International Journal of ChemTech Research
CODEN (USA): IJCRGG, ISSN: 0974-4290, ISSN(Online):2455-9555
Vol.10 No.6, pp 905-909, 2017

Study the Heat Shock Protein 70 gene in Breast Cancer in Iraqi population

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Abstract : Objectives : this study was planned to analysis the effect of HSP70-2 gene A/G polymorphism on the plasma humane level of Hsp70and compare these results that will obtain with healthy control.
Design and methods: The present study was performed on seventy patients which forty-two of them obese, twenty-eight overweight. Thirty was as a control, eighteen of them obese and twelve overweight. Whole blood samples received from study subjects used to extract DNA for the study of polymorphism in Hsp70-2 gene by way of PCR-RFLP technique.
Results : The Hsp70-2 gene polymorphism was detecting by using PCR-RFLP. The alleles were designate as AA, AG and GG. There was statistically no significant difference in each the genotyping distribution and allelic frequency between each patient corporations and healthy control group ($P > 0.05$).The current study shows that subjects with AG,GG and AA genotype had the highest level of Hsp70 in all study groups.
Conclusion : the results indicate that relative risk of breast carcinoma not associated with Hsp70-2 polymorphism in patients.
Key words : Breast Cancer, Heat Shock Protein 70, polymerase chain reaction, and polymorphism.

Introduction

Breast cancer has become a major threat to female health in Iraq, where it is the main cause of death after cardiovascular diseases between women, with a cancer-related mortality rate of 23% [1-4]. The Hsp70s are an important part of the cell's machinery for protein folding, and help to protect cells from stress [5, 6].Expression of hsp70 in breast cancer tissue has been examined using Western blotting in patients with breast cancer[7]. In human HSP 70-2 encodes the major heat inducible HSP 70, this gene has been shown to be polymorphic by Milner and Campbell [8]. Who identified the polymorphic A to G transition PstI site at position +1267 in the coding region of HSP 70-2 [9]. The position 1267 of the hsp70-2 gene lies in the coding region, but corresponds to a silent mutation[10]. The 70Kdal HSP70 family includes three intron less genes, Hsp701, Hsp702, Hsp70hom, that have been mapped within the class III region of MHC complex on 6p21.3[11]. Hsp70-1 and Hsp70-2 encode identical 641 amino acid proteins [8,12]. While as the Hsp70hom encode a 641 amino acid protein that shares a 90% sequence identity with other Hsp70 proteins [13]. Genetic polymorphism in HSP70genes may influence its antiapoptotic and immune modulator function and, therefore, may have consequences on predisposition to and prognosis of the disease [14].

Methods

This study was carried out at the laboratories of Biochemistry Department, College of Medicine/ University of Babylon.The collection of samples was conducted during the period from 10 thof February 2016 until 30th of May 2016.The present study was conducted on (70) patients, (42) of them obese in the age group ranging from 20-70 years (this group comprised of 2 males 3 % and 68 females 97 %). The study also included (30) apparently healthy individuals were taken as a control group of the age ranging from 20 - 70 years (this group comprised of females 100%), the age of this group were matchedof patient groups.

DNA extraction

The Favor Prep™ Genomic DNA Extraction Mini Kit to extract of DNA from whole blood samples was used for the study of the extraction of DNA [15,16]and polymorphism in Hsp70 gene.

HSP 70 gene amplification for PCR-RFLP analysis

Amplification of Hsp 70 gene was done by polymerase chain reaction (PCR). Amplification was performed in a programmable thermal cycler gradient PCR system. The forward primer:(5′-TCCGAAGGACTGAGCTCTTG-3′),and the reverse primer, (5′-CAGCAAAGTCCTTGAGTCCC-3') where used[8].

