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A Cytomorphometric study of Cervical Cancer and Pre-cancer with special reference to West Bengal

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Abstract: *The purpose of this study was to measure some cytomorphometric parameters in different female uterine cervical conditions to study the progression of pathology from normal to neoplastic conditions. Using a computerised cytomorphometry cell measurement pro- gram, the study was based on a cross sectional study of smear cases diagnosed with different uterine cervical conditions from 2014 to 2015.From different kinds of microscopic image (Differential Interference contrast, Phase contrast, bright field microscopy) 100 pictures of normal cells and 100 pictures of pathological cells are selected. Then some cytoplasmic and nuclear parameters(Area, Perimeter, Convex area, Eccentricity, Major axis length, Minor axis length, Equivalent diameter, Compactness, Mean RGB value, Minimum RGB value, Maximum RGB value, Standard deviation of RGB value, Entropy of RGB value, Cooc features (Stats, contrast, stats, correlation, stats, energy, stats, homogeneity) are assessed. Among the cytoplasmic features area is lowest in dysplasia and highest in neoplasia, major axis length is lowest in dysplasia and highest in hypertrophied cervix, minor axis length is lowest in dysplasia and highest in hypertrophied cervix, Equivalent diameter is lowest in cervicitis and highest in neoplasia. Among the nuclear features area is lowest in hypertrophied cervix and highest in neoplasia, major axis length is lowest in cervicitis and highest in neoplasia, minor axis length is lowest in cervicitis and highest in neoplasia, Equivalent diameter is lowest in cervicitis and highest in neoplasia.*

I. Introduction

Cervical cancer (CC) is the 2nd most common cause of cancer death among women globally. Worldwide, out of 7.6 million cancer deaths, 3.6% was due to CC in 2008 and out of 12.7 million new cancer cases, 4.2% were CC in 2008 and 84% of it occurs in less developed countries.**(1)**

One of the most important achievements of modern medicine was to understand the pathogenesis of cervical cancer, that is its development from precursor lesions, the cervical intraepithelial neoplasia, CIN. Precursor lesions occur almost exclusive in women that had sexual relationships, the association between cervical carcinoma and sexual activity being known for more than 150 years.

Sexual life and promiscuity are markers of high relative risk for causing cervical lesions by the increased risk of achieving an oncogenic factor,(Human Papillomavirus, HPV) in the majority of cases.

It should be mentioned that 80% of cervical cancers occur in women from underdeveloped countries. Screening programs using Papanicolaou (Pap) smear test have reduced significantly the number of invasive cancers, by early diagnosis and treatment of precancerous lesions, the cervical intraepithelial neoplasia, CIN.

The period of time necessary for an intraepithelial lesion to transform into an invasive carcinoma varies from a few months to a few years, depending on the tumors aggressivity and the host specific resistance. Due to the slow progression of this disease, cervical cancer may be diagnosed in early stages of intraepithelial lesion.

When precancerous lesions are diagnosed before their progression towards a cancerous lesion, by applying early therapeutical measures adequate for each stage, the rate of survival may reach almost 100%. Thus, prognosis in invasive cancer depends on the stage of diagnosed precancerous lesion.

Anatomo-pathological studies, through histopathological and morphological methods still have an important role in establishing the prognostic factors in cervical cancer, and implicit decisive role in developing early diagnosis methods for an efficient treatment of cervical cancer.

In this context, for histopathological evaluation of cervical tumors a series of quantitative pathology techniques have been used, like morphometric and stereological measurements, that together with conventional, histopathological or semiquantitative (grading), descriptive methods permitted an approach of these prognostic factors.**(2)**

.Cytomorphometry is a quantitative technique to measure the cells of interest in different conditions. It is useful to classify the different disease condition by calculating different cellular parameters e.g. Area, Perimeter, Convex area, Eccentricity, Major axis length, Minor axis length, Equivalent diameter, Compactness, Mean RGB value, Minimum RGB value, Maximum RGB value, Standard deviation of RGB value, Entropy of RGB value, Cooc features (Stats, contrast, stats, correlation, stats, energy ,stats, homogeneity). Here it is done on cervical smear through DIC, Phase contrast, Bright field microscopy of PAP stain to understand the progression of pathology from normal to neoplastic condition (e.g. normal, cervicitis, hypertrophied cervix, dysplasia, neoplasia etc.).

