EXFOLIATIVE CYTOLOGY – A PREDICTIVE DIAGNOSTIC TOOL

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ABSTRACT

Oral mucosa undergoes continuous rapid turnover and this result in exfoliation of cells. These cells play a vital role in pre-diagnostic assays as they reflect the systemic conditions which induce cyto-morphological and nucleo-morphological variations in the cell. Though it was initially used for diagnosis of potentially malignant disorders and other malignancies, its use has extended to diagnose hormonal activities, iron deficiency anemia, diabetes and many more systemic disorders. They are usually directly stained and visualized under a light microscope. However molecular analysis, DNA analysis and other immune histochemical studies are also performed using this technique. Its rapidity, simplicity and specificity makes it a test of high diagnostic value. This review discusses the concepts, historical aspects and recent advancements of exfoliative cytology and its use in clinical scenario.

Keywords: Cytology, Exfoliation, Prediagnosis, Techniques.

INTRODUCTION

Oral mucosa exhibits a rapid turnover of cells and these exfoliated cells have a valuable role in diagnosis of certain local and systemic diseases. Oral cavity reflects the various events occurring in the body and this is reflected by cytomorphological and nucleomorphological variations in the exfoliated cells. Exfoliative cytology is based on the monitoring the exfoliated cells or cells flake off the mucosa wither through natural or artificial means [1].

It is implemented a prediagnostic tool in potentially malignant disorders like leukoplakia and oral cancers chiefly oral squamous cell carcinoma. Recently, its role has extended into predicting systemic conditions like determination of diabetes, iron deficiency anaemia and hormonal changes [2]. Cytological specimens are recently analysed for nuclear DNA content, immunohistochemical tumour cell marker identification and molecular analysis [3]. It is also used in extraction of RNA to which markers are exposed. Epigenetic alterations (promoter hypermethylation), genomic instability and loss of heterozygosity (LOH), microsatellite instability (MSI), and restriction fragment length polymorphism (RFLP) are other molecular markers that are being used [4].

Historical aspects of exfoliative cytology

The history of exfoliative cytology dates back to 1860 when Bhale described the morphology of malignant cells in sputum of oropharyngeal carcinoma [2]. The use of exfoliative cytology was restricted to gynaecological diagnosis. However Papianacolaou and Traut introduced new methods of staining and collection of specimens [5,6]. The use of exfoliative cytology extended into oral cavity when comparative studies were conducted to study the cervical and oral cytology in menstrual cycles [7].

Table 1: Milestones in the development of Oral exfoliative cytology

Authors	Year	Landmarks
Beale	1860	Cytological examination of sputum in a case of pharyngeal carcinoma 1940
Weinmann	1940	Cytological examination of oral cellular keratinization
Ziskin et al.	1941	Effects of the menstrual cycle on oral cellular morphology
Papanicolaou	1942	Introduction of a staining procedure for cytological smears
Papanicolaou andTraut	1943	Cytological diagnosis of uterine cancer
Morrison et al.	1949	Cytological diagnosis of nasopharyngeal malignancies

Table 2: Methodolgies and its modifications of exfoliative cytology

Authors	Year	Landmarks
Gladstone	1951	Improved quantities of obtained cells by use of a ''sponge biopsy''
Schneider	1952	Modifications of staining
Cawson	1960	Modifications of staining
King	1963	Use of frosted glass slides
Staats and Goldsby	1963	Comparison of wooden and metal spatula. Recommendation of the metal spatula
Sandler	1964	Removal of keratotic layers with a sharp curette
Dumbach et al	1981	Smear curettage'. Inclusion of deeper cell layers by use of a curette

Montgomery and Von Haam were the first to examine the application of cytology in oral cavity [8]. Later, various other techniques were applied in the upcoming years to improvise on the quality of smears and to obtain large number of cells [9]. This prompted new sampling tools, modified object slides and staining dyes. Light microscopy was first used to visulize the cytonucleo-morphological changes. With the advent of fluorescent stains, Fluorescent DNA specific dyes such as acridine orange has been used to measure the cellular DNA[10]. Additional parameters like nuclear and cytological morphometry are based on Image amalysis methods [11]. The

sensitivity of cytology was improvised later with modifications in the collecting devices. However, to collect the parabasal cells, a sharper collecting tool was employed to remove the atypical keratotic cell layers. Sharper tools included metal spatula, curette which gave 100% specificity and 76.9 – 95% sensitivity. However it was not a successful method as it had an invasive approach [12].

