*www.cenresinpub.org*
*ISSN 2277-0143*

# A COMPARATIVE STUDY ON THE USE OF LIQUID BASED CYTOLOGY AND CONVENTIONAL PAP SMEAR IN CERVICAL SCREENING

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## ABSTRACT

The high rate of cervical cancer in women and the inadequacy in its results during diagnosis has necessitated the comparison of the two major techniques used in its diagnosis which is the Liquid based technique and conventional Pap smear method. The aim of this thesis is to compare the accuracy of conventional cytology with liquid based cytology for primary screening of cervical cancers. The two cytological techniques were compared in a group of 300 women who visited Ebonyi State University Teaching Hospital for cervical screening. Outcome of the two screening methods was compared with regard to the determination of the specificity and sensitivity of both methods using histopathology as gold standard. Out of the 300 cases screened 38 and 30 cases were diagnosed as Low grade squamous intraepithelial lesion and High grade squamous intraepithelial lesion respectively by Liquid based cytology and about 32 and 24 cases were diagnosed as Low grade squamous intraepithelial lesion and High grade squamous intraepithelial lesion respectively on conventional cytology. 250 cases were satisfactory for evaluation using the LBC and 140 cases were found satisfactory on conventional cytology. Sensitivity and specificity of LBC was 100% and that of conventional cytology 86% and 97% respectively. From the result above it showed that LBC gives more accurate results. Though both methods have high sensitivity and specificity, LBC still has a higher sensitivity and specificity when compared to conventional Pap smear. On the other hand, the unit cost of LBC method is substantially more than that of conventional cytology.

## INTRODUCTION

Cervical cancer is cancer of the cells lining the cervix which is the passageway between the uterus and the vagina (Arbyn, 2004). Cervical cancer occurs when normal cells in the cervix change into cancer cells. It is usually caused by a chronic and persistent cancer-causing type of human papilloma virus (HPV) infection that leads to pre-malignant changes and progress to cancer. Screening is looking for cancer before a person has any symptom. Cervical screening is a method of preventing cancer by detecting and treating early abnormalities which, if left Untreated could lead to cancer in a woman's cervix (Davey *et al.,* 2006). Two screening tests can help prevent cervical cancer or detect it early. They include; the pap test (or pap smear) which looks for precancers, cell changes on the cervix that might become cervical cancer if they are not treated and the HPV test which looks for the virus (human papilloma virus) that can cause these cell changes (Colgan *et al.,* 2004).Today there are two types of pap tests; The regular pap test in which cells from a woman's cervix are smeared on a microscope slide and the liquid based pap test, in which the cells are placed in a special liquid first and then onto the slide. In both types, cells from the cervix are checked under a microscope in order to find cervical cancer at a stage that is easy to cure. They can also find early changes in the cells which

can be treated to stop cancer from developing. Cervical cytology was introduced by George Papanicolaou into clinical practice in 1940 (Papanicolaou; 1940). In 1945, the papanicolaou smear received the endorsement of the American cancer society as an effective method for the prevention of cervical cancer. Centre of cytology in Vancouver, British Columbia published data which confirmed that cytological screening leads to a reduction in the rate of invasive cancer of the uterine cervix. (Sweeney, 1967).

Although organized screening programs based on the papanicolaous (Pap) smear have been very successful in reducing mortality a major problem emerged. Cervical cancer has not been eradicated and its incidence has remained virtually constant for several years (Nance *et al.,* 1990). In Switzerland, cervical cancer is still among the leading cause of cancer with 400 new cases and 1000 death annually, mostly occurring in women over 65 years old. The majority of cases ever had a pap test or had a false negative results from pap test, leading to death in routinely screened women. Results of one study showed that 14% of women with an invasive cervical cancer or HSIL had received a negative smear result within the two years prior to diagnosis (Hutchinson *et al.,* 1999). Approximate 2/3 of the false negative smears were related to sampling errors and the remaining were due to screening and interpretative errors mainly due to the small number of diagnostic cell present in suboptimal smear (Vassilako's ,1998). Several limitations of the conventional smear has been identified including inadequate transfer of cells to slide (Hutchinson *et al.,* 1992), inhomogeneous distribution of abnormal cells, presence of obscuring blood, inflammation or thick areas of overlapping epithelia cells (Bolick, 1998) and low sensitivity and specificity (Nando, 2000).

Liquid based cytology was developed as an alternative to address the limitations of Pap smear. It was developed to improve the diagnostic reliability of papanicolaous smears. For the LBC the cervical cells are collected with a sampling device and rinsed into a vial with preservation solution rather than being smeared on a slide. Liquid based cytology, rinses cervical cells in preservatives so that blood and other potentially obscuring material can be separated. Because only a representative portion of the sample is used, residual material in the vial may be used for ancillary testing such as HPV testing and other molecular tests (Parker *et al.,* 2001). The remarkable feature of LBC is that it reduces the number of inadequate tests and hence the number of women who have to be recalled for repeat testing. It will also reduce pressure on the cytoscreeners as they will have fewer inadequate smears to look at and cleaner samples to report (Luthra *et al.,* 2002). Several studies comprising of more than 5,000 subjects have been carried out with a preponderance of data indicating a significant benefit of LBC in the detection of cervical cancer precursor lesions and in the improvement of specimen adequacy (Richard *et al.,* 1990). The present study was carried out to evaluate the LBC technique and to compare LBC with the conventional Pap smear.

