

# ***Dedication***

- *To my parents*
- *To my wife & my children*
- *To my family*
- *To my teachers*
- *To my friends*

## ***Acknowledgement***

*I thank the Almighty Allah for bestowing on me the needed strength, clarity of mind and perseverance to complete this work.*

*I would like to express my deep sense of gratitude and thanks to my supervisor Prof. Dr. Hussain Gadelkarim Ahmed, for his mentoring, supervision and motivation since I was a student of B.Sc., and for his patience and encouragement to make this work becomes a reality. I sincerely appreciate his significant comments, suggestions and guidance for organizing this research, the generous support of his reading, amending, and editing of the research several times before it saw the light. If it were not for his understanding and expanded chest, this work would not have been this way.*

*My thanks are also extent to my co-supervisors Dr. Salim Saeed Bafakeer and Dr. Mohammed Siddig Abd-al-Aziz for their support, useful comments and suggestions, guidance and valuable assistance to achieve optimum outcome of this research and help in my research through reading the results and comment on all slides.*

*Special thanks to Dr. Abuelgassim Abbas for his assistance in statistical analysis, useful comments and suggestions.*

*I am grateful to the teachers of Faculty of Medical Laboratory Science (SUST) for their faith and help during my study in this College.*

*My thanks are also extent to Laboratories research members, Sohair Ramadan, Fatima Majzoob, Rasha Yousif and Umsalama Baballa for their supporting to complete this research.*

*I would like to thank the staff of all National Cancer Centers, Hospitals and Histopathology Laboratories in Yemen who assisted me in sample collection.*

*Further I thank all staff of International Medical Diagnostic Center in Mukalla for their support and help in sample processing.*

*I am especially indebted to my country (Republic of Yemen) and Al-awn Foundation for Development for the confidence they have bestowed on me and for the support they have afforded me which enable me to finish my study and complete my dissertation.*

## Abstract

This is a retrospective analytical study was conducted in Yemen during the period from January 2012 to January 2015. The study aimed to determine the most frequent HPV genotypes among Yemeni women diagnosed with cervical cancer.

One hundred and fifty Formalin-fixed paraffin-embedded (FFPE) tissue blocks of patients previously diagnosed with cervical cancer and fifty FFPE tissue blocks of patients with non neoplastic lesions who had undergone hysterectomy for any causes other than cervical cancer as (control group) were included in this study and investigated for the presence of HR-HPV infection by immunohistochemistry techniques and molecularbiology technique using PCR. All samples and data were obtained from the archive of national cancer centers, different hospitals and private histopathology laboratories in Sana'a, Aden and Hadhramout during the period from 2009 to 2013. In these three governorates exist the only three national cancer centers in Yemen and all cancer patients coming from different governorates of Yemen are referred to these centers for diagnosis and treatment. The age of patients ranged from 21 to 75 years with the mean ages of 46.73 years, most of patients were in the age group 41-50 representing 88/200 (44%), squamous cell carcinoma (SCC) was the most common among cases representing 80/150 (53.3%) and Most of them 35/80 (43.7%) were found in the age group 41-50.

Three sections from each sample were prepared, one stained with haematoxyline and eosin (H&E) to confirm the histopathological diagnosis, the second section was immunostained using avidin biotin technique to detect the HPV

by the use of monoclonal mouse anti-human papillomavirus clone (K1H8) from Dako company which is used to demonstrate HPV type 6, 11, 16, 18, 31, 33, 42, 51, 52, 56 and 58 and the third section was prepared for PCR in which the paraffin removed, then the DNA was extracted from each sample according to the steps described in DNA extraction kit purchased from Sacace biotechnologies-Casera –Italy. The obtained DNA was amplified by PCR according to HPV High Risk Typing Kit from Sacace biotechnologies. Type specific primers (primer for HR-HPV 16, 18, 31, 33, 35, 39, 45, 52, 56, 58, 59, 66) were used. The PCR products were visualized in 3% Agarose gel with 0.5 µg/ml Ethidium bromide staining.

