FOXP3 gene promoter polymorphism in breast cancer patients in Basra city / Iraq

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Abstract- this research was designed to investigate a polymorphism (rs3761548) and incidence of breast cancer in Basra city, southern of Iraq, Genetic polymorphism was evaluated in 100 blood samples were collected from breast cancer patients females only, from Al-sader teaching Hospital and in 100 controls by allele-specific PCR, we noticed that the genotype frequency was 63% and 57% for CC homozygote, 26% and 14% for CA heterozygote, and 11% and 33% for AA homozygote in controls and patients, respectively, It was also found by statistical analysis that there is a positive association for homozygous AA (OR = 3.9851, 95% CI = 1.8778 to 8.4569) in relation to breast cancer susceptibility.

KEYWORDS – FOXP3 GENE, BREAST CANCER, RS3761548 POLYMORPHISM.

I. INTRODUCTION

Foxp3 is a member of the P subfamily of the forkhead family of transcription factors sharing a number of common features including a C2H2 zinc finger, a leucine zipper, and a forkhead DNA-binding domain (Hu *et al.,* 2007). These domains are involved in DNA binding, nuclear transport (Hancock and Ozkaynak, 2009),and transcriptional repressor activity (Lopes *et al*., 2006), The expression pattern and role of *Foxp3* in breast cancer has been more difficult to elucidate. Zuo et al (Zuo *et al*., 2007) demonstrated that *Foxp3* is an X-linked breast cancer suppressor gene and an important regulator of the epidermal growth factor receptor (*HER2/ErbB2*) oncogene. They also reported that *Foxp3* is a novel transcriptional repressor for the oncogene *SKP2* in breast cancer cells that do not overexpress *HER2/ErbB2* (Zuo *et al*., 2007). *Foxp3* also induces expression of several tumor suppressors including *p18 (CDKN2C), p21 (CDKN1A), LATS2, and ARHGAPS* (Liu *et al*., 2009a). However, *Foxp3* expression does not show a clear differential pattern in breast cancer cells and several reports have also shown that FOXP3 expression correlates with unfavorable prognosis in breast cancer (Jaberipour *et al*., 2010), Promoter regions are potential candidates for the presence of functional single nucleotide polymorphism (SNPs), as they are involved in transcription initiation, and many of the cis-acting elements that regulate gene expression possibly harbor functional polymorphisms (Hoogendoorn *et al.,* 2003). As recently reviewed by Oda et al., *Foxp3* polymorphisms occur with high frequency in the general population and have been studied in common multifactorial human diseases, like diabetes, allergic rhinitis, and breast cancer (Oda *et al.,* 2013). It is known that SNPs in the promoter region of *Foxp3* gene may affect its expression (Bassuny *et al.,* 2003). Since it has been previously shown that *Foxp3* is involved in breast cancer development (Zuo *et al.,* 2007), several studies have been conducted to investigate a SNP (*rs3761548, C/A)* in the promoter region of *Foxp3* in patients with this neoplasia(Raskin *et al.,* 2009 , Liu and Zheng, 2007), but its exact role is not yet well understood.

II. MATERIAL AND METHODS

A. Sampling –

100 blood samples were collected from breast cancer patients from Al-Sadr Teaching Hospital in Basra city / Iraq, their ages range between 28 to 73 years old from females only . Other 100 blood samples were collected from volunteers as control , their ages range between 25 to 76 years old. Two ml of peripheral blood was drawn by sterilized syringe from the two groups then they have been kept in sterilized EDTA tubes at 4C° for DNA extraction .

B. Extraction of DNA from peripheral Blood –

DNA was extracted by Genomic DNA Mini Kit (Geneaid) according to the manufactured instruction and kept for PCR .

C. Polymerase chine reaction –

DNA was amplified by using thermocycle, FOXp3 gene alleles ware amplified by using isolated DNA and specific primers (Lopes *et al.,* 2014) as in Table (1) .

