

## DEDICATION

### ***I dedicate this work***

I would be very grateful if anybody can help me in writing the dedication of my thesis , I would like to dedicate it to my Mother and my Father but my Mother is dead so how can I say it ... Who offers me the inspiration of success and keenness throughout my life...

### ***To my lovely family..... My wife and my Daughters...***

Who always encourage me with passion and endless support. I am so Lucky to have a woman Love me so much and stand beside me the way you have!

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## List of Abbreviations

ABs	Antibodies
bp	Base pair
CK19	Cytokeratin 19
CA	Cytoplasmic Area
CD	Cytoplasmic diameter
CI	Confidence interval
CM	Centimeter
CT	Computed Tomography
CMV	Cytomegalovirus
DAB	Diaminobenzidine
DW	Distilled water
DPX	Disterne Plasticizer Xylene
DNA	Deoxyribonucleic acid
EBV	Epstein- barr virus
FFPW	Formalin- fixed, paraffin- wax
FNA	Fine needle aspiration
FNAB	Fine needle aspiration biopsy
FISH	Fluorescence in situ hybridization
HC II	Hybrid capture II
HR-HPV	High Risk-Human Papillomaviruses
HIV	Human immunodeficiency virus
HNSCC	Head and Neck squamous cell carcinoma



HPV	Human Papillomaviruses
HSV	Herpes Simplex virus
IARC	International agency for research on cancer
IHC	Immunohistochemical
ISH	In Situ hybridization
LR-HPV	Low Risk-Human Papillomaviruses
μL	Micro-Liter
mL	Mili-Liter
MRI	Magnetic Resonance Imaging
mRNA	Massenger ribonucleic acid
NA	Nuclear Area
NA/CA	Nucleus-to-cytoplasm area ratio
NCR	Non-coding region
OCs	Oral cancers
OPLs	Oral premalignant lesions
OPSCCs	Oropharyngeal squamous cell carcinomas
OR	Odds Ratio
ORFS	Open- reading frame sequence
OSCCs	Oral squamous Cell Carcinomas
PBS	Phosphate Buffer Saline
PCR	Polymerase Chain Reaction
RT-PCR	Real time-polymerase chain reaction
SGT	Salivary gland tumor
SPSS	Statistic Package for Social Sciences

TSN	Toombak use Tobacco Specific Nitrose amine
WHO	World Health Organization
VC	Verrucous carcinoma
VZV	Varicella zoster viru

# Abstract

## Background

High Risk human papillomaviruses (HR-HPV), subtypes are strongly linked to etiology of many human cancers including oral cancer. The epidemiology of infection with different HPV genotypes greatly varies in different countries. In Sudan, there are considerable numbers of oral cancers, most of them are attributed to the use of Tobacco (toombak) use and alcohol consumption is the main risk factors for oral cancers (OCs). Other risk factor includes the viral infection, particularly with Human papilloma virus (HPV).

## Aim

The aim of this study was to identify and genotype the HR-HPV subtypes in oral tissues obtained from Khartoum State with oral lesions.

## Material and methods

This is a prospective analytical case control study conducted at Khartoum State during the period from June 2010 to December 2013. Two hundred tissue blocks were retrieved from different histopathology laboratories in Khartoum State. Of the 200 formalin fixed paraffin wax processed tissue blocks, 100 were obtained from patients diagnosed with oral cancer and 100 samples were obtained from patients diagnosed with non-neoplastic lesions. Infection with HPV was initially determined using p16<sup>INK4A</sup> as a biomarker immunohistochemistry (IHC) method, and then genotyping was subsequently assessed applying polymerase chain reaction (PCR). Tumor DNA was amplified using PCR with HR-HPV consensus and multiplex primers. Cytokeratin 19 (CK19) antibodies was used to study the expression pattern in normal mucosa, dysplasias, and oral squamous cell carcinomas (OSCC), using IHC.

## Results

HPV genomic materials using A6 and A7 primers were detected in 12/200 (6%) of oral lesions. Of these, 6/12 (50%) HPV-16, 4/12 (34%) HPV-18, 1/12 (8%) HPV-31, and 1/12(8%) HPV-33. Out of the 12 HPV; 8/12(66.7%) HPV were found in malignant lesions, whereas, 4/12(33.3%) HPV were found in benign lesions. Consequently, the risk associated with HPV infection was found to be statistically significant ( $P<0.001$ ).

The age group 40-49 years was the most susceptible to HPV infection. According to relation between HPV and lesion sites, the most affected sites were buccal mucosa and oropharynx, particularly, SCCs, hence, most of benign lesions were seen in the salivary glands. The detected HR-HPV were type 16, 18, 31 and 33. The total number of cases detected by the PCR HPV genotyping were 12 cases, out of these 11(91.7%), were detected by p16, Statistically, P16 was significantly associated with HPV the ( $P<0.005$ ).

## Conclusion

In conclusion, the findings of this study provide strong association between HPV and OCs, amongst Sudanese patients. HPV particularly subtypes 16 and 18 play a role in the etiology of oral cancer in the Sudan. On the other hand, in this study P16INK4A immunostaining was used to screen tissue samples obtained from patients with oral lesions to indicate the presence of HPV infection. But the exact role of CK19 in the genesis of HPV requires further assessment.



