

Naif Arab University for Security Sciences

College of Forensic Sciences

Department of Forensic Biology



**Assessment of *HER2/neu* Gene
Amplification Status in Breast Cancer
Using Droplet Digital PCR Technique**

By:

Wessal Ramadan Ahmad Fayyad Abdullatif

Supervision

Main Supervisor

CO- Supervisor

Dr. Mourad Tahar Assidi

Dr. Safia Abdulsalam Messaoudi

**A thesis submitted in Partial fulfillment of the requirements
for the Master's degree in Molecular genetics**

**Riyadh
2019 - 1440**

جامعة نايف العربية للعلوم الأمنية

كلية علوم الأدلة الجنائية

قسم الأحياء الجنائية



تقييم حالة تزايد عدد مستقبل عامل النمو الجلدي
البشري (*HER2 gene*) في سرطان الثدي باستخدام
تقنية تفاعل البلمرة المتسلسل الرقمي

إعداد:

وصال رمضان عبد اللطيف

المشرف المساعد :

د. صافية عبدالسلام مسعودي

المشرف الرئيسي:

د. مراد الطاهر عصيدي

رسالة مقدمة استكمالاً لمتطلبات الحصول على درجة الماجستير في تخصص
الوراثة الجزيئية

الرياض

٢٠١٩-١٤٤٠

List of Abbreviations

Abbrevi	Description
BC	Breast Cancer
<i>HER2</i>	Human Epidermal Growth Factor Receptor 2
PCR	Polymerase Chain Reaction
CNV	Copy Number Variation
qPCR	Quantitative Real-Time PCR
dddPCR	Droplet Digital Polymerase Chain Reaction
ISH	in situ Hybridization
FISH	Fluorescence in situ Hybridization
IHC	Immunohistochemistry
IDC	Invasive Ductal Carcinoma
ILC	Invasive Lobular Carcinoma
DCIS	Ductal Carcinoma in situ
SPC	Solid Papillary Carcinoma
BRCA1	Breast Cancer Type 1 Susceptibility Protein Gene
BRCA2	Breast Cancer Type 2 Susceptibility Protein Gene
HRT	Hormone Replacement Therapy
AJCC	The American Joint Committee On Cancer
NCCN	National Comprehensive Cancer Network
CP	Complex Physiotherapy
MLD	Manual Lymph Drainage
ER	Estrogen Receptor
PR	Progesterone Receptor
FFPE	Formalin-Fixed Paraffin-Embedded
OD	Optical Density
ECD	Extracellular Domain
ADCC	Antibody-Dependent Cell-Mediated Cytotoxicity
CEP17	Chromosome Enumeration Probe 17
WHO	World Health Organization
NAUSS	Naif Arab University For Security Sciences
ACOG	American College Of Obstetricians And Gynecologists Guidelines For Breast Cancer
KSA	Kingdome of Saudi Arabia
SCR	Saudi Cancer Registry

DES	Diethylstilbestrol
UICC	Union For International Cancer Control
TNBC	The Triple-Negative Breast Cancer
IRB	Institutional Review Boar
ATL	a tissue lysis buffer
GMO	Genetically Modified Organisms
IARC	International Agency for Research on Cancer

List of Tables

Tables		Page No.
Table 1	Patients' clinicopathological details of tumors included in the study	27
Table 2	Primer and probe sequences	35
Table 3	ddPCR reaction mix preparation	38
Table 4	GeneAmp PCR system 9700PCR method	39
Table 5	Relationship between <i>HER2</i> status and the patients' features	46
Table 6	Digital PCR results of FAM/VIC* assay in 106 patients	51
Table 7	Correlation between <i>HER2</i> distribution by IHC and ddPCR	61

List of Figures

Figures Title		Page No.
Fig. 1	Milk duct system and distribution of different tissues within the breast	6
Fig. 2	Normal Anatomy of The Nipple-Areolar Complex	8
Fig. 3	Diagram Shows Changes In Cell Structure And Cellular Activity	10
Fig. 4	Overexpression of human <i>c-erbB-2</i>	15
Fig. 5	Separation and digital counting provide sensitive, absolute quantification	22
Fig. 6	Measurement of DNA concentration by NanoDrop	30
Fig. 7	IHC Reaction	32
Fig. 8	Schematic Representation of The Automated Ventana System	33
Fig. 9	Experiment workflow on the QuantStudio™ 3D ddPCR	37
Fig. 10	QuantStudio 3D Digital PCR System	41
Fig. 11	Picture of ddPCR negative result	49
Fig. 12	Picture of ddPCR positive result	50

List of Appendices

Appendix		Page No.
Appx.1	Commercial Kits & Instrumentation	87
Appx.2	IRB Approval	98

Table of Contents

Title	Page No.
Arabic Abstract	i
English Abstract	ii
Dedication	iii
Acknowledgments	iv
Scientific Abbreviations	v
List of Tables	vii
List of Figures	viii
List of Appendices	ix
Chapter 1: Subject of the Study	
1.1.Introduction	2
1.2.Study Questions	4
1.3.Study Objectives	4
1.4.Hypothesis of the study	4
Chapter 2: Theoretical Background	
2.1.Histology of the breast	6
2.2. Breast cancer origin causes and incidence	8
2.3. Breast cancer symptoms and signs	10
2.4. BC risk factors and genomic instability	11
2.5. Breast cancer diagnosis and staging	11
2.6. Breast cancer treatment strategies	14
2.7. Cancer biomarkers	14
2.8. BC clinicopathological factors	16
2.9. <i>HER2</i> gene as molecular marker in BC	17
2.10.Digital PCR	19
Chapter 3: Research Methodology	
3.1.study design	26
3.2.Study populations	26
3.2.1.Patients	26
3.3.DNA extraction	28
3.4.Isolation of Genomic DNA from FFPE Tissue blocks Sections	28

3.5. DNA quantity and quality Examination	29
3.6. DNA examination by NanoDrop™ One/One ^C UV-Vis Spectrophotometer	29
3.7. DNA quantitation by quantitative Real-Time PCR (qRT-PCR)	31
3.8. Immunohistochemistry (IHC)	31
3.9. Instrumentation of IHC	33
3.10. Evaluation of IHC scores	33
3.11. QuantStudio 3D ddPCR	34
3.12. ddPCR Reagents	34
3.13. Operational workflow	36
3.14. ddPCR reaction mix preparation	38
3.15. Chip loading and ddPCR thermal cycling	39
3.16. Analysis of ddPCR Data	40
3.17. Statistical analysis	42
Chapter 4: Results	
4.1. The subject groups characteristics	44
4.2. The clinical and demographic characteristics	44
4.3. Digital PCR result	48
Chapter 5: Discussions	
5.1. Discussion	63
Chapter 6: Conclusion and Recommendations	
6.1. Conclusions	68
6.2. Future perspectives	69
6.3. Recommendations	69
References	
References	71
Appendices	
	86