# بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

### قال الله تعالى

اللَّهُ نُورُ السَّمَاوَاتِ وَالْأَرْضِ مَثَلُ نُورِهِ كَمِشْكَاةٍ فِيهَا مِصْبَاحُ الْمِصْبَاحُ فِي زُجَاجَةٍ الرُّجَاجَةُ كَأَنَّهَا كَوْكَبُ دُرِّيٌّ يُوقَدُ مِن شَجَرَةٍ مُّبَارَكَةٍ زَيْتُونِةٍ لَّا شَرْقِيَّةٍ وَلَا دُرِّيُّ يُولُدُ مِن شَجَرَةٍ مُّبَارَكَةٍ زَيْتُونِةٍ لَّا شَرْقِيَّةٍ وَلَا غَرْبِيَّةٍ يَكَادُ زَيْتُهَا يُضِيءُ وَلَوْ لَمْ تَمْسَسْهُ نَارُ نُّورُ عَلَى غَرْبِيَّةٍ يَكَادُ زَيْتُهَا يُضِيءُ وَلَوْ لَمْ تَمْسَسْهُ نَارُ نُّورُ عَلَى نُورٍ يَهْدِي اللَّهُ الْأَمْثَالَ نُورٍ يَهْدِي اللَّهُ الْأَمْثَالَ شَيْءٍ عَلِيمٌ لِللَّهُ اللَّهُ الللَّهُ اللَّهُ اللللَّهُ اللَّهُ اللَّهُ

صدق الله العظيم

سورة النورالآية 35

#### **Dedication**

This thesis is dedicated to the soul of my father, who taught me that the best kind of knowledge to have is that which is learned for its own sake. It is also dedicated to my mother, who taught me that even the largest task can be accomplished if it is done one step at a time.

This thesis work is dedicated to my husband, Mohmmed, who has been a constant source of support and encouragement during the challenges of graduate school and life. I am truly thankful for having you in my life. This work is also dedicated to my sons, my sisters and my brothers, who have always loved me

### Acknowledgements

Foremost, I would like to express my sincere gratitude to my supervisor Dr. Nadia Madni for the continuous support of my research, her patience, motivation, enthusiasm, and immense knowledge. Her guidance helped me in all the time of research and writing of this thesis.

Besides my advisor, I would like to thank my colleague Salaheldein Elzaki for his help, advise, encouragement and insightful comments.

My sincere thanks also go to the staff of molecular epidemiology laboratory at tropical medicine research institute for their help and hosting me in their lab to carry out my practical work.

Last would like to thank all of the women who participated in this Study.

#### Abstract

This study was carried out Khartoum State at Khartoum Isotope Radiation Centre (RICK), from the period April –September 2013, to study the methalanetertahydrofloate reductase polymorphism with breast cancer risk, and to compare it with normal population. 96 individuals were included in this study of which 60 patients with breast cancer originating from different ethnic group and part of Sudan, and 36 were women without breast cancer as normal controls.

Five ml EDTA blood was taken for hematological parameters. All of our studies patients had low hemglobin level below 10 gm/dl (p-value=0.127)insignificant, and 15% of the patients had thrombocytopenia(less than 150.000/cum) p-value=0.2) insignificant, 50% of the studies group had normal WBCs(4000-6900), 30% had increased WBC count(more than 7000/cum) ( and 20% had decreased WBC count(less than 4000/cum) P-value=0.365)insignificant.

A cross sectional hospital based study was conducted in this study.

16.7% of breast cancer patients had TC alleles of *MTHFR* and none of the controls had mutation (Chi sequre test=6.96. *P.value*=0.01), the results showed highly significant of the TC mutation compared with normal controls.

The majority of the breast cancer patients whom had TC alleles were women under 40 years, and this could be risk of having breast cancer.

high frequency of breast cancer had been observed among northern State.

#### 

اجريت هذة الدراسة فى الفترة من ابريل -اغسطس 2013 فى ولاية الخرطوم المركز القومى للعلاج بالذرة لدراسة انزيم المياثيلين تتراهادروفوليت ومدى تاثيرة على سرطان الثدى عند النساء فى ولاية الخرطوم وذالك باستخدام تفنية البلمرة الجينية فى حالت وجود طفرة جينية مقارنة بنساء اصحاء

تم اختيار ستون مريضة بسرطان الثدى بعد التشخيص , ست و ثلاثون عبنة من الاصحاء كمجموعة ضبط,وقد تم اخذ خمسة مليميتر من الدم الوريدى من كل مريض في اثنين انبوب به مانع من التجلط واحدة لقياس نسية الهميقلوبين وعدد كريات الم البضاء و الصفائح الدموية و الاخرى لفصل الحمض النووى وكانت النتائج في كل شريحة الدراسة لديهم نقصان في قياس نسبة الهيمقلمبين وهي اقل من عشرة جرام في الديسليتر وهي تعتبر نسبة ضعيفة ,كما وجدت عدد كريات الدم البيضاء عشرون في المائة اقل من الطبيعي (اقل من اربعة الف في المليميتر المكعب) , و ثلاثون في المائة اكثر من الطبيعي (اكثر من سبعة الف) وخمسون في المائة لديهم عدد طبيعي للكريات البيضاء , اما الصفائح الدموية خمسة عشر في المائة في المائة و خمسون الف فلي المليمتر المكعب

