

Sudan University of Science and Technology
College of Graduate Studies

**Molecular and Histopathological Study on Mitochondrial
DNA Mutations in Oral Lesions among Sudanese Patients,**

دراسة جزيئية نسيجية على الطفرات الجينية للحمض النووي
الخاص بالميتوكوندريا لدى المرضى السودانيين على المصابين
بأورام الفم

**A thesis Submitted in fulfillment of requirements for PhD degree in
Medical Laboratory Science (Histopathology and Cytology)**

By/

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(فَكَيْفَ إِذَا جِئْنَا مِنْ كُلِّ أُمَّةٍ بِشَهِيدٍ وَجِئْنَا
بِكَ عَلَى هَؤُلَاءِ شَهِيدًا)

سورة النساء الآية (41)

DEDICATION

I dedicate this work to all Sudanese population

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ABSTRACT

The study was conducted as a retrospective study on oral lesions biopsies in Khartoum – Sudan, to investigate the frequency of mitochondrial DNA (D- loop) mutations in oral lesions and to determine whether the genetic alteration has a role in oral carcinogenesis. The study included one hundred and fifteen (115) subjects, their age ranged from 10 to 90 years with mean age 47 years. Fifty four 54 (47%) were females and sixty one 61(53%) were males. The data were collected from examination of biopsies and patients records. All oral biopsies were examined and classified in to Histopathological pattern by professional pathologist using Hematoxylin & Eosin standard method. The detection of mtDNA4977 bp deletion was performed by PCR amplification with three sets of primers: deletion region, positive and negative control. Of the 115 subjects, 4 (3.5%) showed 4,977 -bp deletion. When characteristics and risk factors including age, sex, tumor cell differentiation status were analyzed cross tabulating to 4,977 -bp deletion, the study showed 3(75%) were females and only one (25%) was male. All the 4,977 -bp deletion (4) cases (100%) occurred among population having malignant lesions, and cases of squamous cell carcinoma constituted 3 (75%) of total mutations. The study provided that not all oral lesions underwent surgical operations were having malignancy. Although, the majority of tumors were squamous cell carcinoma, there was a probability of other types of malignancy, Aggressiveness of tumor increased with age. It could be concluded that there was no obvious correlation between 4.977 bp deletion and prognostic indicators among Sudanese with oral lesions, which may limits it`s utility as prognostic biomarker in oral disease, therefore further studies with wide scope in this topic was recommended.

ملخص الدراسة

اجريت هذه الدراسة التراجعية فى السودان- ولاية الخرطوم, بغرض معرفة تردد انواع الاورام التى تصيب الفم ولتقييم نسبة حدوث طفرات فى الحمض النووى الخاص بالميتوكوندريا فى خلايا الفم لدى السودانين المصابين بتقرحات او اورام تجويف الفم. ولمعرفة ما اذا كان هناك علاقة بين نوع الورم ووجود طفرة فى الحمض النووى المايكوكوندورى0 تم اجراء الدراسة فى الفترة من 2011 الى 2014م اذ شملت عدد مائة وخمسة عشر(115) مريض تراوحت اعمارهم بين 10 الى 90 سنة ومتوسط عمر 47 سنة. عدد الاناث 54(47%) وعدد الذكور 61 (53%). تم جمع المعلومات من سجلات المرضى والفحص المعملى لعينات المرضى التى كانت عبارة عن خزعات مثبتة بالفورمالين. شمل الفحص المعملى شرائح مجهرية لتأكيد التشخيص عن طريق صبغة الهيماتوكسلين ومقاطع اخرى لاجراء الكشف عن الطفرات فى الحمض النووى (mtDNA4977 bp deletion) بطريقة تفاعل البلمرة التسلسلى . اوضحت الدراسة ان 70% من جملة 115 حالة هى اورام سرطانية, اذ تمثل اورام النسيج الطلائى منها 67.9% . بينما 30% هى بين اورام حميدة وحالات التهابية. كما اوضحت الدراسة ان هناك علاقة طردية بين نسبة الاورام وتقدم العمر. فيما يتعلق بالطفرات الجينية خلصت الدراسة الى ان 4 (3.5%) من الحالات بها طفرات فى الحمض النووى (mtDNA4977 bp deletion) . وعند ربطها بعوامل العمر , الجنس , ونوع الورم اوضحت الدراسة ان كل الطفرات (100%) كانت لدى الاشخاص المصابين بحالات سرطانية. و3(75%) منهم اناث. على الرغم من تلك المعدلات فى الطفرات الجينية لكن قد لا تصل لتؤخذ كمؤشر او عامل مؤثر فى سرطنة الاورام مما يدعو الى اهمية دراسات لاحقة اكثر شمولا فى هذا المجال.

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List of Abbreviations

A:	Adenine
ADP:	Adenosine diphosphate
AIDS:	Acquired Immune Deficiency Syndrome
ATP:	Adenosine triphosphate
Bp:	Base pair
C:	Cytosine
COX:	Cytochrome C oxidase
D-loop:	Displacement loop
DNA:	Deoxy ribonucleic acid
D.W:	Distilled Water
EBNA:	Epstein–Barr virus nuclear antigen
EBV:	Epstein Barr Virus
FFPE:	Formalin fixed pre embedded
ER:	Endoplasmic Reticulum
G:	Guanine
HIV:	Human Immunodeficiency Virus
HNSCC:	Head and neck squamous cell carcinoma
HPV:	Human Papilloma Virus
HSV:	Herpes simplex virus
IL:	Interleukin
Kb:	kilo base
MAM:	Mitochondria-associated ER-membrane
MERRF:	myoclonic epilepsy with ragged red fibers
MtDNA:	mitochondrial DNA
NNN:	N-nitrosornicotine

OC: Oral cancer
OSCC: Oral squamous cell carcinoma
OxPhos: Oxidative Phosphorylation pathway
PCR: Polymerase chain reaction
Primer (F): Forward
ROS: Reactive oxygen species
Primer (R): Reverse
RNA: Ribonucleic acid
R.T: Room temperature
r RNA: ribosomal RNA
tRNA: transfer RNA
ST: Smokeless tobacco
T: Thymine
TAE: Tris base Acetic acid EDTA
TCA: tricarboxylic acid
TIM: Translocase of the inner membrane
TNF: Tumor necrosis factors
TNM: Tumor/ Lymphnodes/Metastasis
TSN: Tobacco Specific Nitrosamine
TSNAs: Tobacco-specific nitrosamines
VLDL: Very-low-density lipoprotein
USA: United State of America
U.V: Ultraviolet

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