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Histopathological Features in Development of Cancer of the cervix in relation to Sexually Transmitted Infections among Sexually active women attending Machakos Cancer Care and Research Centre

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Abstract

Background: *Sexually transmitted infections (STIs) diagnosis, treatment and management programmes have existed for many decades worldwide. Cancer of cervix diagnosis, treatment and management is however a newer phenomenon compared to STIs. Globally, it is estimated that 568,847 women in their reproductive age are diagnosed with cancer of the cervix and an estimated 311,365 deaths occur annually. Concurrently 499 million cases of STIs occur annually. This high burden of the infections if intervention measures are not put in place, the cases may increase into many folds in the coming future.*

Objective: *This study sought to investigate the influence of STIs in the histopathological outcomes among sexually active women attending Machakos Cancer Care and Research Centre.*

Methods: *The study design applied cross-sectional design to achieve this objective. It involved women who were sexually active and consented to the study voluntarily. The study involved STIs testing and histopathology analysis.*

Result: *The total number of participants who consented to the study after being informed about the consequences and the importance of the research were one hundred and seventy-six. The mean age of the participants was 40.6 years, a median of 43 and a range of 22-58 years. In overall 55 % of the participants had positive STIs outcomes while 44 % of the same population had an abnormal cervical histopathology outcome. The majority of the abnormal histopathology outcomes were among women aged 40 years and above. STIs were distributed in all ages. HPV and HIV were pathogen of interest because their influence was statistically significant.*

Conclusion: *This current study has revealed that STIs infections are very important agents of interest in establishment and progression of cancer of the cervix within the cervical walls of the sexually active women. HPV and HIV were highest influencers of histopathological outcomes in this study.*

Keywords: *Cancer of the Cervix, Cervical Intraepithelial Neoplasia, Histopathology, Human Immunodeficiency Syndrome (HIV), Human papillomavirus (HPV), Sexually Transmitted Infections (STIs).*

Introduction

In most of the low developed countries, cancer of the cervix, HIV and STIs have been reported to be higher in prevalence than developed countries. In most of the health systems, STIs and Cancer of cervix management are treated and managed in separate Departments hence lack of proper coordination and correlation of the two levels of infections hence they are interrelated. In some settings, cancer of the cervix has been regarded as sexually transmissible.{1} Some studies have expressed the opinion that's if the two infections can be integrated in one Department; the outcome would be better in improving the health of women at their reproductive age.{2}

Sexually transmitted infections have been cited as a major cause of women reproductive system infections and a threat to lives of many women of reproductive age who are sexually active. At an age above thirty-five years, HPV has showed to infect more sexually active women globally and among the mostly detected STI pathogen. HPV subtypes are basically categorized as high risk (HR) and low risk (LR) depending on their immunological effect on cancer of the cervix carcinogenesis. HR variant has been mostly associated with premalignant lesions and invasive cancer of the cervix{3}. The influence of the other STIs in precancerous and cancerous lesions has not been well documented although some of them are most present in HPV infected individuals {4} Chlamydia and HIV have been studied as important factors in cancer of the cervix carcinogenesis in conjunction with HR HPV, likewise *Treponema pallidum* has not be found to have a close association with HPV{5}

In Machakos County such relevant information is currently scarce and unpublished. More over the influence of STIs in histopathological outcomes is not documented and the two departments operate separately. It is with this consideration that the study was carried out to evaluate influence of selected STIs in sexually active women in Machakos County Cancer Care and Research Centre and consequently to assess their associations with histopathological outcomes in cervical biopsies.

Materials and Methods

Study design, setting and population: This study which was a cross sectional was based on Machakos Cancer Care and Research Centre which serves Machakos County and its environments. The study lasted from June 2019 to April 2020. It involved women of reproductive age who are sexually active and consented to participate in the study after signing an informed consent. The total number of participants who consented to the study after being informed about the consequences and the importance of the research were one hundred and seventy-six. The women who had been referred to the hospital for cervical biopsy investigation met the inclusion criteria although though who were in antibiotic were excluded from the study. A questionnaire which contained the demographic characteristics of the participants was filled before the study commenced.

Study population and Sample size determination. The Fisher's formula for cross-sectional studies was used to obtain the required sample size(6,7).

$(SS) = (Z^2 P (100-P) / \varepsilon^2$.

