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Research Article

Histological Characterization of Invasive Cervical Cancer and Precursor Lesions in Malawian Women Presenting at Queen Elizabeth Central Hospital (QECH): A Cross-Sectional Study

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Abstract

Background: Cervical cancer is part of the huge burden of diseases in developing countries and is the second most common cancer in women worldwide. In Malawi, cervical cancer ranks as the first most frequent cancer in women between 15 and 64 years of age. We aimed at providing data about the distribution of different histological types of cervical cancer and precancerous lesions in patients presenting at Queen Elizabeth Central Hospital (QECH) in Malawi.

Methods: A total of 212 patient samples collected from 1st January, 2014 through 31st December, 2014 were included in the study. Tissue Hematoxylin and Eosin staining technique for microscopic histopathological classification of cervical lesions was used. Data was analyzed using IBM SPSS[®] version 20.0 for windows. The chi-square and Multinomial logistic regression test with a p-value of less than 0.05 was used to indicate statistical significance of the results.

Results: Out of 212 cases having precancerous lesions and cervical cancer that were included in the study, cervical lesions comprised 17% (35/212) pre-cancerous lesions, 65% (137/212) cancerous and 18% (39/212) had both pre-cancerous and cervical cancer, making a total of 176 (83%) cancer lesions. The most common cervical lesion was squamous cell carcinoma (SCC) with 72 % (151/212) cases.

Conclusion: Invasive SCC was found to be the commonest type of cervical cancer and high grade squamous intraepithelial lesions (HSIL) the commonest pre-cancerous lesion among women of all ages, with a peak at 35 to 49 (44%) year age category

Keywords

Cervical cancer; Intraepithelial lesions; Histopathology; Malawi

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Introduction

Cervical cancer is one of the huge burden of diseases faced by developing countries and is the second most common cancer in women worldwide [1,2]. Statistics indicate that 2,336,986 (1,811,867 in developing regions and 525,120 in developed regions) women who are 15 years and above are at risk of developing cervical cancer [3]. About 86% of all cervical cancer cases occur in developing countries and 88% of women who die of cervical cancer worldwide every year lived in low income countries [1,4,5]. Malawi has a population of 4.5 million women aged 15 years and older who are at risk of developing cervical cancer [6]. Currently it is indicated that every year 3,684 women are diagnosed with cervical cancer and 2,314 die from the disease [6]. Cervical cancer ranks as the most frequent cancer among women in Malawi and the most frequent cancer among women between 15 and 44 years of age [7,8]. Currently, Malawi has the highest number on cervical cancer indicators worldwide [6,8]. Surprisingly, despite cancer of the cervix being one of the major health hazard to Malawian women, little is known about the types of cervical cancer or precursor lesions in Malawi.

The present study aimed to provide novel and comprehensive data about the distribution of different histological types of cervical cancer and precancerous lesions in patients presenting at QECH in Malawi. This study determined the histological classification of cervical cancer and its precursor lesions in Malawian patients presenting at QECH.

Methods

Study design

A cross-sectional study was designed and used to estimate the prevalence of cervical lesions, either as precancerous or cancerous in tissue blocks of women at QECH submitted in 2014. Paraffinembedded specimens from cases (aged 20 – 90 years) with cervical lesions were obtained from QECH pathology archives. Samples from consecutive cases of Cervical Intraepithelial lesion, (I, II, and III) and invasive cervical cancer with information about age at diagnosis, year of diagnosis, and original histological diagnosis were obtained from the Histopathology Laboratory.

After the blocks were processed, inclusion criteria were a pathological confirmation of pre – cancerous lesion and a primary invasive cervical cancer of epithelial origin in the tissue sample selected for analysis, and information about the year of diagnosis. Reasons for exclusion were absence of confirmation of Cervical Intraepithelial Neoplastic lesion and invasive cervical cancer in the first and fourth sections (n=32); blocks that were mouldy, missing information about year of diagnosis (n=10); squamous or glandular differentiation in which confirmation of the cervical origin was not possible on the basis of histology and therefore the tumour was thought to be metastatic (n=3); cervical origin not confirmed (n=4); and no epithelial histogenesis (n=7). 10 cases were internal controls and excluded from the final analysis.