## MATERIALS AND METHODS

The samples were collected from 300 female patients who came for cervical cancer screening between June 2009 and March 2011 at Ebonyi State University Teaching Hospital Abakaliki. Pap smears were taken from the cervix with Ayres spatula for conventional method and endocervical cytobrush for LBC. Smears were collected, processed and prepared as follows:

**Conventional Method**

Patient was asked to lie down and her feet placed in stirrups to hold the feet in place during the examination. Speculum was inserted into the patient's vagina Using an Ayres spatula, sample was taken from the cervix by gently rotating the spatula at 360 degrees. Sample collected was used to make thin smears on grease free glass slides (4 smears for each patient). Smears were then fixed in 95% ethanol. Smears were allowed to fix for 30 minutes and stained with the Papanicolaou staining technique.

**Liquid Based Method**

The method for collection of sample is almost the same with that of the conventional method, the difference is in the instrument used for the sample collection and the method of preservation. A brush-like device known as the endocervical cytobrush was used to scrape the cervix, it was inserted into the cervix and rotated five times at 360 degrees in clockwise direction. The head of the brush was thoroughly rinsed into the vial containing the fixative (consisting of 95% ethanol -20mls, glacial acetic acid-1ml and conc. HCL-ml).

The sample was mixed, and then centrifuged at 1500rpm for 10 minutes. The sediment collected was resuspended and respun for 5 times. At the end of the centrifugation process, the supernatant was decanted and a drop of the suspension was used to make a thin film on a grease free glass slide (4 slides for each patient) smears were then fixed in Pap fixative and stained with the papanicolaou staining technique.

**STAINING**

The smeared slides were stained using the papanicolaou staining technique.

**Staining Procedure**

**Papanicolaou staining technique**

**Principle:** This is based on the use of Harris haematoxylin as nuclear stain, 0.5% alcoholic orange as cytoplasmic stain for matured cells and EA50 as the cytoplasmic stain for immature cells.

**Procedure**

Smears were hydrated in descending grades of alcohol (absolute I, 90% and 70%) for a minute each and rinsed in distilled water. Smears were stained in Harris haematoxylin for 4 minutes and were rinsed in distilled water. Smears were differentiated in 1% acid alcohol for 15 seconds and rinsed in distilled water. Smears were blued in tap water for 5 minutes. Smears were placed in 70% alcohol and 95% alcohol for 5 seconds each and stained in OG6 for 2 minutes. Smears were placed in 95% alcohol; 2 changes for 10 seconds each. Smears were stained in EA50 for 2 minutes. They were placed in 2 changes of 95% alcohol, 10 seconds each.

They were dehydrated in absolute alcohol I and II for 10 seconds each, they were placed in the hot air oven for a minute for proper dehydration. They were cleared in 3 changes of xylene, 3 minutes each and mounted with DPX mountant.

**RESULT**

Ninety four (94) (31.3%) cases studied belonged to the age group, 41-50. The minimum age of patients screened was 12 years and maximum was 78 years.Out of the 300 cases

studied, cytopathology and histopathology diagnosis confirmed abnormalities in 70 (23%) cases (Table 1) 250 (83.3%) cases were satisfactory for evaluation on LBC, whereas 160 (53.3%) cases were satisfactory on conventional Pap smear 16 (5.3%) cases were unsatisfactory for evaluation on LBC and 20 (6.7%) cases on conventional pap smear. There were only 34 (11.3%) cases which were satisfactory for evaluation but limited by factors like air drying artifact, obscuring blood and inflammation, cytolysis or absence of endocervical component on LBC, whereas 120 (40.0%) cases in the same category on conventional pap smear (Table 2).

The most common cause of unsatisfactory smear on LBC was scanty cellularity in 10 (3.3%) cases and on conventional Pap smear, thick smear was the commonest cause in similar percentage of cases. Infectious agents were detected in 50 (16.6%) cases on LBC and in 24 (8.0%) cases on conventional Pap smear. Candida was the commonest infectious agent in 38 (12.7%) cases, followed by Trichomonas vaginalis in 10 (3.3%) cases. A comparative study of LBC, conventional Pap smear and histopathological findings were performed. 38 (54%), 20 (29%) and 12 (17%) cases were diagnosed as LSIL, HSIL and carcinoma respectively on LBC while on conventional pap smear 32 (46%), 24(34%) and 14(20%) were diagnosed as LSIL, HSIL and carcinoma respectively and histopathology confirmed 40(57%), 18(26%) and 12(17%) as LSIL HSIL and carcinoma respectively. (Tables 3).

A total of 58 cases were diagnosed as benign and 12 cases as malignant by histopathology while a total of 58 cases were classified on as benign and 12 cases as malignant on LBC and 56 as benign and 14 as malignant by conventional Pap smear. (Table 4)

**Statistical Analysis**

Sensitivity and specificity of the 2 techniques were calculated thus, using histopathological results as gold standard.

**Where**

T N = True Negative
T P = True Positive
F P = False Positive
F N = False Negative

Sensitivity = $\frac{= TP}{TP + FN}$ X $\frac{100}{}$

Specificity = $\frac{= TN}{TN + FP}$ X $\frac{100}{}$

For LBC

From table 4, $TP = 12 TN = 38 FP = 0, FN = 0$

Sensitivity is = $\frac{= 12}{12 + 0}$ X $\frac{100}{1} = 100\%$

Specificity is = $\frac{58}{58 + 0} X \frac{100}{1} = 100\%$

For Conventional Pap smear

From table 4, $TP = 12 TN = 58 FP = 2, FN = 2$

Sensitivity is = $\frac{12}{12+2} X \frac{100}{1} = 86\%$

Specificity is = $\frac{58}{58+2} X \frac{100}{1} = 97\%$

In this study, sensitivity and specificity of LBC was 100% each and of conventional pap smear 86% and 97% respectively.

