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COMPARATIVE STUDY OF FIXED AND UN-FIXED DRY CERVICAL SMEAR SLIDES

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ABSTRACT
Introduction: Cervical screening test is advised in order to pick abnormalities early and also to institute early treatment to prevent cervical cancer.
AIM: To determine weather unfixed air dried cervical smears can be transported from remote areas in the resource limited setting to laboratories and still obtain staining qualities.
Method: Sixty (60) paired slides of cervical smears were obtained from women attending routine checkups in Aminu Kano Teaching Hospital Kano. The chemical indicator smearswere taken by gynecologist under direct vision using wooden spatula. The smears were immediately fixed in 95% ethanol for 30 to 45 minutes, one group was left and allowed to air dry. The two air dried slides (fixed and unfixed) smears were rehydrated in water before staining using papanicolaou staining method, and examined under Olympus microscope at magnification of x4 and x10 and x40 objectives respectively.
Result: The slides that were kept for 24 hours were viewed at x10 and x40 objectives and the data obtained were as followed. At x40 objective all the smear slides were visualized, at x10 objectives all the sixty(60) smear slides were hazy and at x40 objectives, all the sixty(60) smear slides have poor magnification.
Conclusion: With the result obtained it will not be possible to use plain air dry smear but rather pre fixed smear prior to staining.
Key Words: Cervical, Pap Smears, Papanoclaou, Exfoliate, Fixative

INTRODUCTION

A cervical screening test involves taking a sample of cells from the transformation zone (TZ) of the cervix. This zone is also called the squamous columnar junction (SCJ) and marks the division between the columnar cells that line the endometrium and the squamous cell of the cervix. At this meeting points the cells are continually dividing and growing, thus the likelihood of these cells developing abnormalities that could become cancerous. The surface of the cervix is normal cells lining with endometrial and the cervical canals are reddish and granular in appearance (CKS, 2010). The cervical ranging from infectious, premalignant changes and institute/invasive cancer in a given population.

The cervical smear also referred to as the papsmear after the Greek Doctor who first used the test. The traditional test involvedre moving cells from Transformation Zone (TZ) of the cervix with a small wooden spatula. The cells was smeared on to a glass slide and stained using papanicolous staining techniques and observed under the microscope (Klinkhamer etal; 2009). The cervical smear test is advised to pick abnormalities early and to institute the treatment to prevent cancer.

It is recommended that women within reproductive age should be timely screened, Females with age group 25-45 years should be screened every 5years. In absence of previous detected abnormalities in the cervical smear, sexually active women are encouraged to have screening done yearly. In the absence of abnormalities, Women over the age of 65years should be screened if:

a. They have not had a cervical screening test has been abnormal cervical screening has had three (3) negative results (bulkmans *et al*, 2006). Fixation preserves a sample of biological material (tissue or cell) as close to its natural state as possible.

In the process of preparing tissue a fixative usually acts to disable intrinsic biomolecules. Particularly proteolytic enzymes which otherwise digested or damages the sample. Secondary, a fixative typically protects a sample from extrinsic damage. Fixatives are toxic to most common microorganisms (bacteria in particular) that might exist in a tissue sample or which might otherwise colonize the fixed tissue many fixatives chemically alter the fixed materials to make it less deteriorate. Finally, fixatives often alter the cells or tissue on a molecular level to increase their mechanical strength or stability (CKS, 2010).

A number of fixatives are used in exploitative cytology; the common ones are 95% ethyl alcohol. This can be used on its own with satisfactory result, but addition of 3% glacial acetic acid increase the nucleon-protein-fixing properties (Bancroft and Steven (1982). This is the standard fixatives in most laboratories, and gives excellent nuclear and cytoplasmic morphology, where slides have to be sent by post, the fixatives are preserved by statute, thus aerosol spray fixatives containing polyethylene glycol in ethyl alcohol were introduced. Smears are collected by the gynecologist or paramedical staff in the clinic, hospital or health centers. The routine practice is to fix the slides immediately in 95% ethanol for 45 minutes to 1 hour, send to the laboratories to be stained and evaluated by cytopathology's unfortunately due to inadequate training and heavy work load we get improper fixation, which leads to repeating of the smears and delayed result (Actacytol, 1994). One alternative method to overcome this problem is the introduction of air drying followed by rehydration with and fixation before staining (Bern tein Stack, 2001).

Wet cervical smear slide and dry fixed cervical smears are good for papanicolaou staining technique. However, can dry unfixed cervical smear give the same staining reaction. This study aimed at evaluating the effect of unfixed air dried cervical smear on stainingquality between 48hours slide and 24hours slides.