II. Materials & Methods

2.1. Polysol Preservation Solution Preparation:

Polysol is a preservative solution which is used for preserving the biological sample. Here We have used the following ingredients(NaCl-4.96 gm, sodium acetate-7.48 gm,KCl-0.74 gm,calcium chloride-0.368 gm,magnesium chloride-0.305 gm). These ingredients were dissolved into 1 litre of distilled water and a small portion of liquefied phenol was dissolved into it. Polysol has pH of 7.4 . (3)

2.2. Sample Collection Method:

Sample was collected from 30 outdoor patients in NRS medical college and hospital. Patient was in the lithotomy position Sample was collected by circular motion of tip of Ayre's spatula and the cells from each patient were dissolved into 5 ml. of polysol solution in a Eppendorf's tube. Then sample was carried to IIEST,Shibpur and DIC and Phase contrast microscopy was done.

2.3. Monolayer Preparation:

Monolayer was prepared by giving a drop of sample on a cover slip and spread the drop gently.

2.4. Dic And Phase Contrast Microscopy :

Both were done in the IIEST, Shibpur

Principle of DIC Microscopy: Differential interference contrast (DIC) microscopy, also known as Nomarski Interference Contrast (NIC) or Nomarski microscopy, is an optical microscopy illumination technique used to enhance the contrast in unstained, transparent samples. DIC works on the principle of interferometry to gain information about the optical path length of the sample, to see otherwise invisible features.

DIC works by separating a polarized light source into two orthogonally polarized mutually coherent parts which are spatially displaced (sheared) at the sample plane, and recombined before observation. In our study, DIC was done with 10x and 20x magnification.**(4)**

Principle Of Phase Contrast Microscopy: Phase contrast microscopy is an optical microscopy technique that converts phase shifts in light passing through a transparent specimen to brightness changes in the image.

When light waves travel through a medium other than vacuum, interaction with the medium causes the wave amplitude and phase to change in a manner dependent on properties of the medium. Changes in amplitude(brightness) arise from the scattering and absorption of light, which is often wavelength dependent and may give rise to colors. Photographic equipment and the human eye are only sensitive to amplitude variations. Without special arrangements, phase changes are therefore invisible. Yet, phase changes often carry important information.

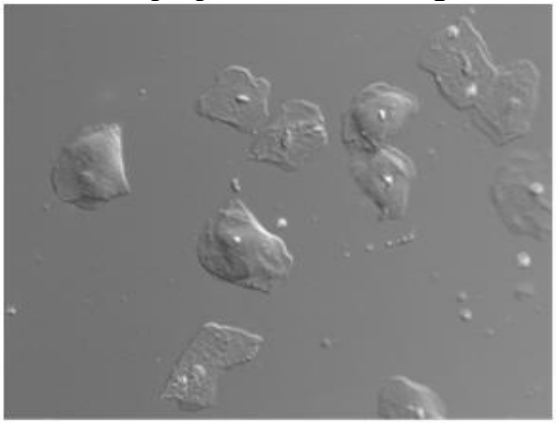
Phase contrast microscopy is particularly important in biology. It reveals many cellular structures that are not visible with a simpler bright field microscope, as exemplified in Figure 1. These structures were made visible to earlier microscopists by staining, but this required additional preparation and killed the cells. The phase contrast microscope made it possible for biologists to study living cells and how they proliferate through cell division. After its invention in the early 1930s, phase contrast microscopy proved to be such an advancement in microscopy, that its inventor Frits Zernike was awarded the Nobel prize (physics) in 1953.In our study, phase contrast was done in 20x magnification.**(5)**

