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A VISUAL PERCEPTION SURVEY: SEEING EYE-TO-EYE AT NUCLEAR CHROMATIN OF NON-NEOPLASTIC CERVICAL SQUAMOUS EPITHELIAL CELLS

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ABSTRACT

OBJECTIVES: Subjective judgement of individual pathologists in visual perception of nuclear chromatin of cervical squamous epithelial cells is well known. Nonetheless, chromatin pattern of cervical squamous epithelial cell forms one of the diagnostic criteria in determining whether such cells have undergone neoplastic transformation. In such background, this study investigates agreement among pathologists regarding visual perception of nuclear chromatin of non-neoplastic cervical squamous epithelial cells. The findings would build a rational basis for future improvement in the diagnostic criteria of cervical Pap smear test. METHODS: A survey of 20 non-neoplastic cervical squamous epithelial cell images captured from Thinprep slides with chromatin regions detected at 5 sensitivity levels by Fuzzy C-Means clustering technique was constructed. This survey was distributed to 10 pathologists. Cohen's and Fleiss' Kappa Tests were performed to investigate inter-observer agreement on the sensitivity levels that best represent the visual perception of the chromatin of each image. RESULTS: Agreement between every two pathologists ranges from poor to moderate (Cohen's Kappa values less than 0 to 0.43). The overall agreement among ten pathologists is poor with Fleiss' Kappa value=-0.0163. The grand mean sensitivity level for the chromatin detection is 3.725, with the standard deviation of 0.378. CONCLUSIONS: Agreement between every two pathologists in perceiving the chromatin of non-neoplastic cervical squamous epithelial cells is fairly low. Nonetheless, on average, the sensitivity level 4 represents the most sufficient level of chromatin detection among all pathologists. This sensitivity level 4 could be set as the optimum level for algorithmic comparison between non-neoplastic versus neoplastic cells in future work.

Keywords: chromatin, non-neoplastic cervical squamous epithelial cell, pathologists, kappa test, thinprep

INTRODUCTION

Papanicolaou-smear test (Pap test) enables early detection of cervical cancer since the screening detects abnormal cells that may progress into cancer if left untreated. As a result, the introduction of Pap-smear screening has significantly reduced the mortality rate due to cervical cancer [1-3].A Pap test result is reported according to the Bethesda System for Reporting Cervical Cytology [4], a worldwide recognised reporting standard. Pathologists or cytotechnologists observe the changes in the morphology of the cell nucleus under light microscope because cancerous cells often displayed characteristics known as malignancy-associated changes (MACs). Changes in chromatin pattern are recognised as one of the MACs [5-8]. For a negative Pap test result, which is reported as negative for intraepithelial lesion or malignancy (NILM), the nuclei of the cervical squamous epithelial cells have been defined as having evenly distributed, finely granular chromatin [9-11].

Before embarking on the effort to elucidate the various chromatin patterns and the ambiguity of their qualitative description in the non-neoplastic cells and neoplastic cells, it is of paramount importance to address the very fundamental issue of the variation among pathologists in perceiving the chromatin itself. It is well known that pathologists perceive the number of chromatin differently, considering that individual pathologists might have different visual acuity.

In such background, this survey investigates the variation among pathologists' agreement on perceiving the number of chromatin. The chromatin in the cervical squamous epithelial cell nuclei was shown at five sensitivity levels, representing five different degree of chromatin detection which may be perceived by pathologists. In addition the average sensitivity level was computed as the best representative sensitivity level for chromatin detection. This sensitivity level would serve as the basis for future work on low- and high-grade squamous intraepithelial lesion.

METHODOLOGY

Acquisition of cervical squamous epithelial cells

The study was approved by the Human Research Ethics Committee of Universiti Sains Malaysia. The ThinPrep slides obtained from Penang General Hospital, Malaysia, had been previously screened by cytotechnologists and formally reported as "negative for intraepithelial lesion or malignancy" by pathologists. Using an Olympus BX43F clinical microscope mounted with a video camera, a pathologist reviewed these slides and captured images of the nucleus of cervical squamous epithelial cell, zooming at 100x objective together with oil immersion.

Processing of cervical squamous epithelial cell images

The single cell images were captured at the size of 2048 x 1536 pixels. The nucleus was manually cropped and the cropped images have constant size of 500 x 500 pixels. The colour nucleus image was further converted into gray scale image and the contrast of the image was enhanced through histogram stretching. Fuzzy C-Means clustering technique [12] is employed to segment the chromatin. The peak of the histogram representing the nucleus region was set as the number of cluster. Intensities of the segmented images were sorted in ascending order. The five smallest intensities were used as the threshold values.