**Table 1: Age Distribution**

| *Age* | *Total no of cases* | *Normal* | *Abnormal* |
|---|---|---|---|
| **11 – 20** | 6 | 4 | **2** |
| **21 – 30** | 32 | 26 | **6** |
| **31 – 40** | 70 | 50 | **20** |
| **41 – 50** | 94 | 80 | **14** |
| **51 – 60** | 32 | 22 | **10** |
| **61 -70** | 46 | 36 | **10** |
| **71 – 80** | 20 | 12 | **8** |
| **Total** | **300** | **230** | **70** |

**Table 2: Cytological Classification**

| *Category* | *LBC* | | *Conventional* | |
|---|---|---|---|---|
| | **No** | **Percent** | **No** | **Percentage** |
| **Satisfactory** | 250 | 83.3 | 160 | **53.3** |
| **Unsatisfactory** | 16 | 5.3 | 20 | **6.7** |
| **Satisfactory but limited by factors** | 34 | 11.3 | 120 | **40.0** |
| **Total** | **300** | **100** | **300** | **100** |

**Table 3: Comparative study of LBC, Conventional Result and Histopathology Result**

| *Category* | *LBC* | *Conventional* | *Histopathology* |
|---|---|---|---|
| **LSIL** | 38 | 32 | **40** |
| **HSIL** | 20 | 24 | **18** |
| **Carcinoma** | 12 | 14 | **12** |
| **Total** | **70** | **70** | **70** |

**Table 4: Benign Versus Malignant**

| *Category* | *LBC* | *Conventional* | *Histopathology* |
|---|---|---|---|
| **Benign** | 58 | 56 | **58** |
| **Malignant** | 12 | 14 | **12** |
| **Total** | **70** | **70** | **70** |

## PHOTOMICROGRAPHY

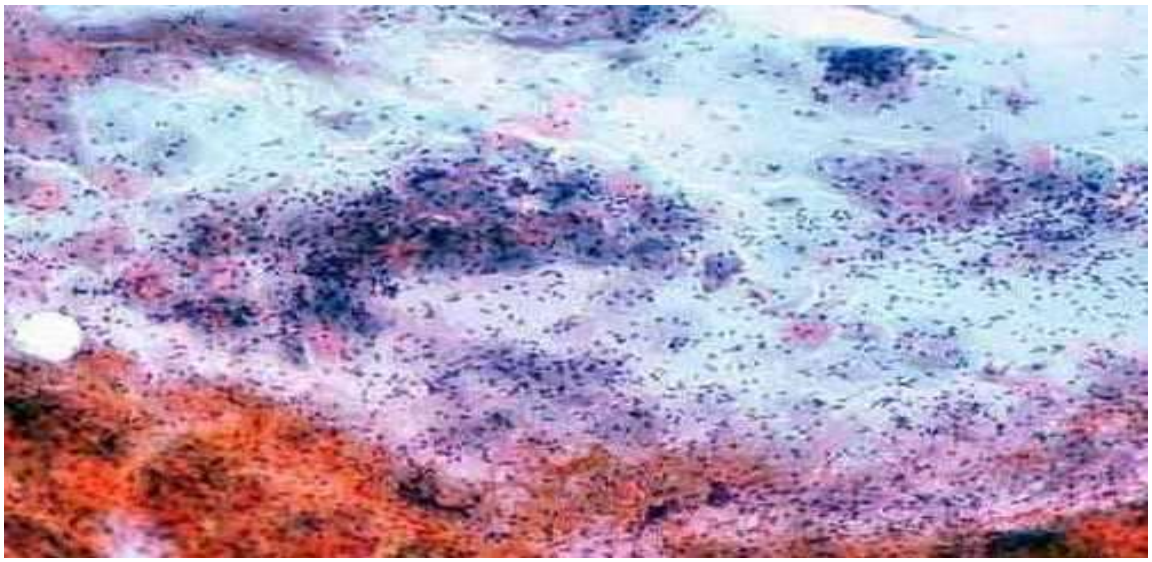

**Plate 1: CONVENTIONAL PAPANICOLAOU SMEAR**

The uneven distribution of cellular material, dirty background and thick clusters of cells (unsatisfactory smear) associated with the conventional papanicolaou pattern

**PAPANICOLAOU STAINING TECHIQUE X10**

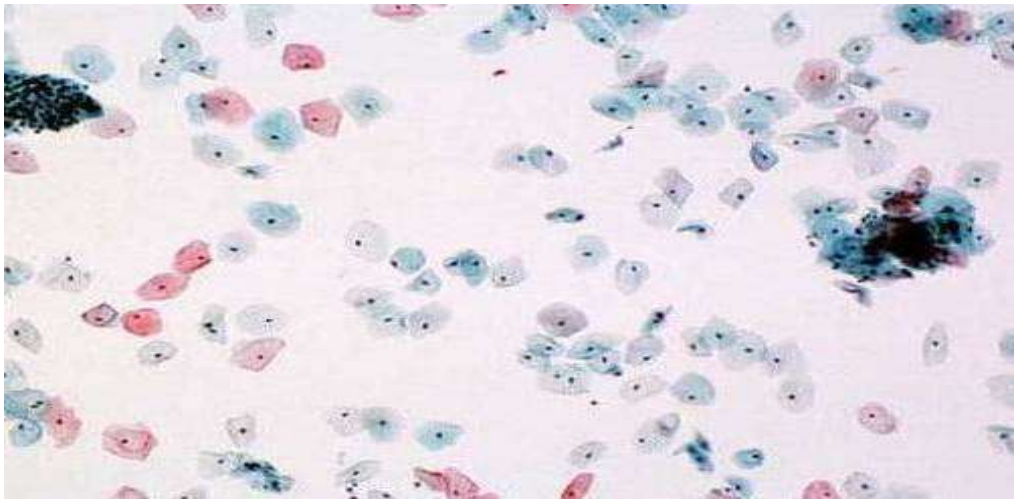

**PLATE 2: LIQUID BASED CYTOLOGY**

Plate made from the same patient as the plate above showing even distribution of cells, clean background with no debris, mucus or cell masking the abnormal cell.

