Comparison of PAP, Modified Pap and Gram Stained Cervico-Vaginal Smears in the Diagnosis of Bacterial Vaginosis in Women Attending Thika District Hospital

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ABSTRACT

Objective: To compare Pap, Modified Pap and Gram stained cervico-vaginal smears in the diagnosis of bacterial vaginosis in women to establish if Modified Pap was a suitable alternative to Pap method.

Design: Descriptive cross-sectional survey of bacterial vaginosis with a comparative evaluation of three methods.

Setting: Thika District Level Five (County) Hospital.

Subjects: A total of 150 female patients who consulted for services at Antenatal Care and Family Planning clinics at Thika district Level 5 (County) hospital between November 2016 and May 2017and who met the inclusion criteria were recruited into the study.

Main outcome measures: Presence or absence of bacterial vaginosis.

Results: The study showed that Pap and Modified Pap methods yielded sensitivity of 47.6% and 26.2%, positive predictive value (PPV) of 80.0% and 68.8%, negative predictive value (NPV) of 82.4% and 76.9%, likelihood ratio of positive result (LR+) of 10.3 and 5.69, likelihood ratio of negative result (LR-) of 0.55 and 0.77 respectively and specificity of 95.4% and overall diagnostic accuracy of 38.9% for both methods.

Conclusion: The Modified Pap staining method has diagnostic value when it is positive in diagnosis of bacterial vaginosis and can therefore be a suitable alternative to Pap method as a confirmatory test for bacterial vaginosis.

INTRODUCTION

Bacterial vaginosis (BV) is a vaginal infection caused by imbalance in the normal vaginal flora. It is characterized by low levels of normally predominant *Lactobacilli species (spp)*,which is replaced by *Gardnerella vaginalis, Prevotella spp, Porphyromona spp, Bacteroides spp, Mobiluncus spp* and *genital Mycoplasma spp*.[1]. It is among the most common reproductive tract infections in women worldwide [2]. Estimated prevalence of BV ranges from 20% to 50% in African populations [3], with higher levels being documented in female sex workers [4].

Until recently, BV which was originally thought to be of little long-term clinical significance, has been implicated in increasing the risks of preterm birth [5], development of pelvic inflammatory disease [6], pregnancy loss, still births, gestational bleeding, preterm birth, preterm labour, premature rupture of membranes, amniotic fluid infection, postpartum endometritis and post caesarean wound infections [7].

Recent studies have shown that Pap stained cervico-vaginal smears can be used to diagnose BV [8-10] and can be a wholly adequate alternative to Gram-stained smears [11] hence the need to validate its use for diagnosis of BV. Therefore, this study sought to establish if Modified Pap method can be used to diagnose BV in cervico-vaginal smears and if Modified Pap method is a suitable alternative to Pap method in diagnosis of BV in cervico-vaginal smears.

MATERIALS AND METHODS

This descriptive cross-sectional study was done at Thika District Level 5 (County) Hospital's family planning (FP) clinic, ante-natal clinic (ANC) and medical laboratory. Thika District Level 5 (County) Hospital is a government hospital located in Thika town, Kenya and serves as a referral hospital for neighbouring districts and also as a teaching hospital.

The inclusion criteria were all females aged 18-45 years (child bearing age), sexually active, had no vaginal bleeding at the time of study and who gave voluntary consent to participate. The exclusion criteria were all females who at the time of the study had not met the inclusion criteria. Ethical clearance was given by Kenyatta National Hospital/University of Nairobi (KNH/UON) Ethics and Research Committee and also by Thika District Hospital, where the study was conducted, as well as by the National Commission for Science, Technology and Innovation (NACOSTI).

A sample size of 150 women was determined statistically and women who met the inclusion criteria were informed about the study and consent obtained through signing informed consent form. A structured questionnaire was then administered to all the subjects to obtain and record socio-demographic information (age, occupation, residence, education, marital status and number of sexual partners), reproductive history, vaginal and menstrual hygiene practices as well as clinical history.

Cervico-vaginal smear was then collected from the posterior fornix and lateral vaginal wall from each participant using a cervical scraper, but in women who were pregnant or suspected to be pregnant, sampling was restricted to lateral vaginal wall. The colour of the discharge (if present) was first noted and documented.

