Al-Quds University

Deanship of Graduate Studies

Molecular diagnosis of Porphyria suspected patients from Hebron District, Palestine

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M.Sc. Thesis

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Dedication

I dedicate this work to my father who didn't scrimp me from anything,

To my mother who flooded me with affection, for her prayers that are always helping me, for her endless support and love.

To my husband Majed, my sisters Anhar and Enmar and to my brother Noor-Aldeen, for their support, encouragement, patience and love.

To anyone who taught me a letter which participated in making me like what I am now.

To the shiny eyes of the patients who look for an answer

To anyone who has a dream, and fights for it.

Thank you all

Nawras Zeidan Hassan Fatouni

Declaration

I declare that the Master Thesis entitled "**Molecular diagnosis of Porphyria suspected patients from Hebron District, Palestine**" is my own original work, and hereby certify that unless stated, all work contained within this thesis is my own independent research and has not been submitted for the award of any other degree at any institution, except where due acknowledgment is made in the text.

Signed:

Nawras Zeidan Hassan Fatouni

Date:22/12/2018

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Abstract

Background: The porphyrias are heterogenous disorders caused by abnormalities in the chemical steps of hemesynthesis, which is a vital molecule for all of the body's organs. Porphyria symptoms arise mostly from effects on either the nervous system or the skin. Effects on the skin occur in the form of cutaneous porphyrias. Proper diagnosis is often delayed because the symptoms are nonspecific. Skin manifestations can include burning, blistering and scarring of sun-exposed areas.

Objectives: To date there have been no reports on the molecular analysis of Porphyria in the Palestinian population. Therefore, the aim of this study was to confirm the clinical diagnosis of porphyria by biochemical and genetic tests in suspected patients from Hebron district and to determine the gene that cause the phenotype and clinical symptoms of cutaneous porphyria.

Method: 40 patients from Tafouh /Hebron city–who have been clinically diagnosed with cutaneous porphyria were recruited**,** Biochemical and genetic studies were performed using different molecular techniques like cloning, Polymerase chain reaction (PCR), Restriction fragment length polymorphism (RFLP), sequencing and next generation sequencing to investigate the causative mutations at DNA and mRNA levels.

Results: According to this study, the biochemical test that involves checking the total porphyrin and porphyrin precursor levels in urine give normal results for all the tested patients. Continually none of the Uroporphyrinogen decarboxylase (*UROD*NC_000001.11), uroporphyrinogen III synthase (*UROS*NC_000010.11), and Ferrochelatase (*FECH*NC_000018.10) genes give any

significant mutation using PCR-based techniques, Restriction fragment length polymorphism (RFLP), Sanger and Next generation sequencing.

In conclusion, deeper molecular tests is needed as doing exome/whole genome sequencing , epigentic modification analysis for these patients or invistigating a novel mutation that causes a new genetic disorder.

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Table of Abbreviations

Abbreviation	Full Word
PCR	Polymerase chain reaction
DNA	Deoxyribonucleic acid
RFLP	Restriction fragment length polymorphism
mRNA	Messenger Ribonucleic Acid
µL	Microliter
M	Molar
PBS	Phosphate buffer saline
EDTA	Ethylene diamintetraacetic acid
TAE	Tris acetate EDTA
V	Volt
Bp	Base pair
°C	Celsius or centigrade degree
BLAST	Basic local alignment search tool
MW	Molecular weight
OMIM	Online Mendelian Inheritance in Man
Mu	Mutant
Wt	Wild type
SNP	Single Nucleotide polymorphism
IVS	Intervening Sequence
UROD	Uroporphyrinogen decarboxylase

التشخيص الجزيئي للمرضى المشتبه باصابتهم بمرض البورفيريافي منطقة الخليل، فلسطين

اعداد الطالبة : نورس زيدان حسن فاتوني

المشرف : د. زياد عابدين

الملخص

البورفيريات هي اضطرابات غير متجانسة تنجم عن تشوهات في الخطوات الكيميائية التي تؤدي إلى إنتاج الهيم، حيث يعتبر من اهم الجزيئات الحيوية الضرورية لجميع أجهزة الجسم ووظائفه الحيوية. تنشأ أعراض البورفيريا في الغالب من آثار قد تتركز على الجهاز العصبي أو الجلد. هناك انواع متعددة للمرض تظهر بصور عدة ولكن الانواع التي تصاحبها اعراض جلدية هي (fPCT, HEP, CEP and EPP). غالبا ما يتأخر التشخيص الصحيح لأن الأعراض غير محددة. يمكن أن تشمل المظاهر الجلدية حرق، وانتفاخات وتندب خاصة في المناطق المعرضة لأشعة الشمس.

حتى الآن لم يكن هناك أي تقرير او دراسة عن التحليل الجزيئي الجيني للبورفيريا في المرضى الفلسطينيين. وكان الهدف من هذه الدراسة هو تأكيد التشخيص السريري للبورفيريا من خلال اختبارات الكيمياء الحيوية والدراسات الوراثية للمرضى المشتبه بهم وتحديد الجين الذي يسبب التوصيف المظهري والأعراض في منطقة الخليل.

وسيشارك التشخيص المؤكد وراثيا في الحد من الآثار الفظيعة للمرض، وسيسهم بشكل كبير في صحة المرضى الفلسطينيين وحالات الطفرة غير المتجانسة سريريا غير المتناظرة داخل الأسر، وسيمكن التشخيص الصحيح والمبكر من وضع خطة لإدارة المشكلة والتقليل من عدد المواليد الجدد المصابين ، وتوجيه المصابين لنمط حياة مناسب للتخفيف من الاعراض وشدتها، وتقديم المشورة الجينية الأفضل للأسر المعرضة لإنجاب اطفال مصابين.

وقد شارك 40 مريضا تقريبا من مدينة تفوح- الخليل في هذه الدراسة، وأجريت الدراسات الوراثية والبيوكيميائية باستخدام تقنيات جزيئية مختلفة للتشخيص والكشف عن الطفرات المسببة للمرض.

واظهرت النتائج وجود عدد من الطفرات دون اثر تشخيصي على المرضى، مما يقودنا الى المزيد من البحث من خلال طرق اكثر تقدما عن طفرات او مؤثرات جينية قد تسبب الاعراض الموجودة عند المرضى او التوجه للكشف عن طفرات تتعلق بمرض اخر سواء كان معروفا او جديدا وذلك استكمالا لما قمنا به من اجل تحقيق كافة الاهداف المرجوة .