**PAPANICOLAOU STAINING TECHIQUE X10**

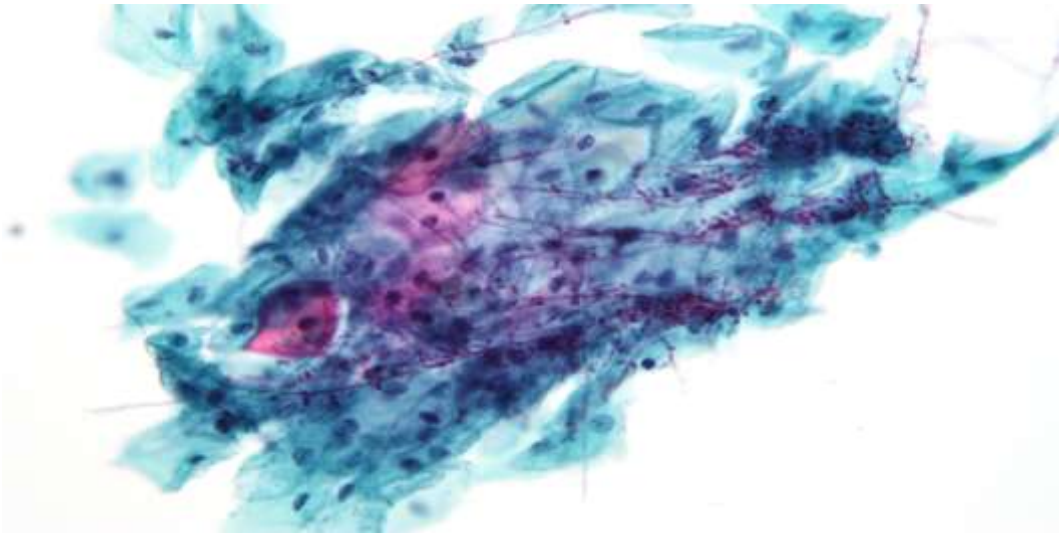

**PLATE 3: CONVENTIONAL PAPANICOLAOU SMEAR**

Smear made from the conventional technique showing candida with hyphea, yeast cells, dirty background and thick cluster of cells.

**PAPANICOLAOU STAINING TECHIQUE X10**

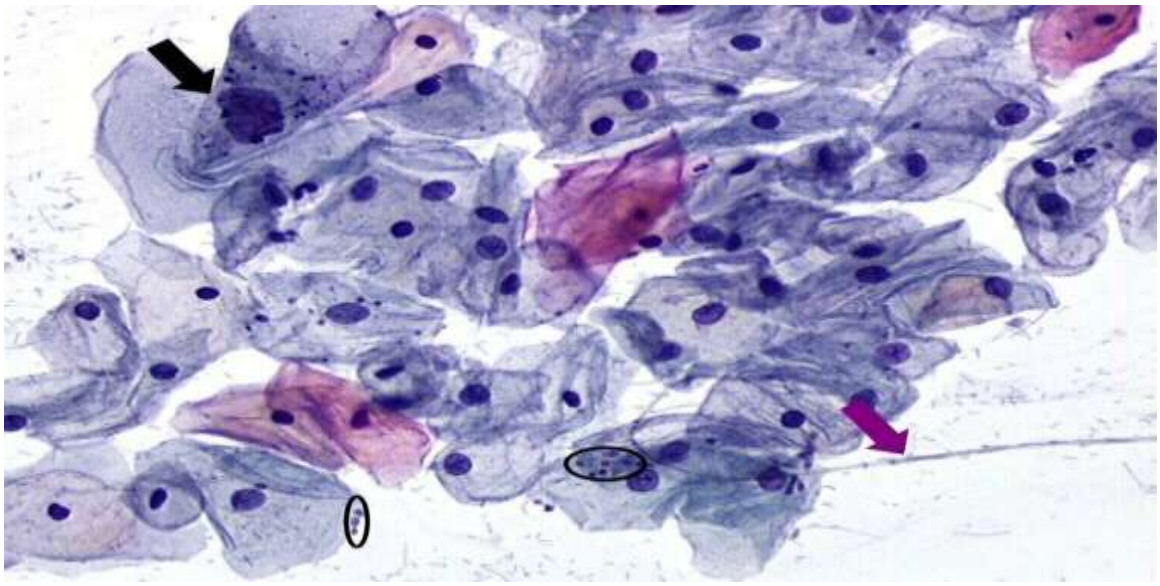

**PLATE 4: CONVENTIONAL PAPANICOLAOU SMEAR**

Smear made from liquid based cytology technique showing yeast cells, hyphea and clear background with no debris, mucus or blood cell masking the abnormal cells.