Three smears were prepared from each cervical scraper; two of the smears were fixed in 95% alcohol for 15 minutes and then stained with Pap and Modified Pap methods respectively. Pap staining method protocol consisted of 15 dishes and Modified

Pap staining method protocol consisted of 14 dishes with the smears held in respective dishes for 10 seconds (dips) with blotting done in between changes from one dish to the next. Mounting was done using DPX mountant and coverslip attached with overnight drying (Table 1). The air dried smear was stained using Gram stain method (Table 2).

Blinding of the staining method used was done using unique codes to label the smears. The three sets of smears were examined using light microscopy with the threshold for a positive BV diagnosis being presence of >20% clue cells in Pap and Modified Pap stained smears and a 7-10 Nugent score of bacterial morphotypes in Gram stained smears.

Primary examination of the smears was done by the principal investigator (PI) using Bethseda System 2001 of reporting cervico-vaginal smears for Pap stained smears and Nugent classification system for bacterial morphotypes in the Gram stained smears. In order to minimize intra- and interobserver variability, two cytologists and two microbiologists confirmed the Pap stained smears and Gram stained smears respectively. It is only at the end of this that the microscopists revealed their reports and any discrepancies resolved.

RESULTS

All the three sets of smears prepared from the sample of 150 were found to be satisfactory for evaluation and data analysis was done using 95% confidence interval (CI) and a statistically significant P-value of less than 0.05.

The mean age of the subjects was 26.9 years with a median of 25 and standard deviation (SD) of 5.9. The minimum and maximum ages were 19 and 42 years respectively. Majority of the subjects, 55.3% were 18-25 years old, followed by 31.3% who were 26-35 years while the least number, 13.3% were 36-45 years old (Figure 1).

Gram stain method using Nugent's scoring system which was the diagnostic gold standard in this study was able to detect forty two (28%) positive cases of BV. There were 6 subjects with intermediate flora and were counted as negative for BV. On the other hand, out of 150 smears stained with both Pap and Modified Pap methods, twenty five (16.7%) and sixteen (10.7%) smears respectively were BV positive with presence of >20% clue cells which was the threshold for positive BV diagnosis. Majority of BV positive cases using Gram stain method were in the age group of 26-35 years with twenty (47.6%) cases. The results indicate that there is a difference in diagnosis of BV in cervico-vaginal smears between the three staining methods, Pap, Modified Pap and Gram stain (Figure 2).

The results of Pap and Modified Pap methods were compared to the results of Gram stain method using Nugent's scoring system which was the confirmatory diagnostic gold standard test in this study and which was administered concurrently with the other two methods to each participant. A positive result in Pap and Modified Pap methods was considered "true positive" if it was confirmed positive using Gram stain method; a negative result in Pap and Modified Pap methods was considered "true negative" if it was confirmed negative using Gram stain method; a positive result in Pap and Modified Pap methods was considered "false positive" if it was confirmed negative using Gram stain method and a negative result in Pap and Modified Pap methods was considered "false negative" if it was confirmed positive using Gram stain method.

Pap method showed BV in twenty (20/42) subjects who tested positive on Gram stain method giving a sensitivity of 47.6%. On the other hand, Pap method showed negative results for BV in twenty two (22/108) subjects with negative results on the Gram stain method giving a specificity of 95.4%. Twenty two subjects who tested negative on Pap method and had BV on Gram stain method gave a false negative rate (FNR) of 52.4% while five subjects who had BV on Pap method and a negative result on Gram stain method gave a false positive rate (FPR) of 4.6% . Positive predictive value (PPV) was 80.0% while the negative predictive value (NPV) was 82.4%. The likelihood ratio for a positive test (LR+) was 10.3 while likelihood ratio for a negative test was 0.55. The overall diagnostic accuracy was 38.9% (Table 3). Modified Pap method showed BV in eleven (11/42) subjects who tested positive on Gram stain method giving a sensitivity of 26.2%. On the other hand, Modified Pap method showed negative results for BV in thirty one (31/108) subjects with negative results on the Gram stain method giving a specificity of 95.4%. Thirty one subjects who tested negative on Modified Pap method and had BV on Gram stain method gave a false negative rate (FNR) of 73.8% while five subjects who had BV on Modified Pap method and a negative result on Gram stain method gave a false positive rate (FPR) of 4.6% . Positive predictive value (PPV) was 68.8% while the negative predictive value (NPV) was 76.9%. The likelihood ratio for a positive test (LR+) was 5.696 while likelihood ratio for a negative test was 0.77. The overall diagnostic accuracy was 38.9% (Table 4).