MATERIAL AND METHODS

Materials used were frosted end slides, 22 x 40 size cover slips, Viginal – Speculum, Arye's Spatula, cotton wool, slides – rocky jar containing 95% ethyl alcohol, request form, hand gloves, diamond pencil, Olympus microscope.

Papanicuolou stain was prepared, 20 paired slides of cervical smears were obtained from women attending routine checkups in Aminu Kano Teaching Hospital Kano. Clinical indicator smears were taken by gynecologist under direct vision using wooden spatula. The smears were immediately fixed in 95% ethanol. For 30 to 45 minutes. One group was left and allowed to air dry. The two air dried slides (fixed and unfixed) smears slides were rehydrated in water before staining the slides and then examined using Olympus microscope at magnification of 40 x 10 and x 40 objectives respectively.

Papanicolaou staining procedure.

The slides rehydrated using tap water, the slides obtained with Harris heamatoxylin for 5 minutes. The slides were rinsed in tap water, blued with amounted water for nuclei staining. The slides were stained in O.G 6 for 4 minutes rinsed in charges of 95% alcohol and transferred to E.A 50 for another 4minites, finally dehydrated in two changes of 95% alcohol allowed to air-dried and cleared in xylene and mounted with DPX, labeled and examined with Olympus microscope. The expected result was based on the criteria used for microscopic observation, which includes the appearance of the nuclei, demonstration of the basic cytoplasmic color clarity of the background, to give optimal contrast and differentiation with these criteria.

RESULTS

The slides collected and stained were examined under Olympus Cx21 microscope by a consultant pathologist to ascertain its suitability for cytological diagnosis. The slides were kept for 24 hours were examined at (x4) panaromic objectives, (x10) objectives and (x40) objectives len and the data is shown below.

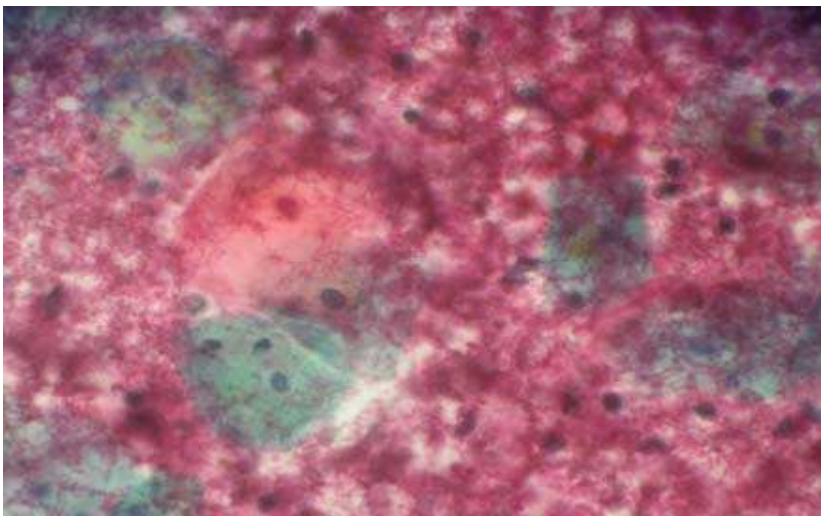

Fig: 1. A wet fixed smear using x 40

Sixty slides fixed in 24hour (cell components) were examined at (x4) objectives, all slides were examined at (x10) objectives and at (x40) objectives there is poor magnification.
Sixty slides in 48hour
At x4, x10 and x40 objective lens all the slides have poor magnification.

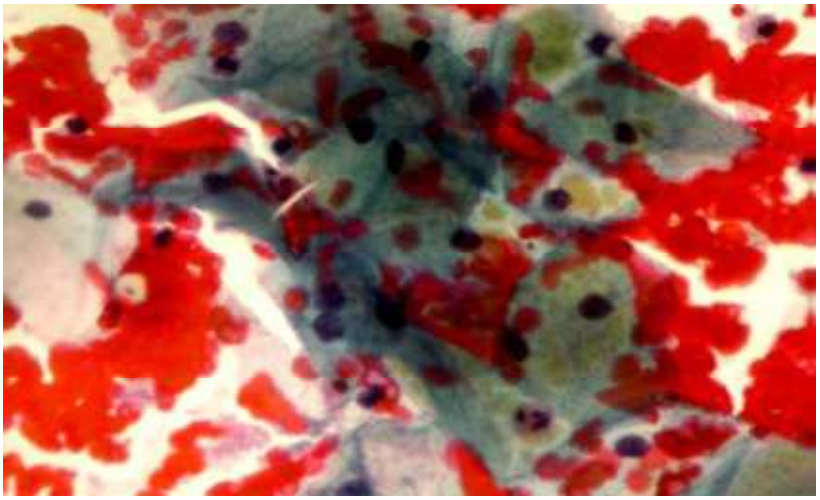

Fig: 2. Properly fixed pap smear with higher magnification

Data Analysis

Criteria for scoring

- Visualize
- Hazy distinction between nucleus and cytoplasm
- Poor magnification