**PAPANICOLAOU STAINING TECHNIQUE X200**

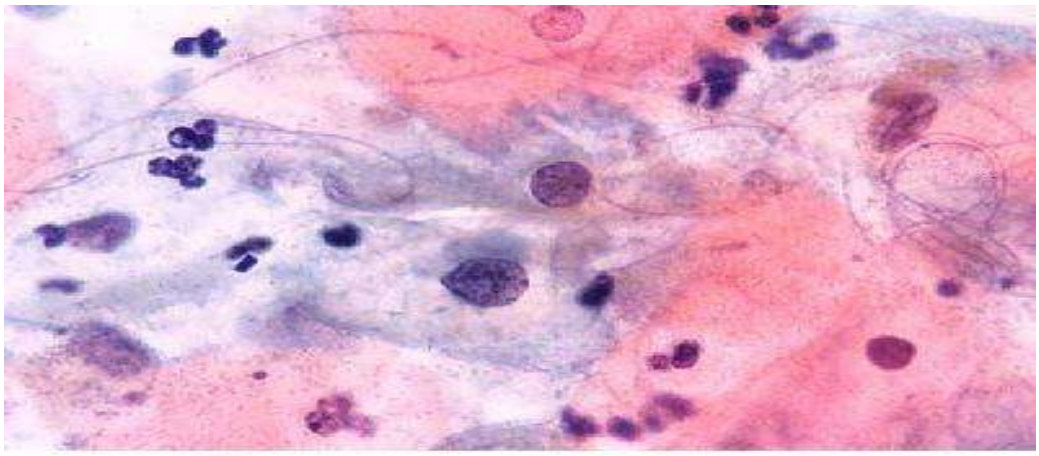

**PLATE 5: CONVENTIONAL PAPANICOLAOU SMEAR**

Smear made from conventional technique showing reactive squamous cells associated with Trichomonas Vaginalis. Cytomorphologic features; minimal nuclear enlargement and cytoplasmic polychromesia

**PAPANICOLAOU STAINING TECHNIQUE X200**

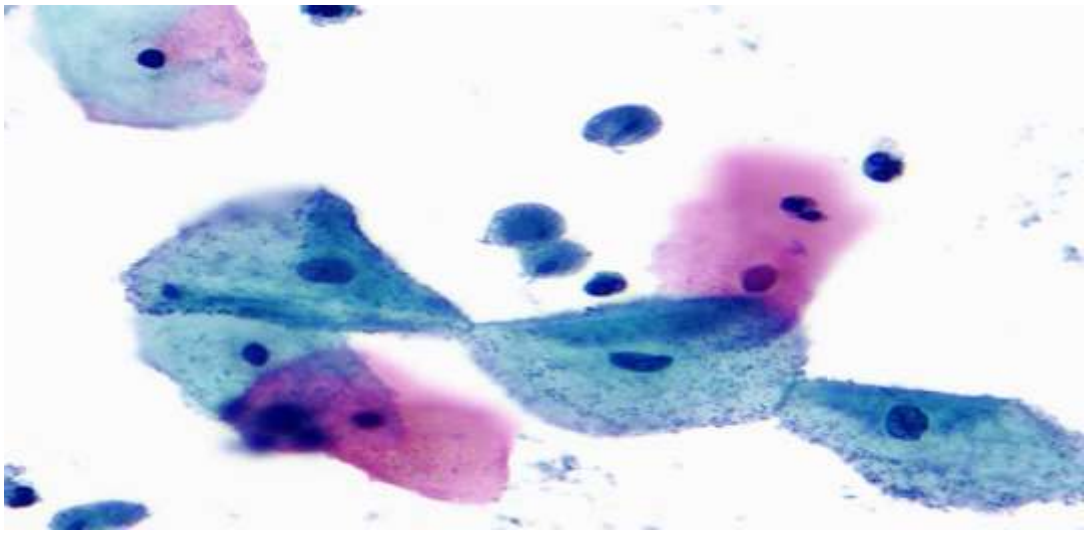

**PLATE 6: LIQUID BASED CYTOLOGY**

Liquid based smear showing trichomonas Vaginalis; A pear shaped oval to round cyanophilic organism that ranges in size from 15-30 microns. The nucleus is pole, vesicular and centrally located. Eosinophilic granules are often visible in cytoplasm.

**PAPANICOLAOU STAINING TECHNIQUE X200**

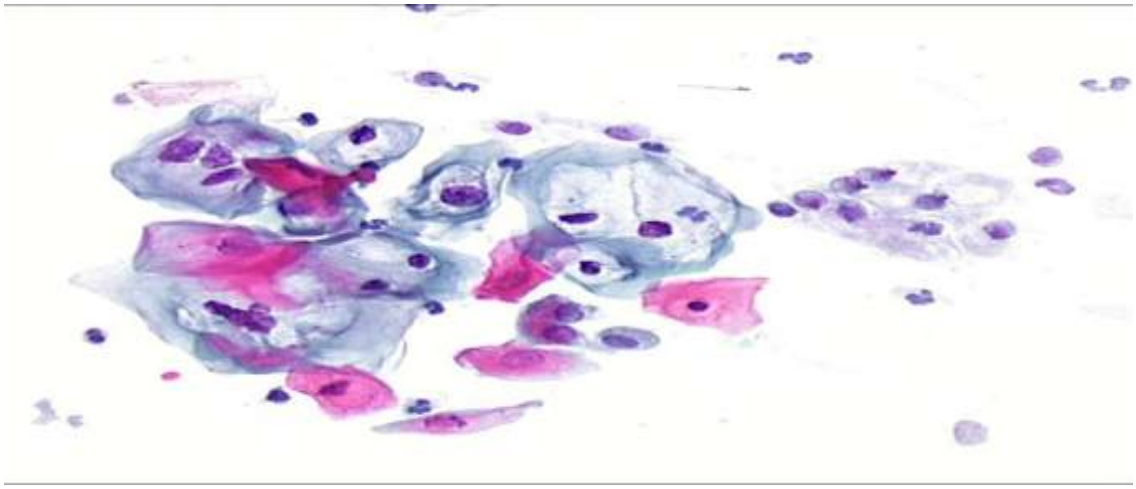

**PLATE 7(a): LIQUID BASED CYTOLOGY**

Basophilic and a few eosinophilic squamous cells with a perinuclear empty cavity surrounded by cytoplasmic thickening and with moderate nuclear enlargement: typical koilocytes.