The results show that Pap and Modified Pap methods vary in sensitivity, 47.6% and 26.2% respectively, but had similar specificity of 95.4%. However, even though their NPV's were fairly close, PPV of Pap method, 80.0% was relatively higher than 68.8% of Modified Pap. This indicates that Modified Pap method can be a suitable alternative to Pap method especially in excluding BV in truly negative cases. The diagnostic capabilities of Pap and Modified Pap methods in the diagnosis of BV in cervico-vaginal smears were analyzed using Cohen kappa statistics to determine the consistency between the two methods to establish if Modified Pap was a suitable alternative to Pap method in this case. The results indicate that there is a statistically significant ($p \leq 0.05$) level of agreement between the two methods with the kappa value of 0.692 representing moderate agreement between Pap and Modified Pap methods in diagnosis of BV indicating that Modified Pap method can be a suitable alternative to Pap method in diagnosis of BV (Table 5).

Table 1: Pap and Modified Pap staining protocols

Pap protocol		Modified Pap protocol	
Tap water	10 dips	1% acetic acid	10 dips
Harris Haematoxylin	10 dips	Pre-heated Harris's	10 dips
Tap water	10 dips	Haematoxylin (60^0C)	10 dips
95% ethanol	10 dips	Tap water	10 dips
OG-6 stain	10 dips	1% acetic acid	10 dips
95% ethanol	10 dips	OG-6	10 dips
EA-50	10 dips	1% acetic acid	10 dips
95% ethanol	10 dips	EA-50	10 dips
95% ethanol	10 dips	1% acetic acid	10 dips
100% ethanol	10 dips	Methanol	10 dips
100% ethanol	10 dips	Methanol	10 dips
100% ethanol	10 dips	Methanol	10 dips
Xylene	10 dips	Xylene	10 dips
Xylene	10 dips	Xylene	10 dips
Xylene	10 dips	Xylene	10 dips
DPX mount and coverslip		DPX mount and coverslip	

Table 2: Gram stain method protocol

Heat fix air dried smear	
Crystal violet stain	1 minute
Tap water	
Gram's iodine	1 minute
Tap water	
Acetone-alcohol	6 seconds
Tap water	
Neutral red	2 minutes
Tap water	
Air dry	