In PCR mixture total volume of 50µl contained Top DNA polymerase 2,5U, Each: Dntp (dATP, dCTP, dGTP, dTTP) 250 µM, Tris-HCI (PH 9.0) 10mM, KCI 30mM, MgCl2 1,5mM ,1 pmoles of each forward and reverse primer and 10 µl template DNA. Total volume of reaction mixture was made 50µl by adding sterile water and dispensed in . PCR was done with an initial denaturation only once at 94° for 3 min and then 30 cycles each of 30 sec denaturation at 94°C, 30 sec annealing at 50.5°C and 1 min extension at 72°C. Final extension was at 72°C for 5 min. The amplified short fragments of FOXp3 gene were run on 1% agarose gel and observed in UV light (Ma *et al*., 2011).

Table (1) : Oligonucleotide primer sequences used for amplification the *FOXP3 gene alleles.*

Gene	Allele	Primer Sequence	PCR Product
FOXP3 (C/A,rs3761548)	A	5'-CTGGCTCTCTCCCCAACGA-3'	334 bp
		5'-ACAGAGCCCATCATCAGACT CTCTA-3'	
	C	5'-TGGCTCTCTCCCCAACTGC-3'	333 bp
		5'-ACAGAGCCCATCATCAGACT CTCTA-3'	

III. RESULT AND DISCUSSION

DNA extraction

The total DNA was extracted from whole blood by using kit from (Geneaid) and then the DNA was run on 0.8% agarose gel and observed in UV light, as shown in figure (1)

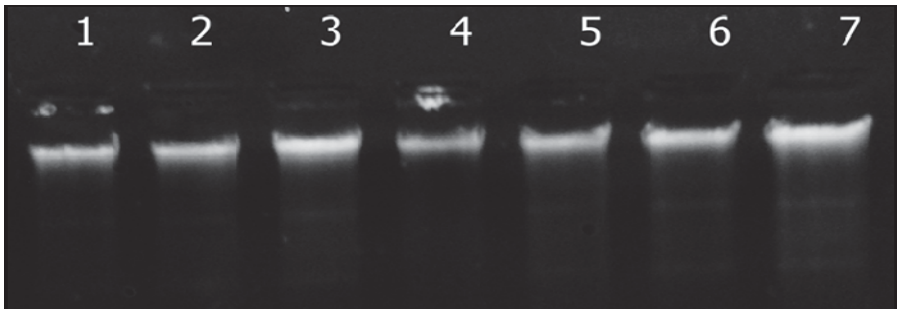

Figure 1. Photograph of agarose (0.8%) gel electrophoresis showing genomic DNA
Lane 1,2,3 : Control sample, Lane 4,5,6,7 : patient sample

PCR amplification of FOXP3 gene alleles :

The extracted DNA for all samples was subjected to PCR for amplifying the *FOXP3* gene alleles, the PCR products from amplification were then electrophoresed on an 1% agarose gel Ethidium Bromide-stain and the result was as in the following figure (2) and figure (3) .

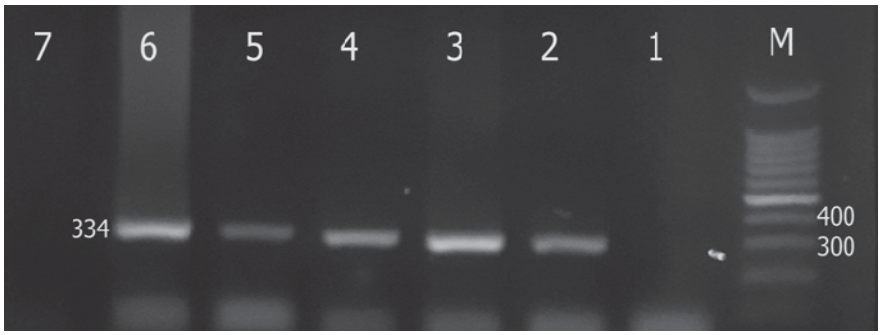

Figure 2. Figure 5. Photograph of agarose (1%) gel electrophoresis showing PCR product of *FOXP3* gene Allele A in patient and control samples .
M : 100bp Ladder**, Lane 1,2,3,4:** patient samples**, Lane 5,6,7:** control samples

