UNIVERSITI PUTRA MALAYSIA

POLYMORPHISMS IN ESTROGEN RECEPTOR-α AND -β GENES AND THEIR CORRELATIONS WITH RISK FACTORS IN IRANIAN BREAST CANCER PATIENTS

SAKINEH ABBASI

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By

SAKINEH ABBASI

Thesis Submitted to the school of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

February 2009

DEDICATION

I would like to dedicate this thesis to:

My parents, I thank you for unconditional love and sacrifices you made for me

To my husband, who has put up with me during working on this thesis

And to my daughters, who bring joy and happiness to my heart.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

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Chairman: **Professor Dr. Patimah Ismail, PhD**

Faculty: **Medicine and Health Sciences**

Receptor-mediated estrogen activation participates in the development and progression of breast cancer. Evidence suggests that alterations in estrogen signaling pathways, including estrogen receptor-α (ER-α) and estrogen receptor-β (ER-β) occur during breast cancer development. Estrogen receptor genes (ERs) polymorphism has been found to be associated with breast cancer and clinical features of the disease in Caucasians. In order to investigate whether polymorphisms in the ER-α and ER-β are associated with breast cancer risk in a case-control study was conducted with 150 Iranian patients newly diagnosed invasive Breast Ductal Carcinoma, and 147 healthy women. PCR single-strand conformation polymorphism method and direct sequencing screened the selected encoding regions exon 4 ER genes for mutation or variant sites were performed. Three silent single nucleotide polymorphisms (SNPs) were found in the ER- α gene

(exon 1, exon 4, exon 8 respectively), as reported previously in other studies, but at significantly different frequencies and one SNP was found in ER- β gene (exon 7). The statistical significance was achieved in the most of demographic characteristics. Age at menarche of less than or equal to twelve years old in codon 594 of ER- α gene and among the eight different races the race of Fars in all four polymorphic sites of ER-α and ER-β genes were revealed statistically significant differences between case and control groups (p=< 0.05). Furthermore, blood group B of all four ABO blood groups, was shown statistically significant differences between case and control groups (p=< 0.05) for all four polymorphic sites of codons 10, 325, 594 of ER-α and 392 in ER-β . The frequency of allele 1 in codon 594 exon 8 was significantly higher in breast cancer patients (48.0%) than in control individuals (1.4%; P = 0.001). The codon 392 polymorphisms were presented only in cases group, in genotypes of heterozygote with statistically significant frequency of 8.7% and in the genotypes of homozygote with statistically significant frequency of 1.3%. Furthermore, in 1he exon 4 we found a novel mutation at codon 323 in Iranian women, and the statistical significance was achieved for the presence and absence of LN metastases at this codon (P = 0.017). Combination of the three SNP markers in *ER-α* may increase the incidence of age at menarche of less than or equal twelve years old, which itself could increase accuracy in predicting developing breast cancer later in their lifetime. Moreover, SNP in codon 392 of ER-β gene is more effective than those SNPs in three polymorphic sites of ER-α gene, in developing familial breast cancer and LN metastases phenotype. This was the first systematic association study in ER-α and ER-β genes polymorphisms and demographic

characteristics for breast cancer risk in Iran. In conclusion, our data suggest that ER-α and ER-β genes polymorphisms are correlated with various aspects of breast cancer risk in Iranian women. Moreover, the greater the frequency of allele 1 in codon 10, codon 325 and codon 392 the lesser the likelihood of LN metastasis in the Iranian breast cancer patients. We also noted that greater the frequency of allele 1 in codon 10 in the form of 01, the more likely in patients with familial breast cancer. Our findings suggest that, SNP in codon 392 of estrogen receptor- β gene is much effective than those SNPs in codons 10, 325, 594, of estrogen receptor-α gene, in developing familial breast cancer. Therefore, ER-α and ER-β genotypes, as determined during pre-surgical evaluation, might represent a surrogate marker for predicting breast cancer in Iran.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

