Cytomorphological Analysis of Uterine Cervical Smears in Women Aged 50 and 50+ Attending Cervical Cancer Screening at Malamulo Adventist Hospital

Chagwa Patrick*, Banda Speak, Katiniche Blessings, Katuah Joel, Munthali Ackim, Majamanda Jonathan, Linje Linly, Kamwendo Chikondi, Siyano Exton, Mlozen Madalitso and Kalumbi Martin

Department of Biomedical Sciences, Malawi Adventist University, Malamulo Campus, R4H5+5CV, Makwasa, Malawi

Abstract

Cervical cancer has highest incidence in developing countries with almost 90% of cervical cancer deaths and Malawi is not spared. Almost all cases of cervical cancer are caused by the persistent infection with "high-risk" types of HPV, with HPV types 16 and 18 being the most common. Cervical cancer screening has emerged as a very effective method for the prevention of cervical carcinoma. This is because the uterine cervix is anatomically accessible, and the prolonged pre-malignant phase can be detected by studying morphology of exfoliated cells through Papanicolaou Smear (Pap smear) evaluation. From a diagnostic perspective, atrophic smears, typical of smears from postmenopausal women, pose a challenge.

Aim: The aim of this study was to assess pattern of cytomorphology of Pap smears in postmenopausal women.

Methods: 98 processed and stained pap smears from postmenopausal women who came for cervical cancer screening at Malamulo Adventist Hospital were examined retrospectively. Cytomorphology was assessed considering nuclear details including staining, chromatin pattern, and cytoplasmic preservation. Specimen adequacy was also considered. Descriptive statistics were used to analyze data.

Results: Age group of 55-60 years had a highest frequency of 38 (38.8%) women and the lowest frequency was for the age group of more than 70 years with a frequency of 8 (8.2.0%). Majority, 82(83.7%) were seropositive for HIV. On specimen adequacy, majority, 86 (87.8%) were considered satisfactory and 12 (12.0%) were unsatisfactory. In terms of the unsatisfactory samples, 8(8.1%) were due to inadequate squamous cellular component and 4 (4.1%) were due to masking of inflammatory cells. Normal chromatin pattern was found in 79 (92%) and abnormal pattern was in 7(8%) and abnormal nuclear staining was found in 7(8%). Abnormal nuclear membrane was found in 6 (7.0%) and increased nuclear to cytoplasmic ratio was found in 8(9.3%). In terms of neoplasia the study found that 81 (94%) women samples were Negative for Intraepithelial Lesion or Malignancy (NILM), Atypical Squamous Cell of Undetermined Significance (ASC-US) were 3 (3.5%), High Grade Squamous for Intraepithelial Lesion (HSIL) 1 (1.2%), and Squamous Cell Carcinoma (SCC), 1 (1.2%).

Conclusion: In this study cytomorphological pattern of pap smears from women aged 50 and above has been characterized and found that small proportion of smears had abnormal pattern.

Study limitation: The researchers had less control over the procedures for collection and processing of the Pap smear as the study involved already processed archived samples. Again no histological confirmation was done hence the results remain those of a screening test.

Keywords: Postmenopausal • Cytomorphology • Pap smear • Cervical cancer screening • Neoplasia

Introduction

Cervical cancer is the fourth most common cancer among women globally. In 2018 it was estimated that there were 580,000 new diagnoses and approximately, 90% of deaths occur in low-income areas with high HIV prevalence and largely this is attributed to limited prevention and screening opportunities and scarce treatment options [1]. The World Health Organization has set standards and goals to eliminate cervical cancer by 2030 by target of 70 per cent coverage of twice lifetime screening (WHO 2020) [2]. There are numerous screening methods available for cervical cancer, these methods include cytology, Human Papilloma Virus (HPV) DNA testing and visual inspection tests. Amongst these molecular and protein biomarkers such as DNA methylation, p16 immunostaining, and HPV mRNA testing are more precise and help to enhance specificity and sensitivity [3]. According to World Health Organization, women who are 50 years and above are not eligible for visual inspection tests like Visual Inspection with Acetic acid (VIA) as their transformation zone is usually invisible hence resorting to testing by pap smear or other alternative.