Table (1): Amplification conditions of HSP 70 gene

Stage	Temp.(C^o)	Time	Function	Cycles
1	95	5 min.	Initial denaturation	
2	95	1 min.	DNA denaturation	35
	56-59	1 min.	Primer annealing	
	72	1.30 min.	Template elongation	
3	72	10 min	Final elongation	
4	8	-	Incubation	Hold

Results and Discussion:

The presence of DNA extracted detected by using agarose gel electrophoresis technique[17-18]. The extracted DNA was colorless, so a bromophenol blue dye was used with DNA to ease the loading step of the electrophoresis procedure, as shown in Fig. (1).

Then the amplification products were separated by electrophoresis through 1% agarose gel stained with SimplySafe™ blue stain. The PCR product length was [189bp] as shown in Fig. (2). The PCR product was digested with the restriction enzyme depending on the methods that described by PstI[8]

HSP70 gene polymorphism analysis:

The results of amplification and digestion by restriction enzyme of HSP70 gene by PCR-RFLP assay were of two alleles (A and G) and three genotypes were digested by restriction enzyme (PstI): AA has two bands 116 and 73bp, and AG has three bands 189, 116, and 73bp, and GG has one band 189 as shown in Fig. (3).

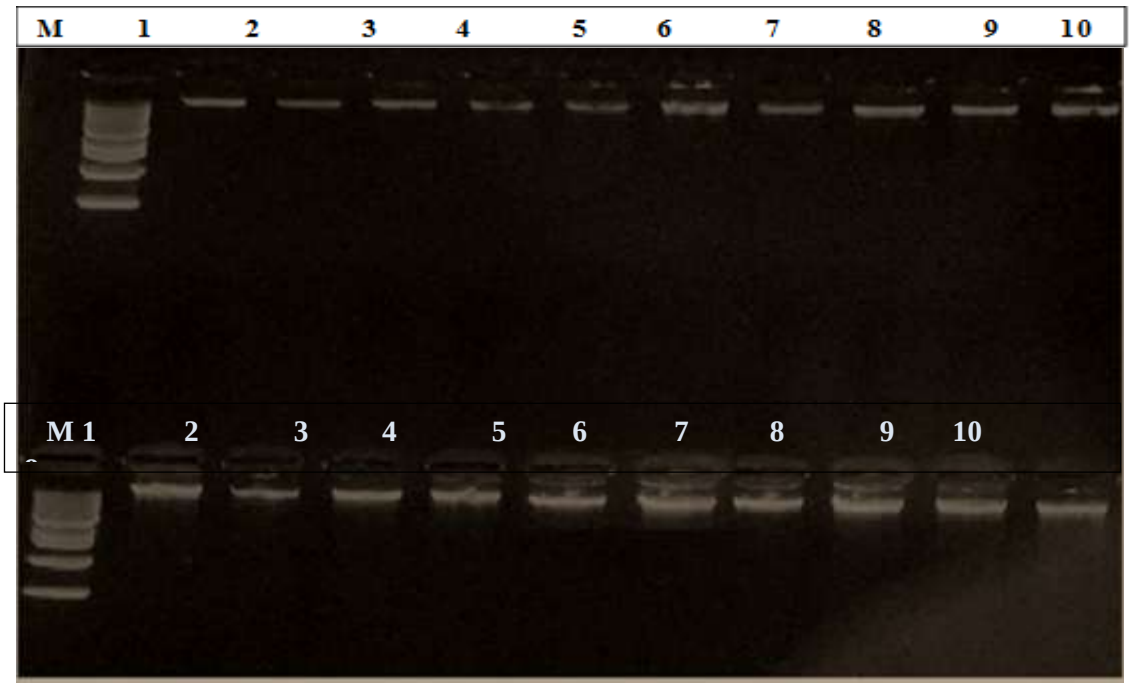

Figure (1): Genomic DNA extracted from whole blood. M DNA ladder Lane up (1-10): DNA extracted from patients, and lanedown (1-10): DNA extracted from controls , 1% agarose.