2.5. Method For Pap Staining :

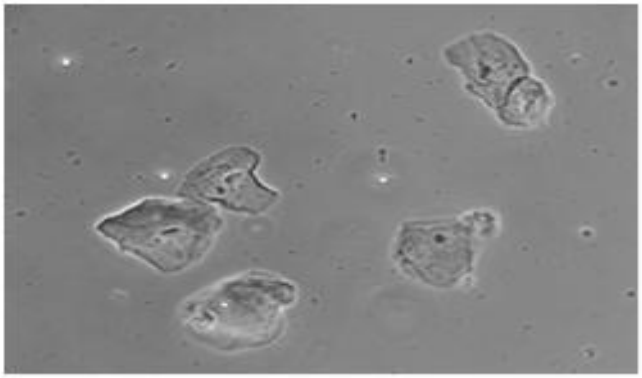
5 ml. of polysol containing cervical cells was taken for exfoliative cytology.Sample is centrifuged. Supernatant is discarded and some amount of polysol is given to it.Vortexing and Funneling is done again. 500 microlitre of sample was given into funnel and again centrifugation is done .Supernatant is discarded and fixation was done for 10 minutes with 95% propanol.supernatant was discarded and slide was put into alcohol**.**Slide was airdried.Isopropyl alcohol was given into the slide and it was kept for 10-15 minutes.Alcohol washing was done.Sample was washed under tap water for 1-2 minutes**.**Harris haema stain was given to sample and kept for 2 mnts.Washing was done with tap water for 3-5 minutes.Slide was rinsed with 1% acid alcohol. Again washing was done with tap water for 5-15 mnts.OG-6 was given for 2 mnts. and water-washing was done. Isopropyl alcohol was given for 2 mnts. and water-washing was done. EA-50 was given for 4 mnts. and water-washing was done.Isopropyl alcohol was given for 2-3 mnts. water-washing was done. Sample was dried and Xylene was given to it**.**Sample was dried and mounting was done with DPX. Sample was examined under Microscope.**(6)**

2.6. Principle of Bright Field Microscopy : Bright-field microscopy is the simplest of all the optical microscopy illumination techniques. Sample illumination is transmitted (i.e., illuminated from below and observed from above) white light and contrast in the sample is caused by absorbance of some of the transmitted light in dense areas of the sample. Bright-field microscopy is the simplest of a range of techniques used for illumination of samples in light microscopes and its simplicity makes it a popular technique. The typical appearance of a bright-field microscopy image is a dark sample on a bright background, hence the name.**(7)**

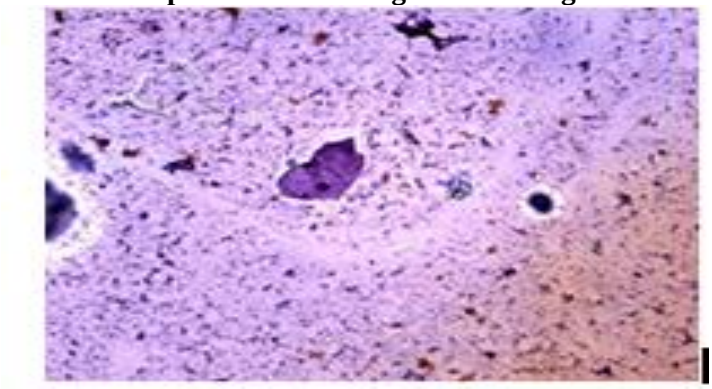
Sample picture of DIC image



Sample Picture Of Phase Contrast Image :



Sample Picture Of Bright Field Image:



2.7. Diagnosis Of Disease From Image:

Diagnosis was made for the DIC, phase contrast, bright field image of pap stained smear by expert cytopathologists.

2.8. Image Analysis :

100 images of normal smear and 100 images of pathological smear are selected. Part of images containing cells of interest were cut out by microsoft paint and they were named accordingly(cell-normal-1,cell-cervicitis-1,cell-hypertrophied cervix-1,cell-dysplasia-1,cell-neoplasia-1 etc.). Pixel values of the region of interest were calculated and they were recorded in a file. Cells of interest are segmented by watershed segmentation and some cellular parameters(area,convex area, perimeter,major axis length,minor axis length,equivalent diameter etc.) were calculated in pixel values. Pixel values are converted into micrometer unit using the above mentioned file after correlating the data with conventional medical knowledge .