Malawi has the highest mortality related to cervical cancer [4,5]. Age-standardized incidence was highest in Malawi (67.9 [95% CI 65.7 -70.1]) and Zambia (65.5 [63.0-67.9]) in Africa [6]. Human Papilloma Virus (HPV), the most common Sexually Transmitted Infection (STI) is a prerequisite for the development of cervical cancer. Almost all cases of cervical cancer are caused by the persistent HPV infection with one or more of the "high-risk" (or oncogenic) types of HPV which may lead to the development of pre-cancer which, if left untreated, can lead to invasive cancer. HPV 16 and 18 are most likely responsible for at least 70% of the invasive cervical cancers [7].

Cervical cancer screening has emerged as a very effective method for the prevention of cervical carcinoma. This is because the uterine cervix is anatomically accessible and the prolonged, premalignant phase has a treatable pre-invasive stage which can be detected by studying the cellular characteristics. Cervical cytology is frequently used as a screening test for Squamous Intraepithelial Lesion (SIL) and early invasive squamous carcinoma. From a diagnostic perspective, atrophic smears may be misinterpreted as positive smears in postmenopausal and occasionally in peri-menopausal women thereby leading to increased false positivity [8]. Similar study done by Kiff JM, et al. reported about 96% of all abnormal pap smears as atypical squamous cells of undetermined significance (ASCUS) [9]. Clearly this was an overall of ASCUS category which is to be avoided. Walboomers JMM, et al. found that the diagnosis of ASC-US (Atypical Squamous Cells - Undetermined Significance) was 2-3 times greater with respect to fertile age women [10].

Evidently, Pap smears from postmenopausal women pose a challenge to the cytologist due to altered maturation in the sense of reduction in both estrogen and progesterone levels, potentially leading to over interpretation of smears as abnormal. Traditionally, the cytologist assesses the cytomorphology mentally and come up with interpretation. This study aims at determining and documenting the cytomorphology of cervical cells which shall be used to make an interpretation. This would make it easier for the cytologist eventually reducing/minimizing over- interpretation. Although this principle may be applicable to all types of smears, it would be more useful in this age group with particularly 'difficult' smear.

Malamulo Adventist Hospital screens women for cervical cancer using 3-5% acetic acid. However, according to World health Organization Recommendation, women who are 50 years of age and above are screened using pap smears. Hence this population was the target of this study.

Methods

This study involved evaluating archived pap smears from the target population. The study was approved by the National Health Sciences Research committee and the slides were accessed with permission from Malamulo Adventist Hospital which is situated in Makwasa, 20 Kilometers from Thyolo District in the southern part of Malawi. Microscopic evaluation was done at Malawi Adventist University, Malamulo campus. Targeted population was Women aged 50 years and above that had undergone cervical cancer screening test (Pap smear) at Malamulo Adventist Hospital and 98 women where included in the study.

Convenient sampling method was used by choosing sample with age group of 50 years and above for the women who attended cervical cancer screening at Malamulo Adventist Hospital. A predesigned laboratory form was used to capture the laboratory results, age and HIV serostatus. The data collected was entered into and analyzed by Statistical Package for the Social Sciences (SPSS) Version 20. Descriptive statistics was used where tables, charts and graphs where generated to summarize data. Any result or information that was collected from this study was treated with privacy and confidentiality.

Results

Age (years) distribution

The mean age of the study participants was 50 years with age range of 20 years. Majority, 38 (39%) of participants were in the age group of 55-60 years followed by 50-54years representing 30% of the participants (Table 1).

HIV sero status

Out of 98 participants, 82 (83.7%) samples were positive, 11 (11.2%) samples were negative for Human Immunodeficiency Virus (HIV) antibody test. Five percent of the participants had unknown HIV serostatus (Figure 1).

Specimen adequency

Out of the 98 samples that were collected, 86 (87.8%) were considered

as satisfactory and 12 (12.0%) unsatisfactory (Figure 2). In terms of the unsatisfactory samples, 8 were due to inadequate squamous cellular components and 4 were due to masking of inflammatory cells.

Chromatin pattern

With the 86 samples that were found satisfactory, the study found that there was a normal chromatin pattern in 79 (92%) and abnormal chromatin pattern with a frequency of 7 (8%) (Figure 3).

Nuclear membrane

Nuclear membrane was categorized as smooth nuclear membrane and irregular nuclear membrane, with smooth membrane with a frequency of 80 (93%) and irregular membrane were 6 (7%) out 86 smears that were examined (Figure 4).

