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ORIGINAL RESEARCH

Influence of Image Manipulation Restricted to Global Alterations on Diagnosis by an Oral Cytologist

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Abstract

Background: Cytology has always proudly been in forefront to absorb new technologic advances for practical use. The extensive and passionate implementation of digital imaging in telecytology, automated screening, education, training and certification, research, and publications is an appropriate example. Digitalization cytology records make its manipulation very simple. Several such manipulations, however, correspond to inappropriate changes and can affect final diagnosis of digital slides.

Aim: To determine if global alterations of digital oral cytology images can affect its interpretation by an individual.

Methodology: 20 digital images of cytological test smears of normal oral epithelium were acquired and stored in JPEG format. These slides were then manipulated restricted to global alterations (modify brightness, contrast and color levels, without focal changes) and were camouflaged from the original. A single power point presentation of 40 slides was created of group A (original) and group B (manipulated) digital slides, 20 each. This presentation was given to 5 experts for categorization on chart provided to them for easy data compilation. Intra-examiner pretest-post test difference was analysed using Wilcoxon rank sums test.

Results: Out of the 5 examiners, diagnosis of three examiners changed significantly ($p < 0.05$) after slide alteration. Percentage of Over-interpretation ranged from 30-85% and under-interpretation 5-15% from unaltered slides for different examiners.

Conclusion: Study clearly demonstrates that global manipulation of a digital image affects its interpretation and suggests that care needs to be taken when digital cytology images are used, to specifically ensure that their alteration does not affect diagnosis.

Keywords

Digital oral cytology, Image manipulation

Introduction

Digital images have grown to be a significant element of oral cytology practice. They are used extensively in tele-cytology, automated screening, educational material, and Web sites [1,2]. Freely available software allows investigators to tweak their digital images as never before. Digital images can be produced either by merging two or more digital images, or by altering an existing image [3]. But, there is a fine line between acceptable enhancements and scientific delinquency. There have been many cases in the past where manipulated images were utilized unethically, the publications of Hwang, et al. being a case in point [4].

To avoid confusion and misconduct some journals provide guidelines for image submission which clearly states that only global manipulations are allowed for acceptance. Thus present study was designed with an objective to determine if global alteration of digital oral cytology images can affect its interpretation.

Aims and Objective

Aim

To determine if global alterations of digital oral cytology images can affect its interpretation by an individual.

Objective

To assess whether changes made in the brightness, contrast and other globally permissible alterations affects interpretation of digital oral cytology slides of normal oral mucosa by assessing the intra-observer

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variations in interpretation.

Materials and method

The present study was conducted at Department of Oral Pathology and Microbiology, Faculty of Dentistry, Jamia Millia Islamia, New Delhi. The study was cross sectional type, of one month duration and involved procurement of cytological smears from normal oral mucosa of 20 volunteers. The samples were collected from volunteers aged between 18-30 years who had a disease free healthy mouth. Any volunteers with oral habits such as smoke/smokeless tobacco, alcohol, beetle nut, other similar oral abuses were excluded from the study. A written consent form was obtained from the study subjects before sample collection [5].

Conventional cytological smears were collected from the normal oral mucosa of right cheek by gently scraping with a metal spatula and directly smearing on clean glass slide. The smear was fixed using 95% ethyl alcohol and then submitted for routine PAP staining to our cytopathology laboratory. The PAP stained slides of all the twenty smears were focused at 40X magnification and their digital images were captured using a 12 Mega Pixel samsung Techwin Camera. White level balancing of camera was set on auto mode to allow camera to read the slides colour temperature and choose a setting from its collection of pre-programmed adjustments. All the digital images were stored in JPEG (Interchange) Format as it is the most widely used, compatible with most image manipulation softwares, saves memory and does not have many appreciable changes after manipulation. The standard image size of 900 × 400 pixels (width × height) was maintained for all the images. These twenty images were stored under name group A.