Survey form

Ten pathologists with 8 of them have working experience from 1 to 5 years and 2 of them with more than 5 years working experience participated in the survey. Pathologists were blinded to patient identity, i.e. no information regarding patient history was provided with the survey. The feedback was collected in June 2015. The survey contained a total of 20 questions. Each question had five sensitivity levels of chromatin detection. Pathologists were instructed to independently select the images which best suit the chromatin as perceived. Examples of two survey questions are demonstrated in Figure-1.

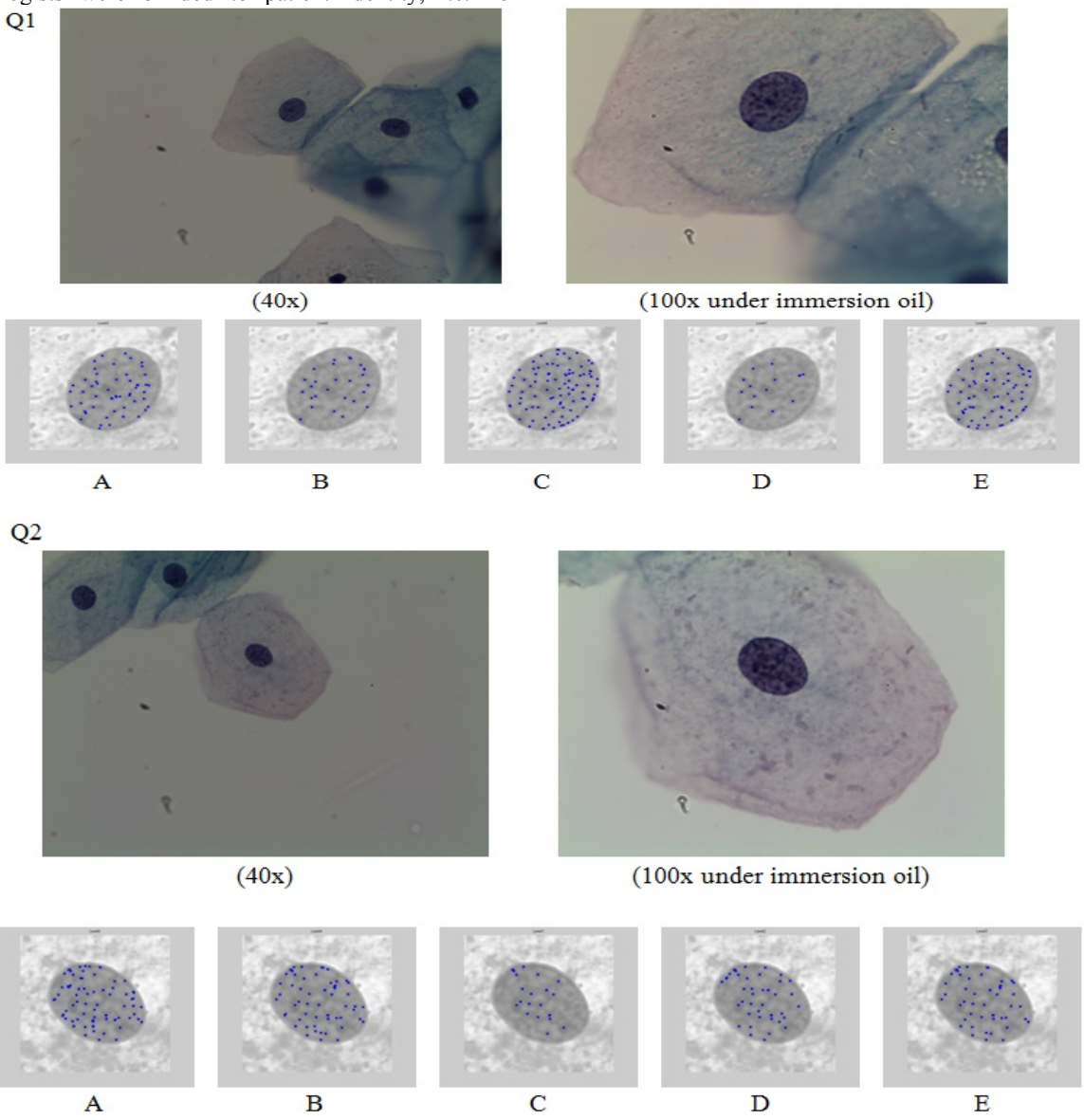

Figure-1. Two sample questions in the survey form.

