بسم الله الرحمن الرحيم

قال تعالى

( وَمَا أُوتِيَتْ مِنَ الْعِلْمِ إِلَّا فَقْلِيْلاً).

حمد الله العظيم

سورah الإسراء الآية (85).
Dedications

To my Husband Muamar

To my daughter Renad.

To my family.

To my supervisors

To my friends.
Acknowledgement

First of all I thank my God who gave me the patience and power to complete this thesis.

My gratitude and appreciations are to my supervisor Dr. Zahir Abbas Hilmi Ibrahim for his consistent follow up, encouragement, guidance, invaluable comments, scientific criticisms and his great efforts to complete this thesis.

I would like thank my co supervisor Dr. Yuosif Fad Allah for his administrative and scientific assistance.

Also my thanks are extended to Ustaza Suhair Ramadan at the Research Lab of Sudan University of Science and Technology, for her great assistances and support.

I would like to express my special appreciation to Professor Moawia Mukhtar at Institute of Endemic Diseases- University of Khartoum for helping in selection of PCR reagents.
Abstract

Human papillomaviruses (HPV) are the principal etiology for cervical cancer. This study aimed to detect mucosal HPVs, HPV16 and HPV18 in cervical cancer in Sudan. The DNA was extracted from sections of paraffin embedded tissues of Sudanese women (n, 63) with cervical squamous cell carcinomas and controls (n, 17). The patients DNA samples (100ng/µl) were amplified by mucosal HPV GP5+/GP6+, HPV16 type specific and HPV18 type specific primers. GP primers detected mucosal HPVs in 75% (n, 47) of the patients, and in none of the controls. HPV16 and HPV18 types were detected in 70% (n, 33) and 9% (n, 4) of the GP positives, respectively. HPV16 was detected from all tumor differentiations degrees specially moderately differentiated tumors, while HPV18 was detected from moderate and well differentiated tumors. HPV16 was detected in all ages but mostly isolated from older patients, while HPV18 was isolated from younger patients. Ten samples were HPV positive but their types were not determined. HPV16 and HPV18 were mostly detected in housewives ethnically from Central Sudan. HPV 16 is the most important determinant for development of cervical cancer in Sudan, with less contribution of HPV18. HPV screening and vaccination programs have to be established in young Sudanese women.
الملخص

فيروسات البالبومة البشرية هي المسبب الرئيسي لسرطان عنق الرحم. هدف هذه الرسالة للكشف عن فيروسات البالبومة البشرية الخاصة بالانسجة المخاطية والأعضاء التناسلية وتحديد الأنواع الخطرة 16 و 18 في سرطان عنق الرحم في السودان. تم استخلاص دنا من عينات سرطان الخلايا الحرفشية عنق الرحم محفوظة بالفورمالين والشع من 63 سودانية مصابة به و17 للمجموعة الضابطة. تم القيام بمسح وتشخيص عينات دنا المرضى بتركيز 100 نانوغرام بالميكروليزر بجهز تفاعل البلمرة السلسلة بالمبتدأت العامة ج ب 5 و 6 للكشف عن فيروسات البالبومة الأنسجة المخاطية والميتادس الخاصة بالكشف عن الأنواع 16 و 18. كشفت مبتدأت ج ب العامة عن إصابة 75% (n=47) من عينات المرضى سرطان عنق الرحم بفируسات البالبومة النوعين 16 و 18 في 70% (n=33) و 9% (n=4) من العينات الإيجابية لمبتدأت ج ب العامة (n=47) بالنتيجة. وجدت فيروسات البالبومة النوعين 16 في كل درجات تميز الأورام وخاصة درجة متوسطة التمايز بينما وجدت فيروسات البالبومة النوع 18 في الأورام متوسطة وجدية التمايز. وجدت فيروسات البالبومة من النوع 16 في كل الأعمار ولكن معظمها في المرضى من كبار السن، في حين وجدت فيروسات البالبومة من النوع 18 في المرضى من صغار السن. تم الكشف عن وجود فيروسات البالبومة لم يتم تحديد انواعها في عشرين عينة. وجدت معظم فيروسات البالبومة النوعين 16 و 18 في ربات منازل من أواسط السودان. خلصت الدراسة إلى أن فيروس البالبومة البشرى النوع 16 هو المسبب الرئيسي لسرطان عنق الرحم ودرجة أقل فيروس البالبومة البشرى النوع 18. توصى الدراسة بإجراء مسح صحى للكشف عن فيروسات البالبومة بالنساء وتطبيق الصغيرات.
Table of contents

Subject                                      Page No.

