IJCRR
Section: Healthcare
Sci. Journal Impact
Factor: 6.1 (2018)
ICV: 90.90 (2018)

Chronicles of Cytopathology-A Review

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ABSTRACT

The review gives brief information on the olden days of cytology beginning from its origin to recent modern advancements. It also gives an outline regarding the basics of cytology. The field of cytology has achieved a marvelous drive all across the world owing to its rapidity, accurateness, and cost-efficacy. The literature was searched using MEDLINE/PubMed to identify relevant articles published in English from the date of inception to date. A brief history of cytology, newer cytological methods, modifications in the sampling tools and the sensitivity and specificity of collecting devices are presented in the article. The review will benefit all the dental practitioners facing challenges during performing exfoliative cytology.

Key Words: Cytology, Collecting devices, Cytomorphometry, Cancer

INTRODUCTION

The field of cytology has attained a great amount of drive throughout the nation with respect to its rapidity, reliability, and inexpensiveness. The branch of cytology was identified and practiced in the 18th and 19th centuries.¹ The advancement of this branch was not standardized until the 20th century. In 1989, the first American Board of examination in the field of cytopathology has commenced.² The field of cytopathology is well established with two main branches exfoliative cytology and fine-needle aspiration biopsy.² The study of exfoliated cells from pre-malignant and malignant lesions of cervical mucosa by Papanicolaou and Traut has strongly swayed the way for Oral cytology.¹ The worth of using a microscope in the 19th century was recognized by Pathologists Italian Morgagni, the British Baillie, and the French Bichat.¹ In the sputum of oropharyngeal carcinoma, the architecture of abnormal cells was first identified in the 19th century. Exfoliative cytology in the oral cavity was

first identified by Montgomery and Von Ham.³ The purpose of performing Cytology is to reach at an accurate diagnosis, screening and follow up. The Advantages are a simple, safe and quick method of collection. It is cost-effective and gives accurate results.⁴ Cytopathological Features of malignancy include cellular pleomorphism, nuclear pleomorphism, altered Nuclear/Cellular ratio, hyperchromatism, enlarged nucleoli, increased and abnormal mitotic activity.⁴ There were many contributions and publications in the field of Cytology (Table 1).

With many breaks and deviations after an arduous 150 years, cytology has become a subspecialty of pathology in the last 25 years. In an attempt to improve the quality of smears and staining of cells, various new tools for sampling with modifications were introduced. The sensitivity and specificity of the tools were identified. (Table 2 & Table 3).

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ISSN: 2231-2196 (Print)

ISSN: 0975-5241 (Online)

