بسم الله الرحمن الرحيم

MONA MAGHRABY

شبكة المعلومات الجامعية

التوثيق الالكتروني والميكروفيلم

MONA MAGHRABY

شبكة المعلومات الجامعية

التوثيق الإلكتروني والميكروفيلم

جامعة عين شمس

التوثيق الإلكتروني والميكروفيلم

قسم

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MONA MAGHRABY

Microbiology Department

Possible Association of Viral Infections with Presence of BRCA1/2Genes Mutations among Breast Cancer Patients: Clinical Relevance and Disease Outcome

A Thesis submitted for the degree of Doctor of Philosophy in Science in Microbiology

By

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To

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بسم الله الرحمن الرحيم

اقْرَأْ بِاسْمِ رَبِّكَ الَّذِي خَلَقَ ﴿١﴾

خَلَقَ الْإِنْسَانَ مِنْ عَلَقٍ ﴿٢﴾

اقْرَأْ وَرَبُّكَ الْأَكْرَمُ ﴿٣﴾

الَّذِي عَلَّمَ بِالْقَلَمِ ﴿٤﴾

عَلَّمَ الْإِنْسَانَ مَا لَمْ يَعْلَمْ ﴿٥﴾

(صدق الله العظيم)

‹‹سورة العلق 1- 5 »

DEDICATION

رب أوزعنى أن أشكر نعمتك التي أنعمت على و على والدى
و ان أعمل صالحا ترضاه و أدخلنى برحمتك فى عبادك الصالحين

«سورة النمل الاية 19»

My work is dedicated to:

Allah and I hope to accept it from me and to my father and my mother

to whom I owe my life.

Also I dedicate this work to my sisters and my brothers. This dissertation has not previously been submitted for any degree at this or at any other university.

Nasra fathy Abd el fattah

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Thank you

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ABSTRACT

Background and objectives. Breast cancer (BC) is a multifactorial disease that is attributed to non-familial factors such as environmental or genetic factors that play a vital role in development of disease. Breast cancer type 1and 2 susceptibility protein *(BRCA1/2*) mutations represent a high risk of breast cancer. The current study focused on exploring relation between the presence of *BRCA1/2* mutations and some clinic-pathological characteristics which might impact the pathogenesis of BC disease. Also, we aimed at evaluating frequency of Human Papillomavirus (HPV) and its association with some clinical and pathological characteristics of BC disease as a possible indicator of BC who are at high risk of development of severe disease.

Material and Methods. Genomic DNA samples were obtained from 19 fresh tissues using Gene JET Extraction Kit (Thermo Scientific/US, Canada), and 29 Formalin-fixed paraffin-embedded (FFPE) using QIAamp DNA FFPE Tissue Kit (Qiagen, Valencia, USA) obtained from pretreatment BC patients. Library preparation was performed using Devyser BRCA NGS kit (DEVYSER, Stockholm, Sweden), according to the manufacturer's instructions. The reagent kit V2, 500 Cycles PE, on the Illumina MiSeq System (Illumina, San Diego, CA, USA). The *BRCA1* and *BRCA2* genes reference were: NM_007300 and NM_000059, respectively. Variants were called using freebayes (1.1.0.46) and annotated using ANNOVAR (version2019Oct24). All samples were subjected for detection of *Human Mammary Tumor Virus (HMTV)* and *Human Papillomavirus (HPV*) DNAs using qualitative PCR assay.

Result. The study identified 40 and 54 different *BRCA1* and *BRCA2* mutations, mostly in the form of frame shift and stop codon mutations. Regarding, *BRCA1*, 14 pathogenic mutations were detected, exon10 and exon 9 showed to be the most

affected exons representing (c.C1612T (25%) and c.C1471T (22.9%), respectively. Thirteen pathogenic mutations were detected, ten of these mutations were not previously reported, including four frame shift deletions mutations (c.7231delA in exon 14, c.4056delT in exon 11, c.4056delT in exon 11, and c.1012delG in exon 10), One frame shift insertion mutation (c.7620dupG in exon 16). Five stop codon mutations (c.C818A in exon 10, c.G8490A in exon 20, c.C5047T in exon 11, c.C7588T in exon 15, and finally c.T9861A in exon 27).

Regarding relation between *BRCA1/2* genes mutations and clinic-pathological parameters, *BRCA1* and *BRCA2* carriers were younger than non-carriers though not significant (p=0.440). Regarding the tumor characteristics, *BRCA1/2* carriers had large tumor size (p=0.091), high tumor grade compared to non-carriers but without significance (P=0.098). Micro-classifications positivity (60%) was also more frequently among *BRCA1/2* carrier than non-carrier (p= 0.082). Regarding detection of *HMTV* and *HPV*, *BRCA1/2* mutation carriers has a skew towards negative results (P=<0.001 and 0.004, respectively).

Conclusion. Our data suggest that *BRCA1/2* mutations might contribute to the pathogenesis among both familial and non-familial Egyptian breast cancer patients, but we need more sample size to confirm the findings. It also identifies the most affected exons in preparation for establishing diagnostic tool like HRM for genetic counseling, which will help when selecting treatment modalities for BC patients. Also high risk HPVs were identified into 10% of studied Egyptian BC patients and were associated with young age.

Keywords. BRCA1, BRCA2, *Human Mammary Tumor Virus*, *Human Papillomavirus*, Next Generation Sequencing