الأية .................................................................................................................. i
Dedication...........................................................................................................ii
Acknowledgments.............................................................................................iii
English Abstract ................................................................. iv

الملخص ..............................................................................................................v
List of contents...............................................................................................vi
List of figures .................................................................................................xiii
List of plates ................................................................................................... .xv
List of abbreviations.........................................................................................xvi
List of appendices............................................................................................xvii
Chapter One and Objectives

Introduction

1.1 Cervical cancer

1.2 Symptoms

1.3 Epidemiology

1.4 Incidence

1.5 Mortality of cervical cancer

1.6 Types of cervical cancer

1.6.1 Cervical Squamous cell carcinoma

1.6.2 Cervical Adenocarcinoma

1.7 WHO and Bethesda classification system

1.8 Stages of cervical cancer

1.9 Cytological diagnosis; Papanicolaou test - Pap test

1.10 Treatment

1.11 Vaccination

1.12 Risk factors

1.12.1 Human papilloma virus infection

1.12.2 Smoking
1.12.3 Alcohol .................................................................17
1.12.4 Sexual activity .....................................................17
1.12.5 Oral contraceptives .............................................18
1.12.6 Genetic susceptibility .........................................18
1.12.7 Nutrition ..............................................................19
1.12.8 Parity .................................................................19
1.12.9 Immunosuppression ...........................................20
1.12.10 Socio-economic factors .................................20

Rationale .............................................................................21
Objectives ...........................................................................22

Chapter Two - II

Literature Review

2 Human papillomaviruses .................................................23
2.1 HPV prevalence .......................................................23
2.2 Taxonomy of papillomavirus ......................................24
2.2.1 Family Papillomaviridae ........................................25
2.2.2 Genus and species ................................................27
2.2.3 HPV types ...........................................................28
2.2.4 Papillomaviruses Types, Subtypes and Variants………………..28
2.3 High and low risk HPVs………………………………………………..29
2.4 HPV genome……………………………………………………………29
2.4.1 E1 protein……………………………………………………………32
2.4.2 E2 Protein………………………………………………………………32
2.4.3 E4 Protein………………………………………………………………33
2.4.5 E5 Protein………………………………………………………………33
2.4.6 E 6 oncprotein…………………………………………………………..34
2.4.7 E7 oncprotein …………………………………………………………..37
2.5 HPV transmission and life cycle………………………………………..41
2.6 Integration of viral genome……………………………………………..43
2.7 HPV and cell immortality, transformation and metastasis………..43

Chapter Three

Materials and Methods

3.1 Materials………………………………………………………………..44
3.1.1 Study approach and ethics…………………………………………..44
3.1.2 Study type & design…………………………………………………..44
3.1.3 Study area and time …………………………………………………..44
3.1.4 Study population.................................................................44
3.1.5 Samples..............................................................................45
3.1.6 Data collection.................................................................45
3.1.7 Data analysis .................................................................45

3.2 Methods.............................................................................45
3.2.1 DNA extraction .............................................................45
  3.2.1.1 Removal of paraffin by Xylene......................................45
  3.2.1.2 Addition of ethanol ....................................................45
3.2.2 DNA extraction kits.........................................................46
3.2.3 Measuring DNA concentration........................................46
3.3 Polymerase chain reaction -PCR........................................47
  3.3.1 PCR Protocol: GP5+/GP6+ for detection of mucosal HPVs....48
  3.3.2 PCR Protocol: Type specific for HPV 16............................49
  3.3.3 PCR Protocol: Type specific for HPV 18............................49
3.4 Agrose gel electrophoresis..................................................51
3.5 Quality control.................................................................51
Chapter Four

Results

4.1 Patients and controls.................................................................53

4.1. Patients with cervical cancer.....................................................53

4.1.2 Controls..................................................................................54

4.2 Detection of mucosal HPVs by PCR.............................................63

4.2.1 Detection of mucosal HPVs using GP5+/GP6+ PCR in sudanese patients with cervical cancer and controls ...........................................63

4.2.2 Identification of HPV16 using type specific PCR primers in patients with cervical cancer .................................................................63

4.2.3 Identification of HPV18 using type specific PCR primers in cervical cancer .....................................................................................64

4.3 HPV and age .............................................................................69

4.4 HPV positivity and geographical sites...........................................71

4.5 HPV and jobs ............................................................................73

4.6 HPV positivity and tumor differentiation.....................................75

Chapter Five

Discussion..........................................................................................79

Conclusion..........................................................................................87
Recommendations

References

References
List of Figures

Fig 1.1  Cervix normal cells and cervical intraepithelial neoplasia (CIN) .................................................................11