POLIMORFISMA DALAM GEN ESTROGEN RESEPTOR-α DAN-β DAN PERKAITANNYA DENGAN FAKTOR RISIKO DI KALANGAN PESAKIT KANSER PAYUDARA PENDUDUK IRAN

Oleh

SAKINEH ABBASI

Febuari 2009

Pengerusi: **Profesor Dr. Patimah Ismail, PhD**

Fakulti: **Perubatan dan Sains Kesihatan**

Pengaktifan reseptor penerima estrogen memainkan peranan dalam pembentukan dan perkembangan kanser payudara. Bukti kajian mencadangkan terdapat perubahan dalam isyarat laluan estrogen, termasuk gen estrogen reseptor-α (ER-α) dan gen estrogen reseptor-β (ER-β) semasa pembentukan kanser payudara. Gen estrogen reseptor (ERs) polimorfisma didapati mempunyai kaitan dengan kanser payudara dan secara klinikalnya ciri ini sering berlaku pada masyarakat Kaukasia (Caucasians). Dengan itu, data genomik bagi gen ER sama ada dalam populasi menunjukkan nilai bagi set klinikal terhadap kumpulan etnik yang dikaji. Kami mengkaji satu kajian kes sama ada polimorfisma dalam ER-α dan ER-β mempengaruhi risiko kanser payudara, dilakukan. Kajian Gen ER-α dan ER-β dilakukan pada pesakit di Iran yang baru menerima dignosis serangan kanser payudara, (150 pesakit) dan penduduk yang sihat (147 orang sebagai

individu kawalan). Pengesahan polimorfisma menggunakan kaedah tindakbalas berantai polimerase dan penjujukan terus terhadap bahagian hujung gen kod ekson 4 ER yang dipilih terlibat dalam mutasi atau bahagian yang berlainan dilakukan. Tiga gen senyap polimorfisma nukleotid (SNPs) telah ditemui dalam gen ER-α, (masing-masing pada ekson 1, ekson 4, ekson 8), seperti yang telah dilaporkan dalam kajian lain tetapi kekerapannya berbeza secara signikan dan satu SNP didapati dalam gen ER-β (ekson 7). Secara statistiknya nilai signifikan didapati dalam kebanyakan ciri-ciri demografik. Didapati pada umur bermulanya awal kematangan bagi perempuan kurang daripada atau bersamaan dengan 12 tahun dalam kodon gen 594 ER-α dan antara 8 perbezaan bangsa Fars dalam 4 bahagian polimorfik gen ER-α dan ER-β menunjukkan perbezaan statistik yang signifikan antara kajian kumpulan kes dan kawalan (p=<0.05). Selain itu, antara kumpulan darah B dari 4 kumpulan darah menunjukkan perbezaan nilai statistik yang signifikan dibandingkan antara kumpulan kes dan kumpulan kawalan (p=<0.05) untuk semua 4 bahagian kodon folimorfik pada 10, 325, 594 dari ER-α dan 392 dalam ER-β). Oleh itu, kami mendapati lebih besar kekerapan bagi alel 1 dalam kodon 325 dan 392, semakin lebih bersamaan kemungkinan berlaku metastasis LN dalam pesakit kanser payudara penduduk Iran. Selain itu, nilai statististik yang signifikan menunujukkan kehadiran dan ketiadaan metastasis LN bagi kodon (P=0.017) Mutasi baru terdapat di kodon 323. Kekerapan bagi alel 1 dalam codon 594, ekson 8 secara signifikannya lebih tinggi bagi pesakit kanser payudara (48.0%) berbanding dengan individu kawalan (1.4%; P=0.001). Alel 1 dalam kodon 594 mempamerkan kekerapan yang lebih tinggi menunjukkan seakan-akan berkurangan kejadian metastasis LN. Hasil kajian memperlihatkan bahawa penanda SNP seperti ini akan meningkatkan ketepatan dalam