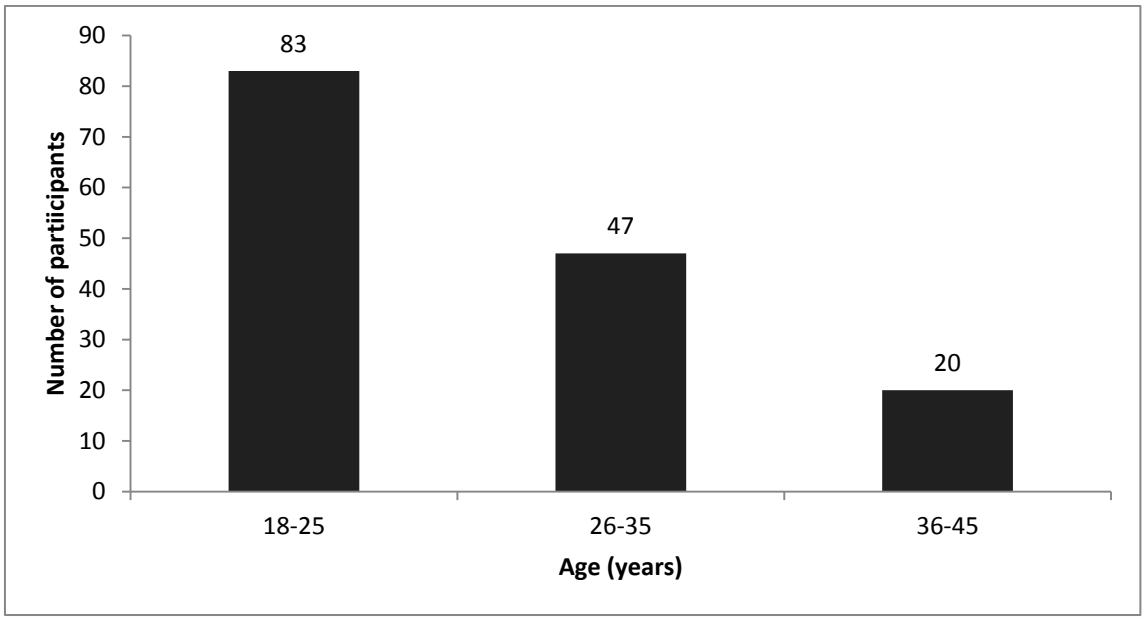

Figure 1: Age distribution of the subjects

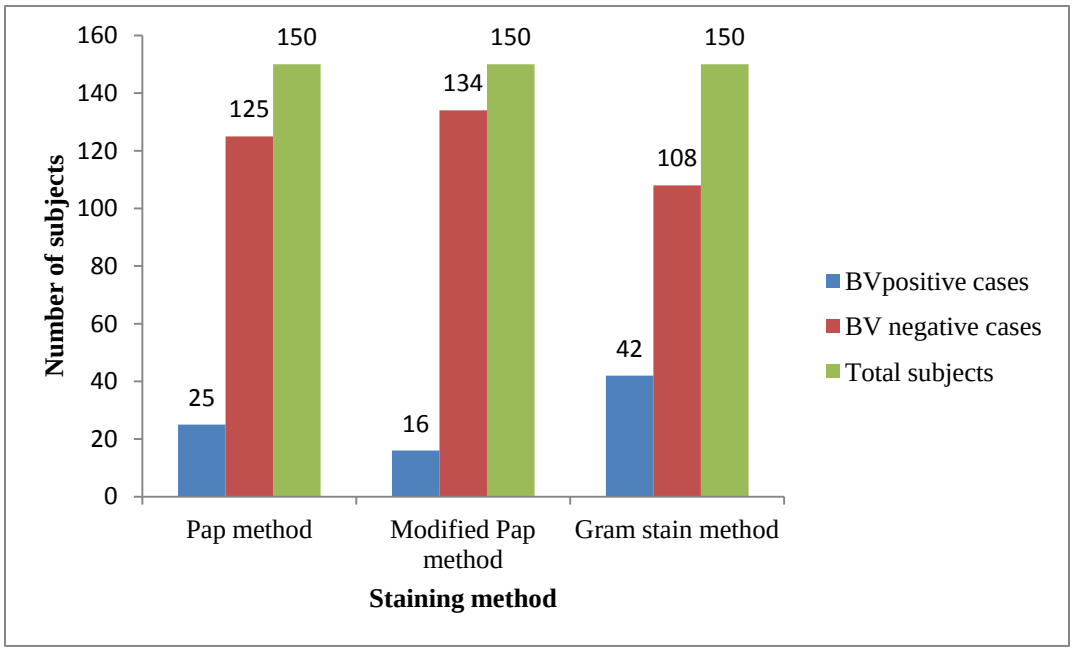

Figure 2: Frequency distribution of BV positive and negative cases in cervico-vaginal smears stained using Pap, Modified pap and Gram stain methods

Table 3: Sensitivity, specificity, Positive Predictive Value (PPV) and Negative Predictive Value (NPV) of Pap method with reference to Gram stain method as the gold standard in the diagnosis of BV

			Gram stain method		
			Positive	Negative	Total
Pap method	Positive	Count	20	5	25
		% within Pap_method	80.0%	20.0%	100.0%
		% within Gram stain method	47.6%	4.6%	16.7%
	Negative	Count	22	103	125
		% within Pap_method	17.6%	82.4%	100.0%
		% within Gram stain method	52.4%	95.4%	83.3%
Total		Count	42	108	150
		% within Pap_method	28.0%	72.0%	100.0%
		% within Gram stain method	100.0%	100.0%	100.0%

*Pap_method * Gram stain method crosstabulation.

Table 4: Sensitivity, specificity, Positive Predictive Value (PPV) and Negative Predictive Value (NPV) of Modified Pap method with reference to Gram stain method as the gold standard in the diagnosis of BV

			Gram stain method		
			Positive	Negative	Total
Modified Pap method	Positive	Count	11	5	16
		% within Modified Pap_method	68.8%	31.2%	100.0%
		% within Gram stain method	26.2%	4.6%	10.7%
	Negative	Count	31	103	134
		% within Modified Pap_method	23.1%	76.9%	100.0%
		% within Gram stain method	73.8%	95.4%	89.3%
Total		Count	42	108	150
		% within Modified Pap_method	28.0%	72.0%	100.0%
		% within Gram stain method	100.0%	100.0%	100.0%

*Modified Pap method * Gram stain method crosstabulation.