Received: 31.03.2020

Revised: 30.04.2020

Accepted: 18.05.2020

Table 1: Contributions and Publications in the field of Cytology

Years	Year	Author	Subject
1830s-1890s	1830	-	Imprint smears and microscopic observation of sediments from body fluids began
	1838	Johannes Muller	First pathologist who identified cancer cells from the cut surfaces of excised mammary carcinoma. ⁵
	1843	Walsh	Identified cancer cells from scrapings of uterine cervical cancer and fistulous parotid tumor. ⁶
	1844	Walsh	Publication in colored prints on Cancerous cells of breast carcinoma, sarcoma of the mandible and soft tissue sarcomas of the leg. ⁷
	1845	Lebert	The cytology atlas of Lebert ⁵
	1846	Paget	Cancer cells were identified in the lung cancer and gastric cancer. ⁸
	1853	Paget	Smears from aspirate of breast cancer were prepared. ⁸
	1856	-	Cancer cells were analysed in samples of urine. ⁵
	1860	Baele	Sputum examination in pharyngeal carcinoma. ⁹
	1869	-	Cancerous cells were identified from Urethra. ¹⁰
	1890	-	Microscopic investigation of urine became a standard method for the diagnosis of urinary bladder tumor. ¹¹
1900s to 1950s	1904	-	Malignant cells were identified from CSF, gastric washings
	1920	-	There came a momentous years and great amount of significance in the field of cytology. ⁵
1950s to 1980s	1928	Papanicolaou	The conclusions at the Third Race Betterment Conference was made. ¹² The results were conferred in the conference proceedings with the title 'New cancer diagnosis'. PAP test for the diagnosis of cancer was described in this paper. ¹³
	1928	Papanicolaou and Babeş	First presented their classic papers on the use of cytologic specimens to diagnose cervical cancer. ⁵
	Mid of 1930s	-	Cytologic examination of all sites of the body was made possible. ¹⁴⁻¹⁹
	1940	Weinmann	Study of oral cellular keratinisation. ²⁰
	1941	Ziskin et al,	Effects of menstrual cycle on oral cellular architecture. ²¹
	1942	Papanicolaou and Traut	Staining procedure for cytologic smears. ²²
	1866-1943	James Ewing	Aspiration cytology was made known. ²³
	1943	Traut and Papanicolaou	Book on 'Diagnosis of uterine cancer by the vaginal smear'. ²⁴
	1948	Papanicolaou and Traut	The epithelia of woman's reproductive organs. ²⁵
	1948	Lombart et al,	Introduced the screening tests of vaginal smear for uterine cervix carcinoma. ⁵
	1949	Morrison et al	Cytological diagnosis of malignancy of nasopharynx. ²⁶
	1950s	Nieburgs and Pund	First result of screening of 10,000 women for occult cancer of the cervix. ²⁷
	1879-1954	Hashime Murayama	The drawings of exfoliated cells were excellently depicted. ⁵
	1956 & 1960	-	Two complementary editions of "The Atlas of exfoliative cytology" was published. ²⁸
	1960s	-	Cytology emerged as a new speciality. ^{29,30}

Table 1: (Continued)

Years	Year	Author	Subject
	1961	Leopold Koss	Text book on cytopathology. ³¹
	1886-1961	Aurel Babes	Published papers on diagnosis of uterine cervical cancers. ¹³
	1883-1962	George Papanicolaou	
	1974	Swedish hematologist-cytologist	Monograph on aspiration cytology was published in cytology. ³²
	1981	Swedish hematologist-cytologist	Guidebook on aspiration cytology. ³³
1980s to Today	By the end of 1980s	-	70% reduction in the mortality from cancer of uterine cervix was recorded in several geographical areas where mass screening was introduced. ³⁴
			Automated screening procedures, monolayer preparations, new sampling instruments such as endocervical brushes were widely developed.

Table 2: Modifications of sampling tools in Exfoliative cytology

Year	Author	Modification
1951	Gladstone ³⁵	Sponge biopsy
1952	Schneider ³⁶	Staining modifications
1960	Cawson ³⁷	Staining modifications
1963	King ³⁸	Metal spatula recommendation
	Staats and Goldsby ³⁹	
1964	Sandler ⁴⁰	Use of sharp curette for removal of keratotic layer
1981	Dumbach et al ³⁶	Smear curettage
1999	Sciubba ⁴¹	Oral brush
2001	Remmerbach et al ⁴²	Conventional oral brush cytology combined with DNA-image-cytometry
2003	Remmerbach et al ⁴³	Conventional oral brush cytology combined with AgNOR-analysis
2007	Gupta et al ⁴⁴	Oral brush cytology with Toluidine blue
2007	Driemel et al ⁴⁵	Conventional oral brush cytology combined with high molecular Tenascin-C antibody
2008	Mehrotra ⁴⁶	Conventional oral brush cytology without computer assistance

Newer cytological methods

The use of Oral brush was recommended for smears of cervical lesions in Gynaecology during the 1980's. This method gave better spreading of exfoliated cells on slides in comparison to smears taken by wooden spatula. The quality and validity of smears were improved by this method.^{47,48} It has an advantage of sampling of deeper cell layers. Thus, the use of a brush was found to be more convenient and accurate when compared to the wooden depressor.

