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COMPARING BETHESDA SYSTEM AND RICHART SCORE IN THE EVALUATION OF CERVICAL LESIONS AMONG WOMEN ON CONTRACEPTIVES ATTENDING MACHAKOS LEVEL V COUNTY HOSPITAL IN KENYA

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ABSTRACT

Background of the study: **Early detection of the precursor lesion before the invasive stage of cervical cancer prevents further damage to the neighbouring tissues and organs. Bethesda system which applies cytological techniques is primarily used as a screening test while Richarts score is based on predicting the biological behaviour of abnormal epithelium. Laboratories in not so well-endowed settings should find the most predictive technique in assessing the cervical lesions.**

Objective: **To Compare Bethesda System and Richart Score in the evaluation of Cervical Lesions among women on contraceptives attending Machakos Level V Hospital.**

Design: **A descriptive cross-sectional study.**

Setting: **Machakos level V Hospital, Machakos County, Kenya**

Subjects/participants: **One hundred and seventy-six (176) women aged ≥22 <60 years in their reproductive age who sought clinical services at the facility.**

Results: **This study evaluated 176 women with an average age of 40.5 years, a median of 43 and a range of 22 to 58 years. Several types of contraceptives evaluated in this study were classified into hormonal and non-hormonal. The sensitivity of Bethesda system was 88%, Specificity 61%,Positive predictive values 49%, Negative predictive values 93%, Kappa 0.810, McNemar 0.035 at 95% CI and Fishers exact <0.001 in comparison to the Richart score.**

Conclusion: **In this study Bethesda system achieved a high level of accuracy in terms of specificity, sensitivity and predictive values.**

INTRODUCTION

Globally, 568,847 women in their reproductive age are diagnosed with cancer of the cervix and an estimated 311,365 die annually. Histo-pathological studies (Richarts Score) forms the basis of clinical and laboratory diagnosis for detection, treatment and management of cancer of the cervix. However this requires resources that may not be available in many settings where cancer of the cervix is rated second after breast cancer among women of reproductive age (1). Conferring to the GLOBOCAN reports on cancer statistics, this disease is of significant mortality burden ranked as the seventh most cause of cancer-related deaths worldwide (2). At the pre cancer stage, affected tissues can be contained with current available management protocols and the affected women can have a better chance of living the full span of their life (3).

Progression of the disease in undetected cases leads to spread of severer form of the disease in affected reproductive tract organs (4-5).

Since the introduction of hormonal contraceptives in early 1960s, several medical concerns have been raised on the effect of the contraceptives in patients with cancer of the cervix. Several epidemiological studies have linked use of hormonal contraceptives with cancer of cervix carcinogenesis. Although other several predisposing factors like sexual debut, smoking ,Human papillomavirus, age and marital status have been seen as confounding, hormonal contraceptives has never been exonerated from the influence (6-7)

Early screening using Bethesda score method, which is commonly done using the Pap smear test is one of the methods of prevention of cervical cancer progression. Richart score test follows thereafter if the Bethesda score results indicate so. Thus if conducted early enough morbidity and mortality associated with cancer of the cervix can reduce significantly (4-5,8).

A major challenge paused in diagnosis of cervical intraepithelial neoplasia is that, majority of the precancerous and cancerous lesions are asymptomatic and mostly become symptomatic after at fairly advanced stage when invasion and destruction to the neighboring structures has occurred. Diagnosis at late stages of the disease show that little success is realized in treatment and management hence leading to the high mortality and morbidity. In consideration of the above factor, advocacy in routine screening would be a major boost in preventing the rising cases of cancer of the cervix in women of reproductive age in countries who track epidemiological data. (3).

Currently, there is no clear consensus on the diagnostic accuracy of Bethesda score and Richart score in diagnosis of cancer of the cervix. The accuracy of the two tests differs from one study setting to another. This is more so because the two tests are observer dependent. Contrastingly most of the documented studies provide a more leniency of acceptability to histology although preferring cytology as a screening test. This preference however differs from one center to the other. In some settings, a cervical biopsy is only recommended for high grade squamous intraepithelial lesion (HGSIL) in Bethesda

score outcomes while in other settings every abnormal cervical smears cytology outcomes, a cervical biopsy is recommended (9–13). Some clinicians prefer Richart score outcomes in Cancer of the cervix management for therapeutic management. It is worth noting that the consensus on when to begin treatment with abnormal Bethesda score results depends on the clinician consulting the patient (14). Even with all these diagnostic and conflicting challenges, the two techniques both in screening and diagnostic levels have made an enormous contribution in reducing the burden of cancer of the cervix in women in their reproductive age. Majorly in countries that have invested in cervical cancer screening programs using pap smears, statistics have shown a decline in mortality and morbidity by over 70%. With the attention given to HPV in developed and developing countries with emphasis on early screening, these numbers may reduce if implementation of the programs is taken with the seriousness it deserves (15).