Table 5: Cohen kappa measure of agreement for Pap and Modified Pap methods

		Value	Asymp. Std. Error[a]	Approx. T[b]	Approx. Sig.
Measure of Agreement	Kappa	.692	.086	8.753	.000
N of Valid Cases		150			

*Modified Pap method * Pap_method Crosstabulation Symmetric Measures
a. Not assuming the null hypothesis.
b. Using the asymptotic standard error assuming the null hypothesis.

DISCUSSION

Pap smear test is a simple cytology screening test primarily used in detection of pre-neoplastic and neoplastic changes in the uterine cervix. In reporting cervical pap smear results, a remark is usually made on presence of cervico-vaginal infection due to bacteria, fungi and candida with the Bethesda system having a class on reporting 'shift in vaginal flora, suggestive of BV' [12]. Conventional Pap method has undergone several modifications to reduce alcohol use to make it cost effective in resource poor settings. One of the modified Pap protocols is Rapid, Economic, Acetic acid, Papanicolaou (REAP) method [13] that has successfully been utilized in screening for cervical cancer in Pap smears with no compromise on staining quality and diagnostic standards.

This study was to compare Pap, Modified Pap and Gram stained cervico-vaginal smears in the diagnosis of BV to establish if Modified Pap was a suitable alternative to Pap method in this regard.

The subjects in the study were 150 (n=150) from whom three sets of smears were prepared and found satisfactory for evaluation. The age range of the female subjects was 19-42years, with majority in the 18-25 years old age group. Mean age of the subjects was 26.9 years with a median of 25 and standard deviation (SD) of 5.9. This is comparable to a study done by Shayo *et al.*, [14] in Mwanza, Tanzania where median age of the subjects was 26 years. In the present study, BV was detected in 28% of the women and this is similar to a study in Ghana by Aubyn *et al.*, [15] that reported BV prevalence of 28%. This present study utilized Gram stain method as the reference diagnostic standard in evaluating diagnostic performance of Pap and Modified Pap methods and reports sensitivity of 47.6% and 26.2%, specificity of 95.4% and 95.4%, PPV of 80% and 68.8% and NPV of 82.4% and 76.9% for Pap and Modified Pap methods respectively. The results for Pap method are comparable to those reported by Platz-Christensen *et al.*, [16] of sensitivity, specificity, PPV and NPV values of 88.2%, 98.6%, 96.8%, 94.7% respectively and concurs with Livengood [17] who reported that Pap test has sensitivity as low as 50% and specificity of about 95% in diagnosis of BV indicating that a positive result is reliable evidence of BV presence but a negative result does not exclude presence of BV. However, these results differ from that of a prospective study done by Karani *et al.*, [18] in Mombasa, Kenya that reported sensitivity, specificity, PPV and NPV values of 59.4%, 83.3%, 67.3% and 78.0% respectively. The varied results may be attributed to interobserver variability, type of population used, environment and other socio-demographic characteristics of the subjects, research design and specimen source site (cervix/endocervix as opposed to posterior fornix and lateral vaginal wall).

In this study, a kappa value of 0.692 showed moderate agreement between Pap and Modified Pap methods with the overall diagnostic accuracy of 38.9% for both methods. Additionally, Filho *et al.*, [19] reported that Pap method would be a valid diagnostic option in comparison to gold standard when it especially gives a positive BV result and a mean specificity of 95% and this criterion has also been fulfilled by Modified Pap method.

CONCLUSION

In conclusion, this study faced several limitations that will affect the generalizability of the results. First, due to limited resources and time in conducting this research, the research subjects were recruited from only one hospital during the study period and this may not be representative of the annual female population served by the hospital. Secondly, only women visiting ANC and FP clinics were recruited and the results may not be applicable to women delivering at the hospital but this will be augmented when the research can be applied to other populations of women. Thirdly, due to recall or social desirability bias, self-reported information may have been misreported or under-reported during the questionnaire interview and lastly, extensive training requirements made it impossible to perform Nugent scoring of Modified Pap and Pap methods .

In spite of these limitations, the greatest strength of this study is that it showed that Modified Pap has diagnostic value for BV diagnosis when it is positive and is therefore suitable as a confirmatory test for BV. Therefore, this study supports the use of Modified Pap as an alternative to Pap method in diagnosis of BV in cervico-vaginal smears.

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