Fig 1.2  Histopathology of the normal cervix and CIN.................11

Fig 2.1  The Human papillomavirus Capsid...............................26
Fig 2.2  The genome of Human papillomavirus..........................31
Fig 2.3  HPV E6 Interactions with P53 ..................................36
Fig 2.4  HPV E7 Actions on PRb ........................................40
Fig 4.1  Age group of cervical cancer patients........................41
Fig4.2  Geographic sites cervical cancer patients ....................56
Fig4.3  Jobs of cervical cancer patients .................................57
Fig4.4  Tumor differentiation of cervical cancer patients.......58
Fig 4.5  Control's clinical conditions .................................59
Fig 4.6  Control's age groups........................................60
Fig 4.7  Control's geographical sites.................................61
Fig4.8  Control's jobs ............................................62
Fig 4.9  HPV16 and HPV18 in cervical cancer patients...........68

Fig 4.10  Positivity of HPV HPV16 and HPV18 in 47 patients aged ≥ 56 & ≤ 55..........................................................70

Fig 4.11  Positivity of HPV, HPV16 and HPV18 and patient's geographical sites..............................................................71

Fig 4.12  HPV and cervical cancer patient's jobs.........................74

Fig 4.13a  HPV and tumor differentiation of cervical cancer patients.................................................................77

Fig 4.13b  HPV16 and HPV18 and tumor differentiation of cervical cancer patients.......................................................78
List of color plates

Plate 1.1A Diagram showing the normal cervix and cervical cancer ....3
Plate 1.1B The normal cervix and cervical cancer..........................3
Plate1.2 Cervical squamous cell carcinoma..................................4
Plate 1.3 Pap test: sample collection, smear preparation and microscopic examination ..................................................12

Plate 4.1 Detection of mucosal HPV s by GP5+/GP6+ PCR in Sudanese women with cervical cancer .................................65

Plate 4.2 Detection of HPV16 by type specific PCR primers in cervical cancer........................................................................66

Plate 4.3 Detection of HPV18 by type specific PCR primers in cervical cancer...........................................................................67
### List of Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPV</td>
<td>Human papilloma virus</td>
</tr>
<tr>
<td>HPV16</td>
<td>Human papilloma virus genotype 16</td>
</tr>
<tr>
<td>HPV18</td>
<td>Human papilloma virus genotype 18</td>
</tr>
<tr>
<td>LCR</td>
<td>Long control religion</td>
</tr>
<tr>
<td>ORF</td>
<td>Open reading frame</td>
</tr>
<tr>
<td>L1</td>
<td>HPV major capsid protein</td>
</tr>
<tr>
<td>E6</td>
<td>HPV early protein 6</td>
</tr>
<tr>
<td>E7</td>
<td>HPV early protein 7</td>
</tr>
<tr>
<td>P&lt;sup&gt;Rb&lt;/sup&gt;</td>
<td>Retinoblastoma tumor suppressor protein</td>
</tr>
<tr>
<td>P&lt;sup&gt;53&lt;/sup&gt;</td>
<td>P&lt;sup&gt;53&lt;/sup&gt; tumor suppressor protein</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase chain reaction</td>
</tr>
<tr>
<td>GP5+/GP6+</td>
<td>HPV general primers GP5+ and GP6+</td>
</tr>
<tr>
<td>CSCCs</td>
<td>Cervical squamous cell carcinomas</td>
</tr>
<tr>
<td>WDSCCs</td>
<td>Well differentiated squamous cell carcinomas</td>
</tr>
<tr>
<td>MDSCCs</td>
<td>Moderately differentiated squamous cell carcinomas</td>
</tr>
<tr>
<td>PDSCCs</td>
<td>Poorly differentiated squamous cell carcinomas</td>
</tr>
<tr>
<td>CIN I</td>
<td>Cervical intraepithelial neoplasia grade I</td>
</tr>
<tr>
<td>CIN II</td>
<td>Cervical intraepithelial neoplasia grade II</td>
</tr>
<tr>
<td>CIN III</td>
<td>Cervical intraepithelial neoplasia grade III</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
</tr>
<tr>
<td>HGSIL</td>
<td>High-grade squamous intraepithelial lesion</td>
</tr>
<tr>
<td>IEL</td>
<td>Intraepithelial lesion of the uterine cervix</td>
</tr>
<tr>
<td>LGSIL</td>
<td>Low-grade squamous intraepithelial lesion</td>
</tr>
</tbody>
</table>
List of appendices

Appendix I  Preparation of Solutions.......................................................101

Appendix II  Questionnaire.................................................................103

Appendix III DNA extraction kit manual..............................................105

Appendix IV MaximePCR PreMix Kit manual.........................................107

Appendix V HPV, HPV16 & HPV18 Primers..........................................108

Appendix VI Sizer. DNA marker ............................................................110