Z is the value (1.96 for 95% confidence level (CI), P represents Estimated prevalence of Cervical intraepithelial neoplasia (15%), and ε is the minimal tolerable error at 95% CI, expressed as a decimal (0.05). This formula yielded a minimum sample size of 176.

Laboratory Procedures

Cervical Biopsy

The need for a cervical biopsy depended directly on the recommendation by the examining gynaecologist according to the clinical manifestation and clinical observations. The cervical biopsy was obtained after careful application of Lugols iodine on the cervical wall. The sites which stained banana yellow were

considered to be suspicious and forceps were used to obtain a punch excision biopsy.

The cervical biopsy obtained was grossly examined, underwent the conventional tissue processing and stained with haematoxylin eosin. A pathologist examined the slides microscopically and the generated report was classified according to Richart guidelines. {8}

Sexually transmitted infections identifications

The screening of STIs in this study involved two samples which was blood and cervical swab. Different rapid diagnostic was used for STIs screening. Alere Determine™ HIV-1/2 was used. One Step Syphillis Rapid Diagnostic Kit for the screening of *Treponema pallidum* antibodies in the participants blood. Three cervical swabs were collected from each participant for detection of HPV 16/18, Gonorrhea and *Chlamydia trichomatis*. The Strong Step® HPV 16/18 Antigen Rapid Test Device was used for qualitative determination of HPV 16/18. Strong Step Test Kit was used for *Chlamydia trachomatis.* DRG® Gonorrhea Rapid (RAP-4867) which is an immunoassay for the detection of gonorrhea in cervical swab was used

Statistical Analysis

The histopathology obtained results were analyzed after classifying them into normal CIN 1, CIN2, CIN 3. The STIs were also classified as positive or negative. The results obtained were tabulated in an excel sheet and descriptive statistics obtained. The tabulated data was exported to SPSS software version 22 (USA, NY - IBM Corp., Armonk,) which was analyzed the data as per the outlined objectives The Pearson's chi square was used to calculate the relationship between STIs and cervical histopathology outcomes. The p value of < 0.05 was termed as significant.

Ethical Considerations

In adherence to the Helsinki declaration {9}, the ethical clearance to carry this study which involved human subjects was granted by Masinde Muliro university of Science and Technology, National Council for Science and Innovation, Machakos County Referral Hospital. The participants also signed an informed consent after accepting to participate in the study. The participants data was coded and kept out of reach unless for research work or clinical management.

Results

Demographic characteristics of the study participants

A total of 176 women were enrolled in the study. A summary of the demographic characteristics of the study participants is shown in Table 1. The participants had a mean age of 40.5 years, a median of 43 years and a range of 22-58 years. Majority of the women were married (69.9%) with the remaining reporting being single (19.9%), divorced (2.3%), widowed (2.8%) or cohabiting (5.1%). Almost a third of the participants (29.5%) had 1-2 children, 0 (28.4%), 3-4 (19.5) and 5 or more (22.7%).

Most of the study participants had sexual activity at the age of 22 years and above (68.8%) while the rest began sexual activities at an age below 22 years (31.2%). Tobacco smoking was not a common practice among the participants. A low percentage was realized to have smoked tobacco at one point in their lives i.e (24%) while the rest had never smoked (76%). Several methods of family planning were employed which included oral contraceptives (27.8%), barrier (19.3%), injectable (28.4%), implants (8%), natural (8%). Half of the participants had attained a primary level of education (50.6%) while the rest which includes post-secondary education (6.3%), secondary education (28.4%) and those with no formal form of education was (14.8%).

Table 1 Demographic characteristics of the study participants

Characteristic	Frequency (%)
Age (range), years	43 (22-58)
Marital status	
Married	123 (69.9)
Single	35 (19.9)
Divorced	4 (2.3)
Widowed	5 (2.8)
Cohabiting	9 (5.1)
Parity, number of children	
0	50 (28.4)
1-2	52 (29.5)
3-4	34 (19.3)
≥5	40 (22.7)
Age of sexual debut, years	
<22	55 (31.2)
≥22	121 (68.8)
Tobacco smoking	
Yes	43 (24)
No	133 (76)
Family planning methods	
None	15 (8.5)
Oral contraceptives	49 (27.8)
Barrier	34 (19.3)
Injectables	50 (28.40)
Implants	14 (8.0)
Natural methods	14 (8.0)
Level of education	
Post-secondary education	11 (6.3)
Secondary	50 (28.4)
Primary	89 (50.6)
No formal education	26 (14.8)

Data presented are characteristics of the findings in terms of frequency (n) and the proportion of the frequency in percentage (%). The age is shown as median (range).

