

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

قال تعالى :

(وَقَضَىٰ رَبُّكَ أَلَّا تَعْبُدُوا إِلَّا إِيَّاهُ وَبِالْوَالِدَيْنِ إِحْسَانًا ۖ إِمَّا يَبُلُغَنَّ عِنْدَكَ الْكِبَرَ أَحَدُهُمَا أَوْ
كِلَاهُمَا فَلَا تَقُلْ لَهُمَا أُفٌ وَلَا تَنْهَرُهُمَا وَقُلْ لَهُمَا قَوْلًا كَرِيمًا (23) وَاخْفِضْ لَهُمَا جَنَاحَ الذُّلِّ مِنَ
الرَّحْمَةِ وَقُلْ رَبِّ ارْحَمْهُمَا كَمَا رَبَّيَانِي صَغِيرًا (24)

صدق الله العظيم

سورة الاسراء 23 - 24

Dedication

To my inspiring father

To my kindle mother (Fatima Ali)

To my lovely sister and blessing brothers

To my special friends and colleagues who were giving me a lot of encouragement

To every person help and support me in this study

I dedicate this work

Acknowledgment

The greatest thanks firstly and finally for Allah ,I would like to present my great thanks for cooperating supervisor Dr. Ibrahim Khider Ibrahim who guided me and solved work problems .

A lot of thanks for Taiba cancer center and Khartoum specialized center for oncology which had good dealing with me .

Great thanks for Molecular laboratory research Alnillin University where practical of this study was done .

Abstract

A common 677 C-T transition (rs1801133) in the MTHFR gene is a well identified genetic determinant of hyperhomocysteinemia and there are some reports have shown an association between MTHFR gene polymorphism with cancer development.

The aim of this study was to detect the presence of MTHFR polymorphism among Sudanese prostate cancer patients by using PCR and explore its relation with hypercoagulable state.

The study is a case control study conducted at Taiba cancer center and Khartoum center for oncology in period from June to December 2017, 38 patients with prostate cancer(diagnosed by histopathology) and 40 healthy male(control group) were enrolled in this study,2.5 ml venous blood was collected after informed consent. RBCS was Hemolysed by alkaline solution (Red Cells lysis buffer) ,then the membranes of WBC were digested by solution containing detergent and proteases (White Cells Lysis buffer),then protein was precipitated out by saturated NaCL and centrifugation , finally DNA was precipitated by absolute ethanol ,washed by 70% ethanol and eluted in 50 µL of 10 mM Tris-HCl, 1 mM EDTA, pH 8.0 .

MTHFR C677T genotype frequencies were detected by PCR, Five µl of the PCR product (ready to load) was electrophoresed on 1.5% agarose gel, and was stained with ethedium bromide, 1X TBE buffer was used as a running buffer. The Voltage applied to the gel was 100 volt with time duration of 30 minutes. 50 bp DNA ladder was used as molecular weight marker with each patch of samples .Finally, PCR product was demonstrated by gel system.

The frequencies of CC and TT genotypes among the patients with prostate cancer were 95 % and 5% respectively, and among the control subjects 97.5 %, and 2.5%, respectively.

In conclusion, there was no statistically significant difference in genotypes distribution when compared in patients with prostate cancer and control so thrombosis for those patients not caused by MTHFR gene mutation.

ملخص الدراسة

تعتبر عملية التحول سي الي تي في الجين ميثيلين رباعي الفوليت المختزل C677T محدد وراثي ينتج عنه فرط الهوموستئين في الدم وهنالك بعض التقارير التي أشارت إلى وجود علاقة بين هذا الجين وتعدد اشكال تطور مرض السرطان.

تهدف الدراسة للكشف عن وجود الانماط الوراثية للميثيلين رباعي الفوليت المختزل C677T لدي المرضى السودانيين المصابين بسرطان البروستاتا من خلال استخدام تقنية مضاعفة الحمض النووي لاكتشاف علاقتها بحالة تخثر الدم المرتفع .

ويعتبر البحث حالة دراسية تم إجراؤها في مركز طبية للسرطان، ومركز الخرطوم لعلاج الأورام في الفترة من يونيو وحتى ديسمبر من العام 2017. ضمت هذه الدراسة ثمانية وثلاثون من المصابين بمرض سرطان البروستاتا الذين تم تشخيصهم بشكل صحيح، واربعون شخصا من الأصحاء، وتم أخذ عينات دم وريدية تبلغ 2.5 ملم بعد موافقة المرضى والأصحاء . وتم خلال الدراسة تحليل خلايا الدم الحمراء بإضافة محلول قلوي ، ومن ثم تم هضم الأعشوية الدقيقة باستخدام محلول يحتوى على مادة البروتياز التي تحلل خلايا الدم البيضاء، بعدها تم ترسيب البروتين بإضافة كلوريد الصوديوم المشبع ، وأخيرا تم ترسيب الحمض النووي باستخدام 100% الإيثان ومن ثم غسله ب 70% إيثان وتصفيتها في وحدة قياس 50 ميكروليتر في محلول TBE ذات الرقم الهيدروجيني 8.

كُشفت ترددات الجين ميثيلين رباعي الفوليت المختزل C677T باستخدام تقنية مضاعفة الحمض النووي ، وتم دمج 5 مايكروليتر من الحمض النووي مع 1.5% من مادة هلامية ممزوجة مع بروميد الإيثيديوم ، تم توصيل تيار كهربائي 100 فولت لمدة ثلاثون دقيقة .تم قياس الاوزان الجزيئية للعينات بواسطة لادر طوله 50 بيس بير

اظهرت نتائج التحليل الاحصائي ان نسب تكرار الانماط الوراثية (سي سي) (وتي تي) بين المرضى 95% و 5% على التوالي ونسب التكرار للأصحاء 97% و 2.5% علي التوالي أي لا يوجد فرق ذو دلالة احصائية في انتشار هذه الانماط الوراثية بين مجموعتي الدراسة (المرضى المصابين بسرطان البروستاتا والأشخاص الأصحاء) وبالتالي حدوث التجلط في هؤلاء المرضى ليس له علاقة جينية وانما يحدث لأسباب اخرى.

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List of Abbreviations

APC	Activated protein C
APTT	Activated partial thromboplastin time
BPH	Benign prostatic hyperplasia
CT	Computerized tomography
DNA	Deoxyribonucleic acid
DRE	Digital rectal examination
DHT	Dihydrotestosterone
DVT	Deep venous thrombosis
EDTA	Ethylin diamin tetra acetic acid
FVL	Factor five ledein
FDA	Food and drug administration
LAC	Lupus anticoagulant
LMWH	Low molecular weight heparin
MTHFR	Methylene tetra hydrofolate reductase
MRI	Magnetic resonance imaging
PCR	Polymerase chain reaction
PET	Positron emission tomography
PNH	Paroxysmal nocturnal haemogolbinurea
PSA	Prostatic specific antigen
PT	Prothrombin time
PTS	Post thrombotic syndrome
RCLB	Red cell lysis buffer
SAM	S adenosyl methionine

SDS	Sodium dodecyl sulfate
TNM	Tumor nodes metastasis
UFH	Unfractionated heparin
VTE	Venous thromboembolism
WCLB	White cell lysis buffer