

بسم الله الرحمن الرحيم

قال الله تعالى:

﴿ اللَّهُ لَا إِلَهَ إِلَّا هُوَ الْحَيُّ الْقَيُّومُ لَا تَأْخُذُهُ سِنَّةٌ وَلَا نَوْمٌ لَهُ مَا فِي السَّمَاوَاتِ وَمَا فِي الْأَرْضِ مَنْ ذَا الَّذِي يَشْفَعُ عِنْدَهُ إِلَّا بِإِذْنِهِ يَعْلَمُ مَا بَيْنَ أَيْدِيهِمْ وَمَا خَلْفَهُمْ وَلَا يُحِيطُونَ بِشَيْءٍ مِنْ عِلْمِهِ إِلَّا بِمَا شَاءَ وَسِعَ كُرْسِيُّهُ السَّمَاوَاتِ وَالْأَرْضَ وَلَا يَئُودُهُ حِفْظُهُمَا وَهُوَ الْعَلِيُّ الْعَظِيمُ ﴾

صدق الله العظيم

الآية 255 سورة البقرة

Dedication

To my father.....who taught me that the best kind of knowledge to have is that
which is learned for its own sake

To my mother..... who taught me that even the largest task can be accomplished
if it is done one step at a time

To my husband for his support and without the stability and security provided by his love
and encouragement, this study would not have been possible.

To my beloved brothers and sister for their endless love, support and encouragement

To my beloved kids: Mohammed and Mostafa, whom I can't force myself to stop loving

To my homeland Sudan, the warmest womb

To all my family and all the people in my life who touch my heart, I dedicate this work

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Finally I would like to thank many friends whom I could not mention by name, for all their support, encouragement and motivation throughout my research study.

This thesis is only a beginning of my journey.

Abstract

A case control study conducted during the period from June 2013 to September 2016 to assess the genetic polymorphisms of thyroid related genes, they were Iodo thyronine deiodinase-1 (*DIO1*) (a & b), Phosphodiesterase 8B (*PDE8B*) and Thyroid Stimulating Hormone Receptor (*TSHR*) and its relationship to pathogenesis of thyroid disorders in White Nile State in Sudan.

One hundred Sudanese women diagnosed with thyroid disorders classified as (30 hypothyroidism, 30 hyperthyroidism and 40 euthyroid goiter) enrolled in this study as a test group whose admitted to the health insurance hospital in White Nile State, age range 22.1-47.5years old and fifty apparently healthy matched individuals age range 22.8-49.4 years old as control group, Blood specimens were collected and the levels of free thyroxin (FT4), free triiothyroinine (FT3) and thyroid stimulating hormone (TSH) in serum were measured by microplate immuno enzymatic assay with commercial kits from Omega Company. From blood sample DNA was extracted using phenol chloroform method and polymerase chain reaction was performed and the PCR products were used for restriction fragment length polymorphism to identify the specific alleles of genes. DNA purification and standard sequencing was performed and analyzed by bioinformatics analysis to confirm the results of RFLP analysis. The Data were computed and analyzed using statistical package for social sciences (SPSS Version 20) computer soft ware.

The results of the study showed that, there was significantly decrease of the mean of TSH levels p -value (0.020) euthyroid goiter when compared with control group.

Also the study indicated that, there was a significant association between *DIO1a* gene polymorphism and euthyroid goiter p -value (0.002). A Novel mutation was detected in five patients with thyroid disorders (3 patients with hypothyroidism, one with hyperthyroidism and one with euthyroid goiter) when standard DNA sequencing was performed. The results also showed a significant association between *DIO1b* gene polymorphism and hypothyroidism p -value (0.001).

On the other hand *PDE8B* gene polymorphism significantly associated with hyperthyroidism, hypothyroidism and euthyroid goiter (p -value = 0.009, 0.010, 0.008), also *TSHR* gene polymorphism was significantly associated with the three types of

thyroid disorders (hyperthyroidism p -value (0.009), hypothyroidism p -value (0.004) and euthyroid goiter p -value (0.016).