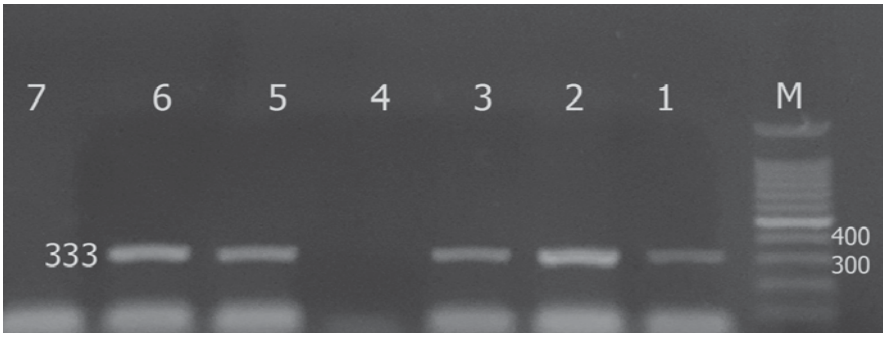

Figure 3. Figure 4 Photograph of agarose (1%) gel electrophoresis showing PCR product of *FOXP3* gene Allele C in patient and control samples .
M : 100bp Ladder**, Lane 1,2,3,4 :** patient samples**, Lane 5,6,7:** control samples

The promoter polymorphisms in the FOXP3 gene are considered to affect FOXP3 produc¬tion and activity. The FOXP3 gene rs3761548 (C→A) polymorphisms, located on the promoter region of the FOXP3 gene, are one of the most common single nucleotide polymorphisms (SNPs) (Jiang and Ruan, 2014).

In this study, the rs3761548 (reference sequence 3761548) polymorphism of FOXP3 gene was evaluated in 100 breast cancer patients and 100 samples for control free of neoplasia. The genotype frequency is 11% and 33% for homozygous (AA), 26% and 14% for heterozygous (CA) , and 63% and 57% for homozygous (CC) , in controls and patients, respectively. The obtained results are illustrated in the table below (Table 2)

Table (2) : distribution of *FOXP3* gene alleles between breast cancer patients and control .

Genotype	Control	patient	OR*	95% CI*
CC	63%	57%	0.7785	_
AA	11%	33%	3.9851	1.8778-8.4569
CA	26%	14%	0.4633	0.225-0.9521
CA+AA	37%	47%	1.5009	0.8583-2.6562

*OR = ODDS RATIO

*95 CI = 95% CONFIDENCE INTERVAL

IV.CONCLUSION

The results indicated a positive association for (AA) homozygous genotype in relation to breast cancer development (OR = 3.9851, 95% CI = 1.8778 to 8.4569) . Therefore, we Concluded that individuals who had inherited both copies (AA) of this allelic variation had a higher susceptibility for developing breast cancer than individuals with other genotypes (CC , CA) . Jahan *et al.* (2014) studded the potential influence of Foxp3 polymorphism (rs3761548) in 202 breast cancer patients and 130 normal healthy women of Indian origin , and found highly significant association with the advanced stage (T3-4) of the tumor (OR = 3.90; 95 % CI = 1.56–9.70) . Lopes *et al.* (2014) investigated the genetic polymorphism of *FOXP3* and TNBC (Triple Negative Breast Cancer) and found a positive association for (AA) homozygous genotype in relation to TNBC development (OR = 3.78, 95% CI = 1.02 to 14.06) . while Raskin *et al.* (2009) investigated three genetic polymorphisms in the *FOXP3* gene in

patients with breast cancer, and found none significant associations . Jiang and Ruan (2014) mentioned that rs3761548 (C→A) polymorphisms were not associated with the risk of breast cancer .

The (AA) genotype of the rs3761548 (C→A) polymorphism causes the loss of binding with some transcription factors, such as E47 and C-Myb, leading to defective transcription of *Foxp3*. (Shen et al. 2010). According to our study considering with the above written this may explain the association between the (AA) genotype and breast cancer .

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