The study found that immunohistochemical detection of HPV gave positive results in 52% (78/150) of cases and 12% (6/50) of controls while by PCR HPV types were detected in 114/150 (76%) of cases and 8/50 (16%) of controls, so the prevalence of HR-HPV was 114/150 (76%) among cases and 8/50 (16%) among control. Among the positive patients, 89/122 (73%) had single type infections, and 33/122 (27%) was multiple HPV types involving mainly HPV 16, HPV 18 and HPV 31. Consequently, the risk associated with HPV infection was found to be statistically significant. Ten HR-HPV types were detected in this study, the most common genotypes among cases were HPV 16: 72/150 (48%), (51/72 as single infection, 21/72 as multiple infections), most common associated with SCC in women less than 50 years old, the second most common was HPV 18: 31/150 (20.7%), (16/31 as single infection and 15/31 as multiple infection) and always in association with adenocarcinoma, followed by HPV 31: 9/150 (6%) (that was mainly detected as co-infection with other types), HPV 45: 9/150 (6%), HPV 58: 6/150 (4%), HPV 33: 5/150 (3.3%), HPV 35: 5/150 (3.3%), HPV 39: 5/150 (3.3%), HPV 59: 4/150 (2.7%) and

HPV52: 1/150 (0.7%). Among the control group HPV 16: 2/50 (4%), HPV 31, 33, 35, 18, 45, and 58 were seen, 1/50 (2%) each.

In this study there was no association between types of cervical cancer and types of HR-HPV with the regions of Yemen. Also there was no statistically significant association between type of cervical cancer, regions and age groups.

The study concluded that the prevalence of HR-HPV infection is high among Yemeni women, with 76% prevalence among cases. So, HR-HPV proved to be the most important factor that leads to cervical cancer. HPV 16 had the highest prevalence and is commonly associated with SCC in women less than 50 years old, followed by HPV 18 that was mainly associated with adenocarcinoma. HPV 16, 18, 45, 31 and 58 were the most common five HPV types among Yemeni patients. Multiple infections with more than one type of HPV were found in about 27% of all positive cases involving mainly HPV16, 18 and 31. Immunohistochemistry using clone K1H8 (anti-HPV) is considerably lower-priced, and has reasonable specificity to be applied in Yemen for screening for HR-HPV, but the PCR is more sensitive than immunohistochemistry in the detection of HPV.

## ملخص الاطروحة

أجريت هذه الدراسة التحليلية الاسترجاعية في اليمن خلال الفتره من يناير 2012 إلى يناير 2015. هدفت الدراسة إلى تحديد الأنماط الجينية عالية الخطورة لفيروس الورم الحليمي البشري الأكثر شيوعاً في اوساط النساء اليمنيات المصابات بسرطان عنق الرحم.

تم أخذ 150 عينة نسيجية شمعية لحالات تم تشخيصها مسبقاً بسرطان عنق الرحم و 50 عينة نسيجية شمعية لحالات غير مصابة بسرطان عنق الرحم وانما أجريت لها عمليات استئصال الرحم لأسباب مرضية أخرى (كمجموعة ضابطة)، تم تحديد وجود الأنواع عالية الخطورة من فيروس الورم الحليمي البشري وذلك باستخدام طريقتين هما: تقنيات الكشف المناعي النسيجي الكيماي وطريقة الأحياء الجزئية (تفاعل البلمرة المتسلسل PCR).

تم جمع العينات والبيانات من المراكز الوطنية للأورام باليمن وعدد من المستشفيات الحكومية والخاصة ومعامل الأنسجة في كل من صنعاء، عدن وحضرموت وذلك للفترة من 2009 إلى 2013 حيث توجد بهذه المحافظات المذكوره أعلاه المراكز الوطنية الثلاثة للأورام الوحيدة في اليمن لذا فان كل مرضي السرطان باليمن يتم تحويلهم لتلك المراكز للتشخيص وتلقي العلاج.