All protocols were approved by University of Zambia biomedical ethics committee (UNZABREC) in Zambia and College of Medicine research and ethics committee (COMREC) in Malawi. Paraffin blocks were processed under strict conditions to avoid contamination. Two

paraffin sections were systematically obtained from each block for histopathological assessment after haematoxylin and eosin staining. The sections were processed and the disease was diagnosed at the reference pathology laboratory at the University Teaching Hospital in Lusaka. Diagnosis included confirmation of invasive cervical cancer and assessment of the histological type (e.g. squamous cell carcinoma, adenocarcinoma, adenosquamous cell carcinoma, non-invasive cervical pre-cancerous lesions); presence of normal mucosa or precancerous lesions adjacent to invasive cervical cancer (Squamous intraepithelial lesion, low and high grade; adenocarcinoma in situ). Diagnosis of adenocarcinoma was confirmed by a panel of three pathologists. A blank paraffin section was cut and processed in between specimens to control for any carryover contamination in addition to the routine controls.

Statistical analysis

Variables analysed were, age at diagnosis, pathological report that included histopathological diagnosis, presence of pre-cancerous lesions (Low grade SIL and High grade SIL), presence of cancerous lesions (as percentage of the total), including squamous cell carcinoma (well, moderately and poorly differentiated), adenocarcinoma, endocervical adenocarcinoma, squamous-adenocarcinoma, SCC with schistosomiasis and SCC with schistosomiasis and cysticercosis. The Chi-square test was used to associate truncated age group and cervical cancer, whilst Multinomial logistic regression test was used to associate different age groups and cervical lesions; The P- value was used to determine the statistical significance with α of 0.05. SPSS statistical software version 20.0 was used for all analysis.

Results

After histological assessment of 584 blocks of archived paraffinembedded tissues that were from 236 women suspected of having cervical lesions, 212 cases having pre-cancerous lesions and invasive cervical cancer were included in the study. Out of 212 cases included in the study, cervical lesions comprised of: 16.5 % (37/212) precancerous lesions, 65% (137/212) cancerous and 18% (38/212) had both pre – cancerous and cervical cancer, making a total of 83% (176/212) cervical cancer lesions.

At least nine cervical lesions were characterized histologically with SCC being the most common cervical lesion (55.2%). Second from the SCC was SCC and HSIL (17%) then, High grade Squamous Intraepithelial lesions, with 13.2% (28/212) among all the cervical lesions. Adenocarcinoma with a 7.1% (15/212), was also further divided into mucinous adenocarcinoma (n=2), endocervical adenocarcinoma (n=9) and adenocarcinoma with perineural invasion(n=4). All the

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lesions showed no association with age (p values=0.085 and above), however, both HSIL & SCC (17%) and HSIL (13.2%) were highest in the 35 – 49 age group. This is the same sexually active group affected by SCC (Table 1 and Figure 1)

The chart below shows Squamous cell carcinoma as the most common among all the cancer lesions, with 86% (151/175). The degree of differentiation was also characterized into three categories, namely; well differentiated SCC 11% (20/175), moderately differentiated SCC 57% (100/175), and poorly differentiated SCC 18% (31/175) as seen in Figure 2. The SCC was further subdivided into keratinizing squamous cell carcinoma (KSCC), 19% (29/151) and non-keratinizing squamous cell carcinoma (NKSCC) 81% (122/151) (Figure 3).