The Society of Gynecologic Oncology (SGO) and American Society for Clinical Pathology (ASCP) however have developed a guideline on the detection, management and treatment of abnormal cytological and histological outcomes (16). According to the WHO 2013 reviewed guidelines in collaboration with American Society for Colposcopy and Cervical Pathology (ASCCP) 2014 reviewed guidelines, the management guidelines may be different in the varying countries. This may depends on their health institution testing capacity, abilities and the clinical diagnosis which surpasses the guidelines recommendations (17). In accordance to the reviewed literature, this study compared Bethesda system against Richart score in diagnosis of cancer of the cervix at an early stage in a County hospital with minimal diagnostic resources.

MATERIALS AND METHODS

Study design: Hospital-based descriptive cross-sectional study conducted between June 2019 and April 2020.

Setting and Population: Women of reproductive age attending the reproductive health clinic at Machakos Level V Hospital, Kenya. This hospital serves as a cancer referral centre in Machakos County residents and its environs. Its cancer care and Research Centre offers cancer screening and testing within a well-established diagnostic laboratory serving more than 9,000 women of reproductive age annually. The study participants whose ages ranged between 22-60 years were enrolled into the study after signing an informed consent.

Inclusion Criteria: Patients with no history of hysterectomy, not on their monthly menstruation circle and not using vaginal suppositories and not having engaged in a sexual activity in the past five days. Consenting to participate was mandatory

Exclusion criteria: The study excluded participants who already diagnosed to have cervical cancer.

Study population and Sample size determination: The Fisher's formula for cross-sectional studies was used to obtain the required sample size (18-19).

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

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.

Demographic information: Demographic information which included marital status, parity, age of sexual debut, tobacco smoking, family planning methods, and level of education were obtained and entered in a structured questionnaire upon enrolment of participants into the study.

Smear specimen and Biopsy tissue collection and preparation: Each participant was taken through the study requirement and consent signing. Then requested to lie on a couch at lithotomy position. The vaginal canal was manipulated using a plastic disposal speculum by a qualified gynaecologist. A cervical brush was used to scrape cells from the squamocolumnar junction. Duplicate smears were prepared from excavated cervical wall material on labeled frosted microscopic slides and immediately preserved using commercially prepared cytofix. The smears were allowed to air dry in preparation for staining. Subsequently, the cervical biopsy tissues were also obtained after careful application of Lugols iodine on the cervical wall. The sites which stained banana yellow were considered suspicious and a cervical biopsy forceps was used to obtain excision punch biopsy. The cervical biopsy tissues were fixed with 10% formalin and taken through tissue processing. Consequently, the tissue pieces were then sectioned and adhered to microscopic slides in preparation for staining.

Staining: The fixed cytological smears were stained using a commercial prepared kit for Papanikalou stain (Atom, Atom Scientific, CHESHIRE, ENGLAND). The cervical biopsies slides were stained using Haematoxylin-Eosin (Atom, Atom Scientific, and CHESHIRE, ENGLAND).

Examination: The cyto-smears were examined by two-independent anatomic pathologists for cytomorphologic features using a microscope at x40 objectives. In cases of greater than 5% discrepancy, a third anatomic pathologist read the slides. The findings were recorded as the final results. The cervical cytomorphologic changes were classified according to 2014 Bethesda classification protocols into normal, ASCUS, LGSIL and HGSIL (20). Likewise, the cervical biopsy slides were examined by two independent anatomic pathologists with a third anatomic pathologist tie breaking the discrepancies. The histopathological changes were classified according to the Richart guidelines into normal , CIN 1,CIN 2 and CIN 3 (21). The Richart score was used as the gold standard in evaluating the Bethesda system performance.