تراوحت أعمار المرضى مابين 21 إلى 75 سنة بمتوسط عمر 46.73 سنة، حيث كانت معظم الحالات في الفئة العمرية 41 – 50 بمايشكل 44% (200/88) من مجموع الحالات وكان سرطان الخلايا الحرشفية (SCC) هو الأكثر شيوعاً بمايشكل 53.3% (150/80) ومعظمها واقعة في الفئة العمرية 41 – 50.

تم اخذ ثلاثة مقاطع نسيجية من كل عينة باستخدام المشراح (الميكروتوم)، المقطع الأول تمت معالجته وتصبيغه بصبغة الهيماتوكسلين والأيوزين (H&E) وذلك لتأكيد التشخيص النسيجي والمقطع النسيجي الثاني تمت معالجته وتصبيغه بتقنية كيمياء الأنسجة المناعية وذلك بإستخدام جسم مضاد من شركة داکو خاص بالكشف عن فيروس الورم الحليمي البشري الأنواع (6، 11، 16، 18، 31، 33، 42، 51، 52، 56 و 58) يعرف ب (K1H8) بحسب تعليمات الشركة المصنعة. وأما المقطع الثالث فقد تم تحضيره لطريقة الأحياء الجزئية (تفاعل البلمرة PCR) حيث تمت إزالة الشمع وإستخلاص الحامض النووي DNA وذلك بإستخدام محاليل من شركة سكا سي الإيطالية بحسب تعليمات الشركة المصنعة، وبعد الحصول على الحامض النووي DNA تمت مضاعفته بإستخدام تفاعل البلمرة المتسلسل (تفاعل سلاطات

إنزيم البوليميريز) باستخدام المحاليل الخاصة بذلك من شركة سكاسي الإيطالية والذي يحتوي على فتايل خاصة بالكشف عن فيروس الورم الحليمي البشري الأنواع (16، 31، 33، 35، 18، 39، 45، 59، 52، 56، 58 و 66) بحسب تعليمات الشركة المصنعة.

فباستخدام طريقة تقنيات الكشف المناعي النسيجي الكيميائي تم إكتشاف فيروس الورم الحليمي البشري في 78/150 (52%) من حالات السرطان و 6/50 (12%) في المجموعة الضابطة، بينما بإستخدام تفاعل البلمرة (PCR) فقد وجد أن معدل ظهور الفيروس في الحالات المصابة بالسرطان كان بنسبة 76% (114/150) وبنسبة 16% (8/50) في المجموعة الضابطة. وقد وجد ان الإصابة بنوع واحد من أنواع فيروس الورم الحليمي البشري في الحالات الإيجابية كان بنسبة 73% (89/122) بينما كانت نسبة الإصابة المتعددة بأكثر من نوع 27% (33/122) معظمها من الأنواع 16، 18 و 31. خلال هذه الدراسة تم إكتشاف عشرة أنواع من فيروس الورم الحليمي البشري عالية الخطورة وقد كان أكثرها شيوعاً هو النوع 16 حيث وجد في 48% (72/150) من حالات السرطان إما منفرداً او مزدوجاً مع أنواع أخرى وقد كان أكثر شيوعاً في حالات سرطان الخلايا الحرشفية (SCC) وفي الفئات العمرية أقل من 50 سنة، متبوعاً بالنوع 18 حيث وجد في 20.7% من حالات السرطان بصورة منفردة او مزدوجة بأحد الأنواع الأخرى وقد كان أكثر ارتباطاً بسرطان الخلايا الغدية (adenocarcinoma). ثم تأتي الأنواع الأخرى بحسب الترتيب التنازلي كما يلي: 31 (6%) وهو في الغالب بصورة مزدوجة مع نوع آخر من أنواع الفيروس، 45 (6%)، 58 (4%)، 33 (3.3%)، 35 (3.3%)، 39 (3.3%)، 59 (2.7%)، 52 (0.7%) بينما لم يتم العثور على أي من النوعين 56 و 66 في هذه الدراسة. أما في المجموعة الضابطة أعطى النوع 16 نتيجة إيجابية بنسبة 4% (2/50) والأنواع 18، 35، 33، 31، 45 و 58 كل نوع منها يشكل فقط 2% (1/50).