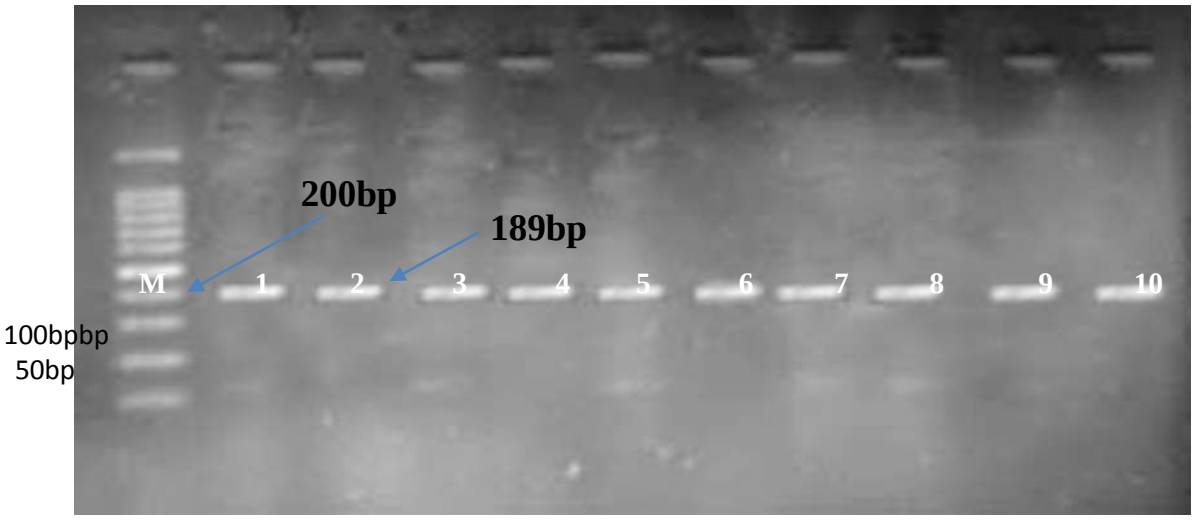

Figure (2): Amplification and PCR product (189 bp) picture of HSP70 gene on 1% agarose. Lane M, DNA ladder 50 bp and lanes (1-10), PCR products (189 bp).

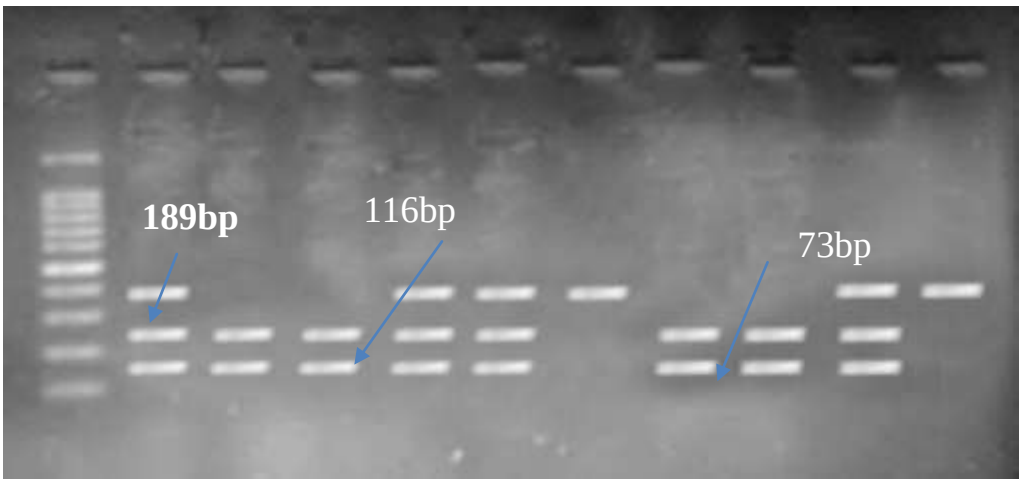

Figure (3): Electrophoretic picture represents the HSP70 genotyping, (3% agarose), where lane M is 50 bp DNA ladder, lane (2, 3,7,8) has two bands at (116bp+73 bp) representing the homozygous of (AA), lane (1,4,5,9) has three band at (189 bp+73 bp+116 bp) representing heterozygous (AG) allele ,and lane (6,10) has one band at (189 bp) representing the homozygous (GG).