The sample size should be adequate to evaluate the histomorphology of the exfoliated cells. The significance of oral brush cytology was revealed in a multicentre study where smears were taken from 5% of benign lesions using cyto-brush and subsequently dysplastic epithelial changes were confirmed by using scalpel biopsy.⁴⁹ The most representative site should be selected for scalpel biopsy in case of a larger lesion.

Table 3: Specificity and Sensitivity of sampling tools for oral cytology

Collecting device	Specificity	Sensitivity
Cotton tipped applicator ^{50,51}	-	93.8-96%
Wooden spatula ⁵²⁻⁵⁶	88.9-100%	86.5-97.5%
Metal spatula ^{24,57-60}	100%	76.9-100%
Curette ⁶¹	99%	87-100%
Conventional brush cytology ^{42,45,62,63,64}	93.3-100%	76.8-94.6%
Collecting device	Specificity	Sensitivity
Cotton tipped applicator ^{50,51}	-	93.8-96%
Wooden spatula ⁵²⁻⁵⁶	88.9-100%	86.5-97.5%
Metal spatula ^{24,57-60}	100%	76.9-100%
Curette ⁶¹	99%	87-100%
Conventional brush cytology ^{42,45,62,63,64}	93.3-100%	76.8-94.6%

In conventional oral brush cytology, the sensitivity and specificity ranges between 79% & 97%, 95.1% to 99.5% respectively. Though it is a promising method, the specificity and the sensitivity of oral brush cytology is increased but is statistically not significant in comparison with conventional cytology (Table 4). Hence it is essential to utilize the accurate devices in order to enhance the defined specificity and sensitivity of conventional oral brush cytology.⁶⁵

Liquid-based cytology

In the mid-1990's, Liquid based cytology (LBC) was introduced to overcome the drawbacks of conventional Papanicolaou (Pap) smears in the screening of cervical cancer. It basically uses the collection fluid that fixes, homogenizes and rinse the cells.

Liquid based cytology was utilized on smears obtained from the oral cavity taken by cytobrush. It had an advantage of easier diagnosis of abnormal cells, better cell spreading and smear thickness. The specificity of 99.0% and sensitivity of 95.1% was reported after the combination of LBC and cytobrush in Oral squamous cell carcinoma.⁶⁶

Improved analytical methods

Cytomorphometry

OralCDx (Oral cell diagnostics) is a computer assisted method that helps in the study of exfoliated cells collected by brush biopsy. The microscopic image of the cells collected by an image processing system is examined by a computer. This system is planned for the detection of Pre-malignant

and malignant cells. Features of atypia at cellular level are cellular keratinization and alterations in cellular, nuclear morphology. 90% of sensitivity and specificity of OralCDx was found in the lesions tested with the scalpel and brush biopsy.^{41,66}

In many articles, it has been shown that the negative and positive predictive values of OralCDx were considerably greater when compared to the Pap test, mammogram, or prostate specific antigen (PSA).^{41,67,68,69}

OralCDx showed magnificent positive predictive values reported by Kosicki et al. as 42% by Scheifele et al. as 35%⁶⁷, by Poate et al. as 44%.⁶⁸, by Svirsky et al. as 38%⁶⁹ and by Sciubba et al. as 30%⁴¹.

Table 4: Specificity and Sensitivity of oral brush cytology

Method	Specificity	Sensitivity	Author
Computer-assisted analysis of oral brush biopsy	93%	100%	Sciubba ⁴¹
Conventional cytology	99.5%	94.6%	Remmerbach et al ⁴²
DNA aneuploidy by image cytometry	100%	96.4%	
Combination of Conventional cytology and DNA image cytometry	100%	98.2%	
Combination of Conventional cytology and DNA image cytometry	97.4%	100%	Maraki et al ⁷⁰
Combination of Conventional cytology and AgNOR analysis	100%	92.5%	Remmerbach et al ⁴³
Oral CDx technique	94.3%	92.3%	Scheifele et al ⁶⁷
Conventional cytology	100%	79%	Driemel et al ⁶⁵
Laminin-5 immunocytochemistry	98%	93%	
Combination of Conventional cytology and gamma 2-chain of Laminin-5 immunochemistry	100%	93%	
Conventional cytology	96%	78%	Driemel et al ⁴⁵
High molecular Tenascin-C antibody	93%	83%	
Combination of Conventional cytology and high molecular Tenascin-C antibody	99%	95%	
Conventional cytology	95.1%	91.3%	Remmerbach et al ⁶⁴
DNA aneuploidy by image cytometry	100%	97.8%	
Conventional cytology	93.3%	76.8%	Mehrotra et al ⁴⁶

