ORIGINAL ARTICLE

ROLE OF PAPANICOLAOU SMEAR IN MAPPING OUT CERVICAL EPITHELIAL MORPHOLOGY IN WOMEN WITH INFERTILITY: MODIFIED BETHESDA CLASSIFICATION

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Background: Papinocolaou smear (PAP) smear has a specificity of 98% in detecting early changes in cervical epithelium in women above 21 years, thus its avocation as a screening tool for cervical cancers worldwide. In Pakistan with a lack of awareness in our population as well as socioeconomic conditions there is no such screening program. Infertility on the other hand affects 22% of women in Pakistan and thus one of the most common cause of physician visits. Both cervical cancers and infertility have sexually transmitted diseases (STD's) as common causes with studies reporting ~75% of women have an STD at-least once during their life, presenting with cervical epithelial cell lesions. Thus, the present study was carried out to identify patterns of cervical cell morphology in women with infertility using PAP smear. **Methods:** Cervical smears were taken from infertile women and fertile women (n=150), stained with H&E and PAP stains, and graded according to Bethesda Classification 2001. Analysis of data was done by using SPSS version 20 and MS Excel. **Results**: The mean age of participants was ~29 years. Epithelial cell morphology of these smears showed significant difference among three groups (*p* value 0.037) with severity in infertile women. Further in the subgroup of secondary infertile women, there were more abnormal smears as compared to primary as well as a higher grade of severity by Pap smear. **Conclusion:** Thus, it is concluded that women presenting with some level of infertility are at a higher risk of having cervical epithelial abnormalities.

Keywords: PAP smear; Infertility; Epithelial lesions

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INTRODUCTION

The World Health Organization (WHO) describes infertility as a disorder of the reproductive system as a "failure to bring about a clinical pregnancy after a year or more of regular unprotected sexual intercourse".[1,2] Though Pakistan has a high population growth rate (~2%),[3] there is also a significantly higher prevalence rate of infertility in women as well (~21.9%). In both cases the commonality exists in the presence of sexually transmitted diseases (STD) as the root causes with chlamydia trachomatis and human papilloma virus (HPV) as leading pathogens.[4,5] Thus there are a number of potentially serious pathologies that are identified as being the root cause of infertility. Further because of increased exposure to exogenous hormones studies have shown that women with infertility may be more prone to develop cervical epithelial cell abnormalities and cervical carcinoma.[6]

PAP smears from the transformation zone (TZ) of the cervix; that is highly susceptible to carcinogens and HPV infections due to the rapid turnover of cells can be used to identify cervical epithelial cell abnormalities (ECA).[7] These ECA include atypical squamous cells (ASC) that maybe atypical with undetermined significance (ASC-US) and those in which high grade lesion cannot be excluded (ASC-H) precede cervical carcinoma.[8] These epithelial cell lesions range from mild dysplastic changes to marked dysplasia and ultimately progress to cervical invasive carcinoma.[9] There are to date hardly any publications on the prevalence of such abnormal cytology in infertile women in Pakistan and so the main aim of this study was to identify abnormal cytology of the cervix on Pap smear as one of the primary causes of infertility. Thus, the main objective of this study was to compare these patterns of cervical cytology of infertile women with those of fertile women.

MATERIAL AND METHODS

This cross-sectional comparative study was carried out after approval from Ethical Review Board of Khyber Medical University on 150 patients (WHO sample size calculator, 6.5% cervical changes) presenting at infertility clinics. Comparison of cervical cytological morphology according to modified Bethesda was done among each of the

three groups of Fertile (Controls, Group A), Primary infertility and Secondary infertility (Group B and Group C, respectively, n=50 each) by quota sampling. Lab work (smear fixation, staining and further grading) was done in the Histopathology Department of Ayub Teaching Hospital, Abbottabad and Institute of Basic Medical Sciences (IBMS), KMU. The inclusion criteria was subjects aged between 20–40 years and showing willingness to have per vaginal examination and Pap smear while the exclusion criteria was subjects with acute pelvic infection or presence of any systemic disease. The Controls (fertile women) were selected from patients presenting to Gynaecology OPDs for minor illnesses (other than infertility treatment) who had delivered a baby within the past one year. Women that had never conceived after trying for a minimum of two years were labelled as Primary infertility and secondary infertile were patients that had conceived and subsequently were unable to conceive after a trial of at least two years. By using the Conventional (Classic) method, cells were gently scrapped from the squamo-columnar junction of the cervix and evenly smeared on a microscopic glass slide, fixed and air dried. These slides were stained with Haematoxylin and Eosin stain (H&E) and Papanicolaou (Pap) stains. The slides were examined for both qualitative (04) and quantitative variables (03) and graded accordingly (Table-1). In the next step these graded variables of cervical cytology were classified into revised Bethesda system, 2001 (mTBS) with triangulation to reduce bias.[10,11]

Descriptive statistical analysis was conducted in SPSS for all the qualitative and quantitative variables included in the study. Variables were represented by mean±standard deviation. Inferential statistical analysis was conducted in SPSS by applying mainly two different tests depending upon the type of variable. Qualitative variables: Kruskal Wallis test, Mann Whitney U test. Quantitative variables: One way ANOVA, Post hoc Tukey test.

