphism was reported in African women with breast cancer (Canter *et al.*, 2005). In another study the mutation was predicted to provide further evidence of association with breast cancer coupled with alcohol consumption (Pezzotti *et al.*, 2009).

Diagnosis of breast cancer in Iraq is based on cytogenetic testing and immunohistochemical assays (Alwan, 2010) lack of advanced diagnostic tools, genetic markers in particular delays early diagnosis of this disease. In this study, we found no evidence of association for the variant (A10398G polymorphism) with malignant and benign breast cancer patients in the sample used in the study even after adjustment with multiple testing of each sample. And so we conclude that the A10398G variant cannot be considered a potential risk marker for breast cancer susceptibility in Iraqi women. To the best of our knowledge, this study represents the first in mtDNA polymorphism screening in breast cancer in Iraq.

The contrast of our results with other studies may be explained by the presence of different risk modifiers that exist in diverse geographical areas. Another possible explanation may be due to an interaction with unknown genetic and environmental risk factors that may cause these differences.

In summary, our results do not support the hypothesis that the mtDNA A10398G polymorphism is a marker of breast cancer risk in women as reported in other studies. There may be other mtDNA polymorphisms that impair the efficiency of mitochondrial electron transport associated with breast cancer, so we conclude that more mtDNA polymorphisms need to be screened to see if there is a relationship of these polymorphisms with breast cancer in Iraqi women.

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