Every image has different threshold value for each sensitivity level. Choices from A to E were generated from the threshold values of the segmented image. They represented different amount of chromatin detection,

which was termed as 'sensitivity level' in this paper. In order to reduce the tendency of bias in selecting the images at a certain sensitivity level, the sequence of the images for each question was randomized, i.e. choice A might not correspond to sensitivity level 1. Besides, to distinctly show the distribution of chromatin, the contrast of the image was adjusted. This further reduced chances of bias by pathologists. Instead of judging whether the blue dots plotted by the computer correctly matched the chromatin, the pathologists were urged to choose the sensitivity level which best matched the distribution as perceived by their eyes.

RESULT AND DISCUSSIONS

This study investigates whether there are variations among pathologists in detecting the chromatin. Kappa test is performed to compute the agreement among pathologists. Furthermore, from the twenty test images, the chosen sensitivity levels will be analysed for the best representative sensitivity level.

The Cohen's Kappa coefficient, κ , is computed to measure the inter-rater agreement [13]. It is preferable to use Weighted Kappa [14] since the sensitivity levels are ordered. The linear weighting function takes into account the degree of disagreement, e.g. the degree of disagreement is more severe when the same image is chosen at sensitivity levels of 1 and 5 by two pathologists than when sensitivity levels of 1 and 2 are selected for the same image. The lower and upper limits of are -1.00 and +1.00 respectively. Interpretation of κ is shown in Table-1 [15]. Weighted Kappa coefficient between every pair of pathologists is shown in Table-2, which measures agreements between two pathologists. Fleiss' Kappa is computed for overall agreements between all the pathologists.

From Table-2, the Kappa coefficients between all the pathologist pairs ranged from negative value to 0.43, indicating that the degree of agreement among pathologists varied from poor agreement to moderate agreement. Fleiss' Kappa revealed poor agreement in overall, with the value of -0.0163 [95% confidence interval, -0.0256 to -0.0069]. From both the Weighted Kappa and Fleiss' Kappa tests, it can be concluded that there is little agreement among the pathologists on how they perceive chromatin.

The distribution of the sensitivity levels is demonstrated by bar chart in Figure-2. The boxplot of the chosen sensitivity levels for each image are demonstrated in Figure-3. The grand mean sensitivity level is computed and shown as the final plot (i.e. labelled as 'GM') on the right side in Figure-3.

From Figure-2, it can be noticed that sensitivity levels 3 to 5 are the mostly selected levels by pathologists. Sensitivity level 1 appeared to be least chosen level. The interquartile range varies for all the images as demonstrated in Figure-3, revealing that the sensitivity levels selected varies for each image. The median value of the selected sensitivity levels lay within 3 and 5. The grand mean sensitivity level, shown as the final plot, has small interquartile range, indicating that the mean sensitivity levels for all the images are not far from each other. The mean sensitivity levels for all the images fall within the range of 3 and 4.5, which are similar to the median sensitivity levels. The grand mean sensitivity level computed is 3.725, with the standard deviation of 0.378.

Table-1. Interpretation of Kappa coefficient, κ.

κ	Interpretation
<0	poor agreement
0.0-0.20	slight agreement
0.21-0.40	fair agreement
0.41-0.60	moderate agreement
0.61-0.80	substantial agreement
0.81-1	almost perfect agreement

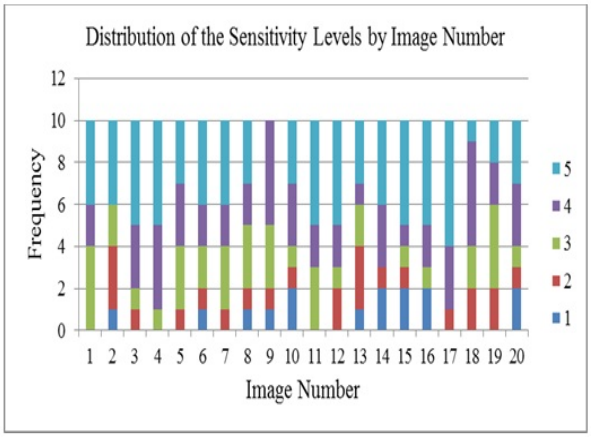

Figure-2. Frequency of the selected sensitivity levels.

Table-2. Linearly weighted Kappa test between all pair combinations of pathologists.