Weigum and et al have introduced a novel lab-on-a-chip (LOC) sensor technique for analysis of oral cancer biomarkers in exfoliative cytology specimens, targeting both biochemical and morphologic changes associated with early oral tumourigenesis [13]. N.shara et al clarified the histological aspect of human deciduous teeth using exfoliative cytology [14]. Greater understanding of the type of mutation present may, in the future, predict notonly tumour behavior, but also its response to both traditional and novel forms of therapy[15].

Cytological Techniques

A biopsy brush was first used in 1980's to demonstrate cervical smears. This had a better spread of cells on the slide as compared to wooden spatula. Sampling of deeper mucosa with minimal invasion was an added advantage [16]. This classical technique of obtaining cells is used in oral candidiasis, Leucoplakia, Oral carcinoma where adequate saple is required to yield representative findings [17].Scraping was done and directly smeared onto the glass slide and fixed in isopropyl alcohol, before staining with the standard papanicolaou stain for cytological demonstration [18]

The LOC sensor utilizes an embedded track-etched membrane, which functions as a micro-sieve, to capture and enrich cells from brush cytology suspensions. Once captured, immunofluorescent assays reveal the presence and phenotype of interrogated cells via automated microscopy and fluorescent image analysis. Here, the epidermal growth factor receptor (EGFR) biomarker was examined due to its high diagnostic, prognostic, and therapeutic potential, with up to 90% of all oral cancers overexpressing EGFR [13]. Cytological smears were stained by papanicolaou technique using commercially available staining kit RAPIDPAP™(Biolab diagnostics, Tarapur, Maharashtra). The slides were mounted with cover glass using DPX mountant [19].

Liquid Based Cytology

Liquid based cytology is commonly used on oral smears collected by cytobrush to show a significant improvement in smear thickness and cellular distribution which leads to easier identification of abnormal cells [20]. This method of improving cell spreading leads to destruction of epithelial fragments which allows better evaluation of epithelial cell architecture [21]. This method has a sensitivity of 95.1% and specificity of 99% which makes it a technique in demand [22].

Cytomorphometry

Oral CDx presents a computer assisted method for analysis of cellular samples sampled by Brush biopsy technique. It is an oral tranepithelial biopsy system that is used along with computer assisted techniques. This technique improves accuracy of the interpretation as it samples the entire thickness of the epithelium. Computer analysis is based on working of a digital microscopic image of the sampled cells through a specialized neural network based image processing system designed to detect oral precancerous and cancerous cells.Oral CDx have shown a specificity and sensitivity over 90% with greater positive and negative predictive values [23,24].

DNA analysis

DNA image cytometry measures the malignant potential of cells by DNA ploidy. This technique involves comparision of the test group with controls consisting of normal epithelial cells following staining with Feulgen dye. A computer assisted programme identifies the deviations in the cellular DNA content thereby giving the results. This method has 100% sensitivity and specificity [25].

Molecular analysis

Oegden et al evaluated the role of immunohistochemistry in Smears from Oral Squamous cell carcinoma by directing antibodies against 8 and 19. However this technique demonstrated low sensitivity [26].

Combining this technique with liquid based cytology helped in visualization of malignant cells using antibodies against cytokeratin AE1 and AE3. Mutations in p53 gene and demonstration of other essential protein structures in DNA replication – the minin chromosome maintenance proteins helps in determination of the tumour prognosis [27].