Seven women, all with squamous cell carcinoma were also found with schistosomal infestation, contributing about 3.3 % (7/212) of all the cervical lesions (Table 2). One case had SCC, Schistosomiasis and cysticercosis, contributing 0.5% (1/212), and 19 cases with SCC had marked eosinophilia which could be suggestive of parasitic infection. Figure 4 below shows parasitic infestation with squamous cell carcinoma

Discussion

Cervical cancer is the most common type of cancer affecting most Malawian women, since 1996 to date [7,8]. Its heterogeneity and high morbidity make it clinically relevant as a research topic. Of all the cases that were examined in our study, 90 percent had cervical lesions, and the majority of cancer lesions were found to be at an advanced stage.

Age distribution

The majority of specimens with cervical cancer and its precursor lesions were from patients below 65 years of age with a peak between 35 and 49 years. This age group represents sexually active women who are at a higher risk of developing cervical cancer [3]. However, in our study population, women with ADCA were not younger than those with SCC, as observed in a study conducted in Peru that reported an increase in the burden of ADCA, particularly among young women [9]. The significant number of ADCAs could be a consequence of well-known limitations of cytology-based screening of ADCA precursor lesions since they are frequently located in the endocervical canal, making them less accessible than SCC precursor lesions for cytological detection [10]. Besides that, sensitization and screening of cervical cancer in Malawi has not been adequate and intensive. A Malawian study which was conducted in 2012, revealed delays in seeking attention hence late diagnosis and poor prognosis. Factors that contributed to their delay in seeking early

Table 1: Age-distribution frequencies and prevalence of cervical cancer and its precursor lesions by histological type.

Age in Years	Pre-cancerous Lesions (LSIL and HSIL / CIN I, II & III)	HSIL and Cancerous (HSIL / CIN III and SCC or ADCA)	Cancerous Lesions (SCC, ADCA and ASC)	Totals per Category
20 - 34	9	7	17	33 (15.6%)
35 - 49	17	13	53	83 (39.2%)
50 - 64	9	10	35	54 (25.5%)
≥ 65	1	4	13	18 (8.5%)
Vissing	1	4	19	24 (11.3%)
Total	37 (17 %)	38 (18%)	137 (65%)	212 (100%)
Key: Low-grade squamou	s intraepithelial lesion (LSIL), high-grade s	squamous intraepithelial lesion (I	HSIL), cervical intraepithelial I	esion (CIN), squamous cel

carcinoma (SCC), adenocarcinoma (ADCA), adenosquamous carcinoma (ASC), and those whose ages were not documented, "Unknown".



(ADCA), Adenosquamous carcinoma (ASC), High grade Squamous Intraepithelial Lesion (HSIL), Low grade Squamous Intraepithelial lesion (LSIL), Squamous Cell Carcinoma (SCC), Cysticercosis.



diagnosis and treatment included; limited knowledge on symptoms and signs and limited financial resources. The health facilities factors included; limited accessibility and unavailability of cancer screening facilities in the health centers. These results show that there is a need to strengthen the screening of cervical cancer among women in the country, in addition, there is a need to create community awareness about the signs and symptoms of cervical cancer and the merits of seeking early diagnosis and treatment [11].

Histological classification of cervical lesions

Comparing the prevalence pattern of cervical lesions in Malawi to that of Africa and worldwide distribution (Figure 5) [12,13], it was found out that the Malawian pattern of Invasive Cervical Cancer [ICC] (84%), HSIL (13%) and LSIL (3%)] had a very similar trend to the worldwide distribution of ICC (66%), HSIL (25%) and LSIL (9%). This suggests that the Malawian population is not unusual with reference to the worldwide distribution of cervical lesions. However, there was a significant increase in the number for ICC and a decrease in pre-cancerous lesions in Malawi as compared to the world distribution. This can be directly attributed to major barriers to seeking preventative screening in Malawi included low knowledge doi: 10.4172/2324-8955.1000168

levels, low perceived susceptibility and low perceived benefits from the service [14]. Cervical cancer screening not viewed as a critical health care [14].