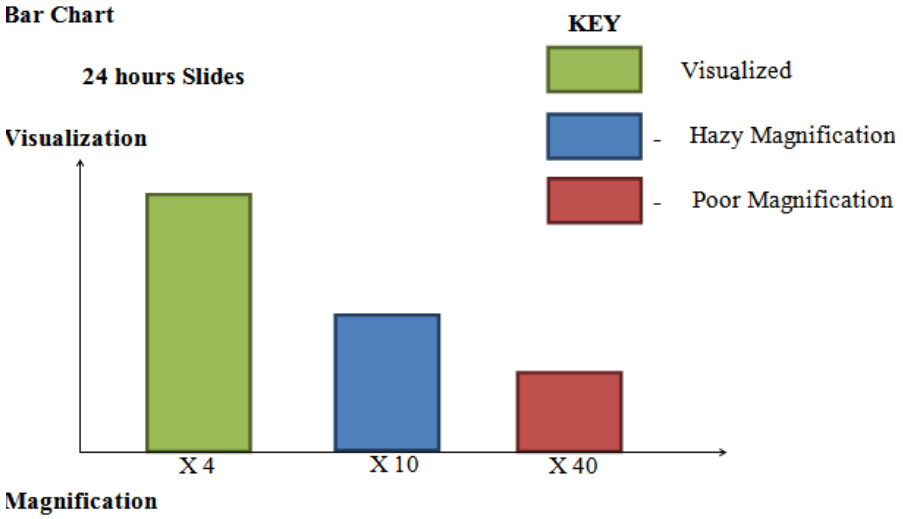

Figure 1: Bar chart of the data collected at 24 hours showing the level of the visualization.

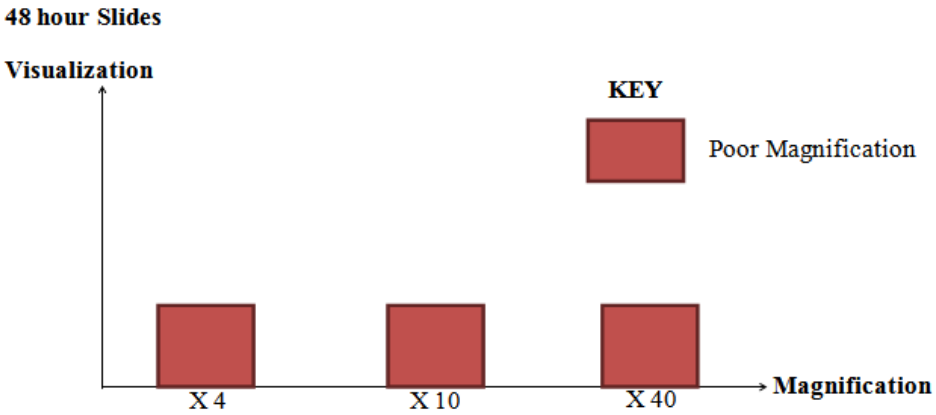

Figure 2: Bar chart of the data at 48 hours showing the level of visualization

DISCUSSION

The requirement of cervical screening for an average women of child bearing age cannot be over emphasized the women in the rural areas deprived of these because of their distance from the source of screening which makes it very necessary to seek for a way to transport collected slides to the laboratory. After carryout the data collection and analysis, it was observed that the cells remain visible at low magnification especially at x4 which is the panaromic view. This is unconnected with the ability to observe details at this magnification. But when viewed at x10 objectives with the view to see details it was observed that hazy appearance showing no distinction between cytoplasm and nuclear were registered, this could be as a result of loss of cellular architecture because of no fixation from the very beginning of collection. Moreover, at x40, distinction in the cellular details is lost which may be as a result of detailed observation of microscopic damage by lack of fixation, this result when compared to previous work done by J.D. Yaro and A. Mairiga published in proceeding of the anatomical society of Nigeria which indicates that pre-fixed slides before air drying and transporting is far superior. Therefore it is important to adopt such with view to continue to check the mechanism of presentation of the pap smear without fixation, therefore, there is possibility of obtaining a good result with shorter time than 24 hours if it could be tried.

CONCLUSION

This work was carried out with the burden of cervical screening to the door post of rural women in order to save them from cancer of the cervix. But with the result obtained it will not be possible to use plain dry smear but rather prefixed smear.

RECOMMENDATION

I strongly recommend a further work to be carried out especially on the reduction of time from 24 to 12 hours to see possibility of a good contrast and visibility.

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