RESULTS

Cervical smears were analysed following the standard scheme of mTBS, 2001 assuring adequacy of specimen with a minimum of 10–15 cells per field for 5 fields (40X) and the presence of transformation zone.

The mean age of the participants was ~29±5.5 years across the groups. The average duration of infertility relative to duration of marriage was 9±5.4 years.

Microscopic variables: Based on the four qualitative parameters (nuclear outline, chromatin distribution, cytoplasm density and hyperchromasia) each sample was graded according to mTBS as illustrated in Figure-1 (A, B, C, D). Group C (Secondary infertile) showed marked hyperchromasia, highly irregular nuclear outline, irregular chromatin distribution and cyanophilic cytoplasm as compared to Group A and B ($p<0.05$, Mann Whitney U test). Microscopic quantitative variables (Figure-1: A", B", C", D") were studied with mean nuclear diameter 8.58±2.47, cell diameter 54.5±6.5 and N:C ratio of 16.13±8.35 (mean±SD). There was no significant difference in these values across all three groups (Figure-2).

Grading: Based upon the microscopic parameters defined above, of all smears analysed, 4.7% showed epithelial cell abnormalities (Figure-3). These abnormalities were then graded into mild, moderate and severe as shown in table-2.

Cervical epithelial lesions: The categorization of these cervical cytology into Normal for Intraepithelial Lesion/malignancy (NIL/NILM) and Epithelial Cell Abnormalities (ECA) using mTBS is illustrated in Figure-4 from lowest severity grade of ASCUS to the highest severity grade of squamous cell carcinoma (SCC).

Normal intermediate squamous cells with a nuclear diameter of ~7–8 µm were differentiated from atypical intermediate cells present in the form of a cluster with a nuclear area of ~2–3 times the area of normal intermediate cell nucleus (i.e., ~23µm) and mild irregularity of nuclear contour (ASCUS). Atypical intermediate cells with enlarged nuclei (1.5–2 times the area of normal intermediate squamous nucleus), high NC ratio, irregular nuclear boundary with coarse granular chromatin (ASC-H) were also seen. Changes of squamous cell carcinoma (SCC), i.e., marked pleomorphism in cell shape and size, irregular nuclear contour, high NC ratio, dense cyanophilic cytoplasm were identified.

Overall comparison of these lesions in the study groups (Figure-5) showed a greater difference between Group C (Secondary infertile) with other 2 groups Group A (Fertile) and B (Primary infertile). When the groups were compared statistically, there was significant difference ($p<0.05$, Kruskal Wallis test). Cross comparisons between the groups gave significant result between Group A (Fertile) and C (Secondary infertile) and between Group B (Primary infertile) and C (Secondary infertile) with p-value <0.05, Mann Whitney U test. Group C (Secondary infertile) showed more epithelial cell abnormalities as compared to the rest of the 2 groups.

Table-1: Grading criteria

S.No	Cyto-morphologic feature	Grading			
		0	1 (Mild)	2 (Moderate)	3 (Marked)
A	**Qualitative**				
1	**Nuclear outline**	Regular	Irregular	Irregular	Irregular
2	**Chromatin distribution**	Fine	Irregular	Irregular	Irregular
3	**Cytoplasm density**	Eosinophilic	Orangeophilic	Amphophilic	Cyanophilic
4	**Hyperchromasia**	Normochromic	Mild	Moderate	Marked
B	**Quantitative**				
1	**Nuclear diameter**	7–8 µm	>1–2 times	>2–3 times	>3 times
2	**Cell diameter**	40–60 µm	>60 µm	25–30µm	<25 µm
3	**N:C ratio**	<25%	25–33%	33–50%	>50%

Table-2: Grading distribution of epithelial cell abnormalities

Cyto-morphologic feature	Grading n=150			
	0	1 (Mild)	2 (Moderate)	3 (Marked)
Qualitative				
Nuclear outline	142	5	1	2
Chromatin distribution	142	5	1	2
Cytoplasm density	142	5	1	2
Hyperchromasia	142	5	2	1
Quantitative				
Nuclear diameter	142	1	7	0
Cell diameter	144	3	2	1
N:C ratio	142	4	1	3

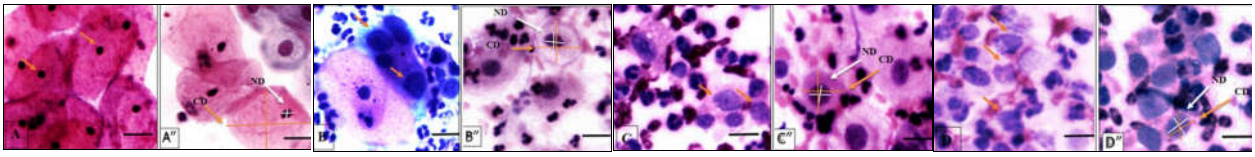

Figure-1: Cervical smears.