Nuclear organizer regions (NOR) measures the cellular proliferation and thereby differentiates a reactive lesion from nonneoplastic lesion. This technique has limitations due to feasibility though it has a superior role in assessing the proliferation rates [28]. A combined method of cytogenetic FISH and cytomorphometric analysis has high specificity for predicting the natire of the lesion [29]. Other studies include the increased expressions of extracellulat matrix proteins namely gamma 2-chain of laminin-5 and high molecular tenascin C for determination of oral cancer [30]. They have a vital role in the cascade of invasion and metastasis of oral squamous cell carcinomas.

Protein- Chip arrays (SELDI) is a recent technique of monitoring oral lesions based on expression of protein levels. The use of exfoliative cytology has also extended into determination of aberrant promoter hypermethylation in exfoliated cells [31].

The use of toluidine blue (tolonium chloride) as a diagnostic aid for the detection of oral cancer has been evaluated in a large number of studies over many decades. It has also been suggested that TB may provide information on lesion margins, accelerate the decision to biopsy, and guide biopsy site selection and the treatment of oral potentially malignant and malignant lesions. Based on data available up to 1989, a meta-analysis assessing the effectiveness of TB for identifying oral squamous cell carcinomas, revealed a sensitivity between 93% and 97% and a specificity between 73% and 92%[18].

CONCLUSION

Oral cytology plays a major role in preventing misdiagnosis of lesions which are interpreted clinically. It is a simple chairside test which provides as a predictive diagnosis. However its limited sensitivity and specificity and high negative values are its limitations. However improved accuracy is obtained by combining cytology with computer assisted morphometry. This method also serves to be used a regular chair monitoring tool in patients who need a routine follow up to diagnose any changes in the early stages of development.

REFERENCES

1. Perez-Sayans M, Somoza-Martin JM, Barros-Angueira F, Reboiras-Lopez MD, Gandara-Vila P, Gándara-Rey JM. Exfoliative cytology for diagnosing oral cancer. Biotech Histochem 2009;25:1–11.
2. Beale LS. Examination of sputum from case of cancer of the pharynx and adjacent parts. Arch Med 1860; 2: 44–6.
3. Mehrotra R, Gupta A, Singh M, Ibrahim R. Application of cytology and molecular biology in diagnosing premalignant or malignant oral lesions. Mol Cancer 2006;5:11.
4. Spivavack SD, Hurteau GJ, Jain R, Kumar SV, Aldous KM, Gierthy JF, et al. Gene– environment interaction signatures by quantitative mRNA profiling in exfoliated buccal mucosal cells. Cancer Res 2004;64:6805–13.
5. Papanicolaou GN, Traut HF. Diagnosis of uterine cancer by the vaginal smear. The Commonwealth Fund, New York, 1943; S1–47.
6. Papanicolaou GN. A survey of the actualities and potentialities of exfoliative cytology in cancer diagnosis. Ann Intern Med 1949; 31: 661–74.
7. Ziskin DE, Moulton R. A comparison of oral and vaginal epithelial smears. J Clin Endocrinol 1948; 8: 146–65.
8. Morrison LF, Hopp ES, Wu R. Diagnosis of malignancy of the nasopharynx. Cytological studies by the smear technic. Ann Otol Rhinol Laryngol 1949; 58: 18–32.
9. Cawson RA. The cytological diagnosis of oral cancer. Br Dent J 1960; 19: 294–8.
10. Caulder SL. Fluorescence microscopy utilizing acridine orange in oral cytodiagnosis. Oral Surg 1967; 23: 343– 50.