Sexually transmitted infections and their classifications

Sexually transmitted infections among the participants was evaluated and the results presented in Table 2. HPV 16/18 was the most common infectious agent identified (59%) while the other infections were as follows: *Chlamydia trachomatis* (27%). HIV 1&2(8%), *Treponema pallidum* (3%) and *Neisseria gonorrhea (*3%).

Table 2 Sexually transmitted infections

STI	n (%)
HPV-16/18	57(59)
HIV-1/2	8(8)
Treponema pallidum	3(3%)
Neisseria gonorrhea	3(3%)
Chlamydia trachomatis	26(27)

The data is presented as number (n) and proportion (%) of subjects. STI, sexually transmitted infections. HIV, human immunodeficiency virus, HPV, Human Papilloma virus

Histopathological patterns of cervical biopsies

The results obtained from the cervical biopsies were analyzed microscopically. The obtained histological results were classified according to the system proposed by Richart (Melnikow et al., 1998). The findings obtained are presented in Table 3. Three-quarters of the population that was tested had normal (75%) histological results while the remaining abnormal results were classified as CIN 1(18%), CIN 2 (5%) and CIN 3(2%).

Table 3 Histopathological patterns of cervical smears

Histopathological patterns	176 (100%)
Normal	132(75.0)
CIN 1	32 (18.0)
CIN 2	9(5.0)
CIN 3	3(2.0)

Data Presented as number of subject specimen (n) and proportion (%) of subjects after microscopic evaluation of the cervical biopsies. CIN, cervical intraepithelial neoplasia.

The association of the Sexually transmitted infections and histological patterns

The selected STIs of interest in this study evaluated during the study among the women of reproductive age who participated in the study as shown in Table 4. Subsequently histopathological features obtained from the cervical biopsies were also evaluated as demonstrated in Table 4. The Pearson Chi square was done to assess the relations between the independent and dependent variable. A *p* value of less than 0.05 was termed as statistically significant. Table 4. Shows the results obtained. In all the STIs analyzed, *Treponema pallidum* (p-value = 0.182), *Neisseria gonorrhea (*p-value = 0.797), and *Chlamydia trichomatis* (p-value = 0.275) there was no statistical significant relationship with abnormal histological results. The *p-value*s obtained were greater than 0.05. Only HIV *(*p-value = 0.043), and HPV 16/18 (p<0.0001) had a statistical significant relationship with histological patterns, with a *p-value* of less than 0.05.

Table 4 The association of the Sexually transmitted infections and histological patterns

	Histology				Total (n=176)	*P-value*
	Normal (n=132)	**CIN I (n=32)**	**CIN II (n=9)**	**CIN III (n=3)**		
HPV 16/18						
Negative	103	11	3	2	119	
Positive	29	21	6	1	57	**0.001***
HIV 1 &2						
Negative	126	32	7	3	168	**0.043***
Positive	6	0	2	0	8	
Treponema pallidum						
Negative	131	30	9	3	173	0.182
Positive	1	2	0	0	3	
Neisseria gonorrhea						
Negative	129	32	9	3	173	0.797
Positive	3	0	0	0	3	
Chlamydia trachomatis						
Negative	111	28	9	2	150	
Positive	21	4	0	1	26	0.275

Data are presented as number (n) of subjects. STI, sexually transmitted infections.HIV, human immunodeficiency virus. Human papillomavirus (HPV). Data presented as CIN, cervical intraepithelial neoplasia. Controls were women whose histological findings were normal. Statistical comparisons were performed using the chi-square test for proportions

Discussion

In this cross sectional study of sexually active women attending Machakos Cancer care and Research Centre, HPV 16/18 was the mostly frequently detected STI pathogen (58%) followed by *Trichomatis chlamydia* (27%), HIV (8%) , *Nesseria gonorhhea* and *Treponema pallidum* each with 3% as seen in Table 2 . Approximately 55 % of the study participants had an encounter with at least an STI. These study findings correspond with other studies done among various STIs. HPV and HIV were the commonly detected STIs in this study. {10}