Frequency distribution of the genotyping and each allele reported among the studied group is shown in table (Table 3)where no statistically significant differences had been found among the three groups regarding the frequencies of genotypes or separated allele, (P>0.05)

Table (2): Distribution of genotyping among the studied groups

Model	Genotype	Patient	Control	OR (95% CI)	P-value
Codominant	A/A	8 (11.8%)	7 (23.3%)	1.00	0.13
	A/G	50 (73.5%)	22 (73.3%)	0.50 (0.16-1.56)	
	G/G	10 (14.7%)	1 (3.3%)	0.11 (0.01-1.13)	

The present study showed the frequency of Hsp70-2 G allele change in patients with breast carcinoma compared to controls. The allelic frequency of Hsp70-2A/G heterozygote was insignificantly more with a relative risk fold in breast carcinoma cases (**p=0.13)**. Although the Hsp70 polymorphism analyzed undergo to silent mutation, it was associated with higher expression of Hsp70. Several studies have shown statistical suggestion of association between specific human leukocyte antigen (HLA) alleles and risk for or protection against various cancers [19].Several reports have indicated that different HLA products and genes may be risk factors for or protective is factors against cancer [20–23]. The relative risk of breast carcinoma not associated with Hsp70-2 polymorphism allele condition is rather a protective allele for breast carcinoma in homozygous condition for breast carcinoma inpatients[24]. But this study indicate no association between HSP70 polymorphism with relative risk of breast cancer, where found increased concentration of HSP70 in BC patients, this may be due to the number of control in this study are less and the comparison between patients and controls not clear. Milner et al [8] who identified a polymorphic PstI site at position 1267 of the hsp70-2 gene have characterized polymorphism within hsp70-2 gene. The position 1267 of the hsp70-2 gene lies in the coding region, but correspond to silent mutation. HSP70 gene was determine by polymerase chain reaction. The result of amplification by PCR process, which was conducted by using specific primer, resulted in one band when visualized on agarose gel electrophoresis. These findings support the hypothesis that this have further highlighted the presence of candidate genes for various cancers within or nearby the human leucocyte antigen (HLA). Given the chromosomal location of Hsp70 genes within HLA; their essential role in multiple steps involved in cancer pathogenesis and their determining role in the immune response to tumor cells, they are rightly associated with cancer susceptibility [25].

References:

1. Globocan, Lyon andFrance, "International Agency for Research on Cancer". World Health Organization International Agency for Research on Cancer, 2013.