Significant decreased of FT4 levels was observed when compared mutant allele (*DIO1a*) with normal allele in hyperthyroidism p -value (0.040) and euthyroid goiter p -value (0.020) while FT3 and TSH levels were unchanged. In addition there was significant increased of FT3 p -value (0.001) and FT4 levels p -value (0.001) and significant decreased of TSH level p -value (0.010) when compare mutant allele (*PDE8B*) with normal in hyperthyroidism.

Finally these genes may contribute to pathogenesis of related disorders. In addition *TSHR* and *PDE8B* genes polymorphisms link with hyperthyroidism, hypothyroidism and euthyroid goiter therefore could be a useful prognostic marker for thyroid disorders.

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المستخلص

أجريت هذه الدراسة التحليلية في الفترة ما بين يونيو 2013 حتى سبتمبر 2016 لتقييم الصور المتعددة الجينية للجينات التي لها علاقة بالغدة الرقية، وهي إنزيم نازع اليود أيودو ثيرونين 1 (*DIO1*) أ و ب، الفوسفات ثنائي استريز 8 بيتا (*PDE8B*) و محفز مستقبلات هرمون الغدة الرقية في حدوث اضطرابات الغدة الرقية في ولاية النيل الأبيض في السودان.

100 من النساء السودانيات المشخصات باضطرابات الغدة الرقية (30 لديهم فرط نشاط الغدة الرقية، 30 قصور الغدة الرقية و 40 تضخم الغدة الرقية السوى) تضمنوا كمجموعة إختبار من مستشفى التأمين الصحى في ولاية النيل الأبيض تتراوح اعمارهم من 22.1 الي 47 سنة، و 50 من المتطوعات الاصحاء تتراوح اعمارهم من 22.8 الي 49.4 سنة كمجموعة مكافئة ضابطة.

تم جمع عينات الدم من كلا المجموعتين ثم تم قياس مستويات هرمون الثيروكسين الحر، يودو ثيرونين الثلاثي الحر و هرمون تحفيز الغدة الرقية في مصل الدم، باستخدام فحص الصفيحة المناعية الأنزيمية و طقم شركة Omega التجارية.

من عينة الدم تم استخلاص الحمض النووي باستخدام طريقة الفينول كلوروفورم لأداء تقنية تفاعل البلمرة المتسلسل لتضاعف الحمض النووي و استخدام تقييد طول القطعة تعدد الأشكال للتعرف على طفرة معينة من الجينات.

تم إجراء تنقية الحمض النووي و التسلسل القياسي وتحليلها بواسطة تحليل المعلوماتية الحيوية لتأكيد نتيجة التنميط الجيني باستخدام تقييد طول القطعة تعدد الأشكال، كما تم استخدام برنامج الحزمة الاحصائية للعلوم الاجتماعية (SPSS) (النسخة 20) لتحليل النتائج.

أظهرت نتائج الدراسة أن هناك أهمية انخفاض في متوسط مستوى الهرمون المحفز للغدة الرقية القيمة الاحتمالية (0.020) لدي مرضي تضخم الغدة الرقية السوي عند مقلرنه مع المجموعة الضابطة. وأشارت نتائج هذه الدراسة إلى أن هناك علاقة ذات دلالة احصائية القيمة الاحتمالية (0.002) للصور المتعددة لجين *DIO1a* و تضخم الغدة الرقية السوي. كما تم الكشف عن طفرة جديدة في *DIO1a* في 5 مرضي باضطرابات الغدة الرقية (3 لديهم قصور الغدة الرقية، 1 مريض بفرط نشاط الغدة الرقية و 1 مريض بتضخم الغدة الرقية السوي) بعد أداء تقنية التسلسل القياسي.

وأظهرت نتائج الدراسة علاقة ذات دلالة احصائية القيمة الاحتمالية (0.001) بين الصور المتعددة لجين *DIO1b* و قصور الغدة الرقية.