11. Abdel-Salam M, Mayall BH, Hansen LS, Chew KL, Greenspan JS. Nuclear DNA analysis of oral hyperplasia and dysplasia using image cytometry. J Oral Pathol 1986; 16: 431–5.
12. Sandler HC. The cytologic diagnosis of tumors of the oral cavity. Acta Cytol 1964; 8: 114–20.
13. S.E. Weiguma,, S.W. Redding, C. Yeh, H.S. McGuff,N. Vigneswaran, J.T. McDevitt. Lab-on-a-chip sensor for analysis of cellular biomarkers in oral exfoliative cytology: A new diagnostic tool for early detection of oral cancer 2009; 06: 251
14. N. Sahara, N. Okafuji, A. Toyoki, Y. Ashizawa, H. Yagasaki, T. Deguchi et al. A Histological Study of the Exfoliation of Human Deciduous Teeth. Journal of Dental Research 1993; 72; 634.
15. G.R. Ogden. The Future Role for Oral Exfoliative Cytology-Bleak or Bright? Oral Oncology, 1997; 33:2-4.
16. Jones AC, Pink FE, Sandow PL, Stewart CM, Migliorati CA, Baughman RA. The cytobrush plus cell collector in oral cytology. Oral Surg Oral Med Oral Pathol 1994; 77: 101–4.
17. Walling DM, Flaitz CM, Adler-Storthz K, Nichols CM. A non-invasive technique for studying oral epithelial Epstein–Barr virus infection and disease. Oral Oncol 2003; 39: 436–44.
18. Ajay Reginald, B. Sivapathasundharam. Oral hairy leukoplakia: An exfoliative cytology study. Contemporary clinical dentistry 2010; 1;1:10-13.
19. Devendra H Palve, Jagdish V Tupkari. Clinico-pathological correction of micronuclei in oral squamous cell carcinoma by exfoliative cytology. Journal of oral and maxillo facial pathology2008;12(1): 2-7.
20. Hayama FH, Motta ACF, de Padua AGS, Migliari DA. Liquid-based preparations versus conventional cytology: specimen adequacy and diagnostic agreement in oral lesions. Med Oral Pathol Oral Cir Bucal 2005; 10: 115–22.
21. Hullmann M, Reichert TE, Dahse R, et al. Orale Zytologie: Historische Entwicklung, aktueller Stand und Ausblick. Mund Kiefer Gesichtschir 2007; 11: 1–9.
22. Navone R, Burlo P, Pich A. The impact of liquidbased oral cytology on the diagnosis of oral squamous dysplasia and carcinoma. Cytopathology 2007; 18: 356–60.
23. Scheifele C, Schmidt-Westhausen AM, Dietrich T, Reichart PA. The sensitivity and specificity of the OralCDx technique. Oral Oncol 2004; 40: 824–8.
24. Sciubba JJ. Improving detection of precancerous and cancerous oral lesions. Computer-assisted analysis of the oral brush biopsy. US Collaborative OralCDx Study Group. J Am Dent Assoc 1999; 130: 1445–57.
25. Remmerbach TW, Mathes SN, Weidenbach H, Hemprich A, Bo¨cking A. Nichtinvasive Bu¨ rstenbiopsie als innovative Methode in der Fru¨ herkennung des Mundho ¨ hlenkarzinoms. Mund Kiefer Gesichtschir 2004; 8: 229– 36.
26. Ogden GR, Cowpe JG, Chisholm DM, Lane EB. DNA and keratin analysis of oral exfoliative cytology in the detection of oral cancer. Eur J Cancer B Oral Oncol 1994; 30B: 405–8.
27. Lopez M, Aguirre JM, Cuevas N, et al. Use of cytological specimens for p53 gene alteration detection in oral squamous cell carcinoma risk patients. Clin Oncol 2004; 16: 366–70.
28. Remmerbach TW, Weidenbach H, Mu¨ ller C, et al. Diagnostic value of nucleolar organizer regions (AgNORs) in brush biopsies of suspicious lesions of the oral cavity. Anal Cell Pathol 2003; 25: 139–46.
29. Hirshberg A, Yarom N, Amariglio N, et al. Detection of non-diploid cells in premalignant and malignant oral lesions using combined morphological and FISH analysis – a new method for early detection of suspicious oral lesions. Cancer Lett 2007; 253: 282–90.
30. Driemel O, Dahse R, Berndt A, et al. High-molecular tenascin-C as an indicator of atypical cells in oral brush biopsies. Clin Oral Invest 2007; 11: 93–9.
31. Driemel O, Murzik U, Escher N, et al. Protein profiling of oral brush biopsies: S100A8 and S100A9 can differentiate between normal, premalignant and tumour cells. Proteomics Clin Appl 2007; 1: 486–93.