Analysis of DNA

DNA (Deoxyribonucleic Acid) image cytometry is used to measure DNA ploidy to detect the risk of malignancy. The smear samples were compared with normal 300 oral exfoliated cells after Fuelgen staining. DNA content is analyzed via a computer endorsed program. The specificity and sensitivity was increased to 100%.⁷⁰

Molecular analysis

Ogden et al assessed immunohistochemical staining for the diagnosis of oral squamous cell carcinoma. Low sensitivity rates were determined with antibodies against cytokeratin 8 and 19. In liquid-based cytology, the assessment of malignant cells is made possible with antibodies AE1 and AE3 (anti-cytokeratin monoclonal antibodies).⁷¹

p53 (TP53 or tumor protein) suppressor gene mutation can be a useful prognostic marker of Oral cancer.⁷² The differentiation of cellular proliferation between reactive and neoplastic lesions was assessed by Nuclear organizer regions (NOR).⁴³ The cost was comparatively more to use it as a method of diagnosis. The specificity of combined cytogenetic FISH (Fluorescence in situ hybridization) and the cytomorphometric analysis was shown to be increased in determining the innocuous lesions of oral cavity.⁷³

The extracellular matrix proteins Laminin and high molecular weight Tenascin-C (TNC) being highly expressed plays a major role in the carcinogenesis of Oral cancers.^{45,63} The false-negative rate could be lowered by the sensitivity of 93-95% of this method. Protein chip arrays were used for the analysis of Oral brush biopsies of normal, inflammatory,

hyperproliferative and malignant lesions. S100A8 and S100A9 (S100 calcium-binding proteins) are the proteins identified for the diagnosis of oral lesions.⁷⁴

DISCUSSION

Exfoliative cytology is the microscopic examination of exfoliated, shed or desquamated epithelial cells from the surface of the epithelium usually the mucous membrane. It also includes the examination of cells that have been collected from body fluids or by scraping the tissue surface.⁷⁵ The fact behind exfoliative cytology lies in the physiology of the epithelium. The continual exfoliation of epithelial cells is a part of physiological turnover. Deeper cells strongly adhere to each other with the help of the attachment apparatus in normal conditions. In case of any abnormal pathology or in case of malignancy, the cells lose their adhesion and exfoliate along with the superficial cells of the epithelium. It was only in very recent years, the concept of screening and early detection of cancer has gained enormously wide acceptance. The concept was discussed in detail by Cochrane and Holland.⁷⁶ To date, mass screening programs only for cervical cancer have been successful. In a country like India where usage of tobacco is very high, mass screening programs must be adopted as a part of national health policy. The search engines reveal a large number of newly introduced techniques that can be performed on oral exfoliated cells like liquid-based cytology, molecular analysis via immunohistochemistry, polymerase chain reaction, flow cytometry, image analysis, neural networking, southern blotting, interphase cytogenetics etc.

CONCLUSION

The branch of Oral cytology has emerged a long way around from its Papanicolaou days. It plays the major role in avoiding misdiagnosis of clinically interpreted lesions. Early screening and detection of potentially malignant conditions have a better prognosis. However surgical biopsy followed by a histopathological diagnosis remains the Goldmark. Recently, a large number of novel techniques have been introduced that help in the early detection of potentially malignant lesions and oral cancer. These newer techniques appear promising and could contribute to the change in the paradigm of oral cancer diagnostics.

ACKNOWLEDGEMENT

I am grateful to all the authors/editors/publishers whose articles are cited and included in references of this manuscript.

Source of Funding- Nil

Conflict of Interest- Nil

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