Nuclear staining characteristics

With the sample size of 86 Satisfactory (100%), nuclear staining was

Table 1. Age distribution.

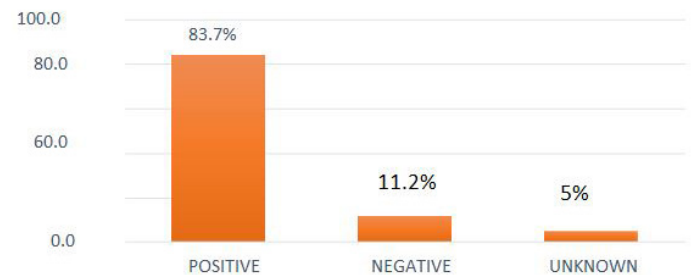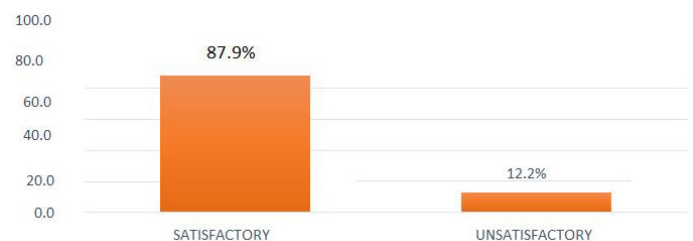| Age Group (Yrs)	Frequency	Percent	Valid Percent	Cumulative Percent
50-54	29	29.6	29.6	29.6
55-60	38	38.8	38.8	68.4
61-64	10	10.2	10.2	78.6
65-70	13	13.3	13.3	91.8
>70	8	8.2	8.2	100
Total	98	100	100	-

Figure 1. HIV serostatus.
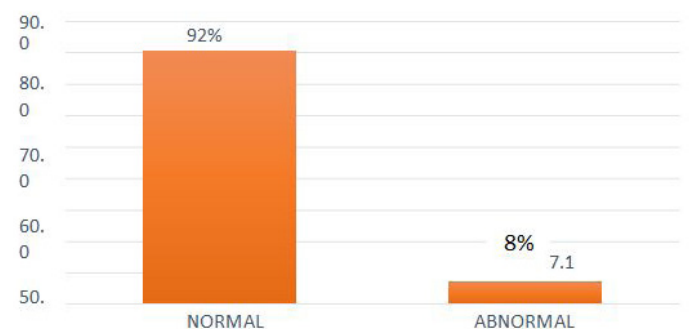

Figure 2. Specimen adequacy.

Figure 3. Chromatin pattern.

categorized as pale staining and hyperchromatic staining characteristics. Out the total 79 (92%) were pale staining characteristics and 7 (8%) with hyperchromic characteristics (Figure 5).

Nuclear to cytoplasmic ratio and cell preservation

According to nuclear to cytoplasmic ratio characteristics; it was grouped as normal nuclear membrane and slightly increased nuclear. Out of 86 smears that were examined, normal nuclear to cytoplasmic ratio was seen in 78 (91%), slightly increased 8 (9%) below is Figure 6 illustrating this information. In terms of cell preservation, all 86 smears had well preserved cells.

Pap smear results

Out of 86 samples (smears) that were found to be well preserved and passed specimen adequacy,81(94.8%) women samples were Negative for Intraepithelial Lesion or Malignancy (NILM), 3 (3.2%) women samples were Atypical Squamous Cell of Undetermined Significance (ASC-US),1 (1.0%) with High Grade Squamous for Intraepithelial Lesion (HSIL) 1 (1.0%) and Squamous Cell Carcinoma (SCC) 1(1.0%) (Figure 7).