Statistical analysis: Data analysis was conducted using the IBM SPSS version 20.0 (IBM Corp, Armonk, NY, USA). The proportion of distribution of categorical variables (smoking, education level, marital status, family planning method) were analysed using the Pearson's Chi square whereas the continuous variables (age, parity, sexual debut) using the ANOVA test. Richart score and Bethesda system results were classified into normal and abnormal. To evaluate the effects of different variables and their influence in diagnosis of cancer of the cervix, their areas under the receiver operating characteristic (ROC) curves were equated. This curves represents a plot of sensitivity of test (Y-axis) against the specificity of the test (X-axis). The study conducted the McNemar to evaluate the inter observer reliability in the two tests conducted. This was also confirmed using Kappa and Fishers exact. Odds ratios were estimated at a 95% confidence interval. In all the statistical tests calculated, one tailed format was applied and a *P value of <0.05* was considered as significant.

Ethical considerations: Ethical approval for the study was obtained from Masinde Muliro University of Science and Technology Institutional Ethics Review Committee (MMU/COR: 403012 Vol 2. Permission for the study were obtained from the National Commission for Science and Innovation (NACOSTI/P/19/37448/31103), Machakos County Director of Medical Services Office, Machakos County Education Office, and the Machakos County Commissioner. The research was conducted in accordance with the Helsinki declarations (22), and all the participants provided informed consent before enrollment into the study with confidentiality ensured throughout the study.

RESULTS

The demographic profiles of the participants illustrate that out of 176 women studied, aged between 22 years to 60 years, 41 years to 60 years (46%) were the majority having a median age of 43 years. Hormonal contraceptive method choice in this cohort was 127 (72%) women participants (Table 1).

Table 1

Aspects of Socio- demographic profiles 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	
Hormonal	127 (72)
Non hormonal	49 (28)
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).

From the processed cervical scrapes cytology results, 77 (49%) had atypical cells as per Bethesda system (15) into ASCUS (28%), LGSIL (15%) and HGSIL (6%) (Table 2).

Table 2

Cyto-pathogical features of the study participants

Cyto-pathological features	**N (%)**
Normal	89(51)
ASCUS	46(27)
LGSIL	25(15)
HGSIL	11(7)

Data presented as number and proportion (%) of subjects after microscopic examination of the pap smears. ASCUS, atypical squamous cells of undetermined significance, LGSIL, low grade squamous intraepithelial lesions, HGSIL, high grade squamous intraepithelial lesion.

The punch excision biopsy tissue histology results were classified according to Richarts guidelines (15), 44 (25%) had abnormal histology scores results as CIN1 (18%), CIN 2 (5%) and CIN 3 (2%) (Table 3).

Table 3

Histo-pathological patterns of cervical smears

Grade	**N (%)**
Normal	134(75)
CIN 1	29(17)
CIN 2	12(7)
CIN 3	2(1)

Key

CIN 1- Confined to the basal epithelium spanning to a third of the epithelium.

CIN 2- Represents moderate dysplasia occupying two thirds of the epithelium.

CIN 3- Represents severe dysplasia spanning to a more than two thirds of the epithelium.

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

Logistic analysis was done to determine the Odds Ratios (OR). During the analysis, the histopathological features were considered as the dependent variables while cytopathological outcomes were considered as independent variables. Confounding factors like age, parity, family planning methods and smoking were controlled for the regression models. Data is presented as odds ratios (OR) at a 95% confidence interval (CI) (Table 4).

Table 4

Comparing Bethesda System and Richart Score in the evaluation Cervical Lesions

Histo-pathological patterns	OR	95% Confidence Interval		P-value
		Lower	Upper	
Normal				
Normal	5.068	2.309	11.125	<.0001
CIN I	0.288	0.152	0.545	<.0001
CIN II	0.095	0.012	0.742	0.005
CIN III	0.344	0.032	3.699	0.362
ASCUS				
Normal	1.032	0.903	1.180	0.411
CIN I	0.803	0.326	1.977	0.411
CIN II	0.281	0.026	3.016	0.294
CIN III	0.988	0.963	1.012	0.650
LGSIL				
Normal	2.308	1.411	3.774	<0.0001
CIN I	0.183	0.095	0.353	<0.0001
CIN II	0.075	0.007	0.765	0.044
CIN III	0.988	0.963	1.012	0.889
HGSIL				
Normal	-	-	-	-
CIN I	0.112	0.063	0.201	<0.0001
CIN II	0.013	0.002	0.088	0.001
CIN III	0.013	0.002	0.088	0.001

The performance of cytology against histology was evaluated by employing the Receiver Operating Curve which gave us the estimate of sensitivity 88% and 61%, Kappa (0.810) which was confirmed by McNemar and Fishers exact (Table 5).