III. Result and Discussion

We have got the following results. It will describe the numerical values of cellular parameters for different diseases. Area and convex area will be expressed in square of micrometer unit. Perimeter,major axis length,minor axis length,equivalent diameter will be expressed in micrometer unit.

Chart 1: Cytoplasmic areas for different cervical conditions(in μm^2)

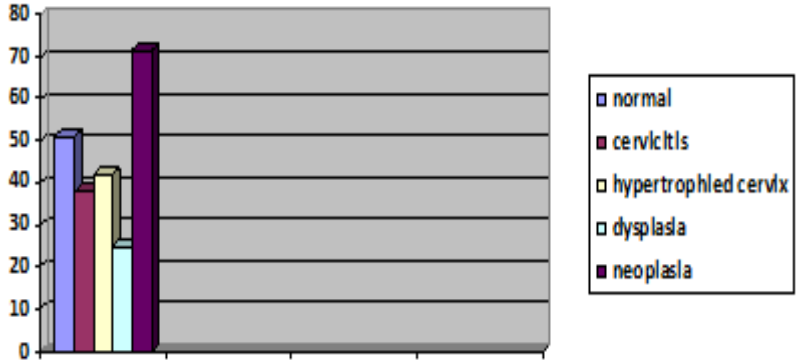


Chart 2: Nuclear areas for different cervical conditions(in μm^2)

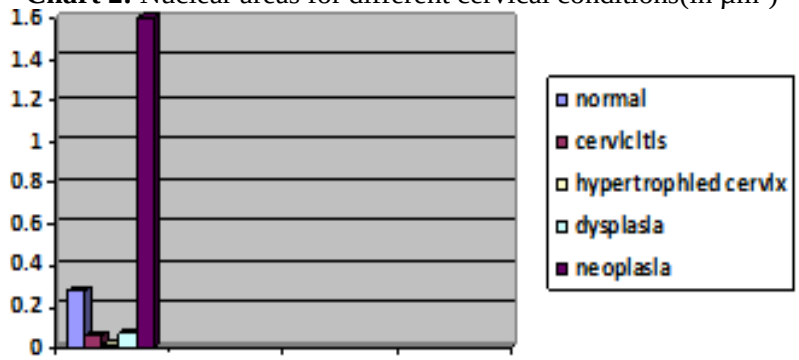


Chart 3: cytoplasmic convex areas for different cervical conditions(in μm^2)

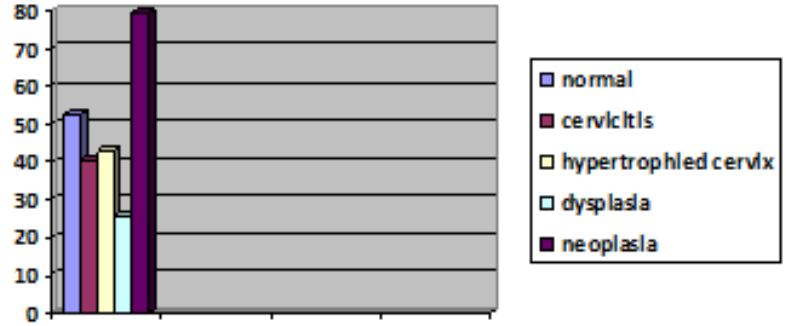


Chart 4: Nuclear convex areas for different cervical conditions(in μm^2)

