Dedication

To the soul of my

parents,

Acknowledgement

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Abbreviations

ESR1	Estrogen receptor α gene
HER-2/neu	Epidermal growth factor receptor gene
P53BP1	P53 binding protein 1 gene
AP1	Amplifed protein 1
AIB1	Amplified in breast cancer gene1
RGS	regulators of G protein signaling
SERM	Selecive estrogen receptor modulater
ERKO	Estrogen receptor knockout mice
MGB	minor groove binder
aa	amino acid(s)
Ile	Isoleucine
Val	valine
Glu	Glutamate
Asp	Asparatate
Gly	Glysine
Ser	Serine
CI	confidence interval
DNA	deoxyribonucleic acid
ER	estrogen receptor
LOH	loss of heterozygosity
HRT	Hormone replacement therapy
OR	odds ratio
p	short arm of a chromosome
q	long arm of a chromosome
PCR	polymerase chain reaction
PR	progesterone receptor
RFLP	restriction fragment length polymorphism
SNP	single nucleotide polymorphism
SSCP	single-strand conformation polymorphism
UTR	untranslated region
wt	wild-type

Abstract

Breast cancer, is a common type of cancer, with over two million newly diagnosed cases annually worldwide. In Sudan breast cancer is the most common cancer comprising 34% of all cancer patients. The functionally defective mutations in BRCA1 and BRCA2 genes are responsible for up to 5% of all breast cancer patients, while other genes (so-called low penetrance genes) account for the remainder of breast cancer patients. Among those possible low penetrance candidate genes for breast cancer are, ESR1, HER-2/neu and P53PB1 genes. Since single-nucleotide polymorphism (SNP) is the most frequent and most subtle genetic variation in the human genome and has great potential for application to association studies of complex diseases such as that of breast cancer the aim of this study was to evaluate the role of ESR1, HER-2/neu and P53BP1 polymorphisms in breast cancer predisposition in Sudanese breast cancer patients and in breast cancer risk at the population level.

This is a case control study where we genotyped a total of 81 breast cancer patients and 91 age matched healthy controls for 4 SNPs, namely, ESR1 variant C325G [db SNP rs1801132] and HER-2/neu codon 665 Ile –Val polymorphism [db SNP rs1136200] as well as 2 SNPs in P53BP1 tumor suppresser gene namely Glu 353 Asp or 1236C→G [db SNP rs560191] and Gly 412 Ser [db SNPrs689647]

The role of these polymorphism in breast cancer susceptibility were investigated using both conventional genotyping technique and high throughput Tag Man allelic discrimination method (SNP scoring methods) using Real-Time PCR technique . Data on clinical features and demographic details were collected. The association between the case –control status and each individual SNP, measured by the odds ratio and its corresponding 95% confidence interval, was estimated using unconditional logistic

regression models. At the second stage, tow-way interactions were investigated using multivariate logistic models. The C allele of ESR1 codon C325G was shown to exhibit significant association of breast cancer risk in the subgroup of women 50 years and younger in the patients group compared to control subjects (P= 0.03) (OR: 2.28, 95%CI: 1.10-4.72). However, the overall susceptibility to breast cancer was not significant, although all estimates were in the direction of a higher risk in women with CC genotypes. Regarding the HER-2/neu codon 655Leu/Val variant we observed a modest positive association for Ile/Val versus Ile/Ile genotype in patients with breast cancer compared to control subjects (OR= 2.95, 95% CI 0.97-8.96), the Ile/Val heterozygous were more common among patients (P= 0.06). No associations of Val allele with breast cancer when stratified by menopausal status or age were observed. Genotypic and allelic frequencies of the P53BP1 Glu325Asp and of P53BP1 Gly412Ser lack association with respect to breast cancer risk when considered in overall, stratification according to menopausal status shows a modest increase of risk among homozygous carrier of P53BP1 412Ser/Ser P=0.08 (OR = 4.00, 95% CI 0.85-18.34) in post menopausal patients compared to postmenopausal control women and of Ser alleles carrier P=.0.05 (OR= 5.71 (95% CI .0.92–5.5). No significant associations were seen among homozygous carrier of P53BP1 353 Asp/Asp neither of Glu alleles versus Asp alleles in the menopausal subgroup. In the haplotype of the 2 SNPs of P53BP1, no significant associations were observed. Nor when the genotype investigated in overall to the breast cancer risk. These results indicate that polymorphisms of these selected breast cancer susceptibility genes vary in their association with breast cancer. Genetic epidemiology study replication and functional assay of these SNPs as well as of haplotypes should permit a better understanding of the role of these genetic variants and breast cancer risk.