A, B, C, D: Cervical smears showing abnormalities in nuclear outline, chromatin distribution, hyperchromasia and cytoplasmic density of squamous cells shown by arrows. A) Normal intermediate squamous cells B) Mild changes. C) Moderate changes D) Severe changes (Scale 25 µm) (100X). A", B", C" and D": Cervical smear with comparison of microscopic quantitative variables (SND= Squamous Nucleus Diameter, SCD= Squamous Cell Diameter, N:C=Nucleus Cytoplasm ratio)

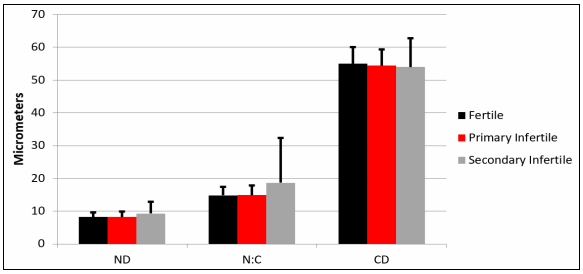

Figure-2: Microscopic quantitative variables

(ND= Nucleus Diameter, CD= Cell Diameter, N:C=Nucleus Cytoplasm ratio) in all 3 Groups. *p*-value >0.05, One way ANOVA, Post hoc Tukey

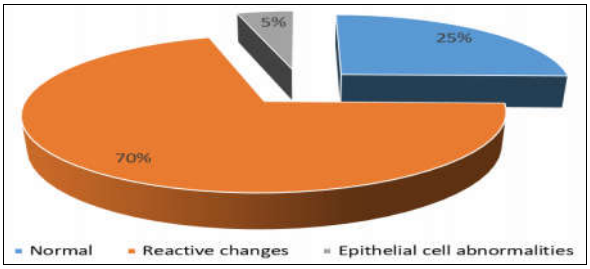

Figure-3: Pattern of distribution of cervical epithelial cell morphology.

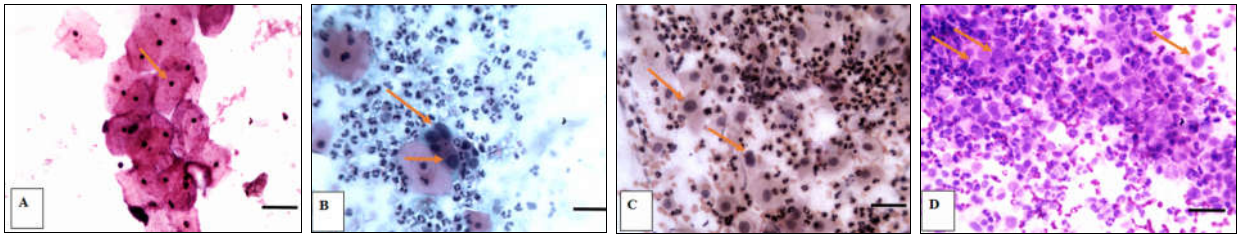

Figure 4: Cervical Epithelial Lesions

A) Normal intermediate squamous cells **B)** Atypical intermediate cells (ASCUS)
C) Isolated atypical intermediate cells (ASC-H) **D)** Changes of Squamous Cell Carcinoma (SCC), Arrows showing nuclear difference in size (Scale 50µm, 40X)

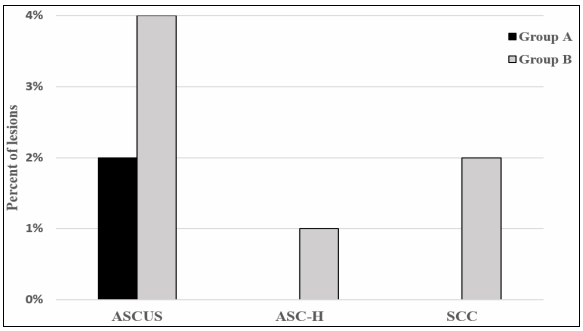

Figure-5: Comparison of various grades (ASCUS=Atypical Squamous Cells of Undetermined Significance,
ASC-H= Atypical Squamous Cells which cannot exclude high grade lesion, SCC= Squamous Cell Carcinoma) of epithelial cell lesions in study groups; (Group A=Fertile, Group B= Infertile)

DISCUSSION

PAP smear is a relatively simple tool to determine cellular changes in the transitional zone of the cervix, strongly advocated as screening for cervical cancers. Grading of these smears via Bethesda we go a step further to identify other cellular changes. Thus, the surmise of this study was to identify changes in the cervical epithelium in infertile women and as a part of the protocol towards infertility treatment.