**PAPANICOLAOU STAINING TECHNIQUE X100**

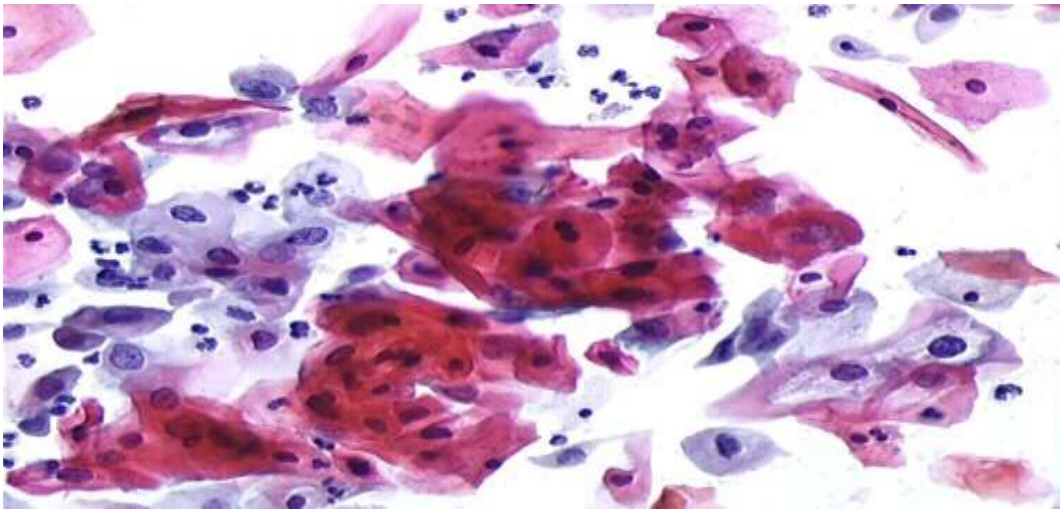

**PLATE 7(b): LIQUID BASED CYTOLOGY**

Eosinophilic squamous cells with dense cytoplasm, parakeratosis and some typical koilocytes.

**PAPANICOLAOU STAINING TECHNIQUE X100**

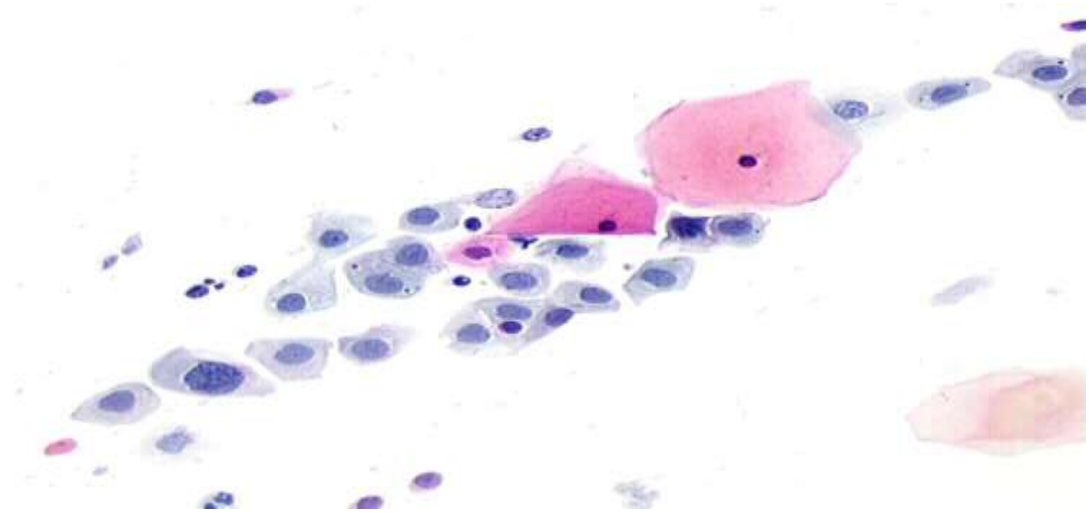

**PLATE 8(a): LIQUID BASED CYTOLOGY**

Parabasal cells with nuclear enlargement, irregular nuclear outlines, with anisokaryosis and anisocytosis in a homogenous cell population.