Discussion

This study assessed cytomorphology of Pap smears from women aged 50 years and above. Out of 98 women included majority, 38 (68.4%) were in the age category of 55-59 years and a lowest 2 (2.0%). being in the age category of greater than 70 years. Majority, 3 (60%) of Pap smear abnormalities were also found to be in the age category of 55-59 years. Data on Human immunodeficiency virus serostatus was also captured and results shows (83.7%) with age category of 55 -59 years having the highest prevalence at 38.8%. HIV prevalence among this age may not be reflective of similar population in Malawi since this study was not designed to assess the same
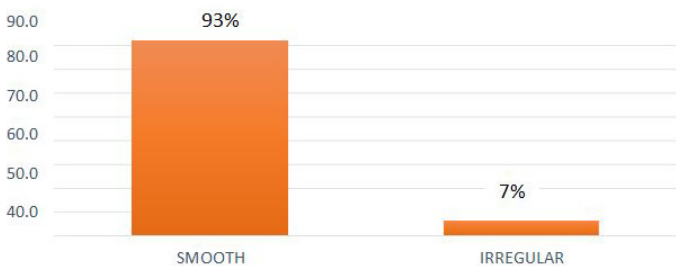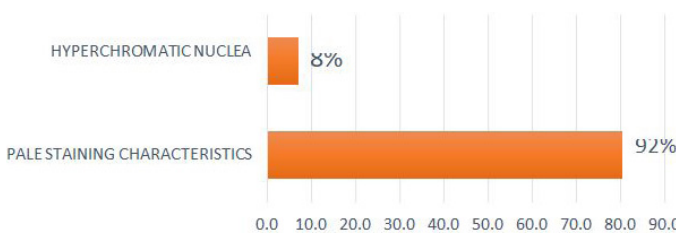

Figure 4. Nuclear membrane characteristics.

Figure 5. Nuclear staining characteristics.
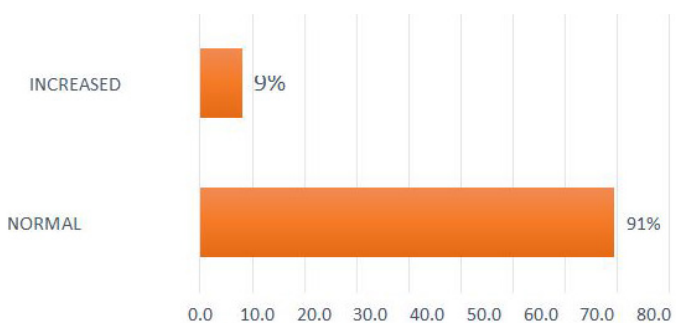

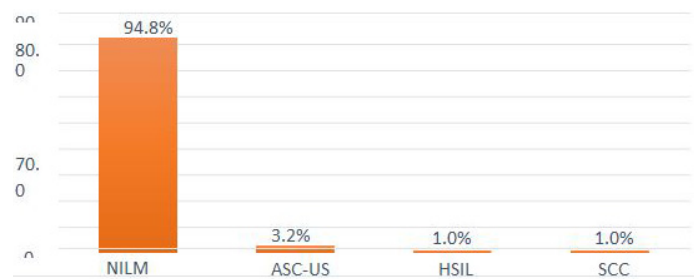**Figure 6.** Nuclear to cytoplasmic ratio.

Figure 7. Pap smear results.

and the higher rates may be due to the fact that people living with HIV are encouraged to undergo cervical cancer screening.

In this study, out of the 98 smears that were examined, 12 (12%) were found to be unsatisfactory for evaluation with inadequate cellular component being the major cause (67%) followed by masking inflammatory cells (33%). Findings of specimen adequacy in the present study are similar to those found in a large study done by Kashiyap V and Sharna S who found a 12% inadequacy rate and Mahadik J, et al. found 14.9% inadequacy rate. Atrophic changes and dryness due to cessation of secretory activity of endometrial and cervical glands, might be culprits in the high rate of inadequate smears in this age.

Additionally atrophic changes of the genital tract are relevant to the migration of the transformation zone, making adequate sampling difficult and morphologically mimicking high grade cervical dysplasia causing misinterpretation. Detection of neoplasia requires the cytologist to examine and visualize the cell in terms of nuclear appearance and often in relation to the whole cell while the type of cell is recognized by the cytoplasmic appearance. Irregular nuclear membrane, unevenly distributed chromatin, hyperchromatic staining of nucleus and increased nuclear to cytoplasmic ratio favor neoplastic although one factor may not be standalone to define neoplasia while others may be strong predictors. This study endeavored to evaluate these parameters and found that all except cell preservation were abnormal in 6-8% of smears that were reported as satisfactory. This is in line with the prevalence of Pap smear abnormality of 6% considering ASCUS and above. Difference in overall cytomorphological abnormality and the overall pap abnormality results is due to the fact that some cytomorphological abnormalities were standalone and could not be used to define neoplasia.