	Pathologist										
Pathologist		1	2	3	4	5	6	7	8	9	10
	1		0.18	<0	0.11	0.05	0.03	<0	<0	0.09	0.05
	2			<0	0.03	0.18	0.13	<0	<0	<0	0.01
	3				0.02	0.43	<0	0.13	0.15	0.03	<0
	4					<0	<0	0.19	0.09	0.08	<0
	5						0.08	<0	0.08	<0	<0
	6							<0	<0	0.10	<0
	7								0.23	0.10	0.04
	8									0.10	<0
	9										0.09

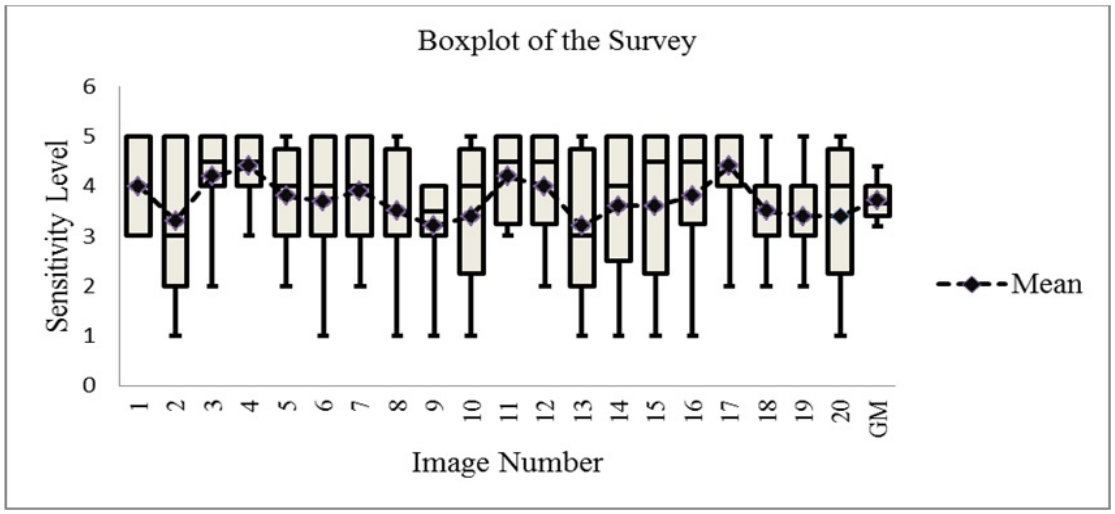

Figure-3. Boxplot of the survey. Label 'GM' stands for grand mean.

Changes in the chromatin pattern are recognised as one of the diagnostic criteria in the Bethesda System for Reporting Cervical Cytology for precancerous and cancerous diagnostic categories [4]. The importance of chromatin pattern in diagnostic of cervical cancer is proven by literatures [16-19]. Nonetheless, the criteria are mostly qualitative in nature. Variation in pathologists or cytotechnologists in observing the slides further devotes to discrepancies between individual pathologists or cytotechnologists and hence results in possibility of different diagnostic judgement [20-23].

However, the above mentioned literatures only highlighted the fact that different pathologists might draw different diagnostic conclusions due to ambiguity of the descriptive terms in the criteria (i.e. homogeneity, clumping and granularity). Little studies (if any) take into consideration on the issue of different judgments by individual pathologist or cytotechnologist due to different sensitivity levels in visual perception of chromatin detection, which is the very first level of discrepancy before reaching a diagnostic conclusion.

Example of survey questions as shown in Figure-1revealed that the detection of chromatin can be presented at different sensitivity levels since there is no precise term to describe the exact intensity level for chromatin. The five different sensitivity levels represent the potential view of individual pathologist in detecting chromatin, which successfully imitate different sensitivity abilities of pathologists.

Poor agreement among pathologists proved that variation did occur in pathologists when perceiving the number of chromatin. Nonetheless, variation in chromatin detection does not suggest different diagnostic judgement from pathologist. The survey is not focusing on how pathologists define the chromatin pattern, such as evenness or coarseness. It is emphasizing on the variation of chromatin detection by individual pathologists or cytotechnologists. Level 4 is found to be the best representative sensitivity level. Finding from this study establishes the basic for further study on non-neoplastic cases, i.e. the cases of low- and high-grade squamous intraepithelial lesion.

CONCLUSIONS

A survey containing 20 cervical squamous epithelial cell images with chromatin detected at 5 sensitivity levels was distributed to 10 pathologists to investigate the degree of agreement among the pathologists. Linearly Weighted Kappa test between all pathologist pairs reported a poor to moderate agreement. Fleiss' Kappa test for overall agreement returned a poor agreement result. Variation in chromatin detection does not imply that pathologists will make different diagnostic judgement because the diagnostic criteria related to chromatin pattern are not studied. The best representative sensitivity level is level 4, which could be set as the optimum level for algorithmic comparison between non-neoplastic versus neoplastic cells in future work.

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