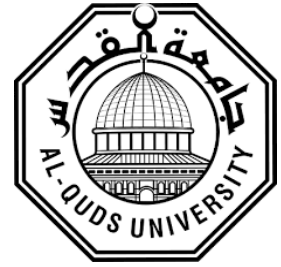


Al-Quds University

Deanship of Graduate Studies



**Molecular diagnosis of Porphyria suspected patients
from Hebron District, Palestine**

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M.Sc. Thesis

Jerusalem-Palestine

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Molecular diagnosis of Porphyria suspected patients
from Hebron District, Palestine

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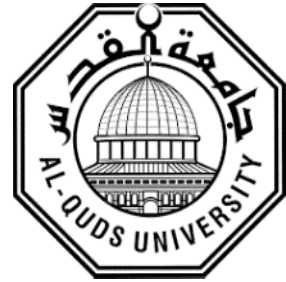
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Thesis Approval

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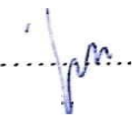
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Dedication

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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Molecular diagnosis of Porphyria suspected patients from Hebron District, Palestine

Prepared by: Nawras Zeidan Hassan Fatouni

Supervisor: Prof. Ziad Abdeen

Co-supervisor: Dr. Abed elmajeed Nasereddin

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 (*URODNC_000001.11*), uroporphyrinogen III synthase (*UROSNC_000010.11*), and Ferrochelatase (*FECHNC_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 , epigenetic modification analysis for these patients or invistigating a novel mutation that causes a new genetic disorder.

Table of Contents	page
Declaration	i
Acknowledgment	ii
Abstract	iii
Table of contents	v
List of tables	xii
List of figures	xiii
List of appendices	xv
Chapter 1: Introduction	1
1.1 Historical background	1
1.2 Biochemistry of Porphyria	1
1.2.1 HemeBiosynthesis (Porphyrin Biosynthesis)	1
1.2.2 Heme Biosynthesis Pathway Regulation	3
1.3 Classification of Porphyria	3
1.3.1 The hepatic porphyria	4
1.3.1.1 Clinical manifestations	4
1.3.1.2 Pathogenesis of Acute Attacks	5
1.3.2 Erythropoieticporphyrias	5
1.3.2.1 Congenital Erythropoietic Porphyria (CEP)	5
1.3.3 Cutaneus Hepatic Porphyria	6
1.3.3.1 Porphyria CutaneaTarda (PCT)	6
1.3.3.1.1 Hepatoerythropoietic Porphyria	8
1.3.3.2 Erythropoieticprotoporphyrin (EPP)	8
1.4 Diagnosis of Porphyria	10
1.5 Research significance	11
Chapter 2: Material and Methods	
2.1 Working Plan	12
2.2 Patients and Controls	13

2.3 Ethical issues	13
2.4 Logistics of Sample Collection	14
2.4.1 Samples for Genetic analysis	14
2.4.2 Samples for Biochemical Studies	14
2.4.3 Preparing DNA samples	15
2.5 Molecular DNA analysis	15
2.5.1 UROD gene analysis on DNA and RNA levels	15
2.5.1.1 Cloning UROD gene in a plasmid vector and Sanger sequencing (UROD gene amplification)	15
2.5.1.2 PCR Master Mix set up and Condition of the PCR reaction	15
2.5.1.3 Detection and Purification of PCR product	16
2.5.1.4 Blunting, ligation reaction and cloning of UROD gene	17
2.5.1.5 Competent cells preparation (DH5 α)	17
2.5.1.6 Transformation	18
2.5.1.7 Positive selection of transformed DH5 α cells	19
2.5.1.8 Plasmid extraction for DNA preparation from clones	20
2.5.1.9 UROD gene and Sanger sequencing	20
2.5.1.10 UROD gene and next generation sequencing	21
2.5.2 UROS gene analysis	22
2.5.2.1 RNA Extraction and cDNA production of UROS gene	22
2.5.2.2 Amplification of cDNA – UROS gene and Sanger sequencing	22
2.5.3 FECH gene analysis	23
2.5.3.1 Restriction fragment length polymorphism for FECHIVS3-48C/T polymorphism detection	24
2.5.3.2 FECH gene and Next generation sequencing	24
2.6 Bioinformatics analysis	26
Chapter 3: Results	
3.1 Patients and Controls	27
3.2 Biochemical Test result	28
3.3 DNA Molecular analysis	29

3.3.1UROD molecular DNA Analysis:	29
3.3.1.1Cloning UROD gene in a plasmid vector	29
3.3.1.2 UROD gene sequencing and screening	30
3.3.1.3 UROD next generation sequencing and screening	34
3.3.2 UROS molecular DNA analysis	34
3.3.3 FECH molecular DNA analysis	35
Chapter 4: Discussion and Conclusions	38
4 .1 Discussion	38
4.2. Conclusions and Recommendations	40
4.3 Limitations	41
References	42
Abstract in Arabic	49

Lists of Tables

Table name	Page
Table 1.1: Classification and Major Features of Human Porphyrrias	4
Table 1.2: Accumulation and excretion patterns of heme precursors and intermediates in body fluids	10
Table 2.1: Setting up PCR reactions using LongAmp Hot Start Taq 2X Master Mix	16
Table 2.2: Primer sets used to amplify the UROD gene	21
Table 2.3 : Primers code, sequences and sizes for each target for UROS gene	23
Table 2.4 : Primers code, sequences ,sizes for each target and the extension time for FECH gene	25
Table 3.1: The porphyrin and porphyrin precursor levels in urine/24 hrs.	28
Table 3.2: cloning selection results for three patients	29
Table 3.3: SNPs in UROD Gene for KZ patient	31
Table 3.4: SNPs in UROD Gene for LKH patient	32
Table 3.5: SNPs in UROD Gene for LT patient	33
Table 3.6: SNPs were detected in UROD by NGS	34

List of Figures

Figure Title	Page
Figure 1.1: The heme biosynthetic pathway	2
Figure 2.1: Flow chart demonstrates the working plan for our study	12
Figure 2.2: Map and Features of Pjet1.2/Blunt Cloning Vector	17
Figure 2.3: Preparation of competent cells by CaCl ₂ treatment and transformation	19
Figure 2.4 : Distribution of primer sets cross UROD gene and the PCR product size	21
Figure 3.1: Family history and the progression of disease	27
Figure 3.2 : Detection of positive clones for three patients	29
Figure 3.3: Full UROD gene (3.6 Kb) gene image	30
Figure 3.4: Three hot spot areas of UROS gene were amplified for sequencing	35
Figure 3.5: cDNA was amplified for UROS gene for four unrelated patients (Lane 1-4)	35
Figure 3.6: : Intron 3 (IVS3-48C/T allele) was amplified from different sample patients 1-5, using specific primers. Lane 6 negative control, PCR product 282 bp.	36
Figure 3.7: Restriction fragment length polymorphism (RFLP) analysis after digestion the PCR product by TseI, enzyme	36
Figure 3.8: PCR product for FECH gene, ready for Next generation sequencing	37

Appendixes

Appendix A:Patients' Photos shows a mild to severe symptoms

47

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
$^{\circ}\text{C}$	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 البورفيريا في منطقة الخليل، فلسطين

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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 أو 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.

وسيشترك 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، وسيتمكن 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.

وأظهرت 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 .