It was also find out that 18% (38/212) had both ICC and HSIL / CIN II & III, and 25% HSIL. These apart from indicating late presentation to the hospital as majority of the cases were in advanced stage of cancer [11], it also cement the fact that invasive cervical cancer is a progression from advanced stages of precancerous lesions. Squamous intraepithelial lesion which represents early stages of cervical cancer had an overall prevalence of 17% of which CIN I / LSIL and CIN III/HSIL were 2.8%, and 13.7% respectively. These findings are in support of the prior published studies done in various countries. Study carried out by Sahasrabuddhe [15] in Zambia and a study done at Ipoh hospital in Malaysia [16], where CIN III was reported as the most prevalent CIN lesion (27.3%). In this study the highest prevalence of CIN was seen in biopsies from patients aged between 20 and 49 years and the lowest prevalence was from patients who were above 50 years. A study in Malaysian women by Karim [16] also found a lower prevalence (6%) in older patients (above 60 years). The low prevalence of CIN in older patients could be related to the fact that CIN lesions may have regressed or transformed into invasive lesions during the time when the patient was presented to the hospital.

Low grade squamous intraepithelial lesion / CIN I is very rare in postmenopausal women [17] and this study agrees with the observation where there was a decrease of CIN l cases beyond the



Figure 3: Histology of Cervical Lesions after Routine Hematoxylin and Eosin Staining (These photomicrographs shows sections of different types of cervical lesions stained with haematoxylin and eosin stain. (A) Shows high- grade squamous intraepithelial lesion or carcinoma in situ, which is characterized by major loss of orderly maturation of the epithelium with the entire epithelium replaced by immature abnormal cells with abnormal nuclei and atypical mitoses, x 40. (B) Invasive SCC consists of nests and irregular clusters of tumor cells, which shows either a basal-like maturation, often with keratinization, x 40. (C) Shows moderately differentiated adenosquamous carcinoma with glandular architecture, x 40. (D) Depicts moderately differentiated adenocarcinoma with perineural invasion in the endocervix forming glandular elements, as demonstrated by the wavy nuclei architecture, x 40 (UTH 2016).

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Variable Frequency Percentage Lesion **Histology results** Intraepithelial lesions 37 17% Intraepithelial lesions & Invasive lesions 38 18% Invasive lesions 137 65% Total 212 100% **Histological classification** LSIL 6 2.8% HSIL 29 13.7% SCC 153 72.1% ADCA 15 7.0% 1 ASC 0.5% SCC & ADCA 1 0.5% SCC & Schistosomiasis 7 3.3% Total 212 100% Intraepithelial lesion / Cervical Intraepithelial Lesion 6 17% I SII HSI 29 83% Total 35 100% Invasive lesions SCC 153 86.9% ADCA 15 8.5% ASC 1 0.6% SCC & ADCA 7 3.9% Total 176 100%

Table 2: Descriptive statistics of histology findings (The table above shows that the SCC was the most prevalent invasive lesion across all the age groups and the HSIL was the most prevalent intraepithelial lesion of the cervix in all the age groups. Unlike HSIL which showed no association with age (p = 0.085), SCC showed association with age category 35-49 years (p=0.04).

Key: Low-grade squamous intraepithelial lesion (LSIL), High-grade squamous intraepithelial lesion (HSIL), Squamous cell carcinoma (SCC), Adenocarcinoma (ADCA), Adenosquamous carcinoma.



differentiated squamous cell carcinoma with Schistosomiasis, X400 magnification, UTH 2016.

age of 60 years. The higher frequencies of CIN lesions which were observed in the young age groups (below 50 years) may be because this group represent sexually active women who are at a higher risk of developing CIN lesions and eventually cancer develops. The early stages of cervical cancer such as CIN I, has an important bearing on the prognosis of cervical cancer, since treatment at an early stage has the best prognosis with the highest cure rates [18].