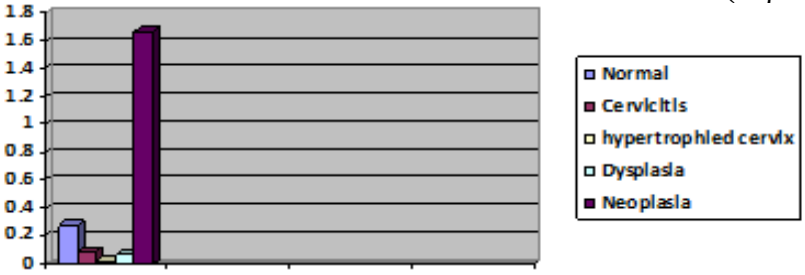


Chart 5: Cytoplasmic perimeters for different cervical conditions(in µm):

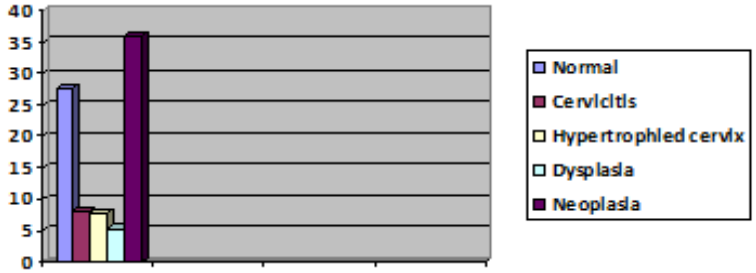


Chart 6: Nuclear perimeters for different cervical conditions(in µm):

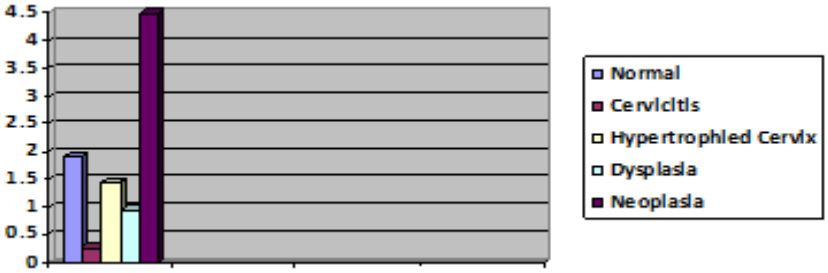


Chart 7 : Cytoplasmic major axis lengths for different cervical conditions(in µm) :

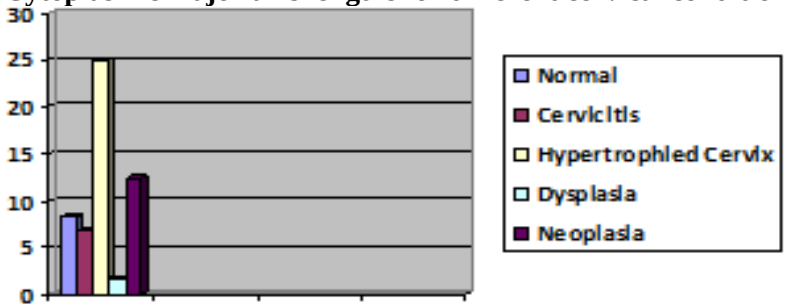


Chart 8: Nuclear major axis lengths for different cervical conditions(in µm)

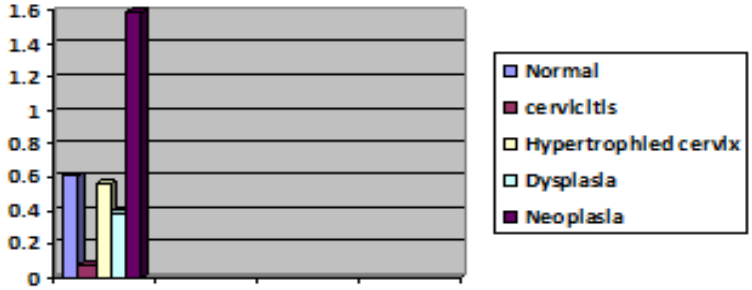


Chart 9: Cytoplasmic minor axis lengths for different cervical conditions(in µm):