مستخلص البحث

يعد سرطان الثدي من أكثر أنواع السرطانات شيوعاً، حيث يتم سنوياً تشخيص أكثر من مليوني حالة حول العالم. وفي السودان يعتبر سرطان الثدي من أكثر السرطانات تفشياً ويشكل حوالي 34% من العدد الكلي للمصابين بمرض السرطان. أثبتت الدراسات أن وجود خلل في الجينات المعروفة بـ ($BRCA_1$ $BRCA_2$) يمثل إحدي العوامل في الإصابة بالمرض في حوالي 5% من المرضى، بينما الجينات التي تعرف بـ(low penetrance genes)، تشكل النسبة المتبقية بالاصابة بمرض سرطان الثدي. أثبتت الدراسات السابقة وجود علاقة بالاصابة بمرض السرطان والتباين في جينات مستقبلات الاستروجين ألفا ESR1، جينات مساعدات النمو الابديرمية HER-2 والجينات المثبطه للنمو P53PBI وتعتبر من العوامل التي تجعل الفرد أكثر قابلية للإصابة بمرض سرطات الثدي.

تهدف هذه الدراسة الي تقويم دور تباين الجينات في القابلية بالإصابة بسرطان الثدي في السودان. شملت الدراسة تنبيط 4 متغيرات variants في 3 جينات في عدد 81 حالة سرطان ثدي و 91 أصحاء. فُحصت المتغيرات الجينية باستخدام التقنيات التقليدية علي سبيل المثال (RFLP و SSCP) بالإضافة الي استخدام تقنيات عالية المستوي علي سبيل المثال (Realtime PCR, Tag Man allelic discrimination) كذلك تم جمع البيانات الإكلينيكية والديمغرافية وقد تم قياس العلاقة بين المتغيرات Variants الجينية في المصابين والأصحاء مستخدماً الطريقة الاحصائية التي تعرف Chi Square.

وقد توصلت الدراسة الي النتائج التالية :

1. إن المتغير Variant C في جين ESRI يوضح فروقات معنوية في مجموعة من النساء اللائي تقل أعمارهن عن 50 عاماً بمقارنة المجموعة المماثلة من الأصحاء (P= 0.03) (OR: 2.28, 95%CI: 1.10-4.72)، بينما لا توجد فروقات ذات دلالة معنوية عند دراسة المجموعة ككل.
2. لوحظ وجود علاقة إيجابية (P= 0.06) بين حاملي المتغير ILe/Val.Variant في المرضي مقارنة بالأصحاء (OR= 2.95, 95% CI 0.97-8.96).
3. لا توجد فروقات ذات دلالة معنوية لحاملي المتغير Variant عندما قُسمت المجموعات علي أساس سن اليأس والعمر) < و > من 50 عاماً(.
4. لا توجد فروقات ذات دلالة معنوية في متغيرات Variants جينات P53BP1 Glu325Asp and P53BP1 Gly412Ser عند دراسة المجموعة ككل في مجملها. عندما درست هذه المتغيرات Variants الآنفة الذكر علي أساس فيما بعد سن اليأس (menopausal status) وجدت فروقات معنوية لحاملي المتغير Ser (P=.0.05 (OR= 5.71 (95% CI .0.92–5.5 .

خلصت هذه الدراسة الي ان المتغيرات Variants في هذه الجينات المذكورة تتفاوت في درجة علاقتها بالإصابة بمرض سرطان الثدي.

إجراء المزيد من الدراسات في الدور الوظيفي لهذه المتغيرات Variants للوصول الي فهم دقيق لمخاطر سرطان الثدي.