#### □□□□□□□□:

ان فيروس الورم الحليمي البشري للسلاسل الخطرة ، وهو السبب الرئيسي للعديد من السرطانات البشرية بما في ذلك سرطان الفم. انتشار وبائيات عدوى فيروس الورم الحليمي البشري يختلف اختلافا كبيرا في مختلف بلدان العالم حسب الأنماط الجينية. وفي السودان هنالك أعداد كبيرة من سرطانات الفم، وتنسب معظمها إلى استخدام التبغ (التمباك) وشرب الكحول وهي عوامل المسببة لسرطانات الفم. وتشمل عوامل خطر فيروسية أخرى، لا سيما فيروس الورم الحليمي البشري.

#### □□□□□□:

هدفت هذه الدراسة الي كشف فيروس الورم الحليمي البشري للسلاسل الخطرة بين المرضى المترددين بأفات الفم في ولاية الخرطوم.

#### □□□□□□ □ □□□□□□□:

الكشف عن هذا الفيروس باستخدام طريقتين هما تقنيات الكشف المناعي النسيجي الكيميائي و طريقة الاحياء الجزيئية. أجريت هذه الدراسة في ولاية الخرطوم خلال الفترة من يونيو 2010 إلى ديسمبر 2013. حيث تم اخذ 200 عينه نسيجه من معامل الأنسجة المريضة المختلفة في ولاية الخرطوم، وقد تم الحصول على 100 عينة من المرضى الذين شخضت حالاتهم على انها سرطان الفم و 100 عينة من المرضى الذين شخضت حالاتهم بغير سرطانية. تم الكشف عن فيروس الورم الحليمي البشري مبدئيا بطريقة كيمياء الانسجة المناعيه و ذلك باستخدام جسم مضاد خاص في شرائح محضره بطريقة شمع البرافين من (p16<sup>INK4A</sup>) لهذا الفيروس يعرف بال انسجه سرطانات الفم ثم لاحقا تم تحديد انواع هذا الفيروس بالكشف عن الحمض النووى الخاص بهذه الانواع و ذلك عن طريق استخدام تفاعل سلاسل انزيم البولمريز. ثم تم تضخيم الحمض النووي الخاص بهذه الانواع باستخدام بادئه خاصه لانواع ذات خطوره عاليه من هذا الفيروس باستخدام طريقة تفاعل سلاسل انزيم البولمريز. كذلك تم الكشف عن انواع معينه من واسمات السرطان مثل سرطانات للأج (CK19) الفم للخلايا الحرشفية باستخدام الكشف المناعي النسيجي الكيميائي لسام المضادة ايضا لدراسة نمط التغير في الغشاء المخاطي.

#### **النتائج:**

تم الكشف عن فيروس الورم الحليمي البشري الجيني باستخدام طريقة الاحياء الجزيئية عن الحمض النووى الخاص بهذه الانواع و ذلك عن طريق استخدام تفاعل سلاسل انزيم البولمريز ، حيث تم تحديد 12/200 (6%) من الآفات الفموية، مقسمة على النواع الفرعية كالاتي 6/12 من فيروس الورم الحليمي البشري رقم 16 (50%) ، 4/12 من فيروس الورم الحليمي البشري رقم 18 (34%)، من 1/12 فيروس الورم الحليمي البشري رقم 31 (8%) واخيرا 1/12 من فيروس الورم الحليمي البشري رقم 33 (8%). وايضا اظهرت النتائج على ان 8/12 (66.7) من فيروس الورم الحليمي البشري في الآفات الخبيثة ، في حين تم العثور على 4/12 )

33.3%) من فيروس الورم الحليمي البشري في آفات الغير سرطانية لتكون ذات دلالة إحصائية ( $P < 0.005$ ).

كانت الفئة العمرية 40-49 سنة الأكثر عرضة للإصابة بفيروس الورم الحليمي البشري . وفقا للعلاقة بين فيروس الورم الحليمي البشري و مواقع الآفات ، حيث كانت المواقع الأكثر تضررا تجاويف الفم و البلعوم و معظم الآفات الغير سرطانية في الغدد اللعابية. كان إجمالي عدد الحالات المكتشفة للحمض النووي الخاص بهذه الانواع و ذلك عن طريق استخدام تفاعل سلاسل انزيم البوليمريز. حيث كان فيروس الورم الحليمي البشري 12 حالة ، من هذه 12 من هذه 11 منها كانت موجبة من الكشف ( $p16^{INK4A}$ ) باستخدام جسم مضاد خاص لهذا الفيروس يعرف بال. المناعي النسيجي الكيميائي.

□□□□□□□□:

تعطي نتائج هذه الدراسة دليل مقنع بوجود ارتباط قوي بين فيروس الورم الحليمي البشري و سرطانات الفم في المرضى السودانيين بالاخص تلك التي تنشأ في منطقة تجويف الفم، البلعوم و المرئ. وان فيروس الورم الحليمي البشري خاصة أنواعه فرعية 16 و 18 تلعب دورا كبيرا في حدوث سرطانات الفم في السودان. من المناعية لفحص عينات الأنسجة P16 ناحية أخرى ، في هذه الدراسة تم استخدام مناعيا كيميائيا حيث يمكن استخدام هذا المضاد المناعي كمؤشر لوجود فيروس الورم الحليمي البشري من المرضى الذين يعانون من آفات الفم. ولكن الدور في نشأة فيروس الورم الحليمي البشري يحتاج إلى مزيد من CK19 الدقيق للدراسات.