وجد أيضاً في هذه الدراسة أنه لا توجد علاقة ذات دلالة إحصائية تربط بين المنطقة الجغرافية في اليمن مع كل من أنواع فيروس الورم الحليمي البشري أو أنواع سرطان عنق الرحم، كما أنه لا توجد علاقة ذات دلالة إحصائية تربط بين الفئات العمرية مع نوع السرطان او المنطقة الجغرافية.

خلصت الدراسة إلى أن معدل إنتشار أنواع فيروس الورم الحليمي البشري عالية الخطورة في أوساط النساء اليمنيات المصابات بسرطان عنق الرحم كان عالياً جداً وذلك بنسبة 76% مما يؤكد أن فيروس الورم الحليمي البشري عالي الخطورة هو العامل الأساسي والأكثر خطورةً في سبب الإصابة بسرطان عنق الرحم في اليمن، وقد وجد أن الأنواع 16، 18، 31، 45 و 58 هي الخمسة أنواع الأكثر إنتشاراً في أوساط النساء اليمنيات.

وخلصت الدراسة أيضاً إلى أن تقنيات الكشف المناعي النسيجي الكيميائي باستخدام الجسم المضاد (K1H8) هي أقل تكلفة وذات تخصصية يمكن تطبيقها لإجراء المسوحات للكشف عن فيروس الورم الحليمي البشري في اليمن وأن تقنية تفاعل البلمرة المتسلسل (PCR) يعتبر الأفضل والأكثر حساسية للكشف عن فيروس الورم الحليمي البشري.



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## List of Abbreviations

Abbreviation	Full name
AIS	Adenocarcinoma <i>in situ</i>
ASR	Age-standardized incidence rate
bp	Base pairs
BV	Bacterial vaginosis
CANSA	Cancer Association of South Africa
CI	Confidence Interval
CIN	Cervical intraepithelial neoplasia
CIS	Carcinoma <i>in situ</i>
CT	Computed Tomography
D.W	Distilled water
DAB	3,3- diaminobenzidinetetrahydrochlorate
DNA	Deoxyriboneocleic Acid
DPX	Dextrin Plasticizer Xylene
DVI	Direct visual inspection
ECC	Endocervical curettage
EDTA	Ethylene DiamineTetraacetic Acid
EIA	Enzyme immunoassay
ELISA	Enzyme linked immunosorbant assay
FDG	2-fluoro-2-deoxy-D-glucose
FFPE	Formalin-fixed paraffin-embedded
FIGO	International Federation of Gynecologists and Oncologists
FISH	Fluorescence In situ Hybridization
FYR	Former Yugoslav Republic
HC	Hybrid Capture
HIV	Human Immunodeficiency Virus
HLA	Human leukocyte antigens
HN	Head and neck

HPV	Human Papilloma Virus
HR-HPV	High Risk-Human Papilloma Virus
HRP	Horseradish peroxidase
HSIL	High-grade squamous intraepithelial lesion
HSV	Herpes Simplex Virus
IARC	International Agency for Research on Cancer
ICC	Invasive cervical cancer
IHC	Immunohistochemical
ISH	In situ hybridization
Kd	kilodalton
LCR	Long control region
LSIL	low-grade squamous intraepithelial lesion
ml	Mili-liter
MRI	Magnetic Resonance Imaging
mRNA	Messenger Riboneocleic Acid
ORF	Open Reading Frames
PBS	Phosphate Buffer Saline
PCR	Polymerase Chain Reaction
PET	Positron Emission Tomography
pRb	Retinoblastoma protein
RFLP	Restriction fragment length polymorphism
RNA	Riboneocleic Acid
RT	Room Temperature
SCC	Squamous cell carcinoma
SIL	Squamous intraepithelial lesion
SPSS	Statistical Package for Social Science Software
TBE	Tris-Buffer EDTA
USA	United States of America
WHO	World Health Organization
μl	Micro-liters