Results of our study revealed significant abnormalities in cervical epithelium in infertile group as measured by using Revised Bethesda grading system (~5:1). Studies across the world have identified cervical epithelial lesions in their populations mostly as pre-requisite for in-vitro fertilization (IVF).[6,12–16] A study performed by Almobarak AO et al, in 2013[6] observed abnormal cervical cytology in a ratio of ~ 2:1 between infertile and fertile women. The difference maybe because our study was specifically trargetted towards the reproductive age and Almobarak *et al* had a wider range including post-menopausal (20-60 years).

Other studies for example a cross sectional hospital based study conducted by Al-Jaroudi *et al*[12], in sub-fertile women of Saudi Arabia identified abnormal findings in cervical cytology in almost 29.5% population of sub-fertile women. The main reason of relatively increased rate of cervical lesions is that they took a larger sample size of infertile women and used a more the liquid-based pap cytology rather than using the classical conventional method of Pap smear. However, they did not compare these lesions with a similarly matched control group of fertile women. Similar abnormal findings were observed by Abdull Gaffar *et al* in 2009[13] who also used thin preparation liquid-based Pap Tests in a retrospective case control study.

Almost consistent results were shown by van Hamont D *et al*, in 2006[14], who observed abnormal cervical cytology at a more frequent rate in patients (women who were seeking in-vitro fertilization (IVF) treatment when compared with controls. Controls were the women taken from the general population enrolled in cervical screening programs, but the incidence of cervical epithelial abnormality was quite low (i.e., ~2:1). Although they also used the liquid-based cytology but the main reason of their lower rate may be that a different cytological system of classification. They classified abnormal cervical cytology using Dutch CISOE-A classification instead of using the more valid and updated revised Bethesda system of cervical cytology classification. The Dutch CISOE-A classification differentiates cervical smear findings into normal cytology, borderline cytology with mild nuclear changes, mild changes of dyskaryosis, moderate dyskaryotic changes, marked dyskaryosis, carcinoma in situ and invasive carcinoma. According to this system of classification borderline nuclear changes are not included in abnormal lesions which are otherwise classified as ASCUS under the category of epithelial cell lesions in revised Bethesda classification.

Furthermore some studies showed contrasting results.[17,18] Lundqvist *et al* in 2002,[17] when studying the significant role of cytologic screening and human papilloma virus test in women receiving artificial fertilization observed abnormal cytology at a lower rate in infertile women admitted for IVF than in healthy control women from a screening program (~1:2). One possibility of these contrasting results may be that the sample size taken was too small and age was not matched between the cases and controls. The cases were in the age group of 20–40 years, while the controls were aged 25–60 years. In contrast to our findings Mbazor JO *et al*[18] who was working on the cervical cytology of infertility population in Nigeria also reported that incidence of abnormal cervical lesions among healthy women (controls) was more than in infertile population (17.7% versus 14.9%). Although the difference was quite small but the probable reason of this higher prevalence in fertile women may be because of the cultural variations of multiple sexual partners.

Further analysis of cervical epithelial cell lesions was done according to the Bethesda classification system revised in 2001. In our study we divided these lesions into 3 grades, i.e., ASCUS, ASC-H and SCC according to their severity. Higher grade squamous intraepithelial lesions were observed more frequently in infertile patients. Our findings suggest that ASCUS was the common lesion which was observed at a higher rate in infertile women.

Infertile women showed higher grades of cervical lesions. We could not find any study which has specifically reported the prevalence of ASCUS or other high grade lesions in infertile women. Though van Hamont D *et al*[14] in their study observed severe dyskaryosis in infertile population.

CONCLUSION

We conclude that cervical epithelial cell abnormalities are found significantly more often in women with infertility as compared with fertile women of similar age and demographic background. On the other hand, women with secondary infertility had a more ominous incidence of squamous intraepithelial lesions than women with primary infertility.

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AUTHORS' CONTRIBUTION

SJ: Acquisition of data, analysis, drafting, critique. ZH: Concept and design, data analysis, interpretation of data, drafting, review and supervision, final approval. AS: Acquisition and interpretation of data, review. NH: Data analysis, review and critique. FS: Data analysis, drafting, proof reading. NM: Data analysis, proof reading, experiments.

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