In this study, overall cytological abnormalities considering Atypical Squamous Cells of Undetermined Significance (ASCUS) cut-off was 6% as stated above. This finding is similar to study by Mahadik J, et al. who found 5.4%, Zubair AA got 9.2% while that of Kashiyap V and Sharna S was 2.3%. Pattern of Pap smear abnormalities considering atypical squamous cells of undetermined significance cut-off were, ASCUS 3(3%), HSIL was 1(1%) and SCC was 1(1%) while majority 94% were reported as Negative for Intraepithelial Lesion or Malignancy (NILM) in this study. Anbumozhi k, et al. compared Pap smear findings in premenopausal and postmenopausal women in India and found similar results i.e. 1.5%, 1.5% and 3.2% respectively for ASCUS, HSIL and SCC [11]. Mahadik J, et al. found similar results with ASCUS about 2%, HSIL (1%) and SCC (<1%).

Established nuclear changes of hyperchromasia, chromatin pattern and nuclear membrane are important factors in determining cellular neoplastic transformation hence systematic characterization of these factors was the approach used in this study and produced comparable results to similar studies.

Conclusion

In this study cytomorphological pattern has revealed few abnormal cytomorphology which corresponds to abnormal Pap results. The study has also found high inadequacy rate similar to findings in similar studies.

Recommendations

This study recommends strict use of diagnostic criteria in order to correctly characterize the smear from postmenopausal women.

Study Limitation

Results of this study should be interpreted bearing in mind that sensitivity and specificity of Pap test was not performed and that study used already processed archived samples.

Conflict of Interest

None for all authors.

References

1. Cubie, Heather A. and Christine Campbell. "Cervical cancer screening-the challenges of complete pathways of care in low-income countries: Focus on Malawi." *Women's Health* 16 (2020): 1745506520914804.

2. World Health Organization. Global strategy to accelerate the elimination of cervical cancer as a public health problem. World Health Organization (2020).

3. Rajaram, Shalini and Bindiya Gupta. "Screening for cervical cancer: Choices & dilemmas." *Indian J Med Res* 154 (2021): 210-220.

4. Gerstl, Sibylle, Lawrence Lee, Robin C. Nesbitt and Christopher Mambula, et al. "Cervical cancer screening coverage and its related knowledge in southern Malawi." *BMC Public Health* 22 (2022): 295.

5. Shrestha, Aamod Dhoj, Dinesh Neupane, Peter Vedsted and Per Kallestrup. "Cervical cancer prevalence, incidence and mortality in low and middle income countries: A systematic review." *Asian Pac J Cancer* 19 (2018): 319.

6. Singh, Deependra, Jerome Vignat, Valentina Lorenzoni and Marzieh Eslahi, et al. "Global estimates of incidence and mortality of cervical cancer in 2020: A baseline analysis of the WHO global cervical cancer elimination initiative." *Lancet Glob Health* 11 (2023): e197-e206.

7. Denny, Lynette, Rosalind Boa, Anna-Lise Williamson and Bruce Allan, et al. "Human papillomavirus infection and cervical disease in Human Immunodeficiency Virus-1-infected women." *Obstet Gynecol* 111 (2008): 1380-1387.

8. Tabrizi, Ali Dastranj. "Atrophic Pap smears, differential diagnosis and pitfalls: A review." *Int J Womens Health Reprod Sci* 6 (2018): 2-5.

9. Kiff, Jaime M., Madisen Cotter, Elizabeth G. Munro and Molly E. Leonard, et al. "Cervical cancer screening in postmenopausal women: Is it time to move toward primary high-risk human papillomavirus screening?." *J Womens Health* 30 (2021): 972-978.

10. Walboomers, Jan MM, Marcel V. Jacobs, M. Michele Manos and F. Xavier Bosch, et al. "Human papillomavirus is a necessary cause of invasive cervical cancer worldwide." *J Pathol* 189 (1999): 12-19.

11. Kannan, Anbumozhi, Wangol Kiyam, Giridharan Bupesh and Matcha Bhaskar, et al. "Comparative analyses of PAP smear data in pre and postmenopause Indian women." *Bioinformation* 16 (2020): 452.