**PAPANICOLAOU STAINING TECHNIQUE X100**

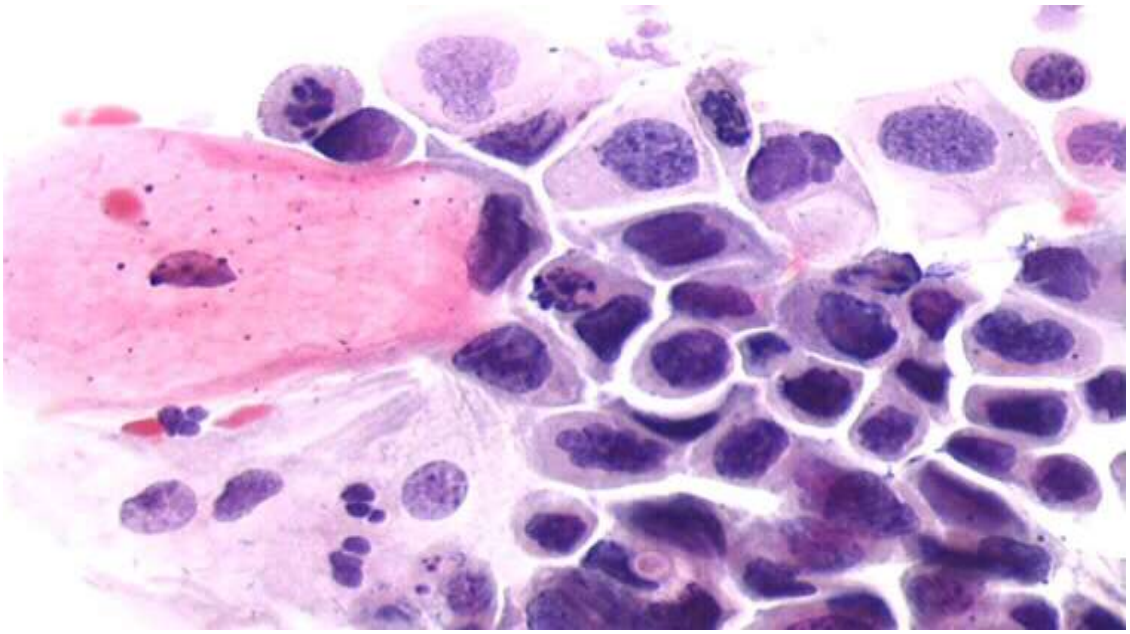

**PLATE 8(a): LIQUID BASED CYTOLOGY**

Parabasal cells with nuclear enlargement, irregular nuclear outlines, with anisokaryosis and anisocytosis in a homogenous cell population.

**PAPANICOLAOU STAINING TECHNIQUE X200**

## DISCUSSION

The Papanicolaou smear has been utilized for cervical cancer screening for more than 50 years. Despite being credited with a 70% reduction in mortality for cervical cancer, the false negative rate is still a cause for concern. It is widely acknowledged that two third of the overall false negative rate can be attributed to sampling errors. Liquid based cytology has been developed to address the sampling problems of conventional Pap smear.

This present research work was done to compare the LBC and Conventional cytology. In this research it was realized that 80% of cells collected by conventional technique were not transferred no to the slide this was similarly noted by Hutchinson *et al*.By rinsing the sample device into a liquid fixture in LBC technique helps the entire sample to be captured into the vial. In this study satisfactory smears on the conventional was 53.3% as compared to 83.3% on the liquid based cytology method. This is quite similar to the work of Weintraub and Morabia, 2000 who have reported an increased num, 1992 and this explains the high prevalence of true false negative rate. ber of satisfactory cases (72.2% - 92%) on liquid based cytology than conventional smears. All drying artifacts and cytolysis is almost absent or minimal with liquid based cytology and specimen adequacy was greatly improved due to absence of limiting factors like blood, mucous and inflammatory cells. Conventional smears had more unsatisfactory smears and this is due to thick smears, which was not a problem with liquid based cytology due to even distribution of cells. The microscopic details of infectious agent like Candida were enhanced on LBC which made it easy to be detected. In this research work sensitivity and specificity of LBC was 100% and 100% respectively and conventional Pap smear 86% and 97%

respectively. This is very similar to the work of Beerman *et al.,* 2009 who reported sensitivity and specificity of LBC as 96.2% and 98.2% respectively, whereas on conventional Pap smear it was 92.0% and 97.8% respectively.

## CONCLUSION

Liquid based cytology was found to have high diagnostic accuracy compared to conventional cytology in this research work. The study confirms previous reports of decreased numbers of unsatisfactory samples, increased satisfactory samples, and increase detection of LSIL, HSIL, Carcinoma and true positive result with liquid based cytology. Liquid based cytology is strongly advocated for the best interest of the public, it improves the quality of samples and reduces the likelihood of false negative result, thereby significantly improves early detection and treatment of cervical lesions.

## RECOMMENDATION

Since there was a significant increase in the rate of detection of cervical lesions using the Liquid based cytology technique, it is recommended that health organization change to this method for better cervical screening results.

## REFERENCES

Arbyn M, Baldauf JJ, Da Silva D.( 2004) Methods and techniques of cervical cancer screening. In European guidelines for quality assurance in cervical cancer screening. Luxemburg European Commission,

Beerman H, Vandorst E.B.C, Kuenen - Boumeester V, Hogendoorn P.C.W (2009) superior performance of based versus conventional cytology in a population based cervical cancer screening program. Gynecol oncol doi 10.1016/J.ygyno 12012.

Bolick D R, Hellman D T. (1998) Laboratory implementation and efficacy assessment of the thin prep cervical cancer screening system. Acta Cytol 42:209-13.

Christopherson WM and Parker JE. (1960) Poor socioeconomic condition and its association with carcinoma cervix. Cancer; 13: 711-5

Colgan TJ, Mclachlin CM, Cotterchio M, et al. (2004) results of the implementation of liquid – based cytology – surepath in the Ontario screening program. Cancer Cytopathol;102:362-7.

Davey E, et al. (2006) effect of study design and quality on unsatisfactory rates, cytological classifications, and accuracy in liquid –based versus conventional cervical cytology: a systematic review. Lancet; 367: 122-32.

Diaz Rosario LA, Kabawat SE.(1999) Performance of a fluid based thin layer Papanicolaou smear method in the clinical setting of an independent lab outpatient screening of population in New England. Arch Path Lab Med, 123:817-21.

Duggan MA, Khalil M, Brasher PM, Nation JG.(2006) Comparative study of the Thin Prep test and conventional cytology results in a Canadian cohort. Cytopathology; 17 (2): 73 – 18

Fathey MT, lrwig L, Macaskill P.(1995)Meta-analysis of Pap test accuracy. Am J Epidemiol; 141:680 – 9.

Hakama M, Chamberlain J, Day NE, Miller AB, Prorok PC (1985) Evaluations of screening programmes of gynaecolgical cancer. Br J Cancer; 52:669-72.