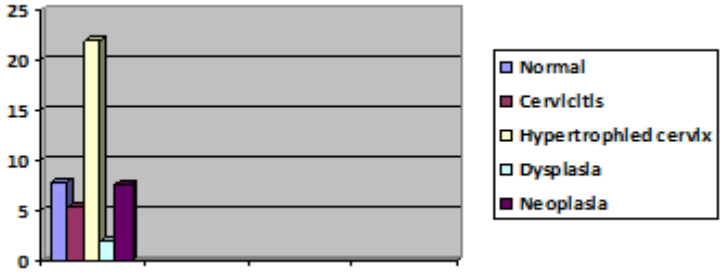


Chart 10: Nuclear minor axis lengths for different cervical conditions(in µm):

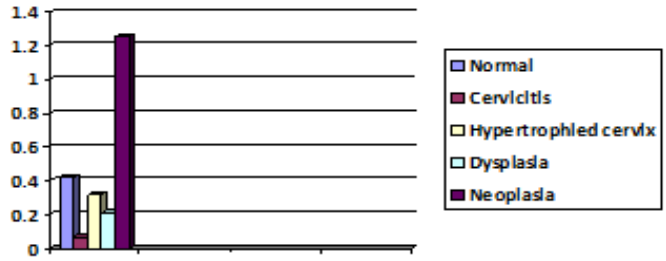


Chart 11: Cytoplasmic equivalent diameters for different cervical conditions(in µm) :

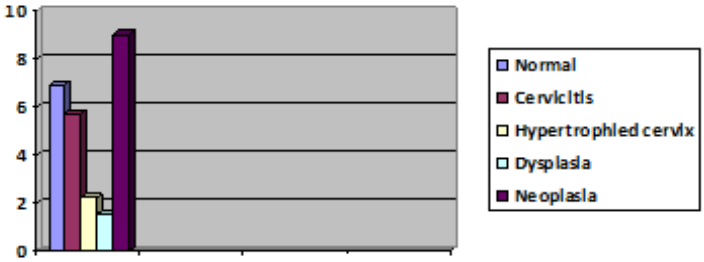
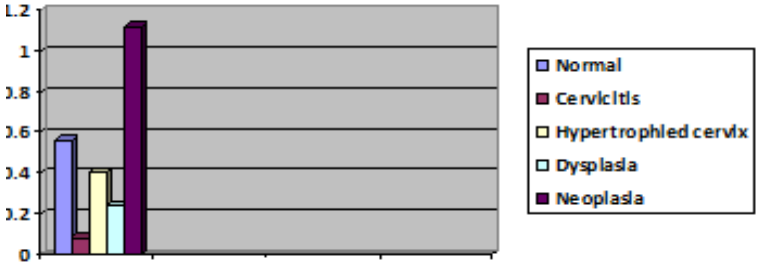


Chart 12: Nuclear equivalent diameters for different cervical conditions(in µm):



IV. Conclusion

Based on the above calculation, we will summarise the cytomorphometric parameters by the following range. Area and convex area will be expressed in square of micrometer unit.Perimeter,major axis length,minor axis length,equivalent diameter will be expressed in micrometer unit.

Parameters concerning with the range	Cytoplasmic range	Nuclear range
Area(µm^2)	25-71.88	0.014-1.6
Convex area(µm^2)	25.7-79.53	0.0145-1.67
Perimeter(µm)	5.27-36.02	0.27-4.49
major axis length(µm)	1.66-25.01	0.08-1.59
Minor axis length(µm)	1.93-21.9	0.069—1.25
Equivalent diameter(µm)	0.57-9.04	0.08-1.12

Inference On Cytoplasmic Range :

Areas,convex areas and perimeters are lowest in dysplasia, highest in neoplasia.Major and minor axis lengths are lowest in dysplasia, highest in hypertrophied cervix. Equivalent diameter is lowest in cervicitis, highest in neoplasia.

Inference On Nuclear Range :

Areas and convex areas are lowest in hypertrophied cervix, highest in neoplasia. Perimeter is lowest in cervicitis, highest in neoplasia. Major axis lengths,minor axis lengths and equivalent diameters are lowest in cervicitis, highest in neoplasia.

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