كما لوحظ ان ستة عشر وسبعة من عشرة فى المائة من المرضى المصابيين بسرطان الثدى لديهم طفرة جنية للانزيم كما لاتوجد هذة الطفرة عند الاصحاء وعند استخدام برنامج الهزم . الاحصائية .كانت قيمة الاختبار واحد من مائة

وجدت ان غالبية المرضى فى الفئة العمرية مابين ثلاثون الى اربعين عام كما ان الولاية و الشمالية هى الاكثر انتشارا للمرض لـذالك نوصى بعمل دراسة تشمل الفئة العمرية و الشمالية هى الاكثر انتشارا للمرض لـذالك نوصى بعمل دراسة تشمل الفئة المركورتين

### **Table of Contents**

Contents	Page No
الاية	I
Dedication	II
Acknowledgements	Ш
Abstract	IV
Abstract Arabic	V
Table of contents	VI
List of Tables	IX
List of figures	X
List of a privations	ΧI
Chapter One	
Introduction and literature review	
1.1 Breast cancer	1
1.2 Risk factors	2 2
1.3 Lifestyle	2
1.4 Association between oral contraceptive breast	2
cancer	
1.5 Chemicals and radiation	2
1.6 Genetics	3
1.7 Pathophysiology and Carcinogenesis	
1.8 Diagnosis	4
1.9 Breast cancer classification	4
1.10 Breast cancer screening	6
1.11 The Burden of Breast cancer in Sudan	7
1.12 Heamglobin	8
1.13 White blood cells or leukocytes	8
1.14 Platelets	9
1.15 Methylenetetrahydrofolate reductase (MTHFR)	9
1.16 Effect of low folate and MTMFR mutation	11
1.17 Folic acid	12
1.18 MTHFR and modulatation the chemosensitivity	13
of cancer cells	
1.19 Rationale	14
1.20 Objectives of the study	15
1.21. 1General objectives	15
1.22.2 Specific objectives	15
Chapter Two Materials and methods	
Materials and methods	1.0
2.1 Study area	16

2.2 Study population	16
2.3 Sample size	16
2.4 Inclusion Criteria	17
2.5 Exclusion Criteria	17
2.6 Ethical consideration:	17
2.7 Blood Sample	17
2.8 Hemoglobin determination	18
2.9 White Blood Cell Count (WBC)	18
2.10 Platelets count	18
2.11 Buffy coat preparation	19
2.12 DNA extraction	19
2.13 DNA quantitation (Gene Quant)	19
2.14 DNA and reagents storage conditions	20
2.15 Polymerase Chain Reaction (PCR)	20
2.16 Master Mix preparation	20
2.17 Tempature profile of the primer sequences	20
flanking region 677	
2.18 DNA visualization	21
2.19 Digestion of <i>mthfr</i> gene by restriction fragment	21
polymorphism (RFLP)	
2.20 Detection of <i>mthfr</i> gene mutation	21
2.21 Statistical Methods	21
Chapter Three	
Results	
3.1 Geographical distribution of the patients and controls	22
3.2 Characteristic of the normal controls	22
	23
3.3 Distribution of the study group according to age	23
3.4 Distributions of the study group according to family history of breast cancer	23
3.5 Hematological parameters	24
3.6 Amplification of <i>MTHFR</i> 677 gene	26
3.7 Restriction Fragment Length Polymorphism of	27
MTHFR 677	21
3.8 Genotype of MTHFR alleles of the patients	27
compared with controls	
3.9 Genotyping and history of breast cancer	28
3.10 Association of <i>Mthfr</i> genotyping and	29
geographical location	
Chapter Four	
4. Discussion, conclusion and recommendat	ions

4.1 Discussion	30
4.2 Conclusions	32
4.3 Recommendations	32
References	33
Appendix	39

## **List of Tables**

content	Page
Table 3.1 showed MTHFR alleles in patients with breast cancer	27
Table 3.2 history of breast cancer and genotype	29
Table3.3 Main characteristics of the MTHFR genetic	29
variants analyzed in this study	
Table 3.4 Allele and genotype frequencies for breast cancer risk factors	29
Table 3.5 of geographical location	29

## **List of Figures**

contents	page
Figure 1.1 Mammograms showing a normal breast (left) and a cancerous breast (right).	7
Figure 1.2 MTHFR gene locations on chromosome 1(NCBI)	11
Figure 2.1 Khartoum State Map	16
Figure 3.1 Geographical Distributation of the patients	22
Figure 3.2 study group according to age	23
Figure 3.3 Distributions of the study group according to family history of breast cancer	23
Figure 3.5 white blood cells count in patients with breast cancer	24
Figure 3.7 showed relation between WBC in the treated and untreated patients	25
Figure 3.8 showed correlation of mean platelets in the treated and untreated patients	62
Figure 3.9 MTHFR 766 PCR products on 1.5% agarose gel, lane 1 molecular weight marker 100 bp, 1-5 were samples.	26
Figure 3.10 Digested with Hinf1: Lane 1 molecular weight marker 100 bp, 2,5 and 7 were TC, and 3,4 and 6 were TT genotype	27
Figure 3.11 <i>MTHFR</i> genotype of patient's breast cancer	28
Figure 3.12 Association ofn TC genotype with age group	28
Appendix (5) Showed Invasive Breast Cancer	44
Appendix (5) stage 1 breast cancer	44

**List of A privation**Fine Needle Spiration cytologyy. FNAC:

Uridine mono phophate Dump:

TMP: Thymidine mono phosphate.

FAD: Flavin adenine dinuclotide.

MTHFR: Methylenetetrahydrofolate Reductase.

FA: Folic Acid.