Different studies have shown that HPV 16/18 variants is a chief contributing factor of precancerous and cancerous infections within the cervical walls. This places it at an advantage of colonizing and affecting the morphology and structure of the squamous epithelium of the cervix. History and development of this disease has it that cancer of the cervix as a unremitting single disease that progress slowly from mild cervical intraepithelial neoplasia (CIN1) to more austere degrees of neoplasia and to micro invasive lesions (CIN2 or CIN3) and ends up to invasive disease {[11]. Additionally, Some investigators have linked HPV type 16/18 with different degrees of CIN and have suggested that CIN1, CIN2 and CIN3 are discrete in their processes, with CIN1 demonstrating as a self-limiting edity. More to that HPV infection and CIN2 or CIN3 have being regarded as the only true cancer of the cervix. Currently, there is no consensus on when treatment should start after the histopathological results. The Society of Gynecologic Oncology (SGO) and American Society for Clinical Pathology (ASCP) however have developed a guideline on the management and treatment of abnormal cytological and histological outcomes regarding prevention, screening, diagnosis and treatment of cancer of the cervix. {[12]} The findings of this current study correlated well with the other previous studies that HPV especially HR 16/18 play a very vital role in cervical cancer carcinogenesis. Majority of the HPV cases in this study had has CIN 1 (65%), CIN 2 (50%) and CIN 3 (50%). The HPV was statistically significance with a p value of < 0.001.

There has been a school of thought that cancer of the cervix in an HIV infected individual becomes an AIDS-defining disorder. HIV infection to as sexually active woman can modestly escalate the risk of cancer of the cervix especially when HPV 16 /18 is also present. There is a scantiness of evidence regarding influences of HIV infections in cervical cancer infected individuals. {[13]} Similarly, if the high cases in new HIV infections in women, especially in their early years of maturity found in the low and middle-income countries can be curbed, the life of women in their reproductive age would be salvaged. {[14]} The cases of HPV and HIV presented above the point at a connection between poverty, gender inequality, poor access to information and poor health infrastructure as a determinant in cancer of the cervix diagnosis, treatment and management. This study showed a low statistical relationship between HIV 1& 2 and histology outcomes (p-value of 0.043). This is consistent with with Muitta 2019 in Nakuru county who found similar findings. {[15]} Although the significance might be low in this study this might be attributed to the low number of HIV positive participants who were enrolled for the study, unlike other studies which were done HIV clinics.{[16]}

Chlamydia trachomatis causes inflammation of the cervix which affects the cells of the endocervix at the transformation zone. This type of inflammation can predispose the women to other STIs by detrimental effect to the squamous epithelial integrity. Some of the studies have indicated that persistent infection with *Chlamydia trachomatis* is highly connected with HR-HPV infections. Thus, continuing cervical exposure to *Chlamydia trachomatis* could intensify the possibility of transformation of cervical cells hence cancer of the cervix development. Although this study never established a statistical significance in relation to influence of *Chlamydia trachomatis* in histopathological outcomes as portrayed in Table 4, this might be due to the high number of who were negative for *Chlamydia trachomatis*. The findings agrees with (Madeleine et al ., 2007) {[16]}. Another study done in Kenya involving commercial sex workers demonstrated a positive effect of cancer of cervix progression among the women infected with HR-HPV coexisting with *Trichomatis chlamydia* {[17]}. Several factors might have contributed to the outcome which includes multiple infections, individual inflammatory response, the infection sequence and the duration of infections. Most of the known STIs are related with inflammatory

reactions in the immune response which enhances the entry of HPV affecting the severity of infection. {18}

Treponema pallidum which is a bacterium associated with syphilis was also evaluated. The participants whose results were positive were very few compared to other pathogens of interest. There was significant statistical influence in histopathological outcome in this current study. This is in consistent with Ferrera et al., 1997 who had also established such findings {19}. Conflictingly, a study done in Spain gave a differing opinion that Treponema *pallidum* influences cervical biopsy outcomes {20}. This disparity may be due to the size of the study participants, the study site and time of the study.

Conclusion

This current study has revealed that STIs infections play a key role in establishment and the progression of pre-cancerous and cancerous lesion within the cervical walls of sexually active women. Although *Treponema pallidum*, *Chlamydia trichomatis* and *Neisseria gonorrhea* did not demonstrate a statistical significance in this study, a bigger population-based study need to be done to access their influence in the histological outcomes from cervical biopsies. There is an urgent need to in cooperate the two levels of Departments that the women who have been referred for cervical histology investigations are also screened for the STIs profiles.

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