2. Alwan N. "Breast cancer: Demographic characteristics and clinico-pathological presentation of patients in Iraq". East Mediterr Health J 16, 2010:1159-1164
3. Iraqi Cancer Board. "Results of the Iraqi Cancer Registry 2012. Baghdad, Iraq", Iraqi Cancer Registry Center, Ministry of Health, 2015.mammary carcinoma. EastMediterr Health J 6:1062-1072, 2000.
4. Alwan NA: "DNA proliferative index as a marker in Iraqi aneuploid mammary carcinoma". East Mediterr Health J 6:1062-1072, 2000.
5. Ritossa F. "A new puffing pattern induced by temperature shock and DNP in drosophila". Cellular and Molecular Life Sciences (CMLS) 18 (12): (1962); 571–573.
6. Ritossa F. "Discovery of the heat shock response". Cell Stress Chaperones 1 (2): (June 1996); 97–8.
7. Ciocca DR. *et. al*.. "Heat shock protein hsp70 in patients with axillary lymph node-negative breast cancer" prognostic implications. J. Natl Cancer Inst. 85, (1 993b) ; 570- 574.
8. Milner CM. and Campbell RD. "Structure and expression of the three MHC linked HSP 70 genes". Immunogenetics 1990;32:242–51
9. Bolla MK. *et. al*. "Analysis of the association of a heat shock protein 701 gene promoter polymorphism with myocardial infarction and coronary risk traits". Dis Markers 13: (1998); 227–35
10. Neve A. "Kangribumepithelioma in Kashmir". Ind Med Gaz. 1900;35:81–3.
11. Taira T, *et. al.* "Cell cycle dependent switch of up and down regulation of human hsp70gene expression by interaction between cmyc and CBF/NFY". J Biol Chem.1999;274:24270–9.
12. Sargent CA, *et. al*. "Human major histocompatibility complex contains genes for the major heat shock protein HSP70". ProcNatlAcadSci U S A. 1989;86:1968–72.
13. Hunt C. and Morimoto RI. "Conserved features of eukaryotic HSP70genes revealed by comparison with the nucleotide sequence of human HSP70". ProcNatlAcadSci U S A. 1985;82:6455–9.
14. Neve A. "Kangribumepithelioma in Kashmir". Ind Med Gaz. 1900;35:81–3
15. Moaed E., Alaa S. , A. S., and Kzar, H. H. (2016). "Assessment of antioxidant status in different genotypes/phenotypes at codon 72 of TP53 gene for patients with sporadic colorectal cancer in Babylon province." International Journal of PharmTech Research 9: 280-286.
16. Maha F. Smaism (2016) .Assessment of leptin levels in the different genotypes and leptin receptor genes in the women with polycystic ovary syndrome and diabetes mellitus type 2 in Iraq population. International Journal of PharmTech Research 9(5):269-276.
17. MoaedE.,Alaa S., and Kzar, H. H. (2016). "Evaluating the Superoxide Dismutase-1 status in Wild Type and Mutant at codons 12 and 13 of KRAS gene Spectrum for the Patients with Sporadic Colorectal Cancer." International Journal of PharmTech Research 9: 272-279.
18. Moaed E., Obed, A. F., and Al-Saadi, A. H. (2016). "Effect of ACE gene polymorphism of Iraqi patients on ischemic stroke." International Journal of ChemTech Research 9(3): 424-429.
19. Chouchane L, Ahmed SB, Baccouche S, Remadi S. Polymorphism in the tumor necrosis factor-α promotor region and in the heat shock protein 70 genes associated with malignant tumors. CANCER. 1997; 80:1489–1496.
20. Terasaki PI, Perdue ST, Mickey MR. HLA frequencies in cancer: a second study. In: Mulvihill JJ, Miller RW, FraumeniJF,tion with sequence specific primers (PCR-SSP) in 2 hours: editors. Genetics of human cancer. New York: Raven Press,an alternative to serological DR typing in clinical practice 1977:321–8.
21. MarkmanM, Braine HG, AbeloffMD. Histocompatibility antigens in small cell carcinoma of the lung. *Cancer*1984;54:2943–5.
22. Prazak J, Mericka O. HLA and bronchogenic carcinoma. *Neo-plasma* 1989;36:599–602.
23. Romano PJ, Bartholomew M, Smith PJ, Kloszewski F, Stryker J, Houk J, et al. HLA antigens influence resistance to lung carcinoma. *Hum Immunol*1991;31:236–240.
24. Rafiqa Eachkoti1*et. al*. "Polymorphic Analysis of MHC-Linked Heat Shock Protein 70 (HSP70-2 AND HSP70-HOM) Genes: Their Susceptibility and Prognostic Implications in Breast Carcinoma Cases of Kashmiri Population", Asian Journal of Biomedical and Pharmaceutical Sciences; 3(25) 2013, 13-20**.**
25. Milner CM. and Campbell RD. "Polymorphic analysis of three MHClinked HSP70genes". Immunogenetics.1992;6:357–62.

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