membuat jangkaaan populasi penduduk Iran. Tambahan lagi, kawasan hujung kod ekson 7 daripada gen ER-β membuktikan kehadiran gen (SNP) pada kodon 392 populasi penduduk Iran. Polimorfisma pada kodon 392 hanya ditunjukkan dalam kumpulan kes dengan heterozigot genotype dengan kekerapan statistik yang signifikan pada 8.7% dan dalam homozigot genotaip dengan kekerapan nilai statistik signifikan 1.3%. Keputusan kajian membuktikan polimorfisma pada kodon 392 dalam ER-β adalah berbeza secara signifikan dalam kumpulan kes dan kawalan. Kombinasi antara 3 penanda SNP dalam ER-α akan meningkatkan kejadian pada umur bermulanya kematangan bagi perempuan kurang atau bersamaan dengan 12 tahun, boleh meningkatkan ketepatan dalam membuat jangkaan pembentukan kanser payudara dalam jangka hayat. Tambahan lagi, SNP pada kodon 392 dari ER-β adalah lebih berkesan dari SNPs dalam 3 kawasan polimorfisma gen ER-α, dalam pembentukan kanser payudara keturunan dan metastasis fenotaip. Ini adalah kajian pertama perkaitan antara polimorfisma gen ER-α dan ER-β dan variasi risiko kanser payudara penduduk Iran. Kesimpulannya, keputusan yang diperolehi mencadangkan polimorfisma gen ER-α dan ER-β adalah berkaitan dengan pelbagai aspek kanser payudara. Penemuan dalam kajian ini mencadangkan SNP pada kodon 392 gen estrogen reseptor-β adalah lebif efektif daripada SNPs pada kodon 10, 325, 594 gen estrogen reseptor-α dalam pembentukan kanser payudara. Dengan itu, genotaip ER-α dan ER-β seperti yang ditentukan semasa penilaian pra-pembedahan, akan mewakili penanda timbalan dalam meramalkan kanser payudara di Iran.

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I certify that an Examination Committee has met on? March 2008 to conduct the final examination of Sakineh Abbasi on her Doctor of Philosophy thesis entitled "Polymorphisms in Estrogen Receptor- α and - β Genes and their Association with Risk Factors in Breast Cancer Patients from Imam Khomeini Hospital Complex" in accordance with Universiti Pertanian Malaysia (Higher degree) Act 1980 and Universiti Pertanian (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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Date: 9 April 2009

DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or other institution.

SAKINEH ABBASI

Date:

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LIST OF ABBREVIATIONS

∞	Limitless or Infinity
bp	Base pair
BMI	Body Mass Index
CI	Confidence Interval
DMSO	Dimethyl sulphoxide
DNA	Deoxyribonucleic Acid
dNTP	Deoxyribonucleotide Phosphate
EDTA	disodium ethylenediaminetetraacetate
ER	Estrogen Receptor
ESR1	Estrogen Receptor Alpha
ESR2	Estrogen Receptor Beta
g	gram
hr	hour
kb	kilo base
KCl	Potassium Chloride
lad	DNA ladder
LN	Lymph Node
mg	miligram
mg/L	milligram/liter
$MgCl_2$	Magnesium chloride
min	minute

ml	millilitre
mM	millimolar
NaOH	Sodium Hydroxide
ng	nanogram
OD	optical Density
OR	Odds Ratio
P mole	Pico mole
P^{53}	53-kilodalton tumour suppressor protein
PAGE	Polyacrylamide Gel Electrophoresis
PCR	Polymerase Chain Reaction
pH	Hydrogen Ion Concentration
Rh	Rhesus blood group system
rpm	rotation per minute
sec	second
SSCP	Single Nucleotide Conformational Polymorphisms
TAE	Tris-Acetate- EDTA buffer
Taq	*Thermus aquaticus*
Tm	melting temperature
und DNA	Undenaturate DNA
UV	Ultraviolet
V	volt
WHO	World Health Organization
α	Alpha

β	Beta
µ	micron
µl	microliter
µ mol / L	micromole per litre
^{o}C	Celsius