من جانب اخر الصور المتعددة لجين *PDE8B* يرتبط ارتباط ذي دلالة احصائية مع فرط نشاط الغدة الرقية القيمة الاحتمالية (0.009)، قصور الغدة الرقية القيمة الاحتمالية (0.010) و تضخم الغدة الرقية السوى القيمة الاحتمالية (0.008) وكذلك الصور المتعددة لجين *TSHR* يرتبط بشكل كبير مع الثلاثة أنواع لاضطرابات الغدة الرقية (فرط نشاط الغدة الرقية القيمة الاحتمالية (0.009) ، قصور الغدة الرقية القيمة الاحتمالية (0.004) و

تضخم الغدة الدرقية السوى القيمة الاحتمالية ((0.016) للمجموعة المختبرة عند مقارنتها مع المجموعة الضابطة.

لوحظ انخفاض ذي دلالة احصائية في مستوى هرمون الثيروكسين الحر في مرضى فرط نشاط الغدة الدرقية القيمة الاحتمالية (0.040) و مرضى تضخم الغدة الدرقية السوى القيمة الاحتمالية ((0.020 بينما مستوى هرموني يودو ثيرونين الثلاثي الحر و هرمون تحفيز الغدة الدرقية لم يتغيروا عند مقارنة الأليل المتحول مع الطبيعي لجين *DIO1a*.

بالإضافة إلى أن هناك زيادة ذات دلالة إحصائية في مستوى هرموني يودو ثيرونين الثلاثي الحر القيمة الاحتمالية (0.001) و الثيروكسين الحر القيمة الاحتمالية (0.001) وانخفاض ذي دلالة إحصائية في هرمون تحفيز الغدة الدرقية القيمة الاحتمالية (0.010) عند مقارنة الأليل المتحول بالطبيعي لجين *PDE8B* في مرضى فرط نشاط الغدة الدرقية.

واخيرا هذه الجينات يمكن ان تساهم في امراضية الاضطرابات المتعلقة بها بالاضافة للصور المتعددة لجيني *PDE8B* و *TSHR* ترتبط بفرط نشاط الغدة الدرقية، قصور الغدة الدرقية و تضخم الغدة الدرقية السوى وبالتالي يمكن أن يكونا علامتي النذير المفيدة لاضطرابات الغدة الدرقية.

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List of Abbreviations

BAT	Brown Adipose Tissue
cAMP	Cyclic Adenosine Monophosphate
CAPZB	F-Actin-Capping Protein Subunit Beta
cDNA	Colony Deoxyribonucleic Acid
CH	Congenital Hypothyroidism
CTLA-4	Cytotoxic T Lymphocytes Antigen -4
D1	Deiodinase Types 1
D2	Deiodinase Types 2
D3	Deiodinase Types 3
DEHAL	Iodotyrosine Dehalogenase

DIO1	Iodothyronine Deiodinase 1
DIT	Diiodotyrosine
DNA	Deoxyribonucleic Acid
DUOX2	Dual Oxidase 2
FNAC	Fine Needle Aspiration Cytology
FT3	Free Triiodothyronine
FT4	Free Thyroxine
GPCRs	G-Protein-Coupled Receptors
GWAS	Genome-Wide Association Studies
HLA	Major Histocompatibility Complex
HPT	Hypothalamus-Pituitary-Thyroid
Mcg	Micrograms
MCT	Monocarboxylate Transporter
MD	Major Depression
MIT	Mono Iodotyrosine
NIS	Sodium Iodide Symporter
OATP	Organic Anion Transporting Polypeptide
PDE8B	Phosphodiesterase 8B
rT3	Reverse Triiodothyronine
SCH	Subclinical Hypothyroidism
SECIS	Sec Insertion Sequence
SNP	Single Nucleotide Polymorphism
TAARs	Trace Amine Associated Receptors
TBG	Thyroxine-Binding Globulin
Tg	Thyroglobulin
THs	Thyroid Hormones
	Tetra Methyl Benzidine

TMB	
TMNG	Toxic Multi Nodular Goiter
TPO	Thyroid Peroxidase
TPOAbs	Thyroid Peroxidase Antibodies
TRH	Thyrotropin-Releasing Hormone
TSH	Thyroid-Stimulating Hormone
TSHR	Thyroid-Stimulating Hormone Receptor
TTR	Transthyretin
UCP	Uncoupling Proteins
UTR	Un Translated Regions
WHO	World Health Organization