Following this, each digital image from group A was then manipulated once using Adobe Photoshop 7.0 (Adobe Systems Inc, San Jose, Calif) by the Principal Investigator (PI). The manipulations were restricted to global alterations as described by Pinco J, et al. that is only the brightness, contrast and colour levels of all elements within the digital image were modified (Table 1). No focal changes were made and before saving the changes each image was rotated by 180°, in order to camouflage it from the original (Figure 1). This set of modified images was saved under the group B.

A total of 40 images comprising of 20 original images (group A) and 20 modified images (group B) were coded by the PI to give each image a specific identity. These 40 coded slides were selected using Random table generator [6] and a single Powe Point (PowerPoint 2003, Microsoft Corp) slide show was created of these 40 images. To preserve the maximum picture quality, picture compression in the PowerPoint presentation was turned off for all pictures.

In the final part of the study the slides for submitted to an expert panel for diagnosis. Expert panel composed of oral pathologist/cytologist from dental colleges of Delhi/NCR with more than 5 years of experience in oral cytology after post-graduation. The slides were administered to the experts on a monitor (with static settings) and no clinical data was shared

Table 1: Manipulations done on the digital images in group B.

Manipulation done	Level of change	Result obtained
Brightness increase	(+50)	To make nuclei appear clearer.
Contrast manipulation	(+50)	To make nuclei darker (hyperchromatic).
Red-green-blue manipulation	• +20 towards Red, blue and green respectively. • Tone Balance (Mid-tones). • Luminosity shall be preserved.	To make squamous cell appear less keratinized along with darker nuclei.

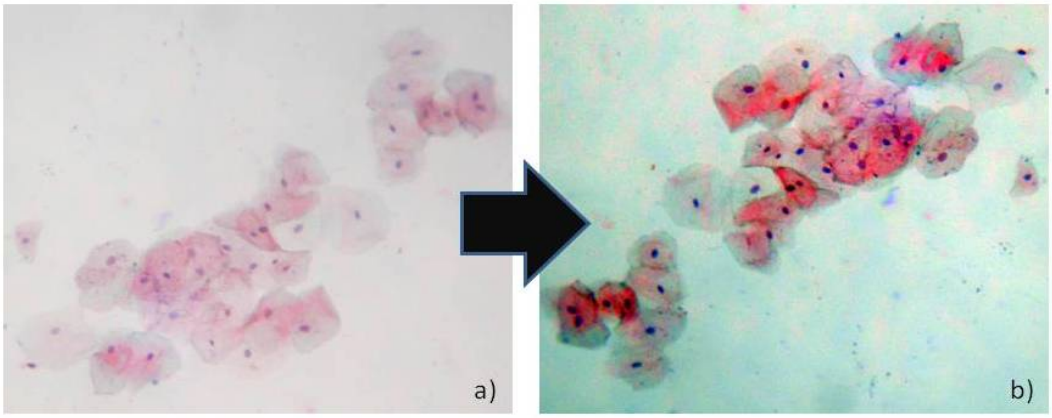

Figure 1: Manipulation of digital image of smear: a) Original image; b) Manipulated image after.

Table 2: Identical, over diagnosed and under diagnosed slides interpreted in group B when compared with group A.

Observer Number	Experience in years after post graduation	Identical interpretation		Over interpreted		Under interpreted		P Value
		Number	Percentage	Number	Percentage	Number	Percentage	
1	10	3	15	17	85	0	0	0.0001[a]
2	27	7	35	11	55	2	10	0.01[b]
3	4	6	30	12	60	2	10	0.009[a]
4	18	12	60	7	35	1	5	0.34[c]
5	6	11	55	6	30	3	15	1[c]
Wilcoxon Ranks Sums Test								

[a]: Highly significant; [b]: Significant; [c]: Not significant.

Table 3: Over diagnosis of original and manipulated slides.