Histologically, the most frequent type of cervical cancer is SCC [17,19]. This fact was also observed in our study, where the majority (72%; n=153) of histological types of cervical cancer were SCC. A similar study done in Malaysia showed the same outcome, non-keratinizing squamous cell carcinomas had a lower frequency

of diagnosis, most women had high grade of CIN lesions (CIN II and III) as well as invasive lesions and few have access to any form of treatment. The high prevalence of intraepithelial lesions such as the CIN III (13%) and invasive lesions such as the KSCC (72%) observed in this

study could be attributed to many risk factors which are associated with cervical cancer. The tendency toward regression of HPV infection correlates inversely with the severity of cervical disease. Only a small proportion of mild and moderate cervical diseases develop into invasive cancer, but the risk of progression from severe cervical cellular abnormality to invasive carcinoma is at least 20% [21-23]. Factors such as genetic predisposition, frequency of reinfection, intratypic genetic variation within HPV type, coinfection with more than one HPV type, hormone levels, and immune response may influence the ability to clear an HPV infection.

of 15.6% [16] just like in our study, the frequency was 19%. The high prevalence of invasive lesions means that most patients present to the hospital with advanced stages of cancerous conditions and this agrees with the literature of Kerr [20] where it was observed that during the time

Squamous cell carcinoma and cervical Schistosomiasis

Cervical Schistosomiasis adds to the disease burden of women in all age groups. Pathological consequences due to the involvement of cervical organ can be damaging for the affected women. Clinical unawareness of cervical Schistosomiasis can lead to misdiagnosis and therefore false and ineffective therapy. The study found that 4% of cases had cervical schistosomiasis. These results supports the previous studies done in Malawi, Tanzania, Mozambique and South Africa where 4.0 to 6.0 percentage of cervical Schistosomiasis was detected [24-26].

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The possible link between chronic cervical schistosomiasis and cervical dysplasia has been the subject of intense controversy. While any association between cervical dysplasia and schistosomiasis is disputed in some studies, others have suggested that schistosomiasis is a possible cofactor, as evidenced by the relatively high incidence of cervical cancer in regions where schistosomiasis is endemic [27-29]. The association between active cervical schistosomiasis and increased risk of infection with high-risk HPV suggests that schistosomiasis not only facilitates the agents of sexually transmitted infections, but may also alter the natural history of such infections [30]. The ova are usually situated in the sub epithelial connective tissue, the majority being present in the squamocolumnar junction [31], the transformation zone where cervical cancer originates. Lesions in the cervix impair the epithelial integrity and thus make it biologically plausible that schistosomiasis can increase the risk for transmission of HPVinfection in the same way as is seen in other genital ulcer disease [24]. As one of the neglected diseases, schistosomiasis may have a large contribution to development of cervical cancer than what is currently known hence need for further investigations.

Limitations

The limitations of this study include the lack of access to original tissues by the reviewing pathologists, variable quality of paraffin embedded tissue samples and self-reporting of ethnicity which might have led to misclassification. Only samples from one histopathology laboratory were used in this study; hence detection rate in this study might therefore underestimate the true prevalence of HPV. The study aimed to be a representative of all women with ICC in Malawi. Some cases had no documentation of age, this may have a negative impact on cervical lesions distribution in different age groups. While these matters must be remembered when drawing conclusions from this retrospective cross-sectional study the authors believe this is the most accurate possible assessment of cervical lesions in Malawi.

Conclusion and Recommendation

Squamous cell carcinoma was the most common cervical lesion affecting women, followed by CIN III / HSIL. The most affected age group by both invasive cervical cancer and high grade squamous intraepithelial lesion was between 35-49 years, which is the sexually active group. Findings from this study will contribute to the body of knowledge on epidemiological distribution of cervical lesions in Malawi. A larger study not restricted to Queen Elizabeth Central Hospital would need to be carried out to generate more representative results, as data from one referral hospital may not give a true picture of cervical lesion prevalence in Malawi.

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Disclosure

The authors report no conflicts of interest in this work.

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