Odds Ratios (OR) were performed to relate selected histo-pathological patterns with cyto-pathological features which were classified according to the Bethesda grading system for squamous intraepithelial lesions. During the analysis the histo-pathological features were considered as the dependent variables, predictors such as Normal, ASCUS, LGSIL and HGSIL were considered. Data are presented as odds ratios (OR) and 95% confidence interval (CI).

Table 5

Statistical indicators of the performance of cytopathology against histopathology

	Cytology	**P value at 95 % CI**
Specificity	61%	<0.0001
Sensitivity	88%	<0.0001
Positive predictive values	49%	
Negative predictive values	93%	
Accuracy (Kappa,MacNemar)		
Kappa	T_{value} =4.265,	0.810
MacNemar	T_{value} =3.68	0.035
Fisher's exact test		<0.0001

Data presented as percentage (%) of subjects. Statistical comparisons were performed using the McNemar test, Kappa and Fisher's exact test at a cut-off point of 95% confidence interval.

DISCUSSION

In this study, the performance of Bethesda Score in detection of pre-cancerous lesions was evaluated using Richarts score as the standard test. Despite Bethesda score being a mainstay in screening for precancerous lesions, it has it short comings(23).The reported false negatives can be attributed to inadequate transfer of the cervical material to the glass slide, inadequate sampling or inter observer challenges. An estimate of 75% of the observer screening test have low specificity and sensitivity. Commonly the cytology has been found to have a sensitivity ranging from 50% to 90% which is demonstrated by the false negatives (23).

This study recorded; sensitivity of 88% and specificity of 61%. This is consistent with other studies done to assess the performance of Bethesda system against the Richart score. Studies by; Gangane Mukerji and Sharma had a Sensitivity 86.3%, Specificity of 100%, Gupta, Halder, Khan & Sodhani had a sensitivity of 87%,specificity of 100%, Diaz (2000) achieved a sensitivity of 50% and a specificity of 100%, Nandini et al. had a sensitivity of 75% and a specificity of 61% (24–26).

Contrastingly, a study done in California among sexual active women demonstrated that 17% of the reported cases with HGSIL and 38% with confirmed dysplasia of the cervix were diagnosed using cytology method(27). The author attributed the reduced association factor of Bethesda score and Richart score to low grade lesions in the study. However, several epidemiological and clinical Studies have indicated that most of the normal cytology results in cytology screening will indicate a normal histology findings. This also depend on the time the patients presents the clinical symptoms(28). For instance, HGSIL has high odds of being either CIN1, CIN 2 or CIN 3. These outcomes demonstrated that positive likelihood of Richarts score increases with increase in positivity in the Bethesda score.

The reasons attributed to the better performance of Bethesda system in this study included: Strict adherence to guidelines in exclusion and inclusion criteria, keen adherence to the set guidelines on sufficient specimen collection, reasonable sample size and good response rate from the participants,

the willingness of the participants, the experience of the anatomic pathologist and in microscopic examination of slides and the experience of the gynaecologist in specimen collection.

The study calculated the Kappa test which measured the agreement of the two tests and obtained a *p* value of 0.810 which indicated a perfect agreement between cytology and histology. This was confirmed by McNemar test which measures the closeness of the outcome of the two tests that obtained a p value of 0.035 which signifies a very close significance in the two tests. This shows though histology is a good diagnostic test, the importance of cytology as a screening test cannot be underestimated.

CONCLUSION

This study has demonstrated that the accuracy of Bethesda system as a screening test is of key value that can help in detection of cervical precancerous lesions. It's therefore recommended that similar studies can be done to shed more light on the pre malignant and malignant conditions in a general population since this was a hospital based study.

LIMITATIONS

This study was done on a single-Centre of which a multi-center setting would be recommended for such a work. Reports presented on this study were observer depended compared to other studies which in cooperated molecular and serological diagnostics. Majority of the participants had symptomatic presentation leaving out precancerous lesions which are asymptomatic. The low numbers of participants who had abnormal Richart score affected the statistical power of the overall analysis.

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