Hutchinson ML, Agarwal P, Denant T, Cibas E, A (1992) new look at cervical cytology: thin prep multicenter trial result. Acta Cytol; 36: 499-54

Hutchinson ML, Zahniser DJ, Shermann ME, et al. (1999) utility of a liquid – based cytology for cervical carcinoma screening: results of a population – based study conducted in a region of Costa Rica with a high incidence of cervical carcinoma. Cancer; 87:48-55.

Hussein T, Desai M, Tomlinson A Kitchencer HC.(2005) The comparative diagnostic accuracy of conventional and liquid-based cytology in a colposcopic setting. BJOG; 112 (11):1542-6.

Kenneth DH, Yao S Fu.(2002) Cervical and vaginal cancer, Novak's Textbook of Obstetrics and Gynaecology, 13th ed. Baltimore: WB Saunders Co;. p. 471 -93.

Klikhamer PJJM, et al. (2003) liquid-based cervical cytology. A review of the literature with methods of evidence-based medicine. Cancer Cytopathol; 99(5):263-71.

Koss diagnostic cytology and its histopathologic bases, volume I by Leopold G Koss 1992

Kreuger FAF, Beerman H. (2000) The screening of women with cervical cancer of the Rotterdam area. Eur J Epidemio; 16 (7): 641-5.

Limaye A, Connor A J, Haung X, Luff R, (2003) comparative analysis of conventional papanicoloau test and a fluid-basxed thin-layer method. Arch pathol Lab Med;127(2) 200-4

Luthra UK, Chrishti M, Dey P, et al. (2002) performance of monolayered cervical smears in a gynecology outpatient setting in Kuwait. Acta Cytol; 46(2):303 –10.

McCrory DC, Matchar DB, Bastian L, Datta S, Hasselblad V, Hickey J, Nanda K, Evaluation of cervical cytology. Evid Rep Tectnol Assess 1999:1-6.

Miller AB, Chamberlain J, Day NE, Hakama M, prorok PC.( 1990) Report on a workshop of the UICC project on evaluation of screening for cancer. Int J Cancer 46:761-9.

Nance K V. (2006) Evolution of Pap testing at a community hospital – a ten year experience. Diagnostic Cytopathol;35:148-53

Nando K, Mccrory D C, Myers E R. Bastian L A, Hasselbald V, Hickey J D, Matchar D B, (2000) Accuracy of the papanicolaou test in screening for and follow-up of cervical cytology abnormalities: a systemic review. Ann int Med: 132:810-9

Papaincolaou G N (1940) Introduction of pap smear in early detection of cervical malignancies Am J clin path; 19 301-8

Parker DM, Pisani P, Ferlay J.(2001) Cancer incidence, mortality and prevalence worldwide. Ver. I,IARC, Cancer No. 5, Lyon Press,.

Payne N, Chilcott J, McGoogan E. (2000) Liquid-based cytology in cervical screening: a rapid and systematic review. Health Technol Assess 4:1-73.

Richard R M, Barron B A. (1969) A follow up study of patients with cervical dysplasia. Am J Obstet Gynecol; 105:386-93

Richard R.M. (1968) Natural history of cervical intraepithelial neoplasia. Clin Obstet Gynaecol; 10: 748-50.

Robert ME, Fu YS. (1990) Squamous cell carcinoma of the uterine cervix – a review with emphasis on prognostic factors. Semin Diagn Pathol; 7:173-89.

Rotkin. Epidermiology on cancer of the cervix. Sexual characteristics of cervical cancer population. Am J Public Health 1973;57:815-29.

Shakarnarayana R, Nene BM, Dinshaw K, Raj Kumar. (2003) Early detection of cervical cancer with visual impaction methods: a summary of completed and ongoing studies in India. Shawad Publica de Mexico;45 291-301.

Stewart B W, Kleihues P. (2003) Cancers of the female reproductive tract. In: World cancer report, WHO. Lyon: IARC Press;. P. 215-22

Sweeney BJ, Haq Z, Happel JF, Weinstein B, Schneider D. (2006) Comparison of the effectiveness of two liquid-based Papanicolauo systems in the handing of adverse limiting factors, such as excessive blood. Cancer Cytopathol; 108(1):27-31.

Taylor S, Kuhn L, Dupree W, et al. (2005) Direct comparison of liquid-based and conventional cytology in a South African screening trial. Int J Cancer 118:957-62.

Wintraub J, Morabia A. (2000) Efficacy of a liquid basxed-thin layer method of cervical cancer screening in a population with a low incidence of cervical cancer. Diagn Cytopathol;22:52-9

**APPENDIX I**
**MATERIALS:**
Frosted slide
Spatula
Cervical
Speculum
Slide
Fixative
Liquid based fixative
Xylene
Ethanol papanicolaou
Centrifuge
Staining rack
Filter paper
Cover slip
Mountant
Oven
Microscope
Distilled water
Slide box
Photo microscope
Weighting balance beaker

**APPENDIX II**
**Preparation of stain Haematoxylin (Harris)**
Haematoxylin – 2.5 g
Absolute alcohol – 50 ml
Potassium alum – 50g
Distilled water –500mal
Mercuric oxide acid - 1.5 g
Glacial acetic acid – 20mal
The haematoxy lin was dissolved in absolute alcohol and alum in distilled water. The two solutions were mixed in a large flask and boiled. Mercuric chloride was added and mixed. It was cooled immediately in cold water before the addition of glatial acetic acid. It was then filtered before use. **OG6**
This was commercially prepared
**EA40**
This was commercially prepared

**APPENDIX III**
**Abbreviations**
LBC: Liquid based cytology
HPV: Human papiloma virus
CIN: Cervical intraepithelial neoplasia
LSIL: Low grade squamous intraepithelial lesion
HSIL: High grade squamous intraepithelial lesion
ASCUS: Atypical squamous cells of undetermined significance