	Original set	Manipulated set	Total
Expert 1	6	20	26
Expert 2	2	11	13
Expert 3	9	20	29
Expert 4	3	6	9
Expert 5	9	11	20

so that the interpretations were without any bias and based purely on the basis of cytological features. Each expert analyzed and interpreted the 40 images in a single sitting. Each expert was instructed to categorize each digital image of smear according to Papanicolaou's classification [7]: Class I (Normal); Class II (atypia); Class III (Intermediate i.e. in-between cytology); Class IV (Suggestive of cancer i.e. border line); Class V (Cancer Positive). The results were then collected from the experts, decoded by the PI and entered in an excel sheet. The data was further analyzed using the SPSS version 21.0. The interpretation for unaltered image was treated as the standard interpretation for that expert and this was then compared to the interpretation of the manipulated corresponding digital image. Wilcoxon rank sums test was used to analyse the differences observed between the original and modified images of slides for each expert. $P < 0.05$ was considered significant.

Results

There was 100% response rate and no column in the chart was left empty by any expert oral pathologist. Number of years of experience after post-graduation of expert panel ranged between 4 to 27 years (n = 5, mean 13 years (SD 9.488). Table 2 summarizes the findings about interpretation by all the experts. The overall over diagnosed slides ranged from 30% to 80%, whereas under diagnosis varied from 5% to 15%. Overall group B images were interpreted as compared to group A images in the order of: over diagnosed (53%) > identical interpretation (39%) > under-diagnosed (8%). Table 3 provides details for amount over diagnosis by each expert. Among the experts, three out of five experts showed statistically significant differences in their interpretation of these digitally modified images.

Discussion

With the widespread and far reaching use digital imaging, more and more pathologists are incorporating digital image data into their practice of medicine [8-10]. There are various easily available software's which are routinely used to achieve cleaner and sharper images. This digital enhancement of images becomes a point of contention as there is a very fine line that delineates acceptable enhancements and digital fraud [3]. The root cause of this debate is the fact that digital imaging and its modifications are being explored extensively but there is paucity of guidelines to standardize the process.

Yagi, et al. have summarized the concepts for pathology imaging standards which encompass a vast area including receiving, preparing and staining the sample, optical image formation, digital image capture by the camera and post processing modifications [11]. Cromey DW proposed 12 guidelines for scientific digital image manipulation with valid reasoning for each of the guidelines and recommended inclusion of these guidelines as part of undergraduate student teaching curriculum in order to sensitize the students about the effects of the so called "digital image beautification" [12]. Indu M, et al. have conducted a survey-based study to assess awareness of Indian oral pathologists about various aspects of medical photography and found out that most students and faculty were using image enhancing softwares but were unaware of their ill effects [13].

Our study demonstrated that performing manipulations limited to adjusting brightness, contrast and colour levels of healthy oral cytology digital images the overall diagnosis was significantly affected. 61% of the slides were either over diagnosed or under diagnosed by the experts. These results are in concordance with earlier work done by Prasad H and Pinco J [14]. Our study reemphasises the need for formulation and implementation of stringent and well formulated guidelines for digital image enhancements, which should be incorporated in regular laboratory meetings and as part of the curriculum in health care institutions.

Conclusion

In conclusion, as our study clearly demonstrates that manipulation of a digital image significantly affects its interpretation. This suggests that care needs to be taken when digital cytology images are used, to specifically ensure that their alteration does not affect diagnosis. Students and researchers should be educated about do's and don'ts of image enhancements. As suggested preventative measures such as attaching metadata and water marking of digital images should be done in order to avert their malicious reuse [3]. Undoubtedly, the entire imaging process in cytology needs to be standardized if digital images are to be fully espoused in the field of diagnostics.

Author Contribution

All the three authors were involved equally in the conceptualization, planning and supervision of work done. All authors discussed the results and contributed to the final manuscript.

Conflict of Interest

None.

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